Chapter 5
Hyperlipidemias and Obesity

The organs require oxygen and other types of nutrients (amino acids, sugars, and lipids) to function, the heart consuming large amounts of fatty acids for oxidation and adenosine triphosphate (ATP) generation.

In the postprandial period, adipocytes take up fatty acids delivered by chylomicrons and lipoproteins synthesized in the gut and liver, which then form triglycerides (TGs). TGs are stored in lipid droplets (LDs) in an inert healthy form.

Adipocytes can also synthesize fatty acids from excess circulating glucose using acetyl-CoA carboxylase, although the liver is the major site of conversion of carbohydrates to lipids.

In the postprandial period, insulin promotes glucose and fatty acid uptake in addition to lipogenesis and suppresses lipolysis. Conversely, secretion of adipokines and lipokines by adipocytes contributes to metabolism regulation. Regulators secreted by the adipose tissue (AT) have local effects, that is, target adipocytes and vascular cells, along with a global systemic impact.

During periods of restricted nutrient supply and prolonged physical activity, organismal survival and muscular activity depend on the ability of adipocytes to process excess triacylglycerols temporarily stored in LDs. Lipolysis by adipose triglyceride lipase (ATGL; or patatin-like phospholipase PnPLA2), hormone-sensitive lipase (lipase-E; HSL), and monoglyceride lipase releases fatty acids and glycerol.

Fatty acids are conveyed in the bloodstream in an esterified form as TGs in lipoproteins and unesterified form bound to albumin. Esterified fatty acids are liberated by lipoprotein lipase on the luminal side of the vascular endothelium. Fatty acids are also carried by lymph. They are then used for ATP synthesis, whereas glycerol can serve as a substrate for gluconeogenesis in the liver [717].

Uptake of circulating lipids, lipogenesis, and lipolysis and hence adipocyte metabolism depend on the balance between anabolism and catabolism. These processes are regulated by endocrine messengers and the sympathetic nervous system.

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When nutrient intake exceeds energetic expenditure during a prolonged period, the AT, liver, and muscle store lipids and engender adipose tissue growth, insulin resistance, and persistent hyperglycemia. Adipose tissue grows by hyperplasia (cell number increase) and hypertrophy (cell size increase). Excess food intake and the modern Western diet, which is rich in carbohydrates (high-carbohydrate diet) and lipids (cholesterol and saturated fatty acids in addition to mono- [MUFA] and polyunsaturated fatty acids [PUFA] connected to hydrogen atoms; high-fat diet [HFD]), that is, high-carbohydrate, high-fat diet enriched in caloric drinks and alcohol overconsumption coupled with a sedentary lifestyle, engender obesity.

On a microscopic scale, chronic intake of excess nutrients enlarges LDs. On a mesoscopic scale, it causes adipocyte hypertrophy and, on a macroscopic scale, a gradual increase in weight.

Products of adipocytic lipid metabolism, *lipokines*, act as beneficial endocrine regulators. In particular, both palmitoleate (C16:1) and palmitic acid–hydroxystearic acid, formed in adipocytes during lipogenesis, can improve systemic glucose metabolism [717].

On the other hand, impaired TG storage in overloaded adipocytes is associated with constitutive fatty acid mobilization, reduced glucose uptake, and lipogenesis, in addition to generation of lipotoxic diacylglycerol and ceramides, which can accumulate in remote organs.

Adipocyte hypertrophy correlates with dyslipidemia, inflammation, and impaired glucose homeostasis in humans, whereas adipocytes are smaller in *metabolically healthy obese* individuals (without metabolic disease) [717]. On the other hand, limited storage capacity and a concomitant increase in ectopic lipidic depots are major contributors to metabolic disease genesis.

Various intermediates of fatty acid metabolism engender *lipotoxicity* in adipocytes in addition to myocytes, hepatocytes, and immunocytes [717]. In particular, diacylglycerol can activate some PKC subtypes, thereby hindering insulin signaling. In addition, saturated fatty acids connect to toll-like receptor TLR4, thereby favoring *inflammation* and *insulin resistance* (InsRce). Ceramides can interfere with insulin signaling and mitochondrial oxidation, provoking endoplasmic reticulum (ER) stress and apoptosis.

Hyperlipidemia,[1] the most common form of *dyslipidemia*,[2] comprises:

- Pure *hypercholesterolemia*
- Isolated *hypertriglyceridemia*
- Mixed hyperlipidemia (combined hypercholesterolemia and triglyceridemia)

Obesity is often associated with insulin resistance and elevated insulin secretion from pancreatic β cells. Hypertrophic adipocytes have a reduced autonomous ability for insulin-stimulated glucose uptake. The lipid mediator leukotriene-B_4 and macrophage-derived galectin-3 are implicated in obesity-linked systemic insulin

[1] That is, high blood concentrations of lipids and cholesterol.

[2] That is, abnormal blood concentrations of lipids and lipoproteins.

resistance [717]. Hyperglycemia is the consequence of the absence of compensation of insulin resistance by increased glucose-stimulated insulin secretion.

The energy sensor AMPK promotes glucose uptake independently of insulin. This kinase constitutes 12 distinct complexes. A potent allosteric activator of all 12 AMPK complexes provokes a robust, sustained insulin-independent glucose uptake and glycogen synthesis in skeletal muscles but causes cardiac hypertrophy and an increased amount of cardiac glycogen [718].

The TORC1–S6K1 axis and its effectors, such as glutamyl prolyl tRNA synthetase (EPRS), are related to metabolism contributing to adiposity and aging [719].[3] Upon phosphorylation (Ser999) by S6K1, EPRS is released from the aminoacyl tRNA multi-synthase complex for execution of tasks other than protein synthesis. In adipocytes, insulin stimulates EPRS phosphorylation. Phosphorylated EPRS binds to SLC27a1 (or fatty acid transport protein FATP1) and provokes its translocation to the plasma membrane for long-chain fatty acid uptake.

Obesity raises the risk for metabolic and cardiovascular disease (CVD). However, excess AT in the trunk (*android obesity*), a compartment of TG-storage white adipose tissue (WAT), exerts more deleterious metabolic effects than fat depots in the limbs (*gynoid obesity*). Furthermore, among subcutaneous (scAT), subfascial, and visceral adipose tissue (vAT), which comprises omental, mesenteric, and peri-organ adipose compartments, omental and mesenteric fat depots drained by the portal circulation have the strongest metabolic effects [720].[4] In addition, local obesity around large blood vessels, perivascular adipose tissue (pvAT), including the perivascular compartment of epicardial adipose tissue (eAT), is involved in cardiovascular system regulation and pathogenesis.

Obesity is associated with a shift from secretion by the AT of vasodilators to release of the vasoconstrictors and promoters of cell proliferation and migration and inflammation. These agents can cause *endothelial dysfunction*, which is reversible by weight loss, which also lowers AT-related inflammation and raises NO availability. In obesity, the endothelium loses its control of the vasomotor tone, vascular smooth myocyte (vSMC) proliferation, blood coagulation, and inflammation, thereby favoring adverse vascular remodeling.

[3] Aminoacyl–tRNA synthetases charge tRNAs with their cognate amino acids, a first step in mRNA translation. At least one synthetase exists for each amino acid. Glu/Pro-tRNA synthetase (GluProRS) is also named bifunctional glutamate– and proline–tRNA ligase and prolyl–tRNA synthetase (PARS). It catalyzes the attachment of the cognate amino acid to the corresponding tRNA. It is also a component of the γ-interferon-activated inhibitor of translation (GAIT) complex that represses Ifnγ-induced translation of diverse selective mRNAs in inflammation [108, 194].

[4] Adipocytes linked to the portal circulation are exquisitely sensitive to stimuli that mobilize free fatty acids (FFAs) owing to a high number of β-adrenoceptors and relatively weak inhibition by α-adrenoceptors.

Exposure of the liver to elevated FFA concentrations increases synthesis and secretion of very low-density lipoproteins (VLDLs) and stimulates gluconeogenesis. It can also cause hepatic insulin resistance and reduce hepatic clearance of insulin [720].

The grade of obesity is currently assessed according to the *body mass index* (BMI) in children and adults [226]:

- Normal weight (BMI = 18.5–24.9 kg/m^2; 5th–<85th percentile in children)
- Overweight (BMI = 25.0–29.9 kg/m^2: 85th–<95th percentile in children)
- Obesity (BMI>30.0 kg/m^2; ≥95th percentile in children) with several categories:
  - Grade-*I* (mild) obesity (BMI = 30.0–34.9 kg/m^2)
  - Grade-*II* (moderate) obesity (BMI = 35.0–39.9 kg/m^2)
  - Grade-*III* (severe) obesity (BMI > 40 kg/m^2)

This index is a height-normalized body mass (body mass [kg] divided by squared height [m]), that is, the sum of body fat and fat-free mass. Obese individuals have both high levels of fat and fat-free mass [721]. This index is thus not an index of adiposity, but instead an index of cardiometabolic risk. Higher fat-free mass is associated with a greater blood volume and hence cardiac load in obese individuals.

Abdominal obesity is defined according to waist circumference (men >94 or 102 cm and women >80 or 88 cm according to national health agencies). Over the past few decades, abdominal obesity has augmented significantly in both sexes.

In CVD and type-2 diabetes mellitus (T2DM), the BMI-related *obesity paradox*[5] refers to overweight patients who have lower cardiovascular morbidity with respect to lean individuals, although obesity is a major risk factor of CVD. Abdominal (omental) adiposity assessed by waist circumference (in men >102 cm and women >88 cm) and waist-to-hip ratio (in men and women ≥0.90 and 0.85, respectively) usually weaken the survival rate with respect to overweight in other body compartments. Overweight and obese people have a higher rate of cardiac events than individuals with a normal weight, but overweight metabolically healthy (and not obese) people can have a higher survival rate than underweight subjects. An inverse correlation may be observed between mild adiposity and cardiorespiratory fitness in subjects with a higher exercise capacity who do not have other risk factors, such as insulin resistance and hypertension. A BMI–mortality plot is U- or J-shaped with a minimum close to a BMI of 25 kg/m^2. However, other types of diseases can explain this observation. Moreover, in aged patients (62–66 years), BMI is a better indicator of lean body mass than adiposity [723]. When data supporting the obesity paradox are adjusted by cardiorespiratory fitness, the paradoxical association between BMI and mortality is blunted [724]. The obesity paradox is questionable because of the insufficient control of cardiorespiratory fitness, inadequate determination of AT location, among other sources of bias.

Low-risk adiposity may be related to a proper adiposecretome, regional AT distribution, and adipocyte turnover rate, in addition to the type of AT expansion (hyperplasia versus hypertrophy), matrix quality without adverse remodeling, angiogenic potential, adipocyte browning capacity, and macrophage density [722].

[5] When other obesity indices are used, the concept of obesity paradox disappears [722].

In any case, weight reduction in CVD patients improves insulin sensitivity, blood pressure, lipidemia, reactivity of platelets, and endothelial function. In addition to weight loss, physical exercise and adequate diet enable prevention of CVDs in obesity.

Exercise training provokes secretion of *myokines* from skeletal muscles and *cardiomyokines* from the myocardium, such as FGF21 and irisin, which act on pvAT and eAT.

5.1 Classification and Etiology of Dyslipoproteinemia

Lipoproteins carry in the bloodstream water-insoluble TGs and cholesterol. They possess a core of hydrophobic cholesterol esters and TGs surrounded by a hydrophilic monolayer of phospholipids, free cholesterol, and apolipoproteins (Apos).

The lipoprotein types include chylomicron; chylomicron remnant; very-low- (VLDL), intermediate- (IDL), low- (LDL), and high-density lipoprotein (HDL); and lipoprotein-A (LPa), which are composed of distinct types of apolipoproteins. Their density depends on their content in TGs and cholesterol. Chylomicrons, chylomicron remnants, VLDLs, and IDLs are rich in both TGs and cholesterol, whereas LDL, HDL, and LPa mainly contain cholesterol and have a higher density.

Lipoproteins are produced by exo- and endogenous pathways:

1. The exogenous pathway is related to chylomicrons produced by enterocytes. Chylomicrons are converted to chylomicron remnants by TG lipolysis in plasma.
2. The endogenous pathway is related to VLDLs assembled in hepatocytes. They are converted to IDLs and LDLs by TG lipolysis on the wetted surface of the capillary endothelium and in the vascular lumen and by exchange of lipids and apolipoproteins with other lipoproteins.

After secretion of chylomicrons from the intestine and VLDLs from the liver, both types of lipoproteins are enriched in ApoE during their degradation to remnants [725]. Apolipoprotein-E, a ligand for receptors LDLR and LRPs, enables, at least partly, remnant uptake in the liver.

Remnant lipoproteins can accumulate in the arterial wall and cause inflammation, especially in patients with familial dysbetalipoproteinemia who have a 7.7- to 14.3-fold increased remnant cholesterol concentration [726]. Monocytes have a higher content of LDs and a greater surface concentration of integrins.

On the other hand, HDLs have an anti-inflammatory, antioxidant, antithrombotic, and vasorelaxant action and promote the reverse cholesterol transport (RCT) from atherosclerotic lesions.

According to the National Center for Biotechnology Information, dyslipidemias, a lipid overproduction or deficiency with abnormal serum lipid profiles including high concentrations of total cholesterol (CS), TGs, LDL^{CS}, and low concentrations of HDL^{CS}, include:

Hyperlipidemias that comprise:

1. *Hypercholesterolemia* (blood cholesterol concentration exceeding the 95th percentile for the population
2. *Familial combined hyperlipidemia* (hypercholesterolemia and hypertriglyceridemia) engendered by multiple gene defects
3. *Hyperlipoproteinemias*
4. *Hypertriglyceridemias*, among which

   - Type-4 hyperlipoproteinemia
   - Type-5 hyperlipoproteinemia
   - Hypertriglyceridemic waist (elevated waist circumference and fasting blood concentrations of TGs)

Hypolipoproteinemias (abnormally low blood concentrations of lipoproteins)[6] with

1. *Hypoalphalipoproteinemia* (low blood HDL concentration), which can be associated with mutations in the genes encoding apolipoprotein-A1, lecithin–cholesterol acyltransferase (LCAT),[7] and ATP-binding cassette transporters
2. *Hypobetalipoproteinemia* (low blood LDL concentration $\leq$ the 5th percentile for the population) consisting of autosomal dominant disorder linked to APOB gene mutations and autosomal recessive disorder resulting from mutation of the gene encoding microsomal TG transfer protein

Smith–Lemli–Opitz syndrome, an autosomal recessive disorder of cholesterol metabolism caused by a deficient 7-dehydrocholesterol reductase

Dyslipoproteinemias are inherited (primary) or acquired (secondary).

Primary dyslipoproteinemias can be genetic or a result of a defect in lipoproteins (Sect. 7.4). Behavioral factors (e.g., diet, alcohol, and drugs) can contribute to primary dyslipoproteinemia.

Secondary dyslipoproteinemias are provoked by sedentary lifestyle with excessive food intake. They can be caused by diseases such as diabetes, hypothyroidism, chronic kidney disease, pancreatitis, cholestatic liver disease, dysglobulinemia, and autoimmune hyperlipoproteinemia, in addition to some drugs. Low HDL concentrations can result from smoking, anabolic steroids, and

[6] Whereas the HDL1 subtype is not associated with CVD risk, concentrations of large and more buoyant HDL2 and small and dense HDL3 significantly decrease in coronary artery disease [727].

[7] Lecithin–cholesterol (phosphatidylcholine–sterol) acyltransferase is a major determinant of the metabolism of plasmatic lipoproteins. It is synthesized mainly in the liver and secreted into plasma, where it converts cholesterol and phosphatidylcholine (PC; lecithin) to cholesteryl esters and lysophosphatidylcholine (LPC) on the surface of HDLs and LDLs. The reciprocal transfer of cholesterol esters and TGs among lipoproteins relies on cholesterol ester transfer protein, which enables movement of cholesteryl ester from HDLs to TG-rich VLDLs and the equimolar motion of TGs from VLDLs to HDLs.

nephrotic syndrome. On the other hand, intestinal postprandial overproduction of atherogenic chylomicrons is observed in diabetic dyslipidemia with insulin resistance.

Acquired combined hyperlipidemia is common in patients with the metabolic syndrome.

Treatment is aimed at correcting levels of LDL^{CS}, TGs, and HDL^{CS} to achieve a reduction in CVD risk. Combination therapy associating statins with niacin, fibrate, and ω3-fatty acids, among other drugs, has been proposed, especially in patients with mixed dyslipidemia and insulin resistance.

5.1.1 Hypercholesterolemia

Hypercholesterolemia is a major cardiovascular risk factor. Atherosclerosis is initiated by retention and accumulation within the arterial intima of cholesterol-rich ApoB+ lipoproteins, that is, LDLs (∼90% of circulating ApoB+ lipoproteins during fasting) and other species (diameter <70 nm), such as VLDLs, VLDL remnants, IDLs, and LPa [225].[8] Lowering blood cholesterol concentration in people with moderate to high cardiovascular risk prevents myocardial infarction and stroke [30]. A rise in total cholesterol from 5.2 to 6.2 mmol/l (200 to 240 mg/dl) in young men (18–39 years old) is associated with a two- to three-fold increased risk of death from a CVD [728].

On the other hand, plasmatic HDL^{CS} concentration is inversely correlated with CVD. However, extremely high and low HDL^{CS} concentrations aggravate CVD [729]. The HDL^{CS} concentration with the lowest mortality is 1.9 mmol/l (73 mg/dl; 1.4–2.0 mmol/l [54–77 mg/dl]) in men and 2.4 mmol/l on average (93 mg/dl; 1.8–2.5 mmol/l [69–97 mg/dl]) in women.

The liver responds to dietary cholesterol uptake and facilitates the release and clearance of lipoproteins. Persistent hypercholesterolemia elevates the intrahepatic regulatory T cell population and expression of TGFβ1 in the liver [730]. Moreover, hypercholesterolemia supports differentiation of intrahepatic T_{H17} cells in mice.

5.1.1.1 Lysosomal Acid Lipase (Lipase-A)

Lysosomal acid lipase, or lipase-A, which is encoded by the LIPA gene at chromosomal site 10q23, hydrolyzes cholesteryl esters and TGs derived from lipoproteins taken up by cells in the lysosome into free cholesterol, glycerol, and FFAs.

[8]Concentrations of circulating ApoB+ lipoproteins rely on the calculation of ApoB content in nanomoles per liter using 500,000 as the molecular mass, that is, for LDL 100 mg/dl (2000 nmol/l); VLDL 5 mg/dl (100 nmol/l); IDL remnants 5 mg/dl (100 nmol/l); and LPa 10 nmol/l [225].

Excess intracellular free cholesterol concentration is cytotoxic; it is avoided by increased cholesterol efflux to ApoA1, the major structural protein of HDL, and re-esterification of free cholesterol to form LDs.

Loss-of-function mutations in LIPA cause rare Mendelian disorders, such as the *Wolman disease* and *cholesteryl ester storage disease* (CESD). The LIPA mutations in CESD are linked to hyperlipidemia and early atherosclerosis [731]. The decayed LipA activity can impair ABCa1 expression in response to LDL loading, diminishing cholesterol efflux [732].

The more common LIPA rs1051338 variant is associated with risk for coronary artery disease. It encodes a LOF Thr16Pro change within the LipA signal peptide, which is necessary for the correct targeting to specific organelles. This single nucleotide polymorphism impairs LipA translocation from the ER, hence reducing lysosomal LipA concentration, as it raises proteasomal degradation at least in macrophages, thereby lowering cholesterol efflux to ApoA1 [732].

5.1.1.2 Prolyl Hydroxylase

In $Ldlr^{-/-}$ mice, plasmatic cholesterol is mainly carried by IDLs and LDLs. In $Phd1^{-/-}$ $Ldlr^{-/-}$ mice, plasmatic VLDL and LDL concentrations and leukocyte density decline, normalizing hypercholesterolemia, owing to enhanced cholesterol excretion by feces [733]. Furthermore, atherosclerotic plaque development is stopped.

Deficiency in the oxygen sensor, prolyl hydroxylase ^{HIF}P4H1[9] not only lowers LDL^{CS} concentration but also reprograms cellular metabolism to glycolysis and reduces circulating density of immunocytes and glucose intolerance [733].

In atherosclerotic plaques, Phd1 mRNA level does not correlate with lesion features (lipid core size, intraplaque hemorrhage, necrotic core T-cell density, or capillary number). Hence, systemic, rather than plaque-derived ^{HIF}P4H1, regulates cholesterolemia.

5.1.1.3 ATP-Binding Cassette Transporter ABCa1

The ATP-binding cassette transporter ABCa1 is the main protein responsible for cellular cholesterol efflux. Its amount and function at the plasma membrane is regulated both transcriptionally and post-translationally. Endocytosis of ABCa1 leads to its degradation or recycling back to the plasma membrane.

[9] A.k.a. prolyl hydroxylase domain-containing protein PHD1. In normoxia, PHDs hydroxylate HIF1α and HIF2α, which are then ubiquitinated by von Hippel–Lindau protein for proteasomal degradation. They hydroxylate the HIF subunit with different affinities, ^{HIF}P4H1 targeting preferentially HIF2α.

The ABCa1 carrier enables HDL genesis in hepatocytes. ABCa1 is phosphorylated by the long form of the PIM1 kinase ($PIM1_L$), hence promoting its location in the plasma membrane and ApoA1-mediated cholesterol efflux, facilitating the NR1h2–ABCa1 interaction, thereby stabilizing ABCa1 and preventing ABCa1 ubiquitination and subsequent lysosomal degradation [734].

In macrophages, the principal pathway responsible for ABCa1 internalization and degradation depends on the small ARF6 GTPase [735]. Inhibition of ARF6 shifts ABCa1 into recycling endosomes, thereby enhancing efflux of intracellular cholesterol. On the other hand, ABCa1 recycling and cholesterol efflux is predominantly controlled by an ARF6-independent mechanism.

A pool of extracellular cholesterol microdomains is deposited by CS-enriched $ABCA1^{+/+}$ macrophages and mobilized by ABCa1 and ApoA1, ABCA1 complexing ApoA1 with phospholipid, a CS solubilizer [736]. On the other hand, cholesterol microdomains deposited by $ABCA1^{-/-}$ macrophages are mobilized by apolipoprotein-A1 complexed by sphingomyelin.

5.1.1.4 ATP-Binding Cassette Transporter ABCa8

High-density lipoproteins facilitate removal of cholesterol from cells. Among regulators of cholesterol efflux, heterozygous ABCA8 SNPs (P609R and T741X) are associated with low HDL^{CS} concentration [737]. In $ABCA8B^{-/-}$ HFD-fed mice, HDL^{CS} concentration is significantly lower than that in wild-type (WT) mice. On the other hand, hepatic overexpression of human ABCa8 in mice markedly raises plasmatic HDL^{CS} concentration and the first steps of macrophage-to-feces RCT.

Overexpression of standard ABCa8 (but not variant) significantly increases (∼2-fold) cholesterol efflux to ApoA1 in vitro. ABCa8 co-localizes and interacts with ApoA1, potentiating ApoA1-mediated cholesterol efflux.

5.1.2 Hypertriglyceridemia

Triglyceride serves as an energy source. It is not directly atherogenic. Triglyceride-rich lipoproteins (TGRLs) (e.g., chylomicrons and VLDLs) favor atherogenesis via their conversion to cholesterol-enriched remnant particles.

Unlike cholesterol, TGs can be degraded in most cell types. Triglycerides carried by chylomicrons and VLDLs secreted from the intestine and liver, respectively, are partly degraded by lipoprotein lipase (LPL).

Triglyceridemia[10] is another independent predictor of CVD. Concentrations of TG-rich lipoproteins are elevated in obesity and type-2 diabetes mellitus (T2DM).

[10] That is, plasmatic TG concentration.

Postprandial hypertriglyceridemia correlates with a cardiovascular risk rather than fasting hypertriglyceridemia, owing to the huge interindividual variability of triglyceridemia.

In individuals with a nonfasting TG concentration of 6.6 mmol/l (580 mg/dl), the CVD risk rises to 5.1-fold for myocardial infarction, 3.2-fold for ischemic heart disease, and 3.2-fold for ischemic stroke with respect to subjects with a TG concentration of 0.8 mmol/l (70 mg/dl) [738].

- Mild-to-moderate hypertriglyceridemia is defined as a concentration range of 2–10 mmol/l (176–880 mg/dl). It is linked to obesity and T2DM, in addition to genetic variations and alcohol intake. Treatment is recommended.
- In severe hypertriglyceridemia (>10 mmol/l [>880 mg/dl]), proper drugs should be administered. It results from severely dysregulated diabetes, alcoholism, and rare cases of homozygous mutations in some genes (Sect. 7.4).

The transcription factor CREB3L3,[11] which may act during ER stress via activation of unfolded protein response target genes, is a determinant of TGs in humans. Rare loss-of-function mutations in the human CREB3L3 gene are associated with hypertriglyceridemia [286].

5.1.2.1 Apolipoprotein-C3

ApoC3 is a VLDL constituent and hence a regulator of TG and TGRL metabolism. In fact, ApoC3 resides on the surface of TGRLs, mainly VLDLs and, to a lesser extent, IDLs. In humans, ApoC3 is much smaller than the other major apolipoproteins regulating lipoprotein metabolism (ApoA1, ApoB100, and ApoE) [739].

In the human genome, the APOA1, APOC3, and APOA4 genes are tandemly organized in a short region on the chromosomal 11q23-q24 region. The APOA1 and APOA4 genes are transcribed from the same strand. The APOA1 and APOC3 are convergently transcribed. The lipid metabolism regulation relies on tissue-specific expression profiles of genes in the APOA1–APOC3–APOA4–APOA5 cluster and DNA demethylation [740].

ApoC3 inhibits lipoprotein (lipase-D) and hepatic lipase (lipase-C) in addition to hepatic lipoprotein receptors, thereby raising TG concentration, delaying clearance of ApoB+ lipoproteins, including large ApoB+ and ApoE+ LDLs and atherogenic remnants, and accelerating conversion of light to smaller dense proatherogenic LDLs [739]. ApoC3 not only inhibits hydrolysis by LipP of TGs in TRLs and counters conversion of VLDLs to IDLs and LDLs by LipC but also lowers hepatic uptake of TRLs through LDLR and LRP1 and, on light LDLs, enhances conversion to dense LDLs [741]. The smaller dense proatherogenic LDLs in addition to VLDL and remnant lipoproteins (small VLDLs and IDLs) enter the arterial intima, where they are oxidized and taken up by macrophages. Macrophages that reside

[11] CREB3L3: cAMP-responsive element-binding protein-3-like protein-3, also dubbed CREBH.

in the arterial intima secrete LipD, which can process TRLs to remnants, which can then be taken up by macrophages, lipid accumulation generating foam cells. Enrichment of ApoC3 in ApoB+ lipoproteins (VLDLs and LDLs) increases the risk for atherosclerosis. Furthermore, ApoC3 favors inflammation, as it activates NFκB and thus elicits vcam1 production [739].

ApoC3 is independently associated with risk for chronic obstructive airway disease (CoAD), especially in subjects with hypertriglyceridemia [741]. ApoC3 concentration correlates positively with concentrations of TGs, VLDLs, IDLs, small dense LDLs, and C-reactive protein, and negatively with the concentration of large LDLs.

5.1.2.2 Very-Low-Density Lipoprotein

The liver stores about 100-fold less TGs than AT but secretes fatty acids and TGs lipidated in VLDLs at a similar rate [742]. A small pool of LDs is permanently and efficiently processed in hepatocytes to produce VLDLs. Kinesin nanomotors are recruited to LDs, which store cholesterol, TGs, and sphingolipids, by the small GTPase ARF1, which also activates lipolysis, upon insulin stimulation. Upon feeding, ARF1 and kinesin localize to TG-rich LDs and transfer them at the periphery of hepatocytes to the smooth endoplasmic reticulum (sER), which contains lipases that process LD content and is the VLDL assembly site [742]. In the fasting period, insulin action drops, ARF1 and kinesin are removed from LDs, and LD–sER contacts disappear, impeding lipid supply to the sER and hence TG availability for VLDL assembly. Adipose tissue-derived fatty acids reach the liver and are esterified into TG in hepatocytes leading to massive accumulation and protecting organs from lipotoxic FAs and TGs, whereas the plasmatic TG concentration remains nearly constant during the feeding–fasting cycle.

Increased hepatic secretion of TG-rich VLDLs is a major determinant of hypertriglyceridemia. Hepatic VLDL overproduction is a common feature of insulin resistance.

Disrupted subdiaphragmatic vagal signaling decreases circulating ^{VLDL}TG concentration, but raises concentration of glucagon-like peptide GLP1, thereby reducing the synthesis of sterol regulatory element-binding protein, SREBP1c, stearoyl-CoA desaturase, SCD1, and fatty acid synthase (FAS), but enhancing hepatic insulin sensitivity [743].

Whereas pre-VLDLs can be degraded, TG-poor VLDLs are converted to mature TG-rich VLDLs by the addition of TGs derived from LDs (Sect. 5.4.4). Lipid droplets are composed of a core surrounded by a phospholipid monolayer and 229 types of protein [744].

Ancient ubiquitous protein AUP1 is the first identified ER- and LD-associated protein. It is implicated in the degradation of ubiquitinated misfolded proteins in the ER (ER-associated protein degradation [ERAD]) of misfolded proteins, that is, in the quality control of proteins in the ER and LD clustering [744]. It is implicated in the retrotranslocation of misfolded proteins from the ER lumen to the cytosol

for proteasomal degradation. Its C-terminus binds to ubiquitin conjugases such as UbE2g2 (UbC7 homolog). It contributes to ubiquitination and degradation of several regulators of lipid synthesis, such as 3-hydroxy 3-methylglutaryl coenzyme-A reductase, thereby affecting the number and size of LDs [744]. It controls formation of apolipoprotein-B100, LD mobilization, and hepatic VLDL assembly and secretion. This determinant of hepatic VLDL metabolism interacts on the LD surface with ApoB100, the main structural protein of hepatic VLDLs, which connects to many chaperones (heat shock proteins) involved in the ERAD pathway.[12] Lipidated ApoB100 translocates from the ER lumen to the LD surface. ApoB is lipidated to form pre-VLDL, whereas incorrectly folded and lipidated ApoB is destroyed. Accumulation of AUP1 on LDs hampers ApoB lipidation and VLDL assembly, independently of the MAP2K1/2–ERK pathway; the average size of the LDs declines; smaller underlipidated ApoB+ lipoproteins are secreted instead of fully lipidated VLDLs. Hepatic AUP1 production lowers in obese subjects and in those with *non-alcoholic fatty liver disease* (NAFLD; Sect. 5.3.4) and T2DM patients (diabetic dyslipidemia) [744].

5.1.2.3 Triglycerides and Protein-C

Triglycerides and LDL^{CS}, but not HDL^{CS}, are significantly associated with protein-C deficiency [745]. They may modulate protein-C synthesis or degradation.

Protein-C is a vitamin-K-dependent glycoproteic zymogen produced by the liver. Once activated by the thrombin–thrombomodulin complex, protein-C is an anti-coagulant, anti-inflammatory, antiapoptotic, and cytoprotective molecule. Activated protein-C inactivates factor-*V*a and factor-*VIII*a, thereby reducing thrombin generation.

Autosomal dominant mutations in the PROC gene cause protein-C deficiency, thereby favoring venous thromboembolism. Single-nucleotide polymorphisms in the chromosomal regions linked to protein-C-influencing genes, that is, in Whites, the BAZ1B[13] and GCKR loci[14] in addition to, in both Whites and Blacks, the

[12]The chaperone HSPa5 of the ER lumen is involved in proteasomal degradation of misfolded apoB100 [744]. The MAP2K1/2–ERK pathway involved in crosstalk with ERAD impedes the synthesis, packaging, and secretion of mature VLDLs. On the other hand, adipose differentiation-related protein, another LD-associated protein, which counters glucose tolerance and insulin sensitivity in the liver and skeletal muscle, can increase VLDL secretion in addition to LD size, but lowers their number. Cell death-inducing 45-kDa DNA-fragmentation factor, DFFα-like effector CIDEb, an apoptosis activator inhibited by DFFα, localizes to both the ER and LDs. It links to ApoB, facilitates LD clustering and fusion, and promotes VLDL assembly and secretion [744].

[13]BAZ1B: gene encoding bromodomain adjacent to zinc finger domain-containing protein-1B (BAZ1b), a kinase that phosphorylates H2a.x.

[14]GCKR: gene encoding glucokinase regulator.

PROC, PROCR–EDEM2,[15] and the CELSR2–PSRC1–SORT1 region on chromosome 1 (1p13.3),[16] especially rs12740374, a variant that influences LDL^{CS}, disturb circulating protein-C concentration [745]. Among the three lipidic fractions, TGs influence protein-C concentration.

5.1.3 Dyslipoproteinemias

Hyperlipidemia is also called *hyperlipoproteinemia*, as it usually results from altered lipoprotein metabolism. Lipoproteinemia thresholds are determined according to age and sex. Hyperlipoproteinemia is defined by abnormally elevated plasmatic concentrations of a given or many lipoprotein species. *Dyslipoproteinemia* is a more relevant term as anomalies of the lipidic checkup encompass not only high concentrations of LDL^{CS} and TGs but also low HDL^{CS} concentration.

In general, dyslipoproteinemia also refers to abnormal plasmatic concentrations of lipoproteins and their associated apolipoproteins above the 90th percentile of the general population for total CS, LDL, TG, ApoB, and LPa or below the 10th percentile for HDL and ApoA (Vol. 11, Chap. 5. "Lipoproteins"). Dyslipoproteinemia is, in general, defined quantitatively by:

1. Elevated concentrations of:
   - Total cholesterol ($\geq$239 mg/dl [6.2 mmol/l])
   - LDL^{CS} ($\geq$3.36 mmol/l [$\geq$130 mg/dl])
   - TGs ($\geq$1.69 mmol/l [$\geq$150 mg/dl])
2. Diminished concentration of HDL^{CS} ($\leq$1.03 mmol/l [$\leq$40 mg/dl] for men and $\leq$1.29 mmol/l [$\leq$50 mg/dl] for women).

Concentration of LDL^{CS} is most often measured in blood sampled during fasting, only when TG concentration is lower than 400 mg/dl. It is defined by the amount of cholesterol that is not contained in HDLs, VLDLs, and chylomicrons but incorporates the content of IDLs and LPa. It is calculated with an estimated cholesterol concentration in VLDLs being one-fifth of the total lipid content in VLDLs:

$$[LDL^{CS}] = [CS_{tot}] - ([HDL^{CS}] + [TG]/5). \tag{5.1}$$

[15] PROCR: gene encoding protein-C receptor; EDEM2: gene encoding ER degradation-enhancing α-mannosidase-like protein-2. EDEM2 initiates the ER-associated degradation that targets misfolded glycoproteins.

[16] CELSR2: gene encoding cadherin EGF LAG seven-pass G-type receptor-2; PSRC1; gene encoding proline- and serine-rich coiled-coil protein-1; SORT1: gene encoding sortilin, a multiligand receptor.

However, concentrations of total cholesterol and TGs, which are related to all types of circulating lipoproteins (chylomicron, VLDL, IDL, LDL, and HDL), in addition to HDL^{CS} and LDL, can also be measured directly.

Remnant cholesterol is defined as the cholesterol content of a subset of TGRL (i.e., VLDLs and IDLs) remnants, that is, chylomicron remnants, VLDLs, and IDLs in the feeding state in addition to VLDLs and IDLs in the fasting state. Remnant cholesterol concentration can be estimated as total cholesterol level minus LDL^{CS} and HDL^{CS} concentrations, concentrations of TGRLs in the fasting state and of chylomicron remnants in the feeding state being included [738]. In most individuals, chylomicrons are very rapidly degraded to chylomicron remnants due to TG hydrolysis by lipoprotein lipase. Remnant cholesterol concentration thus correlates with the TG level [725]. In plasma, TGs and cholesterol are exchanged between HDLs and remnants. Therefore, concentrations of HDL^{CS} and remnant cholesterol are inversely correlated.

An elevated concentration of remnant cholesterol causes cholesterol accumulation in the arterial wall, as does an augmented level of LDL^{CS}. However, although an increased LDL^{CS} level correlates with coronary artery disease, but not with low-grade inflammation, a heightened concentration of remnant cholesterol is associated with both low-grade inflammation and atherosclerosis. An increment of 1 mmol/l (39 mg/dl) in nonfasting remnant cholesterol concentration, which contributes to atherosclerosis, is associated with a 2.8-fold increase in the risk of cardiac ischemia [725].

Lipoproteins enriched in TGs (>2 mmol/l [176 mg/dl]) in addition to cholesterol or remnant cholesterol concentrations (>1 mmol/l [39 mg/dl]) are strong and independent predictors of atherosclerosis [738].

Triglyceride-rich lipoproteins contain both TGs and cholesterol, in addition to phospholipids and proteins. They are associated with low-grade inflammation and atherosclerosis. Circulating ApoB+ TGRLs include intestinal and hepatic lipoproteins (chylomicrons, VLDLs, and their remnants). Augmented TGRL concentration alters LDL and HDL composition and function. An elevated TGRL concentration in the postprandial period can engender endothelial inflammation [746].

Epidemiological studies cannot distinguish cause from mere correlation. On the other hand, genetic analyses and randomized controlled trials can buttress the causal role in atherogenesis of lipid markers, especially proinflammatory and proatherogenic plasmatic lipoproteins (i.e., ApoB+ LDL, LP_A, and TGRLs; Vol. 11, Chap. 5. "Lipoproteins").

Elevated plasmatic concentrations of total cholesterol and LDL^{CS} cause atherosclerosis, as LDLs enter the arterial intima. Mid-sized TGRLs (intermediate size between LDL and chylomicrons) can also penetrate the arterial intima. Once they are entrapped in the intima, lipoprotein lipase at the endothelial surface or in the arterial intima degrades TGs and liberates toxic FFAs and monoacylglycerols. Lipoprotein lipase is also synthesized in macrophages.

5.1.3.1 High-Density Lipoprotein

Circulating HDLs comprise subpopulations according to their size, shape, charge, and lipid and protein composition. These particles can indeed be categorized according to their density and size, particularly in lipid-rich large HDL2 and protein-rich small HDL3 subpopulations. Constituents of HDLs enter the plasma separately and are assembled into HDLs within the plasma [747]. Multiple plasmatic factors remodel HDLs, removing HDL constituents separately from blood rather than as the cellular uptake of intact HDls.

High-density lipoproteins protect against atherosclerosis, as they have antioxidant, anti-inflammatory, and anti-thrombotic effects, favor cholesterol egress from macrophages lodging in the artery wall, enhance endothelial function, promote endothelial repair, and support angiogenesis. Certain HDL constituents are responsible for their antioxidant and anti-inflammatory properties, whereas others serve in HDL transfer, especially in the RCT.

Reverse cholesterol transport comprises cholesterol efflux from ABCa1+ macrophages to apolipoprotein-A1, which creates nascent HDLs followed by esterification of free cholesterol, hepatic extraction of HDLs, and hepatic conversion of HDL^{CS} to bile salts, which are then excreted [748]. Cholesterol esterification by LCAT is a minor process in nascent HDL metabolism. Most free cholesterol of nascent HDLs and phospholipids is rapidly extracted by hepatocytes via ScaRb1, hepatic phospholipid uptake being promoted by phospholipid transfer protein; they are quickly transferred to HDLs and LDLs. On the other hand, ApoA1 is only transferred to HDLs and to the lipid-free form that can be recycled to nascent HDLs.

Large human population studies have demonstrated that low HDL-emia predicts a high probability of a CVD event. On the other hand, in mice and rabbits, intravenous infusions of HDLs or increased synthesis of ApoA1, a major HDL apolipoprotein, markedly decreases susceptibility to atherogenesis. However, in humans, gene variants that raise plasmatic HDL^{CS} concentration are not accompanied by a reduced risk of CVD events. Increased HDL^{CS} concentration via inhibition of cholesteryl ester transfer protein (CETP) fails to reduce CVD events. In addition, the relation between HDL^{CS} concentration and mortality is U-shaped, whatever the gender, both extremely high and low concentrations being associated with high mortality risk [729].

Low HDL-emia represents a strong independent cardiovascular risk marker in subjects with heterozygous familial hypercholesterolemia. Familial hypercholesterolemia is linked to mono- and polygenic defects. Low HDL-emia can be associated with environmental factors and lifestyle, and with primary hypoalphalipoproteinemia. A high $[CS_{tot}]/[HDL^{CS}]$ ratio is related to the extent and severity of atherosclerotic lesions.

Reverse Cholesterol Transport

In addition to other protective effects, HDL permits the elimination of excess cholesterol by RCT. This transfer mode corresponds to the return of an excessive amount of cholesterol from cells to the liver, where cholesterol is used to synthesize bile acids, which are excreted with the bile and partly with the feces. Bile acids facilitate the efficient solubilization and intestinal absorption of dietary lipids, cholesterol, and liposoluble vitamins. This protective metabolic pathway begins with cholesterol efflux from atherosclerotic plaque cells such as macrophage-derived foam cells to HDLs.

On the other hand, inflammation-responsive transcription factor C/EBPδ, which is detected in macrophages of atherosclerotic plaques, responds to modified LDLs via the P38MAPK–CREB pathway and favors lipid accumulation in M1 macrophages (but not M2 macrophages) [749]. It upregulates expression of pentraxin-3, which promotes LDL macropinocytosis, and downregulates expression of ABCa1, which impairs cholesterol efflux from M1 macrophages.

After esterification of cholesterol in the plasma by LCAT, cholesteryl esters can be selectively delivered to the liver via the scavenger receptor ScaRb1.

Alternatively, cholesterol ester transfer protein (CETP) can exchange cholesteryl esters for TGs on ApoB+ lipoproteins, which can deliver cholesterol to hepatocytes by receptor-mediated endocytosis.

The biliary pathway consists of cholesterol conversion to primary bile acids and subsequent elimination from the liver that relies on transport across the canalicular surface by the ABCg5 and ABCg8 transporter or the bile salt export pump (BSEP, or ABCb11), the delivery of gallbladder bile to the intestinal lumen being intermittent and primed by food intake [750].

A fraction of cholesterol is reabsorbed in the proximal small intestine via Niemann–Pick-C1-like protein (NPc1L1) and bile acids in the distal small intestine through the combined actions of the apical sodium-dependent bile acid transporter and intestinal bile acid transporter, thereby limiting neutral and acidic sterol loss from the body [750].

The melanocortin 1 receptor (MC1R, or MC_1)[17] resides on monocytes and macrophages and exerts anti-inflammatory actions, once it is linked to α-melanocyte-stimulating hormone, a peptidic hormone and neuropeptide of the melanocortin family.[18] In atherosclerotic lesional macrophages, activated MC_1 upregulates formation of ABCa1 and ABCg1 involved in initiating reverse cholesterol transport, thereby promoting cholesterol egress and countering foam cell generation [751]. In addition, MC_1 lowers plasmalemmal ScaRb3 concentration and hence cholesterol uptake, further limiting lipid accumulation in macrophages.

[17] A.k.a. α-melanocyte-stimulating hormone receptor (MSHR).

[18] MSH is an unselective ligand of the melanocortin receptors MC_1 and MC_3 to MC_5, but not MC_2, which is exclusive for adrenocorticotropic hormone (ACTH).

Transintestinal Cholesterol Excretion

In addition to biliary cholesterol secretion, transintestinal cholesterol excretion (TICE) contributes to RCT. Liver-derived ApoB+ lipoproteins can deliver cholesterol to the basolateral surface of enterocytes, which is taken up by LDLR and other receptors. After transfer to the apical membrane, cholesterol exits into the intestinal lumen via ABCg5, ABCg8, and possibly ABCb1a and ABCb1b [752]. In mice, TICE (∼35% of fecal cholesterol removal in humans) is stimulated by liver X receptor (LXR) and farnesoid X receptor (FXR) agonists. However, most macrophage-derived cholesterol is excreted via the hepatobiliary route [752].

Endothelial Transcytosis of HDLs and LDLs

The transmembrane class-B scavenger receptor ScaRb3 recognizes multiple ligand types. Its primary function is related to the capture and clearance of modified lipoproteins, advanced glycation products, apoptotic cells, microbial diacylglycerols, and Plasmodium-infected erythrocytes. In addition, it is involved in cellular uptake of long-chain fatty acids, hence its other name fatty acid translocase. Oxidized low-density lipoprotein (oxLDL) is a high-affinity ScaRb3 ligand. Diverse forms of oxidized lysophosphatidylcholine species (oxGPC) reside on the surface of lipoproteins and may serve as recognition elements by ScaRb3 [753].

Nutrients must traverse the endothelium to reach parenchymal cells. Fatty acid transport proteins FATP3 and FATP4 and ScaRb3 can carry fatty acids in endotheliocytes (ECs).

Paracrine messengers secreted by parenchymal cells support nutrient transfer through the adjacent endothelium. For example, vascular endothelial growth factor b (VEGFb) is released from skeletal and cardiac myocytes to promote transendothelial lipid transport adapted to their metabolic need [754].

In ECs, the dynamics of LD genesis and degradation using diacylglycerol acyltransferase DGAT1 and adipocyte TG lipase, respectively, not only provides a fatty acid source for adjacent cells but also regulates endothelial glycolysis and protects ECs from lipotoxic stress [755].

Both LDLs and HDLs cross the vascular endothelium and exert their pro- and antiatherogenic activity, respectively, within the vascular wall. Accumulation of LDLs in the subendothelial layer of the intima causes atherosclerosis. Conversely, removal of cholesterol from the subendothelial space by RCT protects against atherosclerosis.

In ECs, endocytosis of LDLs, which relies on the clathrin-dependent pathway and involves LDLR, leads to lysosomal degradation. The transendothelial LDL transfer implicates caveolae, ScaRb1, and activin-like kinase ALK1 [757]. Transcytosis of HDLs is carried out by ABCg1, ScaRb1, and endothelial lipase, in addition to the ectopic β-ATPase–nucleotide receptor axis [757].

Among modulators of transendothelial LDL and HDL transfer, VEGFa and VEGFR2 significantly lower the binding, uptake, and transcytosis of HDL, but

not LDL, VEGFR1, and VEGFR3 having no impact on this transfer [757]. The VEGFa isoform elicits actin re-organization, hence contributing to HDL uptake. The VEGFa–VEGFR2 couple operates via the PI3K–PKB axis,[19] P38MAPK (but not the Ras–Raf–MAP2K axis), and ScaRb1 in HDL uptake. Moreover, VEGFa promotes ScaRb1 localization in the plasma membrane of ECs; the VEGFa–VEGFR2-launched signaling acts as a rate-limiting factor for the plasmalemmal ScaRb1 concentration, thereby regulating HDL uptake by ECs.

CETP and S1P

HDL particles are composed of apolipoproteins (e.g., ApoA1, ApoA2, and ApoM), antioxidant enzymes such as paraoxonase-1, LCAT, and diverse lipidic species (e.g., cholesterol esters, TGs, phospholipids, and sphingolipids such as sphingosine 1-phosphate [S1P]) [759].

Endothelial function is restored by plasmatic HDLs. However, protection of the endothelium and vasodilation ensured by HDL depends on its S1P carrier. Sphingosine 1-phosphate is a vasoprotective lysophospholipid mediator, which is carried in blood by albumin (Alb) and ApoB+ lipoproteins; yet, plasmatic S1P travels mainly with ApoM+ HDLs, ApoM, a minor apolipoprotein type on HDLs, being a S1P carrier and modulator of its activity.

The properties of S1P depend on its carrier, HDL or albumin. Effects of HDL^{S1P} differ from those of Alb^{S1P} in endothelial inflammation inhibition, barrier function,

[19] PI3K is involved in endosomal transfer. After a meal, insulin released from the pancreatic β cells triggers glucose uptake by target cells, such as skeletal myocytes and adipocytes. Insulin stimulates translocation of the glucose transporter GluT4 to the plasma membrane from specialized intracellular GluT4 storage vesicles via PI3K and a proteic octamer, the exocyst. This complex assembles at the site of exocytosis in response to insulin under the control of RhoJ and tethers GluT4+ vesicles to the plasma membrane via the PI3K–PKB axis and its effector RalA GTPase. The latter interacts with the nanomotor Myo1c complex, which facilitates recruitment of GluT4+ vesicles to the plasma membrane. Moreover, the Ral GAP complex (RGC) is implicated in RalA activation downstream from the PI3K–PKB pathway; it is composed of the RGC1 regulatory and RGC2 (or Akt [PKB] substrate of 250 kDa [AS250]) catalytic subunit [756]. Insulin inhibits the RGC1–RGC2 complex (RhoGAP4–RalGAPα2) via phosphorylation of RGC2 by PKB2, activated PKB, thus relieving inhibition of the RalGAP complex on RalA activity. Upon RalA activation due to inhibition of two GAP types, TBC1D4 (or Akt [PKB] substrate of 160 kDa [AS160]), a RabGAP, which inactivates Rab10 involved in insulin-stimulated GluT4 exocytosis and operates via the RalGEF RGL2, once it is phosphorylated, and the RalGAP complex RGC1–RGC2, RalA can interact with the exocyst subunits Sec5 and Exo84 [758]. Once Sec5 is phosphorylated by PKC, RalA dissociates from Sec5. The kinase TBK1, a member of the IKK family, which is involved in both inflammatory and insulin responses (TBK1 does not play an important role in NFκB activation, but acts in the regulation of type-*I* interferon production via interferon regulatory factor (IRF) phosphorylation and can counter overproduction of inflammatory mediators via IKK inhibition), phosphorylates the exocyst subunit Exo84, thereby reducing its affinity for RalA, enabling its release from the exocyst, and eliciting insulin-stimulated GluT4+ vesicle fusion with the plasma membrane in adipocytes [758]. In hepatocytes, insulin regulates via PI3K the cell surface expression of ScaRb1 and hence lipid ingress.

and lymphopoiesis [760]. ApoM+ HDL promotes receptor activation. S1P carried on ApoM on HDL is more efficient in the maintenance of the endothelial barrier and insulin secretion from pancreatic β cells [762].

- Under normal conditions, HDL3 carries S1P, which interacts with endothelial $S1P_1$ receptor to protect the endothelium [760].
- In type-1 diabetes mellitus (T1DM), the concentration of light lipid-rich, protein-poor HDL2, which has a defective anti-inflammatory function, is higher than that of HDL3 particles. Hence, although plasmatic concentrations of ApoM and S1P in T1DM patients remain similar to those of controls, the ApoM–S1P complex transport shifts from dense to light HDLs [761]. At least in women, the ApoM–S1P complex in light HDLs is less efficient at inhibiting TNFSF1-induced expression of vcam1 than that in denser HDLs. In addition, light HDLs cannot activate PKB, whereas all HDL subfractions are equally as efficient at activating ERK and receptor internalization. The HDL3-to-HDL2 change may lower S1P signaling to the endothelial $S1P_1$ receptor [760]. Reduced HDL^{S1P} level attenuates NOS3 activity.
- In type-2 diabetes mellitus, glycation of HDL significantly lowers the S1P content of HDL, altering protection from redox stress [760].

The quantity of HDLs depends on CETP, which transfers cholesteryl ester from HDLs to ApoB+ lipoproteins and TGs from ApoB+ lipoproteins to HDLs. In addition, CETP modulates the distribution of S1P among lipoproteins [762].

Sphingosine 1-phosphate on VLDL, IDL, and LDL is cleared more rapidly than that on HDL [762]. The ApoE–LDLR couple is involved in the clearance of ApoM+ lipoproteins; LDLR removes S1P and ApoM carried on ApoE-rich HDLs, at least in mice. In addition, ApoM and S1P may be eliminated using the ApoB+ lipoprotein clearance pathway. Therefore, S1P, which abounds on HDL, can be cleared from blood circulation using two routes, the ApoE–LDLR pathway of ApoB+ lipoprotein removal and LDL clearance following transfer from HDL to LDL during CETP processing. CETP can also modulate the lipid composition of LDLs. The pathway involving ApoB+ lipoproteins may be a major clearance route of S1P bound to ApoM+ lipoproteins [762].

Sphingosine 1-phosphate on ApoB+ lipoproteins (i.e., VLDL and LDL) induces phosphorylation of PKB and NOS3 in ECs via $S1P_1$ or $S1P_3$, to a greater extent, than S1P on HDLs [762]. Hence, in subjects with CETP deficiency, HDL activates NOS3 to a lesser degree. On ApoB+ lipoproteins, S1P provokes insulin secretion to a greater magnitude than S1P on HDL. Therefore, short-duration CETP overexpression (but not prolonged CETP overexpression) increases insulin secretion and sensitivity via S1P and $S1P_1$ or $S1P_3$, thereby enhancing glucose tolerance in diabetes mellitus [762].

Cholesterol ester transfer protein does not control plasmatic concentrations of ApoM and S1P, although CETP lowers HDL concentration, but ApoM and S1P are transferred from HDLs to ApoB+ lipoproteins in CETP-overexpressing mice [762]. The quantity of LDL may be important in S1P transfer. Hence, CETP shifts the

distribution of S1P and ApoM from HDL to ApoB+ lipoproteins, on which S1P exert some effects more potently, but is removed more rapidly.

The receptor $S1P_1$ abounds in ECs, where it contributes to the regulation of angiogenesis and maintenance of the microvascular barrier. The endothelial $S1P_1$ receptor stabilizes developing vascular networks, prevents sprouting angiogenesis, and decreases vascular permeability.

Endothelial $S1P_1$ signaling augments in inflamed arterial segments [759]. In cultured human umbilical vein ECs, $HDL^{ApoM--S1P}$, but not Alb^{S1P}, upregulates formation of the plasmalemmal $S1P_1$–β Arr2 complex and attenuates activation of NFκB by TNFSF1 and hence the amount of ICAM1.[20] Although S1P bound to either carrier stimulates the MAPK module, Alb^{S1P} triggers greater Gi signaling and $S1P_1$ endocytosis. Therefore, anti-inflammatory and endothelial protective functions of HDLs complement their role in cholesterol removal from cells and suppress endothelial dysfunction and inflammation characterized by impaired NO release and increased abundance of adhesion molecules for leukocytes, which are early events in CVD. However, a global increase in the amount of HDL does not repress EC inflammation.

ApoM is unstable when it is not tethered to HDLs. An engineered soluble and stable form of ApoM bound to S1P can sustainably activate $S1P_1$ on ECs and attenuates hypertension induced by angiotensin-2 [763].

5.1.3.2 Low-Density Lipoprotein

Elevated plasmatic LDL^{CS} concentration alone does not suffice to provoke vascular lesions. Modifications of LDL such as oxidation in addition to small and dense LDLs are associated with atherosclerosis. Carbamylated LDL stimulates mitochondrial endonuclease G, which may be involved in DNA fragmentation during cell apoptosis.

Plasmatic LDLs enter the endothelium and cross it to reach the subendothelial layer of the intima, where they are trapped. Transcytosis implicates both caveola-mediated endocytosis and SNARE-mediated exocytosis. Caveolae are regulated by cavins.[21]

Endocytosis of LDLs depends on two routes [764]:

1. The first route relies on LDLR and favors LDL degradation. It declines at high LDL concentrations. Leukemia-inhibiting factor upregulates hepatic LDLR formation at least in rabbits, thereby increasing cholesterol clearance.
2. The second route diverts LDL from lysosomal degradation and promotes LDL transcytosis. It is enhanced with hypercholesterolemia.

[20] Activation by ω3-polyunsaturated fatty acids of the receptor GPR120 (FFAR4) primes recruitment of β-arrestins, which prevents activation of the IKK complex and NFκB [759].

[21] A.k.a. polymerase-1 and transcript release factors (PTRFs). Redox stress can raise cavin-1 formation and thus the number of caveolae.

Transmembrane protein TMem97, which under sterol depletion localizes to the endolysosomal compartment, binds to the LDL^{CS} transport regulator, Niemann–Pick type-C protein NPc1, to control cholesterol level [765].[22] Other regulators of cellular cholesterol homeostasis include sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) and LRP6.

Prosaposin (PSap) is a precursor of several non-enzymatic glycoproteins, sphingolipid activator proteins (SAPa–SAPd), related to four domains (A–D) from the N- to the C-terminus, which participate in the lysosomal degradation of sphingolipids.[23] Reduced function of betaine–homocysteine methyltransferase BHMT2 decreases LDL uptake in ECs.

C-reactive protein (pentraxin-1) increases LDL transcytosis, as it generates ROS, which increase endothelial permeability and activate PKC and Src,[24] and translocation of caveolae or SNARE carriers [766].[25]

Endothelial activin-like kinase ALK1 connects to plasmatic LDLs in a noncompetitive manner with a lower affinity than LDLR [764]. This binding is inhibited neither by sterols, nor by PCSK9, a serine peptidase.[26] The ALK1–LDL complex is then internalized and translocates from the apical to the basolateral surface of ECs to be released into the intima.

Oxidized LDLs were originally defined as oxidatively modified LDLs containing protein components modified by aldehyde products creating net negative charges that enable interaction and uptake by macrophages. Formation of oxidation products depends on the oxidant type, the extent of oxidation, and the presence or absence of other agents such as redox metals [767]. Minimally oxidized LDLs possess lipid peroxides or their degradation products (e.g., oxovaleryl PC), without apolipoprotein modification. Some oxidation products, such as malondialdehyde

[22] Cholesterol derived from LDLs is transferred from the endosomal–lysosomal compartments to the endoplasmic reticulum under the control of NPC1 and NPC2.

[23] Saposins SAPa and SAPc (SAP2) stimulate the hydrolysis of glucosylceramide by β-glucosylceramidase and galactosylceramide by β-galactosylceramidase [108]. Saposin-B (SAP1) stimulates the hydrolysis of galactocerebroside sulfate by arylsulfatase-A, GM1 gangliosides by β-galactosidase, and globotriaosylceramide by α-galactosidase-A. Saposin-C is also an activator of β-glucosidase. Saposin-D is a specific sphingomyelin phosphodiesterase (PDE) activator.

Prosaposin behaves as a myelino- and neurotrophic factor via its G-protein-coupled receptors, GPR37 and GPR37L1 [108]. It is internalized and phosphorylated by ERK.

[24] The kinases PKC and Src phosphorylates Cav1 at Ser37 and Tyr14, respectively [766]. Dynamin Dnm2, which mainly localizes at the neck of the caveolae, executing the fission of the caveolae, is a target for PKC and Src; its translocation depends on activation of these kinases. C-reactive protein (CRP) may increase LDL transcytosis via formation of both the endocytotic Cav1–cavin-1–Dnm2 and exocytotic SNARE complexes.

[25] The SNARE proteins (soluble ^{N}ethylmaleimide-sensitive factor-attachment protein receptors) support membrane docking and fusion via interactions with an ATPase, ^{N}ethylmaleimide-sensitive factor (NSF), and its SNAP "receptor" (α SNAP–γ SNAP).

[26] PCSK9 is derived predominantly from the liver. It is secreted into the bloodstream and increases plasmatic LDL^{CS} concentration, as it binds to LDLR both intra- and extracellularly and elicits its lysosomal degradation in hepatocytes.

Table 5.1 Lipid and protein oxidation products in LDLs (Source: [767]; *HODE* hydroxylinoleic acid [octadecadienoic acid], *HPODE* hydroperoxylinoleic acid)

Fatty acid Oxidation products	Free and esterified fatty acid peroxides (e.g., 13HPODE)
	Free and esterified fatty acid hydroxides (e.g., 13HODE)
	Free and esterified isoprostanes
	Aldehydes (MDA, 4-hydroxy nonenal, and hexanal)
	Core aldehydes (e.g., oxovaleryl phosphatidylcholine)
	Pentane and other hydrocarbons
Lipid-derived products	Lysophosphatidylcholine
	Cholesterol oxidation products (e.g., 7-keto-cholesterol)
	Internally modified phosphatidylethanolamine/serine products
Protein Oxidation Products	Protein carbonyls
	Protein crosslinks
	Lipid–protein adducts
	Protein fragmentation
Other changes	Increased buoyant density
	Increased negative charge
	Loss of enzyme activities associated with LDL

(MDA), diffuse out of oxLDLs. Conversely, MDA-modified LDL (^{MDA}LDL) can arise from MDA released by platelets or other sources. Polyunsaturated fatty acids favor LDL oxidation, but not monounsaturated fatty acids. Specific amino acids may propagate oxidation. Oxidized LDL is thus now defined as a particle derived from circulating LDLs that have peroxides or their degradation products.

Oxidized LDLs contain unoxidized and oxidized fatty acid derivatives both in the ester and the free forms, their decomposition products, cholesterol and its oxidized products, proteins with oxidized amino acids and crosslinks, and polypeptides with varying extents of covalent modification with lipid oxidation products (Table 5.1) [767].

Low-density lipoproteins are heterogeneous in density, size, chemical composition, and net charge. The LDL spectrum can be characterized by a predominant peak of LDLs with large (pattern A) and small (pattern B) diameters. Small, dense LDLs have decreased levels of glycosylation of apolipoprotein-B and sialic acid content [768]. Any small LDL with a given valence (net negative charge) has a greater surface charge density than a larger LDL with the same valence. Mid-dense LDLs (1.030–1.039 g/ml) have a lower net negative electrical charge than the small, most buoyant, dense LDLs. On the other hand, mid-dense LDLs bind with a higher affinity to LDLR, thereby having greater rates of uptake and degradation than LDLs of lesser or greater density [768].

In humans, according to the net negative surface charge, plasmatic spherical LDLs are categorized into five subfractions from L1 to L5 with increasing electronegativity, which describes fast relative electrophoretic LDL mobility on agarose gel [769]. Whereas ^{L1}LDL, the most abundant and least negatively

Table 5.2 Composition of plasmatic ^{L1}LDL and ^{L5}LDL (low-density lipoprotein subfractions) from hypercholesterolemic humans (Sources: [769, 771])

^{L1}LDL	^{L5}LDL
Core	
CE (38%), TG (4%)	CE (20%), TG (7%)
Periphery	
Proteins (25%)	Proteins (40%)
ApoB100 (99% LDL proteins)	ApoB100 (60% LDL proteins), ApoA1, ApoC3, ApoE, Apo_A
CS (8%), PLd (25%)	CS (8%), PLd (25%)

Spherical LDLs contain an apolipoproteic (Apo) framework, phospholipids (PLd) and free cholesterol (CS) on their surface, and triglycerides (TG) and cholesteryl esters (CE) at their core. Compared with ^{L1}LDL, ^{L5}LDL has a greater content of proteins and TGs, but a smaller amount of cholesteryl esters

charged LDL subtype, represents harmless normal LDL, the most negatively charged ^{L5}LDL subfraction is atherogenic. The ^{L5}LDL subfraction is isolated using anion-exchange chromatography from plasma of smokers and individuals with hypercholesterolemia, T2DM, and metabolic syndrome, whereas its concentration is negligible in healthy subjects. Its concentration is significantly elevated in STEMI patients [770].[27] Its plasmatic concentration rises in patients with ischemic stroke, as it favors platelet aggregation and platelet–EC interaction. It can also induce endothelial dysfunction and impairs the differentiation of endothelial progenitor cells (EPCs).[28]

^{L5}LDL is neither smaller nor denser than ^{L1}LDL, but has a higher aggregability. Whereas ^{L1}LDL is composed mainly of apolipoprotein-B100 (99% proteic content), concentrations of ApoA1, ApoC3, ApoE, and Apo_A progressively increase from ^{L1}LDL to ^{L5}LDL, the concentration of ApoB100 concomitantly decreasing (Table 5.2) [769]. Other minor proteins in ^{L5}LDL (but not ^{L1}LDL) include albumin, ApoJ, platelet-activating factor acetylhydrolase (PAFAH), paraoxonase POn1, the plasmatic apolipoprotein SAa4,[29] and complement component C3.

The ^{L5}LDL particle does not tether to LDLR, but is endocytosed into vascular ECs via the scavenger receptor ScaRe1 (or CLec8a), the lectin-like oxLDL receptor,

[27] STEMI: ST segment elevation myocardial infarction.

[28] ^{L5}LDL impedes expression of growth factor receptors and favors endothelial progenitor cell senescence, as it suppresses telomerase activity [771].

[29] SAa4: serum amyloid-A4. This major acute phase reactant is an apolipoprotein of HDLs. The group of HDL-associated apolipoproteins and cytokine-induced acute phase molecules is composed of: (1) major apolipoproteins on HDL produced in the liver (SAa1–SAa2, encoded by the SAA1 and SAA2 genes); (2) acute phase reactant SAa3, which is peripherally produced and is a minor HDL apolipoprotein (SAA3 is a pseudogene in humans); and (3) a constitutive acute phase reactant SAa4, which is a minor normal HDL apolipoprotein and is encoded by the SAA4 gene [772].

which has a high affinity for negatively charged ligands [769].[30] It can also signal via the G-protein-coupled platelet-activating factor receptor (PAFR) on ECs and endothelial progenitor cells [771].[31]

^{L5}LDL precludes EC proliferation and favors EC apoptosis; ^{L5}LDL and CuOx-LDLs[32] are equally potent at suppressing the Fgf2 gene transcription and inducing apoptosis in vascular ECs [771]. In addition, ^{L5}LDL upregulates expression of adhesion molecules (e.g., vcam1), cytokines (e.g., IL1β), and chemokines (e.g., CCL2, CXCL1–CXCL3, CXCL5–CXCL6, and CXCL8), thereby favoring inflammation [774]. In macrophages, ^{L5}LDL increases production of IL1β via ScaRe1, NFκB activation being required to produce proIL1β that is subsequently cleaved into IL1β by activated caspase-1, which lodges on the NLRP3 inflammasome, a molecular platform. Therefore, In addition to LDL^{CS} plasmatic surplus, the proportion of ^{L5}LDL that signals distinctly from usual LDLs such as ^{L1}LDL, is an important factor for atherosclerosis.

Atrial natriuretic peptide (ANP) is an endo-, auto-, and paracrine regulator. It acts via its guanylate cyclase receptor GC2a (NPR1)[33] and the second messenger cGMP.[34] It prevents NFκB-mediated proIL1β production and NFκB–NLRP3–Casp1-mediated IL1β release [775].

Natriuretic peptides constitute a family of three structurally related hormones and autacoids. All natriuretic peptides are synthesized as pre-prohormones encoded by the NPPA, NPPB, and NPPC genes. A- (atrial) and B-type natriuretic peptides (BNPs) are secreted from the atria and ventricles,[35] ANP lowering blood pressure and attenuating cardiac hypertrophy, BNP reducing ventricular fibrosis [777]. Both

[30] Low-density lipoprotein is taken up by hepatocytes and vascular cells via the LDL receptor (LDLR). Homo- or heterozygous Ldlr gene defects raise LDL-emia. Whereas ^{L1}LDL to ^{L4}LDL are endocytosed via LDLR, ^{L5}LDL is internalized by ScaRe1 in both ECs and EPCs. Expression of ScaRe1 is induced by ^{L5}LDL (but not by ^{L1}LDL). Via ScaRe1, ^{L5}LDL disturbs the equilibrium between the pro-survival and proapoptotic members of the BCL2 family. Upon ScaRe1 stimulation, P38MAPK, which is countered by the PI3K–PKB–NOS3 pathway, is phosphorylated and activates caspase-3, thereby causing EC apoptosis [773].

[31] It disrupts FGF2 autoregulation via the FGF2–PI3K–PKB loop [771]. Exposure of ^{L5}LDL with PAF acetylhydrolase to degrade PAF and PAF-like lipids removes its capacity to lower FGF2 signaling and induce EC apoptosis.

[32] CuOx-LDL: copper-oxidized LDL.

[33] Also known as NP_1, NPRa, and atrionatriuretic peptide receptor ANP_A. It is produced in the brain, heart, kidney, lung, adrenal gland, adipose tissue, and testis, in addition to vascular smooth myocytes [777].

[34] Increased content of intracellular cGMP activates its effectors, cGMP-dependent PKGs, phosphodiesterases (PDEs), and cyclic nucleotide-gated channels. In addition to intracellular cGMP accumulation, concentrations of cAMP, Ca^{2+}, and IP_3 decline upon ANP exposure, the ANP–GC2a alleviating activity of adenylate cyclase (AC) and phospholipase-C in addition to Na^+ influx and countering activation of PKC and MAPKs, thereby augmenting diuresis, provoking vasodilation, hindering cell proliferation, inflammation, and adverse hypertrophy [776].

[35] Low BNP concentrations are stored with ANP in atrial granules. However, BNP concentrations are greater in ventricles, where it is not stored in granules, but formed under the GATA4 control in response to cardiac stresses such as volume overload.

Table 5.3 Tissular and cellular distribution of natriuretic peptide receptors (Source: [776])

Receptor	Organ	Cell
GC2a (NP_1)	Brain, heart, vasculature, kidney, adrenal glands, pituitary gland, lung, liver, ileum, thymus, ovary, placenta, testis	EC, vSMC
		Renal epitheliocyte, mesangiocyte
		Fibroblast
		Granulosa cell
		Leydig cell
GC2b (NP_2)	Brain, heart, vasculature, adrenal gland, cartilage, lung, pituitary gland, thymus, ovary, placenta, testis	VSMC
		Fibroblast
		Chondrocyte
GC2c (NP_3)	Brain, heart, vasculature, kidney, liver, intestine	EC, vSMC
		Fibroblast, mesangiocytes

ANP and BNP are also produced in the brain; ANP is additionally synthesized in the kidney and AT, whereas C-type natriuretic peptide (CNP), which is produced in the brain, heart, endothelia, and bone, primarily stimulates long bone growth [777]. CNP is not stored in granules. It is secreted upon exposure to growth factors and a stress field in cultured ECs.

In humans, circulating BNP has a significantly longer half-life (∼20 mn) than ANP and CNP (∼2 mn). Membrane metalloendopeptidase (MME)[36] degrades ANP and CNP, in addition to binding to the natriuretic peptide clearance receptor (GC2c), a pseudo-guanylate cyclase that constitutively mediates their internalization and elimination. On the other hand, BNP is initially cleaved by meprin-A in the kidney brush border and then further degraded by MME [777].

Both ANP and BNP activate the transmembrane guanylyl cyclase GC2a[37] whereas CNP stimulates GC2b (Table 5.3).[38] Transforming growth factor (TGF)β1, angiotensin-2, and endothelin-1 reduce GC2a synthesis [776]. A third receptor GC2c (NPR3)[39] clears natriuretic peptides from the circulation via endocytosis for sequestration and degradation. The receptor GC2a lodges on adipocytes and can promote adiponectin production [777].

[36] A.k.a. neutral endopeptidase (NEP).

[37] With a potency order ANP ≥ BNP ≫ CNP [777]. Glycosylation of GC2a may influence receptor stability and ligand binding [776].

[38] Also abbreviated NP_2, NPRb, and ANP_B. It has the following selectivity preference CNP ≫ ANP ≥ BNP [777]. It is produced in the brain, heart, kidney, liver, lung, bone, and uterus, in addition to fibroblasts and vSMCs [777]. Activation of GC2b by CNP generates cGMP, but to a lesser extent than that of GC2a excited by ANP or BNP [776].

[39] Also dubbed NP_3, NPRc, and ANP_C. This most widely and abundantly expressed natriuretic peptide receptor has the following selectivity preference ANP > CNP ≥ BNP [777].

Atrial natriuretic peptide acutely reduces plasmatic volume using three mechanisms: increased renal excretion of salt and water, vasodilation, and augmented vascular permeability via its endothelial receptor GC2a [778]. It is secreted by the heart upon atrial stretch and hence hypertension. ANP is also synthesized and metabolized in ECs.

The natriuretic peptides, ANP, BNP, and CNP, are implicated in obesity and lipid mobilization [779]. The CNP subtype causes endothelium-dependent and -independent vasodilation.

A high-cholesterol diet can prevent efficacy of protein Tyr kinase inhibitors (PTKI), which are used to treat various cancer types, especially renal cell carcinoma (RCC), via suppression of cancerous cell proliferation or tumoral angiogenesis, rendering RCC refractory to PTKI treatment [780]. In cultured RCC and endothelial cells, exposure to LDLs activates the PI3K–PKB pathway, thereby promoting cell survival and proliferation and attenuating PTKI cytotoxicity.

5.1.3.3 Lipoprotein-A

Lipoprotein-A is an LDL-like particle linked to apolipoprotein-$_A$, which is encoded by the APO_A (LP_A) gene on chromosome 6. This ApoB100–Apo_A complex is composed of apolipoprotein-B100 covalently attached to the very large hydrophilic glycosylated apolipoprotein-$_A$. It thus resembles LDL by the presence of ApoB and a high content of cholesterol, but differs from LDL by its content in Apo_A. It is enriched with oxidized phospholipids.

Because Apo_A and ApoB+ lipoproteins are secreted separately, the covalent linkage of Apo_A to ApoB by a disulfide bridge occurs in the extracellular medium [781].

Lipoprotein-A shares structural features with plasminogen (kringle domains and a peptidase domain, which is catalytically inactive in LPa), the number of kringle-4 repeats determining the LPa-emia and hence the cardiovascular risk. Being similar in structure to that of plasminogen may explain its prothrombotic effect.

Concentration of LPa obeys an autosomal dominant pattern of inheritance [727]. In the European population, circulating LPa concentration (LPa-emia) is lower in Northern (mean 4.9 mg/dl) than Central (mean 7.9 mg/dl) and Southern European cohorts (mean 10.9 mg/dl) [782].

Lipoprotein-A concentration is determined by Apo_A synthesis in hepatocytes and catabolism, i.e., clearance. LPa-emia is elevated ($>$30–50 mg/dl; $\geq$75–125 nmol/l) in CVD patients [783], the range of values measured by different techniques in various cohorts being similar and Blacks having a higher LPa-emia than Whites [784].

Standardization of LPa immunoassay is difficult, and LPa-emia threshold depends on the assay used. Sex (estrogens or testosterone) and thyroid hormones in addition to nicotinic acid and PCSK9 and CETP inhibitors lower LPa concentration, in addition to lipid apheresis [781].

Concentration of circulating LPa in a population of individuals (48% women) mainly from North America (50%) and Western Europe (47%), with no previous history of CVD, correlates weakly with several conventional vascular risk factors. It represents a relatively modest risk factor for coronary heart disease and stroke [784].

Nevertheless, LP-a aggravates the risk for adverse cardiovascular events associated with other classical risk factors, such as LDL^{CS}. It represents a risk for atherosclerosis, in particular CoAD, in addition to aortic valve calcification and stenosis and possibly venous thromboembolism [781]. Elevated LPa-emia is robustly associated with an increased CVD risk, in particular in diabetic individuals [782].

In vitro, LPa exerts inflammatory and thrombogenic actions. Lipoprotein-A enters the arterial intima and can favor inflammation, thrombosis, and foam cell formation [784].

Lipoprotein-A and lipoprotein-associated phospholipase-A2 (lpPLA2)[40] act as causal and noncausal markers [784]. Lipoprotein-associated phospholipase-A2, which is encoded by the PLA2G7 gene (group-7 PLA2), circulates in the plasma mainly attached to LDLs. It may also be associated with atherosclerosis, according to some authors (but not all).

The lipidic components of LP_A and the protein Apo_A undergo different intracellular fates in hepatocytes [783]. After uptake, the lipidic components leave the Rab5+ early endosomes for lysosomal degradation. On the other hand, Apo_A dissociates from ApoB and moves to the trans-Golgi network and then possibly to Rab11+ recycling endosomes to be resecreted, about 30% of Apo_A being recycled back to the extracellular space [785]. In the extracellular medium, Apo_A reassociates with newly formed or circulating LDL, hence generating LP_A, and is cleared via various receptors, enters the vessel wall or kidney, or is degraded. Endocytosis of Apo_A and its recycling rely on the plasminogen receptor-KT (PlgRkt).[41] Several hepatic receptors are involved in LP_A catabolism. Its clearance and possible lysosomal degradation can rely on LDLR, VLDLR, LRP1, LRP2, ScaRb1, and syndecan-1 [783]. Once LP_A is connected to LDLR and LRP1, clathrin-mediated endocytosis leads to lysosomal degradation of its lipid moiety and associated Apo_A. The receptor ScaRb1 primes lysosomal degradation of the entire particle or the lipid content.

[40] A.k.a. platelet-activating factor acetylhydrolase (PAFAH).

[41] Plasminogen receptor PlgRkt is not only involved in LP_A catabolism and Apo_A internalization and recycling but also regulates plasminogen activation at the cell surface via urokinase- and tissue-type plasminogen activator. It also controls monocyte migration and matrix metallopeptidase (MMP) activation (e.g., MMP2 and MMP9) [108]. On the other hand, urokinase-type plasminogen activator receptor (uPAR or PlAUR) linked to the plasma membrane by a glycosyl phosphatidylinositol (PI) anchor, regulates plasmalemmal activation of plasminogen. Urokinase plasminogen activator stimulates migration of arterial smooth myocytes via uPAR and the pseudokinase protein Tyr kinase TYK2, which complexes with PI3, RhoA, and Rac1 (but not CDC42). Plasminogen can also carry oxidized phospholipids. In fact, Apo_A may not be internalized by PlgRkt, but rather resorted to PlgRkt in the early endosome for slow recycling and prevention of rapid lysosomal degradation [783].

Plasmatic LPa concentration is primarily determined by variations in the LP_A (APO_A) gene locus. In addition, APO_A gene transcription is controlled by various agents, such as interleukin-6, which increases Apo_A production in hepatocytes, and estrogens and bile acids, which decrease it [786].

5.1.3.4 Apolipoprotein-E

The glycoprotein ApoE, which is synthesized and secreted mainly by the liver, brain, and skin, in addition to macrophages, is a component of VLDLs, remnant lipoproteins, and HDLs. It facilitates their clearance via LDLR, LRP1, and syndecan-1.

The three ApoE isoforms ($ApoE^{\epsilon 2}$–$ApoE^{\epsilon 4}$) associated with three APOE alleles in humans differ in one or two amino acids (i.e., single amino acid substitutions) at two sites (positions 112 and 158):

- $ApoE^{\epsilon 2}$ (ApoE2) possesses Cys112 and Cys158
- $ApoE^{\epsilon 3}$ (ApoE3) Cys112 and Arg158
- $ApoE^{\epsilon 4}$ (ApoE4) Arg112 and Arg158

These amino acid differences modify affinities for TG-rich lipoproteins and clearance receptors.[42] Thus, they affect circulating concentrations of $VLDL^{CS}$, IDL^{CS}, and LDL^{CS}, in addition to the remodeling of VLDL to LDL and receptor-mediated remnant clearance [786]. The ApoE4 protein prefers large TGRLs (VLDLs and chylomicrons), whereas ApoE3 and ApoE2 preferentially connect to small spherical HDLs [786]. Enrichment of ApoE4 on VLDL accelerates its clearance from blood circulation by the liver receptor (LDLR, LRP1, and syndecan-1) and outcompete LDL–LDLR binding, because of the 20-fold greater affinity of ApoE3 and ApoE4 for LDLR than that of ApoB100, elevating LDL^{CS} concentration.

The APOE genotype ($\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, and $\epsilon 4/\epsilon 4$) strongly influences concentrations of ApoB-related lipoproteins including LPa and hence LPa^{CS}.[43] Difference in the affinity of ApoE subtypes for the lipoprotein clearance

[42] Gliocytes produce and secrete ApoE. ApoE can stimulate the MAP3K12–MAP2K7–ERK1/2 pathway in neurons, thereby phosphorylating Fos and activating production of amyloid-β precursor protein (APP) and amyloid-β secretion [787]. ApoE4, the most important genetic risk factor for Alzheimer's disease, is more potent in stimulating APP transcription and amyloid-β secretion than ApoE3, which is more efficient than ApoE2.

[43] In a cohort of more than 430,000 patients, mean LPa concentration rises from subjects with the $\epsilon 2/\epsilon 2$ to those with $\epsilon 4/\epsilon 4$ genotype ($\epsilon 2/\epsilon 2$ [ApoE2/2]: 23.4 ± 29.2; $\epsilon 2/\epsilon 3$ [ApoE2/3]: 31.3 ± 38.0; $\epsilon 2/\epsilon 4$ [ApoE2/4]: 32.8 ± 38.5; $\epsilon 3/\epsilon 3$ [ApoE3/3]: 33.2 ± 39.1; $\epsilon 3/\epsilon 4$ [ApoE3/4]: 35.5 ± 41.6; and $\epsilon 4/\epsilon 4$ [ApoE4/4]: 38.5 ± 44.1 mg/dl) [786]. Individuals with the ApoE4/4 phenotype thus have a 65% higher LPa concentration than those with the ApoE2/2 phenotype.

receptors, such as LDLR and LRP1, and hence competition between LPa and ApoE for a given receptor may thus affect LPa catabolism [786].[44]

Many ApoE receptors connect to other ligands. For example, VLDLR and LRP8 bind reelin; LRP5 and LRP6 are Wnt coreceptors; and LRP2 is an auxiliary receptor for sonic hedgehog [787].

In gliocytes, LDs store lipids for energy production. Mitochondrial dysfunction and redox stress due to elevated ROS formation induce neuronal lipid production and transport of lipids from neurons to gliocytes for storage in LDs that can contribute to the adjustment to redox stress. In neurons, lactate is converted to pyruvate and acetyl-CoA. In addition, lactate accumulation in neurons triggers the production of lipids that are subsequently transferred to gliocytes, in which they form LDs. Conversely, formation of glial LDs requires lactate transfer from gliocytes to neurons using monocarboxylate transporters in addition to fatty acid transporters, and apolipoproteins [788]. Glial lactate is then used for lipid synthesis within neurons. Monocarboxylate transporters enable gliocytes to secrete and neurons to absorb lactate. Lactate metabolites provide substrates for the synthesis of fatty acids, which are processed and transferred to gliocytes by FATP and apolipoproteins. In the presence of high ROS concentrations, impaired lactate transfer and low concentrations of FATP or apolipoproteins decrease glial LD formation. Apolipoproteins ApoD and ApoE participate in the transfer of lipids between neurons and gliocytes. Whereas ApoE2 and ApoE3 promote LD formation, ApoE4 leads only to the weak formation of LDs, even under conditions of overexpression and redox stress. Therefore, stress-primed LD formation, which relies on neuronal lactate, is not supported by ApoE4.

5.1.3.5 Lipoprotein Lipase (Lipase-D)

Lipoprotein lipase and its stimulator ApoA5 and inhibitors ApoC3 and AngptL4, which is secreted by AT during fasting, are implicated in the catabolism of TGRLs. Lipolysis of postprandial TGRLs by lipoprotein lipase augments levels of saturated and unsaturated FFAs in addition to those of hydroxylated linoleates hydroxyoctadecadienoic acids 9HODE and 13HODE, which mediate redox stress, thereby eliciting formation of TNFSF1, adhesion molecules, and ROS in ECs.

Various LPL regulators include ApoA5, ApoC1 to ApoC3, and angiopoietin-like proteins AngptL3, AngptL4, and AngptL8 [789]. ApoC2 is the necessary cofactor for LPL, whereas ApoC1 and ApoC3 inhibit LPL [727].

[44]The ApoE$^{\epsilon 2}$ subtype, which is associated with recessive inheritance and low penetrance, has the weakest binding affinity to LDLR, LDLR binding for ApoE$^{\epsilon 3}$ and ApoE$^{\epsilon 4}$ being normal.

5.1.4 Ceramides

Ceramides are linked to several cardiovascular risk factors (inflammation, insulin resistance, and obesity) in addition to major adverse cardiovascular events in apparently healthy individuals. They serve as markers for assessing the cardiovascular risk.

These molecules and related sphingolipids are involved in lipoprotein uptake and aggregation and hence cholesterol accumulation within macrophages in addition to regulation of NO synthesis and production of superoxide anions and cytokines [790]. Thus, they participate in atherogenesis. Inhibition of glycosphingolipid synthesis counters atherosclerosis in mice.

Among four circulating ceramide species (Cer[d18:1/16:0], Cer[d18:1/18:0], Cer[d18:1/24:0], and Cer[d18:1/24:1]), the strongest association with major adverse cardiovascular events in apparently healthy individuals (primary prevention) is observed for Cer(d18:1/18:0) [790]. In patients with diagnosed disease (secondary prevention), among the culprit ceramides, Cer(d18:1/16:0) and Cer(d18:1/24:1) linkage with cardiovascular events is higher than that of Cer(d18:1/18:0). The role of Cer(d18:1/24:0) mimics that of LDL^{CS}, which is typically associated with cardiovascular risk in primary prevention, whereas in CoAD patients, an inverse relationship or even no association can be observed due likely to hepatic LDLR regulation during inflammation. The Cer(d18:1/24:0) species, which links to lipoproteins, may be less active than other ceramide species.

Adiponectin receptors (AdpnRs) encoded by the ADIPOR1 and ADIPOR2 genes differ according to the mammalian species and tissular distribution. They control glucose and lipid metabolism, at least partly, via ceramidase.

Ceramide, sphingosine, and sphingosine 1-phosphate (S1P) are lipids that participate in regulating cell adhesion, differentiation, proliferation, migration, and apoptosis. Lysosomal and nonlysosomal ceramidases (acid, neutral, and alkaline ^{N}acylsphingosine amidohydrolases ASAH1–ASAH3)[45] cleave ceramide to a FFAs and sphingosine, a precursor of the antiapoptotic factor sphingosine 1-phosphate.

[45] Ceramidases have a maximal activity in acidic, neutral, and alkaline environments, respectively. Alkaline ceramidases include three subtypes: ACer1 (ASAH3), Acer2 (ASAH3l), and ACer3. ASAH1 is a lysosomal ceramidase that works at an optimal pH value of 4.5 [791]. It targets ceramides with saturated medium acyl chains (C10–C14) or unsaturated long acyl chains (C18:1 or C18:2). It not only hydrolyzes ceramide into sphingosine but can also synthesize ceramide from sphingosine and FFAs [792]. This reverse enzymatic activity occurs at a distinct pH (backward reaction at pH 6.0 and forward reaction at pH 4.5). A multi-enzyme complex contains ASAH1, acid sphingomyelinase, and β-galactosidase (but not other lysosomal enzymes, such as α-iduronidase and α-galactosidase). ASAH2 localizes to the plasma membrane and can be secreted. It hydrolyzes various types of ceramides at an optimal pH value of about 7.0 [791]. ACER1 lodges in the ER. It processes ceramides with unsaturated long acyl chains (C18:1 and C20:1) and very long saturated (C24:0) or unsaturated (C24:1) acyl chains. ACER2 resides in the Golgi body and has an optimal pH value of about 9.0. It uses various ceramides. ACER3 is located in the ER and Golgi body. Its preferential substrates are ceramides carrying unsaturated long acyl chains (C18:1 and C20:1). It has an optimal pH of about 9.0.

The zinc-binding GPCR subtype AdpnR2 possesses low basal ceramidase activity that is enhanced by adiponectin (Adpn) [793]. The AdpnR1 isoform also has weak intrinsic ceramidase activity.

Prolonged glucocorticoid-based treatment counters inflammation, but develops insulin resistance. In addition, glucocorticoids induce production of AngptL4 and ceramides [794]. AngptL4 mediates glucocorticoid-induced lipolysis in WAT, induces expression of genes encoding enzymes of ceramide synthesis in the liver of glucocorticoid-treated mice: PP2a and PKCζ. Inhibition of AngptL4 (mainly), PP2a, or PKCζ lessens glucose intolerance observed in WT mice owing to chronic glucocorticoid administration.

5.2 Gluco- and Lipotoxicity

Diabetes mellitus leads to diabetic cardiomyopathy. Mitochondrial dysfunction impairs oxidative metabolism despite the availability of substrates (amino acids, glucose, and fatty acids). Gluco- and lipotoxicity (hyperglycemia and dyslipidemia) provoke cardiomyocyte (CMC) dysfunction and death. Accumulation of metabolic intermediates within the myocardium perturbs gene transcription (e.g., transcription carried out by fatty acid-activated PPARs), mRNA translation (e.g., transcription controlled by nutrient-activated TOR), and protein post-translational modifications, in addition to the amount and action of signaling mediators [795].

In diabetic hearts, glucose uptake and oxidation are impaired, and hence fatty acids are almost exclusively used for ATP synthesis. Chronically elevated FA conversion to potentially toxic metabolites (e.g., ceramides, diacylglycerols, and ion channel-regulating acylcarnitines) and oxidation that increases ROS formation are responsible for lipotoxicity.

Vascular endothelial growth factor is not only implicated in angiogenesis, especially under hypoxia, but also in neurogenesis, immunomodulation, wound healing, and metabolism, via its numerous VEGF and VEGFR isoforms and splice variants.[46] The heart possesses all VEGF isoforms and receptors, VEGFb and VEGFR2 being the most highly abundant in normal conditions, a substantial and

Sphingomyelinases process sphingomyelin to ceramide and ceramidases ceramide to sphingosine, which is phosphorylated by sphingosine kinases (SphK1–SphK2). Sphingosine 1-phosphate is irreversibly cleaved by S1P lyase to ethanolamine phosphate and hexadecenal, which are incorporated into phosphatidylethanolamine (PE) and glycerolipids, respectively [791]. Sphingosine 1-phosphate can also be dephosphorylated to sphingosine by S1P-specific phosphatases (SPP1–SPP2) and broad-specificity lipid phosphate phosphohydrolase (LPP1–LPP3; a.k.a. phospholipid phosphatases, PLPP1–PLPP3, and phosphatidic acid phosphatases, PPAP2a–PPAP2c). Sphingosine 1-phosphate abounds in plasma; it mainly originates from red blood capsules.

[46] For example, $VEGFb_{167}$ (>80% of total VEGFB transcripts) and $VEGFb_{186}$ are VEGFb splice variants. $VEGFb_{186}$, but not $VEGFb_{167}$, possesses the HSPG-binding domain [795].

releasable VEGFb pool lodging on the CMC surface [795].[47] Vascular endothelial growth factor b modulates fatty acid uptake and oxidation, thereby protecting against the consequences of FA ingress and overprocessing. It is regulated by the PGC1α–ERRα (NR3b1) axis. Nutrients influence epigenetic background and hence transcription of the VEGFB gene; some species of dietary fatty acids affect the methylation status of the VEGFB promoter. In addition, VEGFb substantially increases transcription of genes implicated in cardiac contraction (e.g., SERCA and RyR) [799]. It also promotes cell (EC and CMC) survival.

Hyperglycemia induced acute VEGFb release from CMCs in co-cultures with ECs linked to high glucose level-induced heparanase release from ECs [799]. Heparanase[48] is secreted from ECs in response to elevated glucose concentration, releasing VEGFb from heparan sulfate proteoglycan, which then binds to ^{EC}VEGFR1, increasing fatty acid uptake, and ^{CMC}VEGFR1, attenuating apoptosis launched by the ERK–GSK3β pathway [799]. VEGFb activates the ERK–GSK3β axis in both CMCs and ECs, thereby attenuating H_2O_2-primed activation of caspase-3 and poly^{ADP}ribose polymerase and hence the likelihood of cell death. Therefore, ECs sense glucose and then protect both CMCs and ECs via the auto- and paracrine action of VEGFb.

In diabetic rats, cardiac heparanase and ^{CMC}VEGFb concentrations and ERK–GSK3β signaling decline, whereas ^{CMC}VEGFR1 expression rises, defining a state of *VEGFb resistance* [799]. In diabetes, circulating concentrations of glucose and fatty acids are chronically elevated; a depression of VEGF sensitivity may be an adaptation to prevent accumulation of lipotoxic species in the myocardium. Nevertheless, VEGFb resistance can also impair cardioprotection.

In addition, VEGFb promotes transendothelial transport of circulating fatty acids that are subsequently used by cardiac and skeletal myocytes [795]. Resulting increased fatty acid oxidation raises mitochondrial acetyl-CoA concentration, which inhibits pyruvate dehydrogenase, the gatekeeper of pyruvate entry into the

[47] Synthesis of VEGFc and VEGFd is upregulated in heart failure, whereas VEGFb concentration declines [795].

[48] Heparanase is encoded by the HPSE gene under control of early growth response EGR1, among other transcription factors. It is overexpressed in diabetes. This endoglycosidase (endoglucuronidase) resides in the endosomal and lysosomal compartments for a relatively long period (half-life ~30 h). Once it is secreted by degranulation, it degrades heparan sulfate. It thus releases various types of growth factors, cytokines, chemokines, and enzymes sequestered by heparan sulfate. Conversely, heparanase uptake is mediated by plasmalemmal heparan sulfate proteoglycans of the syndecan family, which limits its extracellular accumulation [796]. Under normal conditions, heparanase activity is restricted to some organs in addition to bloodborne cells (e.g., platelets, mastocytes, monocytes, neutrophils, and T lymphocytes). It is also synthesized in neovascular ECs and in some types of cancerous cells. It is implicated in inflammation, leukocyte migration, angiogenesis, and tumor growth and metastasis. It upregulates production of tumor progression agents (e.g., VEGF, HGF, TNFSF11, and MMP9), as it reduces the nuclear amount of syndecan-1, which prevents activity of histone acetyltransferases, thereby raising their activity and transcription of genes that favor an aggressive tumor phenotype [797]. It provokes VEGFc formation, thereby promoting tumoral lymphangiogenesis, which enables cancerous cell migration [798].

tricarboxylic acid cycle, thereby preventing glycolysis (Randle cycle). Imbalance between fatty acid uptake and oxidation causes their accumulation, hence altering cardiac gene expression and signaling, especially the one driving insulin-mediated glucose uptake. Moreover, CMC-specific VEGFb overexpression provokes ceramide accumulation and mitochondrial dysfunction.

Systemic excess lipids affect organismic metabolism and promote cardiometabolic disease with hypothalamic and pancreatic lipotoxicity and inflammation [717]. Local toxic and inflammatory effects of excess lipids in the hypothalamus impair its regulation of food intake, systemic energy expenditure, and peripheral metabolism. Ectopic fat depots and inflammation in the pancreas may alter insulin secretion.

Endothelial dysfunction refers to a maladaptive endothelial phenotype characterized by reduced NO availability, NO being an important determinant of endothelial function, and hence abnormal vasoreactivity, in addition to augmented redox stress and expression of proinflammatory and pro-thrombotic factors.

Nitric oxide is produced in vascular endothelia by the activated NOS3 subtype, which requires availability of its substrate, ^{L}arginine, and enzymatic cofactors (BH_4 [tetrahydrobiopterin], FAD, FMN, and NADPH). Elevated endothelial cytosolic Ca^{2+} concentration, which can be initiated by GPCRs such as that of acetylcholine, promotes binding of calmodulin to and subsequent activation of NOS3. In addition, NOS3 phosphorylation (Ser1177) by AMPK, PKA, and PKB stimulates NO production independently of Ca^{2+} ion. Induced vasodilation results from reduced Ca^{2+} concentration inside adjacent vSMCs upon guanylate cyclase activation and cGMP formation.

Major vasodilators, such as NO and PGi_2, are antiproliferative and anti-inflammatory, whereas important vasoconstrictors, such as ET1 and Agt2, are mitogenic and proinflammatory.

Insulin signals to participate in the vasomotor tone control using the vasodilatory NO-synthesizing PI3K and vasoconstrictory ET1-secreting MAPK branch, the latter also regulating adhesion molecule expression in vascular ECs (Table 5.4) [800].

Insulin tethers to its cognate receptor, which phosphorylates IRS1, which then connects to and activates PI3K. The latter produces PIP_3, which stimulates PDK1, which phosphorylates (activates) PKB. The latter phosphorylates NOS3 (Ser1177), which then produces NO. Insulin also stimulates the MAPK module and hence ET1 production in addition to PAI1, vcam1, and E-selectin in ECs [800]. On the other hand, the PI3K–PKB axis downregulates insulin-induced expression of PAI1 and adhesion molecules.

Glucotoxicity, *lipotoxicity*, and inflammation that contribute to insulin resistance related to altered glucose transport also provoke endothelial dysfunction with increased vascular permeability [800, 801]. Glucotoxicity, lipotoxicity, and various types of cytokines inhibit the PI3K–PKB axis.

A high intake of oils and fats enriched in ω6-fatty acids such as linoleic acid can cause endothelial dysfunction, redox stress, and inflammation [831]. On the other hand, ω3-fatty acids, such as eicosapentaenoic and docosahexaenoic acids, have antioxidant and anti-inflammatory effects.

Table 5.4 Insulin regulates the vasomotor tone and other processes and the effect of gluco- and lipotoxicity (Source: [800]; *cGMP* cyclic guanosine monophosphate, *ERK* extracellular signal-regulated kinase, *ET* endothelin, *InsR* insulin receptor, *IRS* insulin receptor substrate, *JNK* Jun N-terminal kinase, *MAPK* mitogen-activated protein kinase, *NOS* nitric oxide synthase, *PDK* phosphoinositide-dependent kinase, *PI3K* phosphatidylinositol 3-kinase, *PLC* phospholipase-C, *S6K* P70 ribosomal S6 kinase, *sGC* soluble guanylate cyclase)

Process	Pathway
Vasodilation	InsR–IRS1–PI3K–PDK1–PKB–NOS3–NO–sGC–cGMP
Vasoconstriction	InsR–SHC–GRB2/SOS–Ras–Raf–MAPK–ET1–ETR–PLC–Ca^{2+}
Cell adhesion	InsR–MAPK (↑)
Lipogenesis (hepatocyte)	InsR–IRS1–aPKC
Glucotoxicity, lipotoxicity, inflammation	ERK⊖ ⟶ IRS1
	JNK⊖ ⟶ InsR, IRS1
	PKCα⊖ ⟶ IRS1
	S6K⊖ ⟶ PI3K

Insulin binds to its receptor and then controls lipid uptake, lipolysis, and lipogenesis. Atypical protein kinase-C (aPKC) is required for insulin-stimulated glucose transport in myocytes and adipocytes and, in the liver, for activation of lipogenic enzymes

Endothelial lipotoxicity is caused by elevated concentrations of circulating non-esterified (free) fatty acids (neFAs), abbreviated in the present text *neFA-emia*. NeFAs are linked to obesity, insulin resistance, hypertension, and endothelial dysfunction [831]. In AT, neFA production increases in obese subjects; neFAs raise expression of IL6, but lower that of IL10 [802]. Augmented neFA-emia causes endothelial dysfunction, as FFAs: (1) disturb NOS3 activity, prevent prostacyclin production, and inhibit potassium channels, thereby impairing stimulation by insulin of endothelium-dependent vasodilation,[49] (2) support α AR-mediated constriction, (3) provoke redox stress,[50] and (4) trigger vascular cell proliferation and inflammation [831].

The FFA composition is more relevant to the vascular function than the total neFA amount [831]. An acute elevation of long- (lcFAs) but not medium-chain fatty acids (mcFAs) attenuates endothelium-dependent vasodilation. Endothelial function is inversely related to the proportion of saturated fatty acids (e.g., lauric and myristic acid) and positively related to the fraction of α-linolenic acid. The most abundant saturated fatty acid in human plasma, palmitate, can elicit formation of inflammatory cytokines in ECs.

[49] In ECs, insulin triggers the InsR–IRS1–PI3K–PDK1–PKB–NOS3 pathway. Once synthesized in ECs, NO is secreted and activates sGC in neighboring vSMCs.

However, insulin also stimulates endothelin-1 synthesis in and secretion from human endotheliocytes.

[50] In vSMCs, oleic and linoleic acids activate PKC, which stimulates NOx and hence ROS formation.

Stearoyl-CoA desaturase SCD1, the rate-limiting enzyme of lipogenesis, converts saturated to monounsaturated fatty acids for incorporation into neutral lipids, hence lowering lipotoxicity [831]. Palmitic acid, a saturated fatty acid that abounds in HFD, increases SCD1 concentration.

On the other hand, in myeloid cells, the toll-like receptor adaptor, toll–IL1R (TIR) domain-containing adaptor inducing interferon-β (TRIF), not only promotes inflammation to fight infection but also contributes to the hepatic metabolism. Activated TRIF represses SCD1, thereby reducing lipid accumulation in hepatocytes and preventing diet-induced hepatic steatosis [803]. Its effector, interferon regulatory factor IRF3, is a transcriptional suppressor that tethers to the Scd1 promoter.

Small lipid-binding proteins (SLBPs) are receptors and transporters for hydrophobic ligands with distinct ligand selectivity, binding affinity, and action modes, in addition to binding partners,[51] possessing both unique and overlapping functions according to the cell type. Whereas adipocyte FABP4 bound to linoleic acid translocates to the nucleus, once it is linked to oleate or stearate, it remains in the cytosol [831].

Lipidic autacoids, metabolites of arachidonic acids, short-lived endoperoxides, some prostaglandin types, and thromboxane-A_2, mediate endothelium-dependent vasoconstriction, which is exacerbated when NO production is altered, such as when FABP4 expression is triggered [831]. Lipocalin-2 also favors endothelial dysfunction related to aging and obesity. This lipid carrier elicits endothelium-dependent vasoconstriction and attenuates endothelium-dependent vasodilation. Furthermore, both FABP4 and Lnc2 are proinflammatory factors.

Therefore, SLBPs, such as FABP4 and Lnc2, favor obesity-induced endothelial dysfunction, as they modulate various signaling cascades involved in vascular homeostasis maintenance.

5.3 Overweight and Obesity

Imbalance between caloric intake (diet) and energetic expenditure (physical activity) is the major cause of obesity, an excess of body mass and adiposity.

Carbohydrate and fatty acid surplus affects body metabolism and organ functioning. Saturated fatty acids (e.g., palmitate) favor ceramide accumulation and obesity-related insulin resistance more than unsaturated fatty acids (e.g., oleate). Sugar-sweetened beverages is the primary source of added sugars in the diet; sweeteners are high-fructose corn syrup and sucrose, both containing approximately equal amounts of fructose and glucose [804]. Excessive fructose consumption is

[51] Among SLBPs, adipocytic fatty acid-binding protein, FABP4, interacts with HSL (or lipase-E), whereas lipocalin-2 heterodimerizes with MMP9 [831]. The secretory protein FABP4 has an elevated circulating concentration in obese individuals [831].

more detrimental than that of glucose, as it raises hepatic de novo lipogenesis and dyslipidemia, hence favoring insulin resistance and obesity (Sect. 6.5.3).

Adiposopathy designates qualitative anomalies of the AT as a consequence of AT maladaptive remodeling. A lower degree of adiposopathy is associated with a better systemic metabolic profile and vascular function [805]. Macrophage infiltration and redox stress in adipose depots provoke secretion of numerous inflammatory agents and adipocytic hormones that impair functioning of the heart and vasculature. In addition, adipogenesis is associated with angiogenesis.

The AT consists of multiple depots in the body and participates in the maintenance of physiological activities. However, excessive growth of adipose depots and ectopic accumulation of lipids, especially in the liver and skeletal muscles, alter the cardiovascular apparatus.

Increased circulating lipidemia contributes to atherosclerosis, whereas augmented lipid deposition in the AT, liver, and skeletal muscle causes obesity and insulin resistance.

Olfaction influences the anticipation of feeding. Inhibition of olfaction in lean and HFD-fed obese mice lowers nutrient intake and subsequently impedes further weight gain, reduces AT mass, and improves insulin resistance [806]. Olfactory sensory neurons also affect energy regulation. In mice with defective olfaction, fat consumption rises owing to elevated sympathetic nerve activity in AT, activated β-adrenoceptors on white and brown adipocytes promoting lipolysis [806]. In addition, thermogenesis increases in brown and inguinal fat depots. Conversely, ablation of the IGF1 receptor in olfactory sensory neurons enhances olfactory performance in mice, increases adiposity, and provokes insulin resistance.

Bone morphogenetic protein BMP4 supports multipotent mesodermal stem cell differentiation into adipocytes, recruits and activates beige adipocytes in the subcutaneous WAT, increases the number of stromal vascular cells, and launches AT angiogenesis [807].

Pericytes are capable of adipogenic differentiation; these PDGFRβ+ progenitors within neovessels serve as adipocyte progenitors, PDGFRβ expression decaying during adipogenic differentiation. On the other hand, PDGFRα is a marker of adipogenic precursors that only lodge in the vAT. BMP4 represses PDGFRβ activity via lysosomal degradation, hence priming pericyte differentiation into adipocytes during beiging of the subcutaneous WAT, whereas angiogenesis engenders neovessels that maintain the pool of adipocyte progenitors.

Blood concentration of erythritol, which is synthesized from glucose on the pentose phosphate pathway, is associated with increasing adiposity in young adults; it is higher than in individuals with stable adiposity [808].

Obesity can be associated with glomerular hypertrophy (glomerulomegaly) and *focal segmental glomerulosclerosis* (FSGS);[52] it becomes a leading cause of chronic

[52]Lesions are often perihilar. FSGS is defined as a segmental sclerosis (perihilar, cellular, tip, collapsing, and not specified) of the glomerular tuft causing capillary obliteration [809]. Perihilar afferent arterioles and glomerular capillaries are dilated. Podocyte volume rises (podocyte

kidney disease [809]. The glomerulus enlarges in response to obesity-induced elevation of renal plasma flow due to dilation mainly or solely of the afferent arteriole and constriction of the efferent arteriole by Agt2 and aldosterone that increases glomerular filtration rate and hence the amount of filtered and subsequently reabsorbed sodium and engenders a stable or slowly progressive proteinuria, which defines *obesity-related glomerulopathy*. Angiotensin-2 (obesity-mediated overactivation of the renin–angiotensin axis), insulin,[53] the renal sympathetic nervous system,[54] increased postglomerular oncotic pressure due to increased filtration fraction, and mechanosensors of tubular flow rate raise tubular sodium reabsorption. Insulin–PI3K–PKB and TOR signaling are implicated in podocyte hypertrophy [809].[55] In the kidney, adipokines and altered metabolism of fatty acid and cholesterol and resulting lipid accumulation[56] provoke insulin resistance in podocytes, tubular

hypertrophy), but at a lower rate than the increase in glomerular tuft volume, and the podocyte density declines. Because the glomerular volume expands, stress and strain exerted on these cells can detach them and cause a local denudation of the glomerular basement membrane. In addition, approximately 50% of patients with obesity-related glomerulopathy, a hyperfiltering nephropathy that does not manifest the typical signs of the nephrotic syndrome (hyperlipidemia, hypoalbuminemia, and edema) have mild diabetoid changes (focal or diffuse increase in the mesangial matrix and thickening of the glomerular basement membrane). Intracellular lipid vacuoles can accumulate in mesangiocytes, podocytes, and proximal tubular epitheliocytes.

[53] Hyperinsulinemia secondary to insulin resistance increases the tubular reabsorption of sodium, as insulin stimulates epithelial sodium channel (ENaC) activity in the late distal tubule and, to a lesser extent, in the proximal tubule and loop of Henle.

[54] Three factors associated with obesity activate the renal sympathetic nervous system: high leptin concentration, low adiponectin concentration, and obstructive sleep apnea [809].

[55] Podocytes possess insulin receptor and can adjust their morphology to postprandial changes in intracapillary pressure and glomerular filtration rate. Accumulation of non-esterified fatty acids in podocytes is linked to insulin resistance and podocyte apoptosis [809]. Insulin activates TORC1 via the PI3K–PKB axis and hence lipogenesis via PPARγ and SREBP1, angiogenesis via HIF1 and VEGF, and cell growth via S6K and 4eBP1. On the other hand, S6K inhibits TORC2 and IRS1, favoring insulin resistance. Nutrients and growth factors activate TORC2, which provokes actin remodeling via PKCα, Rho, and Rac and supports cell survival via FoxO1 and sodium reabsorption via the SGK1–ENaC axis [809]. Insulin also stimulates VEGF production in podocytes. Podocytes undergo hypertrophy via TOR to cover the enlarging glomerular tuft associated with excess weight. Once it is activated by TORC2, PKB2 favors podocyte survival.

[56] Renal TG accumulation results from increased fatty acid synthesis mediated by SREBP1c, which promotes the formation of acetyl-CoA carboxylase, FAS, and stearoyl-CoA desaturase SCD1, in addition to CHREBP, which upregulates production of liver pyruvate kinase [809]. Moreover, SREBP1 upregulates expression of proinflammatory cytokines, such as TNFSF1, IL1β, and Ifnγ, which activate SREBP1, hence engendering a vicious cycle. Angiotensin-2 elicits SREBP1 activity, which also mediates profibrotic TGFβ signaling. On the other hand, FXR inhibits SREBP1c and ChREBP and stimulates PPARα, thereby impeding lipid accumulation. Renal TG accumulation can also occur by increased uptake via ScaRb3 and/or fatty acid transport protein (FATP) or by decreased fatty acid oxidation mediated by PPARα via peroxisomal acyl-CoA oxidase-1 and carnitine palmitoyltransferase in addition to sirtuin-3 via mitochondrial long- and medium-chain acyl-CoA dehydrogenases [809]. On the other hand, G-protein-coupled bile acid receptor, GPBAR1, decreases inflammation and mitochondrial ROS generation and increases mitochondrial genesis and hence mitochondrial antioxidant generation and fatty acid oxidation.

atrophy, chronic inflammation, redox stress, and interstitial fibrosis; mechanical forces arising from glomerular hyperfiltration cause a maladaptive adaptation.

5.3.1 Epidemiology

Approximately one-third of the human population consists of overweight and obese people [810]. Obesity lowers life expectancy, as it augments the coupled metabolic and cardiovascular risks, that is, the likelihood of developing T2DM and atherosclerosis.

Between 1978 and 2013, the proportion of overweight and obese adults (BMI $\geq 25\,\mathrm{kg/m^2}$) increased from 28.8 to 36.9% in men and from 29.8 to 38.0% in women [809]. The prevalence of obesity has increased to about 40% among adults and approximately 20% among adolescents [811]. In 2014, about 40% of adults were overweight, and 13% were obese worldwide [812].

In some countries in Oceania, North Africa, and the Middle East, the prevalence of obesity in 2013 exceeded 50% of the adult population. The prevalence of obesity is lower but still high in other countries, such as in North America (∼30%) and Western Europe (∼20%) [721].

Overweight and obesity increased at the end of the last century much faster in adults than in children and in women than in men. In children and adolescents (2–19 years), the prevalence rates of overweight and obesity defined as BMI greater than or equal to 85th or 95th percentile for a given age or sex, respectively, increased significantly among Hispanic females and Black males. Adult Black women had the highest rate of increase (annual average increase of 0.88%) compared with other ethnic groups [662].

Ethnic disparities exist in the prevalence of two related multifactorial diseases, obesity and diabetes. In general, in both adults and children, Blacks and Mexican Americans are at a higher risk than non-Hispanic Whites [662]. Among men, Blacks have a lower percentage of body fat than non-Hispanic Whites and Mexican Americans. Among women, Mexican Americans have a higher mean percentage body fat than non-Hispanic Whites and Blacks.

Childhood adiposity is associated with adult left ventricular hypertrophy. In a population of 710 adults (aged 26–48 years), after age, sex, and race adjustments, association between childhood BMI and left ventricular mass index is explained more by adult BMI than systolic blood pressure, adiposity being a major predictor

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Cholesterol accumulation results from augmented cholesterol synthesis by hydroxymethylglutaryl CoA reductase (HMGCR) stimulated by SREBP2 and uptake through LDLR, ScaRa, ScaRb3, and ScaRe1, and lowered cholesterol efflux through ABCa1 and ABCg1 via LXR and catabolism linked to bile acids and bile acid transporters. Activation of SREBP2 is caused by inflammatory cytokines, which interfere with the SCAP–SREBP2–LDLR and HMGCR axes, ER stress, which stimulates SREBP2 release from the ER, and advanced glycation end products (AGEs), which prime abnormal translocation of SCAP from the ER to the Golgi body [809].

of left ventricular hypertrophy, especially the eccentric form, whereas hypertension causes concentric left ventricular hypertrophy [813]. Therefore, as longer exposure aggravates the risk for adverse remodeling, an independent predictor of cardiovascular events, obesity should be evaluated as excess adiposity multiplied by years of exposure (then expressed in kg–year).

5.3.2 Cardiovascular Effects of Obesity

Obesity is strongly related to other cardiovascular risk factors, such as dyslipidemia, T2DM, and hypertension. Diverse fatty depots have a distinct impact on the cardiovascular apparatus. Obesity is linked to *FAA-emia* (plasmatic free fatty acid concentration), which provokes *endothelial dysfunction* and atherosclerosis (e.g., coronary arterial plaques).

Systemic *endothelial dysfunction* and CVD is strongly associated with *visceral adiposity*, which does not represent predominant AT in healthy subjects. On the other hand, the scAT is a minor contributor or plays a neutral or even protective role [805]. Normally, subcutaneous and abdominal vAT represent about 80% and 5–20% of total body fat mass, respectively.

According to the American Heart Association, cardiovascular health relies on a BMI lower than 25 kg/m^2 and a fasting glycemia less than 100 mg/dl. Both the degree and duration of obesity influence CVD prognosis [721].

Metabolically benign obesity is defined by the presence of certain factors and conditions, such as low inflammatory marker concentrations in blood, high adiponectinemia, preserved insulin sensitivity, a low amount of vAT, and proper caloric intake and physical activity [721].

Cardiorespiratory fitness is improved by physical activity and exercise. The *fat-but-fit concept* relies on the following observations: (1) among obese men and women who are relatively fit using the sex- and age-specific quintiles from the Aerobics Center Longitudinal Study, the risk of CVD-induced mortality is reduced and (2) a mild to moderately obese fit man or woman can have a lower CVD-induced mortality risk than an unfit individual with a normal weight. Fit obese people have lower levels of most of the CVD risk factors.

5.3.2.1 Proatherogenic Fatty Acids

Oleic acid is the most abundant circulating proatherogenic ω9-monounsaturated fatty acid. It precludes vasodilation primed by acetylcholine and elicits proliferation of vSMCs. In smooth myocytes, oleic acid represses the formation of antiatherogenic sirtuin-1 and NR1c3 (PPARγ) [814]. Sirtuin-1 deacetylates NR1c3, thereby impeding inflammation, medial SMC proliferation and migration, and hence vascular wall remodeling, and protecting against redox stress. On the other hand, oleic acid activates NFκB and raises TGFβ1 release. Moreover, oleic acid

augments NO production owing to NOS2 overexpression via NR1c3 inhibition and NFκB activation, thereby promoting the secretion of proinflammatory MMP1 and MMP3 [814].

5.3.2.2 Endothelial Dysfunction

Obesity is associated with hypertension secondary to arterial stiffness and vascular dysfunction with reduced endothelium-dependent dilation due to defective interplay between multiple endo- and paracrine messengers that provokes inflammation, redox stress, and subsequent structural modifications of the vessel wall.

In young sedentary obese men and women, acetylcholine-stimulated blood flow declines because of impaired microvascular endothelial function with respect to lean and overweight subjects, whereas interstitial concentrations of hydrogen peroxide and superoxide rise in the vastus lateralis owing to augmented NOx activity [815]. After 8 weeks of exercise, H_2O_2 concentration decreases in obese subjects and the microvascular endothelial function is restored, becoming similar to that in lean humans. In skeletal muscles, synthesis of the NAD(P)H oxidase subunits cytochrome-B245α, NOxO2, and NOxA2 increases in obese subjects, linking excessive NOx-derived ROS to microvascular endothelial dysfunction in obesity. Exercise reduces cytochrome-B245α and NOxA2 formation in the skeletal muscles of obese individuals.

Endothelial dysfunction correlates with altered mitochondrial quality control in aging. Cellular senescence is associated in mitochondria with defective genesis and dynamics (fusion and fission) on a microscopic scale, and defective electron transport chain (ETC) and quality control, in addition to increased superoxide production and decayed activity of antioxidant ^{Mn}SOD2 on a nanoscopic scale [816].[57] NOx4 is implicated in EC senescence.

Although ECs have a low content of mitochondria (2–6% of the cell volume [∼28] and 32% in hepatocytes and CMCs, respectively, in rat ECs [816]),[58] damaged mitochondrial dynamics participates in endothelial dysfunction. Myocardial arteriolar ECs contain mitochondria anchored to the cytoskeleton that release ROS in response to cell deformation caused by hemodynamic stress [816]. Under hypoxia, they form perinuclear clusters using microtubules and dynein.

Hyperglycemia induces ROS overproduction, which provokes mitochondrial fragmentation [817]. In ECs, the ROS-sensitive channel TRPM2 is thus gated, eliciting Ca^{2+} influx, lysosomal permeabilization, and redistribution of lysosomal Zn^{2+} to mitochondria, where Zn^{2+} recruits DRP1, a mitochondrial fission factor (MFF).

[57] Expression of SOD2 is regulated by FoxO and SIRT1.

[58] Mitochondrial content depends on the balance between mitochondrial genesis, which involves transcription of nuclear and mitochondrial genes, and mitophagy. Healthy mitochondria undergo fusion–fission cycles, whereas damaged ones depolarize and are cleared by mitophagy.

Endotheliocytes create a large proportion of their energy from the anaerobic glycolysis.

Overproduced ROS uncouple NOS3 and react with NO,[59] thereby precluding the availability of NO, an antihypertensive, anti-thrombotic, and anti-inflammatory molecule.

In ECs, the integral membrane protein, mitochondrial calcium uniporter regulator ^{Mt}CUR1, in addition to UCP2 and UCP3, support mitochondrial Ca^{2+} uptake by ^{Mt}CU [816]. Mitochondrial calcium at relatively low levels increases PGC1α expression and hence promotes mitochondrial genesis. Moreover, it stimulates mitochondrial NO production in addition to enzymes of the tricarboxylic acid cycle and oxidative phosphorylation. In addition, H_2O_2 increases mitochondrial Ca^{2+} content due to at least partly decreased Ca^{2+} extrusion secondary to inhibited Na^+–Ca^{2+} exchanger.

5.3.2.3 Microvascular Dysfunction

Obesity affects large blood vessels in addition to the microvasculature, not only in vAT but also in the heart, brain, kidney, lung, and skeletal muscle, altering organ perfusion and contributing to impaired release and clearance of metabolites and neurohumoral factors, in particular adipokines, proinflammatory cytokines, and cardiomyokines [818].

A high circulating lipid load, which is linked to the amount and composition of the vAT, even before the onset of obesity, engenders global *microvascular dysfunction* in various organs [818]. Local low-grade inflammation and overall endothelial dysfunction influence vascular structure.

Changes in arteriolar tone by vasoregulators released from perivascular nerves, irrigated organs, and the endothelium, in addition to circulating factors acutely regulates local blood flow resistance and hence supply of oxygen and nutrients.

The endothelium produces and secretes vasodilators, such as NO, prostacyclin (PGi_2), hydrogen peroxide (H_2O_2), and endothelium-derived hyperpolarizing factor (EDHF), vasoconstrictors, such as endothelin-1 (ET1), vasoconstrictory prostanoids, and superoxide ($O_2^{\bullet-}$), in addition to vasoactive substances, the action of which depends on the vascular bed, such as uridine adenosine tetraphosphate (UP_4A).

In obesity, the sympathetic nervous system is activated by leptin [818]. Endocrine messengers, such as the hormone insulin, target ECs. Within ECs, insulin can activate the PI3K–PKB–NOS3 axis, hence producing NO, or the MAPK module, ERK1 and ERK2, which stimulates ET1 formation and release. In the

[59] Superoxide ($O_2^{\bullet-}$) reacts with NO to form peroxynitrite ($ONOO^-$).

Endothelial NO synthesis depends on arginase-2 (Arg2) in mitochondria and ^{L}arginine carriers, in addition to NOS3. In ECs, mitochondrial arginase-2 is constitutively expressed, whereas the cytosolic arginase-1 (Arg1) is barely detectable [816].

Acetylcholine and serotonin activate NOS3 via cytosolic calcium ions and binding of calcium–calmodulin, which activates CamK2, which controls Nos3 gene transcription and the NOS3 phosphorylation state.

healthy vasculature, insulin-induced activation of NOS3 and epoxyeicosatrienoic acids (EETs) predominate, whereas obesity favors insulin-mediated activation of ET1 and vasoconstrictory eicosanoids. Moreover, insulin-mediated capillary recruitment by adiponectin declines with adiponectinemia, whereas FFAs and inflammatory cytokines counter this process.

Visceral Adipose Tissue Microvasculature in Obesity

Blood flow to the vAT rises after a meal in lean, but not obese subjects. Lipid accumulation in the visceral white adipocyte increases in size from 50 up to 200 μm, a length greater than the O_2 diffusion distance and, with accompanying capillary rarefaction, diminishes AT oxygenation [818].

Impaired AT angiogenesis associated with capillary rarefaction, hypoxia, inflammation, and metabolic dysfunction results from overexpression of the antiangiogenic splice variant $VEGFa_{165}b$, which binds, but fails to efficiently activate VEGFRs [819]. Wnt5a is a proinflammatory secreted protein that is associated with metabolic dysfunction in obesity. Concentrations of Wnt5a and $VEGFa_{165}b$ correlate in the scAT and vAT of obese individuals. In scAT, where angiogenic capacity is greater than in visceral depots, Wnt5a raises $VEGFa_{165}b$ formation in vascular ECs. Moreover, Wnt5a can increase secretion of soluble VEGFR1 ($VEGFR1^S$), an inhibitor of angiogenesis. Secreted frizzled-related protein sFRP5, which acts as a Wnt5a decoy receptor, improves capillary sprout formation and reduced $VEGFR1^S$ production. Therefore, Wnt5a hampers AT angiogenesis via $VEGFa_{165}b$ in obese humans.

Acute hypoxia of hypertrophic adipocytes during short periods of high-fat feeding, launches angiogenesis via VEGF and proper matrix remodeling, thereby enabling further expansion [722].

On the other hand, chronic hypoxia occurs in expanded vAT with adipocyte hypertrophy and hyperplasia during prolonged high-fat feeding. Hypoxia lowers adiponectin and raises leptin release from adipocytes [818]. Moreover, chronic hypoxia induces sustained inflammation, inappropriate angiogenesis, and adverse matrix remodeling that becomes stiff and evolves to fibrosis.

Coronary Microcirculation in Obesity

In obesity and hypercholesterolemia, coupling of the coronary blood flow with the myocardial metabolic demand is altered and coronary arterial resistance rises owing to disturbed regulation of the vasomotor tone and capillary rarefaction [818].

Obesity is also associated with the structural remodeling of the coronary microcirculation. Lower capillary density in obese patients is associated with arteriolar hypertrophic inward remodeling and stiffening [818].

Cerebral Microcirculation in Obesity

The brain microvasculature is only surrounded by neurons and gliocytes. Obesity can thus impact it only via modifications of interactions between vascular cells and their neighbors, hemodynamic changes, and circulating endocrine messengers.

Obesity affects the structure of small cerebral arteries, arterioles, and capillaries associated with impaired metabolism, redox stress, and endothelial dysfunction and disrupts the neurovascular coupling [818].

In obese humans, cerebral blood flow declines with impaired vasodilation during hypercapnia due to capillary rarefaction, diminished NO contribution to basal cerebral microvascular tone control, altered release of vasodilatory prostanoids, and the effect of H^+ on vascular smooth myocytic ion channels [818]. Inward remodeling and progressive arterial stiffening can be observed in obese rats with metabolic syndrome.

Renal Microcirculation in Obesity

Obesity is linked to increased perirenal fat deposition, which causes low-grade inflammation, reduced antioxidant capacity, and renovascular endothelial dysfunction [818]. Obesity can be associated with nephrosclerosis and glomerulonephritis. Afferent arteriolar vasodilation increases renal blood flow and hence glomerular filtration. Arteriolar and capillary density in the outer renal cortex does indeed increase in obese animals. However, newly formed vessels are more tortuous and leaky, hence immature and dysfunctional.

Structural alterations in the kidney of obese subjects result from impaired balance between pro- and antiangiogenic factors, concentrations of VEGF, VEGFR2, and AngptL2 increasing and that of the angiogenesis inhibitor TSp1 decreasing owing to redox stress [818].

Pulmonary Microcirculation in Obesity

Obesity is associated with structural changes in the pulmonary microvasculature with increased medial thickness of pulmonary small arteries and veins and muscularization of pulmonary arterioles [818]. In general, vasoconstriction to serotonin and hypoxia is reduced in pulmonary resistance (but not conductance) arteries of obese rats.

Skeletal Muscle Microcirculation in Obesity

At rest, skeletal muscle blood flow is moderately reduced, at least in mild obesity. Because exercise hyperemia involves many redundant regulatory mechanisms, it is relatively well maintained [818].

Resting muscle sympathetic nerve activity is significantly higher in obese patients with metabolic syndrome and does not further increase during exercise [818]. Exercise-induced increase in skeletal muscle blood flow is reduced or at best preserved in obesity. Obesity is linked to a shift in the balance of neurogenic control of skeletal muscle blood flow, with increased α AR-mediated constriction at rest, which disappears during exercise, thereby compensating for a loss of β AR-primed vasodilation.

In humans, endothelium-dependent skeletal muscle microvascular vasodilation in response to acetylcholine is preserved or reduced in obesity [818]. Contribution of both NO and vasodilatory prostaglandins to ACh-induced vasodilation is reduced, but compensated for by EDHF linked to EETs and/or H_2O_2. Superoxide dismutase, which converts superoxide to H_2O_2, is more active in obese individuals. In addition, reduced NO availability is counterbalanced by reduced PDE5 activity and ET1 sensitivity of skeletal muscle arterioles and local ET production.

In addition, insulin resistance is associated with a shift from insulin-primed vasodilation to -constriction [818].

5.3.2.4 Obesity and Thrombosis

The healthy endothelium synthesizes and releases regulators of the coagulation cascade, platelet activation and aggregation, and fibrinolysis. This anti-coagulant barrier produces two transmembrane proteins, thrombomodulin and heparin sulfate proteoglycan, and release tissue factor pathway inhibitor (TFPI) [820]. Thrombomodulin tethers to thrombin to activate protein-C, thereby preventing F$VIII$ and FV activation and thrombin formation. HSPG is a cofactor of anti-thrombin-3. TFPI inhibits both FXa and TF–FVIIa complex. Endotheliocytes also produce tissue-type plasminogen activator, which activates the fibrinolytic cascade.

The endothelium also precludes platelet activation via plasmalemmal ectonucleotide pyrophosphatase–phosphodiesterase, which degrades extracellular ATP and ADP, ENPP1, and ecto-5′-nucleotidase in addition to synthesis and release of prostacyclin and especially NO [820]. NO also hampers platelet aggregation.

Krüppel-like factor, KLF2, an endothelial transcription factor, contributes to maintaining the anti-thrombotic endothelial surface, as it primes thrombomodulin and NOS3 expression [820].

Abdominal obesity is associated with an elevated risk for arterial and venous thrombosis in a context of inflammation and redox stress, which can alter anti-thrombotic endothelium. Obesity is linked to the risk for deep vein thrombosis and pulmonary embolism. Obese patients have chronic intra-abdominal hypertension, decreased blood flow velocity in the common femoral vein, and hence disturbed venous return [820].

Obesity and Platelet Function

The circulating platelet size, which is related to higher susceptibility of platelets to activation, increases in obese subjects [820]. Platelet density also rises in obese women.

Platelets are anuclear fragments of megakaryocytes. Megakaryocyte maturation depends on inflammatory mediators (e.g., IL1, IL3, IL6, IL11, and IL18), NO, and thrombopoietin. In obesity, reduced NO production is compensated by augmented release of cytokines that influence megakaryopoiesis.

Platelet guide inflammation and thrombosis are activated in these processes. Several platelet activation markers are elevated in obese patients, such as the mean platelet volume, circulating concentrations of platelet microparticles, thromboxane-B_2 metabolites, soluble P-selectin, and platelet-derived TNFSF5 [821].

Platelet hyperactivity can be observed in obese women without cardiovascular risk factors [820]. Android obesity is linked to a four-fold higher rate of urinary excretion of 11-dehydroTxB_2 with respect to lean women, a value comparable to that associated with cigarette smoking, hypercholesterolemia, and T2DM [820].

Abdominal obesity with inflammation and redox stress favors lipid peroxidation and hence increases 8-iso$PGf_{2\alpha}$ concentration, a marker of redox stress that amplifies response of platelets to low stimulating agent concentrations.

Platelet activation markers, such as TNFSF5 and P-selectin, have an upregulated expression in obesity. Upon platelet activation, TNFSF5, a trimer stored in α granules of resting platelets, is rapidly exposed on the platelet surface, where it is cleaved into a soluble fragment. $TNFSF5^S$, which has auto-, para-, and endocrine effects, elicits platelet recruitment and vascular inflammation [820]. Selectin-P, which is also stored in α granules, rapidly translocates to the surface of activated platelets, where it facilitates monocyte recruitment to the vessel wall.

Numerous adipokines contribute to endothelial dysfunction and can support platelet activation (Table 5.5).

In addition, the sensitivity of platelets and their response to insulin, PGi_2, and NO decrease. Insulin receptors in the platelet plasma membrane, once they are liganded, autophosphorylate and then trigger insulin anti-aggregatory signaling and lower cytosolic Ca^{2+} concentration via NOS3 activation and PGi_2 production, which launches the cGMP–PKG and cAMP–PKA pathways, respectively [820]. In addition, insulin can reduce platelet sensitivity to various platelet stimulants, such as ADP, adrenaline, collagen, and thrombin. Moreover, insulin regulates transcription factors that launch synthesis of tissue factor and plasminogen activator inhibitor PAI1 in obese individuals.

Microvesicles released from activated platelets exert proinflammatory and procoagulant effects and trigger platelet aggregation. Circulating concentration of platelet-derived microvesicles is elevated in obese subjects compared with age-matched lean subjects [820].

Activated platelets not only aggregate and release proinflammatory mediators but also shed LPC-rich microvesicles. These microvesicles serves as dockers that

Table 5.5 Major adipokines contributing to endothelial dysfunction and platelet activation (Source: [820]; *CCL/CXCL* chemokines, *CRP* C-reactive protein, *IL* interleukin, *PAI* plasminogen activator inhibitor, *PGi_2* prostacyclin, *SAa* serum amyloid-A, *TNFSF* tumor-necrosis factor superfamily member, *VEGF* vascular endothelial growth factor)

Process	Adipokines
Endothelial dysfunction	With decreased concentration: adiponectin, apelin, clusterin, ghrelin, IGF1, IL10, lipocalin-2, omentin, PGi_2, SAa
	With elevated concentration: adipsin, angiotensinogen, calprotectin, cathepsin-S/L/K, CCL2/3/5/7/8/11, CRP, CXCL8, fetuin-A, IL1β/6, leptin, osteopontin, PAI1, resistin, tissue factor, TNFSF1, VEGF, visfatin
Platelet activation	With decreased concentration: apelin, PGi_2, SAa
	With elevated concentration: CRP, IL1β/6, PAI1, tissue factor, TNFSF1

dissociate pentameric C-reactive protein (or Ptx1; ^{5}CRP) into its proinflammatory and pro-thrombotic monomeric isoform (^{1}CRP) [822].

Adiponectin tethers to its receptors AdipoR1 and AdipoR2 on platelets. Although it impedes tissue factor formation in macrophages and activity, it exerts no effects on platelet activation and aggregation [820].

Leptin targets its receptors on ECs, macrophages, and platelets. At obesity-relevant leptinemia, leptin acts synergistically with ADP and thrombin for platelet aggregation [820]. Leptin may also activate platelets via the JaK2–PI3K–PKB–PDE3a–cAMP pathway in addition to PLCγ2, PKC, and PLA2.

Hyperlipidemia enhances susceptibility to thrombosis. In addition, altered *platelet lipidome* may predispose to thrombosis. LDL and oxLDL[60] prime platelet degranulation, $\alpha_{2B}\beta_3$-integrin activation, apoptosis, thrombin generation, and CXCL12 release [823]. The chemokine CXCL12 tethers to its receptors CXCR4 and CXCR7 and facilitates LDL and oxLDL uptake by platelets. Intraplatelet content of oxidized lipid metabolites, cholesteryl esters, sphingomyelin, ceramides, phospholipase metabolites (diacylglycerol), and acylcarnitines is elevated in atherosclerosis [823].

[60]LDLs taken up by platelets are oxidized by mitochondrial ROS, generating oxidized fatty acids (oxFAs), phospholipids (oxPLs), and lysophosphatidylcholine (oxLPC). Oxidized LDLs can activate platelets and promote uptake and generation of lipid metabolites. Lipids can be metabolized into ceramides, diacylglycerol, and sphingomyelin.

Obesity and Coagulation Factors

Obese subjects also have an elevated circulating concentration of von Willebrand factor, tissue factor, clotting factors F*VII* and F*VIII*, and fibrinogen but also of plasminogen activator inhibitor PAI1 and thrombin-activatable fibrinolysis inhibitor (TAFI), having an impact on fibrinolysis and contributing to a pro-thrombotic state [820].

Plasminogen activator inhibitor PAI1, or serpin-E1, is mainly secreted from the liver and AT. It represses plasminogen activators (tPa and uPA), thereby precluding dissolution of the fibrin clot. Obesity is linked to upregulated PAI1 formation in the vAT, hence hampering fibrin clearance and raising thrombosis risk.

Thrombin not only contributes to clot formation but also stabilizes the clot via thrombin-activatable fibrinolysis inhibitor, which protects the fibrin clot against degradation.

In obesity, the AT releases lower amounts of adiponectin, raising platelet susceptibility to aggregation and limiting fibrinolysis via PAI1 [820]. Obesity also impairs platelet sensitivity to insulin and promotes production of isoprostanes, which further render platelets reactive.

In obese individuals, the AT favors immunocyte switch to an inflammatory phenotype, macrophage evolving to an M1 proinflammatory and T_{H2} cells to T_{H1} and T_{H17} polarization [820]. Adipose tissue M1 macrophages secrete tissue factor, which, combined with augmented hepatic synthesis of F*VII* and F*VIII*, heightens the coagulation risk.

Tissue factor, a transmembrane glycoprotein, serves as a receptor for F*VII*a and then activates the extrinsic coagulation cascade.[61] Elevated tissue factor production in obesity is linked to its synthesis in macrophages and neutrophils in addition to adipocytes and stromal vascular cells. It can result from the action of insulin, TNFSF1, TGFβ, and leptin [820].

Production of ROS by NAD(P)H and xanthine oxidases, uncoupled NOS3, lipoxygenases, cyclo-oxygenases, microsomal P450 enzymes,[62] and prooxidant CyP heme molecules augments. Furthermore, ROS production is favored by overactivation of the renin–angiotensin axis (RAA), as several RAA components are produced by dysfunctional adipocytes (Sect. 5.4.5.17).

Concomitantly, endothelial dysfunction is accompanied by a decreased antioxidant defense, particularly NFE2L, in metabolic syndrome patients [820].[63]

[61] Activation of the TF–F*VII*a complex activates F*X*, thereby priming generation of thrombin and conversion of fibrinogen to fibrin. The generation of an insoluble fibrin clot is the final step of the coagulation cascade.

[62] Microsomal P450 enzymes comprise CyP1a1/2, CyP2a6, CyP2b6, CyP2c8/9/19, CyP2d6, CyP2e1, CyP2j2, CyP3a4/5/7/43, and CyP4a11 [824].

[63] Redox stress causes dissociation of NFE2L from keap1, which initiates NFE2L proteasomal degradation. Hence, NFE2L translocates to the nucleus, where it binds to antioxidant response element and triggers transcription of antioxidant genes.

5.3.2.5 Aldosterone and Mineralocorticoid Receptor

Obesity, hypertension, and hyperaldosteronism can be interrelated. The renin–angiotensin–aldosterone axis is activated in obesity and hypertension. In humans, obesity is associated with elevated aldosterone production in addition to metabolic syndrome and cardiac hypertrophy and fibrosis. In obese diabetic mice, concentrations of aldosterone and mineralocorticoid receptor (MR or NR3c2) rise [825]. Blockage of NR3c2 counters the pro-inflammatory cytokine profiles of AT in obese mice.

In addition to the kidney, where aldosterone provokes sodium retention and volume expansion, NR3c2 lodges in the colon, brain, and AT, especially adipocytes in the perivascular adipose depots, and on leukocytes, particularly monocytes and macrophages, vascular smooth muscle and endothelial cells, and cardiac myocytes and fibroblasts. Mineralocorticoid receptor can favor arterial stiffness and insulin resistance [826]. In macrophages and dendrocytes, NR3c2 affect their phenotype and hence cytokine production [489]. In addition, the G-protein-coupled receptor, GPR30, may mediate some of the effects of aldosterone [825].

An early aldosterone-mediated PKCα activation promotes NR3c2 transactivation, rapid (within minutes) nongenomic aldosterone effect, enhancing its genomic action. Once it is liganded, NR3c2 translocates to the nucleus, where it connects to the steroid hormone (SHRE) and negative response element (nSRE) [827]. Among proteins, the synthesis of which is regulated by aldosterone, glucose 6-phosphate dehydrogenase may mediate the effect of NR3c2 on cellular function [825].

NR3c2 interacts with various modulating proteins of its activity, such as AT_1, EGFR, and probably striatin [825]. The small GTPase, Rac1, increases NR3c2 activity. In addition, the epigenetic regulator and demethylase KDM1a and membrane scaffold protein caveolin-1 modulate NR3c2 activity.

Augmented NR3c2 activation in obesity can result from: (1) adipocyte production of aldosterone and aldosterone secretagogues, (2) increased NR3c2 synthesis, and (3) altered activity of NR3c2-interacting proteins [825].

Crosstalk among the AT, heart, and adrenal cortex on a macroscale is linked to interaction between aldosterone and adipokines on a nanoscale, especially between aldosterone and adiponectin [827].

Both AdipoR1 and AdipoR2 are identified in the normal human adrenal cortex. In healthy subjects, a high-salt diet (HSD) decreases concentrations of renin, Agt2, and aldosterone and increases adiponectinemia [827]. On the other hand, dietary sodium intake alters concentrations of almost all of the NR3c2 interactors and favors aldosterone-mediated vascular damage in animal models.

Aldosterone-producing CyP11b2+[64] cell clusters appear with aging; they are distinct from the zona glomerulosa of the young adrenal gland cortex, a continuous outer layer of aldosterone-producing cells, which becomes progressively discontinuous during aging [828].

[64] CyP11b2: adrenal aldosterone synthase. Aldosterone synthesis requires the coordinated activity of several enzymes, in addition to CyP11b2, which converts deoxycorticosterone into aldosterone.

Adrenal Gland

Aldosterone is primarily synthesized in cells of the adrenal cortex. Classical aldosterone secretagogues include Agt2, ACTH, and K^+ ion.

However, adipocytes synthesize and secrete aldosterone in addition to mineralocorticoid-releasing factors that act directly on the adrenal gland and raise aldosterone secretion [826, 827]. Conversely, aldosterone promotes adipogenesis via the mineralocorticoid receptor.

Kidney

The major action of aldosterone and its receptor is situated in the kidney, where they increase the amount of the ENaC on the apical membrane of epitheliocytes in the distal nephron and hence renal sodium reabsorption. Angiotensin-2 is the primary stimulus for aldosterone secretion in response to blood volume depletion.

Adipose Tissue

Adipocytes produce aldosterone synthase and hence aldosterone, basally and upon exposure to angiotensin-2 [825]. Paracrine secretion of aldosterone by the pvAT favors vascular dysfunction in obese db/db mice, whereas NR3c2 blockage prevents endothelial dysfunction in diet-induced obesity (DIO).

Overnutrition facilitates NR3c2 activation, which promotes adipogenesis via the TOR–S6K1–PPARγ and –C/EBPα pathways [826]. In fact, the TORC1 complex activates SREBPs via both TOR–S6K1-dependent and -independent signaling. The TORC2 complex controls PKB activity and thus participates in the early stage of adipogenesis. On the other hand, aldosterone hampers production and activity of UCP1 in brown adipocytes.

Adipocytes can prime the adrenal secretion of aldosterone, as they release para- and endocrine aldosterone-stimulating factors, independently of plasmatic renin and K^+ concentrations. The potent oxidized derivative of linoleic acid, (12,13)-epoxy 9-keto 10(trans)octadecenoic acid (EKODE), stimulates aldosterone genesis in rat adrenal glomerulosa cells. Concentration of EKODE correlates with aldosterone level in human subjects with an elevated BMI and in hypertensive African Americans [827].

Adipocytes also synthesize adiponectin, angiotensinogen, angiotensin, and mineralocorticoid receptor, in addition to aldosterone, in some conditions [827].

Angiotensin-2 produced by adipocytes, elicits expression of aldosterone synthase (CyP11b2) and promotes aldosterone secretion using the PP3–NFAT pathway.[65] Leptin also promotes CyP11b2 formation and hence stimulates adipocytic

[65] Both aldosterone and cortisol can activate mineralocorticoid receptor.

aldosterone synthesis using calcium ion. In addition, CETP inhibitors increase aldosterone synthesis in adipocytes via NOx [826]. Both NOx1 and NOx4 are involved in ROS-sensitive aldosterone production in cultured adipocytes.

Conversely, aldosterone stimulates production of NOx2 and cytochrome-B245α subunit via NR3c2 and of NOx organizer NOxO2 via both AT_1 and NR3c2, at least in rats [826]. NOx-derived ROS in WAT upregulates proinflammatory adipokines, engendering vascular insulin resistance and impaired vasodilation. Altered perivascular AT volume is linked to elevated aorta size and stiffness.

Elevated aldosterone concentration and subsequent mineralocorticoid receptor activation linked to maladaptive AT expansion cause redox stress, inflammation via impaired adiposecretome (e.g., increased formation of TNFSF1, IL6, IL18, CCL2, CXCL8, TLR4, AngptL2, and Wnt5a and linked recruitment to AT of dendrocytes, B and T lymphocytes, macrophages, mastocytes, and neutrophils),[66] and deregulated adipocyte autophagy,[67] alters insulin metabolic signaling, and increases risk for hypertension [826]. Augmented activity of the aldosterone–NR3c2 couple is observed in obese patients with insulin resistance and hypertension.

In adipocytes, NR3c2 activation not only favors dyslipidemia and insulin resistance but also alters arteriolar vSMC contractility via elevated vascular redox-sensitive PKG1 activity and lowered redox-sensitive rock activity in mice overexpressing adipocytic NR3c2 [826].

Heart

In the heart, aldosterone causes inflammation, redox stress via NOxs, insulin resistance, left ventricular hypertrophy and cardiac fibrosis via genomic and nongenomic effects of the mineralocorticoid receptor [827].

Cardiomyocytes also possess AdipoR1, AdipoR2, and NR3c2, which is targeted by circulating aldosterone and cortisol. They can also synthesize aldosterone and adiponectin in pathological situations [827]. In mice, selective deletion of macrophage NR3c2 reduces cardiac fibrosis and blood pressure [825].

In CMCs, NR3c2 induces expression of ANP, a marker of cardiac hypertrophy. It interacts with KAT3b, a GATA4 transcriptional coactivator involved in cardiac hypertrophy [827]. The aldosterone–NR3c2 couple launches expression of connective tissue growth factor. In these cells, NR3c2 also rapidly raises concentration of Na^+–K^+–$2Cl^-$ cotransporter and concomitantly lowers that of Na^+–K^+ pump

[66] Low doses of spironolactone, an MR antagonist, prevent macrophage infiltration and M1 macrophage polarization in aortic and cardiac walls [826]. On the other hand, the Western diet enriched in saturated lipids and refined carbohydrates increases α_M-integrin, a macrophage marker in the cardiac wall.

[67] In obesity, autophagosome maturation can be impaired. In addition, activity of AMPK, sirtuin, and TORC1 is altered [826]. However, in obese mice, defective autophagy may decrease WAT volume and enhance insulin sensitivity. Moreover, adipocytic MR activation by aldosterone promotes adipocyte autophagy.

via PKCϵ, elevating intracellular Na^+ and Ca^{2+} concentration, hence favoring pro-hypertrophic ERK1/2 signaling and phosphorylation of Src, JNK, and NFκB [827]. Moreover, it boosts formation of the pro-hypertrophic cytokines cardiotrophin-1 and IL18, acting via P38MAPK on the one hand and the Rho–rock (Sect. 2.3.3.5) and PPAR–NFκB pathways on the other [827]. Aldosterone stimulates fibroblasts via the Ras–Raf–ERK axis. In these cells, aldosterone increases via NR3c2 expression of TGFβ, which favors the production of matrix proteins, but lessens NOS2 activity, thereby facilitating fibrosis. Aldosterone can also elicit proliferation of cardiofibroblasts via the kRas–ERK1/2 pathway [827].

Vasculature

In the vasculature, aldosterone and NR3c2 favor macrophage infiltration, proinflammatory cytokine and superoxide production, but reduces endothelial progenitor cell migration and NO release, thereby leading to endothelial dysfunction and wall remodeling (Table 5.6) [825].

Endothelial and smooth myocytes possess 11β-hydroxysteroid dehydrogenase 11β HSD2 that allows aldosterone–NR3c2 interaction. Once it is activated, NR3c2 translocates to the nucleus and regulates transcription of genes that encode adipokines and SGK1 [826].

Mineralocorticoid receptor can be activated not only by aldosterone but also by glucocorticoids. Cortisol may participate in obesity-linked vasculopathy, especially when activity of 11β HSD1, which produces cortisol, is altered, or that of 11β HSD2, which converts cortisol to inactive, cortisone is impaired [825].

Aldosterone contributes to regulating not only insulin and redox signaling but also the vasomotor tone. However, whereas short-term exposure to aldosterone promotes vasodilation via NO, prolonged exposure causes vasoconstriction, as it attenuates NO availability, raises endothelin-1 expression and causes H_2O_2 overexpression [826].

In aged mice with a vSMC-specific deletion of NR3c2, redox stress and vasoconstriction decline [825]. In vascular smooth myocytes, NR3c2 contributes to elevation in blood pressure due to aging [489]. In vSMCs, MR activation is involved

Table 5.6 Effects of NR3c2 on the vasculature, that is, endothelial and smooth muscle cells and perivascular adipocytes (Source: [825]; ↑ increase, ↓ decrease, *icam* intercellular adhesion molecule, *NO* nitric oxide)

Cell type	Effects
Endotheliocyte	Redox stress
	↓ NO availability and vasodilation
	↑ icam1 and leukocyte adhesion
Smooth myocyte	Redox stress
	↑ vasoconstriction
Adipocyte	↑ inflammatory factors
	↓ insulin sensitizers
	Altered activity of vasoactive factors

in vascular stiffness, as it raises vSMC proliferation, migration, calcification, in addition to $Ca_V1.2b$ channel activation and hence vasoconstriction [826].

Diet-induced obesity impairs endothelial regulation of the vasomotor tone; maximal acetylcholine-induced relaxation decreases down to 20% [829]. On the other hand, the MR antagonist eplerenone (200 mg/kg/day) of HFD-fed obese mice prevents this effect, as it downregulates the formation of membrane and cytosolic subunits of NAD(P)H oxidase P22PhOx and P40PhOx, but upregulates that of antioxidants (glutathione peroxidase-1 and superoxide dismutase SOD1 and SOD3). Eplerenone does not affect obesity-induced synthesis of prostaglandin synthases, PGhS1 and PtgIS. Therefore, obesity-induced endothelial dysfunction depends on the endothelial NR3c2 and redox stress.

In mice, endothelium-specific ablation of the NR3C2 gene protects against DIO-induced endothelial dysfunction, but not obesity-linked insulin resistance and AT inflammation. Aldosterone infusion into lean mice achieving aldosteronemia similar to that in obese mice provokes endothelial dysfunction in WT mice due to redox stress, but not in $^{EC}NR3C2^{-/-}$ mice [829]. In obese mice, Rac1 activation increases in ECs.

In aortic ECs, the aldosterone–NR3c2 couple raises the plasmalemmal concentration of icam1 and subsequently leukocyte adhesion [825]. In ECs, NR3c2 participates in vascular stiffening, which accompanies obesity [827].

In ECs, NR3c2 activation is facilitated by adipocytic NR3c2 overactivation, increasing endothelial plasmalemmal Na^+ channel (EdNaC) density, actin polymerization, and endothelial cortical stiffness, which decreases NOS3 activity [826]. Decayed NO amount provokes tissue transglutaminase TG2 release in the extracellular space, launching vascular and cardiac wall remodeling.[68]

The aldosterone–NR3c2 axis causes tissular remodeling. In resident vascular macrophages, NR3c2 activation mediates M1 polarization, chemotaxis, and inflammatory response [826]. In addition, ^{AdC}NR3c2 activation increases H_2O_2, affecting the vasomotor tone. Moreover, vascular NR3c2 overactivation stimulates pro-fibrotic TGFβ1, leading to fibrosis [826].

5.3.3 *Obesity-Associated Chronic Inflammation and Insulin Resistance*

Cardiovascular disease is associated with adipocyte dysfunction and moderate chronic sterile inflammation. Inflamed AT is a local pathogenic environment. Impaired pvAT function affects associated blood vessel function in addition to causing systemic disorders via adverse delivery by blood of certain adipokine types.

[68] TG2 is nitrosylated by NO and hence retained in the cytosol. Reduced NO amount causes TG2 secretion in the extracellular medium, where it elicits crosslinking of extracellular matrix proteins and fibrosis.

Table 5.7 Adipokines and inflammation (Source: [802]; ↑ increase, ↓ decrease, *APC* antigen-presenting cell, *DC* dendrocyte, *Eφ* eosinophil, *EC* EC, *Mφ* macrophage, *Mo* monocyte, *NK* natural killer cell, T_H helper T lymphocyte, *CCL* C–C-motif chemokine [ligand], *CD* cluster of differentiation, *CmkLR* chemokine-like receptor, *CXCL* C–X–C motif chemokine, *$CX_3CL(R)$* CX_3C chemokine (receptor), *IL* interleukin, *NFκB* nuclear factor κ light chain enhancer of activated B cells, *RBP* retinol-binding protein, *TGF* transforming growth factor, *TNFSF* tumor-necrosis factor superfamily member)

Adipokine	Immunocyte recruitment	Immunocyte activation
Adiponectin	↓ Eφ chemotaxis	↓ IL17 in γ/δ T cell
	↓ ^{EC}icam1	M1-like Mφ phenotype
	↓ T-cell recruitment	CD4+ T-cell activation
	↓ CXCL	↓ antitumoral DC immunity
Chemerin	Chemotaxis via CmkLR1 especially on DC, NK, Mφ	↑ NKκB, TGFβ
		↑ adiponectin
		↓ TNFSF1, IL6
Leptin	Chemotaxis of Mo/Mφ	↑ $^{Mo/M\phi}$IL6/TNFSF1
	↑ $^{M\phi}$CCL3/4/5	T-cell activation, proliferation
		↑ $T_{H1/17}$
		↓ T_{H2}/T_{Reg}
		↓ NK cytotoxicity
RBP4	ND	Activation of APC, T cell
		Inhibited by TNFSF1
Resistin	CD4+ T-cell chemotaxis	↑ $^{M\phi}$IL6/23/27
	↑ $^{M\phi}$CCL1/3/CXCL1	↑ $T_{H1/17}$
	↑ CX3CL1, CX3CR1	
Visfatin	↑ $^{EC/SMC}$icam1/vcam1	↑ B-cell maturation
		↑ leukocyte activation
		↑ NFκB, TNFSF1, IL1β/6

Upon stressor exposure, adipocytes produce inflammatory cytokines supporting local infiltration and activation of immunocytes (Tables 5.7 and 5.8). Conversely, activated immune leukocytes secrete cytokines that alter adipocyte function and subsequently adipokine secretion pattern.

Obesity-related AT inflammation impairs action of insulin in insulin-sensitive organs, such as the liver and skeletal muscle along with endothelium, in addition to AT. In obesity, many types of immunocytes accumulate in insulin-targeted organs and favor chronic inflammation and hence glucose intolerance and insulin resistance.

Decreased uptake of excess fatty acids and an increased lipolysis rate in adipocytes increase fatty acid flux to the liver. Lipid accumulation in the liver causes NAFLD. Hepatic glucose egress and glycemia regulation are controlled by AT lipolysis rather than by insulin action on the liver. Availability of adipose-derived fatty acids determines acetyl-CoA concentration in the liver, independently

Table 5.8 Immunocytes infiltrating the AT (AT; Source: [802]; *APC* antigen-presenting cell, *epiAT* epididymal AT, *ingAT* inguinal AT, *pvAT* perivascular AT, *scAT* subcutaneous AT, *vAT* visceral AT)

Cell type	Preferential location	Metabolic effects
APC	vAT>scAT	↑ ^{AdC}ROS
		↑ lactate production
		Differentiation of adipocytes via CSF2
B cell	pvAT>vAT	Glucose intolerance via IgG
	vAT>scAT	Higher fasting insulinemia
Eosinophil	vAT>scAT	Insulin sensitivity
		Enhances beiging
Macrophage	vAT>pvAT	Insulin resistance
Neutrophil	vAT>scAT	Insulin resistance
NK cell	vAT>scAT	Insulin resistance
	epiAT>ingAT	Infγ production
		Promotes M1 macrophage phenotype
NKT cell	epiAT>ingAT	Insulin resistance, hepatosteatosis
Cytotoxic CD8+ T cell	vAT>scAT	Insulin resistance, steatohepatitis
		Regulates glucose tolerance via perforin
γ/δ T cell	vAT>scAT	Insulin resistance
T_{H1}	vAT>scAT	Insulin resistance
T_{H2}	vAT>scAT	Improves glucose tolerance via IL13–STAT3 and M2 macrophage phenotype induction
		Enhances beiging
T_{H17}	vAT>scAT	Insulin resistance, obesity
	epiAT>ingAT	Suppresses adipocyte differentiation
T_{Reg}		Insulin sensitivity

of liver insulin sensitivity, but depends on increased gluconeogenesis via activation of pyruvate carboxylase by acetyl-CoA [717].

After food ingestion, nutrients are transported to and stored in the skeletal myocytes and adipocytes and subsequently used in periods of energy deprivation. Nutrient uptake relies on adequate organ perfusion.

In skeletal muscles, insulin dilates arterioles and increases the capillary surface area, thereby facilitating nutrient and insulin delivery [720]. On the other hand, insulin resistance limits postprandial perfusion. Acute overfeeding reduces skeletal muscle perfusion, but not AT irrigation [830].

However, in ECs of the forearm circulation in healthy volunteers, insulin promotes ET1 synthesis and release [720]. In fact, in the skeletal muscle circulation, insulin can stimulate the release of both NO and ET1, thereby priming a neutral response. Insulin stimulates NO production, as it promotes NOS3 phosphorylation at Ser1177 and impedes that at Thr495 [831].

In obesity, activated and inflamed endothelium initiates AT inflammation. In cultured ECs exposed to palmitic acid, which provokes mitochondrial damage and leakage of mitochondrial DNA into the cytosol, cytosolic DNA sensor cGMP–cAMP synthase activates stimulator of interferon genes (sting). Sting then tethers to interferon regulatory factor IRF3, causing its phosphorylation and nuclear translocation [832]. IRF3 connects to the ICAM1 promoter, icam1, eliciting monocyte adhesion on ECs (cGAS–STING–IRF3–icam1 pathway).

Obesity is characterized by adipocyte hypertrophy and augmented infiltration of macrophages in adipose depots. Infiltrated macrophages in the AT generate ROS by NOx2, which causes lipid peroxidation.

In adipocytes, excess nutrient (e.g., glucose and fatty acids such as palmitate) also provokes production by NOx4 of ROS in addition to a transient increase in activity of the pentose phosphate pathway, which is a major source of NADPH. This activates NOx and causes AT inflammation and insulin resistance [833].

The population of CX_3CR1+ sympathetic nerve-associated macrophages (SNAMφs) in AT, which are distinct from adipose tissue macrophages (ATMφs), increases in mouse models of obesity [834]. In humans, SNAMφs also lodge in the paravertebral sympathetic ganglia.

Fasting or cold exposure triggers noradrenaline (NAd) release from sympathetic neurons that innervate the AT. NAd stimulates adipocytic adrenoceptors that launch the Gs–cAMP–PKA axis, thereby promoting lipolysis in the WAT and hence fat mass reduction, in addition to heat production by brown adipose tissue (BAT). On the other hand, obesity and aging support SNAMφ activity, decreasing response to cold and starvation. Fasting-induced lipolysis is also impaired in the elderly due to NAd degradation by ATMφs. With aging, synthesis of NAd-degrading monoamine oxidase-A (MAOa) is indeed upregulated in ATMφs via secreted GDF3, production of which is upregulated by the NLRP3 inflammasome [835]. Furthermore, in aged mice during fasting, adipocytes fail to synthesize hormone-sensitive lipase (HSL or lipase-E) and ATGL (Atgl or patatin-like phospholipase, PnPLA2).

In addition, ^{AT}SNAMφs limit NAd-primed lipolysis during aging and in obesity, clearance by SNAMφs of extracellular NAd released from neurons contributing to obesity. In mice, diet-induced obesity raises the number of SNAMφs [834]. Noradrenaline-scavenging ^{AT}SNAMφs import and degrade NAd, as they possess sodium-dependent NAd transporter SLC6a2 for NAd import as well as MAOa for NAd degradation (clearance).[69] Activated sympathetic nervous system elicits NAd uptake by SNAMφs through SLC6a2, increasing NAd content, and shifts the SNAMφ phenotype to a proinflammatory state. The specific deletion of SLC6A2 in SNAMφs does indeed increase NAd concentration, thermogenesis, browning of white adipocytes and thus BAT mass, and weight loss in obese mice [834].

In the pvAT, recruitment of proinflammatory macrophages is linked to a depletion of the vasodilator hydrogen sulfide in the vessel [836]. In mesenteric arterioles,

[69] MAOa catalyzes oxidative deamination of neuro- and vasoactive amines (e.g., adrenaline, NAd, and serotonin) in the central nervous system (CNS) and peripheral organs.

smooth myocytic and endothelial H_2S concentrations decline in vessels from obese mice with respect to those of lean mice. This effect depends on NOS2 activity of pvAT-resident proinflammatory macrophage.

In skeletal muscles, insulin resistance is linked to a decreased signaling via insulin receptor substrate IRS1 and PI3K, whereas signaling via the MAPK module is maintained [687]. The kinase activity of InsR and, downstream from IRS1, of PI3K lowers in adipocytes of obese individuals, whereas IRS2-mediated PI3K activity remains normal.

In the liver, the number and activity of insulin receptors and the ability of insulin to inhibit glycogenolysis do not change.

Adaptive hyperinsulinemia is a compensatory state. All insulin signaling pathways are not homogeneously affected by insulin resistance that is linked to some pathways, whereas insulin sensitivity is maintained in others [687]. On the one hand, insulin resistance is observed in the brain, liver, skeletal muscle, and AT. On the other, hyperinsulinemia associated with insulin resistance can potentiate insulin action in insulin-sensitive cells. Hyperinsulinemia, which acts on the liver, kidney, and ovary, causes hypertriglyceridemia, increased sodium retention and hypertension, and hyperandrogenism, respectively.

In mice, ablation of the insulin receptor in the vascular endothelium does not affect glucose metabolism, but lowers cardiac frequency and blood pressure in addition to protecting against adverse neovascularization in the retinal vasculature and blunting upregulated expression of VEGF, NOS3, and ET1 upon hypoxia [687].

In insulin resistance, elevated circulating insulin concentration favors production of vasoconstrictors and proinflammatory molecules, such as endothelin-1 and serpin-E1 (PAI1), thereby contributing to endothelial dysfunction [831]. However, endothelium-dependent hyperpolarization is maintained to elicit endothelium-dependent vasodilation in obese animals.

5.3.3.1 PI3K

The lipid and protein kinase PI3K is involved in the regulation of cell metabolism, growth, proliferation, migration, and survival. It produces the lipidic second messenger PIP_3 to regulate cell fate and Ca^{2+} signaling, hence connecting cell contractility to metabolism control. It phosphorylates the lipidic substrates, PIs (PI4P and PIP_2).

Eight PI3K isoforms can be grouped into three categories according to the structure and preferred lipid substrate. Set-*I* PI3K isozymes form PIP_3 from $PI(4,5)P_2$, set-*III* PI3Ks generate $PI(3,4)P_2$ and PI3P, and set-*III* PI3K primarily synthesizes PI3P. Among set-*I* PI3Ks, subset-*I*A heterodimeric PI3Ks (ubiquitous $PI3K_{c1\alpha}$ and $PI3K_{c1\beta}$ and leukocytic $PI3K_{c1\delta}$) are activated by protein Tyr kinase receptors [837]. The single subset-*I*B member, $PI3K_{c1\gamma}$, which is confined to hematopoietic and cardiac and smooth myocytes, mainly signals from GPCRs. Nevertheless, $PI3K_{c1\beta}$ can be activated by GPCRs and $PI3K_{c1\gamma}$ by RTKs via the Ras–$PI3K_{r6}$ axis. PI3K signaling can be terminated by PTen, which dephosphorylates PIP_3.

The latter serves as a docker for lipid-binding domain-containing proteins, such as PKB, which is activated by phosphorylation on Thr308 and Ser473 by PDK1 and TORC2, respectively. Activated PKB phosphorylates TSC2 and TORC1 involved in protein synthesis and cell growth, GSK3 implicated in glucose metabolism, and the FoxOS and P53 regulator of cell survival.

In addition, both $PI3K_{c1\alpha}$ and $PI3K_{c1\beta}$ are required for the maintenance of T-tubule network integrity via the correct location of junctophilin-2 and hence Ca^{2+} handling. Moreover, $PI3K_{c1\alpha}$ participates in controlling myofiber maturation and Z-disc alignment.

PI3K mediates insulin action in the heart, especially insulin- and IGF1-responsive $PI3K_{c1\alpha}$. The latter controls CMC growth and contractility, as PKB phosphorylates (potentiates) $Ca_V1.2a$ action.[70]

In addition, PI3K can control Ca^{2+} signaling in response to catecholamines; it prevents positive inotropic action of β-adrenoceptors. β1-Adrenoceptor, the predominant subtype, and β2AR increase PI3K activity in adult rat CMCs [837]. Once β AR is activated by NAd and circulating adrenaline, it stimulates PKA that then phosphorylates Ca^{2+}-handling proteins. PI3K does not affect Ca^{2+} effectors directly, but operates via β ARK1, which phosphorylate liganded GPCRs, leading to their desensitization and internalization, thereby reducing the plasmalemmal density and β1AR-mediated positive inotropy. On the other hand, $PI3K_{c1\gamma}$ is implicated in signaling triggered by β2AR using the cAMP–PKA axis confined to plasmalemmal nanodomains, hence controlling Ca^{2+} transient amplitude and decay kinetics, limiting the Ca^{2+} spike magnitude, but keeping a proper gating duration [837]. Calcium transient amplitude elevates and decay kinetics lowers in $PIK3CG^{-/-}$ adult ventriculomyocytes, which are more sensitive to stimulation by β2AR, which are compartmentalized in plasmalemmal compartments, via a kinase-unrelated mechanism. $PI3K_{c1\gamma}$ serves as an anchoring protein that maintains within the same complexes PKA and PDE3a and PDE4a–PDE4b, thereby favoring PDE activation by PKA and subsequent cAMP degradation in restricted compartments. Whereas PI3Kγ-regulated PDE4 limits cAMP owing to subplasmalemmal domains close to $Ca_V1.2a$, restraining Ca^{2+} influx and inotropic response, PI3Kγ-regulated PDE3 and PDE4 may control phospholamban activity and thus a cAMP pool at the ER for Ca^{2+} re-uptake during diastole, hence yielding proper lusitropy.

In insulin resistance, the PI3K–PKB signaling activation is impaired, eventually causing contractile dysfunction and diabetic cardiomyopathy. It also prevents

[70] In CMCs, Ca^{2+} enters the cell mainly through $Ca_V1.2a$ in T tubules and triggers a massive Ca^{2+} release from the ER through RyR2 and binds to troponin-C, enabling myosin–actin interaction and CMC contraction.

Calcium signaling is controlled by activating and inhibiting kinases and phosphatases, which target Ca^{2+} channels and their adaptors. In CMCs, PKA phosphorylates (activates) $Ca_Vv1.2a$ and RyR2 in addition to phospholamban, thereby having positive inotropic and lusitropic effects. Once it is activated by cytosolic Ca^{2+} and diacylglycerol, PKC phosphorylates Na^+–Ca^{2+} exchanger, transient receptor potential channels, and PLC [837].

hypertension-induced fibrosis. On the other hand, angiotensin-2 has a rapid negative inotropic effect, likely via PKCα and $PI3K_{c1\alpha}$ inhibition [837].

In ECs, VEGF and FGF regulate angiogenesis via different PI3K subtypes ($PI3K_{c1\alpha}$ or hRas-activated $PI3K_{c1\gamma}$), Ca^{2+}, and NO [837]. Both FGFR and VEGFR provoke NOS3 phosphorylation via the PI3K–PKB and PLCγ–Ca^{2+}–Cam–CamK2 pathways. In addition, Ca^{2+}-dependent cytosolic cysteine peptidases calpain-1 and -2 control cytoskeleton dynamics and hence cell migration, calpain-2 being involved in VEGF-induced angiogenesis.

In vSMCs, the PI3K–PKB axis is required for correct $Ca_V1.2b$ functioning.[71] $PI3K_{c1\gamma}$ is the major isozyme, which is preferentially linked to Ca^{2+} signaling [837]. It is implicated in Agt2-mediated Ca^{2+} entry through $Ca_V1.2b$ and subsequent vasoconstriction. Set-*I* PI3Ks act both in the contractile and the synthetic phenotype. $PI3K_{c1\gamma}$ participates in SMC migration induced by chemokine receptors such as CCR2 in addition to PDGFR [837]. In addition, PI3K raises TRPC6 translocation to the plasma membrane, especially in pulmonary arterial smooth myocytes in some cases of idiopathic pulmonary hypertension. Intimal hyperplasia is also related to interferon-γ released by T lymphocytes using $PI3K_{c1\gamma}$.

$PI3K_{c1\gamma}$ supports activation of macrophages in obesity, in addition to oxLDL internalization during atherogenesis. In a nonhematopoietic cell type, its action in HFD-induced inflammation and insulin resistance depends on its role in the control of adiposity [839]. In leukocytes, PI3Kγ recruits neutrophils to the AT, which is associated with macrophage activation in the AT and inflammation, hence triggering insulin resistance in the early stage of obesity.

5.3.3.2 PKB

Both acetylation and phosphorylation intervene in the initiation of insulin signaling. In the heart, insulin-stimulated glucose oxidation decreases in obesity. Impaired signaling by the Ins–PKB–GSK3β axis and hence phosphorylation of its substrates reduces cardiac glucose use [840]. Cardiac insulin resistance in obese mice is associated with increased PKB acetylation and decreased phosphorylation. Acetylation of PKB is increased by HFD, which decreases its phosphorylation state and activity. Deacetylation of PKB is a prerequisite for its activation, which enhances insulin signaling in obesity.

[71] Smooth myocyte contraction only partly relies on $Ca_Vv1.2b$ and RyR; it primarily depends on PLC–DAG/IP_3 pathways that trigger Ca^{2+} influx through receptor-operated channels and Ca^{2+} release through IP_3R, respectively. Activated calmodulin stimulates MLCK that phosphorylates MLC20, hence launching contraction.

Vasoconstriction is countered by vasodilation. In arterioles, Ca^{2+}, rather than IP_3 crossing gap junctions of myoendothelial junctions, is the intercellular signal that provokes endothelium-mediated feedback vasodilation [838]. Calcium ion enters vascular smooth myocytes through $Ca_V1.2b$ channels and subsequently ECs through gap junctions. However, the magnitude of Ca^{2+} signals in ECs depends on IP_3Rs, which amplify Ca^{2+} cues to propagating waves that activate IK channels to suppress vasoconstriction.

5.3.3.3 Sirtuins

Sirtuins (SIRT1–SIRT7) are type-*III* histone and protein deacetylases and ^{ADP}ribosyltransferases that require NAD^+ as a cosubstrate. The founding member of the SIRT family is the yeast silence information regulator-two (SIR2; i.e., SIRT), a factor that silences the mating-type locus, in addition to its participation in telomere regulation, maintenance of DNA integrity, and lifespan extension. Three sirtuin isozymes (SIRT1 and SIRT6–SIRT7) localize to the nucleus.

In mammalian cells, instead of gene silencing, SIRT1 often promotes gene transcription by deacetylating specific transcription factors, corepressors, and coactivators (e.g., FoxOs, MyoD, NFκB, P53, and PGC1α).

Sirtuins rapidly adjust the activity of histones, transcription factors, metabolic enzymes, and structural proteins to cellular needs. They operate in chromatin silencing, cell cycle regulation, cellular differentiation, and metabolism. They also enable organisms to cope with different stressor types and prevent aging. Lysine acetylation regulates the activity of enzymes involved in both fatty acid and glucose metabolism.

Their specific functions differ, but different types of sirtuins cooperate to control cellular processes. In addition to their synergistic actions, sirtuins have also antagonistic effects. Members of the sirtuin family can set up cross-regulation.

Obesity augments reliance of the heart on fatty acid oxidation, a major energy source in the adult heart. This phenomenon involves increased circulating fatty acid concentrations, altered transcriptional control of fatty acid oxidative enzymes in addition to post-translational control of fatty acid and glucose oxidation factors, and the development of cardiac insulin resistance, which decreases glucose oxidation [840].

Sirtuin-1

Sirtuin-1 autodeacetylates (self-activates; K230), similar to autocatalytic modifications of other types of enzymes (acetyltransferases, kinases, and phosphatases) [841]. On the other hand, the HDAC6 deacetylase is acetylated (inhibited) by KAT3b.

Once it is autodeacetylated, sirtuin-1 efficiently deacetylates its substrates, such as P53 and histone lysines H1K26, H3K9, H3K56, and H4K16 [841]. The transcription factor P53 stimulates adipogenesis, as it activates the nuclear receptor NR1c3 (PPARγ) via KAT2b and C/EBPβ.

Sirtuin-1 prevents adipogenesis, as it interacts with corepressors NCoR1 and NCoR2, thereby repressing NR1c3 (PPARγ) activity.

The deacetylase sirtuin-1 protects against metabolic disorders engendered by overnutrition and aging in many cell types, such as adipocytes and macrophages [842]. Sirtuin-1 activity is reduced in adipocytes of obese and aged subjects. HFD primes sirtuin-1 cleavage by caspase-1 in the AT.

Sirtuin-1 controls synthesis and secretion of several types of adipokines, cytokines, and chemokines (e.g., adiponectin, IL4, and CCL2). It deacetylates (inhibits) NFκB, thereby hampering its binding to its target gene promoters (e.g., Il2 and Ccl2). It also supports macrophage M2 phenotype, hence blocking infiltration of macrophages, limiting AT inflammation, and protecting against the onset and progression of obesity-related insulin resistance [842].

In mice, selective ablation of the Sirt1 gene in adipocytes accelerates glucose intolerance and hence HFD-induced InsRce during obesity onset with respect to specific Sirt1 depletion in myeloid cells such as macrophages [842]:

- In adipocytes, SIRT1 targets NR1c3 (PPARγ). It plays a prominent role in glucose and lipid homeostasis during the early stage of obesity.
- In macrophages, which interfere with adipocytes in obesity, SIRT1 counters the action of inflammatory mediators. Sirtuin-1 alleviates aggravation of metabolic disorders only at a later stage.

Selective deletion of Sirt1 in adipocytes, but not in macrophages, exacerbates infiltration of macrophages in the AT at the early DIO stage, at least partly because of the increased formation of CCL2 and reduced production of adiponectin, macrophage migration being countered by CCL2 neutralization or adiponectin supplementation [842]. In $^{AdC}Sirt1^{-/-}$ mice, the density of AT-resident macrophages rises and they evolve from the anti-inflammatory M2 to the proinflammatory M1 phenotype.

Both CD4+ and CD8+ T lymphocytes control inflammation in visceral adipose depots. Infiltration of CD8+ T lymphocytes precedes accumulation of macrophages in WT and adipocyte- Sirt1-depleted mice [842]. Cytotoxic CD8+ T lymphocytes secrete TNFSF1, IL2, Ifnγ, and CCL5; CD4+ T_{H1} cells TNFSF1, IL12, and INFγ, these cytokines affecting adipocyte function and favoring the M1 macrophage phenotype.

In the AT of obese subjects, activated natural killer T cells favor polarized M2 macrophages via the IL4–STAT6 axis [842]. Adipocytes release T_{H2}-type cytokines (e.g., IL4 and IL13) that are responsible for macrophage M2 polarization in the AT. However, IL4 is produced at a much higher level in eosinophils and other immunocytes than in adipocytes.

Adiponectin primes change of peritoneal macrophages and Kupffer cells to the anti-inflammatory M2 phenotype also using the IL4–STAT6 pathway [842].

Sirtuin-1 and NFAT

In adipocytes, sirtuin-1 deacetylates the transcription factor NFATc1 (or NFAT2), thereby controlling its binding to the Il4 gene promoter. The transcription factors of the NFAT family tether to the promoter and enhancer of the genes encoding IL2, IL4, and IL5, among other cytokines and plasmalemmal receptors.

The NFAT factors share the Rel homology domain (RHD), a DNA-binding and dimerization sequence, with the transcription factors of the REL family, Rel[72] and the NFκB heterodimer. All Rel proteins form homo- or heterodimers, except RelB, which only heterodimerizes.

The PP3-dependent proteins NFATc1 to NFATc4, which are activated by cytosolic Ca^{2+}, bind to DNA as monomers, whereas tonicity-responsive NFAT5 tethers to DNA as a dimer and has a slower dissociation rate than the NFκB dimer.[73]

At rest, NFATcs are phosphorylated and sequestered in the cytosol. Upon stimulation, they are dephosphorylated by PP3 and translocate to the nucleus, where they regulate gene transcription alone or in cooperation with other transcription factors (e.g., AP1 and FoxP3).[74]

The NFAT factors are synthesized in T, B, and NK cells in addition to mastocytes. In particular, NFATc1 elicits Il4 gene transcription in T cells. In NFATC2-deficient CD4+ T_{H2} cells, chromatin accessibility is enhanced owing to permissive histone modification and DNA demethylation in the Il4 promoter region [843]. Deletion of NFATC1 and NFATC2 in T cells causes over- and underproduction of IL4, respectively, because of the differential regulation of these two factors [844]. In fact, both NFATc1 and NFATc2 are activators of Il4 gene transcription.[75]

Sirtuin-2

Among sirtuins, cytoplasmic sirtuin-2 is the most abundant in adipocytes [845]. Like sirtuin-1, sirtuin-2 hampers adipogenesis in addition to accumulation of lipids in adipocytes, as it deacetylates FoxO1, an inhibitor of adipogenesis that then localizes to the nucleus and represses activity of the nuclear receptor NR1c3 (PPARγ) [841]. The anti-adipogenic factors SIRT2 and asymmetric dimethylarginine (ADMA) countering the effects of pro-adipogenic factors that convert pre-adipocytes to mature adipocytes, such as adipokines and adipogenic transcription factors (e.g., NR1c3 a0nd C/EBPβ) [846]. During pre-adipocyte differentiation, SIRT2 synthesis is downregulated due to insulin-stimulated FoxO1 phosphorylation (inhibition) by the PI3K–PKB axis, FoxO1 translocating from the nucleus to cytosol,[76] whereas the formation of NR1c3, C/EBPα, FABP4, GluT4, and FAS is upregulated [845].

[72] Rel: reticulo-endotheliosis viral homolog proto-oncogene.

[73] The affinity of NFAT5 for DNA is much lower than that of NFATc1 to NFATc4. The Nfat5 gene encodes multiple isoforms, among which NFAT5a and NFAT5c are the most well-known. The NFAT5c isoform has an additional N-terminal sequence of 76 amino acids with respect to NFAT5a.

[74] VEGF signals via the PP3–NFATc1 axis in vascular ECs. It regulates endotheliocyte proliferation during valvuloseptal- and angiogenesis.

[75] Demethylation at H3K4 by KDM1a engenders transcription of the NFATC1 gene, whereas an aging-associated increase in H3K9 methylation owing to reduced KDM3a activity represses NFATc1 synthesis.

[76] The extent of deacetylation of FoxO1 by sirtuins affects its phosphorylation and DNA binding. FoxO1 is acetylated by KAT3a.

Sirtuin-3

In mitochondria, the major NAD^+-dependent deacetylase sirtuin-3 targets numerous enzymes involved in fatty acid oxidation, such as long-chain acyl-CoA dehydrogenase (lcACDH) and β-hydroxyacyl-CoA dehydrogenase subunit (β HADH) [840]. Sirtuin-3 also deacetylates ETC complexes-*I* and -*I I* in addition to activating isocitrate dehydrogenase during caloric restriction.

The cardiac fatty acid oxidation rate is higher (mean ∼14.2 nmol/g/s) in HFD-fed mice (60% fat) than in mice (mean ∼9.2 nmol/g/s) fed with a low-fat diet (LFD; 4% fat) [840]. A high fatty acid oxidation rate can decrease heart beat efficiency. Activity of lcACDH and β HADH rises with their acetylation state. Hyperacetylation observed in HFD-fed mice is linked to a decreased sirtuin-3 synthesis, whereas KAT2b formation does not change. In addition, HFD and Sirt3 deletion diminish glucose oxidation, but acetylation of pyruvate dehydrogenase, the rate-limiting enzyme of glucose oxidation, is not altered. The inverse relation between fatty acid and glucose oxidation may be the main determinant for the low glucose oxidation in HFD-fed mouse hearts. Although sirtuin-1 expression does not change with the diet type in mice, that of sirtuin-6 is augmented in HFD-fed obese mice.

Sirtuins SIRT4 and SIRT5

Sirtuins cooperate to improve the mitochondrial metabolism. Sirtuin-1 stimulates mitochondrial gene transcription, whereas SIRT3, SIRT4, and SIRT5 modify various mitochondrial enzymes [841].

Sirtuin-6

The PGC1α factor, which regulates transcription of genes encoding enzymes of fatty acid oxidation, is acetylated (inhibited) by the nuclear acetyltransferase, KAT2a. On the other hand, the mitochondrial acetyltransferase KAT2b acetylates mitochondrial proteins.

Sirtuin-6 deacetylates (activates) KAT2a, which acetylates PGC1α [840]. On the other hand, sirtuin-1 deacetylates (activates) PGC1α.

In $Sirt6^{-/-}$ mice, the amount of AT is reduced, whereas SIRT6 overexpressing mice are protected against HFD-induced obesity [841].

Sirtuin-7

Sirtuin-7 specifically deacetylates histone lysine H3K18 and a small amount of other targets primarily involved in the activation of rDNA transcription. Whereas SIRT7 activates rDNA transcription, SIRT1 inhibits it [841].

Sirtuin-7 tethers to SIRT1 and prevents its autodeacetylation, avoiding SIRT1 overactivity [841]. In Sirt7$^{-/-}$ mice, augmented SIRT1 activity represses activity of the nuclear receptor NR1c3 (PPARγ), thereby suppressing adipocyte differentiation and countering growth and maintenance of white adipose tissue and even diminishing WAT size; reduced SIRT1 activity restores adipogenesis.

5.3.3.4 Interferons

In obese individuals, NAFLD (Sect. 5.3.4) is linked to the development of insulin resistance. In NAFLD, both intrahepatic CD8+ and CD4+ T lymphocytes are activated.

In humans, intrahepatic CD8+ T lymphocytes comprise conventional CD8+ T cells, which are usually activated by antigens, and CD8+ mucosal-associated invariant innate-like lymphocytes, which detect bacterial metabolites and constitute from 20 to 50% of the hepatic population of lymphocytes. In addition, innate-like lymphocytes, such as group-*I* innate lymphoid cells and natural killer (NK) and NK T cells reside in the liver; they rapidly respond to cytokines [812].

In mouse models of obesity (HFD-fed leptin-deficient [ob/ob] mice) and NAFLD patients, accumulation and activation of pathogenic CD8+ T lymphocytes in the liver by type-*I* interferon (Ifnα) is linked to a dysregulated glucose metabolism (augmented gluconeogenesis) and hepatic insulin resistance [847]. HFD-fed IFNAR1$^{-/-}$ mice are protected against NAFLD and hepatosteatosis.

Hepatic CD8+ T lymphocytes augment their synthesis and release of the type-*II* interferon Ifnγ and TNFSF1,[77] provoking insulin desensitization, Ifnγ and TNFSF1 impairing insulin signaling in hepatocytes.

In the liver of obese mice, production of interferon regulatory factors IRF3 and IRF7 is upregulated and transcription of interferon stimulatory genes increases.

The gut flora participates in low-grade chronic hepatic inflammation of obese mice. Broad-spectrum anti-biotic treatment depletes commensal bacterial population and reduces the density of intrahepatic CD8+ T lymphocytes. Hepatic response to Ifnα may result from increased bacterial products entering the liver because of elevated intestinal permeability [847]. In addition, hepatic mitochondrial DNA, the concentration of which rises in NASH mice and patients, may act as TLR9 ligands to promote Ifn*I* production. Intrahepatic immunocytes such as plasmacytoid dendrocytes, may be Ifn*I* sources, CD8+ T lymphocytes forming the dominant Ifn*I*-responsive cell types, the expanding population of which favors systemic insulin resistance in obesity.

[77] TNFSF1 is involved in fibrosis in nonalcoholic steatohepatitis (NASH).

5.3.3.5 Adiponectin

The anti-inflammatory adiponectin (Sect. 5.4.5.1) increases the activity of AMPK[78] and reduces that of the NAD(P)H oxidase subtype NOx4. Ablation of the NOX4 gene raises adiponectinemia.

Adiponectin is secreted from AT in response to metabolic cues to sensitize the liver and muscle to insulin. Reduced adiponectinemia, which is usually observed in obesity, contributes to insulin resistance.

Adiponectin prevents macrophage recruitment via its inhibition of several proinflammatory cytokines and chemokines (IL6, CCL2, and CXCL8).

Adiponectin monomers multimerize, multimers being stabilized by covalent and noncovalent linkages. The largest high-molecular-weight (hMW) adiponectin resists peptidases.

Sirtuin-1 supports formation of the FoxO1–C/EBPα complex, thereby upregulating the ADIPOQ gene transcription, but represses $Adpn_{hMW}$ secretion,[79] as it inhibits the ER oxidoreductase paralog ERO1Lα, a catalyzer of disulfide bond formation with disulfide isomerase. Adiponectin secretion is indeed regulated by SIRT1, NR1c3 regulators, and ERO1Lα [848].[80] Inhibition of SIRT1 and activation of NR1c3, especially during adipogenesis, enhances ERO1Lα synthesis and stimulates $Adpn_{hMW}$ secretion in mature adipocytes. Hence, SIRT1 precludes ERO1Lα action and hence $Adpn_{hMW}$ release. In adipocytes, ablation of Sirt1 raises ERO1Lα production without a marked change in synthesis of Adpn and C/EBPα and only a modest increase in the expression of FABP4 [848].

Methylation of DNA and expression of genes related to metabolism and inflammation in AT are altered in patients with T2DM. Low adiponectin concentrations predispose to T2DM, but high $Adpn_{hMW}$ concentrations predict increased mortality in T2DM subjects [849]. Plasmatic concentrations of FABP4 and $Adpn_{hMW}$ correlate positively with CVD-induced death [850]. FABP4, which is formed in adipocytes and macrophages and in foam cells, is an early predictor of adverse cardio- and cerebrovascular events, developing metabolic syndrome and T2DM, in addition to heart failure. In macrophages, FABP4 significantly increases content in triacylglycerol and cholesterol, as it downregulates transcription of genes involved in cholesterol egress and cholesterol ester hydrolysis. In addition, BNP and the N-terminal fragment of its prohormone contribute to AT metabolism and influence adiponectin secretion.

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[78] In the AT, adenosine monophosphate (AMP)-activated protein kinase phosphorylates hormone-sensitive lipase and acetyl-CoA carboxylase at inhibitory sites, repressing lipolysis and -genesis.

[79] Circulating adiponectin consists of a set of multimers of the 30-kDa polypeptide via disulfide bonds (low- [$Adpn_{lMW}$ (trimers)], middle- [$Adpn_{mMW}$ (hexamers)], and high-molecular-weight complexes [$Adpn_{hMW}$]). Insulin sensitizers of the thiazolidinedione class increase the circulating $Adpn_{hMW}$ concentration [848]. The circulating $Adpn_{hMW}$ complex is the main mediator of insulin-sensitizing activity of adiponectin.

[80] In mammals, ERO1Lα and ERO1Lβ are encoded by the ERO1LA (ERO1L) and ERO1LB genes. The ERO1Lβ isoform is produced primarily in secretory cells. Its synthesis is induced by the unfolded protein response (UPR). The ERO1Lα isoform is formed in most cell types.

5.3.3.6 Galectin

Galectin-3 (GaL3),[81] is mainly secreted by macrophages and microgliocytes; it mediates interaction of macrophages with the extracellular matrix. In addition, GaL3 causes macrophage chemotaxis; the AT macrophage number falls in LGalS3$^{-/-}$ mice.

Blood concentration of GaL3 rises in obese subjects, connecting inflammation with accumulation of AT macrophages, especially proinflammatory M1 CD11c+ (α_XItg+) macrophages, to obesity-induced insulin resistance [851]. Galectin-3 does indeed decrease insulin signaling and promotes AT inflammation.

Galectin-3 impairs insulin action in adipocytes, hepatocytes, and myocytes. Administration of GaL3 reduces insulin-stimulated glucose uptake in these cell types in vitro. On the other hand, inhibition of GaL3 improves insulin sensitivity in obese mice.

In obese mice, switching from an HFD to a standard diet (SD) lowers the density of AT α_XItg+ macrophages. Both heterozygous (LGalS3$^{-/+}$) and homozygous (LGalS3$^{-/-}$) mice are protected from HFD-induced InsRce in the liver, skeletal muscle, and AT and from age-related insulin resistance, the diet being standard.

The messenger GaL3 binds directly to insulin receptor and inhibits its signaling, the InsR–IRS1–PI3K–PDK1–PKB axis enabling transfer of the insulin-responsive glucose transporter GluT4 to the plasma membrane and repressing the transcription of lipolytic and gluconeogenic genes.

5.3.3.7 Hydroxyisobutyrate

Branched-chain amino acids (BCAAs) are implicated in the development of insulin resistance caused by excess lipid accumulation, once blood-borne lipids have crossed the blood vessel wall.

Hydroxyisobutyrate (HIB), a catabolite of valine (a BCAA),[82] is secreted from skeletal myocytes and serves as a paracrine regulator of transendothelial fatty acid transfer [852]. It thus stimulates fatty acid uptake by the skeletal myocyte and subsequent lipid accumulation. It works via PGC1α, which promotes endothelial uptake of fatty acids and regulates their consumption in addition to mitochondrial formation and angiogenesis.

Increased BCAA catabolism can cause HIB secretion from skeletal muscles, raising transendothelial fatty acid transfer and subsequent muscular import, accu-

[81] Galectin: laminin-binding galactose-specific soluble lectin, which is encoded by the LGALS3 gene. It is also termed carbohydrate-binding protein CBP35 and galactoside-binding protein (GalBP). Galectin-3 is weakly expressed in mouse lymphocytes and neutrophils.

[82] Hydroxyisobutyrate derives from hydroxyisobutyryl-CoA (HIBC) using HIBC hydrolase (HIBCH) and is subsequently processed by HIB dehydrogenase (HIBADH) [852]. The transcriptional coactivator PGC1α launches production of nearly every enzyme of BCAA catabolism. Valine is one of three BCAAs, which are essential dietary components.

mulation of incompletely esterified lipotoxic intermediates such as diacylglycerol, and repressing insulin signaling. In diabetic subjects, the concentration of HIB in the skeletal muscle rises [852].

5.3.3.8 Optic Atrophy OpA1

Insulin resistance can be associated with mitochondrial dysfunction via direct effects, transcriptional repression of mitochondrial genes, and lipotoxicity. On the other hand, limited mitochondrial function in skeletal muscles can improve insulin sensitivity and protect against diet-induced obesity and insulin resistance via ROS, AMPK, and ER stress pathway, in addition to secretion of myokines (irisin, IL6, and FGF21) [853].

Mitochondrial dynamics, that is, repeated cycles of mitochondrial fusion and fission,[83] leads to the exchange of mitochondrial genetic content, ions, metabolites, and proteins. Independently of its role in mitochondrial fusion, the mitochondrial fusion protein optic atrophy OpA1 maintains morphology of mitochondrial cristae and stabilizes ETC complexes [853].

Concentrations of Opa1 and Mfn2 decline in elderly insulin-resistant humans and T2DM patients [853]. In cardiac and skeletal myocytes exposed to hyperinsulinemia, OpA1 synthesis rises, promoting mitochondrial fusion and increasing mitochondrial oxygen consumption and ATP generation. On the other hand, OpA1 deficiency in muscles increases MuRF1 concentration,[84] reduces ATP synthesis and the maintenance of mitochondrial membrane potential capacity, and causes glycolytic compensation, but also muscle atrophy, the severity of which depends on the degree of the progressive mitochondrial dysfunction.

In mice, specific ablation in skeletal myocytes of the OPA1 gene not only provokes progressive mitochondrial dysfunction but also increases entire-body metabolic rate, improves global insulin sensitivity, and enables resistance to DIO via integrated stress response (Vol. 9, Chap. 2. "Hypoxia and Stress Response"), that is, activation of ER stress and secretion of FGF21 via activation of eIF2α and AMPK [853].[85] Circulating FGF21 reduces weight gain and insulin sensitivity in response to HFD.

[83] Fission is regulated by dynamin-related protein DRP1 and its partners fission protein FiS1, MFF, and mitochondrial dynamics protein. Mitochondrial fusion is regulated by outer mitochondrial membrane (OMM) GTPases mitofusins Mfn1 and Mfn2 and inner mitochondrial membrane optic atrophy OpA1.

[84] The ubiquitin ligase MuRF1 favors skeletal muscle atrophy. OpA1 represses MuRF1 expression in skeletal muscles.

[85] In muscles, FGF21 is produced upon ATF4, PKB, and TORC1 activation in addition to ER and redox stress [853].

5.3.3.9 Receptor for Advanced Glycation End Products

The receptor for advanced glycation end products (RAGEs), a member of the immunoglobulin superfamily of plasmalemmal molecules encoded by the AGER gene, interacts with a set of ligands that form in organs and circulate in blood. It especially binds to and transduces the action of end products (AGEs), which accumulate with aging, redox stress, hyperglycemia, inflammation, obesity, T2DM, and renal failure [854].

The RAGE is expressed on numerous cell types, that is, vascular endothelial and smooth muscle cells, and immunocytes, such as B and T lymphocytes, monocytes, macrophages, and neutrophils [854].

The RAGE can also tether to non-AGE ligands and then trigger generalized inflammation and redox stress, particularly via a sustained activation of the proinflammatory transcription factor NFκB and augmented production of microsomal prostaglandin-E synthase-1 (mPGeS1)[86] and prostaglandin endoperoxide synthase PGhS1[87] in addition to MMPs [854]. Hyperglycemia accelerates RAGE ligand formation. The density of AT macrophages correlates with obesity and insulin resistance, at least in mice.

The RAGE also transmits signaling from members of the S100 and calgranulin category,[88] high-mobility group box-containing protein, HMGB1, which connects to toll-like receptors, macrophage differentiation antigen Mac1 (or integrin-$\alpha_M\beta_2$), and lysophosphatidic acid [854]. The RAGE ligand SAa favors occurrence of an uremic environment in addition to ROS production in SMCs.

The RAGE affects migration of monocytes and macrophages, cholesterol efflux, and pro- and anti-inflammatory cytokines. It activates diverse signaling cascades (Table 5.9). Its cytoplasmic domain interacts with the formin diaphanous-1, thereby influencing actin cytoskeleton dynamics, signaling particularly via GTPases of the RHO set (CDC42 and Rac1), and contributes to regulating serum response factor activity [854]. It also promotes PKB action and SMC migration. In vascular cells and macrophages, RAGE elicits hypoxia-stimulated expression of early growth response transcription factor EGR1 via Dia1 and hence proinflammatory and -thrombotic gene expression. Binding of Dia1 to RAGE and hence signaling is suppressed upon phosphorylation by PKB, ERK1, and ERK2. In addition, RAGE downregulates glyoxalase-1 synthesis; GlO1 detoxifies the pre-AGE intermediate, methylglyoxal.

In bone marrow-derived macrophages from diabetic mice, cholesterol efflux to ApoA1 and HDL is higher in mice devoid of the AGER gene than in WT mice.

[86] Prostaglandin-E synthase, which is encoded by the PTGES gene, is also dubbed mPGeS1. It catalyzes oxidoreduction of prostaglandin endoperoxide-H_2 (PGh_2) to prostaglandin-E_2 (PGe_2).

[87] Prostaglandin-G/H synthase-1 encoded by the PTGS1 gene converts arachidonate to prostaglandin-H_2 (PGh_2). It is also labeled cyclo-oxygenase-1 and prostaglandin-H_2 synthase-1.

[88] Expression of RAGE and its ligand S100a12 in macrophages and apoptotic vSMCs is significantly greater in atherosclerotic lesions of diabetic patients [854].

Table 5.9 Effects of the receptor for advanced glycation end products (RAGE; Source: [854])

Process	Mediators
Inflammation	↑ ROS, NFκB
	↑ Dia1, EGR1
Leukocyte migration	↑ icam1, vcam1
and adhesion	↑ CCL2
Cholesterol efflux	↓ PPARγ, ABCa1/g1
SMC migration	PKB

RAGE reduces reverse cholesterol transport from macrophages to plasma, liver, and feces owing to the decreased formation of cholesterol transporters ABCa1 and ABCg1 by PPARγ [854].

In obese individuals, insulin-related metabolism is defective. Expression of RAGE in the AT (i.e., on adipocytes, macrophages, and ECs) is significantly higher in obese than lean people and more in vAT than in scAT.

Soluble RAGE ($RAGE^{S}$), which is composed of the extracellular ligand-binding domains of RAGE that is cleaved by MMPs or adam10, diminishes inflammation and redox stress [854]. The RAGE splice variant also produces a soluble form ($RAGE^{ES}$).

5.3.4 Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD), also named hepatosteatosis and steatohepatitis, is related to insulin resistance. Insulin resistance in the AT causes excess supply of free fatty acids to the liver, which provokes lipotoxicity, redox stress, and apoptosis. It is now considered a hepatic manifestation of the metabolic syndrome. It results from cytoplasmic accumulation of lipids in hepatocytes. It is usually observed in overweight and obese individuals. NAFLD can affect up to 30% of the general adult population and up to 70% of diabetic and obese patients [855].

The main evolving stages range from simple steatosis, non-alcoholic steatohepatitis (NASH) with inflammation, to fibrosis and ultimately cirrhosis. Hence, NAFLD represents a disease spectrum. NASH is characterized by the co-existence of hepatic inflammation and fibrosis. Whereas hepatic steatosis has a benign clinical consequence, NASH has a much higher risk of evolving to cirrhosis and hepatocellular carcinoma.

Simple steatosis is characterized by the ectopic accumulation of lipid droplets in the cytoplasm of hepatocytes without hepatocyte injury, inflammation, and fibrosis.

Increased ingress in hepatocytes or the synthesis of triacylglycerol linked to augmented dietary intake, lipolysis in adipocytes, and de novo lipogenesis in hepatocytes in addition to decreased egress of TGs due to reduced mitochondrial β-oxidation and transport of TGs by VLDLs favor the progression of NAFLD [855].

Evolution to NASH marked by hepatocyte injury, inflammation, and fibrosis results from redox stress, ER stress, and defective UPR, lipotoxicity, and gut dysbiosis [855].

A transition (pre-NASH stage) can be defined during the progression from hepatosteatosis to NASH [856]. The correlations between blood and liver concentrations of lipid species strongly decrease after this transition phase. Fluctuation of gene expression related to hepatic pathways of triacylglycerol synthesis and degradation precedes the transition from simple steatosis to NASH. In particular, diacylglycerol acyltransferase, lipoprotein lipase, and adipocytic triacylglycerol lipase play a crucial role in the transition.

Liver ultrasound, computed tomography, and magnetic resonance imaging are used to diagnose hepatic steatosis with a relative high degree of accuracy. However, these techniques cannot distinguish NASH from simple steatosis. Liver biopsy is required for NAFLD diagnosis and staging. The term dynamical network markers (DNMs), based on nonlinear dynamic theory, refers to a group of molecules such as a set of metabolites with strong collective fluctuations [856]. A DNB criterion index measures the differential correlations and deviations of molecular expressions.

Patatin-like phospholipase domain-containing PnPLA3 (or adiponutrin) is involved in lipid anabolism and catabolism. PnPLA3 is a lipase for TGs; it also acts as an acyl hydrolase (acylglycerol transacetylase). Transcription of the PNPLA3 gene on human chromosome 22 in adipocytes and hepatocytes is regulated by SREBP1c and hence the nutritional state, as SREBP1c is influenced by glucose and insulin. Protein PnPLA3 variant (I148M) is linked to NAFLD in women, not in men; the association varies among different ethnic groups [857]. The PnPLA3 I148M variant is more common in Hispanics.

The PnPLA3 I148M variant may promote fibrosis via the hedgehog pathway, which primes activation and proliferation of hepatic stellate cells and excessive deposition of matrix constituents. According to genome-wide association studies, the TG hydrolase PnPLA3 is one of the major genetic modifiers influencing NAFLD progression [858].

Non-alcoholic fatty liver disease is independently associated with atherosclerosis in nondiabetic individuals. It is linked to a proinflammatory marker profile (low HDL-emia and adiponectinemia, but high triglyceridemia and CRP-emia [high plasmatic concentration of pentraxin-1]). In T2DM patients, hepatic lipidic content assessed by 1H magnetic resonance spectroscopy is higher in the absence of carotid plaques [859].

The type of cytokines implicated in the AT and liver differs. Obesity involves chronic low-intensity inflammation in the AT related to type-1 cytokines, that is, type-1 inflammation and a marked loss of eosinophils. However, obese mice prone to type-1 cytokine response exhibit more pronounced fibrosis. Hepatic fibrosis engendered by NAFLD is characterized by type-2 inflammation and recruitment of eosinophils [860]. Obese IL4- and IL10-deficient mice are resistant to NASH, protection being ensured by hepatic interferon-γ. In Ifnγ-deficient mice, hepatosteatosis progresses rapidly, fibrosis depending on both the TGFβ and IL13 signaling.

Hypothyroidism is an independent risk factor for NAFLD. Hypothyroidism causes hypercholesterolemia, which favors NAFLD. However, cholesterol does not contribute to hypothyroidism-induced NAFLD [861].

Mild hypothyroidism, a metabolic disease characterized by low and high plasmatic concentrations of thyroid hormone[89] and thyroid-stimulating hormone, respectively, is associated with NAFLD owing to the reduced insulin secretion in addition to insulin resistance in the AT [861]. The resulting lack of suppression of lipolysis raises the shuttle of fatty acids to the liver, where they are esterified and form TGs.[90] Triglyceride accumulation in hepatocytes is associated with reduced fatty acid oxidation and VLDL assembly, provoking hepatic insulin resistance, TG accumulation synergizing reduced insulin secretion. Because glucose production is not suppressed after feeding, the resulting hyperglycemia stimulates de novo lipogenesis in the liver.

On the other hand, in mice with severe hypothyroidism, the delivery of fatty acids to the liver decreases, protecting against NAFLD. Severely hypothyroidic SLC5A5$^{-/-}$ mice have hypercholesterolemia, but are protected against NAFLD.[91]

Drugs such as berberine,[92] are aimed at improving mitochondrial function,[93] sugar and lipid metabolism, AMPK signaling,[94] increasing fatty acid β-oxidation

[89]Thyroid hormones (T_3 and T_4) bind to thyroid hormone receptors TRα and TRβ (NR1a1–NR1a2), which interact with other transcription factors to activate or inhibit the transcription of thyroid hormone-regulated genes. In the liver, NR1a1 and NR1a2 cooperate with NR1c1 (PPARα) to activate transcription of genes involved in the fatty acid β-oxidation [861].

[90]In NAFLD patients, de novo lipogenesis accounts for only about 25% of liver TGs, the main source (∼60%) of substrates for hepatic TG synthesis being fatty acids generated by AT lipolysis [861].

[91]The sodium–iodide symporter SLC5a5 (NIS) enables iodide ingress in the thyroid gland, the first step in thyroid hormone synthesis. In SLC5A5$^{-/-}$ mice, the marked reduction in plasmatic thyroid hormone concentrations suffices to lessen the response of AT to adrenergic signaling. Adrenoceptor-stimulated lipolysis and hence a circulating pool of glycerol and fatty acids in addition to activity of hormone-sensitive lipase (lipase-E) after overnight fasting decline [861]. Altered adrenergic stimulation of lipolysis in AT alleviates fatty acid delivery to the liver and subsequently hepatic TG synthesis. Hence, SLC5A5$^{-/-}$ mice are protected against NAFLD despite lowered fatty acid oxidation and thyroid hormone signaling in their livers.

[92]Berberine is an isoquinoline quaternary alkaloid extracted from Coptis chinensis, Rhizoma coptidis, and Hydrastis canadensis, among other plants.

[93]Berberine may increase sirtuin-3 expression, improving mitochondrial function and decreasing mitochondrial ROS generation [855].

[94]Berberine may inhibit mitochondrial ^{ETC}complex-*I* and activate AMPK, thereby diminishing lipogenesis, elevating energy consumption, and promoting FA oxidation [855]. It may elicit adiponectin multimerization and thus enhance insulin sensitivity via AMPK. It may also reduce lipid accumulation via AMPK phosphorylation (activation), thereby repressing transcription of genes targeted by SREBP1c (acetyl-CoA carboxylase ACC1, FAS, and stearoyl-CoA desaturase SCD1) and SREBP2 (hydroxymethylglutaryl-CoA synthetase [HMGCS] and reductase [HMGCR]) and hence TG and cholesterol synthesis.

and insulin sensitivity,[95] and lessening cholesterolemia and lipogenesis,[96] in addition to inflammation[97] and redox and ER stress.[98]

5.4 Adipose Tissue

Adipose tissue includes mature adipocytes (>90% of the AT cell population), and pre-adipocytes, in addition to various other stromal cell types,[99] such as fibroblasts, adipose tissue mesenchymal stem cells (ADMSCs; adipocyte stem and progenitor cells),[100] vascular cells of microvessels (especially ECs), and resident (predominantly M2 macrophages[101] and regulatory T cells [T_{R1}] in as addition to protective eosinophils and, to a lesser extent, neutrophils [802])[102] and infiltrating

[95] Berberine may trigger insulin secretion and synthesis of insulin receptor (via PKC) and insulin receptor substrate IRS2 in addition to supporting the IRS1–PKB axis and GluT4 translocation to the plasma membrane [855].

[96] Berberine may also upregulate LDLR expression owing to Ldlr mRNA stabilization and ERK activation in addition to downregulating that of C/EBPα, adiponectin, and leptin in metabolic syndrome [855]. In addition, it may reduce the production of PPARγ (NR1c3), PCSK9, and fatty acid translocase.

[97] Berberine may alleviate the action of TNFSF1 and IL6 induced by palmitate, COx2 formation, and JNK1 phosphorylation [855].

[98] Redox stress induces expression of uncoupling protein UPC2 in NAFLD mice. Administration of berberine reduces UCP2 synthesis. Berberine may prime ER stress response partly via the ATF6–SREBP1c pathway [855].

[99] The stromal–vascular cell fraction corresponds to the non-adipocyte cell populations of AT. It encompasses AT stem cells, committed pre-adipocytes, fibroblasts, and vascular and immune cells.

Adipose-derived stem cells contribute to cell renewal and AT repair [862]. They are ENPP1+ (CD39), epican+ (HSPG or CD44), Nt5E+ (CD73), Thy1+ (CD90), endoglin+ (CD105), pecam1− (CD31), and PTPRc− (CD45) cells. In fact, different subpopulations of adipose-derived stem cells can exist, expressing distinct surface markers such as the stem cell CD34 marker. They have the different ability to grow and differentiate according to the fat depot type. The adipogenic differentiation capacity of scAT-derived stem cells is higher than that of visceral AT-derived stem cells. Hence, the scAT and vAT can evolve to hyperplasia and hypertrophy in adverse conditions, respectively.

Adipose-derived stem cells can differentiate into multiple cell lineages, such as CMCs, myoblasts, osteoblasts, chondrocytes, hepatocytes, pancreatocytes, ECs, and hematopoietic-supporting cells [862].

[100] Mesenchymal stem cells (MSCs) are multipotent stem cells that can engender adipocytes, osteoblasts, chondrocytes, and myocytes. They reside in almost all postnatal organs. AT stem cells can differentiate into cells of meso-, endo-, and ectodermal origin. They may or not be multipotent.

[101] Macrophages are the most abundant immunocytes in vAT and scAT (>50% of all leukocytes) [802]. In lean humans, AT macrophages characterized by the surface markers macrosialin (CD68+) constitute less than 5% of all AT cells. Increased density of AT macrophages (up to 40% of all AT cells) in metabolic stress is associated with their phenotype changes (from M2 to M1).

[102] In lean AT, both M2 macrophages and regulatory T cells release anti-inflammatory cytokines, such as interleukin-10 and TGF-β, which favor insulin sensitivity and prevents fat depot inflammation. In addition to regulatory T cells, insulin-sensitive fat depot-resident T lymphocytes encompass primarily T_{H2} cells.

inflammatory cells (i.e., effector and memory T cells [e.g., T_{C1}, T_{H1}, and T_{H17}],[103] but also NK and NKT cells and granulocytes) [863]. All cell types contribute to the AT secretome, which comprises adipokines, gaseous messengers, various types of metabolites, and microRNAs.

The nutritional status influences the adipocyte number. Overfeeding and hypercaloric diet with a high fat content cause AT expansion. Obesity from childhood is characterized by AT hyperplasia (elevated adipocyte number) due to the formation of new adipocytes from progenitor differentiation. The signaling effector PKB2 in adipocyte progenitors is activated by pro-adipogenic insulin and IGF1 in HFD-induced adipocyte proliferation [717].

A $5HT_{2A}+$ neuronal cluster in the central nucleus of the amygdala, which is involved in feeding and reward, promotes food consumption, whereas the anorexigenic PKCδ+ neuronal cluster of the amygdala suppresses appetite [864]. Inhibitory $5HT_{2A}+$ neurons innervate the parabrachial nucleus, which is implicated in appetite suppression.

In AT, creatine metabolism is necessary for diet-induced thermogenesis, which limits weight gain, increased caloric intake being balanced by augmented heat production and, conversely, a long-term decreased food intake, reducing energy consumption. Creatine is synthesized by mitochondrial glycine amidinotransferase encoded by the GATM gene, in adipocytes and in other cell types. It promotes glucose tolerance and stimulates mitochondrial ATP turnover. Creatine supports cold- and β3-adrenoceptor-stimulated adaptive thermogenesis [865]. Adipocyte-specific deletion of GATM depletes creatine and phosphocreatine. $^{AdC}GATM^{-/-}$ mice are prone to DIO.

Interleukin-10 modulates insulin signaling via the InsR–IRS1/2–PI3K–PKB–FoxO1 axis used in hepatic gluconeogenesis and lipid synthesis [802].

M2 macrophages control adipocytic lipolysis. In co-operation with eosinophils, M2 macrophages can manage generations of beige adipocytes [802].

Unlike resident macrophages and dendrocytes, neutrophils reside transiently in the AT [802]. They secrete IL4 and IL13. They contribute to the anti-inflammatory insulin-sensitive AT phenotype.

In normal AT, regulatory T cells synthesize IL2 receptor subunit-α (CD25), tumor-necrosis factor superfamily members TNFSF4 and TNFSF18, cytotoxic T-lymphocyte antigen CTLA4, killer cell lectin-like receptor KLRg1 (CLec15a), in addition to FoxP3 [802].

[103] CD4+ and CD8+ T lymphocytes constitute clones defined by their functional and differentiated states. Interferon-γ and interleukin-4 represent type-1 and -2 cytokines, respectively. CD8+ memory T lymphocytes produce Ifnγ and TNFSF1.

CD4+ effector T lymphocytes comprise helper T_{H0}, T_{H1}, and T_{H2} cells in particular. T_{H1} lymphocytes secrete interleukin-2, interferon-γ, and TNFSF2 implicated in adaptive immunity. T_{H2} lymphocytes release IL4 to IL6, IL10, and IL13 involved in humoral and allergic immunity.

Action of cytolytic CD8+ effector T lymphocytes depends on CD4+ effector T lymphocytes. Cytotoxic CD8+ T lymphocytes are also classified according to cytokine type secretion. CD8+ T lymphocytes differentiate into subsets similar to CD4+ T_{H0}, T_{H1}, T_{H2}, and T_{H17} cells, which are referred to as T_{C0}, T_{C1}, T_{C2}, and T_{C17} cells, respectively, which are defined by expression of the same characteristic cytokines as their CD4+ counterparts. Although T_{C1} cells produce high amounts of interferon-γ, T_{C2} cells synthesize interleukins IL4, IL5, IL10, and IL13, but low levels of Ifnγ, T_{C0} cells forming IL4 in addition to Ifnγ and other cytokines and T_{C17} cells IL17.

The diet-dependent AT hyperplasia is heterogeneous among fat depots. In humans, AT hyperplasia is observed in the femoral scAT, but not in the upper abdominal scAT [717]. Whereas expansion of intra-abdominal AT is associated with insulin resistance, a larger scAT improves insulin sensitivity.

The number of adipocytes depends on the balance between cell death and the generation of new adipocytes from progenitor cells. Adipocyte progenitors are immature mesenchymal stromal cells associated with the AT vasculature. In adult humans, the turnover rate is estimated at 10% per year, a low value with respect to other organs [717].

Adipose-derived stem cells can secrete angiogenic growth factors and antiapoptotic cytokines (e.g., HGF, IGF, TGFβ1, and VEGF) [862]. Thus, they have immunomodulatory properties. On the other hand, inflammation can play an important role in adipose-derived stem cell differentiation. Some proinflammatory cytokines can suppress PPARγ expression, thereby impeding adipogenic differentiation, whereas an increase in proinflammatory cytokines usually induces adipocyte hypertrophy and hyperplasia (Table 5.10). Sphingosine kinase SphK1 participates in the regulation of the proinflammatory activity of adipose-derived stem cells [862].

Aging is associated with a loss of adipose-derived stem cell proliferation and differentiation potential. In older individuals, the differentiation rate is only high in the arm and thigh scAT [862].

The main local determinants of AT functionality, that is, the capacity of AT to store excess nutrients without adipocyte overloading, are the number and size

Table 5.10 Inflammation induced by metabolic disturbances affects adipose-derived stem cell behavior, inflammatory stimuli activating or attenuating their signaling (Source: [862]; ↑ increase, ↓ decrease)

Process	Impact of inflammation on signaling mediators
Self-renewal	↓ FGF, notch, Wnt
Proliferation	↑ TNFSF1, IL1β
Adipogenic differentiation	↓ PPARγ (NR1c3)
Migration	↑ MMP2/9
Senescence	↑ TNFSF1
Angiogenesis	↓ notch, Wnt
Immunomodulation	↓ FGF, notch, Wnt
	↑ IL1β (NLRP3 inflammasome)

Altered self-renewal potential and augmented commitment to the adipogenic lineage relies on a coordinated inhibition of FGF, notch, and Wnt. In obese animals, these cells have a higher senescence rate and adipo- and osteogenic potential than those in lean animals. In obese patients, they have a higher migration and phagocytosis capacity and increased metabolic activity (increased glycolysis and succinate release and decreased succinate dehydrogenase activity), expression of proinflammatory cytokines and chemokines in addition to MMP2 and MMP9, and activation of the NLRP3 inflammasome compared with cells in lean subjects. Their angiogenic potential decreases in obesity

of adipocytes. Metabolically healthy obese individuals have AT hyperplasia, the adipocyte number being higher than that of weight-matched insulin-resistant subjects [717].

Obesity is also associated with changes in the extracellular matrix. Adipocyte progenitor response is driven by M2-type macrophages and type-2 innate immunocytes during adaptive tissue remodeling [717]. Transient adaptive local inflammation triggers AT remodeling during AT hyperplasia in addition to WAT browning. On the other hand, chronic inflammation due to adipocyte overloading and lipotoxicity alters adipocyte metabolism.

Extracellular matrix with its fibrillar and laminar structures contributes to the regulation of cell differentiation, metabolism, and inflammation in the AT. It consists of structural and adhesive proteins, collagen and elastin fibers, fibronectin, laminin, proteoglycans, among other species. Adipocytes, pre-adipocytes, and inflammatory leukocytes produce matrix metallopeptidases that degrade and remodel the matrix to regulate its rheology in addition to adipogenesis [717].

Chronic inflammation causes excess matrix, i.e., fibrosis. Collagen accumulation in the interstitial and pericellular spaces is up to four times higher in obese subjects than in lean people [717]. Hypoxia is one of the main causes of fibrosis in obesity. Activated HIF1α stimulates matrix constituent synthesis and crosslinking. It also launches inflammatory gene expression, leading to immunocyte recruitment. Adipocytes, macrophages, and mastocytes contribute to fibrosis in obesity. Fibrosis provokes AT stiffness and limits adipocyte proliferation. In addition to inflammation, lysophosphatidic acid, adiponectin, and leptin intervene in fibrosis.

Adipokines released by adipocytes and stromal cells of the pvAT and eAT exert a paracrine action on blood vessels and myocardium, respectively, in addition to endocrine effects via the circulating adipokine pool. In particular, they participate in controlling blood coagulation.

Conversely, the vascular wall and myocardium send paracrine or endocrine messengers to the AT. In addition, the sensory innervation of the pvAT influences adiposecretome. In particular, innervation of the AT affects responsiveness of the medial smooth muscle to NAd [866].

Adipose tissue dysfunction is characterized by a decreased release of homeostatic protective factors (e.g., adiponectin, NO, and some prostaglandin types) and increased secretion of stress adipokines (e.g., leptin, resistin, and visfatin) and development of low-grade chronic inflammation, which favors metabolic and vascular dysfunction [802].

5.4.1 Structural and Functional Types of Adipose Depots

Functionally, the BAT, which is involved in the body temperature maintenance, oxidizes glucose and fatty acids to fulfill thermogenesis, whereas the *white adipose tissue* (WAT) stores TGs in LDs.

White adipose tissue exists as vAT and scAT. These fat depots differ functionally and immunologically [802]. The former is metabolically more active than the latter, with higher levels of glucose uptake and fatty acid generation, the scAT taking up circulating FFAs and TGs. Moreover, the vAT has a greater sympathetic innervation. The vAT thus participates in the regulation of insulin sensitivity. It also contains more immunocytes, even under healthy conditions. However, excessive immunocyte infiltration and resulting chronic low-grade inflammation, especially in the retroperitoneal compartment, is linked to cardiovascular risk.

5.4.1.1 Brown Adipose Tissue

Energy uptake relies on nutrient absorption and energy expenditure is determined by basal metabolism, physical activity, and thermogenesis. In moderately cold temperatures, heat is generated by the BAT owing to increased uptake of nutrients, particularly carbohydrates (glucose), lipoprotein-derived TGs, cholesterol, and FFAs.

Positron emission tomography coupled with X-ray computed tomography (PET-CT) enables imaging of AT to assess both its volume from CT and metabolic activity using deoxy-(18F)fluoro-glucose uptake triggered by acute cold exposure. However, measurement of entire body BAT volume, distribution, and thermogenic activity is not obvious because brown adipocytes are structurally commingled with WAT, muscle, and blood vessels, forming narrow fascial layers adjacent to muscles, bones, and organs. The human BAT is indeed composed of stromal tissue, white adipocytes, and UCP1+ thermogenic brown and beige adipocytes, whereas, in rodents, BAT is homogeneous [867].

After 5-h tolerable cold exposure, BAT volume and activity measured by PET-CT are lower on average in obese than in lean young men, although several obese young men have a substantial BAT volume [867]. Six activated BAT regions include cervical, supraclavicular, axillary, mediastinal, paraspinal, and abdominal (volume $\sim$1100 ml) with a potential for BAT genesis within anatomically inferior and posterior depots. However, less than one-half of these depots are stimulated by acute cold exposure [867].

Most young individuals, even obese ones, have a potential for BAT expansion, as they do not reach their maximal BAT content [867]. Source of BAT-stimulating NAd can vary. Whereas sympathetic stimulation preferentially activates superior depots, NAd released from pelvic tumors, such as pheochromocytoma and paraganglioma, in plasma targets nearby inferior retroperitoneal abdominal depots.

The BAT increases the body's energy expenditure not only upon cold exposure but also after overfeeding. The extent and activity of BAT are positively correlated, with energy expenditure during cold exposure, and negatively with age, BMI, and fasting glycemia [868]. In humans, BAT activation improves insulin sensitivity.

The BAT, which exists not only in newborns and during childhood but also in adults, primarily localizes to the supraclavicular and paravertebral regions [805]. In newborn human infants, BAT is relatively well defined and resides in the inter-

scapular, axillary, posterior cervical, supra-iliac, anterior mediastinal, and perirenal regions [869]. The BAT of human newborns is interspersed with WAT, the amount of BAT gradually declining in adulthood. In adult humans, in whom homeothermy does not rely on BAT thermogenesis, the BAT is less well defined. The main human depots lodge in the supraclavicular regions and the neck, with some additional paravertebral, mediastinal, para-aortic, and suprarenal sites [869].[104]

The BAT can be detected more or less easily according to age, gender, adiposity, and thermal environment [869]. It is observed most frequently in young lean women. It is much less well identified in response to cold and insulin in obese individuals.

The BAT is highly vascularized to accommodate the greater demand for oxygen and nutrients. The dense vasculature enables a suitable amount of fatty acids and glucose to be delivered upon activation and warm blood irrigating it.

The BAT is endowed not only with a rich blood and nerve supply but also a dense niche of perivascular mesenchymal cells, which serve as a source of pre-adipocytes. Brown adipocytes arise from specific adipogenic progenitors that are closer to skeletal muscle progenitors than to white adipocyte progenitors [869].

Brown adipocytes are characterized by multilocular LDs, whereas white adipocytes contain a unilocular large lipid vesicle.

The gut microbiota can facilitate adaptive thermogenesis via a cold-induced switch in the cholesterol metabolism [870]. A greater proportion of absorbed dietary cholesterol is converted to bile acids using CyP7b1 involved in the alternative pathway of bile acid synthesis in the liver. Cold exposure also affects the bacterial composition of the gut flora.

Mitochondria not only create ATP from oxidative phosphorylation but also orchestrate production of metabolites used for synthesis of nucleotides, lipids, and proteins, generate signaling ROS mediators, participate in regulating calcium concentration in addition to cellular proliferation, immune response, and cell death [816]. In vascular diseases, mitochondria change morphologically and functionally.

Mitochondria abound in brown adipocytes. These cells have a high content of iron and cytochrome and a brownish color. Numerous mitochondria in brown adipocytes are large and possess laminar cristae, whereas they are small and elongated with randomly oriented cristae in white adipocytes [869].

Uncoupling Protein UCP1

In mitochondria of brown adipocytes, the ETC is uncoupled from ATP formation by uncoupling protein UCP1 in the inner mitochondrial membrane [868]. Fatty acid and glucose are processed as energy substrates, chemical energy being dissipated by acute adaptive thermogenesis. Energy of these nutrients is converted into a proton gradient, UCP1 catalyzing the inducible proton leak to release the energy of the

[104] In adult humans, BAT localizes to cervical, supraclavicular, mediastinal, paravertebral, suprarenal, and perirenal regions [818].

proton gradient, that is, dissipating the mitochondrial proton motive force generated by the ETC as heat, instead of storing the energy via ATP generation [872]. In ^{BAdC}mitochondria, the large quantity of ETC complexes is associated with a weak amount of ATP synthase [871].

In addition, release of the proton motive force by the mitochondrial proton leak can alter the redox state of the ETC chain and reduce ROS production [871, 872]. The ETC substrate glycerol 3-phosphate (G3P) and resulting mitochondrial energization generate ROS via reverse electron transport or mitochondrial G3P dehydrogenase GPD2 [872]. Whereas ^{ETC}complex-*I* forms superoxide in the mitochondrial matrix via succinate, GPD2 synthesizes ROS in the mitochondrial intermembrane space.

Cold-triggered sympathetic stimulation of brown adipocytes activates lipolysis, glucose uptake, and mitochondrial genesis. Acute adrenoceptor stimulation supports UCP1-dependent thermogenesis via ROS production by reverse electron transport through ^{ETC}complex-*I*. In mice depleted in the Ucp1 gene, not only is the ETC amount alleviated, but cold-activated metabolism also primes innate immunity via ROS and cell death in BAT [872]. Deficiency in UCP1 in ^{BAdC}mitochondria blunts their ROS-linked Ca^{2+} buffering capacity; ^{BAdC}mitochondria become highly sensitive to permeability transition induced by ROS and Ca^{2+} overload.

The UCP1 protein, which is activated by FFAs and NAd (β3AR-mediated thermogenesis), allows protons to reenter the mitochondrion, bypass ATP synthesis, uncoupling the ETC, thereby, generating heat by nonshivering thermogenesis. Proton leak at the inner mitochondrial membrane via UCP1 is enhanced by long-chain fatty acids and inhibited at rest by cytosolic purine nucleotides, mainly ATP [869]. Long-chain fatty acids are unable to dissociate from UCP1 until they are oxidized. UCP1 cotransports a fatty acid anion and H^+ across the inner mitochondrial membrane. Proton is then released, whereas the fatty acid anion remains bound to UCP1, which can initiate another H^+ transfer. Like other mitochondrial transporters, UCP1 is stabilized by its tethering to cardiolipin (CL).

Fatty acids derived from TGs in LDs are metabolized by β-oxidation enzymes in the mitochondrial matrix and activate UCP1, which then generates heat using the electrons derived from β-oxidation. Replenishment of the intracellular TG stores depends on uptake from blood of FFAs bound to albumin or VLDLs and chylomicrons, and of insulin-dependent and -independent glucose followed by lipogenesis, along with glycolysis for ATP generation [869].

Human BAT takes up large amounts of glucose under thermoneutral conditions and rapidly adapts this uptake upon BAT stimulation. Insulin increases BAT glucose uptake five-fold via GluT4 (mainly) and GluT1. Uptake of circulating glucose by BAT after cold exposure is lower in insulin-resistant than in -sensitive individuals [869]. Activation of BAT by cold exposure (5–8 h) in healthy lean volunteers with large BAT depots (but not in those with small ones) increases insulin sensitivity. Prolonged cold exposure (10 d) markedly raises insulin sensitivity in diabetic subjects, but induces only a minor increase in BAT glucose uptake [869].

Mitofusin Mfn2

The ubiquitous GTPase mitofusin Mfn2 is a major determinant of BAT thermogenesis. Mitofusin-2 controls OMM fusion, and mitochondrial fusion and fission, serving as quality control mechanisms to ensure mitochondrial function.

In skeletal myocytes, defective mitofusin-2 formation and subsequent augmented mitochondrial fission are linked to insulin resistance. Liver-specific deletion of the MFN2 gene engenders glucose intolerance and enhances hepatic gluconeogenesis [873]. Deficiency of Mfn2 in hypothalamic POMC+[105] neurons decreases energy expenditure and causes hyperphagia and hence obesity [874]. Ablation of the MFN2 gene in both white and brown adipocytes of SD- and LFD-fed mice provokes BAT dysfunction with lipid accumulation in the BAT, impaired ETC activity, and a blunted response to adrenoceptor stimulation [873]. Selective ablation of MFN2 in BAT improves glucose tolerance under HFD, but alters cold-stimulated thermogenesis. In HFD-fed obese mice with ^{BAT}MFN2 deletion, improved insulin sensitivity results from a gender-specific rewiring of the mitochondrial function. In females, mitochondria of brown adipocytes have a higher efficiency for ATP synthesis by FA oxidation, whereas in males, ^{ETC}complex-*I* activity declines and glycolysis to lactate rises linked to increased expression of the glycolytic enzyme isoform PKM2 in BAT [875]. Hence, selective excision of MFN2 in brown adipocytes improves insulin sensitivity in obese mice via increased glycolysis in male mouse BAT and coupled lipid import capacity and fatty acid oxidation in female mouse BAT, thereby preventing, whatever the gender, obesity-linked insulin resistance. On the other hand, Ucp1 deletion affects neither the ETC activity nor fatty acid oxidation capacity coupled with ATP synthesis.

The presence or absence of adipose Mfn2 effects on liver parameters correlate with the existence or not of circulating FGF21 concentrations, respectively. Mitofusin-2 may differentially control FGF21 expression in white and brown adipocytes [874].

Mitofusin-2 is highly produced in BAT; its deficiency impairs ^{ETC}complex-*I* activity, destabilizing the connection between ^{ETC}complex-*I* and ^{ETC}complex-*III*, and disturbing the response to adrenoceptor stimulation, but protecting against HFD-induced insulin resistance [873]. In BAT, Mfn2 is required to sustain body temperature after cold exposure, Mfn2 expression induction being an element of the sympathetic response induced by thermal stress under obesity, but contributes to insulin resistance [875]. In response to cold exposure, thermogenesis in BAT is activated by adrenoceptor signaling, which raises lipolysis and fatty acid oxidation linked to proton leak through UCP1. Expression of Mfn2 and UCP1 is upregulated in BAT after acute cold exposure or treatment with β3 AR agonist in addition to feeding. On the other hand, NAd induces mitochondrial fission in brown adipocytes for proper activation of uncoupled ETC activity. Therefore, Mfn2 in BAT promotes cold tolerance via thermogenesis linked to proper lipid mobilization, fatty acid

[105] POMC: pro-opiomelanocortin.

oxidation, and UCP1-dependent mitochondrial ETC activity, rather than favoring glucose uptake and glycolysis, processes improving glucose tolerance and insulin sensitivity.

Mitofusin-2 (but not Mfn1) at the OMM interacts with the lipid droplet-associated protein perilipin-1, facilitating docking of mitochondria to LDs in BAT in response to adrenoceptor stimulation and hence heat production, TG lipolysis in LDs yielding fatty acids that enter the mitochondrion for β-oxidation to feed the ETC [873]. Mitofusin-2 also promotes between-mitochondrion and mitochondrion–ER contacts. As interaction of mitofusin-2 with perilipin-1 ensures proper lipolysis and subsequently fatty acid oxidation in response to adrenoceptor stimulation and food intake, it launches cold- and diet-induced thermogenesis in mice.

Upon cold exposure, activated BAT reduces *TGRL-emia* (Plasmatic concentration of TGRLs) in lean mice and correct hyperlipidemia in ApoA5$^{-/-}$ mice, whereas denervation of mouse BAT causes hypertriglyceridemia [869]. Cold also normalizes the response to glucose and reduces insulin resistance in HFD-fed obese mice. Transplantation of BAT increases glucose tolerance.

The BAT oxidative function declines with obesity and aging. Decreased thermogenic capacity, which fully depends on UCP1, favors obesity and can cause hyperphagia in rodents [869]. Conversely, augmented UCP1 activity ameliorates obesity, at least in mice. Activators of BAT (e.g., β3AR agonists and thyroid hormones) lowers lipidemia and glycemia. Acute BAT activation initially relies on the catabolism of intracellular triglycerides, whereas prolonged BAT activation increases TG clearance from plasma.

The sympathetic nervous system stimulates BAT and contributes to its growth and hence activates thermogenesis. Three subtypes of β-adrenoceptors exist in brown adipocytes. However, their relative quantity varies according to the location and the mammalian species. In rodents, β3AR is predominant, whereas β1AR is generally preponderant in humans [869]. Although β1AR and β3AR regulate brown adipocyte proliferation and differentiation, respectively, β2AR alone may suffice for maintenance of BAT function [868].

Adrenergic stimulation of brown adipocytes enhances the lipolysis of TGs in LDs by ATGL and hormone-sensitive lipase (HSL or lipase-E) and releases fatty acids. The signaling cascade primed by β AR relies on cAMP, a target of hormone-sensitive lipase.

The BAT can also be stimulated by natriuretic peptides, thyroid hormone (e.g., triiodothyronine [T_3]), retinoids, bone morphogenetic proteins BMP7 and BMP8b, fibroblast growth factor FGF21, VEGF, leptin, hypocretin (or orexin),[106] and irisin [869].

In obese individuals, circulating natriuretic peptide concentrations decrease. These blood pressure modifiers also exert metabolic effects. Cardiac natriuretic peptides (ANP–BNP), which tether to GC2a and then activate the GC–cGMP–PKG pathway, can stimulate lipolysis in adipocytes with a potency similar to that of β AR agonist and promote WAT browning [876].

Natriuretic peptides can also increase the oxidative capacity of skeletal muscles. In HFD-fed mice, elevated natriuretic peptide signaling in muscles causes insulin resistance [876]. Overfed mice with a muscle-specific deficiency in the clearance receptor NPR3 ($^{SkM}Npr3^{-/-}$) gain weight. However, in AT, enhanced NP signaling protects against DIO, raising energy expenditure, insulin sensitivity, and glucose uptake into BAT [876]. Mice with an AT-specific NPR3 deficiency ($^{AT}Npr3^{-/-}$) are protected against detrimental effects of HFD-induced obesity (insulin resistance, inflammation, and hepatic steatosis). Oxygen consumption and carbon dioxide production increase without altering food intake and physical activity. In addition, expression of UCP1 and other mitochondrial proteins (and hence thermogenesis) along with that of the batokines FGF21, Nrg4, and BMP8b[107] rise in the BAT of $^{AT}Nprc^{-/-}$ mice. Overexpression of BNP or PKG also augments expression of genes involved in thermogenesis in proper chow- or HFD-fed mice [876].

Both FGF21 and BMP9, which are predominantly produced in the liver, activate BAT thermogenesis in addition to WAT browning [877]. FGF21, which is also synthesized in BAT and WAT, although to a lesser extent than in the liver, and in the skeletal muscle and pancreas, does not mediate cell proliferation, unlike other members of the FGF category. Under chronic cold exposure in mice, FGF21 expression decreases in the liver, but increases under PPARγ transcriptional control in BAT and WAT, where it markedly raises UCP1 production [877].

[106] ορεξις: appetency, conation.

[107] Fibroblast growth factor-21 operates via adiponectin [876]. Neuregulin-4 abounds in the BAT, from which it is secreted; it decreases hepatic lipogenesis. Bone morphogenetic protein-8B increases thermogenesis via its central and peripheral actions.

Irisin is secreted by skeletal muscles during physical exercise, at least in rodents [877]. It is formed by cleavage of fibronectin type-*III* domain-containing protein FNDC5.[108] It promotes the genesis of beige cells in white adipose depots, as it stimulates UCP1 production, at least in mice. It correlates with insulin desensitization.

Thyroid hormones, which mediate overall energy expenditure, act as para- and endocrine messengers to regulate BAT [868]. Brown pre-adipocytes and adipocytes express a high level of iodothyronine deiodinase, DIo2, which converts thyroxine (T_4) to the more active triiodothyronine (T_3), which upregulates UCP1 production. In addition, thyroid hormone regulates energy balance in some types of hypothalamic neurons. Thyroid receptor isoforms specifically regulate UCP1 expression and thermogenesis. The TRα (NR1a1) isoform regulates adaptive thermogenesis, whereas TRβ (NR1a2) modulates UCP1 expression [877].

Among members of the BMP family of the TGF superfamily, BMP7 enhances thermogenesis in BAT [877]. It is produced in several hypothalamic nuclei and may regulate BAT via a central mechanism. BMP8b is also formed in BAT and in the hypothalamus, where it activates the sympathetic signaling to BAT, without changing the feeding behavior. BMP4 promotes differentiation of MSCs into white adipocytes. In primary human adipose stem cells, both BMP4 and BMP7 induce a white-to-brown adipocyte transdifferentiation.

Phosphatase and tensin homolog deleted on chromosome 10, which counters PI3K action and hence reduces levels of phosphorylated PKB and FoxO1, supports the expression of UCP1 in BAT and WAT, and of its transcriptional regulator PGC1α [877]. In humans, PTEN haploinsufficiency increases the risk for obesity, but decreases that for T2DM, as it markedly improves insulin sensitivity.

Brown adipose tissue produces more heat in female rats exposed to cold than in males [868]. Sex steroids, estrogens, and androgens have receptors on brown adipocytes. Testosterone and 17β-estradiol inhibits and stimulates mitochondrogenesis, UCP1 synthesis, and lipolysis, respectively. In humans, BAT is better developed in women than in men [868].

Excess weight gain during the early postnatal period, mainly due to overnutrition, can significantly alter body weight in adulthood. Excessive stimulation of energy expenditure at an early age of life can desensitize or reprogram thermogenesis regulation and subsequently impair energy expenditure in adulthood [878].

A set of 190 kinases enables brown adipocyte formation (i.e., UCP1 synthesis), pre-adipocyte differentiation, proliferation, and activation [879].[109] Among these

[108] In muscles, PGC1α upregulates Fndc5 gene transcription.

[109] Classical brown adipocytes develop from myogenic factor MyF5+ cells, whereas white and beige adipocytes originate from MyF5− precursors. However, brown pre-adipocytes can also derive from MyF5− precursors [879]. Transcriptional control of development and activation of brown adipocytes rely on early B-cell factor EBF2 and PR domain-containing protein PRDm16.

kinases[110] (e.g., PKA,[111] ERK1, ERK2, and Src), AMPK[112] regulates brown adipocyte formation and promotes UCP1 production.

The BAT participates in regulating the vasculomotor tone and cardiovascular metabolism, in addition to thermogenesis [869]. In mice, it increases lipid clearance and improves glucose tolerance, thereby attenuating insulin resistance.

Batokines and MicroRNAs

The BAT releases constitutive or inducible auto-, para-, and endocrine messengers, the so-called *batokines*, which are involved in between-organ crosstalk. Locally acting batokines operate on the BAT and its surrounding tissues.

Endocrine batokines can improve metabolism in the heart and vasculature (Table 5.11). IGF1 secreted by BAT into the circulation ameliorates T1DM without changing insulin concentration [869]. Interleukin-6 and FGF21 reverse metabolic anomalies in HFD-fed and Ob/Ob insulin-resistant mice. However, secretion of T_3 and FGF21 is not specific to BAT, rendering action of BAT uncertain with respect to these substances produced by several other organs; in particular, the liver and skeletal muscle also release FGF21 in the bloodstream.

[110] Casein kinase CsnK2 abounds in white adipocytes. Ablation of the CSNK2A1 and CSNK2B genes suppresses brown adipocyte formation. CK2 may affect maturation of white or brown adipocytes, but not differentiation [879]. Inhibition of CK2 elicits beige adipocyte development in response to β-adrenergic stimulation. Inhibition of JAK3 diminishes brown adipocyte formation; JAK3 may also play a distinct role in brown precursors and brown mature adipocytes [879]. The kinases PKG1, PI3K, and PKB are implicated in brown adipocyte formation and brown mature adipocyte activation [879].

[111] Activated PKA phosphorylates (activates) P38MAPK, which phosphorylates activating transcription factor ATF2, which interacts with PGC1α, coordinating the transcriptional program that activates brown adipocytes, and initiates transcription of the Ucp1 gene [879]. Its ubiquitous regulatory subunits $PKA_{r1\alpha}$ and $PKA_{r2\alpha}$, which are encoded by the PRKAR1A and PRKAR2A genes, promote brown adipocyte formation. On the other hand, $PKA_{r1\beta}$ and $PKA_{r2\beta}$, which are encoded by the PRKAR1B and PRKAR2B genes, play an opposite role. P38MAPK has a dual function in brown adipocyte formation and mature brown adipocyte function.

[112] Among AMPKα catalytic subunits, AMPKα1 is more abundant than the AMPKα2 isoform. In mice, chronic cold exposure raises AMPKα1 concentration in BAT and epididymal WAT (epiWAT) [879]. β3-Adrenergic stimulation does not influence AMPKα1 activity in BAT, but only in epiWAT. Inhibition of AMPK reduces brown adipocyte survival and differentiation. In brown pre-adipocytes, AMPKα1 suffices for cell proliferation and differentiation, as elimination of AMPKα2 does not affect brown adipocyte formation, differentiation, and proliferation. The AMPKβ subunit influences the stability of the AMPK heterotrimer. Both AMPKβ1 and AMPKβ2 subunits are important for brown adipocyte formation [879]. The AMPKγ1 and AMPKγ2 subunits are widespread, the latter being the most abundant in the heart; AMPKγ3, which lodges in the skeletal muscle, is also present in intermediate amounts in brown pre-adipocytes.

Table 5.11 Examples of batokines and their effects (Source: [869])

Batokine	Effects
Fibroblast growth factor-21	Improved metabolism
	Cardioprotection, antihypertrophic action
Free fatty acids	Improved metabolism
Interleukin-6	Improved metabolism
	Cardioprotection (acutely), maladaptive remodeling (prolonged presence)
Nerve growth factor	Pro-survival in cardiac ischemia
	Improved cardiac function
Neuregulin-4	Decreased insulin resistance
	Cardioprotection
Triiodothyronine	Improved metabolism
	Cardioprotection

Neuregulin Nrg4 is formed in BAT activated by adrenoceptors under cold conditions. It protects against insulin resistance, hepatic steatosis, and myocardial ischemia [869].

Interleukin-6 improves insulin sensitivity, but contributes to adverse cardiac remodeling after sustained exposure [869].

Nerve growth factor may promote sympathetic innervation of BAT. It exerts a pro-survival activity in ischemic CMCs [869].

Certain types of adipocyte-specific branched fatty acids enhance the glucose effect and reduce AT inflammation in obesity [869].

MicroRNAs operate in AT formation and function. They are involved in the differentiation of white, beige, and brown adipocytes. They intervene in obesity, T2DM, and cardiovascular disease [880].

Certain microRNAs regulate brown adipocyte differentiation, such as miR26, miR34, miR133, miR155, miR378, and miR455 [880]. On the other hand, BAT activation can also regulate miR expression profiles.

The CNS integrates afferent cues that regulate food intake and energy expenditure in a coordinated manner. In rodents, UCP1 expression in BAT is controlled by changes in metabolic state related to cold exposure or changes in food intake. These stimuli distinctly control sympathetic nervous system activity, which activates adrenoceptors and UCP1 expression in brown adipocytes, its signaling to BAT increasing in hypermetabolic conditions of lean animals [881]. In ADAM17$^{-/-}$ mice, elevated UCP1 concentration in BAT is linked to increased sympathetic outflow.

Members of the MIR26 family, miR26a and miR26b, regulate human white and beige adipocyte differentiation, both types oxidizing nutrients at very high rates via nonshivering thermogenesis, their formation increasing in early adipogenesis. In adipose-derived stem cells, synthesis of mitochondrial UCP1 is upregulated, as miR26a and miR26b target ADAM17 [882].

MiR34a is an anti-adipogenic agent with a dual role in both brown and beige adipocyte formation that has increased expression in obesity [880]. In HFD-fed obese mice, its inhibition reduces adiposity and improves oxidative function in AT. In addition, its reduced expression increases formation of the BeAT -specific marker TNFRSF9 and UCP1 in WAT (vAT) and promotes additional browning in BAT. Furthermore, inhibition of miR34 raises adipocytic SIRT1 level and hence deacetylation of PGC1α, which functions in WAT browning. MiR34a targets FGFR1 [880].

MiR133 targets PRDm16, thereby repressing brown adipogenesis in white adipocyte progenitors [880].

MiR155 operates both in brown adipocyte differentiation and WAT browning [880]. It targets C/EBPβ. It lowers UCP1 and PGC1α expression

Synthesis of miR378 in BAT is induced by cold exposure; it promotes BAT (but not WAT) adipogenesis [880]. MiR378 targets PDE1B, the product of which yields cAMP and cGMP turnover.

Formation of miR455 in BAT is induced by cold temperature exposure and BMP7, which is needed for the development of both BAT and recruitable BeAT [883]. It targets brown adipogenic inhibitors, such as Runx1T1 and necdin homolog, two adipogenic transcriptional repressors, in addition to HIF1α inhibitor (HIF1an), an hydroxylase that processes (inhibits) the AMPKα1 subunit. MiR455 suppresses necdin and Runx1T1 to initiate an adipogenic program and HIF1an to activate AMPKα1, which triggers a brown adipogenic program. Therefore, miR455 induces brown and beige adipogenesis via activation of the HIF1an–AMPK–PGC1α axis [883].

The WAT, and especially BAT, release microRNAs in exosomes that enter blood circulation, thereby supporting glucose tolerance and reducing hepatic synthesis and circulating level of FGF21 [884]. The AT is an important source of circulating exosomal miRs in both mice and humans. These exosomal miRs secreted by AT can act as both para- and endocrine messengers. These circulating miRs do indeed regulate body metabolism and mRNA translation in other organs such as the liver. In particular, exosomal BAT-derived miR99b controls FGF21 activity.

Although the majority of ^{BAT}microRNAs in the bloodstream are contained in exosomes, the AT can also secrete miRs in microvesicles or are associated with Argonaute and HDLs [884].

The pvAT is endowed with a microvasculature. Insulin resistance is associated with the reduced formation of the anti-inflammatory microRNA, miR181b, in AT ECs [880]. MiR181b supports insulin-mediated PKB phosphorylation and hence reduces endothelial dysfunction. It targets Phlpp2 mRNA.[113]

[113] PHLPP2: pleckstrin homology (PH) domain and leucine-rich repeat-containing protein phosphatase. It dephosphorylates PKB (Ser473).

5.4.1.2 Beige Adipose Tissue

Beige adipocytes (brown-like or "brite," i.e., "brown in white" adipocytes) have some of the characteristics of brown adipocytes, such as abundant mitochondria and thermogenic activity They develop with WAT (*browning*) in response to various activators, such as NR1c3 (PPARγ) ligands, FGF21, irisin, cold, and β3-adrenoceptor agonists (Vol. 10, Chap. 1. Architecture and Structure of the Vasculature). Whereas β1AR recruits pre-adipocytes in WAT, β3AR provokes the emergence of beige adipocytes within WAT [868]. In humans, β1AR and β2AR may also regulate BAT activity. Upon removal of these stimuli, the beige adipocytes behave more like white adipocytes.

Mitophagy determines the identity and function of adipocytes and its repression enables maintenance of the beige adipocyte phenotype, whereas enhanced mitophagy shifts the adipocyte phenotypes from beige to white. Therefore, autophagy can be beneficial or detrimental according to the context [885].

The WAT is distributed in visceral regions, whereas scAT is a mixture of white and beige adipocytes.

Beige adipocytes may originate from multipotent pre-adipocytes located in various white adipose depots or from transdifferentiation of white adipocytes into beige adipocytes [869].

A set of markers defines brown, beige, and white adipocytes,

- LHx8[114] and ZiC1[115] being related to brown.
- TBx15[116] to brown and beige.
- HoxC9 (or Hox3b)[117] and Shox2[118] to beige.

[114]LHx8: LIM domain and homeobox-containing protein-8 (also called LHx7). This transcription factor is involved in the differentiation of certain neurons and mesenchymal cells [108]. The ZiC1 transcriptional activator is involved in neurogenesis.

[115]ZiC1: zinc finger protein of cerebellum. The ZiC proteins ZiC1 and ZiC2 launch transcriptional activation of the APOE gene [194].

[116]TBx: T-box transcription factor. TBx15 may be a transcriptional regulator involved in the development of the skeleton, which controls the number of mesenchymal precursor cells and chondrocytes [108].

[117]Hox: homeobox gene product. HoxC9 specifies cell position on the anterior–posterior axis [108].

[118]Shox: short stature homeobox gene product. It may act as a growth regulator, in processing somatosensory information, and body structure formation [108].

Table 5.12 Stimulators of BAT and BeAT mass (Source: [868]; *BDNF* brain-derived neurotrophic factor, *BMP* bone morphogenetic protein, *DBC1* deleted in bladder cancer protein-1 [Fam5a], *TBx* T-box protein, *VEGF* vascular endothelial growth factor)

Agent	Main function
Increases BAT mass and browns WAT	
FGF21	Regulation of fasting substrate metabolism
Cardiac natriuretic peptides	Regulation of sodium homeostasis
Tbx15	Transcription
Glucagon-like peptide	Regulation of postprandial glucose metabolism
PRDm16	Bidirectional switch between myogenesis and brown adipogenesis
Increases BAT mass only	
BMP7	Pleiotropic actions
Myostatin	Inhibition of myogenesis
Twist-1	Organogenesis, apoptosis
Browns WAT only	
Irisin	Myokine stimulated by exercise
BDNF	Neurotrophin (neuronal survival and axonogenesis
Cyclooxygenase-2 (PGhS2)	Rate-limiting enzyme in prostaglandin synthesis
VEGFa	Angiogenesis
Sirtuin-1	Deacetylation
DBC1	Sirtuin-1 inhibition
Increases BAT activity only	
BMP8b	Pleiotropic activity

- HoxC8 (or Hox3a), Inhbb,[119] and Dpt[120] for beige and white.
- TcF21 (bHLHa23) to white adipocytes [868].

Many factors increase BAT and/or BeAT mass (Table 5.12) [868].

MicroRNAs regulate BeAT differentiation, that is, WAT browning [880]. Let7i-5p causes differentiation of white adipocytes to a beige type, as it prevents UCP1

[119]Inhβ b (or Inhβ2): inhibin-β b. The activins, which are dimers of β A or β B subunits encoded by the genes INHBA and INHBB, respectively, are TGFβ superfamily members implicated in reproduction and development. Inhibins and activins inhibit and activate the secretion of follitropin by the pituitary gland, respectively. They are also involved in regulating hypothalamic, pituitary, and gonadal hormone secretion, germ cell development and maturation, erythroid differentiation, insulin secretion, nerve cell survival, embryonic axial development, and bone growth, according to their subunit composition [108]. Activin ligands act as growth and differentiation factors in many cell types [194]. Activins are composed of β subunits only. Activin-β C can dimerize with activin-β A and -β B (activin-AC and-BC heterodimers), but not with inhibin-α. Inhibins are heterodimeric glycoproteins consisting of an α and β A or β B subunit. Inhibin- and activin-β B subunit is expressed in adrenal medullary cells.

[120]DPt: dermatopontin. It is also called tyrosine-rich acidic matrix protein. It may mediate cell adhesion via integrin. It enhances TGFβ1 activity, hampers cell proliferation, accelerates collagen fibril formation, and stabilizes collagen fibrils.

expression. Its overexpression in scWAT alters the formation and function of brite adipocytes via partial inhibition of Gs-coupled β3-adrenoceptor signaling of browning via the AC–cAMP–PKA–P38MAPK–PGC1α/ATF2–UCP1 pathway.

MiR30b and miR30c control not only BAT function and hence energy homeostasis but also beige adipogenesis [880]. Their production rises in response to cold exposure in addition to stimulated β3AR signaling. They target the nuclear corepressor NRIP1.[121]

In scWAT and interscapular BAT, β3AR stimulation attenuates miR125b-5p expression. Overexpression of miR125-5p precludes beige adipogenesis in WAT.

5.4.1.3 White Adipose Tissue

The WAT is devoted to energy storage, fatty acids being exported for oxidation in other organs when energy is required during periods of food restriction or great exertion. The size of the WAT heightens when the energy balance is positive and declines when energy expenditure is in excess of intake. It receives a modest blood supply. Furthermore, WAT represents an endocrine tissue that secretes adipokines, which control eating behavior and insulin sensitivity, among other tasks. Brown adipokines can have more distinct effects than white adipokines [886].

The WAT is composed of white adipocytes ($\leq$75%), fibroblasts, ECs, pericytes, macrophages, and pre-adipocytes, among other cell types. New white adipocytes are formed from a pool of precursors.

White adipocytes store energy, whereas brown adipocytes dissipate energy, converting chemical energy into heat for thermoregulation. White adipocytes contain a single large LD (unilocular adipocytes) and few mitochondria.

Certain hormones (e.g., adrenaline, NAd, glucagon, and adrenocorticotropin) bind to their receptors on adipocytes and trigger lipolysis by activated lipases, that is, the hydrolysis of triacylglycerol into fatty acids and glycerol. The pair of enzymes, hormone-sensitive (LipE) and monoacylglycerol (monoglyceride) lipase (MGL) convert diacylglycerol into fatty acids and glycerol [887]. Hormone-sensitive lipase is inhibited by insulin and activated by glucagon and adrenaline. Glycerol is exported from adipocytes via an aquaporin-type transporter and conveyed to the liver for oxidation or gluconeogenesis.

Various regulators of lipid and lipoprotein metabolism are released from white adipocytes. Lipoprotein lipase secreted by adipocytes processes circulating triacylglycerols into chylomicrons and VLDLs to fatty acids. Other secreted proteins from WAT involved in lipid and lipoprotein metabolism include cholesteryl ester transfer protein and apolipoprotein-E [888].

The WAT also contributes to glucose homeostasis. Lipodystrophic mice, which possess only a small quantity of adipose depots, are diabetic; they exhibit both hyperglycemia and marked hyperinsulinemia [888].

[121] NRIP: nuclear receptor-interacting protein.

The WAT participates in inflammation, some adipokines being inflammatory cytokines, whereas others are involved in the complement system [888]. Adipsin, a serine peptidase, is a constituent of the alternative complement pathway, which is also named complement factor-D; it is secreted from white adipocytes. Acylation-stimulating protein (ASP or $C3a^{desArg}$; Sect. 5.4.5.21) is synthesized by WAT, which stimulates TG synthesis and glucose transport in adipocytes. In humans, its concentration rises in obesity, T2DM, and CVD, whereas exercise or weight loss lowers it.

The WAT also stores cholesterol and is involved in the metabolism of steroid hormones. It does not synthesize steroid hormones, but it does express enzymes converting both glucocorticoids and sex hormones (Sects. 5.4.5.14 and 5.4.5.15) [888].

Adipokines can serve as auto-, para-, and endocrine messengers, which serve in metabolism and remote regulation (Sect. 5.4.5; Table 5.13)). vAT and scAT are characterized by a unique adipokine production pattern. The vAT synthesizes higher amounts of IL6 and PAI1, whereas the scAT produces larger quantities of adiponectin and leptin [887]. The leptin synthesis rate is indeed greater in scAT than in vAT.

The amount of microRNAs synthesized by dicer in white adipose depots declines with aging and in humans with congenital generalized and HIV-associated lipodystrophy [884].

Lipodystrophies are acquired or genetic diseases with various degrees of AT deficiency and hence frequently altered adipokine and cytokine profiles (very low adiponectinemia and leptinemia), which causes ectopic TG accumulation, such as in the skeletal muscle and liver, and reduces insulin sensitivity [717]. Defective AT storage engenders elevated circulating concentrations of lipid metabolites, cholesterol, and TGs. They are often accompanied by dyslipidemia, insulin resistance, and CVD, especially in congenital generalized lipodystrophy (CGLD) patients.

Type-1 to -4 *CGLDs* are autosomal recessive disorders caused by mutations in the AGPAT2 (CGLD1),[122] Bscl2 (CGLD2),[123] CAV1 (CGLD3),[124] and CAVIN1

[122] AGPAT2: acylglycerol 3-phosphate acyltransferase-2 (or lysophosphatidic acid acyltransferase-β). AGPAT2 affects adipocyte differentiation via PKB and PPARγ [717]. It catalyzes the second step of phospholipid synthesis. It operates in TG and glycerophospholipid formation from glycerol 3-phosphate. Mutations in the AGPAT2 gene cause defective TG storage and accumulation of intermediates such as lysophosphatidic acid. Increased LPA concentration hampers adipogenesis and AT expansion and increases AT fibrosis in obesity.

[123] BSCL2: type-2 Berardinelli–Seip congenital lipodystrophy (LD genesis-associated seipin). Congenital generalized lipodystrophy is also dubbed Berardinelli–Seip congenital lipodystrophy. Mutations in the Bscl2 gene cause type-2 CGLD (CGLD2).

[124] Cav: caveolin, a major component of caveolae involved in lipid regulation and endocytosis.

Table 5.13 Adipokines secreted by the AT into the bloodstream and their effects (Sources: [826, 887]; *ADRF* adipocyte-derived relaxing factor, *ASP* acylation-stimulating protein, *FFA* free fatty acid, *IGF* insulin-like growth factor, *PAI* plasminogen activator inhibitor, *TNFSF* tumor-necrosis factor superfamily member [TNFSF1 being TNFα], *VEGF* vascular endothelial growth factor)

Adipokine	Effects
Adiponectin	Protection against inflammation, T2DM and CVD
	Vasodilation
Adipsin	Complement pathway
ADRF	Vasodilation
Aldosterone	Vasodilation and vasoconstriction according to dose and exposure duration
	Inflammation, insulin resistance, hypertension
Angiotensinogen Angiotensin-2	Regulation of blood pressure and electrolyte homeostasis Vasoconstriction
Apelin	Vasodilation and vasoconstriction
ASP	Influences the TG synthesis rate in AT
H_2O_2	Vasodilation and vasoconstriction
IGF1	Cell proliferation
Interleukin-6	Inflammation, lipid and glucose metabolism, regulation of body weight
Leptin	Regulation of appetite and energy expenditure
	Inflammation
	Vasodilation and vasoconstriction
Omentin	Protection against inflammation, vasodilation
Osteopontin	Inflammation
Resistin	Insulin resistance, vasoconstriction
Serpin-E1 (PAI1)	Inhibition of plasminogen
TNFSF1	Affects insulin receptor signaling
	Vasoconstriction and -dilation
	Inflammation
VEGF	Angiogenesis
FFA	Energy source
Glycerol	Gluconeogenic precursor

(PTRF) genes (CGLD4),[125] which encode lysophosphatidic acid acyltransferase-β, seipin, caveolin-1, and cavin-1.[126]

Partial lipodystrophies include *familial partial lipodystrophy* (FPLD). It engenders partial AT loss, mainly from the subcutaneous depots in the extremities

[125] PTRF: polymerase-1 and transcript release factor, also labeled cavin-1. Cavin stands for caveola-associated protein.

[126] The three last-mentioned genes encode proteins involved in vesicular transfer and affect the formation or maturation of LDs.

and upper trunk region.[127] and acquired forms, most commonly occurring in patients with human immunodeficiency virus (HIV) receiving highly active antiretroviral therapy with protease inhibitors that reduce the levels of factors involved in adipocyte differentiation and function (e.g., ATF4, C/EBPα, DDIT3, PPARγ, and XBP1), and raises ROS concentration [717].

Congenital generalized lipodystrophy is characterized by a generalized loss of AT.[128] HIV-associated lipodystrophy is associated with a decreased dicer concentration in AT. MiR16, miR201, miR221, and miR222 are highly expressed in fat depots. Defective miR processing in AT by dicer provokes whitening of BAT, InsRce, and altered circulating lipid levels.

5.4.1.4 Perivascular Adipose Tissue

The pvAT surrounds large arteries and veins, except in the cerebral circulation. It is accompanied by lymphatics and nerves. The pVAT has a unique embryonical origin from Tagln+ precursors of vSMCs.[129]

It is an intermediate between BAT and WAT, that is, beige AT (BeAT) in rodents, although it remains mainly a WAT in larger mammals (rabbits, pigs, and humans) [805]. It is structurally and functionally heterogeneous, not only among mammalian species but also according to its location, i.e., the vascular bed. It is separated from the vascular wall of large arteries, but is integrated into the wall of smaller vessels [889]. The pvAT surrounding large arteries contains its own vasculature, the vasa vasorum. It has beige or white AT-like characteristics in proximal and distal arteries, respectively. Its thermogenic function varies according to the vascular compartment. The pvAT can change its phenotype from WAT to BeAT and conversely, according to exposure to temperature and the nutritional status [805].

The pvAT interferes with the surrounding vascular wall via paracrine and endocrine effects, as pvAT-derived adipokines can reach the vascular lumen, hence the so-called *vasocrine signaling*. For example, the pvAT facilitates insulin effects and glucose uptake in lean (but not obese) mouse skeletal muscles [889].

The coronary vasa vasorum may convey harmful adipokines between pvAT and the vascular wall. The increased density of the coronary vasa vasorum precedes coronary endothelial dysfunction [890]. Adipokines, such as leptin, resistin,

[127] In FPLD3, the PPARG gene, which encodes PPARγ, is mutated. mutations in the Pkb2 (AKT2) and PLIN1 genes impair adipocyte function and also cause FPLD. In FPLD2, mutations in the LMNA gene, which encodes lamin-A/C, weaken the nuclear envelope and cause cell death.

[128] Despite the quasi-complete lack of metabolically active storage AT, mechanical fat in joints, orbits, palms, and soles is present [717].

[129] Tagln: transgelin. This actin crosslinker (gelling protein) is involved in calcium signaling. It is also called 22-kDa smooth muscle protein SM22α, whereas transgelin-2 is named SM22α homolog.

Table 5.14 Perivascular and epicardial adiposecretome, obesity, and atherosclerosis (Sources: [890, 891] *icam1*^{S} soluble intercellular adhesion molecule-1, *IL* interleukin, *MIF* macrophage migration inhibitory factor, *NPR* natriuretic peptide receptor, *PAI* plasminogen activator inhibitor, *PGC* peroxisome proliferator-activated receptorγ coactivator, *sPLA2* secretory phospholipase-A2, *TNFSF* tumor-necrosis factor superfamily member)

Augmented synthesis and secretion	Chemerin, leptin, resistin, visfatin, adrenomedullin, angiotensinogen, TNFSF1, IL1β, IL1Rα, IL6, IL13, IL16, CCL2, CCL5, NPRc, sPLA2, PAI1, icam1^{S}, PGC1α
Decreased synthesis	Adiponectin, adipsin, omentin, MIF

Table 5.15 Processes in which adipokines are involved (Source: [892]; *ADRF* adipocyte-derived relaxing factor, *HGF* hepatocyte growth factor, *IL* interleukin, *PAI* plasminogen activator inhibitor, *ROS* reactive oxygen species, *TNFSF* tumor-necrosis factor superfamily member, *VEGF* vascular endothelial growth factor)

Process	Involved adipokines
Glucose metabolism	Adiponectin, resistin
Lipid metabolism	Retinol-binding protein, cholesteryl ester transfer protein
Angiogenesis	Leptin, VEGF, HGF
Vasodilation	Adiponectin, ADRF, omentin, visfatin
Vasoconstriction	Resistin, angiotensin-2
Vasodilation or -constriction	Apelin, leptin, TNFSF1, IL6, ROS
Immunity	Adipsin
Inflammation	TNFSF1, IL6
Blood coagulation	PAI1
Pancreatic β-cell function	Adiponectin, visfatin, IL6
Feeding behavior	Leptin
Maintenance of reproduction	Leptin, ghrelin

TNFSF1, and IL6, augment ROS formation directly or indirectly via production of endothelin-1 and angiotensin-2 (Table 5.14).

The pvAT operates in various processes, such as angiogenesis, mesenchymal stem cell recruitment, vSMC proliferation, vEC function, vasomotor tone control, and CMC electrochemical activity (Table 5.15) [890].

Regulation of the Vasomotor Tone

Endothelial regulators of the vasomotor tone include the vasodilating gases (e.g., NO), oxygen-derived free radicals (e.g., superoxide [$O_2^{\bullet -}$] and hydroxyl radical [$OH^{\bullet}$]), and peptides (e.g., endothelins and angiotensins), contractant and relaxant

eicosanoids comprising prostanoids (prostaglandins, prostacyclins, and thromboxanes), in addition to EDHF. Among peptides, whereas endothelin-1 and angiotensin-2 cause vasoconstriction, endothelium-derived neuropeptide-Y (NPy)[130] and ANP prime vasodilation.

These regulators are also synthesized in vSMCs, inflammatory leukocytes, mesangiocytes, and adipocytes [779]. Vasoconstrictors include vasocontracting prostanoids[131] and superoxide anion,[132] in addition to angiotensin-2[133] and endothelins (ET1–ET3).[134]

Obese patients have an elevated sympathetic signaling and vasomotor tone, that is, arteries present a stronger vasoconstriction than that observed in normal conditions.

Sensory nerves comprising C and Aδ neural fibers surround vascular walls. They contain and release peptides, among which substance-P and calcitonin gene-related peptide (CGRP) are the principal vasodilators [893]. Substance-P is an endothelial-dependent vasodilator, which also increases microvascular permeability, whereas CGRP operates as both endothelial-dependent and -independent vasodilator. However, sensory nerves are linked to the sympathetic and angiotensin signaling and hence substance-P and CGRP do not affect blood pressure at rest in normal conditions.

Sensory nerves also localize to the pvAT, especially around small mesenteric resistance arteries, and cause vasodilation, despite their interaction with the sympathetic system [893, 894]. Stimulants of the pvAT sensory nerves (e.g., TRPV1 agonists) are thus vasodilators. Among TRPV1 agonists, capsaicin releases CGRP from sensory nerves [894]. Leptin can also be released to potentiate CGRP-mediated pvAT-derived sensory neurogenic vasodilation, leptin release being abolished by hypoxia [894]. Leptin receptors reside on sensory neurons. Deletion of leptin signaling in vagal afferent neurons causes hyperphagia and obesity. In WAT, leptin secreted from adipocytes increases the activity of innervating sensory nerves.

However, other neurotransmitters can regulate the vasomotor tone. Angiotensin-2 secreted by the pvAT augments sympathetic signal transmission in rat mesenteric arteries, which inhibits CGRP release from sensory nerves in this arterial bed [894].

[130] Neuropeptide-Y is involved in appetite regulation and stimulates adipogenesis [779]. It binds to its receptor on ECs, priming NO-dependent vasodilation, stimulating EC proliferation, and affecting endothelial permeability. It also strengthens thromboxane-mediated vasoconstriction.

[131] Prostaglandin-H synthase PGhS1 (or cyclo-oxygenase COx1) is the sole enzyme forming vasocontracting prostanoids, at least in mice [779]. Aging and obesity are associated with PGhS activation.

[132] This short-lived by-product of oxidative metabolism is mainly produced by vascular NAD(P)H oxidase and cytochrome-P450 epoxygenase, in addition to uncoupled NOS [779]. Superoxide inactivates NO via formation of peroxynitrite.

[133] Obesity is also associated with activation of the RAA [779].

[134] Endothelin and the RAA interact, constituting a positive feedback loop, especially in obesity [779].

Table 5.16 Adipokines regulating the vasomotor tone (*ADRF* adipocyte-derived relaxing factor, *Agt* angiotensin, *APPL* adaptor containing phosphoTyr interaction, PH, and Leu zipper domain, *H_2S* hydrogen sulfide, *NO* nitric oxide, *PGi_2* prostaglandin-I_2 [prostacyclin])

Target cell	Messengers (effectors)
EC	ADRF (via BK, IK, SK)
SMC	ADRF (via K_V, K_{ATP}, BK)
	H_2O_2 (via sGC and Src–MAPK axis)
	Adiponectin
	NO (using the PI3K–PKB–NOS3 and APPL1–AMPK–NOS3 axes)
	H_2S
	$Agt_{(1–7)}$ (via K_V)
	PGi_2

Superoxide ions can be generated by pvAT promote sensory neurogenic relaxation in the rat isolated mesenteric arterial bed.

In lean individuals, pvAT is a paracrine regulator of the vasomotor tone and vasoprotector. It does indeed counter vasoconstriction, especially in the microvasculature.[135] This anticontractile property is lost in obese subjects [896, 897].

The pvAT secretes adventitial vasodilators (adiponectin, adipocyte-derived relaxing factor [ADRF], $angiotensin_{(1–7)}$, hydrogen peroxide, hydrogen sulfide, nitric oxide, palmitic methyl ester, and prostacyclin; Table 5.16). These ^{pvAT}vasodilators are counteracted by vasoconstrictors, such as angiotensin-2, superoxide anion, and TNFSF1.

The pvAT operates via endothelium-dependent and -independent mechanisms. An endothelium-independent process relies on hydrogen peroxide and subsequent activation of soluble guanylate cyclase. At low concentrations (10–100 µmol/l) of H_2O_2, pre-constricted mesenteric vessels undergo further constriction, but higher concentrations (0.3–1 mmol/l) provoke a biphasic response, with an early constriction followed by dilation [891]. Hydrogen peroxide released from both adipocytes and macrophages can act via the Raf–ERK pathway in vascular smooth myocytes.

Adipocyte-derived relaxing factor may hyperpolarize ECs. This hyperpolarization is then transmitted to adjacent smooth myocytes via myoendothelial junctions, thereby relaxing the arterial and arteriolar wall. Release of ADRF by pvAT relies on Ca^{2+} ion [866].

ADRF is linked to vascular potassium channels on vascular ECs and smooth myocytes (K_V, possibly K_V7 [866],[136] K_{ATP},[137] $BK_{V,Ca}$, IK_{Ca}, and SK_{Ca}), the types of which depend on the vascular compartment and mammalian species [891]. In human internal mammary arteries, it acts via BK channels. Stimulated

[135] Microvascular changes are rare in obesity before the development of hyperglycemia and T2DM [895].

[136] The K_V7 channel is targeted by vasopressin in aortic smooth myocytes [866].

[137] The K_{ATP} channel at most plays a minor role [866].

β3-adrenoceptors also operate via ^{SMC}BK channels.[138] The effect of ADRF may also be mediated by hydrogen sulfide, a gasotransmitter generated in the pvAT [866, 892].

In addition to ADRF, adipokines such as adiponectin, omentin, and visfatin are vasodilators. On the other hand, angiotensin-2 and resistin are vasoconstrictors released by adipocytes. Apelin, leptin, ROS, TNFSF1, and IL6 have both vasorelaxing and -contracting effects [892].

Plasmalemmal hyperpolarization linked to a product of arachidonic acid metabolism by phospholipase-A2 and cytochrome-P450 is the major contributor to endothelium-dependent vasodilation in human subcutaneous resistance arteries [898]. In these arteries, EDHF is thus a more important vasodilator than NO and PGi_2.

In humans, small arteries and arterioles of the scAT are endowed with a myogenic tone, an increase in pressure triggering the PLC–DAG–PKC pathway [895]. In resistance arteries and arterioles of gluteal scAT from healthy volunteers, vasodilation caused by histamine, which targets myocytic H_2 and endothelial H_1 receptors, provokes endothelial NO release.

In several vascular beds, nitric oxide- and prostacyclin-independent vasodilation is linked to activation of cytochrome-P450 epoxygenases in ECs, which generate vasodilatory EETs, but also oxygen-derived free radicals that lower NO availability [899]. CYP2c9 prevents endothelium-dependent NO-mediated enhancement in forearm blood flow in healthy volunteers and CoAD patients.

Acetylcholine operates not only via NO but also in small arteries and arterioles of obese rodents via EDHF, that is, via K_{Ca} channels [895]. In healthy individuals, acetylcholine primes SMC hyperpolarization. In obesity, EDHF can compensate for impaired NO synthesis. Moreover, in scAT arteries of healthy subjects, insulin can reduce NAd-induced vasoconstriction [895].

Venous pvAT releases angiotensin$_{(1–7)}$ which activates K_V channels and relaxes vSMCs using NO [891].

The pvAT counters vasoconstriction caused of thromboxane TxA_2 and its stable metabolite TxB_2, which prime perioperative spasm, via ADRF and BK [891].

On the other hand, the pvAT enhances vasoconstriction induced by α1-adrenoceptors stimulated by the perivascular sympathetic nerves, as they prime superoxide generation and activation of Src–MAPK pathway. In addition, angiotensin-2 derived from adipocytes potentiates vasoconstriction.

The pvAT undergoes browning upon β3AR agonist exposure. In healthy organisms, pvBeAT regulates the vasomotor tone via autacoids.

The final determinant of the vasomotor tone is the phosphorylation status of myosin light chain MLC20, which is regulated by myosin light chain kinase and phosphatase, MLCK being stimulated by elevated cytosolic Ca^{2+} concentration

[138] Obesity is characterized by an overactive sympathetic nervous system, which releases NAd at nerve terminals in pvAT. NAd can bind to β3-adrenoceptors, albeit with a lower affinity than to β1- and β2-adrenoceptors [891].

and MLCP being inhibited by the $PP1_{r12a}$ subunit. Classically, PKG1 activated by cGMP decreases cytosolic Ca^{2+} content and phosphorylates BK channel and RhoA, thereby reducing rock activity and $PP1_{r12a}$ phosphorylation.

The mesenteric pvAT around small resistance arteries is WAT; its anti-contractile effect can result from vascular potassium channels, such as K_{ATP} and K_V channels (e.g., K_V7 [889]), in addition to messengers, such as H_2S, NO, PGi_2, and $ATn_{(1–7)}$ [897].

The aortic pvAT, a BeAT around a large highly distensible artery, also has anti-contractile and -inflammatory properties. It secretes H_2O_2, which is predominantly synthesized by NOx4, the major isoform in adipocytes, without affecting NOS3 activity [897]. Whereas NOx4 produces mainly H_2O_2, the other Nox isoforms synthesize detrimental superoxide.

Action of epididymal visceral (evWAT), interscapular (isBAT), inguinal subcutaneous (mixed WAT–BAT iscW/BAT), and pvAT (mixed WAT–BeAT pvW/BeAT) on arteries was investigated in WT and $NOX4^{-/-}$ mice [897]. In $NOX4^{-/-}$ mice, isBAT releases a reduced H_2O_2 amount and does not exert an anti-contractile effect; BAT from WT mice mediates an anti-contractile action on vessels of $NOX4^{-/-}$ mice, the perivascular H_2O_2 production being involved rather than that from local medial smooth myocytes. In BAT and BeAT (but not WAT), hydrogen peroxide activates PKA and mainly PKG1α via oxidant-induced dimerization in vSMCs, subsequently phosphorylating BK, which is implicated in the anti-contractile effect of BAT, and reduces phosphorylation of $PP1_{r12a}$ and MLC20 [897].

The pvAT, in particular, epicardial pericoronary adipose tissue, which is characterized by high rates of lipid deposition and lipolysis and high UCP1 expression, expands with obesity and diabetes [869]. A transition of pvAT happens from a protective form in lean healthy subjects to a damaging type in obese and diabetic individuals, pvAT containing more inflammatory cells (e.g., macrophages) and secreting fewer anti-inflammatory substances (e.g., adiponectin) and more proinflammatory adipokines (e.g., angiotensinogen, leptin, resistin, TNFSF1, IL6, chemokines CCL2 and CXCL8, and reactive oxygen and nitrogen species).

In WT mice, 6-month high-sucrose, high-fat diet (HFHSD) raises body weight, fasting glycemia, postprandial insulinemia, and ornithinemia, and causes hypertension and arterial stiffening and fibrosis, but lowers argininemia [900]. *Arginase*-1 produced by vascular ECs is involved in obesity-induced vasculopathies via redox stress and declined availability in arginine and nitric oxide. In HFHSD-fed mice in addition to isolated vessels immersed in palmitate- and glucose-enriched media, vascular arginase activity rises and is coupled with redox stress. In mice, vascular dysfunction in DIO can be countered by arginase-1 inhibition and deletion of the ARG gene in vascular ECs ($^{EC}ARG1^{-/-}$).

Elevated arginase activity can cause depletion in arginine and subsequently in NO, hence impairing endothelial-dependent vasodilation. Arginase, a urea cycle enzyme, catalyzes hydrolysis of ^{L}arginine to urea and ornithine. It thus competes with nitric oxide synthase for their common substrate, ^{L}arginine. Ornithine is processed into polyamines by ornithine decarboxylase and proline by ornithine aminotransferase. Arginase has two isozymes; arginase-1 localizes to the cytoplasm

Table 5.17 Regulators of vascular smooth myocyte (vSMC) proliferation (Source: [901]; *Agt* angiotensin, *C3a*^{desArg} desarginated complement factor C3 fragment [or acylation-stimulating protein], *CCL* chemokine CC-motif ligand, *FFA* free fatty acid, H_2S hydrogen sulfide, *HBEGF* heparin-binding EGF-like growth factor, *IL* interleukin, *MIF* macrophage migration-inhibitory factor, *NO* nitric oxide, *PAI* plasminogen activator inhibitor, *ROS* reactive oxygen species)

Stimulators	C3a^{desArg}, CCL2, CCL3, HBEGF, IL1β, IL6, leptin, MIF, PAI1, resistin, ROS, visfatin
Inhibitors	Adiponectin, $Agt_{(1–7)}$, androgens, glucocorticoids, H_2S, IL10
Dual regulators	Adrenomedullin, angiotensin-2, estrogens, FFAs, NO

and abounds in the liver; mitochondrial arginase-2 is the primary isoform in the kidney. Both isozymes lodge in vascular endothelial and smooth muscle cells; their synthesis can be upregulated by glucose and ROS [900]. Excessive arginase activity can also shift arginine metabolism to the production of ornithine, polyamines, and proline, polyamines promoting vascular smooth myocyte proliferation and proline collagen formation, thereby leading to perivascular fibrosis.

Vascular Smooth Myocyte Proliferation

The pvAT releases tissue growth stimulators and inhibitors that operate on vSMCs (Table 5.17).

Decreased concentration of adiponectin and increased concentration of TNFSF1 in PVAT favor intimal hyperplasia after endovascular injury, which relies on abnormal proliferation and migration of medial SMCs [901].

Visfatin secreted by the pvAT is a growth factor for vSMCs. It stimulates vSMC proliferation via ERK1, ERK2, and P38MAPK rather than JNK and the PI3K–PKB axis [901]. In addition, it can counter vSMC apoptosis induced by H_2O_2 at nonphysiological concentrations.

Pathophysiological Role

Obesity is associated with a reduced dilatory capacity of both conduit and resistance arteries. Hyperglycemia and hypertension as well as insulin resistance and obesity cause redox stress, which decreases NO availability and hence acetylcholine- and histamine-induced NO-mediated dilations of skeletal muscle arterioles [895].

In a pathological context, pvWAT is subjected to redox stress and inflammation. Factors derived from pvAT then provoke endothelial dysfunction and vascular remodeling with vSMC proliferation.

Dysregulated synthesis and secretion of adipokines associated with immunocyte infiltration in the AT is linked to fat depot inflammation. Dysfunctional AT synthesizes harmful proinflammatory adipokines (e.g., leptin) rather than vasodilatory adipokines [892].

Dyslipidemia, insulin resistance, and hyperinsulinemia impair the vasomotor tone control in arteries [895]. Perfusion of the myocardium and skeletal muscles is altered in obese subjects, primarily because of the vasomotor dysfunction of resistance arteries. Hyperemia-induced forearm blood flow during exercise, local heating, or upon acetylcholine stimulation falls, even in obese children [895]. In pre-pubertal boys without insulin resistance, flow-mediated dilation in the brachial artery drops. In the mesenteric and skeletal muscle microcirculation, vasodilation mediated by the endothelium and induced by acetylcholine and sodium nitroprusside (SNP) via NO declines,[139] increasing peripheral vascular resistance in obese people [895].

On the other hand, vasoconstriction by endothelin-1 in the forearm circulation of overweight and obese individuals is potentiated with respect to lean subjects [720].

Constriction of scAT arterioles engendered by NAd is not affected in obesity, but dilation induced by acetylcholine is impaired [895]. Arteriolar dilation mediated by EDHF, which is linked to BK, SK, and IK channels, may be less sensitive to redox stress than that induced by NO [895].

Insufficient AT perfusion increases formation of hypoxia inducible factor, HIF1α in adipocytes, which launches synthesis of several proinflammatory adipokines (TNFSF1, IL6, and CCL2) [895]. On the other hand, hypoxia promotes angiogenesis, as it triggers synthesis of angiogenic adipokines (e.g., VEGF, HGF, FGF2, leptin, PAI1, and IL6) [892]. Under hypoxia in obese subjects, the pvAT elicits formation of the vasa vasorum [889]. The vasa vasorum conveys macrophages from the systemic circulation to the pvAT.

Inflammatory AT deregulates its effect on the vasomotor tone and recruits monocytes and induces local insulin resistance, vascular remodeling, and endothelial dysfunction [892].

The pvAT can not only affect endothelial function, decreasing NO availability and hence endothelium-dependent vasodilation and impairing the ability of the endothelium to respond to circulating messengers, but also the redox state, and can prime vascular wall inflammation. Conversely, endovascular injury engenders rapidly an overexpression of proinflammatory adipokines within the pvAT [890].

Several types of adipokines at pathophysiologically relevant concentrations alter endothelial function. Leptin, resistin, and TNFSF1 diminish endothelial NO production [890]. On the other hand, the pvAT exaggerates vasoconstriction in response to stimulation by the perivascular nerves via angiotensin-2 and increased superoxide production by NOx. In vSMCs of HFD-fed mice, overexpression and deletion of P22PhOx, a subunit of NOx1, NOx2, and NOx4, worsen the obesity state [889].

[139] Sodium nitroprusside, which is processed into NO, is a vasodilator of arterioles and venules.

In metabolic syndrome, the pvAT exacerbates endothelial dysfunction via leptin and PKCβ in aortas, coronary, and mesenteric arteries [890]. Leptin mediates endothelial-dependent vasodilation via NO, but only at pharmacological concentrations (>160 ng/ml), whereas at relevant obese concentrations (<90 ng/ml), it impairs endothelial function [890]. Furthermore, leptin has a sympatho-excitatory effect [891].

In mouse peri-aortic AT (paoAT), endovascular injury downregulates expression of the ADIPOQ gene, which encodes adiponectin. In humans, expression of the ADIPOQ gene in AT is downregulated by low-grade inflammation in the absence of patent CoAD, but is upregulated in advanced CoAD stages (*adiponectin paradox*); in other words, decreased and increased adiponectinemia predict the early onset of inflammation in the cardiovascular system and adverse cardiovascular outcomes in patients with advanced lesions [889]. Adiponectin release regulation depends on the state of the cardiovascular apparatus. Elevated adiponectinemia is associated with reduced vascular redox stress and better endothelial function in CoAD patients. However, adiponectin release from pvAT and eAT is associated with greater superoxide generation in neighboring vessels and the myocardium. In humans with increased vascular redox stress, products of peroxidation produced in vascular walls that are transferred to the pvAT and eAT trigger adiponectin expression in pvAT via PPARγ (NR1c3). In addition, BNP stimulates PPARγ in the AT, thereby increasing adiponectin secretion.

Adiponectin secreted by pvAT or remotely by other adipose depots plays an antioxidant role in the vasculature, as it impedes activity of NOxs [889]. It also stimulates NOS3 activity via AMPK and PKB, thereby increasing NO production.

Adipokines are implicated in adverse vascular wall remodeling. Inflammation of the pvAT is associated with maladaptive hypertrophy in pvAT-enclosed resistance arteries of obese mice with metabolic syndrome [890]. Complement component C3 derived from pvAT stimulates adventitial fibroblast migration and differentiation via JNK in hypertensive rats. Leptin and visfatin can trigger vSMC proliferation. Endovascular injury causes pvAT inflammation and intimal hyperplasia.

In 16-week HFD-fed $Lrp1^{+/+}$ and $^{AdC}Lrp1^{-/-}$ mice, the pvAT participates in atherogenesis. Adipocytes specifically depleted of LRP1 are smaller, but pvAT inflammation is more pronounced [902]. Therefore, pvAT signals through the adventitia to the intima contribute to atherosclerosis progression linked to hypercholesterolemia.

A group of lipid-binding adipokines includes FABP4, RBP4, and lipocalin-2 (Sects. 5.4.5.20, 5.4.5.11, and 5.4.5.8, respectively),[140] the production of which is upregulated in obese humans [831]. Small lipid-binding proteins (SLBPs) promote cellular transfer of hydrophobic ligands and facilitate their subsequent metabolism

[140] Plasmatic concentration of lipocalin-2 is related to augmented production in the AT and liver. It correlates positively with adiposity parameters, systolic blood pressure, and concentrations of fasting glucose, insulin, TGs, and CRP [831]. It can represent an independent risk factor for insulin resistance and hyperglycemia in obese individuals.

or sequestrate them. These ligands include saturated and unsaturated long-chain fatty acids (lcFAs) and eicosanoids that are involved in energy metabolism and inflammation. The lipid chaperones FABP4 and lipocalin-2 favor lipotoxicity in ECs, engendering endothelial dysfunction in obesity.

5.4.1.5 Epicardial Adipose Tissue

The pericardial adipose tissue and eAT, a visceral thoracic fat depot, lies on the surface of the heart, especially the ventricles, and between the myocardium and visceral pericardium [889]. The epicardial perivascular AT (epvAT) surrounds the coronary arteries.

This natural adipose depot expands with obesity. The severity of atherosclerotic lesions, which predominantly lodge in epicardial coronary arteries sheathed in pvAT, is linked to the volume of epvAT, especially fat depot with macrophage infiltration and augmented synthesis of adipokines (chemerin, leptin, resistin, visfatin, TNFSF1, IL6, and IL16) [890]. Epi- and pericardial adipose depot volume represents a risk marker for coronary artery disease.

The eAT controls cardiac metabolism in health and disease [889]. Free fatty acids are a major source of energy in the myocardium, the supply of which can be regulated to avoid FFA overload.

In humans, the pericoronary adipocytes have reduced differentiation, a more irregular shape, and a smaller size than perirenal adipocytes, and contains smaller LDs and higher synthetic capacity [802].

In humans, epvAT is characterized by the augmented expression of the brown adipocyte markers uncoupling protein UCP1, the transcriptional coregulator PR domain, zinc finger-containing protein PRDm16 (KMT8f), and the peroxisome proliferator-activated receptor-γ coactivator PGC1α [890].

Changes in eAT size correlate with coronary artery disease. Pericoronary AT (pceAT) is characterized by augmented macrophage polarization to the M1 phenotype with respect to other eAT regions [889].

Certain adipokines, *fibrokines*, such as activin-A and matrix metalloproteinase, MMP8, favor myocardial fibrosis.

5.4.2 Adipogenesis: A Brief Overview

Brown and white adipocytes originate from MSCs, which can also differentiate into osteoblasts, chondrocytes, and myoblasts.[141] Adipogenesis comprises two stages, commitment and differentiation [868]. During mesenchymal stem cell commitment,

[141] Tripotent stem cells that contain homeobox transcription factor engrailed-1 engender cells of the BAT, dermis, and skeletal muscle [868]. Inhibition of myostatin, an inhibitor of myogenesis,

pre-adipocytes are engendered and their pool can expand; they then differentiate into mature adipocytes. The AT then contributes to the regulation of metabolism and lifespan.

Brown and white adipocyte commitment and differentiation are controlled by a set of transcription factors, mainly C/EBPα, C/EBPβ, and C/EBPδ, PPARγ (NR1c3), and SREBP1c [868].

Brown and beige adipocytes, which produce heat, develop in response to certain stimuli such as cold. Beige and brown adipocytes are related to vascular smooth and skeletal myocytes, respectively. In addition, other cell types of the AT, such as neural, vascular, and immune cells, regulate differentiation of brown and beige adipocytes [903].

Brown and beige adipocytes are enriched with the transcriptional coactivator PGC1α, which controls the formation of mitochondrial genesis regulators. Numerous other nuclear receptor coregulators participate in the generation of brown adipocytes (e.g., nuclear receptor coactivators NCoA1 [bHLHe42] and NCoA2 [bHLHe75], which stimulate and repress brown adipocyte induction within WAT, respectively, and Twist-1).

The transcription factor PRDm16, which abounds in brown adipocytes, complexes with C/EBPβ and induces synthesis of NR1c3, PGC1α, and UCP1 [868]. PRDm16 also acts in beige adipocyte differentiation. Both NR1c3 and PGC1α play an important role in the browning of WAT in humans, which counteracts obesity.

In addition, PRDm16 suppresses the transcription of type-*I* interferon-stimulated genes, such as the Stat1 gene, thereby ensuring maintenance of the mitochondrial function in brown and beige adipocytes [904]. Ectopic activation of type-*I* Ifn in brown adipocytes provokes mitochondrial dysfunction and reduces UCP1 synthesis.

Obesity also leads to subcutaneous adipocyte hypertrophy (but limited hyperplasia), vAT expansion, and ectopic fat depots. In humans, the prototypical visceral depot is the omental AT.

In addition to adipose depots, adipocytes also reside in many organs, such as the epicardium, kidney, lung, bone marrow, and perivascular spaces. The degree of adiposity of these adipose clusters varies with obesity and aging. In addition, lipids can accumulate in an ectopic position, such as in the liver, in metabolically dysfunctional organisms.

Visceral AT and scAT arise from different origins. This organogenic difference may explain distinct metabolic, inflammatory, angiogenic, and lipolytic properties of various sites of AT.

Adipose depot expands by *hyperplasia* (increased adipocyte number) and *hypertrophy* (adipocyte enlargement). Although hyperplasia corresponds to a healthy expansion during the formation of functional adipocytes from progenitor cells, hypertrophy leads to dysfunctional adipocytes.

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promotes brown adipogenesis. The pool of MyF5− precursor cells form white and beige adipocytes in white adipose depots.

Adipogenesis is controlled not only by the transcription factors C/EBPs, NR1c3, and SREBP but also corepressor complexes, which contain histone deacetylases or other chromatin enzymatic modifiers and regulate activity of these transcription factors (Vol. 11, Chap. 1. "Gene Transcription and Epigenetic Control"). The histone deacetylase HDAC3 is a component of the repressive complex that regulates adipogenic genes.

Numerous substances influence adipogenesis. They include lipoprotein lipase, cholesterol ester transfer protein, angiotensinogen, prostaglandins, complement factors, such as adipsin (or complement factor-D), the complement-related C1q and collagen domain-containing protein adiponectin, and desarginated complement factor C3 fragment ($C3a^{desArg}$ or ASP [ASP]),[142] cytokines, such as IL6 and TNFSF1, adipocyte differentiation factor (ADF), and nitric oxide [908].

Adipocyte differentiation factor secreted by mature adipocytes may act as a paracrine messenger that regulates differentiation of pre-adipocytes. The latter then produces up to a four-fold increase in triacylglycerol content and nine-fold elevation in glycerol 3-phosphate dehydrogenase activity, a marker of the late phase of pre-adipocyte differentiation [909].

Sterol regulatory element-binding protein, SREBP1[143] mediates insulin signaling and controls the formation of PPARγ (NR1c3), an important adipocyte differentiation factor [910].

Adiponectin also functions as an adipocyte differentiation factor that precludes adipocyte differentiation and expression of adipogenic marker genes, but activates that of lipolytic marker genes. It stimulates the P38MAPK–ATF2 pathway and inhibits the TOR–S6K axis [911]. P38MAPK hampers adipogenesis, as it represses C/EBPβ and PPARγ.

[142] Complement factor C3 is cleaved by convertases into C3b and C3a. It is activated by pathogens, immune complexes, and apoptotic cells in three pathways (classical, lectin, and alternative). The opsonin C3b mediates phagocytosis and cytotoxicity. It is also a component of the alternative pathway amplification loop that mediates inflammation via the anaphylatoxins C3a and C5a [905]. Carboxypeptidase-B cleaves C3, removing the terminal arginine of C3a (desargination), to ASP ($C3a^{desArg}$) [906]. In the alternate complement system axis, this process involves C3, complement factor-B, and adipsin (complement factor, CFd), which are all synthesized and secreted by adipocytes. C3 is spontaneously transformed into activated C3⋆, which then combines with CFb to form C3⋆B. The latter is cleaved by adipsin to C3⋆Ba and the convertase C3⋆Bb. $C3a^{desArg}$ may be generated by the alternate complement pathways. Multiple proximal components of the alternate complement pathway are indeed also produced by the adipose tissue, such as C3, Cfb, CFh, CFi, properdin, Crry, and complement receptors CR1 and CR2. Both C3a and ASP are potent stimulators of TG synthesis in adipocytes and pre-adipocytes. It is a glucose-dependent stimulator of insulin secretion [907].

[143] A.k.a. adipocyte determination and differentiation-dependent factor ADD1. This transcription factor is a member of the basic helix–loop–helix leucine zipper (bHLH–LZ) category (bHLHd1).

In humans, pre-adipocyte factor PreF1[144] hinders adipocyte differentiation. The extracellular domain of PreF1 is cleaved by TNFSF1 convertase to generate the active soluble PreF1 form [912]. It activates ERK and upregulates expression of Sox9, which binds to the promoter regions of C/EBPβ and C/EBPδ to impede adipogenesis.

Production of the cytokine pleiotrophin via the Wnt–β Ctnn pathway promotes proliferation rather than differentiation of adipocyte precursors. Hyperplasia results from increased differentiation of adipocyte precursors in adipose depots into mature adipocytes.

Adipocytes secrete adamts1 [913]. In mice, glucocorticoid exposure diminishes the amount of adamts1 and raises adipogenesis. In humans with excessive caloric intake, production of adamts1 and pleiotrophin rises in AT. The peptidase adamts1 increases adipocyte precursor proliferation, but prevents their differentiation.

The protein WDTC1 prevents adipogenesis.[145] Loss of a single Wdtc1 allele causes obesity in mice. In the nucleus, the anti-obesity factor WDTC1 binds to histones H2b and H4 in addition to HDAC3 and precludes NR1c3 activity, thereby regulating chromatin dynamics and gene transcription [914]. The anti-adipogenic protein WDTC1 is a component of the ubiquitin ligase CRL complex (Cul4–DDB1–Roc1) [915]. It promotes H2aK119 mono-ubiquitination and hence transcriptional repression.

MicroRNAs participate in adipogenesis control, acting as pro- and anti-adipogenesis regulators. Pro-adipogenic microRNAs include miR33b, miR103, miR143, miR146b, and miR148 [880]. Overexpression of miR103 or miR143 in pre-adipocytes accelerates adipogenesis, as they upregulate expression of transcription factors such as PPARγ, cell cycle regulators, such as G0S2,[146] FABP4, GluT4, and adiponectin. MiR33b, which targets Hmga2[147] and Cdk6 transcripts, and its host gene Srebp1 are highly expressed during preadipocyte differentiation. In obese mice, both miR103 and miR143 levels significantly decay in adipocytes, whereas expression of miR146b and miR148 rises. MiR146b inhibits SIRT1, thereby increasing FOXO1 acetylation and favoring obesity. MiR148 targets Wnt1, an inhibitor of adipogenesis.

MiR34a, miR125-5p, and members of the miR200a–miR200b–miR429 cluster prevent adipogenesis [880]. Mir34a$^{-/-}$ mouse inflamed epiWAT, which increases in HFD-fed mice, is accompanied by elevated expression of FAS, HMGCR,

[144] In mice, Delta homolog DLk1.

[145] WDTC1: WD40 and tetratricopeptide repeat (TPR)-containing protein-1, an ortholog of the Drosophila Adp (adipose) gene.

[146] G0S2: G0–G1 switch regulatory protein-2. It promotes apoptosis via its binding to BCL2 [108]. Its expression is significantly upregulated in human fibroblasts when NFκB is activated or when they are exposed to TNFSF1 [194].

[147] HMGA2: high-mobility group AT-hook protein-2 (the AT-hook is a DNA-binding motif). It is also called high-mobility group protein HMG1c. It acts in adipogenesis, favoring obesity. This transcriptional regulator operates via cyclin-A2 [108]. The miR Let7 also targets HMGA2 [194].

ScaRb3, NR1h3 (LXRα),[148] and PGC1α. Adipocyte-specific depletion of the miR200a–miR200b–miR429 cluster in mice causes HFD-induced weight gain, lowers glucose tolerance and insulin sensitivity, and impairs lipolysis due to targeted EPS8[149] and GLIS2 transcripts.[150]

5.4.3 Adipose Tissue Remodeling

Adipose tissue contains adipocytes in addition to pre-adipocytes, ECs, smooth myocytes, fibroblasts, and leukocytes, which constitute the stromovascular fraction of the AT cellular population. Obesity is associated with an increased infiltration of macrophages.

Obesity induces an adverse remodeling of AT that expands to accommodate the excessive caloric intake. The AT structure and cellular composition change. In general, obesity is associated with adipocyte dysfunction and degeneration.

Abdominal obesity is linked to defective insulin-mediated PKB phosphorylation and hence impaired suppression of hepatic gluconeogenesis and uptake of glucose by myocytes and adipocytes.

Adipose tissue remodeling generates a systemic proinflammatory state, in addition to a chronic low-grade inflammation of the obese AT. In the enlarged AT, redox stress, altered oxygen supply, and inflammation cause adipocyte degeneration. In addition, although hypertrophy and proliferation of adipocytes, angiogenesis, and action of immunocytes are originally coordinated, obesity disrupts this orchestrated process and causes apoptosis and eventually necrosis of adipocytes.

Obesity-induced degenerated adipocytes release DNA, which promotes macrophage accumulation in AT via the TLR9, a sensor of DNA fragments [916]. In obese mice, plasmatic concentrations of single- and double-stranded DNA increase. In humans, the plasmatic concentration of single-stranded DNA is significantly higher in patients with visceral obesity. On the other hand, TLR2 and TLR4 bound to fatty acids released from damaged adipocytes launch AT inflammation.

[148] In macrophages, activation of LXRs promotes cholesterol efflux. In response to activation of bile acid receptors, FXRs, miR144 regulates the expression of ABCa1 and HDL^{CS} and protects against insulin resistance and atherosclerosis [880]. On the other hand, NR1h3 activation reduces miR206 expression.

[149] EPS8: epidermal growth factor receptor (EGFR) pathway substrate-8. This adaptor controls cellular protrusions, as it regulates actin cytoskeleton dynamics and architecture. It participates in signaling from Ras to Rac, as the EPS8–AbI1–SOS1 trimer (AbI: Abl interactor) is a RacGEF [108, 194].

[150] GLIS2: GLIS (Gli-similar) family Krüppel-like zinc finger-containing protein-2. This bifunctional transcriptional regulator, which functions as an activator and repressor. As a transcriptional repressor, it operates in the Hedgehog and Wnt pathways [108, 194].

Extracellular DNA favors CCL2 production via TLR9 in macrophages. Administration of a TLR9 inhibitory oligonucleotide reduces the accumulation of macrophages in fat depots and improves insulin action [916].

Obesity also provokes EC activation in the adipose tissue, thereby favoring recruitment of immunocytes and macrophage infiltration into AT in addition to impairing local NO-dependent vasodilation [805].

The AT is infiltrated by many types of immunocytes (macrophages, mastocytes, neutrophils, B and T lymphocytes, and NK T cells, but not eosinophils and helper-2 and regulatory T lymphocytes) [805].

In obese organisms, AT M1 macrophages may promote insulin resistance [805]. On the other hand, M2 macrophages within the lean AT may protect against inflammation. Eosinophils and T_{H2} and CD4+ FoxP3+ T_{Reg} cells abound in the lean AT, in which T_{H2}-type cytokines are produced (e.g., IL4 and IL13), hence promoting the M2 phenotype. In obesity, CD8+ effector T cells and CD4+ T_{H1} cells accumulate in the AT and promote the M1 phenotype. In addition, macrophages, which are dispersed in the lean AT, cluster in dysfunctional ATs, lipid-scavenging macrophages surrounding free LDs of dead adipocytes.

Neutrophils that accumulate rapidly in the AT after HFD feeding secrete neutrophil elastase and can promote macrophage recruitment and AT inflammation [805].

Obesity reduces AT perfusion [805]. Capillary rarefaction favors adipocyte dysfunction, AT inflammation, and systemic metabolic dysfunction. Deficiency in VEGF leads to a similar decline in capillary densities in WAT and BAT, the impact being greater in BAT than in WAT. Deficient angiogenesis triggers ischemia, necrosis, and inflammation in the AT, the scAT having a higher capillary density and angiogenic capacity than the visceral depot. Pro-angiogenic AngptL4 has a reduced activity in visceral fat, whereas the antiangiogenic splice variant $VEGFa_{165}b$, is expressed at higher levels in human visceral than in subcutaneous fat depots [805].

Obesity increases the synthesis of several matrix components, in particular collagen-6, hence causing fibrosis in both scAT and pvAT, at least partly because of the upregulated formation of hypoxia-inducible factor, HIF1α [805].

5.4.4 Lipid Droplets

Lipid droplets are ubiquitous organelles that store and supply lipids. They consist of a core of neutral lipids surrounded by a phospholipid monolayer. They store lipids in excess and release them upon demand. The balance between lipid supply and consumption determines the number of LDs. In fact, they not only store but also regulate synthesis, metabolism, and transfer of lipids. They are also linked to protein degradation (Sect. 5.1.2.2) and immunity.

Intra- and extracellular stresses trigger LD formation [917]. Accumulation of LDs occurs during progression of some pathological conditions such as cardiomyopathies.

Table 5.18 Glycerophospholipid synthesis (Source: [918]; *DAG* diacylglycerol, *CDP–DAG* cytidine diphosphoDAG, *CL* cardiolipin, *PA* phosphatidic acid, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PS* phosphatidylserine, *PAF* platelet-activating factor, *LCL* lysoCL, *LPA* lysoPA, *LPC* lysoPC, *LPE* lysoPE, *LPG* lysoPG, *LPI* lysoPI, *LPS* lysoPS, *PLA2* phospholipase A2, *GPAT* glycerol 3-phosphate acyltransferase, *LCLAT* lysoCL acetyltransferase, *LPAAT* lysoPA acetyltransferase, *LPCAT* lysoPC acyltransferase, *LPEAT* lysoPE acetyltransferase, *LPGAT* lysoPG acetyltransferase, *LPIAT* lysoPI acyltransferase)

Reaction	Enzymes
G3P ⟶ LPA	GPAT1–GPAT4
LPA ⟶ PA	LPAAT1–LPAAT2, LPCAT1
PA ⟶ DAG ⟶ PC, PE	
PA ⟶ CDP–DAG ⟶ PG, PI, PS	
PC ⟶ LPC	LPCAT1–LPCAT4; PLA2
PE ⟶ LPE	LPEAT1–LPEAT2, LPCAT3–LPCAT4; PLA2
PG ⟶ LPG	LPGAT1, LPCAT1; PLA2
PI ⟶ LPI	LPIAT1; PLA2
PS ⟶ LPS	LPCAT3/4; PLA2
CL ⟶ LCL	LCLAT1; PLA2
AlkylG3P ⟶ alkylPC ⟶ lysoPAF ⟶ PAF: LPCAT1/2, PLA2	

5.4.4.1 Lipid Droplet Formation and Composition

Originating from the ER, LDs are composed of a hydrophobic core of neutral lipids surrounded by a phospholipid monolayer, to which various types of proteins are linked. Although their relative number varies according to the cell type, the most abundant neutral lipids in LDs are triacylglycerol and cholesterol esters.

Lipid droplets are created de novo by progressive accumulation of neutral lipids in the ER owing to multiple enzymatic reactions and synthesis and transformation of many active lipid intermediates [917]. At least 11 acyl-CoA synthetases (ACSs) can form major long-chain fatty acids, thereby initiating the synthesis of neutral lipids. Among long-chain acyl-CoA synthetases, LD-bound ACSLs include ACSL1, ACSL3, and ACSL4. At least 12 acyltransferase types catalyze the last step of neutral lipid synthesis. Some ACS proteins also serve as fatty acid transporters and some regulate fatty acid uptake. Four reactions of de novo triacylglycerol synthesis, which can also occur locally on LDs, are catalyzed by glycerol 3-phosphate ^{O}acyltransferases (GPAT1–GAPT4),[151] 1-acylglycerol 3-phosphate ^{O}acyltransferases (AGPATs), phosphatidic acid (PA) phosphatases (lipins), which link to LDs from the cytosol (whereas other enzymes arrive from the ER), and diacylglycerol ^{O}acyltransferases (DGATs; Table 5.18) [917].

[151] Whereas GPAT1 and GPAT2 localize to the OMM, GPAT3 (also called AGPAT8 and AGPAT9) and GPAT4 (or AGPAT6) reside on the ER.

Construction of LDs requires coordination of: (1) fatty acid activation, (2) synthesis of neutral lipids, (3) remodeling of phospholipids, (4) formation of new phospholipids, (5) activity of accessory proteins [917].

5.4.4.2 Lipid Droplet Morphology: Phosphatidylcholine Transfer Protein

Phosphatidylcholine transfer protein (PCTP), a member of the steroidogenic acute regulatory protein-related transfer domain-containing protein superfamily, which abounds in the liver, carries PC between membranes. This intermembrane PC transfer activity contributes to lipid homeostasis. It interacts with acyl-CoA thioesterase ACoT13[152] to suppress hepatic insulin signaling and promote lipogenesis. It regulates LD morphology during hepatic steatosis development via surfacic PC concentration, but the absence of PCTP does not alter the severity of NASH [919].

The major hematopoietic transcription factor Runx1 connects to the Pctp gene promoter. Haplodeficiency of Runx1 is associated with thrombocytopenia and impaired platelet response. A RUNX1 loss-of-function mutation downregulates the expression of the PCTP gene. However, several RUNX1 mRNA isoforms are generated via two alternative promoters, a distal P1 and proximal P2 promoter, and alternative splicing. They differ by their temporal expression pattern and cell specificity, the P1-derived isoform being restricted to hematopoietic lineage, whereas the P2-derived isoform is ubiquitous. Promoter P1-derived Runx1 correlates negatively with PCTP expression [920]. Synthesis of PCTP is higher in Black individuals than in White subjects.

Higher platelet PCTP expression is linked to increased platelet response upon activation of peptidase-activated receptor PAR4, a thrombin receptor.[153] Three common single nucleotide polymorphisms in the PAR4 gene are associated with PAR4 reactivity to thrombin and hence induced platelet aggregation. In Black individuals, the frequency of the PAR4 gene variant Ala120Thr is higher than in White people and induces a greater PAR4 reactivity and hence Ca^{2+} signaling and platelet aggregation [921]. The combination of PAR4 SNPs Ala120Thr and Phe296Val generates more IP_3.

5.4.4.3 Lands Cycle and Membrane Remodeling

Cell membranes contain several categories of glycerophospholipids. Polyunsaturated fatty acids, such as arachidonic and eicosapentaenoic acid, are linked to sn2 (but not sn1) position of glycerophospholipids [918]. Using acyl-CoAs as

[152] A.k.a. thioesterase superfamily member Them2.

[153] Thrombin signals via platelet Gq-coupled PAR1 and PAR4 receptors. PAR1 has a higher affinity for thrombin; Ca^{2+} transients rise sharply; and inactivation is rapid [921]. PAR4 provokes a gradual sustained elevation in cytosolic Ca^{2+} concentration, yielding most of the calcium flux.

donors, glycerophospholipids are formed by a de novo pathway (Kennedy pathway; Sect. 5.4.4.4) and modified in a remodeling pathway (Lands cycle) to generate membrane asymmetry and diversity.

Each cell type has a distinct content and composition of various phospholipids, PA, PC, PE, phosphatidylglycerol (PG), CL, PI, and phosphatidylserine (PS).

Synthesis of glycerophospholipids starts with fatty acid transformation to acyl-CoAs, which serve as donors. Phospholipids are formed from glycerol 3-phosphate using the Kennedy pathway. However, the acyl groups of glycerophospholipids are diverse and are distributed in an asymmetrical manner [918]. Saturated and monounsaturated fatty acids are usually esterified at the sn1 position, whereas polyunsaturated acyl groups are esterified at the sn2 position. Rapid turnover of the sn2-acyl moiety of glycerophospholipids is carried out in the remodeling Lands pathway, or Lands cycle.

The Lands cycle of phospholipid remodeling defines the turnover of glycerophospholipid acyl chains in deacylation–reacylation cycles catalyzed by phospholipase-A and lysophospholipid acyltransferases. It is an alternative source of PC and triacylglycerol that contributes to membrane remodeling and contributes to LD genesis [917]. Lipid droplets also contain lysophosphatidic acid acyltransferase (or AGAPT3) and LPC acyltransferase.

The Lands cycle depends on enzymes of the acylglycerol phosphate ^{O}acyltransferase and membrane-bound ^{O}acyltransferase family [918]. On the one hand, PC engenders LPC owing to the concerted and coordinated actions of PLA2s and LPCATs (LPCAT1–LPCAT4) in a reversible reaction and, on the other, PA using PLD. Two successive reversible reactions converts LPC to LPA and then to PA using lysoPLD and AGPAT, respectively [917].

5.4.4.4 Kennedy Pathway of Phospholipid Synthesis

The zwitterionic glycerophospholipids PC and PE are the two most abundant phospholipid species (>50% of total phospholipids in cellular membranes [922]. Both PC and PE are synthesized by an amino-alcohol phosphotransferase reaction that uses diradylglycerol and either cytidine diphosphate (CDP)–choline or CDP–ethanolamine, respectively, in the last step of a synthesis route,the Kennedy pathway.[154] The pathway that forms phosphatidylcholine and phosphatidyl etha-

[154]Named after E.P. Kennedy who, in collaboration with S.B. Weiss, elucidated this reaction established in 1956 [923]. A set of enzymes involved in the three steps of the synthesis of PC and PE:

(Step 1) choline kinases, ChKα and ChKβ and ethanolamine kinases, EtnK1 and EtnK2, which phosphorylate choline and ethanolamine, respectively.

(Step 2) phosphocholine (PCyT1α–PCyT1β; or choline–phosphate cytidylyltransferases CCTα–CCTβ) and phosphoethanolamine cytidylyltransferase (PCyT2), which form cytidine diphosphate (CDP)–choline and CDP–ethanolamine from phosphocholine and -ethanolamine (or choline and ethanolamine phosphate), respectively, using cytidine triphosphate (CTP).

(Step 3) choline and ethanolamine phosphotransferase CEPT1 and CDP–ethanolamine-specific ethanolamine phosphotransferase EPT1, which generate PC and PE, respectively.

nolamine from choline and ethanolamine consists of three enzymatic steps and two branches, the CDP-choline and -ethanolamine subaxis. It relies on: (1) choline and ethanolamine kinases; (2) phosphocholine and -ethanolamine cytidylyltransferases, the rate-limiting enzymes; and (3) choline and ethanolamine phosphotransferases.

The pathway that forms phosphatidylcholine and phosphatidylethanolamine from choline and ethanolamine consists of 3 enzymatic steps and 2 branches, the CDP–choline and –ethanolamine subaxis. It relies on: (1) choline and ethanolamine kinases; (2) phosphocholine and -ethanolamine cytidylyltransferases, the rate-limiting enzymes; and, (3) choline and ethanolamine phosphotransferases.

Upon uptake of fatty acids, choline– and ethanolamine–phosphate cytidylyltransferases (but neither choline kinase nor choline phosphotransferase) localize to LDs [917]. In vitro, cytidylyltransferase is activated by diacylglycerol and phosphatidic acid. It binds to membranes depleted of PC. As a LD grows, a relative depletion of PC and enrichment in diacylglycerol and phosphatidic acid promote its binding and activation.

5.4.4.5 Neutral Lipid Synthesis Linked to Lipid Droplets

In adipocytes, LDs store neutral triacylglycerols. Triacylglycerol synthesis, i.e., *lipogenesis*, results from esterification of acyl-CoA derived from fatty acids and glycerol 3-phosphate. Fatty acids originate from three sources: blood circulation, lipolysis of intracellular TGs, and de novo fatty acid synthesis from glucose. Glycerol 3-phosphate arises from glycerol, glucose, and amino acids.

In most cell types, neutral lipids are synthesized by enzymes permanently or transiently located in the ER.

Phospholipids are produced for LD expansion and to avoid coalescence.

5.4.4.6 Lipid Droplet Proteins

Proteic constituents of LDs include components of COP1 coatomer and monomeric ARF GTPases. Both ARF1 and COP1 components act directly on the LD surfaces to remove phospholipids, as the Arf1–COP1 machinery increases the local surface tension and promotes budding of nano-LDs, promoting establishment of membrane bridges between LDs and the ER and allowing rapid displacement of ER-bound proteins, such as PnPLA2[155] the rate-limiting enzyme in TG hydrolysis, which is involved in LD turnover, and GPAT4, to LD surface [924].

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[155]Intracellular Ca^{2+}-independent patatin-like phospholipase domain-containing protein is also called desnutrin, calcium-independent phospholipase-A2, and ATGL.

The COP1 complex may stimulate lipolysis via inhibition of perilipin-2[156] binding to LD surfaces and hence recruit PnPLA2 to lipid droplets [924].

At least five Rab isozymes are connected to LDs, especially Rab18, which mediates apposition of the LD phospholipid monolayer and ER membrane and may control lipolysis. In adipocytes, insulin-stimulated lipogenesis increases the residence of Rab18 into LDs [924].[157] $Rab18^{GTP}$ interacts with the ER-tethering NRZ complex,[158] which facilitates the fusion of vesicles from the Golgi body.

Seipin is a ubiquitous oligomeric ER transmembrane protein implicated in LD genesis. Seipin is involved in nascent ER–LD contacts and the delivery of lipidic and proteic constituents of LDs from the ER [925]. Seipin is dispensable for the initial synthesis of neutral lipids from fatty acids and their aggregation inside the ER membrane, but mandatory for fatty acid incorporation into neutral lipids in pre-existing LDs, hence intervening after the initial nucleation. Seipin helps to anchor newly formed LDs to the ER and stabilizes ER–LD tethering, thereby facilitating the incorporation of proteins and lipids through membrane bridges into growing (maturing) lipid droplets. During LD formation, seipin also assists the transfer of long-chain fatty acid–CoA ligase (synthetase) ACSL3 from endoplasmic reticulum to LDs.

Numerous membrane transfer components, such as ArfGEF, coatomer COP1 components,[159] and Rab GTPases (Rab1, Rab5, Rab7, Rab8a, Rab18, Rab32, and Rab40c) pertain to LDs and/or are involved in their creation [929].

[156] A.k.a. adipophilin and adipose differentiation-related protein (factor [ADRP (ADFP)]).

[157] The small GTPase Rab18 lodges on membranes of the ER, Golgi body, endosomes, and peroxisomes [924].

[158] NRZ: NAG–RINT1–ZW10 (NAG: neuroblastoma-amplified gene product; RINT1: Rad50 interactor-1; ZW10: centromere- and kinetochore-associated protein zw10 homolog). Both ZW10 and RINT1 are cell cycle checkpoint proteins.

[159] The Golgi body-derived coat protomer (coatomer) COP1 in the cytosol and cellular membrane operates along the early secretory pathway, whereas COP2+ vesicles export proteins from the ER and clathrin-coated vesicles transfer cargo along the late secretory and endocytic pathways [926]. COP1 contains ARF1, which controls CoP1 genesis, Arf1GEF (GBF1), and ArfGAPS such as ArfGAP1, with which most cargo interacts. The CoP1 complex carries cargo, such as the KDEL receptors, certain members of the P24 family of cargo proteins, and SNAREs [927]. Type-*I* transmembrane proteins of the P24 family (P24a–P24e) become major constituents [928].

In LDs, the transport protein particle complex TRAPPC2[160] is a Rab1GEF and Rab18GEF [924].[161] The small GTPase Rab18 linked to LDs may contribute to the connection and exchange between the ER and lipid droplets. It is also involved in the maintenance of the ER and the Golgi body [929]. The COP1–TRAPPC2–Rab18 signaling cascade puts LD and ER in close proximity to facilitate the lipogenic and/or lipolytic activities of Rab18 [924].

Lipolysis liberates fatty acids and glycerol from stored lipids. It is primed by hormones such as catecholamines that stimulate cAMP production and subsequent phosphorylation by PKA of LipE (HSL) and perilipins, which coat LDs, protecting them from LipE.

5.4.5 Adipose Tissue Secretome: Adipokines

The AT serves not only as a triacylglycerol store and source of FFAs but also as an endocrine organ. It releases many endocrines in addition to auto-, juxta-, and paracrine messengers that intervene in metabolism and fat depot size and redistribution, and modulates diverse biological processes (Tables 5.19 and 5.20).

[160] Several types of TRAPP complexes (TRAPPC1–TRAPPC4) share the same core subunits, but contain distinct accessory subunits in Saccharomyces cerevisiae. In yeast, the different TRAPP complexes participate in distinct transfer processes: endoplasmic reticulum-to-Golgi body (ER–GB) transport (TRAPPC1), late transport steps in the GB (TRAPPC2), and autophagy (TRAPPC3 and TRAPPC4) [929]. Two TRAPP complexes exist in mammalian cells, TRAPPC2 and TRAPPC3, which share common core subunits (TraPP1–TraPP5), but differ in the peripheral subunits, TRAPPC2 contains TraPP9 and TraPP10, whereas TRAPPC3 possesses TraPP8 and TraPP11 to TraPP13 [929]. In mammals, TRAPP2 is implicated in intra-GB and/or GB-to-plasma membrane (GB–PM) transport and in ciliogenesis in addition to the ER export of fibrillar procollagen; TRAPP3 controls ER–GB transport and autophagy. In mammals, both TRAPPC2 and TRAPPC3 act as a guanine nucleotide exchange factor (GEF) for Rab1 [929]. TRAPPC3 contributes to the control of autophagy via interaction between TraPP8 and TBC1D14, a RabGAP. TRAPPC3 also interacts with the COP2 vesicular coat [924]. TRAPPC2 is recruited to LDs upon lipid load via its interaction with COP1, the latter also mediating the establishment of tubular connections between the ER and the LD. Inactivation of TRAPPC2-specific subunits reduces lipolysis and provokes aberrantly large LDs [924].

[161] Recruitment of Rab18 onto the LD surface is impaired upon TRAPPC2 deletion, but the localization of Rab1 on the GB is not affected. The COP1–TRAPPC2 supercomplex recruits Rab18 onto the surface of small LDs. In addition to the Rab18GEF TRAPPC2, the Rab3GAP complex is a specific GEF for Rab18 [930]. Rab3GAP1 and Rab3GAP2, which elicit GTP hydrolysis by Rab3, work as a Rab18GEF complex. The Rab3GAP1–Rab3GAP2 complex is required for Rab18 linkage to the ER. Among the TBC1 domain-containing protein of the TBC1D set, TBC1D20 functions as a GTPase-activating protein for Rab1, Rab2, and Rab18 (Rab18GAP) [931, 932]. Warburg micro syndrome constitutes a spectrum of disorders characterized by severe eye, brain, and endocrine abnormalities. It is a rare autosomal recessive disorder caused by loss-of-function mutations in the RAB18, RAB3GAP1, RAB3GAP2, or Tbc1d20 genes. Enlarged GB and aberrant LD formation result from TBC1D20 LOF mutations [931]. In addition, fibroblasts with defective Rab18 and Rab3GAP1 exhibit aberrant LD genesis.

Table 5.19 Adiposecretome and main adipokines (**Part 1**; Source: [889])

Adipocytokine	Major source	Receptor(s) Role
Adiponectin	Adipocyte	AdipoR1/2
		Antioxidant and -inflammatory
		Precludes NFκB and NOx activity
		NO synthesis via NOS3 phosphorylation by AMPK
Apelin	Adipocyte	AplnR
		May reduce redox stress and inflammation
		May stimulate NOS3 (vasodilation)
		Positive inotropic effect
Leptin	Adipocyte (mainly subcutaneous)	LepR
		Regulates energy storage
		Induces NOx activity
		Proinflammatory
		Stimulates NOS expression
		Acts via JaK–STAT, SOCS, PI3K–PKB, and MAPK axes
Omentin	Adipocyte	Increases insulin sensitivity
		Antioxidant, -inflammatory
		Reduces vSMC migration
		Raises NO availability
		Hampers intimal hyperplasia
Resistin	Monocytes, macrophages (adipocytes)	CAP1
		Prooxidant, -inflammatory
		Decreases NO production
Visfatin	Stromal cells	Prooxidant, -inflammatory
		May increase NO production

Adipokines include protective anti-inflammatory (e.g., adiponectin, apelin, omentin, and interleukin-10) and detrimental and prooxidant and -inflammatory species (e.g., leptin, resistin, and visfatin) in metabolically healthy individuals and obese subjects, respectively (*AMPK* AMP-activated kinase, *AT* adipose tissue, *CAP* adenylate cyclase-associated protein, *NF* nuclear factor, *NO* nitric oxide, *NOS* nitric oxide synthase, *NOx* NAD(P)H oxidase, *vSMC* vascular smooth muscle cells)

The WAT is implicated not only in glucose metabolism (e.g., via adiponectin, resistin, and visfatin), lipid metabolism (e.g., via cholesteryl ester transfer protein, FABP4, lipoprotein lipase, retinol-binding protein, and sirtuins), and control of vasomotor tone (via adipokines and classical vasodilators such as NO and H_2S and vasoconstrictors such as endothelin-1 and angiotensinogen and its derivatives) along with medial SMC proliferation and migration (e.g., via omentin), but also in appetite regulation (e.g., via leptin), energy expenditure, angiogenesis (e.g.,

Table 5.20 Adiposecretome and main adipokines (**Part 2**; Source: [889]; *ET* endothelin, *IGF* insulin-like growth factor, *IL* interleukin, *PAME* palmitic acid methyl ester, *TNFSF* TNF superfamily member [TNFSF1 is TNFα])

Adipocytokine	Major source	Receptor(s) Role
ET1	All AT cell types	$ET_{A/B}$ Prooxidant, -inflammatory
IGF1	All AT cell types	IGF1R Regulates adipocyte and vSMC differentiation
IL6	Monocytes, macrophages	IL6R Prooxidant, -inflammatory
TNFSF1	Monocytes, macrophages	TNFRSF1a Prooxidant, -inflammatory
NO, H_2S		Vasodilation Antioxidant and -inflammatory
$Agt_{(1–7)}$		Vasodilation
PGi_2		Vasodilation
PAME		Vasodilation
H_2O_2		Vasodilation
MicroRNAs		MiR103-3b is linked to CCL13 MiR29a/143 regulate AT browning, inflammation MiR29a/143 are involved in adverse remodeling

via VEGF), blood coagulation and fibrinolysis (e.g., via plasminogen activator inhibitor-1 [serpin-E1]), immunity and hence inflammation (via cytokines and chemokines), and reproduction.

Adipokines have a hemodynamic, metabolic, and immunological impact. They can influence vasomotor tone (adiponectin, leptin, omentin, resistin, visfatin, and TNFSF1), insulin sensitivity (adiponectin and resistin), and inflammation (leptin, chemerin, CCL2, and CXCL8) [890].

The AT also produces and secretes other types of auto-, para-, and endocrine regulators, such as VEGF and insulin-like growth factor IGF1, sex steroids, angiotensinogen, and ROS [866].

White and brown adipocytes synthesize and secrete AngptL4[162] upon exposure to PPARα (or NR1c1).

They also release the metal-binding and stress-response *metallothioneins* MT1 and MT2, which can play an antioxidant role [888].

[162] A.k.a. fasting-induced adipose factor (FIAF) and hepatic fibrinogen- and angiopoietin-related protein (HFARP).

Table 5.21 Adipokines and metabolic syndrome (Source: [908])

Inhibitors	Adiponectin, nitric oxide
Stimulators	Angiotensinogen, leptin, pentraxin-1, resistin, serpin-E1, TNFSF1
Associated anomalies	Context
Endothelial dysfunction	↓ NO
	↑ ATn2, ET1, icam1, vcam1, CCL2, TNFSF5, TNFRSF5, oxLDL
Insulin resistance	Obesity, hypertension, glucose intolerance, low HDL concentration

Proinflammatory mediators favor endothelial dysfunction and insulin resistance, whereas amounts of protective adipocyte products decline with obesity (↑ increase, ↓ decrease)

Adipokines (or adipocytokines, i.e., adipocyte-derived cytokines)[163] are sources of crosstalk between the AT and cardiovascular apparatus and explain the existence of intertwined metabolic and cardiovascular alterations [810]. Adipokines include hormones, growth factors, cytokines, chemokines, complement factors, enzymes, and constituents of the extracellular matrix. Adipocytes possess receptors for most of these substances. AT environment affect its secretome. Adipokines act on the hypothalamus and other organs, and they regulate metabolism in the liver, skeletal muscles, and heart.

Energetic metabolism relies on metabolic circuits resulting from communications between various organs controlled by hormones (e.g., glucagon and insulin), growth factors (e.g., FGF21), and cytokines, among which adipokines (adipocyte-derived cytokines; e.g., adiponectin, adipsin, 15 C1q and TNF-related proteins [CTRP], leptin, omentin, resistin, retinol-binding protein RBP4, vaspin, and visfatin, in addition to the adipose tissue-derived cytokines IL1β and TNFSF1).

Metabolism and immunity are linked by proteins of dual function. The AT produces and secretes proinflammatory adipokines, such as TNFSF1, interleukins IL1β and IL6, chemokine CCL2, leptin, serpin-E1 (Tables 5.21, 5.22 and 5.23).[164] angiotensinogen, resistin, and pentraxin-1 (or CRP). Obesity and T2DM are characterized by high levels of proinflammatory cytokines.

Obesity typically upregulates expression of proinflammatory adipokines (leptin, TNFSF1, IL6, and resistin) and downregulates that of anti-inflammatory adipokines (adiponectin, omentin, CTRP9, and secreted frizzled-related protein sFRP5) [805]. The cardiovascular risk associated with adipokine imbalance results from the paracrine action of adipokines released from epvATs and the endocrine effect of adipokines secreted by other fat depots.

[163] Originally, the term *adipokine* defines adipocyte-derived secreted immunomodulatory proteins. The broader meaning includes immunomodulatory proteins secreted by adipocytes and other cell types of AT.

[164] A.k.a. plasminogen-activator inhibitor PAI1.

Table 5.22 Effects of adipokines (**Part 1**; Source: [908]; *CSF* colony-stimulating factor, *ET* endothelin, *EC* EC, *icam* intracellular adhesion molecule, *IL* interleukin, *LDL* low-density lipoprotein, *NF* nuclear factor, *NO* nitric oxide, *NOS* NO synthase, *PAI* plasminogen-activator inhibitor, *ROS* reactive oxygen species, *vSMC* vascular smooth myocyte, *TRAF* tumor-necrosis factor receptor-associated factor, *vcam* vascular cell adhesion molecule)

Adipokine	Effects
Adiponectin	↓ icam1, vcam1, E-selectin
	↓ NFκB
	↓ vSMC proliferation and migration
	↑ insulin sensitivity
	↓ foam cell formation
Leptin	↑ NOS3 production
	↑ glucose transport
	↑ ET1, ROS
	↑ CSF1 release
	↑ EC and vSMC proliferation and migration
	↑ vSMC apoptosis
	↑ angiogenesis
	↑ sympathetic tone
	↑ cholesterol accumulation under hyperglycemia
Resistin	↑ ET1 release
	↑ formation of adhesion molecules and chemokines
	↓ glucose uptake and insulin action
	↓ TRAF3

Several adipokines promote insulin sensitivity (e.g., adiponectin, leptin, and visfatin), whereas others induce insulin resistance (e.g., lipocalin-2, resistin, and RBP4, in addition to serpin-E1 (PAI1) along with TNFSF1 and IL6). However, adipokines, such as resistin and visfatin, play different roles in rodents and humans.

5.4.5.1 Adiponectin

Adiponectin[165] is a member of the CTRP family,[166] as it is structurally similar to complement factor C1q and tumor-necrosis factor. It is one of the most effective adipokines in correcting obesity-linked insulin resistance.

[165] Adiponectin (Adpn) is encoded by the ADIPOQ gene, which localizes to a susceptibility locus for T2DM and metabolic syndrome in chromosomal locus 3q27. It is also called AdipoQ (adiponectin C1q and collagen domain-containing protein), ACRP30 (30-kDa adipocyte complement-related protein), adipocyte C1q and collagen domain-containing protein (ACDC), adipose most abundant transcript product-1 (ApM1 and AdipQTL1, and gelatin-binding protein GBP28.

[166] CTRP: C1q–TNF-related protein.

Table 5.23 Effects of adipokines (**Part 2**; Source: [908]; AT_1, angiotensin type-1 receptor, *ATn* angiotensin, *FFA* free fatty acid, *TNFSF* tumor-necrosis factor superfamily member)

Adipokine	Effects
Angiotensinogen	↓ NO availability
	↑ NFκB
	↑ icam1, vcam1, CCL2, and CSF3
	↓ angiogenesis
Interleukin-6	↑ icam1, vcam1, E-selectin, CCL2
	↑ pre-adipocyte differentiation
	↑ SMC proliferation and migration
	↓ insulin receptor signaling
	↑ hepatic CRP production
Pentraxin-1	↓ NOS3 expression
(CRP)	↑ PAI1 expression in ECs
	↑ release of ET1 and IL6
	↑ icam1, vcam1, selectins, CCL2 in ECs
	↑ LDL uptake in ECs
	↑ ROS
	↑ AT_1 on vSMCs
	↑ SMC proliferation and migration
	↓ angiogenesis
	↑ EC apoptosis
Serpin-E1	↑ thrombogenesis
(PAI1)	↑ TNFSF1, ATn2, FFAs
	↑ restenosis
TNFSF1	↓ NO availability
(TNFα)	↓ adipocyte differentiation
	↓ insulin signaling
	↑ NFκB via ROS
	↑ icam1, vcam1, E-selectin, CCL2 in EC and vSMC
	↑ lipolysis and FFA level
	↑ EC apoptosis

Adiponectin is produced in the brown and WAT. Adiponectin expression is launched by PGC1α, PPARγ (NR1c3), and BNP, GC2a residing on adipocytes [849]. In addition, NAD^+-SIRT and catecholamine–β-adrenoceptor axes may participate in determining adiponectin concentration. Transcription of the ADIPOQ gene in the human vAT is inhibited by glucocorticoids and TNFSF1, but stimulated by insulin and IGF1 [908]. Adiponectinemia ranges from 5 to 30 μg/ml in humans, representing up to 0.05% of total plasmatic proteins [933]. However, adiponectinemia depends on ethnicity.

Adiponectin has an insulin-sensitizing effect [934]. Non-esterified fatty acids potentiate glucose-stimulated insulin secretion. However, adiposity often occurs

Table 5.24 Adiponectin effects (Source: [934] ↑ increase, ↓ decrease)

Glucose metabolism	↑ glucose uptake
	↓ gluconeogenesis
	↑ insulin sensitivity
	⊕ ⟶ glycogen synthase
Lipid catabolism	Fatty acid β-oxidation, generation of energy
	Triglyceride and LDL clearance
	⊖ ⟶ LDLR synthesis
	⊕ ⟶ cholesterol efflux
	Weight loss
Miscellaneous	Control of energy metabolism
	↓ TNFSF1 concentration
	Brown adipocyte differentiation
	⊕ ⟶ AMPK and PKA
	⊖ ⟶ PDGFRα and ERK1/2 signaling
	Detection of redox stress
	↓ cell migration and proliferation
	⊖ ⟶ granulocyte differentiation
	⊖ ⟶ NFκB and inflammation
	⊖ ⟶ foam cell formation from macrophages

⊕ ⟶ stimulation, ⊖ ⟶ inhibition

independently of plasmatic neFA concentration. Among adipokines that regulate β-cell function, concentration of adiponectin, a potentiator of glucose-stimulated insulin secretion, decreases with obesity and leptin, the concentration of which rises with adiposity, hampers insulin release [995]. Hypo-adiponectinemia contributes to the pathological conditions associated with overweight.

Adiponectin represses endothelial inflammation and vSMC proliferation [934].

This vasodilator raises NO production via AdipoR1 and PI3K [720, 892]. In addition, adiponectin can decrease TNFSF1-induced production of ADMA, an inhibitor of NO synthesis, and improve the endothelial redox state, as it suppresses superoxide generation by NAD(P)H oxidase [720].

Adiponectin operates in the control of glucose and lipid metabolism and insulin sensitivity (Table 5.24). It has anti-inflammatory, antidiabetic, and antiatherogenic effects.

Adiponectin exists in two forms, full length and a smaller globular fragment, which target the AdipoR2 and AdipoR1 receptors, respectively [891]. In human adipocytes, the expression of AdipoR1 is about 15-fold higher than that of AdipoR2. AdipoR1 supports the pro-angiogenic action of adiponectin in cultured ECs [939]. AdipoR2 can lead to insulin resistance, but helps revascularization. Both globular and full-length adiponectin induces vasodilation via NO in resistance arteries, at least in lean rats [933].

Adiponectin monomers are assembled into multimers that can be categorized into three groups: high ($Adpn_{hMW}$), mid- ($Adpn_{mMW}$), and low-molecular-weight form ($Adpn_{lMW}$). The largest high-molecular-weight (HMW) adiponectin is resistant to peptidases.

Adiponectin circulates in the bloodstream as oligomers (trimers, hexamers, and HMW forms that contain at least 18 monomers). Hexamer and HMW oligomers are assembled from the basic adiponectin trimer. Post-translational modifications (Lys hydroxylation and glycosylation) enable the formation of HMW oligomers.

Adiponectin is also modified by sialic acids via O-linked glycosylation of threonine residues, which determines its clearance from the bloodstream [933]. In addition, Cys36 is succinylated, thereby impeding disulfide bond formation and Adpn oligomerization. In diabetes, adiponectin is succinylated.

Its cognate receptors AdipoR1 and AdipoR2 belong to the 11-member PAQR family of transmembrane proteins (Table 5.25). In addition to AdipoR1, AdipoR2, and T-cadherin, the plasmalemmal calreticulin–CD91 complex may bind adiponectin on macrophages to facilitate the removal of apoptotic cells [939].

Both AdipoR1 and AdipoR2 activate AMPK, PPARα (NR1c1), and P38MAPK in the liver and skeletal muscle in addition to ECs [933]. However, AdipoR1 and AdipoR2 are the main effectors for AMPK and NR1c1 activation, respectively.

The APPL1 adaptor is a direct interactor of both AdipoR1 and AdipoR2. It promotes the translocation of STK11 (LKB1) from the nucleus to the cytosol, where it activates AMPK [933]. In addition, adiponectin activates AMPK via the PLC–Ca^{2+}–Cam2Kβ pathway.

The APPL1 adaptor also activates P38MAPK, triggers the STK11–AMPK–SIRT1 axis, and in cooperation with NR1c1, promotes glucose uptake, fatty acid oxidation, and mitochondrion genesis via PGC1α.

APPL2 also interacts with both AdipoR1 and AdipoR2, but hinders adiponectin signaling in myocytes [933].

Other AdipoR1 signaling mediators include RACK1, CsnK2β, ERP46, and TNFSF3 [933].

In the liver, AMPK is activated by $Adpn_{FL}$ and, in the AT and skeletal muscle, by ^{3}Adpn [891]. Adiponectin thus exerts its metabolic effects (increased glucose uptake, glycogen deposition, and enhanced fatty acid oxidation in muscles following phosphorylation by AMPK of acetyl-CoA carboxylase, inhibition of triacylglycerol and fatty acid synthesis, and reduced glucose output in the liver) via AMPK.

Whereas ^{6}Adpn and $Adpn_{hMW}$ activate NFκB in myocytes, ^{3}Adpn stimulates AMPK and enhances fatty acid oxidation in muscles [935]. $Adpn_{hMW}$, but neither trimer nor hexamer, activates AMPK in the liver to reduce glucose production.

Adiponectin localizes to the heart and vascular endothelium, where it interacts with T-cadherin (Cdh13), a glycosylphosphatidylinositol-anchored glycoprotein, which mediates the cardiovascular effects of adiponectin [805]. Cadherin-13 specifically bind ^{6}Adpn and $Adpn_{hMW}$ [933].

In pancreatic β cells and CMCs, adiponectin stimulates ceramidase, which processes ceramide into sphingosine, which is phosphorylated by sphingosine kinase to form sphingosine 1-phosphate [933]. Ceramidase also inhibits caspase-8

Table 5.25 Members of the progestin and adipoQ receptor (PAQR) family with eleven genes and two pseudogenes

Type	Other aliases
PAQR1	AdipoR1, ACDCR1
PAQR2	AdipoR2, ACDCR2
PAQR3	RKTG
PAQR4	
PAQR5	MPRγ,
PAQR6	
PAQR7	MPRα, Pγ LP
PAQR8	MPRβ, LMPB1
PAQR9	
PAQR10	MMD2
PAQR11	MMD1

Adiponectin binds to its receptors AdipoR1 (PAQR1) and AdipoR2 (PAQR2) in addition to cadherin-13 (or T-cadherin). Both PAQR1 and PAQR2 enhance ceramidase activity; adiponectin lowers ceramide concentration via these receptors. Accumulation of ceramide and glucosylceramide and hence hypo-adiponectinemia are involved in insulin resistance and atherosclerosis [936]. Both G-protein-coupled PAQR1 and PAQR2 tether to globular and full-length adiponectin; PAQR1 and PAQR2 have a high and intermediate affinity for globular adiponectin, respectively. The nuclear receptors NR1c1 and NR1c3 regulate PAQR1 and PAQR2 formation in adipocytes (but not in myocytes). PAQR1 and PAQR2 abound in the muscle and liver, respectively. PAQR1 and PAQR2 mainly target AMPK and NR1c1, respectively, and elicit fatty acid oxidation and glucose uptake upon adiponectin binding. Cadherin-13, a GPI-anchored cadherin, can compete with AdipoR1 and AdipoR2 for adiponectin binding or interfere with adiponectin signaling. It is a LDL receptor; LDL binding to Cdh13 activates ERK1, ERK2, and NFκB (*ACDCR* adipocyte C1q and collagen domain-containing protein receptor, *LMPB* lysosomal membrane protein in the brain, *MMD* monocyte-to-macrophage differentiation-associated factor or monocyte-to-macrophage differentiation protein, *MPR* membrane progestin receptor, *Pγ LP* PPARγ-induced liver protein, *RKTG* Raf kinase trapping to the Golgi body)

and hence CMC apoptosis. This effect strengthens CMC apoptosis inhibition resulting from PKB and AMPK action. The SphK1 kinase activates PGHs2, thereby repressing inflammation primed by TNFSF1.

In CMCs, where it is synthesized and secreted, adiponectin increases ScaRb3 translocation and fatty acid uptake via AMPK [933]. It enhances insulin-stimulated glucose transport via PKB. It stimulates lipoprotein lipase via actin remodeling boosted by the RhoA–rock axis. In addition, adiponectin promotes angiogenesis, as it elicits VEGF production via AMPK [933].

In the aorta of HFD-fed obese rats, adiponectin improves endothelial function, as it increases NO production via NOS3 phosphorylation by AMPK and PKB, once it connects AdipoR1 and AdipoR2, which cooperate [891, 933]. Moreover, adiponectin can repress basal and oxLDL-induced superoxide generation in addition to ROS production linked to hyperglycemia via the antioxidant cAMP–PKA pathway in ECs [891]. Furthermore, adiponectin inhibits NOS2, hence further reducing redox stress caused by excess NO radical.

Adiponectin also impedes peroxynitrite-induced nitrosative stress. It can opsonize apoptotic cells and facilitate their clearance.

Adiponectin promotes endothelial repair and angiogenesis after vascular injury via EPC mobilization from the bone marrow and spleen into the bloodstream and their recruitment to the injured vascular wall [933]. It stimulates survival, proliferation, and differentiation of bone marrow-derived EPCs and supports their migration via the PI3K–CDC42–Rac1 cascade, whereas activated AMPK is required for EPC recruitment by adiponectin to the vascular injury site. In diabetic patients, the circulating EPC density is altered partly because of redox stress [933].

Adiponectin suppresses proliferation and migration of human vSMCs induced by PDGFbb, FGF2, and HBEGF, as it tethers to these growth factors, in addition to those launched by IGF1, as AMPK inhibits ERK1 and ERK2 [933].

In macrophages, both globular and full-length adiponectin prevents production of proinflammatory cytokines induced by leptin and resistin [933]. It also suppresses NFκB signaling via the cAMP–PKA axis. It promotes the anti-inflammatory phenotype in macrophages and has an anti-inflammatory effect on vascular ECs. In human monocyte-derived macrophages, globular adiponectin upregulates the formation of the anti-inflammatory cytokine, IL10 [933].

Therefore, adiponectin exerts anti-oxidative, anti-inflammatory, and hence vasculoprotective actions. It impedes TNFSF1 production in adipocytes and macrophages. It can stimulate the synthesis of PGhS2 (or COx2) and thus prostaglandin-E_2, a vasculoprotective autacoid [805]. Activation of AMPK by adiponectin in the myocardium protects mice against cardiac hypertrophy and ischemia–reperfusion injury. Its cognate receptors AdipoR1 and AdipoR2 mediate the antihypertrophic effect of adiponectin in CMCs.

Hypoadiponectinemia is observed in obese subjects [805]. Adiponectin synthesis in adipocytes is hindered by ER and redox stresses.

Adiponectin protects against the metabolic syndrome characterized by dyslipidemia (hypercholesterolemia, hypertriglyceridemia, and elevated LDL/HDL ratio), endothelial dysfunction, hyperglycemia (decreased glucose tolerance), hypertension, inflammation, insulin resistance, obesity, and redox stress.

Insulin activates the PI3K–PKB pathway, thereby phosphorylating (inactivating) glycogen synthase kinase GSK3, a regulator of metabolism (and of cell fate and immunity), which stimulates adiponectin production. Indeed, GSK3 phosphorylates the transcription factor C/EBPα which is required for the synthesis of adiponectin, thereby impeding adiponectin formation [937].

Mice fed with an HFD and expressing PI3K-insensitive GSK3 are protected against the metabolic syndrome, adiponectin synthesis in the AT being significantly higher than in WT mice [937]. However, these mice, which exhibit hypocorticosteronemia and hypo-aldosteronemia, have a higher renal sodium excretion and hypertension.

Adiponectin protects the ovarian function. Reduced adiponectin concentration and augmented adipocyte size are associated with insulin resistance in women with polycystic ovary syndrome (PCOS), who have decreased fertility and increased T2DM risk [938]. Altered AT function provokes metabolic dysfunction in women with PCOS.

5.4.5.2 C1q and Tumor-Necrosis Factor-Related Proteins

The C1q subclass of proteins includes immune complement C1q, C1qDC1 (or caprin-2), adiponectin, CTRPs, cerebellins, emilins, multimerins, otolin, and collagen-8 and -10. The C1q–TNF superfamily comprises more than 30 secreted multimeric proteins, which operate in the endocrine, immune, neuronal, reproductive, sensory, skeletal, and vascular systems.

Members of the CTRP superfamily (i.e., adiponectin and C1q–TNF-related proteins, CTRP1–CTRP15), which are encoded by the genes of the C1QTNF group, are paralogs of adiponectin that contribute to the regulation of glucose and fatty acid metabolism (Tables 5.26 and 5.27) [939].

Adiponectin is synthesized in the pvAT, among other sites. Certain CTRPs are produced primarily in the stromal vascular cells of the AT.

Table 5.26 Properties of the CTRP proteins (**Part 1**; Source: [939]; $\ominus \longrightarrow$ inhibition)

CTRP	Tissue distribution	Signaling	Action sites	Function
CTRP1	Adipose tissue, placenta	AMPK, ERK1/2	Skeletal muscle	Glucose uptake
				Lipid oxidation
				Insulin sensitivity
CTRP2	Adipose tissue, also lung, liver, testis, uterus	AMPK	Skeletal muscle	fatty acid oxidation glycogen deposition
CTRP3	Adipose tissue, kidney, bone, testis, uterus	PKB	Liver	$\ominus \longrightarrow$ gluconeogenesis and triglyceride synthesis
		MAPK	Endothelium	EC migration and proliferation
		PKB	Heart	Cell survival
			Monocytes	Anti-inflammatory
CTRP5	Adipose tissue, eye, brain, spleen, skeletal muscle, testis, uterus	AMPK	Skeletal muscle, eye	GluT4 translocation
				Lipid oxidation
CTRP6	Placenta, also adipose tissue, spleen, lung, testis, prostate, uterus		Synoviocytes	Complement regulation
			Adipocytes	Adipogenesis

Table 5.27 Properties of the CTRPs (**Part 2**; Source: [939])

CTRP	Tissue distribution	Signaling	Action sites	Function
CTRP7	Adipose tissue lung			
CTRP9	Adipose tissue	AMPK	Skeletal muscle	Lipid oxidation
		AMPK–NOS3	Endothelium	Vasodilation
		AMPK	Heart	Improved function after injury
		MAPK, PKA	VSMC	$\ominus \longrightarrow$ migration and proliferation
CTRP11	Adipose tissue, testis; also brain, kidney	MAPK	Pre-adipocyte	$\ominus \longrightarrow$ adipogenesis
CTRP12	Adipose tissue	PKB	Adipose tissue	Glucose uptake
			Liver	$\ominus \longrightarrow$ gluconeogenesis
			Pancreatic β cells	Insulin secretion
			Adipose tissue	$\ominus \longrightarrow$ inflammation
CTRP13	Adipose tissue, brain, kidney	AMPK	Adipocytes, hepatocytes	Glucose uptake
			Liver	$\ominus \longrightarrow$ gluconeogenesis
			Hypothalamus	$\ominus \longrightarrow$ food intake
CTRP15	Skeletal muscle		Adipocytes, hepatocytes	Lipid uptake

Cq1–TNF-related proteins circulate as monomers and heterotrimers [810]. They target the endocrine, immune, vascular, skeletal, and sensory systems. They can ensure cardioprotection [939].

Most members of the CTRP group are synthesized in the AT, which predominantly produces CTRP1 to CTRP3, CTRP5, CTRP7, CTRP9, CTRP12, and CTRP13 [939]. All CTRPs trimerize, hence forming their basic structural unit; some assemble into hexamer and high-molecular-weight oligomers that may have distinct biological and signaling properties; CTRP3, CTRP5, CTRP6, CTRP9, CTRP10, CTRP12, CTRP13, and CTRP15 multimerize. They also combine, thereby generating distinct functional ligands. In addition to homo-oligomers, the heterotrimers CTRP1–CTRP6, CTRP2–CTRP7, and adiponectin–CTRP2 are also secreted.

Synthesis of CTRP3 and CTRP9 primarily in AT is downregulated in obesity [805]. Their circulating concentrations vary according to the metabolic state, sex, and genetic background [940].

CTRP1 (C1qTNF1)

The adipokine CTRP1 is produced in AT and in oxLDL- or IL1β-stimulated macrophages and ECs of atherosclerotic plaques [810]. Plasmatic CTRP1 concentration is elevated in hypertensive patients; CTRP1 mediates angiotensin-2-induced aldosterone production [941]. CTRP1 also stimulates P38MAPK and hence likely the P38MAPK–Arg pathway that mediates ATn2–primed endothelial dysfunction.

In the plaque, CTRP1 activates ECs and macrophages that then produce adhesion molecules and inflammatory cytokines such as TNFSF1, thereby augmenting leukocyte infiltration and inflammation. Plasmatic CTRP1 originates from visceral AT in addition to monocytes and inflammatory sites; it is also secreted by vascular cells such as ECs [941].

In a small patient population, plasmatic CTRP1 concentration is linked to coronary atherosclerosis and impaired collateralization. In atherosclerotic lesions, CTRP1 co-localizes with macrophages and ECs of plaque microvessels. Exposure to CTRP1 and deletion of the Ctrp1 gene in ApoE-deficient mice facilitate and reduce the progression of atherosclerosis, respectively [941]. Plasmatic CTRP1 concentration rises significantly in coronary endarterectomy samples, atherosclerotic plaques, and in blood and peripheral blood mononuclear cells from patients with severe CoAD [941]. Therefore, CTRP1 can serve as a marker of atherosclerosis.

As for adiponectin, CRTP1 synthesis is induced by the nuclear receptor NR1c3 (transcription factor PPARγ). In skeletal muscles, CTRP1 signals via AMPK; AMPKα is phosphorylated (activated) and hence phosphorylates (inactivates) acetyl-CoA carboxylase (ACC) [939]. In addition, CTRP1 activates PKB, ERK1, and ERK2 in differentiated mouse myotubes.

As does adiponectin with its insulin-sensitizing effect, which also promotes NO synthesis and counters endothelin activity in vascular endothelia, CTRP1 induces glucose uptake and hence reduces glycemia and CTRP3, CTRP5, and CTRP9 (but not the other group members) support NOS3 activity via the AdipoR1–AMPK–PI3K–PKB–NOS3 axis [810].[167] However, whereas adiponectin concentration declines in obese individuals, CTRP1 concentration rises in diabetic patients.

Cq1–TNF-related protein 1 reduces damage after myocardial infarction in mice and platelet aggregation in primates [810]. However, CTRP1 concentration elevates in stable coronary atherosclerosis; it correlates positively with blood pressure and negatively with HDL concentration; it is not related to the BMI; it is also linked only to a low level of arteriogenesis.

Endotheliocytes produce CTRP1 and respond to CTRP1, thereby activating the P38MAPK–NFκB pathway and increasing synthesis of adhesion molecules (selE, icam1, and vcam1) in ECs in addition to inflammatory cytokines and chemokines [941].

[167] In addition, CTRP3 and CTRP9 inhibit the proinflammatory LPS–TLR4 pathway [941].

CTRP2 (C1qTNF2)

The secreted protein CTRP2 enhances glycogen deposition and lipid oxidation in cultured myotubes. Fasting and high-fat feeding upregulate transcription of the Ctrp2 gene in WAT [942].

Cq1–TNF-related protein 2 activates AMPK in a dose-dependent manner. Both the full-length and truncated globular form enhance fatty acid oxidation.

CTRP3 (C1qTNF3)

The adipokine CTRP3[168] is synthesized in adipocytes, adipose stromal cells, and other cell types. In humans, circulating CTRP3 concentration correlates positively with adiponectin and negatively with waist circumference, blood pressure, and concentrations of fasting glucose, TGs, and cholesterol [939].

It lowers glycemia, as it suppresses the expression of gluconeogenic enzymes and hence hepatic glucose output. It also hampers hepatic TG synthesis, as it inhibits the formation of GPATs, AGPATs, and DGATs [943].[169] In hepatocytes, it suppresses production of glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) involved in gluconeogenesis [940]. It also represses gluconeogenesis via activated PKB [939].

It protects against NAFLD and hepatic steatosis (or NASH) in mouse DIO models.[170] Hepatic steatosis is caused by augmented FFA uptake and lipogenesis, especially hepatic TG synthesis, in addition to decreased β-oxidation[171] and export of TGs and fatty acids [945]. Lipogenesis is regulated by nutritional and hormonal cues.[172] Transcription of genes encoding lipogenic enzymes is primed by transcrip-

[168] A.k.a. cartducin and cartonectin.

[169] Triacylglycerol is synthesized and stored in AT and liver. Enzymes that catalyze steps in TG synthesis from glycerol 3-phosphate encompass members of the GPAT (glycerol phosphate acyltransferase [GPAT1–GPAT4]; mitochondrial glycerol 3-phosphate acyltransferase GPAM is also known as GPAT1 and GPAT3 as AGPAT8 and AGPAT9), AGPAT (acylglycerolphosphate acyltransferase [AGPAT1–AGPAT9]; AGPAT7 and AGPAT8 corresponding to LPC acyltransferase LPCAT4 and lysocardiolipin acyltransferase [LyCAT], respectively), lipin ([Lpin1–Lpin3]; LPIN) or phosphatidate phosphatase (PAP), and diacylglycerol acyltransferase (DGAT; [DGAT1–DGAT2]) families [944]. In the liver, TGs are synthesized via the *glycerol phosphate pathway* (glycerol 3-phosphate–LPA–PA–DAG–TG). Glycerol 3-phosphate, lysophosphatidic acid, phosphatidic acid, and diacylglycerol are sequentially processed. Their acylation is achieved by multiple isoforms of GPATs, AGPATs, and DGATs.

[170] Hepatic steatosis, i.e., lipid accumulation in the liver, often develops in the metabolic syndrome. Steatohepatitis results from alcoholic liver disease and NAFLD. The latter can lead to NASH, cirrhosis, and hepatic carcinoma. Hepatosteatosis is linked to redox stress, inflammation of the liver and AT, and hepatocyte death.

[171] The nuclear receptor NR1c1 (PPARα) and PGC1α regulate transcription of the gene encoding carnitine palmitoyltransferase, CPT1α, which is responsible for fatty acid oxidation.

[172] The genes that encode lipogenic enzymes, such as FAS, are activated by insulin. The transcription factor USF1 stimulates FAS formation via the mediator subunit Med17 [945].

tion factors, such as bHLHb11[173] and members of the SREBP family.[174] Production of the antiproliferative transcription cofactor B-cell translocation gene product BTG1 declines in obesity [946].[175] In hepatocytes stimulated with insulin, the mediator complex subunit Med17, a transcriptional coactivator, is phosphorylated by CsnK2, enabling bHLHb11 to activate lipogenic enzyme-encoding genes [945].[176]

Hepatic synthesis of lipids is also precluded by the PAP lipin-1,[177] which suppresses the activity of transcription factors of the SREBP family, thereby reducing production of TGs.[178] Glutathione synthesis and NAD^+ metabolism are altered in NAFLD; plasmatic and hepatic concentrations of glycine, betaine, and serine decline [948]. Supplementation of precursors of NAD^+ and G^{SH} significantly attenuate hepatosteatosis.

Mediator of RNA polymerase-2 transcription recruits ^{RNA}Pol2 and other elements of the general transcription machinery.

[173] Also known as upstream stimulatory factor, USF1. This ubiquitous transcription factor belongs to the MYC family of DNA-binding proteins, which connects to DNA as a dimer (mainly as USF1–USF2a (bHLHb11–bHLHb12a) heterodimers but also as USF1–USF2b heterodimers in addition to USF1 and USF2a homodimers [194]. Food intake stimulates the release of insulin, which triggers anabolism, in particular, lipogenesis in the liver. In hepatocytes, insulin triggers phosphorylation of the transcriptional coactivator Med17, which activates USF1 for lipogenic factor synthesis.

[174] SREBP (bHLHd1–bHLHd2).

[175] The ubiquitous BTG1 protein was initially identified as a translocation gene in B-cell chronic lymphocytic leukemia. It is involved in cell proliferation, differentiation, and survival [946]. At least in adult mice, it is required to generate new neurons. It is a cofactor for various transcription factors. In macrophages, it represses the activity of NFκB. Hepatic overexpression of BTG1 reduces hepatic steatosis in db/db mice, a model of obesity. The BTG1 factor is inhibited by the ATF4 factor, which is implicated in glucose metabolism, autophagy, apoptosis, ER stress, and inflammation, especially in the liver and WAT. Conversely, overexpression of ATF4 precludes BTG1 action. It protects against diet-induced hepatic steatosis, as it regulates hepatic lipid metabolism and prevents activity of ATF4 and SCD1, the rate-limiting enzyme, in the synthesis of monounsaturated fatty acids. This FoxO3a target suppresses transcription of the gene encoding stearoyl-CoA desaturase SCD1, which is involved in fatty acid synthesis [946]. A high-carbohydrate diet favors BTG1 production via the TOR–S6K1–CREB pathway.

[176] This phosphorylation (at Ser53) occurs in the liver of fed mice and insulin-stimulated hepatocytes, only if Med17 is not previously phosphorylated by P38MAPK, which is activated during fasting [945].

[177] Lipin-1 is also a transcriptional coregulator that enhances fatty acid oxidation via PPARα and PGC1α [947]. This PAP catalyzes the synthesis of diacylglycerol.

[178] On the other hand, upon phosphorylation of lipin-1 by TORC1 and casein kinase CsnK1, the RING-type ubiquitin ligase complex $SCF^{\beta TRCP}$ (β TRCP: β-transducin repeat-containing protein) polyubiquitinates lipin-1 for degradation [947]. This ligase also operates in the cell cycle, apoptosis, and metabolism. The SCF complex comprises four core subunits: the RING box protein RBx1, scaffold cullin-1, adaptor S-phase kinase-associated protein Skp1, and substrate receptor F-box protein. F-box proteins were first identified as components of SCF ubiquitin ligase complexes, in which they bind substrates for ubiquitin-mediated proteolysis. In humans, numerous F-box proteins exist that also function in other types of proteic complexes. The F-box protein β TRCP has two distinct paralogs: β TRCP1 (or F-box/WD repeat-containing protein FBxW1) and β TRCP2 (or FBxW11).

In addition, CTRP3 stimulates chondrogenic precursor cell proliferation [940]. In monocytes, adipocytes, and colonic fibroblasts, it can reduce the secretion of proinflammatory cytokines IL6 and TNFSF1 in response to lipopolysaccharide stimulation.

In the vasculature, CTRP3 promotes angiogenesis and thus increases capillary density [940]. It promotes VEGFa formation via the PKB–HIF1α pathway. In vitro, it induces EC proliferation and migration, as it activates ERK1, ERK2, and P38MAPK [939].

In the heart, CTRP3 activates PKB (but not AMPK), attenuating CMC apoptosis in ischemic cardiac regions in addition to increasing revascularization and reducing fibrosis in mice after myocardial infarction [805]. In vitro, CTRP3 inhibits TGFβ-induced profibrotic gene expression in cardiofibroblasts.

CTRP4 (C1qTNF4)

The CTRP4 protein is synthesized and secreted in the CNS by neurons, but not astrocytes, in addition to other organs [949]. It oligomerizes and its circulating concentration increases in leptin-deficient obese mice. Refeeding after overnight fasting triggers CTRP4 formation in the hypothalamus. Suppression of food intake by CTRP4 correlates with a decreased transcription of the genes encoding orexigenic neuropeptides NPy and AgRP in the hypothalamus.

CTRP5 (C1qTNF5)

The protein CTRP5 is widespread, with highest levels detected in the eye and AT [939]. Saturated fatty acids upregulate CTRP5 expression in adipocytes, where it operates in an autocrine fashion to reduce adiponectin and resistin secretion. In muscles, this autocrine regulator acts in response to reduced mitochondrial content. It stimulates AMPK, hence inducing GluT4 translocation. It enhances lipid oxidation using via the AMPK–ACC axis.

CTRP6 (C1qTNF6)

The adipose-tissue factor CTRP6 is involved in adipogenesis via the marker lipogenic genes and ERK1/2 pathway [950]. The complement system is implicated in the host defense against infection and in inflammatory diseases (Vol. 12, Chap. 2. Chronic Inflammation). CTRP6 suppresses the alternative pathway of the complement system, as it competes with complement factor-B [951].

CTRP7 (C1qTNF7)

Like CTRP9, CTRP7 expression is induced in the human failing heart. Whereas CTRP9 is produced in adult human CMCs, CTRP7 mainly originates from cardiofibroblasts. Unlike CTRP9 in adult rat CMCs, CTRP7 does not have an anti-oxidative action via AMPK, adiponectin receptors, and calreticulin.

CTRP8 (C1qTNF8)

The CTRP8 messenger is expressed predominantly in the lung and testis. It homotrimerizes and heteromerizes with the complement component-1-like agent C1qL1,[179] a secreted multimeric protein that also heteromerizes with CTRP1, CTRP9, and CTRP10 [952]. It connects to leucine-rich G-protein-coupled relaxin and insulin-like family peptide receptor RXFP1, at least in human glioblastoma cells. The CTRP8–RXFP1 complex launches cell migration via PI3K, PKC, and increased production and secretion of the lysosomal peptidase cathepsin-B [953].

CTRP9 (C1qTNF9)

Among all CTRP paralogs, CTRP9, a secreted glycoprotein that experiences multiple post-translational modifications, has the highest degree of amino acid identity to adiponectin. It forms predominantly trimers; adiponectin and CTRP9 form heterotrimers [935]. In humans, CTRRP9a and CTRP9b are encoded by distinct genes. CTRP9a is secreted as a multimer. CTRP9b interacts with CTRP9a or adiponectin to be released [952].

The action of CTRP9 is initiated from both AdipoR1 and AdipoR2 in addition to calreticulin. CTRP9 activates AMPK, PKB, ERK1, and ERK2. In the skeletal muscle, activated AMPK increases mitochondrial content and upregulates the transcription of genes that enable lipid oxidation. CTRP9 also improves insulin function and affects glucose metabolism and insulin activity.

The CTRP9 protein also controls the functioning of cardiac and endothelial cells. It increases NO production using the AMPK–NOS3 pathway, thereby priming vasodilation [939]. In a model of femoral artery injury, CTRP9 overexpression reduces intimal hyperplasia, as it represses vSMC proliferation and migration using the cAMP–PKA–ERK pathway.

C1q–TNF-related protein 9 activates synthesis TRdx1 and SOD2 via AMPK and SIRT3, hence attenuating cell death in response to H_2O_2. It attenuates apoptosis and fibrosis via the AdipoR1–AMPK axis in addition to redox stress in diabetic mice after ischemia–reperfusion injury [805].

[179] A.k.a. C1q-related factor (CRF) and C1qTNF14.

On the other hand, in response to hypertension, myocardial capillary ECs can favor adverse hypertrophy of CMCs. CTRP9 secreted by ECs primes the activation of the pro-hypertrophic kinase ERK5, which phosphorylates (activates) the pro-hypertrophic transcription factor GATA4 [954].

Formation of the pro-survival cardiokine, CTRP9, is markedly downregulated after myocardial infarction. It promotes implanted stem cell survival and ensures cardioprotection [955]. In vitro, CTRP9 supports proliferation and migration of adipose tissue-derived mesenchymal stem cells (ADMSCs) and protects them against death induced by hydrogen peroxide. C1q–TNF-related protein 9 promotes superoxide dismutase SOD3 synthesis and secretion from ADMSCs, protecting CMCs against redox stress. At physiological doses, C1q–TNF-related protein 9 prolongs transplanted ADMSC retention and survival. In mice subjected to myocardial infarction, implantation of ADMSCs within the myocardium does not exert significant cardioprotection. On the other hand, once their transplantation is combined with CTRP9, cardioprotection yielded by CTRP9 alone is potentiated. C1q–TNF-related protein 9 promotes ADMSC proliferation and migration via the ERK1/2–MMP9 axis and cell survival via the ERK1/2–NFE2L2 axis and antioxidant production. N-Cadherin is a CTRP9 receptor that mediates ADMSC signaling [955].

CTRP10 (C1qTNF10–C1qL2)

C1q–TNF-related protein 10 corresponds to complement component C1q subcomponent-like protein C1qL2. Whereas C1QTNF1 to C1QTNF3, C1QTNF5, and C1QTNF7 transcripts are predominantly formed in the AT, the eye and placenta produce the highest levels of C1QTNF10 and C1QTNF6 transcripts, respectively [956]. CTRP10 trimers further assemble into oligomers via disulfide bond in their N-terminal cysteine residues.

CTRP11 (C1qTNF11)

The CTRP11 protein is predominantly formed in WAT and BAT and in the former primarily in stromal vascular cells [939]. Overnight fasting and refeeding upregulates CTRP11 production. It can impede adipogenesis in a paracrine manner between adipocytes and stromal vascular cells, as it precludes expression of the two major transcriptional regulators of adipogenesis NR1c3 and C/EBPα.

CTRP12 (C1qTNF12)

Expression of the antidiabetic adipokine CTRP12[180] is downregulated in obesity [957]. It is synthesized more in subcutaneous than in visceral adipose depots and more in adipocytes than in the stromal vascular cells. In humans, AT predominantly produces CTRP12, whereas its expression is more widespread in mice [939].

Krüppel-like factors, KLF3 and KLF15, down- and upregulate CTRP12 production, respectively. In the scAT, insulin elicits CTRP12 synthesis and secretion [958]. Conversely, CTRP12, which acts in an insulin-independent manner, improves insulin signaling in the liver and AT and hence insulin sensitivity [957]. It activates the PI3K–PKB pathway to suppress gluconeogenesis and promote glucose uptake.

The CTRP12 adipokine has at least two isoforms, a full-length and a cleaved globular isoform, which build distinct oligomers and have different functions. Furin (or PCSK3) cleaves CTRP12 (Lys91) [939]. The full-length isoform forms trimers and larger complexes [957]. The cleaved globular isoform mainly dimerizes. Whereas $CTRP12_L$ activates PKB in hepatocytes and adipocytes, $CTRP12_S$ stimulates ERK1, ERK2, and P38MAPK. Only full-length CTRP12 support insulin-induced glucose uptake in adipocytes.

In addition, CTRP12 suppresses gluconeogenesis in cultured hepatocytes. A modest increase in circulating CTRP12 concentration suffices to lower glycemia and improve insulin sensitivity in mice, as CTRP12 enhances insulin signaling in the liver and AT, but not in skeletal muscle [939].

CTRP13 (C1qTNF13)

In humans, CTRP13 is preferentially expressed in AT and, in mice, in the brain and AT (mainly by stromal vascular cells) [939]. In cultured adipocytes, hepatocytes, and myotubes, CTRP13 activates AMPK and hence promotes glucose uptake. In vitro, it activates AMPK and inhibits G6Pase and PEPCK, thereby lowering gluconeogenesis in hepatocytes. It also partly reverses lipid-induced insulin resistance in hepatocytes, as it suppresses the JNK signal.

In the brain, CTRP13 serves as an anorexigenic factor. A reciprocal regulation exists in the hypothalamus between CTRP13 and the orexigenic neuropeptide agouti-related protein (AgRP) homolog, CTRP13 repressing AGRP gene transcription and AgRP upregulating Ctrp13 gene transcription (food intake modulatory

[180] A.k.a. adipolin.

hypothalamic feedback loop) [939]. Food restriction in mice lowers the CTRP13 level and raises those of orexigenic neuropeptides NPy and AgRP in the hypothalamus. However, when food restriction is coupled with physical activity, hypothalamic expression of both CTRP13 and AgRP augments [939]. This neural circuit may be disrupted in anorexia.

CTRP14 (C1qTNF14–C1qL1)

Whereas CTRP10 corresponds to C1qL2, the C1QTNF14 gene product is the complement component C1q subcomponent-like protein C1qL1, which is also termed C1q-related factor (C1qRF or simply CRF), the transcripts of which are most abundant in regions of the nervous system involved in motor function (e.g., cerebellar Purkinje cells in addition to the accessory olivary nucleus, pons, and red nucleus [959].

CTRP15 (C1qTNF15)

The CTRP15 protein[181] is predominantly produced in skeletal muscles, more in oxidative slow-twitch fibers than in glycolytic fast-twitch fibers [939]. Its circulating concentration rises upon refeeding after fasting. It may serve as a postprandial hormone produced and secreted by skeletal muscles in response to nutrient flux. It diminishes FFA-emia, as it upregulates the expression of ScaRb3, FABPs, and FATPs, thereby promoting FFA uptake in hepatocytes.

5.4.5.3 Adipsin (Complement Factor-D)

Type-2 diabetes mellitus linked to a pancreatic β-cell failure, which engenders insulinopenia and hyperglycemia, is associated with adipsin deficiency. Its production declines in many animal models of obesity and diabetes. The adipokine adipsin, or complement factor-D, secreted mainly or exclusively by the AT, maintains β-cell functioning [960].

It catalyzes the rate-limiting step of the alternative pathway of complement activation and hence supports the formation of the C5–C9 membrane attack complex. It also elicits the generation of numerous signaling molecules, such as the anaphylatoxins C3a and C5a [960].

The CFd–C3b complex cleaves complement factor-B and catalyzes the formation of the C3 convertase (C3bBb), which can act on C3 to liberate C3a. The C3a peptide generated by adipsin is a potent insulin secretagogue via the C3a receptor C3aR1 only when coupled to hyperglycemia [960]. C3a augments concentrations

[181] A.k.a. myonectin.

of the messenger and energy provider ATP as well as the signaling mediator Ca^{2+}, thereby supporting mitochondrial ETC. The receptor C3aR1 primes Ca^{2+} influx and activates ERK, Rho, and NFκB.

5.4.5.4 Apelin

The ubiquitous peptide apelin and its Gi/Gq-protein-coupled receptor APJ are formed in the CNS, particularly in the hypothalamus. It is considered an adipokine because it is synthesized and secreted by adipocytes. The PI3K–PKB pathway is involved in apelin synthesis and secretion in adipocytes. Insulin stimulates apelin production and activity via the PI3K–PKB axis and MAPK module [961]. Proinflammatory cytokines launch apelin production via PI3K and JNK in adipocytes.

The apelin gene encodes a 77-amino acid preproprotein and apelin propeptide. Several active apelin forms exist (e.g., $apelin_{36}$ [$apelin_{(42–77)}$], $apelin_{19}$ [$apelin_{(59–77)}$], $apelin_{17}$ [$apelin_{(61–77)}$], and $apelin_{13}$ [$apelin_{(65–77)}$]), and the pyroglutamated form of $apelin_{13}$ ($^{pGlu}apelin_{13}$), which is protected from peptidase degradation [962].

Apelin is involved in the regulation of food intake and energy metabolism in addition to fluid homeostasis, cell proliferation, and angiogenesis.

Apelin receptor, which is encoded by the APLNR gene,[182] predominantly localizes on ECs in organs with a high metabolism rate, such as the skeletal muscle, myocardium, and AT. It inactivates FoxO1 and hence FABP4, thereby avoiding excessive fatty acid accumulation and improving fatty acid uptake, glucose utilization, and insulin sensitivity [963].

It is a potent vasodilator via NO. It also represses vasoconstriction mediated by angiotensin-2 [961]. Conversely, ATn2 downregulates the formation of apelin and APJ, as it hampers ERK1/2 and P38MAPK signaling.[183]

Apelin decreases glycemia mainly because of increased glucose uptake in the myocardium, skeletal muscles, and AT via AMPK and NOS3 (Table 5.28) [962]. In addition, apelin increases the insulin-stimulated glucose transport in insulin-resistant adipocytes. Moreover, ingested glucose rapidly induces the paracrine secretion of apelin in the mouse gut; the amount of glucose transporter SLC5a1[184] declines in enterocytes, whereas that of GluT2 rises upon AMPK activation, thereby priming glucose absorption. A transient increase in glycemia in the portal vein may rapidly induce insulin secretion. Apelin may improve insulin sensitivity. Apelin also launches the secretion of glucagon-like peptide, GLP1 [962].

In adipocytes, apelin inhibits lipolysis induced by β-adrenoceptor (but not basal lipolysis) via the Gq/Gi–AMPK pathway [962]. Apelin injection for 2 weeks decreases the size of fat depots in SD- and HFD-fed mice. Chronic apelin

[182] A.k.a. angiotensin receptor like-protein AgtRL1.

[183] The APJ receptor resides on ECs, vSMCs, and CMCs, among other cell types.

[184] The solute carrier family member SLC5a1 is also named sodium–glucose cotransporter SGLT1.

Table 5.28 Apelin signaling and effect (Source: [962])

Pathway	Effects
AMPK	Intestinal glucose absorption
	Lipolysis, fatty acid oxidation
	Mitochondrial genesis
AMPK–NOS3	Glucose uptake
PDE3b	Insulin secretion
PDE3b–PI3K–PKB	Glucose uptake

It plays a beneficial role in energy metabolism and insulin sensitivity

administration in obese and insulin-resistant mice increases fatty acid oxidation in muscles via AMPK in addition to mitochondrial genesis in skeletal and cardiac myocytes via PGC1α [962]. Apelin may also prevent the development of obesity via the maintenance of proper vascular permeability.

Apelinemia rises in obesity and T2DM, but does not correlate with BMI, compensating for defective insulin signaling [964]. Apelin is implicated in obesity-related hypertension owing to a crosstalk between ATn2–AT_1 and apelin–APJ signaling via ERK1, ERK2, and P38MAPK [961]. In a rat model of obesity-related hypertension, apelinemia and transcription of apelin and APJ in perirenal AT decrease because of ATn2 inhibition.

5.4.5.5 Asprosin

Asprosin[185] is a fasting-induced glucogenic protein secreted by WAT, which circulates in the bloodstream at nanomolar levels. During fasting, it binds to the surface of hepatocytes, activates PKA, and elicits a rapid release of glucose from the liver, especially for cerebral functioning. Glucose may serve as a suppressor of asprosin release via a negative feedback loop. Asprosin also primes hepatic glucose production using the second messenger cAMP [965]. It is the C-terminal cleavage product of profibrillin encoded by the FBN1 gene.[186] Humans with insulin resistance have elevated asprosinemia, the FBN1 mRNA sources including WAT, BAT, and the skeletal muscles. Inappropriately elevated glucose production by the insulin-resistant liver is a major factor of the metabolic syndrome.

[185] αστρον: white.

[186] Fibrillin-1 is a structural component of microfibrils of the load-bearing extracellular matrix (diameter 10–12 nm), which provides structural support against sustained mechanical forces in organs, such as the lung and arteries, where these microfibrils form the periphery of elastic fibers. These microfibrils also form elastin-independent networks in tendon and renal glomerulus, among others, in which they yield tensile strength and play an anchoring role. Fibrillin-1 also interacts with growth factors, such as BMPs, GDFs, and latent TGF-β-binding proteins, in addition to integrins and proteoglycans and other proteic constituents of the extracellular matrix [108].

5.4.5.6 Chemerin

Chemerin is involved in immunity as a chemokine that targets macrophages and dendrocytes. This adipokine is also implicated in lipid and glucose metabolism in the liver and skeletal muscles, yielding a link between chronic inflammation and obesity [966]. Hyperchemerinemia is indeed associated with obesity, insulin resistance, and systemic inflammation in obese adults and children [967].[187] In children, chemerinemia is associated with the amount of soluble adhesion molecules icam1 and E-selectin (but not soluble vcam1 and P-selectin) in the circulation. In human subjects, levels of TGs, total cholesterol, CRP protein, and leptin, in addition to BMI and waist circumference, correlate positively with chemerin, whereas systolic and diastolic blood pressure, LDL^{CS}, and HDL^{CS} do not correlate significantly and adiponectin correlates negatively [968].

Chemerin is synthesized at high levels in the WAT and liver. It is secreted as inactive prochemerin. It is cleaved (activated) by serine peptidases of the inflammatory and coagulation cascade.

Chemerin acts via its cognate chemokine-like receptor CmkLR1 and regulates adipogenesis via NR1c3 in addition to inflammation and glucose metabolism [969]. ECs possess CmkRL1 that activate icam1 and E-selectin expression. The auto- and paracrine messenger chemerin operates in adipocyte differentiation. In adipocytes, it also stimulates lipolysis and modulates the expression of genes involved in glucose and lipid metabolism (glucose transporter GluT4, adiponectin, and leptin) [968].

5.4.5.7 Leptin

Leptin[188] is almost exclusively secreted by white and brown adipocytes. Leptin formation is upregulated by glucocorticoids and proinflammatory cytokines and downregulated by cold exposure, adrenoceptor activation, stimulation by growth and thyroid hormone, and melatonin, in addition to smoking [887]. Obesity is associated with elevated leptinemia and hypothalamic leptin resistance.

This endocrine agent participates in the regulation of energetic balance, as it suppresses appetite and promotes energy expenditure. Its secretion increases or decreases under conditions of a positive or negative energy balance, respectively. Leptin serves as an *adipostat* that informs on the status of energy storage in the AT

[187] Chemerinemia correlates negatively with age, decreasing from the pre-pubertal, pubertal, to post-pubertal period in healthy lean children (only in boys, not in girls) [967]. In aged adults, it correlates positively with age.

[188] λεπτοτης: thinness, leanness; λεπτυνω: make thin (λεπτον: mite, tiny coin; λεπτος: peeled [thin covering removed]). Blood leptin concentration rises with feeding and declines with fasting. In rodents, its absence is a strong feeding inducer, but its presence is not a potent feeding inhibitor. Excessive feeding upregulates leptin synthesis, whereas fasting downregulates it. Various stimuli (e.g., cytokines, glucocorticoids, and insulin) acutely adjust leptin production.

to adapt appetite and metabolism via the leptin receptor [970]. In humans, leptin concentration increases with AT mass and decreases during weight loss.

This hormone targets the brain to establish a negative feedback loop for weight control. However, obese humans exhibit hyperleptinemia without hypophagia (*leptin resistance*).

Leptin impairs several metabolic actions of insulin (i.e., stimulation of glucose transport, glycogen synthase and PKA, lipogenesis, and protein synthesis, in addition to inhibition of lipolysis) [971]. Conversely, insulin potentiates leptin-induced NO release. Whereas leptin impedes lipogenesis, angiotensin and acylation-stimulating protein stimulate it, glucose being a substrate for lipogenesis [887].

Leptin exerts a paracrine effect on adipocytes. Its synthesis and secretion by adipocytes is induced by IL6 and inhibited by TNFSF1 [908].

Leptin stimulates proliferation and migration of ECs and vSMCs. In ECs, leptin increases production of NO and ROS in addition to CCL2 [908]. It stimulates angiogenesis.

Leptin also increases the peripheral sympathetic tone. Therefore, leptin regulates blood pressure by two opposing mechanisms [866, 892]. On the one hand, vasodilation via NO release and stimulation of EDHF, which involves neither K_{ATP} nor BK channels, and attenuation of angiotensin-2 action; on the other, leptin provokes vasoconstriction via central excitation of the sympathetic nervous system.

Leptin represses intimal hyperplasia. It targets its endothelial receptor, which activates STAT3 and prevents synthesis via PPARγ (NR1c3) and release of endothelin-1 and subsequent vSMC proliferation [972]. On the other hand, obesity is linked to *endothelial leptin resistance*. In vSMCs, leptin elicits MMP2 formation [805].

Leptin favors platelet aggregation and arterial thrombosis via its receptor [908].

Leptin is a proinflammatory adipokine for monocytes, macrophages, neutrophils, NK cells, and T lymphocytes [805]. On macrophages, leptin elicits release of monocyte colony-stimulating factor (CSF1) [908]. Leptin facilitates cholesterol accumulation in macrophages, especially in hyperglycemia. Hyperleptinemia in obese individuals supports atherosclerosis, but protects against ischemia-induced cardiac damage [805].

5.4.5.8 Lipocalin

Lipocalin-2[189] is highly expressed in adipocytes [973]. It is widespread and exists as monomers and homo- and heterodimers with gelatinase [831]. It can undergo N-linked glycosylation (Asn65).

Lipocalin-2 belongs to the lipocalin superfamily, which comprises RBP4, FABP4, ApoD, and prostaglandin-D synthase (PGdS). Lipocalins generally bind small hydrophobic ligands, but can also tether to soluble extracellular

[189] A.k.a. growth factor-stimulated superinducible protein-24, neutrophil gelatinase-associated lipocalin, and siderocalin.

macromolecules and specific plasmalemmal receptors. Lipocalin-2 binds to the receptors LRP2 (megalin) for its cellular uptake and brain-type organic cation transporter SLC22a17 [831]. It is implicated in cell survival and apoptosis.

The formation of lipocalin-2 is induced by TNFSF1, an insulin resistance-inducing factor, in cultured adipocytes. Its production elevates upon exposure to insulin resistance-mediating agents in obese subjects via CCAAT/enhancer-binding protein. The proinflammatory transcription factor NFκB upregulates lipocalin-2 synthesis. In mice, WAT is a dominant site of Lcn2 formation, especially in obesity.

Ligands of lipocalins include retinoids, arachidonic acid, leukotriene-B_4, platelet-activating factor, steroids, odorants, pheromones, and, bacterial siderophores [973].[190] The 25-kDa secretory glycoprotein lipocalin-2 can bind weakly to some common ligands of lipocalins (e.g., leukotriene-B_4 and platelet-activating factor) [831].

Lipocalin-2 deficiency attenuates the metabolism of arachidonic acid, the concentration of which elevates with aging and obesity, at least in mice [831]. Inflammatory lipid species processed by arachidonate lipoxygenase are significantly reduced in WAT of $LCN2^{-/-}$ mice.

Lipocalin-2 elicits the production of inflammatory lipid species and adipokines in the AT and magnifies systemic insulin resistance [831]. However, its depletion impairs adaptive thermogenesis and hence cold tolerance, uncoupling protein UCP1 level decreasing in the BAT in $LCN2^{-/-}$ mice.

Lipocalin-2 causes aging- and obesity-associated insulin resistance, hyperglycemia, and hyperinsulinemia, as it upregulates formation and activity of arachidonate 12-lipoxygenase and stimulates TNFSF1 production in the AT [974].

5.4.5.9 Omentin

Omentin-1, or intelectin-1, is abundantly produced in human vAT. Omentinemia decreases in obese subjects and in patients with T2DM [805]. Omentin has two isoforms, omentin-1 being the major isoform in human blood [892].

Omentin-1 ensures protection of the cardiovascular apparatus. It causes vasodilation via NO [892] and suppresses TNFSF1-induced inflammation of vascular endothelia via the AMPK–NOS3 pathway [805].

[190] Lcn2 is used by cells of the innate immunity to sequester siderophores and thus deprive bacteria of iron.

5.4.5.10 Resistin

In rodents, resistin[191] is mainly synthesized in adipocytes, but, in humans, the main sources are monocytes and macrophages. Resistin production in monocytes and macrophages rises in response to proinflammatory stimuli such as IL6 [805]. Adipocytes also produce resistin-like molecule RetnLα [888].

Release of resistin is stimulated by hyperglycemia and growth and gonadal hormones in addition to inflammation [887]. Circulating concentration increases in diet-induced and genetic forms of obesity in rodents [908]. Resistinemia also rises in obese humans.

Adipose tissue-specific resistin affects glucose metabolism and insulin sensitivity. It causes insulin resistance in the liver and skeletal muscle [908]. Neutralization of resistin by specific antibodies decreases glycemia and improves insulin sensitivity.

Resistin impairs endothelial function and thus reduces expression of NOS3 and hence NO concentration [892].

Resistin and Inflammation

Resistin has a proinflammatory effect on smooth myocytes. It induces human aortic smooth myocyte proliferation [908].

Resistin elicits ET1 release and activates ECs. It upregulates production and secretion by ECs of chemokines (e.g., CCL2) and adhesion molecules (e.g., icam1 and vcam1). It thus promotes monocyte–EC interaction in addition to activation of macrophages [805]. Furthermore, it primes synthesis in ECs of pentraxin-3, an inflammatory mediator that contributes to the regulation of innate immunity and is involved in atherosclerosis.

Resistin induces C/EBPβ- and NFκB-dependent production of cytokines (e.g., TNFSF1, IL1α, and IL1β) and chemokines (e.g., CCL2–CCL3, CCL3L1, CCL4–CCL5, CCL8, and CXCL1–CXCL3), at least in human articular chondrocytes [975].

[191] Resistin: resistance to insulin. It suppresses the ability of insulin to stimulate glucose uptake into adipocytes. It is encoded by the RETN gene, which is transcribed during adipocyte differentiation. It homodimerizes or multimerizes, circulating in blood in low- and high-molecular-weight isoforms (7–22 ng/ml) [975]. Patients with acute coronary syndrome have significantly higher resistinemia (1.18 ± 48 μ g/l) than patients with stable angina pectoris (0.66 ± 0.40 μ g/l).

It is also termed C/EBPϵ-regulated myeloid-specific secreted cysteine-rich protein XCP1, CysH-rich secreted protein-A12α-like protein-2, protein found in inflammatory zone 3 (FIZZ3), and adipocyte-secreted factor (AdSF).

It belongs to the RELM family of resistin-like molecules that contains ReLMα and ReLMβ secreted by the AT and the gastrointestinal tract, respectively.

Resistin competes with lipopolysaccharide for binding to TLR4 in human myeloid and epithelial cells and launches the NFκB and MAPK pathways, thereby initiating the expression of TNFSF1, IL1β, and IL6 [975].

Chronic inflammatory atherosclerosis is primed by migration of circulating monocytes into the subendothelial space, where they differentiate into macrophages. Macrophages take up modified lipoproteins (VLDL, IDL, and LDL) and transform into foam cells. Macrophage class-A (ScaRa1–ScaRa3) and -B scavenger receptors (ScaRb1–ScaRb3) internalize oxLDLs. Macrophages infiltrating atherosclerotic lesions secrete resistin, which affects EC function and vSMC migration, especially during plaque rupture [975]. Resistin causes lipid accumulation, as it promotes oxLDL uptake via ScaRa and ScaRb3 by macrophages (and hence foam cell formation) and lowers concentration of ABCa1 used for cholesterol efflux. In addition, resistin affects fatty acid metabolism, as it raises cholesterol esterification and the availability of non-esterified (free) fatty acids in macrophages.

Resistin and Endothelial and Smooth Myocytic Function

Resistin is involved in angiogenesis, possibly via TNFSF12, along with endothelial and smooth myocytic dysfunction, thrombosis, and inflammation [975].

Resistin activates ECs and triggers endothelin-1 synthesis and release, in addition to adhesion molecules and chemokines [908]. On the other hand, it downregulates the formation of TRAF3, an inhibitor of TNFSF5-mediated EC activation [908, 975].

At high concentrations, resistin from eAT of ACS patients increases endothelial permeability [975]. In addition, at clinically relevant concentrations, resistin significantly lowers NOS3 synthesis. On the other hand, it raises ROS production. It operates via the P38MAPK and JNK kinases.

Resistin can provoke vSMC proliferation via ERK1, ERK2, and PKB in addition to migration via integrin activation [975].

5.4.5.11 Retinol-Binding Protein-4

Retinol-binding protein RBP4 belongs to the lipocalin family of proteic carriers of small hydrophobic molecules. It transports retinol (vitamin A) in the blood circulation from the liver to other organs. It binds to transthyretin, a carrier of thyroid hormones. Its circulating concentration is influenced by iron and ferritin. The liver has the highest RBP4 level; AT has the second highest RBP4 production rate [976]. Its synthesis is controlled by many agents (Table 5.29).

The adipokine and plasmatic retinol carrier RBP4 tethers to plasmalemmal receptors in addition to retinoic acid receptors NR1b1 to NR1b3 and NR2b1 to NR2b3. It is involved in glucose metabolism and insulin sensitivity. Circulating concentration of RBP4 correlates with insulin resistance and metabolic syndrome

Table 5.29 Regulation of RBP4 production in human adipocytes (Source: [976]; ↑ increase, ↓ decrease)

Regulator	RBP4 production and secretion
Estradiol	↑
Iron donor	↑
Iron buffer	↓
Leptin	↑
NR1c3 (PPARγ)	↑
TNFSF1 (TNFα)	↓

and serves as an independent predictor of adverse cardiovascular events. It links obesity to the metabolic syndrome.

Omental AT is an important RBP4 source in severely obese patients. Increased *rbp4emia* (plasmatic RBP4 concentration), which is linked to increased vAT mass (i.e., overweight and obese subjects) in addition to T2DM, provokes synthesis of the gluconeogenic enzyme PEPCK in the liver and impairs insulin signaling in the skeletal muscle [977]. However, transcription of the Rbp4 gene is downregulated in scAT of obese post-menopausal women and Rbp4-emia is similar in normal, overweight, and obese women [978]. A weight loss of 5% engenders only a small decline in adipose RBP4 production without marked change in Rbp4-emia.

In the AT, synthesis of RBP4 and GluT4 are reciprocally related. In mice, they are inversely correlated. In adipocytes, GluT4 concentration declines in overweight individuals and more so in obese subjects; it correlates positively with that of RBP4 [978]. Formation of RBP4 thus appears to be distinctly regulated in mice and humans.

In skeletal muscles, RBP4 decreases insulin sensitivity, at least partly via a decreased phosphorylation of IRS1 [978].

Concentration of RBP4 is elevated in atherosclerotic lesions, especially in regions enriched with macrophage-derived foam cells [979]. RBP4 favors macrophage-derived foam cell formation owing to upregulation of ScaRb3 synthesis via TLR4 and the Src–JNK–STAT1 axis and cholesterol uptake.

5.4.5.12 Vaspin (Serpin-A12)

Visceral AT-derived serpin (vaspin or serpin-A12) targets various peptidases such as kallikrein-7. It is predominantly secreted from vAT [980], and originates mainly in non-adipocytes. Vaspin is also expressed in the hypothalamus, pancreatic islets, stomach, and skin [981].

Vaspin synthesis depends on both age and gender [981]. Insulin sensitizers enhance vaspin synthesis. Glucose significantly increases vaspin formation in omental fat depots. Vaspinemia evolves with a daily profile, with a peak during the early morning fasting period and a significant postprandial drop 2 h after breakfast.

Vaspin plays a local and endocrine role. In the liver, it binds to the HSPa5[192] (GRP78)–DnaJc1 receptor complex.[193] In ECs, it targets a plasmalemmal complex made up of HSPa5 and voltage-dependent anion channel and exerts protective and proliferative effects. It inhibits NFκB in this cell type.

Vaspinemia correlates with the degree of obesity and insulin resistance. It can compensate for falling insulin sensitivity. It normalizes glycemia and modifies the transcription of genes involved in the genesis of insulin resistance, such as those encoding adiponectin, leptin, resistin, TNFSF1, and GluT4 [981]. This insulin sensitizer has an anti-inflammatory action.

In humans, obesity, insulin resistance, and T2DM are associated with increased vaspin transcription in the AT. In obese subjects and T2DM patients, vaspin concentration is 0.36 to 0.52 ng/ml higher than in healthy controls [983]. Higher vaspinemia is observed after physical activity in untrained individuals [981]. In obese mice, vaspin administration reduces food intake and improves glucose tolerance and insulin sensitivity [980].

[192] The molecular chaperone 70-kDa heat shock protein HSPa5 is also termed 78-kDa glucose-regulated protein (GRP78), a typical marker of ER stress. The ER is sensitive to stressors that reduce its protein folding capacity and can cause accumulation and aggregation of unfolded proteins, in particular, redox stress. An inflammatory response induces ER stress and UPR to recover a proper ER function or activate apoptosis. Proinflammatory mediators, such as TNFSF1, IL1, and IL6, provoke ER stress via ROS production. HSPa5 triggers UPR. Transcription of the genes encoding the ER chaperones HSPa5 and protein disulfide isomerase PDIa3 (a.k.a. endoplasmic reticulum protein ERP57; ERP60, and ERP61, in addition to glucose-regulated protein GRP57 and GRP58, is upregulated. Several factors are required for optimal protein folding, such as ATP, Ca^{2+}, and an oxidizing environment to allow disulfide bond formation. The protein chaperone PDIa3 catalyzes isomerization, reduction, and oxidation of disulfides. It interacts with the lectin chaperones calreticulin and calnexin to modulate folding of newly synthesized glycoproteins. HSPa5 is involved in the translocation of newly synthesized polypeptides across the ER membrane and their subsequent folding, maturation, transport, or retrotranslocation. It binds transiently to newly synthesized proteins in the ER and with higher affinity to misfolded, underglycosylated, or unassembled proteins. HSPa5 also localizes to the plasma membrane with MHC class-*I* molecules. It is a receptor for the uptake of certain viruses, and is also the receptor of α2-macroglobulin, in addition to LRP1 [982].

[193] DnaJc1: DnaJ (Hsp40) homolog, subfamily-C, member-1, which is also named murine tumoral cell DnaJ-like protein MTJ1. DnaJ-like proteins collaborate with members of the HSP70 family in protein conformation and oligomerization. The transmembrane protein DnaJc1 is a co-chaperone for HSPa5 ATPase activity.

5.4.5.13 Visfatin (NAmPT)

Visfatin[194] is a nicotinamide phosphoribosyltransferase[195] (NAmPT) and adipokine mainly produced in and secreted from vAT rather than scAT [901].[196] It is also released from pvAT. In the rat thoracic aorta, visfatin expression in pvAT is about four-fold and two-fold higher than that in scAT and vAT, respectively [901].

Intracellular visfatin works as a NAmPT and is implicated in the differentiation and survival of vSMCs [984]. Extracellular visfatin may operate as a paracrine messenger.

Visfatin provokes vasodilation via NO production. It also stimulates angiogenesis via VEGF and MMPs [892].

Visfatin, which acts as a NAmPT, synthesizing NMN from nicotinamide, stimulates vSMC proliferation via NMN-mediated activation of ERK1, ERK2, and P38MAPK [984].

Visfatin mimics insulin in cultured adipocytes, myocytes, and hepatocytes. In mice, it activates the insulin receptor and lowers glycemia [984].

Visfatin is implicated in obesity. Visfatinemia rises in overweight and obese individuals in addition to T2DM patients [985]. In Asian Indians, visfatinemia correlates with obesity, even after adjusting for age, sex, and diabetes [986]. Visfatinemia is associated with vAT, but not with subcutaneous fat depot. Among Iranian children and adolescents (aged 7–16 years), obese subjects have significantly higher insulinemia and leptinemia and lower adiponectinemia and visfatinemia [987].

5.4.5.14 Glucocorticoids

Adipocytes synthesize 11β-hydroxysteroid dehydrogenase 11β HSDH1, which converts inactive glucocorticoid precursors (cortisone) to active glucocorticoids (cortisol) [901]. Glucocorticoids hinder vSMC proliferation and migration and hence restenosis after angioplasty. Concentration of the HSD11B1 mRNA increases in eAT near the proximal tract of the right coronary artery in CoAD patients.

5.4.5.15 Sex Hormones

Initiation and progression of CVDs differ according to the gender owing to distinct protective and harmful effects of sex chromosomes and gonadal hormones, especially estrogens and androgens [988].

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[194] A.k.a. pre-B-cell colony-enhancing factor PBEF (PBEF1).

[195] It catalyzes the condensation of nicotinamide with 5-phosphoribosyl 1-pyrophosphate to synthesize nicotinamide mononucleotide (NMN), an intermediate in the synthesis of nicotinamide adenine dinucleotide.

[196] The name visfatin refers to its major synthesis site, visceral fat.

Adipose tissue produces sex hormones from precursors. Estrogens suppress or support vSMC proliferation according to the vSMC phenotype [901]. Androgens reduce intimal hyperplasia in rabbits and exert an antiproliferative, proapoptotic, and anti-migrative action on cultured vSMCs.

However, testosterone concentration declines with aging (i.e., mano- or andropause [a drop of 1–2% per year]), hypogonadism being defined by a testosterone concentration lower than 8–11 nmol/l (<2.30–3.19 ng/ml) [989]. In addition, conversion of testosterone to estradiol by aromatase rises with aging.

The inactive form of testosterone represents 68% of total testosterone. It tethers to sex hormone-binding globulin (SHBG) [989]. Concentration of SHBG increases with aging. Its active form circulates loosely bound to albumin (30%) or freely (1–2%). Testosterone is associated with vasodilation in addition to -constriction.

At a similar age, men are at a greater CVD risk than women [990]. Testosterone triggers calcifications of vSMCs, a predictor of CVD-primed morbidity and mortality, via androgen receptor. Testosterone and dihydrotestosterone increase the concentration of tissue-nonspecific alkaline phosphatase. A low testosterone concentration correlates with a cardiometabolic risk (hypertension, dyslipidemia, and insulin resistance, in addition to atherosclerosis and thrombosis) [989].

5.4.5.16 Adrenomedullin

Adrenomedullin (Adm) is synthesized by the adrenal gland, heart, and vascular endothelial and smooth muscle cells in addition to white adipocytes in humans [891]. It primes vasodilation of coronary and skeletal muscle arteries via NO. It also reduces ROS levels in vSMCs.

Stimulated β3-adrenoceptors on adipocytes provoke lipolysis and release of stored adipokines (but impedes that of leptin), whereas adrenomedullin, the secretion of which is caused by lipolysis, prevents lipolysis via NO [891].

Epicardial AT from CoAD patients exhibits a higher adrenomedullin concentration than that in subjects without patent CoAD [891].

5.4.5.17 Renin–Angiotensin Axis

Angiotensinogen, the precursor of the vasoconstrictor angiotensin-2 (Agt2; Sect. 3.9.4), is synthesized in adipocytes. Brown and white adipocytes are not only sources of angiotensinogen but also possess enzymes necessary to produce Agt2 [892]. Effectors of the renin–angiotensin axis, such as the convertase ACE and AT_1 and AT_2 receptors, are indeed identified in human adipocytes [891]. Synthesis of angiotensinogen and Agt1 is similar between white and brown adipocytes, but that of Agt2 differs in brown and white perivascular adipocytes.

In the rat AT, almost all RAA genes are transcribed, except for those that encode renin, AT_2, and insulin-regulated aminopeptidase (or AT_4 receptor), thereby producing angiotensinogen in addition to RAA enzymes (angiotensin convertases

ACE1 and ACE2, chymase, and aminopeptidases APa and APn) and receptors (AT_{1A}, AT_{1B}, Mas [AT_3 receptor], renin and prorenin receptor (PRR) encoded by the Atp6ap2 gene,[197] and renin and prorenin clearance receptor IGF2R) [901].[198]

Angiotensin-2 provokes vSMC hypertrophy, hyperplasia, and migration via AT_1 and hence adverse vascular remodeling in response to hypertension and vascular injury, whereas AT_2, which is expressed in the developing vasculature and re-expressed in adult vSMCs in response to vascular injury, has opposite effects [901]. $Angiotensin_{(1–7)}$ prevents Agt2-induced vSMC proliferation and migration partly via inhibition of Agt2-mediated activation of ERK1 and ERK2.

Angiotensin-2 secreted by the pvAT potentiates vasoconstriction launched by perivascular neurons in rat mesenteric arteries [901]. $Angiotensin_{(1–7)}$ is a pvAT-generated diffusible RAA product that stimulates NO release from the endothelium, which hyperpolarizes SMC via K_{Ca} channels [891].

However, Agt2 favors NO metabolism into reactive nitrogen species, promoting expression of adhesion molecules and proinflammatory cytokines in ECs [892].

Angiotensin-2 stimulates NFκB, which activates the transcription of genes encoding CSF1, icam1, vcam1, and CCL2 in vascular cells [908]. Angiotensin-2 also launches the formation of free radicals from NAD(P)H oxidase.

[197] ATP6AP2: ATPase H^+-transporting lysosomal accessory protein-2. Renin and prorenin receptor bind prorenin with higher affinity than renin [991]. It does not internalize these proteins. Binding of prorenin to the PRR receptor triggers prorenin activation without cleavage of the prosegment its catalytic activity results from a conformational change. Both PRR and vesicular proton ATPase (vesicular ATP synthase membrane sector-associated protein M8.9 [vATPase M8.9 subunit]) derive from the same transcript. The M8.9 fragment corresponds to the cytoplasmic, transmembrane, and a part of the extracellular domain of the receptor.

Both renin and prorenin activate ERK1 and ERK2 and trigger TGFβ1 release in mesangiocytes [991]. TGFβ1 increases concentrations of plasminogen-activator inhibitor PAI1, fibronectin, and collagen-1. Hence, prorenin directly provokes fibrosis in rats overexpressing PRR [991]. In CMCs, prorenin activates P38MAPK, which phosphorylates heat shock proteinβ1 (HSP27), acting independently of angiotensin-2 [992]. HSPβ1 regulates actin filament dynamics. In rats with hepatic prorenin overexpression, prorenin-induced stimulation of the P38MAPK–HSPβ1 pathway alters actin filament dynamics and contributes to severe adverse cardiac hypertrophy.

[198] The cation-independent mannose 6-phosphate and insulin-like growth factor-2 receptor ([CI]M6PR and IGF2R, also aliased M6P/IGF2R), binds phosphomannosylated (M6P-containing) proteins, such as renin and prorenin. It thus internalizes renin and prorenin. It does not serve for intra- and extracellular angiotensin generation, but instead as a clearance receptor for prorenin and renin, the prorenin and renin receptor being used for uptake of circulating prorenin and renin [991]. It also transports phosphorylated lysosomal enzymes from the GB and the cell surface to lysosomes [108]. Lysosomal enzymes bearing phosphomannosyl residues bind specifically to M6PR in the GB and the resulting receptor–ligand complex is transported to an acidic prelysosomal compartment, where it is dissociated. This receptor also binds IGF2 in addition to dipeptidyl peptidase DPP4, a plasmalemmal glycoproteic receptor involved in the T-cell receptor-mediated T-cell coactivation.

5.4.5.18 Agouti Signaling Protein

Agouti signaling protein (ASiP), the human homolog of murine agouti, is a paracrine factor secreted by dermal papillae cells adjacent to melanocytes that is produced at its highest levels in human adipocytes. It promotes insulin sensitivity. It elicits formation of the transcription factors STAT1, STAT3, and PPARγ (NR1c3) [993]. Agouti-related protein and ASiP compete with POMC-derived peptides for binding to melanocortin receptors to regulate food intake and energy expenditure. Agouti signaling protein participates in adipogenesis regulation coordinated with food intake and energy expenditure, as melanocortin receptor in adipocytes regulates both pre-adipocyte proliferation and differentiation [994]. Thus, it increases adiposity.

5.4.5.19 Secreted Frizzled-Related Protein-5

The sFRP proteins are soluble modulators that sequester Wnt messengers in the extracellular medium. The protein SFRP5 is formed in WAT in lean mice. Its synthesis in the vAT and its circulating concentration decrease in obese humans, particularly in individuals with impaired glucose tolerance and insulin resistance [805].

SFRP5 hampers Wnt5a signaling, which contributes to proinflammatory cytokine production upon JNK activation [805]. Moreover, Wnt5a contributes to endothelial dysfunction in diabetic patients and supports inflammatory reactions in macrophages and ECs.

5.4.5.20 Fatty Acid-Binding Protein-4

Fatty acid-binding proteins comprise nine organ-specific species (Table 5.30). They bind lipophilic molecules intracellularly and hence influence lipid transport and metabolism.

Table 5.30 Fatty acid-binding proteins (FABPs)

Subtype	Other name(s)
FABP1	Liver FABP (lFABP)
FABP2	Intestinal FABP (iFABP)
FABP3	Muscle and heart FABP (hFABP)
FABP4	Adipose tissue FABP (aFABP)
FABP5	Epidermal FABP (eFABP)
FABP6	Ileal FABP (ilFABP)
FABP7	Brain FABP (bFABP)
FABP8	Myelin FABP (mFABP), peripheral myelin protein PMP2
FABP9	Testis FABP (tFABP)

The FABP4 subtype is an adipokine released from adipocytes in obese subjects in addition to activated macrophages. It is also produced in ECs.

FABP4 is one of the most abundant intracellular lipid transporters in adipocytes and macrophages [831]. Once it is liganded, FABP4 translocates from the cytosol to the nucleus, where it delivers its ligand to the nuclear receptor NR1c3 (PPARγ). It supports both basal and hormone-stimulated lipolysis in response to β AR stimulation [831]. It also enhances hepatic glucose production [717].

It is implicated in the regulation of energetic metabolism and inflammation and in the genesis of chronic metabolic diseases (e.g., T2DM, atherosclerosis, steatosis, asthma, and cancer).

It augments insulin release [995]. Circulating FABP4 concentration correlates with glucose-stimulated insulin secretion, which is linked to adipocyte-to-β-cell signaling via insulinotropic FABP4 action. Conversely, insulin precludes FABP4 secretion from adipocytes in humans, hence determining a negative feedback loop.

Adipose triglyceride lipase (ATGL)[199] and, to a lesser extent, LipE (or HSL)[200] assist FABP4 secretion in exosome-like vesicles from adipocytes upon stimulation of lipolysis [997]. Lipolysis increases in prolonged fasting in addition to in obesity, insulin resistance, and diabetes.

In T2DM patients, among the adipokines FABP4, RBP4, and $Adpn_{hMW}$, greater concentrations of FABP4 and $Adpn_{hMW}$ correlate significantly with a higher CVD-linked mortality [850].

5.4.5.21 Desarginated Complement Component C3 (ASP)

Desarginated complement component C3[201] ($C3a^{desArg}$) is a protein of the alternative complement pathway and messenger produced by adipocytes. It derives from the complement C3 complex cleavage, which relies on adipsin (a serine peptidase and a constituent of the alternative complement pathway, also named complement factor-D), complement factor-B, and a carboxypeptidase [888]. It connects to C5aR2.

Desarginated complement component C3 derived from pvAT stimulates TG synthesis via PLC, PKB, and MAPK in addition to glucose transport in adipocytes, thereby regulating lipid storage and contributing to postprandial TG

[199] A.k.a. desnutrin, patatin-like phospholipase PnPLA2, and calcium-independent phospholipase-A2ζ (iPLA2ζ). Adipose triglyceride lipase is a TG-specific lipase. It shares the greatest sequence homology with adiponutrin (a.k.a. PnPLA3 and iPLA2ϵ). In AT, during fasting, activity of PnPLA2 rises and that of PnPLA3 lowers [996]. In adipocytes, insulin decreases the formation of PnPLA2 and increases that of PnPLA3. PnPLA2 provokes glycerol and non-esterified fatty acid release, but not PnPLA3. PnPLA2, but not PnPLA3, contributes to lipolysis in adipocytes.

[200] HSL is an important lipolytic enzyme in adipocytes. Lipolytic hormones such as catecholamines stimulate lipolysis primarily via PKA, which phosphorylates HSL and perilipin-As [996]. The former translocates from the cytosol to the LD, where it processes tri-, di-, and monoglycerides in addition to cholesteryl esters. The latter ensures a protective coating of LDs; its phosphorylation alleviates its barrier function.

[201] A.k.a. ASP.

clearance. It augments glucose-stimulated insulin secretion by β cells via glucose phosphorylation, calcium uptake, and PKC [907].

It is also implicated in the postprandial clearance of triacylglycerol and stimulates uptake of fatty acids into white adipocytes and their esterification. It increases lipogenesis via translocation of GluT4, glycerol 3-phosphate, and diacylglycerol acyltransferase [887].

In hypertensive rats, it stimulates adventitial fibroblast migration and differentiation via JNK, causing adventitial thickening and myofibroblast clustering around pvAT [901].

5.4.5.22 Pentraxin-1 (C-Reactive Protein)

Pentraxin-1 is an acute-phase reactant synthesized mainly in the liver upon exposure to IL6, adipocyte-derived IL6 being a major regulator of hepatic Ptx1 production, but also IL1 and TNFSF1. In healthy subjects, about 30% of circulating IL6 originates from the AT [908]. It likely rises to a much higher level in obese subjects.

Pentraxin-1 is an inflammatory proatherogenic adipokine. Its plasmatic concentration is elevated in obese subjects; it correlates with visceral obesity and the metabolic syndrome.

The AT can be a source of Ptx1. A two-fold increase in PTx1 expression can be observed in fatty depots of obese leptin receptor-deficient db/db mice and cp/cp rats with respect to lean controls [908]. Pentraxin-1 is also synthesized in human AT.

In vSMCs, Ptx1 upregulates formation of angiotensin receptor AT_1, hence facilitating ATn2-induced ROS production and vSMC migration and proliferation, and vascular remodeling [908].

In ECs, pentraxin-1 induces the expression of icam1, vcam1, selectins, and CCL2 via ET1 and IL-6 [908]. It also attenuates basal and stimulated endothelial NO production, hence impeding angiogenesis. The effect of Ptx1 on endothelial dysfunction is potentiated by hyperglycemia.

Pentraxin-1 coordinates and amplifies the pro-.inflammatory activity of other adipokines. In ECs, it supports the synthesis of serpin-E1 (PAI1), a prothrombotic acute phase protein that hampers plasminogen activation and hence fibrinolysis.

5.4.5.23 Serpin-E1 (PAI1)

ECs are major sources with platelets of the serine peptidase inhibitor serpin-E1 (or plasminogen activator inhibitor-1), a potent and fast inhibitor of both types of plasminogen activators (tissue- and urokinase-type plasminogen activators).[202]

[202] Both tPA and uPA (or simply urokinase) convert inactive plasminogen, a hepatic glycoprotein, to plasmin, a serine peptidase that cleaves fibrin, extracellular matrix components, and MMPs.

Serpin-E1 is also produced in the AT that can become an important contributor to obesity. Higher serpin-E1 amounts are formed in omental than in scAT. In the AT, synthesis of serpin-E1 is upregulated by TNFSF1, TGFβ, ATn2, FFAs, hyperinsulinemia, and hypertriglyceridemia [908].

Organismal aging and associated cellular senescence are accompanied by a marked increase in the synthesis of serpin-E1, a marker and mediator of cellular senescence [998]. In addition, sustained redox stress causes DNA damage, which initiates cellular senescence; this *stress-induced premature senescence* accelerates aging. Nevertheless, senescent cells are active, as they produce and release numerous factors such as serpin-E1 (*senescence-messaging secretome*).

Regulation of replicative senescence by serpin-E1 involves the PI3K–PKB–GSK3–CcnD1 pathway [998]. Serpin-E1 is also involved in TGFβ-induced senescence, TGFβ upregulating PAI1 expression.

Serpin-E1 may impede proteolytic degradation of IGF-binding protein IGFBP3, which stimulates cellular senescence [998].

In ECs, sirtuin-1 suppresses formation of serpin-E1, P53, and CsnKI1a, but induces that of ^{Mn}SOD2, preventing senescence and enhancing NOS3 expression [998].

Consumption of vitamin C, vegetables, and fruits diminishes serpin-E1 concentration, thereby improving fibrinolysis and reducing the thrombosis risk [998].

5.4.5.24 Serum Amyloid-A

Serum amyloid-A (SAa) is another acute-phase reactant that can be used as an atherosclerosis marker. Its plasmatic concentration correlates significantly with insulin resistance and obesity in patients with type-2 diabetes mellitus.

Adipose tissue produces SAa at low concentrations under normal conditions. Its synthesis in AT is upregulated in diabetes [908]. It displaces apolipoprotein-A1 from HDL^{CS}, increasing HDL binding to macrophages and decreasing the protective effect of HDLs.

5.4.5.25 Interleukin-6

Interleukin-6 is secreted from several organs and circulates in blood, but AT is a major source of this endocrine proinflammatory cytokine. It is released by adipocytes, pre-adipocytes, and macrophages to a 2–3 times greater extent in vAT than in scAT in humans.

Serpin-E1 thus stabilizes fibrin. Furthermore, it promotes formation of a lysis-resistant crosslinked fibrin clot.

In addition, tPA mediates conversion of proapoptotic pre-BDNF to antiapoptotic BDNF. Serpin-E1 thus impairs BDNF processing and hence normal neuronal function [998]. In mice, uPA overexpression in the brain reduces feeding behavior, but prolongs life duration.

In classical signaling, IL6 stimulates target cells via the IL6 receptor, which lodges in the plasma membrane of few cell types. In trans-signaling, IL6 bound to a naturally occurring soluble form of IL6R stimulates cells via ubiquitous glycoprotein-130.

Interleukin-6 promotes synthesis of adhesion molecules on ECs and lymphocytes, monocyte-to-macrophage differentiation, antibody production by B lymphocytes, and recruitment of T lymphocytes to sites of injury [805]. It induces insulin resistance in hepatocytes and ECs, but supports insulin sensitivity in skeletal myocytes under some circumstances. It prevents cholesterol deposition in the vessel wall, as it increases cholesterol efflux in macrophages and HDL translocation through the endothelium, but favors wall inflammation [805].

Acute exposure of IL6 relaxes isolated aortas in addition to in vivo skeletal muscle resistance vessels, possibly via prostacyclin [892].

5.4.5.26 TNFSF1 (TNFα)

The inflammatory cytokine tumor-necrosis factor-α (TNFSF1) is released in relatively large quantities in obese humans and subjects with insulin resistance [908].

TNFSF1 is synthesized as a transmembrane monomer (^{mono}TNFSF1) that is cleaved by the sheddase adam17 to a soluble molecule (TNFSF1^{S}). Both ^{mono}TNFSF1 and TNFSF1^{S} have an impact on metabolism, the former via auto- and paracrine effects, the latter as an endocrine messenger [716]. TNFSF1 operates via two receptors (TNFR1–TNFR2). Local chronic production of TNFSF1 enables remote effects.

TNFSF1 activates the transcription factor NFκB, which primes production of adhesion molecules and chemokines in endothelial and vascular smooth muscle cells. It lowers NO availability in ECs, hence altering endothelium-dependent vasodilation [908]. Furthermore, TNFSF1 can activate NOx in vSMCs, thus increasing ROS formation and decreasing NO availability [720]. In addition, it stimulates lipolysis and FFA release.[203]

It can also impair metabolic signaling via the insulin receptor and its substrates [908].[204] Many factors implicated in the development of insulin resistance such as TNFSF1, FFAs, and Ser phosphatase inhibitors can activate inhibitor of NFκB (IKK) and its effector, NFκB. The IKKβ–NFκB pathway hence mediates insulin resistance. The kinase IKKβ phosphorylates (inhibits) InsR and IRS1 (Ser312) in response to TNFSF1, impairing IRS1 ability to activate PI3K [999].

[203] Obesity is characterized by an elevated circulating FFA concentration that impairs endothelium-dependent vasodilation via activation of the renin–angiotensin axis and ET1 release [720].

[204] Four IRS isoforms (IRS1–IRS4) are expressed in a tissue-specific manner. Activated insulin receptor phosphorylates IRS on multiple Tyr residues that serve as docking sites for effectors with metabolic actions. They are also phosphorylated on Ser residues to regulate their function. Phosphorylation of IRS1 (Ser312 and Ser616 in humans) impairs insulin signaling. IRS1 is a substrate for CsnK2, ERK, JNK, GSK3, PKB, PKCζ, and TOR [999].

It is a vasoconstrictor and vasodilator via both endothelium-dependent and -independent mechanisms using NO, prostaglandins, endothelin-1, angiotensin, and hydrogen peroxide [892].

5.4.5.27 Reactive Oxygen Species

Reactive oxygen species are intracellular signaling mediators (second messengers) that regulate vSMC hypertrophy, proliferation, and migration. Normal ROS concentrations favor cell growth and proliferation, whereas excess concentrations cause cell death. Migration of vSMCs is triggered by IGF1, PDGF, VEGF, CCL2, and thrombin depends on ROS [901].

In obese mice, superoxide anion and hydrogen peroxide production in adipose tissue rises. Excess vascular superoxide quantities are mainly synthesized by NAD(P)H and xanthine oxidases, uncoupled NOS, and mitochondrial ETC complexes [895]. In obesity, altered ROS production can arise from the adverse effects of adipokines liberated from pvAT, adiponectin secretion being reduced and that of leptin, resistin, TNFSF1, and IL6 elevated.

Leptin favors redox stress in ECs. The eAT suffers greater redox stress than the scAT in CVD patients [901].

Reactive oxygen species produced by pvAT in addition to vascular endothelial and smooth muscle cells modulate the vasomotor tone [892]. They interact with endothelium-produced NO, diminishing vasodilation in resistance arteries and arterioles. In addition, ROS upregulate expression of adhesion and chemoattractants, thereby contributing to endothelial dysfunction and inflammation.

Pathological concentrations of leptin (625 pmol/l) attenuate vasodilation primed by ACh of coronary arteries in normal dogs, but not physiological concentrations (250 pmol/l) [895]. Resistin lowers or not bradykinin-induced dilation of canine coronary arteries via ROS.

Superoxide anions can induce vasoconstriction using Ca^{2+} ions. It can operate via H_2O_2 [892]. Furthermore, it lowers NO availability via peroxynitrite formation.

Hydrogen peroxide, a more stable and less reactive agent, causes vasodilation or -constriction according to its concentration, mammalian species,[205] vascular compartment, membrane potential, and degree of obesity [892]. Vasodilation induced by H_2O_2 relies on the release of NO and ^{SMC}soluble guanylate cyclase[206] in addition to vasodilatory PGhS-processed products and endothelium-independent mechanism involving potassium channels (K_{Ca}) of smooth myocytes. Vasoconstriction launched by H_2O_2 depends on Ca^{2+}, vasoconstrictory PGhS products, PKC, PLC, among other agents.

[205] Hydrogen peroxide is a major vasodilator released from the endothelium of porcine coronary arterioles. In humans, ECs of coronary arterioles also generate H_2O_2 [895].

[206] The BK channel is strongly activated by the sGC–cGMP–PKG pathway [866].

5.4.5.28 Gaseous Messengers

Nitric oxide is released by adipocytes and ECs of the vasa vasorum in AT [901]. The subtype NOS3 is the most abundant in pvAT, at least in the saphenous vein. Its production rises in mesenteric pvAT during early DIO. In AT of obese subjects that experiences a low-grade chronic inflammation, NOS2 expression is upregulated.

In addition, NO regulates vSMC migration and proliferation, favoring or hampering these processes according to the vSMC phenotype [901]. It can indeed prevent migration and proliferation of relatively dedifferentiated vSMCs, but potentiates the effect of growth factors on migration and proliferation of differentiated vSMCs. The NOS subtype also influence the response type [901]. NOS3 represses and NOS2 supports intimal hyperplasia.

Hydrogen sulfide is also synthesized and secreted in the pvAT, but its production decays with aging [901]. Hydrogen sulfide can also preclude vSMC proliferation and apoptosis, thereby countering intimal hyperplasia.

5.5 Nuclear Receptors FXRs, LXRs, PXR, and RXRs

Ligand-activated transcription factors of the nuclear receptor class are implicated in the metabolism of cholesterol and fatty acids, synthesis, transport, and catabolism of bile acids, and the processing of lipoproteins in addition to drugs and xenobiotics, especially liver X-activated receptors[207] (LXRα–LXRβ [NR1h3–NR1h2]), farnesoid X-activated receptors (FXRα–LXRβ [NR1h4–NR1h5]), and pregnane X receptor (PXR [NR1i2]; Tables 5.31 and 5.32) [1000]. The receptors of the FXR, LXR, and PXR sets heterodimerize with the retinoid X receptors (RXRα–RXRγ [NR2b1–NR2b3]) for DNA binding.

Bile acids not only serve as detergents for lipid digestion and absorption but also exert signaling effects in lipid and glucose homeostasis, thermogenesis, liver regeneration, and immunomodulation [1001]. However, bile acids are cytotoxic. Therefore, their concentration is controlled by transcriptional programs activated by bile acid-activated nuclear receptors.

The primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA) are synthesized in the liver from cholesterol or oxysterols and then transported across the basement membrane of the hepatocyte into bile canaliculi and stored in the gallbladder. After their excretion into the intestinal lumen, bile acids can be further metabolized by bacteria into secondary (deoxycholic acid [DCA] and lithocholic acid [LCA]) and tertiary bile acids before being resorbed in the distal ileum [1000]. Bile acids then return to the liver to complete their enterohepatic travel. Secondary

[207] Natural ligands of these receptors were unknown (hence the term "X"); these receptors were originally termed orphan nuclear receptors.

Table 5.31 Regulation of gene transcription in the hepatocyte FXR, LXR, and PXR (Source: [1000]; $\oplus \longrightarrow$ stimulation, $\ominus \longrightarrow$ inhibition, *BA* bile acid, *CS* cholesterol, *FA* fatty acid, *PL* phospholipid, *TG* triglyceride)

Nuclear receptor	Effect
FXR	$\oplus \longrightarrow$ NR0b2$\ominus \longrightarrow$ CyP7a1
	$\oplus \longrightarrow$ ABCb11, ABCc2
	$\oplus \longrightarrow$ ApoC2
	$\oplus \longrightarrow$ PLTP
LXR	$\oplus \longrightarrow$ ABCa1 (CS and PL efflux)
	$\oplus \longrightarrow$ CETP
	$\oplus \longrightarrow$ CyP7a1 (BA synthesis)
	$\oplus \longrightarrow$ SREBP1c (TG and PL synthesis from FAs)
	$\oplus \longrightarrow$ conversion of acetyl-CoA to FAs
PXR	$\oplus \longrightarrow$ CyP3a
	$\oplus \longrightarrow$ ABCb1, ABCc2
	$\oplus \longrightarrow$ SLCO1b1

Table 5.32 Lipoprotein processing in blood and regulation by FXR and LXR of transcription of genes that encode its major agents (Source: [1000]; *CETP* cholesterol ester transfer protein, *CM* ApoC2+ ApoE+ chylomicron [FXR and LXR target Apoc2 and ApoE, respectively], *HDL* high-density lipoprotein, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoprotein, *LPL* lipoprotein lipase, *PLTP* phospholipid transfer protein, *VLDL* very-low-density lipoprotein)

Factor	Role	Regulators
CETP	Conversion of HDL to LDL	LXR
	Conversion of VLDL to HDL	
PLTP	Conversion of CM to preHDL	FXR
	Conversion of VLDL to preHDL	
LPL	FA delivery to cell from VLDL, CM	LXR

Chylomicrons are also transformed to chylomicron remnants, pre-HDLs to HDLs, and VLDLs to IDLs and then LDLs

bile acids are weakly absorbed in the distal ileum. Cholestasis (i.e., reduction or cessation of the intra- or extrahepatic bile flow) can cause hepatic accumulation of both toxic bile acids (e.g., LCA and 3-ketoLCA) and toxins.

Water-soluble bile acids are synthesized from water-insoluble cholesterol exclusively by the liver. The neutral and acidic pathways are involved in bile acid synthesis. The rate-controlling enzyme CyP7a1[208] is the first processor of the neutral pathway, which is partly controlled by a negative bile acid feedback loop. The main enzyme of the acidic pathway, CyP27a1,[209] is not regulated by bile acids.

[208] A.k.a. cholesterol 7α-hydroxylase.

[209] A.k.a. mitochondrial sterol 26- and 27-hydroxylase and vitamin-D_3 25-hydroxylase.

Once they are conjugated with glycine or taurine, bile acids are actively secreted by hepatocytes into the bile canaliculi, which drain into the intrahepatic bile ducts, are stored in the gallbladder, and expelled into the intestinal lumen in response to a fatty meal.

Bile acids preclude the synthesis of further bile acids, as they indirectly downregulate the synthesis of CyP7a1 and hence of chenodeoxycholic acid [1000]. Bile acids also repress CA production, as they prevent transcription of the CYP8B1 gene.[210] Furthermore, bile acids provoke cytokine formation in Kupffer cells, which, once they are released, suppress CyP7a1 activity in adjacent hepatocytes.

ATP-binding cassette transporters for bile acids and organic anions at the canalicular membrane of hepatocytes represent the rate-limiting step in bile formation [1001]. Bile acids promote canalicular excretion of phospholipids and cholesterol for subsequent formation of mixed biliary micelles and osmotically drag water via aquaporins and tight junctions. Canalicular bile is also constituted by excreted reduced glutathione and bicarbonate. Ductal bile, that is, bile inside biliary ductules and ducts, represents a modified canalicular bile due to further material secretion and absorption.

The ABCb11 carrier[211] on the canalicular membrane of hepatocytes, the synthesis of which is stimulated by FXR, enables secretion of bile acids and salts from the hepatocyte into the biliary canaliculus (Table 5.33). Monovalent bile acids such as glycine- or taurine-amidates of cholic, chenodeoxycholic, and ursodeoxycholic acid are excreted into the bile canaliculus through ABCb11 [1001]. Divalent bile acid anions, such as sulfated tauro- and glycolithocholate, are carried by ABCc2, in addition to organic anions, mostly conjugates with glutathione, glucuronidate, and sulfate in addition to reduced glutathione (G^{SH}). ABCb1 transfers amphipathic organic cations (e.g., various drugs) and ABCc4 PC. ABCg5 and ABCg8 export sitosterol and cholesterol. In addition, ATP8b1 and SLC4a2 are involved in bile formation. Bile acids stimulate ABCb11 and ABCc2 expression in a feedforward manner, thereby promoting their biliary elimination [1001].

Alternative bile acid export through the basolateral membrane of the hepatocyte back into portal blood represents an alternative elimination route for accumulating hepatic bile acid during cholestasis when canalicular excretion is impaired [1001].

In the small intestine, bile acids emulsify and facilitate absorption of dietary lipids and lipid-soluble vitamins. Subsequently, they are reabsorbed from the terminal ileum using specific transporters and about 95% return to the liver to be secreted again into the bile, that is, via the *enterohepatic circulation*, the remaining 5% escaping reabsorption being removed with feces [1002]).

The fraction lost per enterohepatic circulation cycle is compensated for by hepatic synthesis from cholesterol, which keeps the bile acid pool constant.

[210] Cytochrome-P450-8B1 is also named 7α-hydroxy 4-cholesten 3-one 12α-hydroxylase and sterol 12α-hydroxylase.

[211] ATP-binding cassette carrier-B11 is also termed BSEP.

Table 5.33 Hepatic bile acid transport through apical (canalicular) membrane and the basolateral (sinusoidal) membrane (alternative export and uptake) and its regulation by nuclear receptors (Source: [1001])

Involved carriers	Regulatory nuclear receptors
Canalicular export	
Bile acid export	
ABCb11	FXR
ABCc2	FXR, NR1i2–NR1i3, RAR
Cholesterol export	
ABCg5/8	FXR, LXR
Phospholipid export	
ABCb4	FXR, NR1c1
Alternative export	
ABCc3	NR1i3
ABCc4	NR1i1–NR1i3, NR5a2
OST	FXR
Import	
SLC10a1	NR2a1, NR3c1, RAR
OATP1a1/1a4/1b2 (rodents)	NR1i2 (OATP1a4), NR2a1, RAR (OATP1b2)
OATP1b1 (humans)	

Systemic bile acids, which escape the enterohepatic circulation, are filtered and excreted into urine and again reabsorbed by transporters in the proximal convoluted tubule [1000].

The bile acid *cholehepatic shunting* from the bile duct lumen consists of a transfer via cholangiocytes and the periductular capillary plexus back to hepatocytes.

In the ileum, bile acids are taken up by enterocytes through SLC10a2[212] and then carried by the shuttle FABP6[213] from the apical to the basolateral membrane to be secreted into the portal venous circuit blood by a truncated form of SLC10a2 ($_t$SLC10a2), ABCc3, or heteromeric organic solute transporter OSTα–OSTβ, which is involved in sterol transport and may be the primary bile acid efflux transporter in the intestine [1002]).

Bile salts returning to the liver after intestinal absorption are mainly taken up (∼75%) by SLC10a1[214] and by SLCO1b1 (or SLC21a6) [1002]).[215]

[212] A.k.a. apical sodium-dependent bile acid transporter and intestinal or ileal apical bile acid transporter (iBAT).

[213] Fatty acid-binding protein-6 is also called gastrotropin, intestinal bile acid-binding protein, and ileal lipid-binding protein.

[214] A.k.a. Na^+–taurocholate cotransporter. Bile acids downregulate SLC10a1 expression via NR0b2 activation, which inhibits RXR–NR1b1 transactivation [1002].

[215] Solute carrier organic anion transporter SLCO1b1 is also termed liver-specific organic anion transporter, LST1, and organic anion transporter polypeptide, OATP2, OATP1b1, and OATPC.

Bile acids operate as hormone-like ligands, in addition to solubilization and absorption of lipids and lipid-soluble vitamins from the intestinal lumen. Both the FXR and pregnane X receptor are activated by bile acids.

5.5.1 FXRs

The FXR [216] regulates bile acid synthesis, conjugation, and transport, in addition to lipid and glucose metabolism. It is activated by bile acids with the rank order of potency CDCA > DCA = LCA > CA [1003]. Polyunsaturated fatty acids, such as arachidonic, linolenic, or docosahexaenoic acid, in addition to intermediates of bile acid synthesis, are also FXR ligands [1002]). In cholestasis or inborn metabolic disorders, bile acid intermediates, which exist in large amounts, can be FXR agonists.

On the one hand, FXR can launch gene transcription as a monomer (e.g., the UGT2B4 gene) or a heterodimer with RXR (e.g., the Pltp gene). On the other, FXR can repress gene transcription indirectly via NR0b2 (e.g., the CYP7A1 gene), or directly as a monomer (e.g., the APOA1 gene) or heterodimer (e.g., the APOC3 gene) [1002]). PGC1α is a FXR cofactor. Four isoforms (FXRα1–FXRα4) do not play the same role in gene transactivation.

The FXR regulates bile acid synthesis, transport, and detoxification (Table 5.34). Its target genes include NR0B2,[217] Fabp6,[218] ABCB11, Pltp[219] and APOC2. Activated FXR reduces triglyceridemia, inhibits bile acid synthesis, and raises transport of bile acids from the intestinal lumen into the enterocytes and back to the liver.

Activated FXR induces expression of NR0b2,[220] which interacts with two other nuclear receptors that transactivate the CYP7A1 gene, NR2a1,[221] and NR5a2.[222] NR0b2 represses CYP7A1 gene transcription, as it causes dissociation of coactivators linked to NR2a1 and NR5a2 in addition to histone deacetylation of the promoter [1002]).

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[216] The isoprenoid farnesol derives from the hydrolysis of farnesyl diphosphate. It participates in the control of HMGCR stability. However, it does not directly interact with FXR [1000].

[217] A.k.a. small heterodimer partner (SHP).

[218] Fatty acid-binding protein-6 is involved in enterohepatic bile acid metabolism. It is required for efficient apical to basolateral transport of conjugated bile acids in ileal enterocytes with the affinity order of potency taurine-conjugated > glycine-conjugated > unconjugated bile acids. It binds to bile acids with the order of potency DCA > CA > CDCA [108]. It stimulates gastric acid and pepsinogen secretion. It functions as the cytosolic receptor for bile acids that have undergone sodium-dependent active transport using SLC10a2 (or iBAT) [194].

[219] PLTP: phospholipid transfer protein.

[220] A.k.a. SHP.

[221] A.k.a. hepatic nuclear factor HNF4.

[222] A.k.a. liver receptor homolog LRH1.

Table 5.34 Effects of FXR on bile acid (BA) processing (Source: [1002]; $\oplus \longrightarrow$ stimulation, $\ominus \longrightarrow$ inhibition, *BAAT* bile acid–CoA:amino acid ^{N}acetyltransferase, *BACS* bile acid coenzyme A (CoA) synthetase, *SLCO* solute carrier organic anion transporter, *SulT2a1* bile salt sulfotransferase, *UGT* UDP-glucuronosyl transferase)

Hepatocyte	
BA synthesis	$\ominus \longrightarrow$ synthesis of CyP7a1
	FXR$\oplus \longrightarrow$ NR0b2; NR0b2–NR5a2$\ominus \longrightarrow$ CyP7a1
	NR0b2–NR2a1$\ominus \longrightarrow$ CyP8b1
	FXR$\oplus \longrightarrow$ FGF19; FGF19–FGFR4$\oplus \longrightarrow$ JNK; JNK$\ominus \longrightarrow$ CyP7a1, CyP8b1
BA conjugaton	$\oplus \longrightarrow$ synthesis BAAT, BACS
BA export	$\oplus \longrightarrow$ synthesis ABCb11, ABCc2
BA import	$\ominus \longrightarrow$ synthesis SLC10a1, SLCO1b1
BA detoxification	$\oplus \longrightarrow$ synthesis CyP3a4, SulT2a1, UGT2b4
Enterocyte	
BA uptake	$\oplus \longrightarrow$ synthesis SLC10a2
BA endocytosis	$\oplus \longrightarrow$ synthesis FABP6
BA egress	$\oplus \longrightarrow$ synthesis OSTα/β

Table 5.35 Impact of the FXR on lipid metabolism (Source: [1002]; $\oplus \longrightarrow$ stimulation, $\ominus \longrightarrow$ inhibition, $\uparrow$ increase, $\downarrow$ decrease, CETP [exchanges cholesterol esters from HDLs with TGs in TGRLs (e.g., VLDLs and chylomicrons)], *PLTP* phospholipid transfer protein, *SREBP* sterol regulatory element-binding protein, *TG* triglyceride)

TG metabolism	$\oplus \longrightarrow$ synthesis NR1c1 (in humans)
	$\oplus \longrightarrow$ synthesis SREBP1c (in mice)
	$\oplus \longrightarrow$ synthesis ApoC2, VLDLR, Sdc1$\oplus \longrightarrow$ LPL
	$\ominus \longrightarrow$ synthesis ApoC3$\ominus \longrightarrow$ LPL
	$\uparrow$ TG clearance
HDL metabolism	$\ominus \longrightarrow$ synthesis ApoA1 ($\downarrow$ [HDL])
	$\oplus \longrightarrow$ synthesis PLTP (HDL remodeling)
	$\uparrow$ CETP activity

Lipoprotein lipase (LPL) is involved in the degradation of TG-rich lipoproteins. Apolipoprotein-C3 (ApoC3) inhibits LPL, whereas ApoC2 and ApoA5 activate LPL. The VLDLR receptor enhances TG hydrolysis by LPL. Syndecan-1 (Sdc1) binds remnant particles before their transfer to receptors

The FXR has an impact on lipid metabolism (Table 5.35). Activated FXR decreases plasmatic concentrations of ApoA1 and HDL^{CS}.[223]

In cultured hepatocytes, glucose induces Fxr gene expression, probably via metabolites of the pentose phosphate pathway, hence reducing cholesterolemia, whereas insulin counters this effect [1002]). Glucose also represses APOC3 gene expression. On the other hand, bile acids modulate gluconeogenesis, as they prevent

[223] HDL carries cholesterol from cells to hepatocytes, where it can be excreted into the bile as either free cholesterol or after conversion into bile acids.

formation of PEPCK, glucose 6-phosphatase, and fructose (1,6)-bisphophatase via NR0b2 and NR2a1.

The FXR participates in the lipoprotein metabolism. It targets the genes encoding phospholipid transfer protein, which permits the transfer of phospholipids and cholesterol from TGRLs to HDLs, in addition to ApoC2 and ApoE [1000].

In hepatocytes, the FXR successively undergoes phosphorylation by casein kinase CsnK2 (Ser327), sumoylation by UbC9 and pias1 (Lys325), and ubiquitination by RNF4, these post-translational modifications define a CsnK2–RNF4 activation–degradation axis [1004]. Sumoylation enhances ligand-primed FXR activation and transcriptional coactivation. Subsequent ubiquitination enables a maximal FXR activation for optimal up- or downregulation of responsive genes involved in bile acid homeostasis and liver regeneration, but leads to FXR proteasomal degradation.

5.5.2 LXRs

The sterol-responsive nuclear receptors, LXRs, are major determinants of cellular cholesterol homeostasis. Whereas NR1h3 (LXRα) is expressed in a cell-specific manner, NR1h2 (LXRβ) is ubiquitous. The LXR ligands comprise oxysterols ([24S,25]-epoxycholesterol and 20(S)-, 22(R)-, 24(S)-, and 27-hydroxycholesterol) and intermediates of cholesterol synthesis, particularly desmosterol [1000].

They regulate transcription of genes involved in lipid absorption, metabolism, and excretion. They are activated by high cellular sterol content; they then induce formation of the cholesterol efflux transporters ABCa1 and ABCg1 in addition to ApoE to export excess cellular cholesterol. They also limit the uptake of lipoprotein-derived cholesterol, as they induce formation of the ubiquitin ligase MyLIP,[224] which ubiquitinates LDLR for degradation [1005].

In the intestine, LXR activates transcription of the genes encoding FABP6 and three ATP-binding cassette transporters (ABCa1, ABCg5, and ABCg8) [1000]. Both ABCg5 and ABCg8, which may form a functional heterodimer, limit intestinal absorption of sterols. On the other hand, ABCa1, which facilitates export of phospholipids and cholesterol from various cell types, may promote efflux of cholesterol from the enterocyte back into the lumen. In addition, ABCa1, ABCg5, and ABCg8 may remove phospholipids and/or sterols out of hepatocytes and macrophages, in addition to enterocytes.

In the liver, LXR activates transcription of the genes encoding controllers of bile acid synthesis and metabolism (e.g., CyP7a1), cholesterol esterification (ACAT) and flux, fatty acid synthesis and esterification (LXR activates SREBP1c (bHLHd1c), which raises production of acetyl-CoA synthase and carboxylase, FAS, stearoyl-CoA desaturase, glycerol phosphate acyltransferase, and CTP:phosphocholine

[224] MyLIP: myosin regulatory light chain-interacting protein. It is also named inducible degrader of LDLR.

cytidylyltransferase), and lipoprotein metabolism [1000]. Other hepatic LXR target genes include ABCA1, ABCG5, and ABCG8. Hepatic ABCa1, ABCg5, and ABCg8 can enable efflux of cholesterol and phospholipids into blood or bile. Oxysterols repress SREBP2 (bHLHd2) activity and hence formation of its targets, such as LDLR and HMGCR, whereas hepatic SREBP1c-dependent genes are transcribed.

Macrophages express LXRα and LXRβ, but neither FXR nor PXR [1000]. Agonists of LXR increase production of ABCa1, ABCg1, ApoE, and LXRα. ABCa1 enables efflux of phospholipids and cholesterol to acceptors, such as ApoA1 and ApoE. ABCg1 may be involved in controlling the efflux of cellular cholesterol to HDL and/or secretion of ApoE.

In macrophages, upon endocytosis of modified lipoproteins or efferocytosis (uptake of dying and dead cells [e.g., apoptotic and necrotic cells]), that is, when cellular pools of oxysterol inflate, LXRs promote cholesterol efflux via ABCa1 and ABCg1, thereby suppressing TLR-mediated inflammation. Moreover, LXRs provoke transcription of genes that encode proteins that are involved in elongation and unsaturation of fatty acids and hence synthesis of long-chain polyunsaturated fatty acids (lcpuFAs; e.g., ω3-fatty acids), in addition to pro-resolving lipid mediators [1006]. LcpuFAs counter transcription primed by NFκB via altered histone acetylation in enhancer and/or promoter regions.[225] In addition, LXRs increase production of the receptor kinase MerTK, which supports efferocytosis by macrophages, hence suppressing TLR4-mediated inflammation in addition to further upregulating ABCA1 and ABCG1 gene transcription [1006].

In macrophages, LXR targets the gene encoding endo- and exonuclease and phosphatase family domain-containing protein, EEPD1,[226] which localizes to the plasma membrane and promotes cholesterol egress, as it controls ABCa1 amount and activity [1007].

The LXR participates in the lipoprotein metabolism. Synthesis of CETP and LPL is primed by oxysterols and the LXR [1000]. CETP is implicated in the transfer of cholesteryl esters between plasma lipoproteins. Lipoprotein lipase catalyzes hydrolysis of lipoprotein TGs. FXR induces production of ApoC2, the LPL cofactor.

[225] Activation of LXRs by desmosterol and oxysterols causes sumoylation of LXRs, leading to LXR binding (without RXRs) to NFκB and AP1 response elements.

[226] Members of the EEPD family cleave phosphodiester bonds in nucleic acids and phospholipids [1007]. Moreover, several members operate as lipid phosphatase, targeting mainly inositol phosphates, which regulate intracellular molecular transfer. The DNA 5′-endonuclease EEPD1 promotes 5′-end resection at DNA double-strand breaks at stalled replication forks [194]. It is required for DNA repair by homologous recombination, but prevents DNA damage repair by nonhomologous end joining. EEPD1 may prevent phosphorylation of ABCa1, which primes degradation by calpain of ABCa1 and attenuates ApoA1-dependent cholesterol efflux, thereby stabilizing ABCa1 and favoring cholesterol export [1007].

5.5.3 PXR

The PXR is also activated by bile acids with the rank order of potency 3-ketoLCA > LCA > DCA = CA [1003]. Other ligands comprise pregnenolone, progesterone, androstanol, and hyperforin, in addition to the drug dexamethasone and various xenobiotics (e.g., rifampicin and phenobarbital) [1000]. Activated PXR increases the formation of numerous cytochrome-P450 (CyP3a11, CyP3a4, and various CyP2b group members) involved in the metabolism of many xenobiotics and endogenous substrates before their excretion into the bile. On the other hand, activated PXR represses CyP7a1 production.

In the liver, PXR ligands (e.g., LCA and 3-keto LCA) elicit formation of SLC01b1, which imports organic anions and sulfated and glucuronidated bile acids from blood into the hepatocyte, in addition to CyP2b and CyP3a [1000]. Many of these compounds are excreted into the bile through ABCb1 on the canalicular membrane.

5.5.4 RXRs

Retinoid X receptors (RXRα–RXRγ [NR2b1–NR2b3]) are activated by retinoids (vitamin A derivatives). They regulate transcription of genes involved in cell proliferation and differentiation and glucose, TG, cholesterol, and bile acid metabolism. They heterodimerize with several other types of nuclear receptors (PPARs, LXRs, and FXRs) to control gene expression. In addition, they sensitize cells to insulin. They also protect against atherosclerosis.

Platelets possess intracellular retinoid X receptors. Stimulation of platelets by PPAR ligands slightly raise cytosolic cAMP concentration, thereby activating PKA [1008]. Ligands of RXRs prevent platelet response (i.e., platelet aggregation, granule secretion, integrin activation, and calcium mobilization) and hence thrombosis upon exposure to ADP and thromboxane-A_2 via inhibition of Gq in addition to thrombin and docosahexaenoic acid, a ligand for the glycoprotein receptor for collagen GP6 [1008]. The RXR ligands activate PKA via cAMP and activated NFκB, the inactive NFκB–Iκ Bα complex tethering to and inactivating the PKA catalytic subunit and this inhibition being relieved after platelet activation by collagen or thrombin. Activated PKA inhibits the RhoA and Rac1 GTPases

5.6 Control of Body Weight and Energy Homeostasis

The control mechanism of body weight involves numerous regulators of food intake and metabolism, especially *peptidic hormones* (e.g., ghrelin and leptin) and *neuropeptides* (e.g., NPy and agouti gene-related peptide [AgRP]), which target their cognate receptors in the cerebral feeding centers, particularly in the hypothalamus (Table 5.36).

Table 5.36 Orexigenic (i.e., appetite stimulators) and anorexigenic messengers (i.e., satietogenic mediators and appetite suppressors)

Agent	Alias
Orexigenic messengers	
Agouti gene-related peptide	AgRP
Ghrelin	Ghrl
Hypocretin-1/2 (orexin-A/B)	Hcrt1/2
Melanin-concentrating hormone	MCH
Neuropeptide-Y	NPy
Neuropeptide LENSSPQAPARRLLPP	LEN
Neuropeptide AVDQDLGPEVPPENVLGALLRV	PEN
Uridine	U
Anorexigenic messengers	
Adiponectin	Adpn
Amylin	Amy
Apolipoprotein-A4	ApoA4
C1q and tumor-necrosis factor-related protein-3	CTRP3
Cholecystokinin	Cck
Cocaine- and amphetamine-regulated transcript-derived peptide	CART
Enterostatin	Est
Gastrin-releasing peptide	GRP
Glucagon-like peptide-1	GLP1
Growth and differentiation factor-15	GDF15
Insulin	Ins
Leptin	Lep
Melanocyte-stimulating hormone-α/β/γ	MSH
Neuromedin-B	NMb
Oxyntomodulin	Oxm
Peptide Tyr–Tyr	PYY
Pro-opiomelanocortin	POMC
Resistin	Retn
Thyrotropin-releasing hormone	TRH

The satiety informant enterostatin is formed in the stomach and intestine by cleavage of secreted pancreatic procolipase (proClps), the remaining Clps part serving as a cofactor for pancreatic lipase during lipid processing. High-fat diet increases procolipase production and subsequently enterostatin release into the gastrointestinal lumen

Nutrients provide energy and building blocks for organismal growth. Cell growth is coordinated with nutrient availability by a central controller TOR, in particular in response to amino acids (Vols. 2, Chap. 2. Cell Growth and Proliferation and 9, Chap. 2. Hypoxia and Stress Response). In response to nutrients, TOR stimulates anabolism (protein, lipid, and nucleotide synthesis) and represses catabolism,

especially autophagy.[227] The TOR kinase forms two structurally and functionally different complexes, TORC1 and TORC2. Nutrients, growth factors, and cellular energy status coregulate TORC1 activity. The active RAG heterodimer binds to raptor and then recruits TORC1 to the lysosome, where it is activated by RHEB, itself stimulated by the PI3K–PDK1–PKB pathway, which dissociates TSC2 from the RhebGAP TSC complex (Tables 5.37 and 5.38).

Upon growth factor and/or amino acid deprivation or stressor exposure, TSC2 moves to the lysosomal surface, where it inhibits RHEB and hence TORC1 [1009].

The intergenic 2p25.3 region of chromosome 2 close to the TMEM18 gene possesses genetic variants that are strongly associated with obesity in children and adults. The transmembrane protein-18 localizes to the nuclear envelope, interacts with components of the nuclear pore complex, nucleoporin NDC1, and the nucleocytoplasmic transport component aladin,[228] and may thus be involved in the transport of molecules across the nuclear envelope [1010]. Moreover, it participates in the control of appetite. Its expression in the murine hypothalamic paraventricular nucleus (PVN), which is implicated in feeding behavior and energy expenditure, influences the nutritional state. Its ablation, both globally and within the hypothalamus in mice, increases food intake and hence body weight with gender-specific changes, whereas its selective overexpression in the PVN of WT mice raises energy expenditure and reduces food intake and adipose depot mass [1010].

The FTO gene[229] that encodes α-ketoglutarate–dependent dioxygenase and its neighboring genes IRX3[230] and RPGRIP1L[231] also contributes to controlling energy balance [1010].

5.6.1 Signal Integration by the Brain

Energy homeostasis requires the transmission to the brain of information on energy fluxes in and from organs, especially nutrient levels in blood and stores, and energy needs of organs to regulate food intake and maintain energy store at appropriate levels in a given condition.

The autonomic nervous system responds via control of activity of organs that play an important role in energy homeostasis in addition to secretion of metabolic

[227] In the budding yeast, Saccharomyces cerevisiae, Tor gene mutations confer resistance to growth inhibition provoked by rapamycin.

[228] Aladin: `al`acrima, achalasia, adrenal `in`sufficiency `n`eurologic disorder.

[229] FtO: fat mass and obesity-associated protein.

[230] IRX3: iroquois homeobox-containing gene-3.

[231] RPGRIP1L: RPGR-interacting protein-1-like protein, also called nephrocystin-8 (NphP8) and fantom (Ftm), which is involved in the organization of apical junctions in addition to regulation of proteasomal activity in the primary cilium.

Table 5.37 Nutrients, growth factors, TOR kinase, and TORC1 (Source: [1009])

Agent	Features
Flcn, FnIP1/2	GAPs for RagC/D
	Stimulated by AA
ARF1	Stimulated by Gln
RHEB	Stimulated by the GF–PI3K–PDK1–PKB axis
Ragulator–RAgA/B–RagC/D–SLC38a9–vATPase at lysosomal surface	
Ragulator	GEF
Gator-2	MIOS–Sec13–Seh1L–WDR24–WDR59 pentamer)
	Inhibits gator-1
SLC1a5	Sodium-dependent AA (Gln) transporter
SLC3a2–SLC7a5	Dimeric antiporter: imports Leu, exports Gln
	Activate TORC1
Arginine	Activates TORC1
	Binds to castor1–castor1 and castor1–castor2, disrupting $(castor)_2$–gator2
SLC38A9	Lysosomal AA transporter
	Arg sensor
	Binds to ragulator and Rags
Glutamine	Stimulates TORC1 lysosomal translocation via ARF1
Glutaminase	De-amination of glutamine to glutamate
Glutamate dehydrogenase	Leu cofactor
	Converts glutamate to α KG which activates TORC1 via PHD
SLC15a4	Lysosomal H^+-coupled histidine transporter
	His export
SLC36a1	Proton and AA symporter, mainly in endosomes
	Interacts with RagC/D
SLC36a4	Proton and AA symporter, mainly in the Golgi body
	Interacts with TOR, raptor, and Rab1a
Rab1a	Stimulates TORC1 interaction with ^{GB}RHEB
TasR1/3	AA-induced TORC1 lysosomal translocation

(**Part 1**) Stimulators involved when nutrients are available and upon growth factor stimulation (*AA* amino acid, *ARF* ADP ribosylation factor, *Flcn* folliculin, *FnIP* folliculin-interacting protein, *gator* GAP activity toward Rag, *KG* ketoglutarate, *Mios* WD repeat-containing protein Mio, *PHD* prolyl hydroxylase, *ragulator* Rag and TORC1 regulator complex, *RHEB* Ras homolog enriched in brain, *Tas1R1[3]* taste GPCR1 member 1[3], *WDR* WD repeat-containing protein)

Table 5.38 Nutrients, growth factors, TOR kinase, and TORC1 (Source: [1009])

Agent	Features
TSC2	Inhibits RHEB
	Activated upon phosphorylation by AMPK
	Repressed upon phosphorylation by PKB
AMPK	Stimulated by glucose deprivation
	Activated by sestrins
	Activated by Axin–STK11 at the lysosome
	Inhibits RHEB via TSC2
	Phosphorylates raptor to inhibit TORC1
Axin	Axin–STK11 phosphorylates (activates) AMPK at the lysosome via vATPase–ragulator
	Inhibits ragulator (RagA/B-GEF) and then TORC1
Castor-1	
Sestrin-2	Stimulated by AA depletion
	GDI for RagA/B
	Binds to and inhibits gator-2
	Cytosolic Leu sensor that links to Leu
Gator-1	DEPDC5—-NPRL2–NPRL3 trimer
	GAP for RagA/B
eIF2α K4	phosphorylates (inhibits) eIF2, repressing protein synthesis, but enabling selective production of ATF4, which elicits synthesis of AA transporters, AA metabolic enzymes, and autophagic factors

(**Part 2**) Inhibitors involved upon nutrient deprivation and growth factor depletion (*AMPK* AMP-activated protein kinase, *Axin* axis inhibition protein-1, *castor* cellular arginine sensor for TORC1, *DEPDC* DEP domain-containing protein, *gator* GAP activity toward Rag, *NPRL* nitrogen permease regulator-like protein, *PKB* protein kinase-B, *STK* protein Ser/Thr kinase, *TSC* tuberous sclerosis complex)

hormones. Control of chemical messengers that regulate hunger and food intake enables handling of metabolic disorders and obesity.

Messengers, such as leptin and ghrelin, act on certain brain regions, using NPy, agouti-related peptide, melanocortins, hypocretins, and melanin-concentrating hormone (MCH), among other mediators [1011].

Feeding behavior is regulated by the CNS, especially some regions of the *forebrain*[232] and *brainstem*[233] participate in regulating feeding [1012].

[232]The forebrain, also called the prosencephalon, is the anterior part of the brain. It comprises the cerebral hemispheres, thalamus, and hypothalamus.

[233]The brainstem is the trunk connecting the spinal cord to the brain. It consists of the medulla oblongata, pons, and midbrain.

In addition, the hypothalamus is a major hub controlling energy homeostasis that integrates nutritional, metabolic, endocrine, and thermal signals. Numerous neuropeptides are released by the hypothalamus.

5.6.1.1 Hypothalamus

Two major opposing hypothalamic clusters encompass the *lateral hypothalamus*, including the perifornical region, which drives feeding and the ventromedial hypothalamic (VMH) nucleus that inhibits it, provoking satiety. In fact, many interconnected hypothalamic clusters regulate food intake and energy homeostasis, such as the arcuate (ArcN),[234] paraventricular,[235] dorsomedial hypothalamus (DMH), and VMH nuclei, and the lateral hypothalamic area (LHA).[236]

In the hypothalamus, hormonal signals that control appetite and meal size and frequency in addition to status of primary storage organs (e.g., lipids in AT and glycogen in the liver) regulate the energy balance.

Arcuate Nucleus

Energy homeostasis is regulated by neuronal populations of the ArcN. In particular, insulin and leptin liberated in blood proportionally to nutrient levels interact with their cognate neuronal receptors, principally in the hypothalamus. Both basal and meal-stimulated insulin secretion depend on available lipids [1014].

[234] Neurons of the ArcN are sensitive to concentrations of ghrelin, Cck, GLP1, NMb, and ApoA4, most of these peptides being made in the brain. However, their activity relies on ghrelinemia, insulinemia, and leptinemia. In addition, these neurons are sensitive to local concentrations of glucose, some long-chain fatty acids, and some amino acids (e.g., leucine) [1014].

[235] Neurons of the PVN possess MC_3, MC_4, and various types of NPy receptors. NPy receptors are targeted not only by NPy but also by peptide-YY and pancreatic polypeptide. They are involved in the control of appetite and circadian rhythm, among other behavioral processes. Neurons of the PVN synthesize and secrete neuropeptides that have a catabolic action, such as corticotropin-releasing hormone (CRH) and oxytocin, which reduces food intake.

[236] Neurons of the lateral hypothalamus synthesize and secrete anabolic peptides, such as MCH,an orexigenic peptide, and hypocretins (Hcrt1–Hcrt2; or orexins [OxA–OxB]; from Greek ορεξις: appetency), which both favor food intake. Hypocretin+ neurons of the LHA belong to the dopamine+ mesolimbic circuit.

In the ArcN, medial NPy+ AgRP+ neurons stimulate feeding,[237] whereas lateral POMC+[238] and CART+[239] neurons cause hypophagia (Table 5.39) [1012]. These neurons project to the PVN that controls feeding and yields preganglionic autonomic output to the brainstem. POMC+ CART+ neurons also innervate MCH+ and Hcrt+ neurons in the LHA and sympathetic preganglionic neurons in the spinal cord [1011]. The NPy+ AgRP+ neurons innervate many of the POMC+ CART+ neuron targets.

Feeding regulators, such as ghrelin, glucocorticoids, leptin, and melanocortins operate at least partly via the NPYergic circuit. Neuropeptide-Y, one of the most powerful orexigenic agents, provokes food intake via its receptors (Y_1–Y_5 [in mice, also Y_6]). The combined action of hypothalamic Y_1 and Y_5 receptors in the PVN, DMH, and VMH mediates hyperphagia [1018].[240]

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[237] Fasting increases the hypothalamic formation of orexigenic NPy and agouti-related peptide and decreases that of anorexigenic pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript-derived peptide.

[238] POMC+ neurons regulate orexinergic signaling by hormones, such as cholecystokinin, ghrelin, insulin, and leptin. POMC is a precursor of three melanocortins, α-, β-, and γ-melanocyte-stimulating hormone (α MSH–γ MSH). Pre-POMC is sequentially cleaved in the hypothalamus; prohormone convertase PC1 (or PCSK1) produces ACTH that is processed into α MSH to γ MSH by PC2 (or PCSK2). All three melanocortins activate the arcuate, ventromedial, paraventricular, periventricular, and supra-optic nuclei, in addition to the pre-optic area [1015]. In humans, α MSH is the predominant POMC-derived neuropeptide in the central regulation of body weight. α MSH and β MSH activate melanocortin receptors in the arcuate (MC_3) and paraventricular nuclei (MC_4) [1016]. Activation of these two GPCRs is antagonized by agouti-related peptide.

α MSH and β MSH bind to MC_4 with high affinity, ACTH to MC_4 with a lower affinity, and γ MSH exclusively to MC_3 [1017].

α MSH and γ_2 MSH are anorexigenic, the latter subtype with a slower time course of action, but not β MSH [1015]. On the other hand, α MSH and β MSH activate the dorsomedial nucleus, but γ_2MSH is weakly active in this cluster. Therefore, in addition to common neural circuits, distinct hypothalamic circuits are activated by different subtypes of POMC products.

Many neuroendocrine cells activated by MSH in the arcuate, paraventricular, periventricular, and supra-optic nuclei (but neither DMH nor VMH) project outside the blood–brain barrier [1015].

The POMC+ neurons of the ArcN project to other hypothalamic nuclei, in particular PVN, where stimulation of the postsynaptic melanocortin receptor MC_4 decreases body weight [1017]. In humans, heterozygous loss-of-function mutations in the Pomc or MC4R gene cause overweight and moderate obesity. Deficiency of POMC also reduces glucocorticoid levels and provokes red-hair pigmentation, as pituitary POMC is the precursor of ACTH, which stimulates glucocorticoid secretion via MC_2 in the adrenal cortex, in addition to MSH, which, via MC_1, controls hair pigmentation.

[239] CART: cocaine- and amphetamine-regulated transcript-derived peptide.

[240] The genes encoding the Y_1 and Y_5 receptors, which are predominant Y receptors in the PVN, are coexpressed in the same neuron types and regulated in a coordinated manner by the same promoter [1018].

Double germline and adult-onset hypothalamus-specific deletions of the genes encoding the Y_1 and Y_5 receptors lower spontaneous hypophagia in male mice and fasting-induced food intake in both male and female mice, hypophagia being stronger in germline than in adult-onset deletions. The receptors Y_1 and Y_5 play a redundant role in feeding regulation. Single germline Y_1 deficiency in male and female mice attenuates fasting-induced intake, but not spontaneous food intake. Single

Table 5.39 Neuronal populations of the arcuate nucleus and their regulators

Neuron type	Stimulators	Inhibitors
NPy+ AgRP+ neurons	(orexigens) Ghrelin	(anorexigens) Insulin, PYY, adiponectin, leptin, resistin, TNFSF1, IL1β
POMC+ CART+ neurons	(anorexigens) Insulin	(orexigens) PYY
	Adiponectin, leptin, resistin	
	IL6/10 (exercise)	TNFSF1

Neuropeptide-Y (NPy) reduces energy expenditure and stimulates food intake via the Y_1 and Y_5 receptors in the PVN. Pro-opiomelanocortin and its products such as α-melanocyte-stimulating hormone (α MSH) preclude feeding. Agouti-related protein homolog antagonizes α MSH, thereby increasing feeding and body weight. Cocaine- and amphetamine-regulated transcript-derived peptide inhibits NPy-induced feeding. Other hypothalamic orexigenic peptides, such as MCH and hypocretins, are produced in neurons of the lateral hypothalamus. In the brainstem connected to the hypothalamus, neurons of the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMNV) receive and integrate inputs from the vagus nerve upon sensing nutrient accumulation in the stomach and duodenum. The brainstem regulates responses to fasting via ascending projections to the hypothalamus and short-term satiety via descending projections from the hypothalamus

In the hypothalamus, insulin and leptin decrease anabolic (pro-feeding) and increase catabolic neuropeptides, whereas ghrelin has opposite effects [1019]. These hormones target hypothalamic neuropeptides to regulate food intake and modulate metabolism via the efferent autonomic nervous system.

Adiponectin and leptin synergistically activate POMC+ neurons in the ArcN [1020]. Conversely, NPy+ AgRP+ neurons are inhibited by adiponectin via PI3K, independently of AMPK, PI3K being a substrate for both adiponectin and leptin in the regulation of energy balance and glucose metabolism via melanocortin activity. Adiponectin stimulates POMC neurons at various glucose concentrations. Adiponectin (AdpnR1–(AdpnR2) and leptin receptors (LepR) localize to POMC+ and NPy+ AgRP+ neurons of the ArcN involved in glucose and fatty acid

germline Y_5 deficiency augments spontaneous and fasting-induced feeding in both genders. The remaining receptor compensates to protect against hypophagia. Resulting hypophagia is associated with decreased NPy synthesis in the ArcN, whereas that of POMC remains unchanged. In male (but not female) $Y1^{-/-}$ $Y5^{-/-}$ mice, body weight elevates; adiposity rises in both genders, but in females, lean tissue mass decreases. Adult-onset PVN-specific $Y1^{-/-}$ $Y5^{-/-}$ chow diet-fed mice have a trend toward reduced fasting-induced food intake, but no significant differences in spontaneous food intake. On the other hand, in HFD-fed mice, spontaneous and fasting-induced food intake diminishes significantly.

Despite hypophagia, these mice become obese. Obesity is more obvious in germline $^{HT}Y_1$- and Y_5-deficient mice than in adult-onset $^{PVN}Y_1$- and Y_5-deficient mice, the latter model exhibiting obesity only with HFD and not normal chow. Other neural circuits can compensate for double ablation, whether germline or adult-onset, of the genes encoding the Y_1 and Y_5 receptors.

metabolism regulation. Whereas AdpnR1 is widespread, at least in the rat brain, AdpnR2 lodges in specific brain regions, such as the cortex, hypothalamus, and hippocampus [1021]. In the arcuate and paraventricular nuclei, Adipor1 and Adipor2 reside on neurons and astrocytes. AdpnRs and LepRs initiate several overlapping signaling cascades that involve the JaK2–STAT3, insulin receptor substrates IRS1 and IRS2, FoxO1, AMPK, and PI3K [1020]. Adiponectin potentiates leptin effects on melanocortin-dependent thermogenesis and AT mass.

In the rodent brain, resistin lowers food intake and controls the formation and the enzymatic activity of various hypothalamic molecules implicated in feeding, likely via auto- and paracrine loops [1022]. It reduces hypothalamic production of NPy and POMC (but not AgRP) and Adpn. On the other hand, it increases the activity of metabolic enzymes (e.g., AMPKα). Both resistin and melanocortin agonists may influence AT 11β HSD1, which converts inactive into active glucocorticoids, thereby favoring obesity and insulin resistance. Resistin stimulates hepatic glucose production and inhibits muscle and AT glucose utilization.

The activity of the hypothalamus–pituitary–adrenal (HTA) axis and the plasmatic concentration of cortisol increase in obese humans [1019]. In obese rats, negative feedback control of the HPA axis is impaired, owing to a lower concentration of mineralocorticoid receptors in the hippocampus, but an unchanged number of glucocorticoid receptors. In obese humans, forward drive to the HPA axis increases and sensitivity to the HPA axis feedback falls. Glucocorticoids participate in the regulation of metabolic homeostasis and can provoke hyperphagia, hyperinsulinemia, and insulin resistance via augmented NPy content in the arcuate nucleus [1019].

Peptide-$Y_{3–36}$, a cleavage product of PYY released after a meal by endocrine cells of the gut (L cells of the distal gastrointestinal tract), is the most common circulating PYY form that targets the Y_2 receptor, which serves as inhibitory receptors on both orexinergic NPy+ neurons and anorexinergic POMC+ neurons. Peptide-$Y_{3–36}$ potently and reversibly inhibits POMC+ neurons via postsynaptic Y_2 receptors [1023]. In fact, $PYY_{3–36}$ inhibits both POMC+ and NPy+ neurons [1024]. It increases G-protein-gated inwardly rectifying K^+ channel flux, decreasing inward calcium current, hyperpolarizing the membrane potential, and inhibits the presynaptic release of excitatory glutamate. It reduces feeding in obese humans; its basal concentration drops in obese subjects.

In the hypothalamus, overeating is associated with *insulin* and *leptin resistance* via aberrant production of proinflammatory molecules, such as TLR4 and IKK, activation of the IKKβ–NFκB axis, dysfunction of insulin and leptin signaling via IKKβ, and endoplasmic reticulum stress. On the other hand, physical exercise suppresses hyperphagia and associated hypothalamic activation of the IKKβ–NFκB axis via anti-inflammatory interleukins IL6 and IL10 [1025]. The cytokine IL6 has both pro- and anti-inflammatory effects; it inhibits acute phase inflammation, as it causes the formation of IL10, IL1R antagonist, and soluble TNFSF1 receptors. During exercise, IL6, which is released by contracting skeletal muscles, provokes metabolic changes in the liver, AT, and hypothalamus.

Activated microgliocytes synthesize and release cytokines that consequently launch inflammation. According to the circumstances, microglial TNFSF1 and can promote neural survival or cause neuronal dysfunction. TNFSF1 operates via widespread TNFR1 and TNFR2 expressed mainly in immunocytes. High-carbohydrate and -fat diet stimulates microglial reactivity in the mediobasal hypothalamus (MBH). Chronic overfeeding and DIO decreases the number of POMC+ neurons [1026]. Microgliocytes in the MBH hypersecrete TNFSF1, which stimulates ATP production in POMC+ neurons, favoring mitochondrial fusion, increasing the firing rate and excitability, triggering mitochondrial stress, and contributing to obesity.

In the hypothalamus of rats, TNFSF1 launches proinflammatory signaling, triggering production of cytokines and cytokine-responsive protein, such as IL1β, IL6, IL10, and SOCS3 [1027]. TNFSF1 activates gene transcription via activator protein-1 in the hypothalamus. In addition, TNFSF1 induces expression of neurotransmitters involved in the control of feeding and thermogenesis. It provokes expression of orexigenic NPy and MCH (up to 1.3-fold) in addition to anorexigenic POMC (up to 8.0-fold) and CRH (up to 1.8-fold) [1027]. Both insulin and TNFSF1 reduces feeding (45 and 25%, respectively) during the same time window, but TNFSF1 does not modulate the anorexigenic effect of insulin in the hypothalamus.

Lateral Hypothalamic Nucleus

Mingled neurons of the lateral hypothalamus release melanin-concentrating hormone or hypocretins (which are not coexpressed). Hcrt+ and MCH+ neurons have similar projection patterns. Major targets comprise brainstem motor centers such as nuclei of cranial motor neurons in the trigeminal, hypoglossal, and facial nerves, implicated in chewing, licking, and swallowing, in addition to the sympathetic and parasympathetic preganglionic nuclei in the medulla and spinal cord, involved in salivation, esophageal and gastric motility, gastric acid secretion, and the regulation of pancreatic hormone secretion of pancreatic hormones, and the ascending arousal system, which includes noradrenergic locus ceruleus,[241] serotoninergic dorsal and median raphe nuclei,[242] and histaminergic tuberomammillary nucleus of the posterior hypothalamus [1011].

In addition, Hcrt+ and MCH+ neurons are connected to the cerebral cortex. MCH+ neurons also project and stimulate to the nucleus accumbens, which receives sensory and feeding behavioral cues from the cerebral cortex. Moreover, the NAcc is involved in the GABAergic circuit via the GABAergic ventral pallidum (VP), thereby disinhibiting feeding [1011].

Food restriction elicits synthesis of MCH and hypocretins. Leptin secreted by the WAT during times of plenty is a necessary (but not sufficient) satiety stimulus.

[241] From Latin *cæruleus*: azure, blue, dark blue.

[242] ραφη: seam.

5.6.1.2 Hedonic Stimuli

Feeding, which provides substrates for metabolism, relies not only on homeostatic signals by sensors of energetic balance transmitted to the brain but also circadian cues and rewarding behavior (Tables 5.40 and 5.41) [1011]. Feeding is not only a necessary element of existence, but it relies on hedonic mechanisms; humans tend to eat large amounts of palatable food (beyond homeostatic need), taste and smell representing major palatability or food aversion signals. Gustatory and olfactory information modulates feeding behavior.

Table 5.40 Major neural circuits linked to circadian, homeostatic, and sensory afferent feeding inputs to the brain and neurotransmitters (Source: [1011] *AgRP* agouti-related protein, *AIC* agranular insular cortex, *ArcN* hypothalamic arcuate nucleus, *CART* cocaine- and amphetamine-regulated transcript-derived peptide, *CGRP* calcitonin-gene related peptide, *DMH* dorsomedial hypothalamic nucleus, *GIC* granular insular cortex, *ILC* infralimbic cortex, *LHA* lateral hypothalamic area, *NAcc* nucleus accumbens, *NPy* neuropeptide-Y, *NTS* nucleus of the solitary tract, *PBN* pontine parabrachial nucleus, *POMC* pro-opiomelanocortin, *PVN* hypothalamic paraventricular nucleus, *SCN* suprachiasmatic nucleus, *SPVZ* subparaventricular zone, *VMH* ventromedial hypothalamic nucleus, *VPMpcTN* ventral posteromedial parvicellular thalamic nucleus [subparafascicular nucleus])

Input	Neural circuits
Circadian	SCN–SPVZ–DMH/PVH
Leptin	ArcN–LHA (NPy/AgRP, POMC/CART)
Vagal, taste	NTS–PBN
Connectivity	PBN–LHA/DMH/VMH
	PBN–PVN
	PBN–VPMpcTN–(CGRP)–GIC
	GIC–AIC/ILC/NAcc

Table 5.41 Major output circuits regulating feeding (Source: [1011])

	Source
Output	Neural circuit (neurotransmitter)
Autonomic	ArcN–(POMC)–NAcc
	LHA–(MCH)–NAcc–(GABA)–VP–(GABA)–LHA
Endocrine	PVH (ADH, CRH, TRH)
Motor	LHA (Hcrt)
Cortex	VP–MDTN–AIC/ILC

The hypothalamic paraventricular (PVH) and arcuate nuclei (ArcN) and the lateral area (LHA) yield the main autonomic, endocrine, and motor responses contributing to feeding, using melanin-concentrating hormone (MCH) antidiuretic hormone (ADH; or vasopressin), corticotropin- (CRH) and thyrotropin-releasing hormone (TRH), and hypocretin (Hcrt: or orexin), respectively. Projections to the prefrontal cortex originate from the LHA and the nucleus accumbens (NAcc) via the ventral pallidum (VP) and mediodorsal thalamic nucleus (MDTN) to the agranular insular (AIC) and infralimbic (ILC) cortical regions (*CART* cocaine- and amphetamine-regulated transcript-derived peptide, *GABA* γ-aminobutyric acid, *POMC* pro-opiomelanocortin)

Food and drink flavor provides a major gustatory hedonic value or taste aversion. Flavors are discriminated by taste receptors, which recognize the four classic tastes (bitter, salty, sour, and sweet). Most of the taste cells in the tongue respond to more than one of these four tastes. Taste information is relayed by the NTS and the parabrachial nucleus [1011]. The latter projects to a gustatory nucleus in the thalamus, the lateral frontal cerebral cortex, central nucleus of the amygdala, and several hypothalamic clusters such as the lateral hypothalamus.

Odorants are detected by GPCRs of olfactory receptors, including the vomeronasal organ, the peripheral sensory organ of the accessory olfactory system at the base of the nasal septum. Olfactory signals are transmitted by bipolar olfactory receptor neurons in the olfactory neuroepithelium, which also contains microvillar, sustentacular, and globose and horizontal basal cells.

5.6.2 Brain–Gut Axis

The autonomic regulation of digestion involves the central, parasympathetic, sympathetic, and enteric nervous systems (ENS) in addition to neuroendocrine messengers originating from the cerebral cortex and adrenal medulla.

The brain–gut or gut–brain axis refers to bidirectional communication between the gut and brain via vagal and spinal afferent neurons and humoral factors, such as intestinal hormones, gut flora-derived messengers, and cytokines, which transmit information from the gut to the brain and from the brain to the gut via autonomic neurons and neuroendocrine factors [1028].

The gut–brain axis thus uses four major communication supports: nervous influx carried by vagal and spinal afferent neurons, immune messages by cytokines, endocrine signals by gut hormones (>20 hormone types), and intestinal flora-derived cues, as lipopolysaccharide and peptidoglycan components can act on the CNS [1028].

5.6.2.1 Vagus Nerve and Nucleus of the Solitary Tract

The brain receives various signals from the gastrointestinal tract via sensory afferents and blood circulation. Afferent signals transmitted by the vagus nerve inform of gastric stretch in addition to hepatic concentrations of glucose and lipids [1011]. Sensory terminals terminate in the medial and dorsomedial parts of the NTS, which is protected from hormones by a blood–brain barrier, and directly in the gastromotor vagal neurons, whereas others relay to the dorsal motor vagal nucleus, which innervates the entire gastrointestinal tract. Direct projections from the NTS and relayed fibers via the parabrachial nucleus innervate the paraventricular, dorsomedial, lateral hypothalamic and arcuate nuclei of the hypothalamus, central nucleus of the amygdala, bed nucleus of the stria terminalis, and visceral sensory thalamus [1011].

5.6.2.2 Area Postrema

The area postrema is a circumventricular organ close to the NTS in the dorsomedial medulla oblongata outside the blood–brain barrier. In humans, this bilateral structure resides immediately dorsal to the subpostremal nucleus, which separates the NTS from the area postrema. The area postrema receives visceral afferent inputs from the glossopharyngeal and vagus nerves. Neurons in the area postrema can respond to circulating gastrointestinal hormones, which also serve as neuropeptides, such as amylin, cholecystokinin, and glucagon-like peptide GLP1, and transmit them to the medullary NTS and pontine parabrachial nucleus [1011].

5.6.2.3 Secretin Set

The secretin (SCT) set of the brain–gut axis[243] consists of structurally and functionally related peptidic hormones, such as secretin (Sct), glucagon (Gcg), glucagon-like peptides GLP1 and GLP2, gastric inhibitory polypeptide (GIP; or glucose-dependent insulinotropic polypeptide), adenylate cyclase-activating polypeptide AdCyAP1 (a.k.a. pituitary adenylate cyclase-activating peptide [PACAP]), PACAP27, PACAP38, and helodermin-like peptide (Table 5.42) [1012]. Other SCT set members, vasoactive intestinal peptide (VIP), peptide histidine–isoleucine (PHI) and its human analog peptide histidine–methionine (PHM), and peptide histidine–valine (PHV) are co-synthesized from the same precursor.[244] These peptidic hormones are widespread, being detected in the CNS and in the gastrointestinal, respiratory, and reproductive tracts. Peptides of the SCT category and their receptors are detected in various brain regions, especially the hypothalamus. They operate via common and distinct class-*II*, subclass-B1 GPCRs.

Class-*II* G-Protein-Coupled Receptors

G-protein-coupled receptors are key players in cellular communication. Class-A rhodopsin-like receptors represent the majority; class B includes secretin-like and adhesion GPCRs, F frizzled, and C (22 members), the main neurotransmitters, glutamate (mGlu1–mGlu8) and GABA ($GABA_A$–$GABA_B$), and sweet and umami taste (T1R1–T1R3) and calcium-sensing receptors (CaS) [1029]. Class-C GPCRs

[243] The first hormone discovered in humans (in 1902), secretin, a 27-amino acid-containing peptide encoded by the SCT gene, provides the name of this hormone category. This hormone category is also named the SCT–VIP set.

[244] Peptide histidine–isoleucine was originally isolated from the porcine upper intestine. It activates AC in rats and inhibits VIP binding to its receptors. PHI exists in the body in two forms: shorter (27 amino acids) and longer peptide (42-amino acid), which is named peptide histidine–valine.

Table 5.42 Peptides of the secretin (Sct) family and appetite regulation (Source: [1012]; *ND* not determined, *AdCyAP1* adenylate cyclase-activating polypeptide-1 [or pituitary adenylate cyclase-activating polypeptide (PACAP)], *GLP* glucagon-like peptide, *GHRH* growth hormone-releasing hormone, *PHI* peptide histidine–isoleucine, *VIP* vasoactive intestinal peptide, MC_4 melanocortin-4 receptor, *POMC* pro-opiomelanocortin, *AP* area postrema, *ArcN* arcuate nucleus, *CeA* central amygdala, *DMH* dorsomedial hypothalamus, *DMNV* dorsal motor nucleus of the vagus, *DVC* dorsal vagal complex, *lPBN* lateral parabrachial nucleus, *LHA* lateral hypothalamic area, *MlPON* medial pre-optic nucleus, *NAcc* nucleus accumbens, *NTS* nucleus tractus solitarius, *PVN* paraventricular nucleus, *SCN* suprachiasmatic nucleus, *vHpc* ventral hippocampus, *VMH* ventromedial hypothalamus, *VTA* ventral tegmental area)

Peptide	Primary function	Feeding behavior	Brain targets
AdCyAP1	Hypothalamic formation of POMC, MSH, MC_4	Anorexigenic	Hypothalamus (ArcN, PVN, VMH)
			Forebrain [striatum] (Nacc)
Glucagon	Glucose release	Anorexigenic	Hypothalamus (ArcN, LHA)
			Brainstem (DVC)
GLP1	Incretin	Anorexigenic	Hypothalamus (ArcN, DMH, LHA, PVN, VMH)
			Brainstem (NTS, lPBN, AP, DMV)
			Mesolimbic reward system (VTA, NAcc)
			Amygdala (CeA)
			Hippocampus (vHpc)
GLP2	Intestinal mucosal growth	Anorexigenic	Hypothalamus (ArcN)
GHRH	GH release	Orexigenic	Hypothalamus (ArcN, DMH, SCN/MlPON)
PHI	Prolactin regulation	Anorexigenic	Hypothalamus (PVN)
			Amygdala (CeA)
Secretin	Bicarbonate and water release from pancreas	Anorexigenic	Hypothalamus (ArcN, PVN)
			Brainstem (NTS)
			Amygdala (CeA)
VIP	Vasodilation	ND	Hypothalamus (PVN, SCN)

are homo- or heterodimers, dimerization being mandatory for signaling and binding to both protomers required for full activity (e.g., $GABA_{B1}$–$GABA_{B2}$, T1R3–T1R1, and T1R3–T1R2).

Class-*I I* GPCRs couple mainly to the Gs–AC–cAMP pathway but also with Gq and Gi, thereby activating PLC. They are stimulated by neuropeptides, such as STN, GLP1 and GLP2, growth hormone-releasing hormone (GHRH),[245] AdCyAP1, CRH, VIP, parathyroid hormone (PTH), and calcitonin-related peptides [1030].

[245] A.k.a. growth hormone-releasing factor, somatocrinin, somatoliberin, and somatorelin.

They promote neuronal survival, especially during stroke, as they support cell response to oxidative, metabolic, and excitotoxic stress.

Corticotropin-Releasing Hormone and Related Neuropeptides

Corticotropin-releasing hormone is a potent mediator of endocrine, autonomic, and immune responses to stress [1030]. It also modulates behavior (motor function, arousal, feeding, and anxiety-related behavior).

It is released from hypothalamic parvocellular neurons of the paraventricular nucleus into portal vessels to activate the HTA via ACTH secreted from the anterior pituitary gland. ACTH stimulates glucocorticoid secretion from the adrenal gland. Other regions with high concentrations of CRH+ neurons include the bed nucleus of the stria terminalis, with projections to brainstem areas involved in autonomic function, and interneurons of prefrontal, cingulate, and insular cortical areas.

The effects of CRH are mediated by two receptors CRHR1 and CRHR2. The former abounds in cerebellar, neocortical, hippocampal, and sensory relay structures of the rat brain. The latter can also modulate stress response upon HPA activation.

Urocortin-1 and -2 are members of the CRH family of neuroprotective neuropeptides. They are produced in multiple regions of the brain, with high levels in certain neuronal populations of the midbrain and brainstem [1030].

Parathyroid Hormone and Related Neuropeptides

Parathyroid hormone (PTH) is a glycoprotein that primarily functions in controlling calcemia, its secretion from the parathyroid glands being regulated by feedback via calcemia. Parathyroid hormone and two related peptides, parathyroid hormone-related peptide (PTHRP) and 39-amino acid tuberoinfundibular peptide TIP39, target three receptors (PTH_1–PTH_3). PTH and PTHRP primarily interact with PTH_1 and TIP39 PTH_2. The receptors PTH_1 and PTH_2 in addition to their cognate hormones are widely expressed in the CNS (PTH_2: limbic, hypothalamic, and sensory areas, especially PVN, nerve terminals in the median eminence, superficial layers of the spinal cord dorsal horn, and caudal part of the sensory trigeminal nucleus) [1030].

The calcitonin family of peptidic neuroprotectors comprises amylin (or islet amyloid polypeptide [IAPP]), calcitonin, α- and β-CGRPs by alternate splicing of a single transcript type, and adrenomedullin (Adm). These members are widespread in peripheral organs and in the peripheral and CNS. Adrenomedullin and CGRP cause vasodilation, calcitonin decrease bone resorption, amylin reduces nutrient intake (Sect. 5.6.7.1). The CGRP and adrenomedullin-2 receptor requires the single transmembrane domain-containing protein RAMP1 coupled with calcitonin receptor and adrenomedullin-1 RAMP2 or RAMP3, and amylin RAMP1, RAMP2, or RAMP3 [1030]. The calcitonin receptor stimulates AC and phospholipase-C in addition to phospholipase-D and inhibition of AC.

Secretin

Secretin is a gastrointestinal hormone released by S cells of the duodenum and proximal jejunum in response to acid milieu to stimulate bicarbonate secretion from the pancreas to neutralize gastric chyme acidity [1012]. It thus acts on epitheliocytes of the pancreatic and biliary ducts, launching secretion of alkaline bicarbonate-rich fluid. It also slows gastric emptying [1030].

Secretin is also a neuropeptide synthesized in multiple brain regions (hypothalamic ArcN, supra-optic nucleus [SON], and PVN, NTS of brainstem, cerebellum, area postrema, central amygdala, hippocampus, and cerebral cortex), being an element of the brain–gut axis that controls digestion and feeding behavior [1012, 1030].

The Sct receptor (SctR) localizes to gastric and intestinal epitheliocytes, pancreatic acinar cells, Brunner's glands, gastric and intestinal smooth myocytes [1030]. It is also identified in the hypothalamic ArcN, PVN, and SON [1012], and in the cerebellum, cerebral cortex, thalamus, striatum, hippocampus, and brainstem [1030].

Secretin stimulates lipolysis and fatty acid uptake in adipocytes [1031]. Secretin is also a neurohypophysial factor detected throughout the hypothalamo-neurohypophysial axis and released from the posterior pituitary gland (neurohypophysis) upon exposure to plasmatic hyperosmolality. In the hypothalamus, it stimulates vasopressin production and release, thereby regulating body water content, as vasopressin elicits water reabsorption in the renal collecting duct via the formation and translocation of aquaporin-2 [1032]. In addition, it has a direct effect on renal water reabsorption and modulates water and electrolyte transfer in pancreatic ductal cells, cholangiocytes, and epididymal epitheliocytes. Secretin stimulates the synthesis of aquaporin-2 under hyperosmotic conditions and its translocation from intracellular vesicles to the plasma membrane [1033].

Peripheral and central secretin exert an anorexigenic effect via SctR on intestinal vagal afferents and the central melanocortin system [1012]. Secretin stimulates the dorsal division of the parvocellular neurons of the PVN, which are involved in the control of central autonomic outflow.[246] In addition, central and peripheral secretin upregulates synthesis of the melanocortin receptor MC_4 in the PVN, which supports secretin action. In the ArcN, POMC+ neurons are endowed with SctR, hence contributing to the secretin effect.

[246]The PVN is composed of magno- (from Latin *magnus*: large) and parvocellular neurons (from Latin *parvus*: small). Vasopressin (AVP; or antidiuretic hormone [ADH]) and oxytocin (Oxt) are synthesized from the precursors, propressophysin (i.e., ADH–neurophysin-2) and pro-oxyphysin (i.e., vasopressin–neurophysin-2–copeptin), in separate magnocellular neurons of the supra-optic and paraventricular nuclei of the hypothalamus, these neurosecretory cells forming the supra-optico- and paraventriculohypophyseal tracts, and secreted by the neurohypophysis (posterior pituitary gland). Parvocellular neurons consist of neurosecretory cells projecting to the median eminence in addition to caudally projecting pre-autonomic cells (neurons projecting to either the medullary [nucleus tractus solitarius and rostral ventrolateral medulla] or the spinal autonomic control centers [intermediolateral cell column]) [1034].

The neurotransmitter secretin, a regulator of feeding behavior and neuroendocrine signaling in the hypothalamus, is released upon membrane depolarization of hypothalamic cells via voltage-gated sodium and calcium channels [1012].

Adenylate Cyclase-Activating Polypeptide AdCyAP1

Adenylate cyclase-activating polypeptide, AdCyAP1, or PACAP, is widespread. Pituitary adenylate cyclase-activating peptide was first identified as a 38-amino acid peptide (PACAP38) from ovine hypothalamus in rat anterior pituitary cells, a C-terminally truncated form, PACAP27, being subsequently identified [1030].

Adenylate cyclase-activating polypeptide is detected in various regions of the brain (central thalamic nuclei, amygdala, olfactory bulb, frontal cortex, basal ganglia, nucleus accumbens, dentate gyrus, superior colliculus, substantia nigra, pituitary gland, locus ceruleus, pontine and raphe nuclei, and hippocampus), its highest expression occurring in the hypothalamic ArcN, PVN, VMH, and SON, adrenal glands, and gonads [1012, 1030]. It can localize to POMC+ neurons of the ArcN, but not POMC+ neurons within the brainstem.

Receptors of AdCyAP1 include AC-activating polypeptide receptor AdCyAP1R1 (PAC1 or PACAPR1) and the VIP receptors VIP_1 ($VPAC_1$ or PACAPR2) and VIP_2 ($VPAC_2$ or PACAPR3). The AdCyAP1 receptor has a high affinity for AdCyAP and a much lower affinity for VIP [1030]. On the other hand, VIP_1 has a similar affinity for VIP and PACAP27 and a lower affinity for PACAP38 and VIP_2 has a similar affinity for AdCyAP1 and VIP. AdCyAP1R1, VIP_1, and VIP_2 activate AC and the IP_3–Ca^{2+} axis.

Adenylate cyclase-activating polypeptide abounds in the brain, especially in the magnocellular region of the hypothalamic PVN and SON, and pituitary and adrenal glands, whereas VIP_1 and VIP_2 reside primarily in the lung, liver, and testis [1030]. These receptors are identified in approximately half of POMC+ neurons and a significant proportion of NPy+ neurons [1012]. AdCyAP1 can stimulate release of both oxytocin and vasopressin and modulate the activity of other hypothalamic neuronal populations, augmenting production of GnRH, somatostatin, and CRH [1030].

This anorexigenic mediator promotes hypothalamic production of POMC and α MSH, in addition to MC_4, and α MSH secretion [1012]. Hypophagia is caused by AdCyAP1 primarily via VIP_1 in the PVN and then the melanocortin system. In the VMH, the AdCyAP1–VIP_1 couple may chiefly stimulate energy expenditure; hypophagia exerted by intra-VMH AdCyAP1 results from activation of ionotropic NMDA-type glutamate receptors.

Binge (i.e., excessive indulgence in eating and drinking alcohol during a given period) transiently separates homeostatic feeding from hedonic feeding behavior. Within the NAcc, AdCyAP1 mimics GABA, reducing the intake of palatable food without altering homeostatic feeding [1012]. On the other hand, within the VMH, AdCyAP1 mimics AMPA, diminishing homeostatic feeding without altering hedonic feeding. Fasting and HFD decreases and increases AdCyAP1 synthesis in VMH, respectively.

Adenylate cyclase-activating polypeptide can have both an- and orexigenic effects. ^{VMH}AdCyAP1+ neurons interact with anorexinergic ^{Arc}POMC+ neurons [1012]. Orexinergic ^{Arc}AgRP+ neurons communicate with excitatory afferent ^{PVN}AdCyAP1+ neurons. Autonomic ^{PVN}neurons projecting to preganglionic sympathetic neurons in the spinal cord are involved in hepatic glucose production and subsequent elevation in glycemia [1012]. In the brain, exposure to PACAP38 also significantly increases glycemia. In addition, the AdCyAP1–VIP_1 couple is involved in light-regulated feeding behavior.

Adenylate cyclase-activating polypeptide interacts with other peptides to modulate feeding. Central administration of AdCyAP1 increases the concentrations of hypothalamic hypophysiotropic neurohormones, such as vasopressin (ADH), gonadotropin-releasing hormone (GnRH1–GnRH2), somatostatin (SSt), and CRH [1012]. AdCyAP1, which counters ghrelin action, but favors activity of GLP1 and leptin, supports leptin-stimulated hypophagia and hypothermia.

Vasoactive Intestinal Peptide

Vasoactive intestinal peptide is distributed throughout the gastrointestinal tract and CNS (e.g., cerebral cortex, thalamus, and hypothalamic suprachiasmatic nucleus [SCN] and PVN) [1012]. It operates as a neuroendocrine hormone and a neurocotransmitter.

Its precursor, pre-proVIP, engenders PHI, PHM, the human equivalent of PHI, and PHV, a C-terminally extended form of PHM [1030].

This hormone causes vasodilation via the VIP_1 and VIP_2 receptors. The former is produced in the cerebral cortex and hippocampus and the latter in the thalamus, midbrain, and in the magnocellular regions of the PVN and SON in addition to SCN of the hypothalamus [1012].

Neuroprotection by VIP relies on activity-dependent neurotrophic factor (ADNF), a 14-kDa gliocyte-derived neuroprotective protein homolog to the chaperone heat shock protein HSP60, and its truncated products, ADNF14 (14-amino acid [VLGGGALLRSIPA]) and ADNF9 (9-amino acid [SALLRSIPA]), neuroprotective protein (ADNP or ADNP1)[247] and its derived protein, novel ADNF9-like active peptide (NAP, which contains 8-amino acids [NAPVSIPQ]), the most potent neuroprotector, in addition to IGF1 [1030, 1035]. Neuroprotection ensured by VIP is thus indirect; it requires astrocytes, which, once they are stimulated by VIP, secrete the neuroprotectors ADNF and ADNP. In fact, the structurally related peptides expressed in the central and peripheral nervous

[247] Activity-dependent neuroprotective protein, or activity-dependent homeobox gene-derived neuroprotector, ADNP1 is a VIP-responsive protein and homeodomain- and zinc finger-containing transcription factor encoded by the ADNP gene in astrocytes, which interacts with components of the BAF complex. The ADNP protein is also involved in erythropoiesis [1036]. The homologous protein ADNP2, which is encoded by the Adnp2 gene, is also dubbed homeodomain- and zinc finger-containing protein ZNF508. Both ADPN1 and ADPN2 regulate globin synthesis.

system, AdCyAP1, PHM, and VIP,[248] act as neuroprotectors via the Gs–AC–cAMP–PKA–MAP2K–ERK pathway and operate via a similar mechanism, priming ADNP1 synthesis [1037]. Moreover, VIP triggers the release of a set of astroglial neuroprotective factors (e.g., interleukins IL1 and IL6, neurotrophin-3 peptidase nexin-1 [serpin-E2], chemokines CCL3 and CCL5, ADNF, and ADNP). In astrocytes, ADNP1 production is modified by VIP via VIP_2 [1038].

Plasmatic VIP concentration increases after a carbohydrate-rich meal or water loading [1012]. VIP impedes food intake and contributes to circadian feeding behavior and control of release of anorexigenic hormones, such as GLP1, insulin, leptin, and PYY in both the fasting and postprandial periods. Moreover, it can stimulate α MSH release. Intracranial injection of VIP suppresses insulin secretion and elicits secretion of adrenaline and glucagon in addition to sympathetic nerve activity [1030]. Other VIP effects include electrolyte secretion, smooth myocyte relaxation, and protection against redox stress.

Glucagon

Proglucagon is synthesized in pancreatic α cells and processed into glucagon (Gcg) (Sect. 5.6.7.10). Proglucagon also contains glucagon-like peptides, GLP1 and GLP2.

Glucagon is a hyperglycemic factor. Peripheral Gcg induces satiety via the NTS, area postrema, and central nucleus of the amygdala (CeNA), influencing meal size rather than between-meal interval via hepatic vagal afferents [1012]. It counters hypoglycemia and insulin action, as it stimulates hepatic glucose synthesis and secretion. On the other hand, its hypothalamic action prevents hepatic glucose production [1012]. Diet-induced obesity abolishes the anorexigenic effects of glucagon.

The glucagon receptor (GcgR) triggers the Gs–AC–cAMP and PLC–IP_3–Ca^{2+} pathways [1030]. It is expressed mainly in the liver and kidney and, to a lesser extent, in the heart, AT, adrenal glands, pancreas, spleen, thymus, and throughout the gastrointestinal tract of rats [1030].

The glucagon receptor is also detected in the cerebral cortex, olfactory tubercle and bulb, amygdala, hippocampus, hypothalamus, thalamus, medulla, lateral septum, subfornical organ, and anterior pituitary gland of rats [1030]. It is observed

[248] AdCyAP1, PHM, and VIP are widespread, with a similar distribution (cerebral cortex, pituitary and adrenal glands, nerve endings of the respiratory system, gastrointestinal tract, and reproductive system). PACAP also lodges in the amygdala, septum, thalamus, brainstem, and spinal cord; PHI in the temporal lobes, striatum, and medulla; VIP and PACAP in immocytes; and VIP and PHI in the suprachiasmatic nuclei and hippocampus [1037].

They regulate the pituitary and adrenal glands and pancreas, relax smooth myocytes in blood vessels in addition to the respiratory, gastrointestinal, and reproductive tracts, and influence immunity. In the CNS, AdCyAP1, PHM, and VIP function as neurotransmitters, neuromodulators, and neurotrophic factors.

in AgRP+ neurons of the ArcN and in the dorsal vagal complex (DVC) of the brainstem [1012], that is, a collection of three neighboring medullary nuclei: (1) the viscerosensory NTS, (2) the area postrema, and (3) the DMNV. Elevated circulating Gcg concentration linked to high-protein diet results from the GcgR–PKA–ERK1/2–K_{ATP} cascade in the DVC [1012]. In addition, circulating Gcg suppresses glucose sensing via hypothalamic neurons (LHA, DMH, and VMH). Central Gcg reduces concentrations of Cam2Kβ and its affector AMPK in the ArcN, where central Gcg exerts an acute anorexigenic action via the PKA–Cam2Kβ–AMPK pathway [1012].

Glucagon-Like Peptide-1

Also processed from the pre-proglucagon, GLP1, is a hormone secreted from the intestinal L cells in response to nutrient intake (Sect. 5.6.7.8). It acts as an incretin with GIP, stimulating glucose-dependent insulinotropic action [1012]. It can induce closure of the K_{ATP} channel, yielding glucose sensitivity in β cells. In addition, GLP1 provokes calcium influx via Rab3, Gi, and Gq [1030]. Moreover, GLP1 is one of the leptin effectors; leptin may enhance the central GLP1 activity [1012].

Glucagon-like peptin-1 operates via the GLP1 receptor (GLP1R). The coordinated action of GLP1R, GIPR, and GcgR reduces obesity in rodents [1012].

The GLP1 receptor, a prototypical member of the family-B GPCRs, is a major target for T2DM treatment. It can launch cAMP production by AC via the Gs subunit, Ca^{2+} mobilization, and G-protein-independent ERK1/2 signaling. Glucagon-like peptide-1 and oxyntomodulin initiate ligand-directed signaling. *Ligand-determined signaling*, i.e., ligand-induced differential signaling, refers to functional selectivity, i.e., the ability of different ligands (full and partial agonists, inverse agonists, antagonists, and allosteric modulators) of a given receptor to elicit distinct cellular responses due to ligand- and effector-binding specificity of the receptor. In other words, receptors trigger *biased signaling*, a given agonist preferentially activating a given signaling cascade over others (*biased agonism*). Biased GPCR agonists distinctly activate signaling pathways with different potencies, whereas unbiased agonists activate these pathways with equal efficiency. In addition, biased ligands stabilize receptor conformations that differ from those primed by unbiased ligands. Distinct biased ligands of the same receptor can stabilize different receptor conformations, which engage different intracellular effectors. Multiple subtypes of G proteins can be engaged with distinct efficacies and kinetics and different types of GPCR ligands and allosteric modulators bias the G-protein coupling, which then depends not only on messenger type but also on the relative amounts of the GPCR subunits and intracellular regulators. G-protein-coupling profiles of GPCRs are related to the variety of α (16 subtypes with distinct properties), β, and γ subunits of G proteins, a given receptor being coupled with multiple types of G protein subunits with varying potency and kinetics [1039]. Furthermore, GPCRs trigger canonical and alternative signaling cascades. They signal not only via heterotrimeric GTP-binding proteins but also

via other effectors such as β-arrestins. Biased ligands selectively change the propensity of GPCR coupling to either G proteins or β-arrestin. On the one hand, liganded GPCRs are phosphorylated by kinases and connect to arrestin, which triggers GPCR desensitization and endocytosis, thereby hindering G-protein coupling with the receptor. Arrestin attracts adaptors and clathrin for GPCR internalization and desensitization. Nevertheless, inhibition of endocytosis does not reduce the initial cAMP production, but the later cAMP accumulation, agonist-bound GPCR signaling, such as that primed by Gs-coupled β2-adrenoceptor, PTH, TSH, sphingosine 1-phosphate, and dopamine, continuing once it is internalized after a short delay (arrestin-based endocytic signaling) [1040]. On the other hand, G-protein-independent signaling uses arrestin, which recruits and activates the ERK cascade. β-Arrestin thus generates its own signaling via the cRaf–MAP2K–ERK axis when the GPCR–Arr complex resides on endosomes (arrestin-based cortical signaling). Arrestin tethers to GPCRs according to two modes [1041]. Stable binding of arrestin to phosphorylated amino acids in the intracellular loop of the M1 muscarinic acetylcholine receptor, which persists for minutes after agonist removal, upregulates ERK, whereas transient binding of arrestin to the unphosphorylated receptor lessens ERK signaling, as it attracts a protein phosphatase. Therefore, the ERK signaling bias is determined by binding modes of arrestin to the receptor.

Polar transmembrane residues of family-B GPCRs function not only for protein folding, but they also control activation transition, ligand-biased signaling, clusters of residues within the receptor stabilizing receptor conformation [1042].[249]

In the rat brain, GLP1R is identified in the lateral septum, subfornical organ, thalamus, hypothalamus, interpeduncular nucleus, posterodorsal tegmental nucleus, area postrema, inferior olive, and NTS [1030].

In rodents, peripheral GLP1 activates the sympathetic nervous system, increasing tyrosine hydroxylase synthesis, sympathetic outflow, and hence cardiac frequency and blood pressure [1030]. Upon nutrient entry into the gastrointestinal tract, peripherally secreted GLP1 connects to GLP1R on adjacent celiac and gastric neurons, which form branches of the vagal afferents [1012]. Peripheral GLP1 can also cross the blood–brain barrier and activate central GLP1R in the NTS. GLP1 sends satiation cues and triggers insulin secretion via the *vagovagal reflex*.

The GLP1 receptor alters feeding behavior using various neural relays, such as hypothalamus (ArcN, PVN, and LHA), hindbrain nuclei (parabrachial nucleus, area postrema, medial NTS), ventral hippocampus (vHpc), and nuclei embedded within the mesolimbic reward circuitry (VTA and NAcc) [1012]. It delays gastric emptying

[249] Polar transmembrane residues avoid the core of the hydrophobic lipidic bilayer; they are buried within the interior of the protein, often lining internal water-filled cavities and forming hydrogen bonds with buried water molecules and other polar residues [1042]. The fine control of GLP1R signaling is linked to changes in interactions formed by these buried polar residues. Family-B GPCRs (e.g., GPCRs of GLP1, AdCyAP1 and VIP (VIP_1–VIP_2), secretin, and PTH) do not share the conserved polar residues that are essential for the functioning of family-A GPCRs (e.g., β2AR); they possess their own unique set of conserved intramembranous polar residues that play a role analogous to those in family-A GPCRs.

and has a hypophagic effect. Central GLP1 modulates utilization of circulating glucose via the PKA–AMPK axis, ERK1, and ERK2 [1012].

Central GLP1 is produced in the caudal nucleus of the NTS and ventrolateral medulla. Its receptor GLP1R lodges in the hypothalamus (ArcN, PVN, and DMH) in addition to the medullary dorsal vagal complex (area postrema, viscerosensory NTS, and DMNV) and pontine parabrachial nucleus in the brainstem. GLP1 activates CRH+ Nucb2+ (nesfatin-1+) neurons and, to a lesser extent, Oxt+ neurons in the PVN [1012]. In the PVN, GLP1 stimulates the hypothalamic–pituitary–adrenal axis and catecholamine release.

The calcium-binding glucose-responsive insulinotropic and anorexigenic hormone and neuropeptide nesfatin-1[250] operates in hypothalamic circuits regulating feeding and energy homeostasis. It may also directly modulate peripheral arterial resistance. It increases preproinsulin formation and insulin secretion and hampers feeding, at least in rodents. Glucose stimulates prepronesfatin synthesis and nesfatin-1 release. In DIO and T2DM, prepronesfatin production is upregulated.

Nucb2+ neurons in the PVN regulate feeding via the hypothalamus and brainstem [1043]. Axon collateral branches target multiple neurons that can be located in different nuclei, for example, a single Nucb2+ neuron in the PVN projects to both the DVC and the ArcN, which are involved in feeding regulation.

In the hindbrain, GLP1+ neurons project to the GLP1R+ nucleus accumbens and ventral tegmentum of the mesolimbic reward system [1012]. In the NAcc, activated GLP1R represses feeding via AMPA- (GluRs) and kainate-type glutamate (GluKs) receptors, and subsequent stimulation of GABAergic medium spiny neurons.

In addition, central GLP1 increases dopamine signaling in amygdala. Activated GLP1R in the CNS increases activity of tyrosine hydroxylase, the rate-limiting enzyme of dopamine synthesis in the ventral tegmental area. ^{VTA}Dopaminergic neurons also project to the amygdala.

Both GLP1 and GLP2 can prevent apoptosis and thus promote gut mucosa enlargement [1030].

Glucagon-Like Peptide-2

Also derived from preproglucagon, GLP2 is an anorexigen (Sect. 5.6.7.9). GLP2 stimulates PKA and then reduces glutamate-induced excitotoxic injury in hippocampal cells [1030].

Central, but not peripheral, administration of GLP2 stimulates hypothalamic nuclei (ArcN, PVN, DMH, VMH, and LH) via GLP2R in cooperation with the melanocortin system (MC_4) [1012].

[250] A.k.a. DNA-binding neuropeptide NEFA and nucleobindin-2. It is encoded by the NUCB2 gene. Prepronesfatin is cleaved into nesfatin-1.

Gastric Inhibitory Peptide

Gastric inhibitory peptide promotes energy expenditure, but does not affect food intake.

Growth Hormone-Releasing Hormone

Growth hormone-releasing hormone (GHRH; or GHR factor [GHRF]) derives from a precursor. It is released from neurosecretory cells in the hypothalamic ArcN. The predominant site of its receptor GHRHR is the pituitary gland, the hypothalamic peptide GHRH stimulating secretion of the pituitary growth hormone (GH).

Hypothalamic hormones control the activity of target cells in the anterior pituitary gland. Hypothalamic GHRH in cooperation with somatostatin regulates via GHRHR the production of GH by pituitary somatotroph cells (or somatotropes) and secretion via the AC–cAMP–PKA axis and calcium influx [1030].

Growth hormone contributes to regulating cellular metabolism, proliferation, and differentiation, many of its effects being mediated by insulin-like growth factor, IGF1 [1030]. The IGF1 receptor is observed in the hypothalamus and pituitary gland. In the hypothalamus, IGF1 can regulate GH secretion, as it stimulates somatostatin release. In the pituitary gland, IGF1 reduces GHRHR synthesis and hence GH secretion.

The GHRH+ neurons localize primarily to the ArcN and also DMH and VMH [1012]. ^{Arc}GHRH+ neurons project to the perifornical region, the lateral pre-optic area, and hypothalamic suprachiasmatic and medial pre-optic nuclei (MlPON). The latter two areas are major central sites of GHRH orexigenic action. This action depends on the circadian rhythm, as it favors and impedes feeding during the light and dark phases respectively, in rats, selectively reducing protein intake from dark onset feeding, without affecting carbohydrate intake [1012]. Hence, GHRH participates in regulating the circadian feeding rhythm. Somatostatin may inhibit GHRH+ neurons.

5.6.2.4 Melanocortin Pathway

Melanocortins are a group of small anorexigenic peptidic hormones derived by the cleavage of POMC synthesized in the pituitary gland and hypothalamic neurons. Melanocortins include melanocyte-stimulating hormone (α MSH, β MSH, and γ MSH) and, in the anterior pituitary gland, ACTH.

After feeding and energy excess, POMC+ neurons are activated by circulating hormones, such as leptin and insulin, and neurotransmitters, and then inhibit feeding and stimulate metabolism. Signaling by POMC+ neurons is controlled by neighboring neurons that synthesize GABA, NPy, and AgRP. Pro-opiomelanocortin is cleaved not only to ACTH and MSH but also to endorphins [1044].

In the brain, POMC processing engenders essentially to α MSH and γ MSH. POMC neurons localize in particular to the ArcN of the hypothalamus, and project to multiple brain regions. Loss of α MSH production by POMC-deficient neurons favors obesity.

In hypothalamic neurons that produce the polypeptide hormone precursor POMC, cleavage of POMC by PCSK1 creates $ACTH_{1–39}$ from the middle part of the POMC precursor; $ACTH_{1–39}$ is further processed by PCSK2 to $ACTH_{1–17}$ [1044]. $ACTH_{1–17}$ generates mature α $MSH_{1–13}$, which, once it is released into the synaptic cleft, stimulates the postsynaptic melanocortin Gs-coupled receptor MC_4 in the hypothalamus and suppresses appetite. $ACTH_{1–17}$ is processed during transit of POMC along the secretory pathway within hypothalamic neurons and α $MSH_{1–13}$ is stored in vesicles. $ACTH_{1–17}$ is sequentially cleaved by carboxypeptidase-E (CPe), peptidyl glycine α-amidating mono-oxygenase (PAM) to desacetyl α $MSH_{1–13}$, the newly exposed C-terminal glycine being converted to an amide, and ^{N}acetyltransferase, which acetylates the N-terminal serine residue. Short-lived α $MSH_{1–13}$ is inactivated to α $MSH_{1–12}$ by proline carboxypeptidase (prCP) in the hypothalamus.[251] Proline carboxypeptidase concentration is linked to inflammation, hypertension, and metabolic disorders (obesity and diabetes).

Melanocortins and their receptors are implicated in feeding, lipolysis, thermogenesis, hyperalgesia, pigmentation, memory, sexual behavior, and central regulation of cardiovascular activity and inflammation. They stimulate melanogenesis in melanocytes and steroidogenesis in adrenal cortical cells.

Five melanocortin receptors (MC_1–MC_5) are characterized by their cell-specific expression pattern and binding affinity, MC_2 being the ACTH receptor. Agouti-related protein, which is involved in controlling feeding behavior via the central melanocortin axis, and agouti signaling peptide antagonize melanocortin receptors; they prevent cAMP production mediated by stimulated melanocortin receptors especially in the hypothalamus and adrenal glands. Agouti-related protein suppresses the activity of MC_3 and MC_4, as it promotes their endocytosis using arrestin; ASiP targets MC_1 and hinders α MSH signaling.

The orexigenic hormone ghrelin produced by the stomach and released into the blood circulation during fasting, involves melanocortin signaling. Ghrelin stimulates the liberation of NPy and AGRP, inhibiting anorexigenic POMC+ neurons and increasing food intake. Furthermore, it upregulates hypothalamic prCP formation [1045].

The feeding-inhibitory melanocortin pathway based on leptin-responsive POMC+ CART+ neurons in the ArcN operates via α MSH on pre- and postsynaptic melanocortin receptors MC_3 and MC_4, AgRP antagonizing α MSH at these receptors [1011]. The MC_4 receptor is synthesized in several hypothalamic nuclei (PVN, LHA, and ArcN) in addition to other brain regions, such as parasympathetic and sympathetic preganglionic neurons in the medulla and spinal cord.

[251] The serine peptidase prolyl carboxypeptidase (prCP) operates in the renin–angiotensin and kallikrein–kinin axes. In hypothalamic POMC+ neurons, it processes α $MSH_{1–13}$ [1045].

Leptin activates POMC+ neurons that innervate sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord, hence contributing to increasing arterial pressure and cardiac frequency as well as regulating energy expenditure and insulin secretion and sensitivity [1011]. Elevated leptin concentration in DIO causes hypertension via LepR of neuronal circuits in the DMH [1046].

The MC_4 receptor is detected in several other brain regions involved in feeding regulation such as the nucleus accumbens, which possesses GABAergic neurons projecting to the LHA [1011]. Therefore, the melanocortin system contributes not only to the homeostatic control of feeding, but also to its hedonic aspects.

5.6.2.5 Neuropeptide-Y Family

Members of the NPY family, NPy, peptide Tyr–Tyr (PYY), and pancreatic polypeptide (PPy), are produced by cells at distinct levels of the gut–brain axis [1028].

Neuropeptide-Y Synthesis Sites

Neuropeptide-Y is produced at all levels of the brain–gut axis, that is, enteric neurons (enteric neural plexus, interneurons, descending inhibitory motoneurons of the myenteric plexus [where it co-localizes with vasoactive intestinal polypeptide and nitric oxide synthase], and noncholinergic secretomotor neurons [SMNs] of the submucosal plexus), primary afferent neurons originating in the dorsal root ganglia (particularly injured mid-sized and large sensory neurons), several neuronal circuits of the brain, and postganglionic sympathetic neurons (where it co-localizes with NAd and ATP) [1028]. It preferentially resides in sympathetic neurons supplying the vasculature, but is absent from sympathetic neurons innervating the gastrointestinal mucosa.

In the spinal cord, an abundant NPy+ neuropil is made of at least three different neuronal populations: (1) NPy+ GABA+ inhibitory interneurons, (2) descending noradrenergic neurons originating in the locus ceruleus, and (3) primary afferent nerve endings. In the spinal cord of rats and mice, the NPy receptor Y_1 lodges on seven distinct neuron types of the dorsal horn and Y_2 exclusively on the central terminals of primary afferent neurons in the superficial laminae [1028].

In the brain, NPy is the most abundant neuropeptide, especially in the NTS, ventrolateral medulla, periaqueductal grey, locus ceruleus, thalamus, hypothalamus (e.g., the arcuate and paraventricular nuclei), septum, hippocampus, amygdala, basal ganglia, nucleus accumbens, and cerebral cortex [1028]. This neurotransmitter is used by noradrenergic neurons originating in the locus ceruleus and issuing both ascending and descending projections in the CNS, NPy+ AgRP+ neurons of the ArcN, and various circuits of the limbic system.

Production of Peptide-YY and Pancreatic Polypeptide

On the other hand, PYY and PPy are exclusively formed by endocrine cells of the digestive tract. Peptide-YY is produced by enteric neurons of the stomach, pancreatic endocrine cells, and endocrine L cells that abound in the lower gastrointestinal tract in proportion to nutrient intake [1028]. It is postprandially released from intestinal L cells. Gastric acid secretion, cholecystokinin, bile acids, and C12-length and short-chain FFAs (butyrate) stimulate PYY secretion. PYY+ L cells also produce glicentin and glucagon-like peptides GLP1 and GLP2. These cells possess bitter and sweet taste receptors in addition to taste-related gustducin and thus serve as chemosensors. In the rodent brain, PYY+ neurons localize to the hypothalamus, pons, medulla and spinal cord [1028].

Pancreatic polypeptide is synthesized by pancreatic endocrine F cells in addition to endocrine cells in the small and large intestines, distinct from PYY+ cells [1028]. It is also postprandially secreted. Its release and actions on feeding and digestion require the parasympathetic vagus nerve.

Cleavage of Neuropeptide-Y and Peptide-YY

Once released, the N-terminus of NPy and PYY is truncated by dipeptidyl peptidase DPP4 and other enzymes (e.g., aminopeptidase-P and membrane metalloendopeptidase [MME]),[252] yielding $NPy_{3–36}$ and $PYY_{3–36}$ fragments [1028]. $PYY_{3–36}$ is a preferential agonist of the Y_2 receptor.

Neuropeptide-Y Receptors

The NPy receptors (Y_1–Y_2 and Y_4–Y_5 in humans), which are involved in circadian rhythm control, are also activated by PYY and pancreatic polypeptide, with given affinities for the different members of the NPY family and their fragments. Whereas NPy and PYY do not markedly differ in their affinities for Y_1, Y_2, and Y_5, $PYY_{3–36}$, the most common circulating form, is a preferred agonist of Y_2 and PPy binds preferentially to the Y_4 receptor [1028]. Whereas full-length $PYY_{1–36}$ activates Y_1, Y_2, and Y_5, $PYY_{3–36}$ preferentially operates via Y_2 and accessorily Y_5. The major NPy receptor subtypes in the brain are the widespread Y_1 and Y_2 receptors, whereas Y_4 and Y_5 are restricted to some brain regions, such as the NTS, area postrema, nucleus ambiguus, hypothalamus, thalamus, and amygdala.

In the brain, NPy binds to its Gi/o-coupled receptors, which trigger AC inhibition. It increases feeding and lipid (energy) storage facilitates learning and memory, as it modulates hippocampal activity, impedes anxiety, and regulates mood

[252] A.k.a. atriopeptidase, enkephalinase, neprilysin, and neutral endopeptidase-24.11.

and stress resilience. Both PPy and PYY signal to the brain, thereby attenuating food intake and depression-related behavior [1028].

Neuropeptide-Y stimulates vSMC contraction, modulates release of pituitary hormones, and hinders insulin release. Its signaling mediators encompass Ca^{2+} channels in neurons and K^+ channels in CMCs and vSMCs.

Neuropeptide-Y and Nociception

In the gut–brain axis, the neurotransmitter NPy, an important regulator of emotion processing, inhibits nociceptive transmission in the spinal cord and brainstem arising from intestinal obstruction, inflammation, and damage [1028]. Its Y_1, Y_2, and Y_5 receptors are widely expressed in cerebral areas involved in anxiety, mood, cognition, and stress resilience regulation. NPy acts as a stress mediator in the central and peripheral nervous systems and in the cardiovascular apparatus and gastrointestinal tract (e.g., stress-induced defecation and feeding), in addition to metabolism, immunity, and stress adaptation.

The sensory innervation of the gastrointestinal tract (mesentery, serosa, muscularis, and mucosa) relies on two major neuronal populations: (1) spinal afferent neurons originating from the dorsal root ganglia and (2) vagal afferent neurons originating primarily from the nodose ganglion.

Spinal afferent neurons, which contain low NPy amounts, terminate in the spinal cord, where interneurons and descending noradrenergic neurons have a higher NPy content. NPy tethers to Y_1 and Y_2 in the spinal cord and counters thermal, chemical, and mechanical hyperalgesia. NPy can control pain transmission in the spinal cord primarily via Y_2 and subsequent inhibition of transmitter release from the terminals of primary afferent neurons and via Y_1 and subsequent inhibition of postsynaptic neurons in the dorsal horn [1028].

Vagal afferent neurons also contribute to visceral nociception, particularly visceral chemonociception. In the gut–brain axis, NPy may function as both a transmitter and modulator of the communication between vagal afferents and their projection neurons in the NTS. NPy operates via Y_2 (most likely presynaptic) and Y_4 in the NTS, which control the chemonociceptive input from the stomach to the brainstem [1028].

Neuropeptide Y can limit the impact of psychological stress on the gut–brain axis. The amygdala, a major brain region coordinating behavioral stress responses, contains high concentrations of NPy, anxiolytic excitatory postsynaptic Y_1, emotion-supporting presynaptic Y_2 and Y_4, and Y_5 [1028].

Intestinal hormones, such as ghrelin, PYY, PPy, GLP1, and GLP2 also influence emotion, anxiety, and mood. Ghrelin released from the upper gastrointestinal tract upon hunger functions as an anxiolytic and antidepressant [1028]. $PYY_{3–36}$ promotes hedonic sensation via direct access to the brain and activation of vagal afferents to the brainstem. The effect of PPy preferentially mediated by Y_4 diminishes anxiety-like and depression-related behaviors via peripheral action and

the area postrema [1028]. On the other hand, GLP1 favors anxiety-related behavior, whereas GLP2 attenuates a depression-like state [1028].

Neuropeptide-Y and Peptide-YY Effects on Feeding and Digestion

The gastrointestinal tract produces and releases multiple para- and endocrine messengers that regulate feeding and satiety to ensure energy homeostasis. In gut–brain signaling, the satiety factors PPy and PYY slow the gastrointestinal transit of chyme and prevent further food intake.

Pancreatic polypeptide release is under vagal cholinergic control. It activates Y_4 on neurons in the brainstem, hypothalamus, and amygdala [1028]. It can enter the brain preferentially in circumventricular organs, which are structures in the brain characterized by their extensive vasculature and lack of a normal blood–brain barrier, such as the area postrema and subfornical organ. Pancreatic polypeptide decreases food intake and increases energy expenditure via the vagus nerve.

Peptide-YY is a relatively selective Y_2 receptor agonist. Both $PYY_{1–36}$ and $PYY_{3–36}$ hamper gastric acid secretion, gastrointestinal transit, and food intake in both lean and obese subjects via stimulation of Y_2 on vagal afferent neurons and in the hypothalamus [1028]. In the brain, $PYY_{3–36}$ reduces feeding primarily via presynaptic Y_2 on orexinergic NPy+ AgRP+ neurons in the ArcN, inhibiting their action and hence disinhibiting POMC+ neurons, as orexinergic and anorexinergic neurons exert a mutual inhibition.

In the brain, Npy mainly targets Y_1 (also Y_5) in the hypothalamus, brainstem, nucleus accumbens, and corticolimbic system implicated in the regulation of appetite.

In the digestive tract, NPy and PYY preclude gastrointestinal motility and water and electrolyte (e.g., Cl^-) secretion [1028]. Both PYY and NPy exert a tonic gastrointestinal anti-secretory activity via epithelial Y_1 and neural Y_2 receptors. Antagonism of Y_1 and Y_2 explains colonic transit acceleration by Y_1 and inhibition by Y_2. Although Y_2 plays a major role in the anti-secretory and pro-absorptive action of NPy and PYY, Y_1 on SMNs and epitheliocytes and Y_4 on enterocytes also contribute to these effects.

The enteric and cerebral neuropeptide NPy and the gut hormones PYY and PPy play specific roles at various levels of the brain–gut axis. They inhibit gastrointestinal motility and secretion, thereby affecting signals sent to the brain via a neural route or the bloodstream [1028].

Polypeptide Y acts preferentially via Y_4 and Y_5. It hampers gastric emptying via the vagus nerve and acid secretion via Y_1 and Y_2 and its action in the brainstem and stomach, hence reducing appetite, in addition to intestinal electrolyte and water secretion and intestinal peristalsis by neural and non-neural mechanisms [1028]. Inhibition of $PYY_{1–36}$ and $PYY_{3–36}$ on gastrointestinal motility and secretion is mediated by Y_1 on enterocytes, myenteric, and submucosal neurons and ECs, Y_2 on myenteric and submucosal neurons and extrinsic primary afferent nerve fibers, and Y_4 on enterocytes [1028]. PYY may act as an ileal and colonic brake set

into operation when lipids reach the lower gut that slows gastric emptying and intestinal transit, which, in coordination with its anti-secretory action, facilitates nutrient absorption.

The receptor of ethanolamide oleoylethanolamide (OEA) and LPC, glucose-dependent insulinotropic receptor GPR119 (or GPCR2) respond to luminal nutrients and release PYY and GLP1 from L cells [1028].

The NPy–PYY system has an impact on the composition and function of the gut flora. The appetite stimulator, NPy, has an anti-bacterial effect against Escherichia coli, Enterococcus faecalis, and Lactobacillus acidophilus [1028].

In addition, NPy released from sympathetic nerves in lymphoid tissues in contact with immunocytes (e.g., dendrocytes, granulocytes, B, T, and NK lymphocytes, monocytes, and macrophages), acts as a neuro-immune transmitter that activates or represses immunity according to its concentration, activated Y receptor subtype, and immunocyte types [1028]. It modulates immunocyte transfer, helper T-cell differentiation, NK-cell activity, phagocytosis, cytokine secretion, and ROS production. It activates antigen-presenting cell function. It stimulates Y_1 and exerts a gastrointestinal proinflammatory action. NPy promotes NOS2 expression and subsequent redox stress and inflammation. In the mouse gastrointestinal tract, NPy+ nerves contact immunocytes such as IgA-producing lymphocytes in the ileum lamina propria. In humans, it stimulates proliferation of lymphocytes in the colonic lamina propria. Nonetheless, the proinflammatory NPy effect may be countered by its induced vasoconstriction, the sympathetic action on splanchnic resistance arteries being co-mediated by the sympathetic triad, that is, ATP, NAd, and NPy. NPy potentiates NAd- and ATP-primed mesenteric vasoconstriction via postjunctional Y_1 receptor [1028]. On the other hand, colonic PYY concentration declines in patients with inflammatory bowel disease.

Conversely, the gut microbiota influences the gut–brain axis via PYY and NPy. For example, dysbiosis of the intestinal flora upregulates the formation of brain-derived neurotrophic factor (BDNF) in the hippocampus [1028]. Long-term treatment of mice with the probiotic Lactobacillus rhamnosus reduces anxiety and improves stress coping via the vagus nerve. Neuropeptide Y participates in the regulation of emotion and affective behavior. NPy+ neurons in the arcuate and paraventricular nuclei protect against behavioral disturbances in response to infection and immunity stimulation. Proinflammatory cytokines reach the brain via the bloodstream in addition to exciting vagal afferent neurons; they then elicit cytokine formation by microgliocytes and astrocytes.

Although the human gut has a limited repertoire of glycoside hydrolases, the gut microbiota synthesizes these enzymes, which process dietary carbohydrates to acetate, propionate, and butyrate [1028]. ScFAs can interact with FFAR3+ PYY+ enteroendocrine cells in the intestinal epithelium.

5.6.3 *Enteric Nervous System*

The digestive tract from the esophagus, stomach, small intestine, to the colon is innervated by the CNS and its walls are endowed with the ENS, which regulates locally the behavior of digestion organs independently of the CNS, both the ENS and the CNS sharing the structure and neurochemistry [1047].

The ENS, a quasi-autonomous component of the nervous system that participates in digestion control (food degradation, nutrient absorption, and waste removal, in addition to mechanical mixing and rhythmic propulsive muscular contractions), cooperates with the sympathetic and parasympathetic branches of the autonomic nervous system (remote control). It communicates with CNS reflex and command centers and sympathetic ganglia-associated neural circuits via a bidirectional information flux between the ENS and CNS through the vagus and pelvic and sympathetic nerves in addition to between the ENS and sympathetic prevertebral ganglia [1048].

The ENS (neuron number $\mathcal{O}[10^8]$) consists of a huge number ($\mathcal{O}[10^3]$) of small ganglia, the great majority of which form the myenteric and submucosal plexuses [1048]:

1. The *myenteric plexus* is a continuous circuit from the esophagus to the internal anal sphincter. It resides between the outer longitudinal and inner circular smooth muscle.
2. The *submucosal plexus* exist only in the small and large intestines.

These plexuses also contain gliocytes, which nourish neurons, mastocytes, which ensure protective inflammation, and the blood–ENS barrier, which prevents entry of harmful substances. They contain sensors of chemical species of the alimentary bolus during the oro-aboral transit and physicochemical properties of the digestive tract environment. These sensors monitor the state of food processing and propulsion within the digestive tract. The nervous system controls not only food transit time and mucus secretion but also bacterial species of the digestive ecosystem. Conversely, the intestinal biota influences neurotransmitter levels. Neurons also project from the ENS to the prevertebral ganglia in addition to organs implicated in food intake and processing, the trachea, gallbladder, and pancreas.

The digestive tract smooth muscle is organized in layers of smooth myocytes that operate as mechanical units, as they are interconnected via gap junctions, under the control of pacemaker cells, interstitial cells of Cajal.

The relative roles of the ENS and CNS differ considerably along the digestive tract [1048]:

- The CNS determines the movements of the esophagus via neural pattern generators and plays a major role in monitoring the state of the stomach and in controlling its contractile activity and acid secretion via the vagovagal reflexes. Voluntary control of defecation is exerted via the CNS, with its defecation centers in the lumbosacral spinal cord and pelvic connections.

- The ENS in the small intestine and colon involved in the sensory-motor control contains reflex circuits with their sensory afferent neurons, which are sensitive to chemical and mechanical stimuli, interneurons, and motor neurons that regulate muscular activity, transmucosal fluxes, local blood flow, and endocrine and immune functions.

Major neurotransmitters of the ENS include dopamine, glutamate, NAd, NO, and serotonin, which triggers peristalsis. The gastrointestinal tract is also a source of neuropeptides, enkephalins, and benzodiazepines.

Networks of enteric ganglia are connected by interganglionic bundles. Most enteric motor neurons, which act on effector cells (epitheliocytes, smooth myocytes of the digestive tract walls, pacemaker, neuroendocrine, mucosal gland and vascular cells, and immunocytes) localize to the myenteric plexus [1049]. Hence, the myenteric plexus comprises intestinofugal (IFNs) and enteric primary afferent neurons (EPANs), ascending (AINs) and descending interneurons (DINs), excitatory (ECMMNs) and inhibitory circular (ICMMNs) and longitudinal muscle motoneurons (LMMNs), SMNs and vasomotor neurons (VMNs), in addition to projections of EPANs of the submucous plexus.

Enteric primary afferent neurons of myenteric and submucous plexuses respond to luminal chemical stimuli and wall stretch [1049]. These neurons receive a slow synaptic input, probably mediated by tachykinins from other EPANs, and are connected to AINs, DINs, LMMNs, ECMMNs, and ICMMNs:

- Excitatory circular muscle motoneurons employ acetylcholine and tachykinins [1049].
- Inhibitory circular muscle motoneurons contain NO, ATP, VIP, and AdCyAP1 (or PACAP).
- Ascending interneurons possess synthases for acetylcholine, tachykinins, and opioids.
- Descending interneurons include cholinergic, somatostatin and ChAT+ (choline acetyltransferase), serotonin and ChAT+, NOS, VIP, and ChAT+, in addition to NOS and VIP+ DINs.
- Secretomotor neurons in the myenteric ganglia comprise two groups, cholinergic and VIP+ SMNs.
- Intestinofugal cholinergic neurons project from the myenteric ganglia to the prevertebral ganglia. They form short intestino-intestinal inhibitory reflex pathways, activation of which reduces both motor and secretomotor activity.

In addition to the reflex motor response of the longitudinal muscle, many polarized enteric motor pathways control the motor activity of the intestinal circular smooth muscle [1049]. Small rings of circular smooth muscle can contract independently with a spatiotemporal coordination due to interconnected neural modules with repeated and overlapping circumferential, ascending, and descending circuits, peristalsis corresponding to the sequential contraction of the circular smooth muscle initiated by local stretch due to the intraluminal content that propels the alimentary bolus.

The circumferential circuit, which is activated by mechanical stimulation of EPANs, synapses with local inhibitory and excitatory motoneurons.
The ascending excitatory circuit involves EPANs, AINs, and ECMMNs.
The descending inhibitory circuit relies on a different EPAN type with long anal projections connected to ICMMNs.
The descending excitatory circuit includes mechanosensitive EPANs in addition to ECMMNs.

The secreto- and vasomotor neural pathways comprise EPANs of both the submucosal and myenteric plexuses and cholinergic and noncholinergic SMNs and VMNs in the submucosal ganglia.

In mice, the ENS neuroglial network is composed of overlapping clonal units. Neurons and gliocytes, which regulate gut function, derive from neural crest progenitors [1050].

The ENS regulates the gut flora, in addition to food flux and processing. Peristalsis influences gut microbiota composition, as it suppresses the growth of the proinflammatory bacterial community, at least in zebrafish [1051]. For example, an expanded population of Vibrio combined with a loss of Faecalibacterium prausnitzii or Escherichia can cause neutrophil recruitment. Conversely, the introduction of Escherichia or Shewanella ameliorates the intestinal state.

The intestinal microbiota is linked to the body metabolism and circadian clock via the circadian transcription factor, nuclear factor interleukin-3-regulated protein NFIL3 [1052]. The epitheliocyte circadian transcription factor NFIL3 activates transcription from the IL3 promoter, but represses the transcriptional activity of Per1 and Per2. Its activity oscillates diurnally in intestinal epitheliocytes. It controls a circadian program of lipid metabolism, as it is involved in regulating lipid uptake in and export from intestinal epitheliocytes. Flagellin and lipopolysaccharide produced by certain intestinal bacteria control the amplitude of the circadian oscillation of NFIL3 via group-3 innate lymphoid cells (ILC3), STAT3, and the epithelial clock [1052].

5.6.4 Peptidic Messengers: Maturation by Cleavage

Neuropeptides constitute the largest class of messengers (>100 neuropeptides). Most peptidic hormones and neurotransmitters are synthesized as precursors that undergo proteolysis.

A *carboxypeptidase* cleaves a peptide bond at the carboxy (C)-terminus of a peptide or protein to liberate a single amino acid residue, whereas an *aminopeptidase* hydrolyzes peptide bonds at the opposite amino [N]-terminus of the protein.

The pancreatic carboxypeptidases CPa1, CPa2, and CPb are involved in food digestion. However, most carboxypeptidases operate in protein maturation, especially the synthesis of neuroendocrine peptides, in addition to growth factor production, blood coagulation, and wound healing, among other processes.

Carboxypeptidases are usually classified into three major categories:

1. *Metallocarboxypeptidases* utilize a metal in their active site.
2. *Cysteine (thiol) carboxypeptidases* use cysteine residues as active sites.
3. *Serine carboxypeptidases* employ serine residues as active sites.

For example, the zymogens[253] and apoenzymes,[254] carboxypeptidases, CPa and CPb, which are formed from precursors (pre-procarboxypeptidases) and activated by trypsin, in addition to CPd are *metallocarboxypeptidases* ($CPx^{Zn^{2+}}$, x: a, b, d).

Another type of classification refers to their substrate preference[255]:

- Carboxypeptidase-A (A: aromatic or aliphatic) targets amino acids containing aromatic or branched hydrocarbon chains.
- Carboxypeptidase-B (B: basic) cleaves positively charged amino acids (Arg or Lys).
- Carboxypeptidase-C has a preference for hydrophobic residues.
- Carboxypeptidase-D processes basic amino acids in addition to peptides with hydrophobic residues.
- Carboxypeptidase-E (CPe), or carboxypeptidase-H (CPh), also called enkephalin convertase, is a prohormone processor.

The membrane-bound metallocarboxypeptidase CPd of the trans-Golgi network removes the C-terminal Arg and Lys residues from peptides with an optimal pH ranging from 5 to 7. It participates in the synthesis of neuropeptides and peptide hormones following the action of furin, an endopeptidase of the trans-Golgi network.

Carboxypeptidase-E, or CPh, is responsible for C-terminal trimming of peptidic hormones and neurotransmitters [1053]. Hence, many substrates of CPe are neuropeptides.

Pro-protein convertases cut the precursor at specific sites to generate intermediates containing a C-terminal basic residue (lysine or arginine). These intermediates are then cleaved by CPe to remove the basic residues. This sequential processing suffices to produce the active peptide for many peptides, but, for some peptides, additional processing steps, such as C-terminal amidation, are subsequently required.

Conversion of C-terminally Gly-extended peptides into C-terminally α-amidated peptides is achieved by a sequential two-step process catalyzed by PAM.[256]

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[253] A zymogen is an inactive molecule that is converted into an enzyme when activated by another enzyme.

[254] An apoenzyme is a protein that forms an active enzyme upon combination with an additional small molecule, the cofactor, either an inorganic molecule such as metal (e.g., Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}) or a small organic molecule, a coenzyme (e.g., biotin [vitamin-B7 (or -H) or coenzyme-R] or coenzyme-A).

[255] For example, cathepsin-A is a lysosomal carboxypeptidase in addition to β-galactosidase and neuraminidase.

[256] A.k.a. PHM and peptidyl α-hydroxyglycine α-amidating lyase (PAL), as it has two enzymatically active domains. These catalytic domains work sequentially to mature neuroendocrine

Table 5.43 Peptidases of the PCSK family (*LPC* lymphoma pro-protein convertase, *MBTPS1* membrane-bound transcription factor peptidase site-1, *NARC* neural apoptosis-regulated convertase, *NEC* neuroendocrine convertase, *PACE* paired basic amino acid cleaving enzyme, *PC* prohormone (pro-protein) convertase, *SKI* subtilisin/kexin isozyme, *SPC* subtilisin-like pro-protein convertase)

Type	Other aliases
PCSK1	PC1, PC3, SPC3, NEC1
PCSK2	PC2, SPC2, NEC2
PCSK3	Furin, SPC1, PACE
PCSK4	PC4, SPC5
PCSK5	PC5, PC6, SPC6
PCSK6	SPC4, PACE4
PCSK7	PC7, PC8, SPC7, LPC
PCSK8	MBTPS1, S1P, SKI1
PCSK9	PC9, NARC1

The isozyme PCSK3 corresponds to furin and the subtype PCSK8 to site-1 peptidase S1P (i.e., membrane-bound transcription factor peptidase, site 1 [MBTPS1]). The serine peptidase PCSK8 catalyzes the first activation step of the membrane-bound transcription factors SREBPs and hence controls the lipid composition of cells. In response to cholesterol deprivation, PCSK8 cleaves SREBPs between two membrane-spanning domains, releasing them from membranes of the ER and GB in co-operation with SCAP, sterols blocking the ER–GB transport and cleavage using the sterol-sensing escort SCAP. Other MBTPS1 substrates include BDNF, ^{N}acetylglucosamine 1-phosphotransferase α–β subunits (GNPTαβ), and activating transcription factor ATF6, another membrane-bound transcription factor that activates genes in the ER stress response

The precursors contain multibasic amino acid cleavage sites with the consensus Lys(Arg)–X_n–Arg (X: any amino acid, except Cys; $n = 0, 2, 4, 6$). These multibasic sites are often cleaved by peptidases of the PCSK family (PCSK1–PCSK9),[257] PCSK1 and PCSK2 (Table 5.43).[258] The production of active PCSK2 requires the chaperone secretogranin-5, which also inhibits this enzyme [1053].

The major peptidases that cleave basic sites include the trans-Golgi network enzyme PCSK3 (or furin), and two secretory vesicle enzymes, PCSK1 and PCSK2. Other furin-like peptidases of the trans-Golgi network include PCSK7[259] and

peptides. This bifunctional enzyme is engendered from a pre-proprotein. Alternative splicing leads to soluble and integral membrane bifunctional PAMs in addition to a soluble monofunctional mono-oxygenase [1054]. Inclusion of exon A between the PHM and PAL domains (PAM1) in addition to monofunctional PAM4 is associated with multiple sulfated ^{O}glycans. The transmembrane domain is eliminated with Tyr965 sulfation (soluble bifunctional PAM3).

[257] PCSK: pro-protein convertase subtilisin/kexin. Members of the PCSK family are related to the yeast peptidase kexin and bacterial subtilisin.

[258] PCSK2 abounds in the intermediate lobe of the pituitary gland (hypophysis), where it may process β-lipotropin to β-endorphin and cleave ACTH into α-melanocyte-stimulating hormone precursor.

[259] A.k.a. pro-protein convertase PC7 or PC8.

PCSK5.[260] These peptidases produce intermediates that contain C-terminal Lys and/or Arg residues. These C-terminal basic residues are removed by CPd in the trans-Golgi network or by CPe in secretory vesicles.

Pro-protein convertase subtilisin/kexin 1 cleaves prodynorphin at a monobasic site, but cleaves the monobasic processing site of prosomatostatin inefficiently [1055]. Dynorphin convertase, a thiol peptidase present at high concentrations in the brain, pituitary gland, and ileum, may be involved in the monobasic processing of precursors other than prodynorphin. PCSK6 cleaves prosomatostatin at its monobasic site in the secretory pathway [1055]. Among four PCSK6 isoforms produced by mRNA alternative splicing, PCSK6c is formed in β cells, but not in α cells of the islets of Langerhans.

Both PCSK1 and PCSK2 process pro-insulin to insulin in the pancreatic β cells in addition to POMC to ACTH and other products in the anterior and intermediate lobes of the hypophysis (pituitary gland) [1055].[261]

Several peptidic regulators of food intake derive from a single precursor, proPCSK1n,[262] or proSAAS,[263] PCSK1n being a potent inhibitor of PCSK1 [1056].

Proteolytic cleavage of proPCSK1n at a K–X–X–R site engenders the fragments proPCSK1n$_{(34–41)}$ and proPCSK1n$_{(42–61)}$ [1053]. The C-terminal peptide corresponds to the proPCSK1n$_{(221–260)}$ fragment. PCSK1n is initially cleaved in the Golgi body or trans-Golgi network by furin and/or furin-like enzymes and carboxypeptidase-D. Resulting fragments are sorted into distinct vesicles and further processed by additional enzymes into the mature peptides. The small forms are generated by secretory granule prohormone convertases and CPe..

Pro-protein convertase subtilisin/kexin 1n has similar characteristics to chromo- and secretogranins, which form a category of neuroendocrine proteins,[264] which are cleaved in neuroendocrine tissues to generate neuroendocrine hormones and neurotransmitters. ProPCSK1n is cleaved in the brain and pituitary gland.

A different post-translational processing of *proglucagon* (proGcg) generates distinct sets of peptides with opposing activities, the corresponding sequences

[260] A.k.a. proprotein convertase PC5 or PC6.

[261] In cells overexpressing the PCSK1 inhibitor PCSK1n, processing of POMC (241 amino acids), itself synthesized from the 285-amino acid pre-pro-opiomelanocortin (pre-POMC), into either ACTH, or β-lipotropin, or β-endorphin is greatly reduced.

In the ArcN, POMC is cleaved to α α MSH, a neurotransmitter, which acts via melanocortin receptors MC_3 and MC_4 on neurons of other hypothalamic regions to cause hypophagia. POMC+ neurons have a catabolic effect; they raise energy expenditure.

[262] PCSK1n: pro-protein convertase subtilisin/kexin type-1 inhibitor.

[263] A protein precursor that contains the amino acid sequence Ser–Ala–Ala–Ser.

[264] Granins are regulated neuroendocrine secretory proteins lodging in dense-core secretory vesicles of endocrine and neuroendocrine cells storing amine and peptide hormones and neurotransmitters. They include chromogranin-A, -B, also called secretogranin-1, and -C, or secretogranin-2, and secretogranin-3, and -5, some of which can be precursors of auto-, para-, and endocrine messengers. Chromogranins can also be involved with the sorting of proteins into the regulated secretory pathway.

in proglucagon being linked by pairs of basic amino acids (Lys–Arg or Arg–Arg) [1055, 1057]. Proglucagon is initially cleaved at the Lys79–Arg80 site and engenders *glicentin* and *major proglucagon fragment* (MPGF). The amino acid sequence of glicentin contains that of glucagon; hence, in the pancreas, glicentin serves as an intermediate in the formation of glucagon.

- In the pancreas, proglucagon engenders *glicentin-related pancreatic polypeptide* (GRPP; $\text{proGcg}_{(1–30)}$), *glucagon* ($\text{proGcg}_{(33–61)}$), intervening peptide IP1 ($\text{proGcg}_{(64–69)}$), and MPGF ($\text{proGcg}_{(72–158)}$). Glicentin ($\text{proGcg}_{(1–69)}$) is cleaved at the Lys31–Arg32 and Lys62–Arg63 sites in GRPP, glucagon, and IP1, whereas MPGF accumulates and is slowly and partly processed to GLP1 after cleavage at the Arg109–Arg110 site. In pancreatic islet α cells, proglucagon is cleaved by PCSK1 to *glucagon-like peptide*-1 (GLP1 [$\text{proGcg}_{(72–107/108)}$]) and by PCSK2 to glucagon. These cells possess a high concentration of PCSK2, which processes proglucagon to glucagon, but a very low PCSK1 concentration.[265]
- In the gut and brain, proglucagon generates *glicentin* (enteroglucagon [$\text{proGcg}_{(1–69)}$]), truncated GLP1 ($_{t}\text{GLP1}$ [$\text{proGcg}_{(78–107)}$]), intervening peptide IP2 ($\text{proGcg}_{(111–122/123)}$), and *glucagon-like peptide*-2 (GLP2 [$\text{proGcg}_{(126–158)}$]). Hence, in intestinal endocrine L cells, proglucagon cleaved by PCSK1, which is both necessary and sufficient for complete proglucagon processing, engenders GLP1, a potent incretin hormone, and GLP2, a regulator of gut mucosal growth and integrity. An additional cleavage of GLP1 at Arg109 yields short active forms of GLP1, collectively termed truncated GLP1, $\text{GLP1}_{(7–37)}$ ($\text{proGcg}_{(78–108)}$) and its desglycyl C-terminally amidated counterpart, $\text{GLP1}_{(1–36)}$–amide ($\text{GLP1}^{\text{NH}_2}_{(1–36)}$ [$\text{proGcg}_{(78–107)}$]). Production of active $_{t}\text{GLP1}$ involves the cleavage of the proglucagon at the monobasic Arg109 processing site. The amidated and Gly-extended forms have similar activities and overall metabolism [1057]. In humans, almost all of GLP1 secreted from the gut is amidated. Glicentin engenders GRPP and *oxyntomodulin* (enteroglucagon [$\text{proGcg}_{(33–69])}$]) using PCSK2.

The pancreatic β cell secretes glucagon that counterbalances the hypoglycemic action of insulin, whereas the intestinal L cell releases a potent insulinotropic hormone, $_{t}\text{GLP1}_{(7–36)}$, the active truncated GLP1 form produced from $\text{GLP1}_{(1–36)}$ cleavage by PCSK1 or PCSK3.

The proglucagon gene engenders various related peptides that target distinct class-*II* GPCRs for specific effects (Table 5.44).

[265] PCSK1 can cleave the Lys31–Arg32, Lys79–Arg80, and Arg109–Arg110 processing sites of proglucagon [1055].

Table 5.44 Class-*II* peptide hormone receptors (Source: [1030] *AdCyAP* adenylate cyclase-activating polypeptide, *CalcRL* calcitonin receptor-like receptor, *CRH* corticotropin-releasing hormone, *CGRP* calcitonin gene-related peptide, *GHRH* growth hormone-releasing hormone, *GIP* gastric inhibitory peptide, *PTH* parathyroid hormone, *PTHRP* PTH-related peptide, *VIP* vasoactive intestinal peptide)

Receptor	Ligand	Primary function
SctR	Secretin	Pancreatic secretion
GcgR	Glucagon	Glycogenolysis and gluconeogenesis, insulin secretion
GLP1R	GLP1	Secretion of insulin and glucagon
GLP2R	GLP2	Proliferation of intestinal epitheliocytes
GHRHR	GHRH	Growth hormone secretion
AdCyAP1R1	AdCyAP1	Glucose homeostasis, nociception, learning, memory, circadian rhythm
VIP_1 (VIPR1)	VIP, AdCyAP1	Neuromodulation, T-cell differentiation
VIP_2 (VIPR2)	VIP, AdCyAP1	Circadian rhythm
CRH_1 (CRHR1)	CRH, urocortin	Secretion of adrenocorticotropin hormone (ACTH)
CRH_2 (CRHR2)	Urocortin-1/2/3	Stress-related behavior, neuroendocrine function
PTH_1 (PTHR1)	PTH1, PTHRP	Ca^{2+} homeostasis (bone and kidney), tissue differentiation
PTH_2 (PTHR2)	PTH2	Renal vasodilation
CalcR	Calcitonin, amylin, CGRP	Ca^{2+} homeostasis (calcitonin)
		Glucagon secretion (amylin)
CalcRL	CGRP, adrenomedullin	Microvascular tone
GIPR	GIP	Secretion of insulin

The proglucagon gene engenders various related peptides, glicentin, glicentin-related pancreatic polypeptide (GRPP), intervening peptide IP1, major proglucagon fragment (MPGF), glucagon, and the glucagon-like peptides, GLP1 and GLP2, which target distinct receptors for specific effects

5.6.5 *Gastrointestinal Epithelial Barrier*

The intestinal mucosa is covered by a single layer of epitheliocytes fastened by tight junctions that limits intestinal permeability. The intestinal epithelium is structurally organized in crypts and villi.

Intestinal cells of crypts and villi include absorptive enterocytes and secretory cells, such as Paneth, goblet, tuft, and enteroendocrine cells in the small intestine (Table 5.45). Thus, the intestinal epithelium consists of [1058]:

Table 5.45 Intestinal epitheliocytes (Source: [1059])

Intestinal epitheliocyte type	Role
Intestinal epithelial stem cell	Renewal of the epithelium
Enterocyte	Nutrient absorption
	Transcytosis of IgA from the lamina propria to the lumen side, secretion of antimicrobial proteins
Paneth cell	Construction of stem cell niche
	Secretion of mucus, antimicrobial agents (α-defensins, cathelicidins, lysozyme, Reg3γ)
Goblet cell	Secretion of mucus, RELMβ, TFF3
	Transport of luminal antigens across epithelia
Enteroendocrine cell	Secretion of regulators of appetite and digestion Cck (I cells), GLP1, GLP2 (L cells), 5HT (enterochromaffin cells)
Tuft cell	Induction of type-2 immune response (secretion of IL25)
Microfold cell (M cell)	Transport of luminal antigens to dendrocytes across epithelia

Enterocytes, which are implicated in nutrient absorption, represent the vast majority of villous cells in the small intestine.

Goblet cells, which are scattered throughout the epithelium and form a protective mucus layer.

Enteroendocrine cells (∼1% of epitheliocytes), which produce hormones and hence regulate various functions of the intestinal epithelium.

Paneth cells (lifespan ∼2 months), which are clustered in the crypt bottom, where they represent the single type of differentiated cells; they produce antimicrobial peptides, which control the gut microbiota, in addition to growth factors for the maintenance of the neighboring stem cells.

Microfold or membranous (M) cells, which cover the surface of the gut-associated lymphoid follicles.

Cup cells (up to 6% of ileal epitheliocytes).

Tuft cells (0.4% of intestinal epitheliocytes).

In the large intestine, secretory cells, mainly goblet cells, are more abundant than absorptive enterocytes.

This epithelial barrier constitutes the first line of defense against intruding microorganisms via secretion of mucins and antimicrobial proteins and transport of secretory IgA.

The intestine is one of the organs with the highest self-renewal capacity. The entire epithelium is renewed in 4–7 days [1058]. Multipotent LGR5+[266] bHLHa45+, and OlfM4+[267] intestinal stem cells (ISCs) localize to the base of intestinal crypts [1060, 1061]. They continuously proliferate to maintain their pool and to engender differentiated intestinal cell types. Regeneration of the intestinal epithelium is initiated by ISCs, which engender transit-amplifying precursor cells. These can generate all intestinal cell types, which move toward the lumen until they are eventually shed [1062]. Wnt, BMP, notch, and hedgehog regulate the ISC fate.

The differentiation status of a cell is determined by its position along the villus axis, which is tightly regulated by opposing gradients of morphogens such as Wnt and BMP, Wnt–β Ctnn signaling being the highest at the crypt base, where it promotes stem cell expansion and transit-amplifying cells proliferate, whereas BMPs abound near the lumen axis and inhibit proliferation [1062]. During intestinal regeneration, opposing gradients of Wnt and BMP signaling ensure proper differentiation along the villus axis.

Intestinal subepithelial myofibroblasts (ISEMFs), which are adjacent to ISCs near the crypt base, secrete BMP antagonists, such as gremlins Grem1 and Grem2 and noggin, thereby promoting ISC proliferation at the crypt base. ISEMFs influence cell fate in the regenerating intestine, as they secrete AngptL2, which primes an autocrine positive feedback loop via integrin-$\alpha_5\beta_1$ and NFκB, which downregulates formation of BMP2 and BMP7 [1063]. Angiopoietin-like protein AngptL2 maintains the ISC niche and enables regeneration of intestinal epithelium after injury, as it prevents BMP synthesis. It does not operate via the PI3K–PKB–β Ctnn and PI3K–NFκB axes [1063].[268]

Upon damage, DLL1+ committed cycling or quiescent progenitors, mainly from the secretory lineage lodging at the border of the stem cell niche dedifferentiate and repopulate the stem cell niche, regenerating LGR5+ cells [1061].

Stem cells rapidly divide to engender highly proliferative progenitor cells, *transit-amplifying cells*, which are transient indispensable integrators of stem cell niche components. Transit-amplifying cells have a finite lifespan [1066]. They arise from stem cells, proliferate, and then gradually differentiate, hence being related to progenitor and precursor cells. Stem, transit-amplifying, progenitor, and precursor cells are all characterized by a gene expression pattern linked to an epigenetic

[266] LGR5: leucine-rich repeat-containing GPCR-5, a R-spondin receptor.

[267] OlfM4: olfactomedin-4. It supports cell adhesion, probably via lectins and cadherins [108]. Overexpression of OlfM4 can induce cell differentiation and apoptosis [194].

[268] The Wnt–β Ctnn and PTen–PI3K–PKB pathways interact to drive long-term hematopoietic stem cell proliferation and simultaneously inhibit differentiation and apoptosis via upregulation of inhibitor of differentiation ID2 (bHLHb26) [1064]. Maintenance of self-renewal and induction of differentiation in embryonic stem cells in response to Wnt signaling relies on a regulatory circuit involving β-catenin, cadherin-1, the PI3K–PKB axis, and Snai2 in a time-dependent manner. Short-term upregulation of β-catenin enhances ESC self-renewal via the PI3K–PKB cascade [1065]. Long-term Wnt activation promotes ESC differentiation via the β Ctnn-Snai2 axis.

signature. Intestinal transit-amplifying cells migrate toward the tip of the villus axis, where they undergo apoptosis and are shed into the lumen of the healthy intestine. Whereas primed stem cells generate transit-amplifying cells, quiescent stem cells only proliferate when transit-amplifying cells are formed and begin to synthesize sonic hedgehog [1067]. Generation of transit-amplifying cells is thus independent of autocrine SHh signaling, which instead promotes proliferation of quiescent stem cells.

The intestinal lineage fate is mainly regulated by two transcription factors, bHLHb39[269] and bHLHa14,[270] which are controlled by notch signaling. The bHLHa14 factor commits cells to the secretory lineage; it controls transcription factors linked to secretory cell types.[271] On the other hand, notch represses bHLHa14 via HES1 and HES5, thereby directing cells to the absorptive lineage.

Transcriptional repression by chromatin modifiers is a mechanism that establishes and maintains cell identity. The chromatin polycomb repressive complex, PRC1 monoubiquitinates histone H2a (Lys119) owing to its Ub ligase RNF51, the activity of which is enhanced by Ring1b. The complex PRC2 methylates H3 (Lys27) because of its methyltransferase subunits KTM6a and KTM6b; it prevents CDKN2A gene transcription. Loss of PRC1 impairs ISC function and causes intestinal stem cell exhaustion. Deletion of PRC2 specifically affects the proliferation of cryptic transit-amplifying cells, provoking the accumulation of intestinal cells of the secretory lineage, especially goblet and enteroendocrine cells. This accumulation does not result from impaired proliferation of progenitor cells upon CDKN2A activation, but from regulation of the transcription factors responsible for secretory lineage commitment [1061]. The complex PRC2 does indeed control the equilibrium between secretory and absorptive lineage differentiation programs, that is, the cell fate balance between secretory cells and enterocytes. It controls proliferation of cells within the crypt and represses the transcription factor bHLHa14, thereby favoring the generation of enterocytes rather than secretory cell types in the adult intestine [1061]. Therefore, PRC2 exerts a dual role in the intestinal epithelium, as it represses CDKN2A expression, hence preserving progenitor cell proliferation, and restricts secretory commitment by targeting master regulators of cell differentiation, thus coordinating cell differentiation, avoiding erroneous activation of secretory lineage differentiation.

The ligand-activated transcription factor aryl hydrocarbon receptor (AHR or bHLHe76) recognizes xenobiotics, such as environmental toxins, dietary com-

[269] BHLHb39: class-B basic helix–loop–helix protein-39. It is also called hairy and enhancer of split protein HES1.

[270] Also known as atonal homolog AtoH1 or AtH1. It is required for secretory commitment downstream from the notch signal.

[271] Indeed, it does activate specific transcription factors that induce terminal differentiation into endocrine, Paneth, or goblet cells. Growth factor independent-1 transcriptional repressor GFI1 and SAM pointed domain-containing ETS factor SPDEF specify the goblet cells' fate.

pounds,[272] and microbial virulence factors,[273] in addition to natural compounds, such as tryptophan metabolites (e.g., kynurenines) and microbiota-derived factors. Activated bHLHe76 primes CYP1 gene transcription and hence production of inducible CyP1a1, CyP1a2, and CyP1b1,which metabolize xenobiotics, in addition to UGT1a6. Cytochrome-P450-1, which are detoxifying mono-oxygenases, oxygenate bHLHe76 ligands for clearance, the resulting metabolites having decreased activity and increased water solubility, thereby controlling bHLHe76 ligand availability and terminating bHLHe76 activation [1070]. On the other hand, inhibition of CyP1a1 increases the presence of bHLHe76 ligands and prolongs bHLHe76 activation. Sustained bHLHe76 activation by ligands resisting clearance or by constitutively active bHLHe76 has deleterious effects. Constitutive expression of CyP1a1 in intestinal epitheliocytes causes a loss of type-3 innate lymphoid cells (ILC3) and T_{H17} cells, which depend on bHLHe76 for survival, increasing susceptibility to enteric infection. In addition, environmental factors, such as redox stress and chemical pollutants, modulate CyP1 activity. Constitutive CyP1a1 activity may be counter-balanced by increased intake of dietary bHLHe76 ligands [1070].

Host–microbia interactions rely on simple metabolites from lipids, sugars, and peptides that act as signaling mediators (e.g., glycans, lipidic messengers, and neurotransmitters).

Commensal bacteria produce GPCR ligands, such as ^{N}acyl amides (e.g., endocannabinoids and long-chain ^{N}acyl amide commendamide), eicosanoids (prostaglandins and leukotrienes), and sphingolipids, that act as messengers. ^{N}acyl amide synthase genes abound in the gastrointestinal bacteria and the resulting lipids interact with GPCRs to regulate gastrointestinal tract function [1072].

A set of GPCRs exist for fatty acids (FFARs). They differ according to their specificity for FAs of various chain lengths and degrees of saturation and for FA derivatives such as OEA and oxidized FAs (Table 5.46) [1071].

The FFARs form a subset of nutrient sensors in the gastrointestinal tract, pancreas, AT, and regions of the CNS such as the hippocampus, in addition to leukocytes.

The FFAR1 receptor (GPR40) is activated by medium- and long-chain saturated and unsaturated FAs [1071]. The FFAR2 receptor (GPR43) reacts to short-chain FAs produced in the gut by microbial fermentation of carbohydrates. The FFAR3 receptor (GPR41) is also activated by short-chain FAs also formed from the fermentation of complex carbohydrates in the colon. The GPR84 receptor for medium-chain FFAs is activated particularly by capric (C10), undecanoic (C11), and lauric (C12) acids. Glucose-dependent insulinotropic receptor GPR119 tethers to ethanolamide OEA, an FA derivative rather than an FFA, and LPC. The GPR120

[272]For example, indole 3-carbinol produced by the breakdown of the glucosinolate glucobrassicin existing at relatively high levels in cruciferous vegetables (e.g., broccoli, cabbage, cauliflower, brussels sprouts, collard greens, and kale) [1068].

[273]For example, phenazines from *Pseudomonas aeruginosa* and the naphthoquinone phthiocol from *Mycobacterium tuberculosis* [1069].

Table 5.46 Fatty acid receptors (Source: [1071]; *HODE* hydroxyoctadecadienoic acid, *LPC* lysophosphatidylcholine)

Type	Ligand	Gα subunit	Cellular expression
FFAR1	C16–C22	Gq/11	Pancreatic α/β cell, K cell of the small intestine, L cell of the large intestine, splenocytes, dendrocytes, monocytes, B/T lymphocytes, natural killer cells
FFAR2	C2–C4	Gq/11, Gi/o	Adipocyte, enteroendocrine cells (L cell of the ileum and colon), immunocytes
FFAR3	C3>C4>C2	Gi/o	Adipocyte
GPR84	C9–C14	Gi/o	Monocytes, macrophages
GPR119	LPC	Gs	Pancreatic β cell, enteroendocrine cells
GPR120	C14–C22	Gq/11	Enteroendocrine cells
GPR132	9HODE	Gq/11, Gi/o, G12/13	Macrophages

Free fatty acids (FFAs) stimulate insulin secretion acutely, but chronic hyper-FFA-emia causes lipotoxicity and hence decreased β-cell function and insulin resistance

receptor is optimally activated by saturated and unsaturated FAs with chains containing 14–18 C and 16–22 C, respectively. The GPR132 receptor is targeted by oxidized FFAs derived from linoleic and arachidonic acids and thus may be a sensor of lipid overload and redox stress.

Lipidic ligand-activated GPCRs, such as $S1P_4$, PGi_2R, PGe_2R4, GPR119, and GPR132 intervene in pathogenesis correlated with changes in the gut flora, in particular obesity, diabetes, and atherosclerosis [1072].

The orphan G-protein-coupled receptor, GPR15, or brother of bonzo, a chemoattractant receptor that assists the homing of T cells to the colon, lodges on lymphocytes. It mediates their transfer to the lamina propria of the colon in addition to the skin and recruitment of effector T lymphocytes to the inflamed intestinal region [1073]. Secreted protein and GPR15 ligand GPR15L, which is encoded by the C10ORF99 gene in epitheliocytes exposed to the environment, especially after immunological challenge, is a potential cytokine, which is also a ligand of the transmembrane and secreted sushi domain-containing protein SusD2.[274]

[274]SusD2 interacts with the secreted protein galectin-1, which is synthesized by cancerous cells and promotes immunity evasion, angiogenesis, and metastasis [1074].

5.6.6 *Endocrine Cells of the Gastrointestinal Tract Mucosae and the Pancreas*

Electrochemically excitable enteroendocrine cells constitute the *enteric endocrine system*. They produce gastrointestinal hormones or peptides in response to various types of stimuli [1075]. A majority of enteroendocrine cells synthesize and secrete multiple types of peptidic hormones, coexpressing for example, GIP, neurotensin, peptide-YY, proglucagon, and secretin. However, in humans, ghrelin and somatostatin are generally not co-produced with other peptidic hormones in the small intestine. As autacoids, they target the *ENS*; as endocrine messengers, they are liberated into the bloodstream.

The reflex circuitry of the ENS comprises sensory transducers in the mucosa (entero-endocrine cells), afferent neurons, interneurons, and motor neurons. The ENS is involved in nutrient absorption, as it controls gastrointestinal peristalsis,[275] segmentation,[276] secretion, and blood flow. It also participates in the response to injury. It adapts the neuronal morphology and function and pattern of released neurotransmitters in response to inflammation.

Luminal nutrients also excite enteroendocrine cells. Satiety is modulated by sensory signals arising from the inner surface of the digestive tract [1076]. Enteroendocrine cells possess a *neuropod* (i.e., a cytoplasmic process elongating toward the neuron) that serves as an enteroendocrine cell–neuron contact. The resulting *neuro-epithelial circuit* contains elements for neurotransmission, that is, pre-, post-, and transsynaptic proteins.

Enteroendocrine cells relay information to local sensory neurons via synthesis and release of neurotransmitters that target their cognate receptors on nearby sensory neurons. These cells form DOPA decarboxylase and Tyr hydroxylase for dopamine synthesis. Therefore, in addition to paracrine transmission, innervation of enteroendocrine cells enables a fast transmission of sensory signals from the digestive tract lumen with precise topographical representation of sensory signals and feedback onto enteroendocrine cells.

Gastric enteroendocrine cells (**G** cells), which release cholecystokinin, α and γ-endorphin, gastrin, somatostatin, substance-P, and vasoactive intestinal peptide. Enterochromaffin-like cells secrete histamine.

Intestinal enteroendocrine cells, which are spread throughout the intestine, although their population in the large bowel is generally less diverse than in the small intestine, include [1075]:

[275] The smooth muscle of the gastrointestinal tract undergoes contraction–relaxation cycles, thereby generating peristaltic waves that propel a food ball (i.e., food bolus in the esophagus, once food is chewed and swallowed, and liquid chyme, once it is processed and digested in the stomach).

[276] Segmentation occurs during feeding, hence mixing food without propelling nutrients, and between meals. In the large intestine, several times per day, mass movements push the chyme toward the rectum.

- **D** cells, which synthesize somatostatin, the major inhibitory hormone of the digestive tract[277]
- **I** cells, which secrete cholecystokinin
- **K** cells, which free gastric inhibitory peptide[278]
- **L** cells, which release proglucagon-derived peptides (glicentin, GLP1, GLP2, and oxyntomodulin) and peptide-YY[279]
- **M** cells, which produce motilin
- **N** cells, which liberate neurotensin
- **S** cells, which secrete secretin
- Enterochromaffin cells, which release serotonin[280]

They contain enolase-2+ secretory vesicles, which encompass chromogranin-A+ large dense-core vesicles and synaptophysin+ small synaptic-like microvesicles.

Pancreatic enteroendocrine cells in the islets of Langerhans produce insulin and glucagon, the parasympathetic nervous system stimulating insulin secretion and inhibiting glucagon secretion and the sympathetic nervous system having the opposite effect. They also synthesize amylin, ghrelin, pancreatic polypeptide,[281] and somatostatin.

[277] Somatostatin also localizes to neurons of the myenteric plexus.

[278] Despite differences in production of peptidic hormones, K cells are highly similar to L cells. Secretion of GIP is stimulated by glucose, cAMP, and linoleic acid.

[279] The L cell is an open-type endocrine cell with a long cytoplasmic process reaching the gut lumen equipped with microvilli that protrude into the lumen. These microvilli can sense nutrients in the lumen and then trigger secretion. L cells are sensitive to glucose. The L cells are most dense in the ileum. They contain multiple types of ion channels, AC isozymes, and PDE subtypes [1077], in addition to glucose transporters and glucokinase, a pancreatic, but not intestinal glucose sensor [1078]. Glucokinase activation potentiates GLP1 secretion from murine enteroendocrine GLUTag cells (derived from a murine colonic tumor), but not from primary murine L cells. Secretion of GLP1 depends on a transmembrane difference in electrical potential at the cell surface, linked to activity of Na_V, Ca_V1, and $Ca_V2.1$ channels [1079]. Elevation of cAMP concentration is an effective stimulus for GLP1 secretion from L cells; the cAMP level depends on the activity of cAMP-producing (ACs) and cAMP-hydrolyzing enzymes (PDEs). All membrane-bound ACs are stimulated by Gs; AC1, AC3, and AC8 are activated and AC5 and AC6 are inhibited by Ca^{2+}–calmodulin, and AC2, AC4, and AC7 are activated by $G\beta\gamma$ in addition to phosphorylation by PKC. Among the ACs, only AC2 abounds in enteroendocrine cells. PDE2a and PDE4d are the predominant isoforms in L cells, with moderate levels of PDE1c, PDE3a, PDE8a, PDE8b, and PDE11a; GLP1 secretion depending on PDE2a, PDE3a, PDE3b, and PDE4d. Inhibition of PDE2 to PDE4 stimulates GLP1 secretion. Inhibition of PDE3, but not PDE2, prevents GLP1 secretion in response to guanylin [1077].

[280] The secretory stimulation pathway is launched from β-adrenergic and PACAP receptors. The secretory inhibition pathway is elicited by ionotropic $GABA_A$ and cholinergic receptors.

[281] In humans, pancreatic polypeptide (PP) is formed from a precursor protein PPy encoded by the PPY gene. The precursor engenders carboxyamidated PP and an icosapeptide. is secreted by pancreatic PP cells of Langerhans islets. The vagus nerve, gastrin, secretin, and cholecystokinin provoke its secretion. It regulates pancreatic endo- and exocrine secretion in addition to gastrointestinal secretion and hepatic glycogen concentration.

Incretins are metabolic hormones that have an incretin effect, that is, they stimulate insulin release from pancreatic β cells of the islets of Langerhans after eating in response to ingested glucose, and inhibit glucagon secretion from α cells. The two major incretins are *glucagon-like peptide* GLP1 and *gastric inhibitory peptide*.[282] GIP-secreting K cells and GLP1-secreting L cells reside throughout the small intestine. In addition, in humans, enteroendocrine cells can produce both GIP and GLP1 [1057]. The sodium–glucose cotransporter SGlT1 (SLC5a1) may be involved in glucose-induced GLP1 secretion. Both insulinotropic GLP1 and GIP are rapidly inactivated by dipeptidyl peptidase DPP4.

Upon lipid ingestion, enteroendocrine cells of the small intestine release *neurotensin* (NTs). Neurotensin and its receptors (NTs_1–NTs_3) favor uptake of dietary fatty acids. They inhibit AMPK, which stimulates lipolysis, reduces fatty acid absorption, and impedes lipid storage, in response to oleate [1081]. Increased fasting plasmatic concentration of proNTs, a stable neurotensin precursor fragment produced in equimolar amounts to neurotensin, are associated with increased T2DM and CVD risk.

Serotonin regulates gastrointestinal motility. Various enteric luminal stimuli act on excitable serotonin-secreting enterochromaffin cells via voltage-gated Na^+ and Ca^{2+} channels, thereby participating in the gut–brain signaling axis. Serotoninergic enterochromaffin cells in the gut epithelium are chemosensors for catecholamines, irritants, and metabolites [1082]. Catecholamines, such as adrenaline and NAd, target α2a-adrenoceptor of the epithelial basolateral surface, activate Ca^{2+}-permeable channel TRPC4 via $G\alpha_i$, and prime Ca^{2+} signaling in enterochromaffin cells. The chemical irritant allyl isothiocyanate, a compound of wasabi, activates the Ca^{2+}-permeable channel TRPA1. The microbial product isovalerate stimulates the olfactory receptor OlfR558 in Mus musculus. Once enterochromaffin cells are activated, voltage-gated Ca^{2+} channels trigger release of serotonin, which excites serotonin-sensitive primary afferent sensory nerves via serotonin receptors at synaptic connections. The response of the ENS to dietary and microbial metabolites then influences gastrointestinal activity.

5.6.7 Satiation Signals

Hunger and appetite are major triggers of eating decisions; satiation and satiety are main stop signals [1014]:

[282] A.k.a. glucose-dependent insulinotropic polypeptide. GIP+ K cells lodge in the duodenal and jejunal epithelia. Its secretion, which depends on sodium–glucose cotransporter SGlT1 (SLC5a1) and is modulated by the K_{ATP} channel, is triggered by the ingestion of carbohydrates or lipids. It has an antiapoptotic effect on pancreatic β cells, which form at high levels $K_{IR}6.2$, ABCc8, SLC5a1, GPR40 (or FFAR1), GPR119, and GPR120 [1080].

Table 5.47 Gastrointestinal hormones that affect satiation (Source: [1014])

Peptide	Effect on food intake
Amylin	Decrease
Apolipoprotein-A4	Decrease
Cholecystokinin	Decrease
Enterostatin	Decrease
Gastrin-releasing peptide	Decrease
Ghrelin	Increase
Glucagon-like peptide-1	Decrease
Neuromedin-B	Decrease
Oxyntomodulin	Decrease
Peptide-YY	Decrease

Satiation factors respond to specific nutrient stimuli (e.g., cholecystokinin to proteins and lipids, glucagon-like peptide, GLP1, to carbohydrates and lipids, and peptide-YY primarily to lipids), mixed meals of different contents eliciting release of diverse cocktails of gastrointestinal hormones. Most of these peptides (Cck, GLP1, GLP2, oxyntomodulin, ApoA4, GRP, NMB, PYY, and ghrelin) are also synthesized in the brain

- *Satiation* is related to the feeling of fullness that initiates the decision to stop eating.
- *Satiety* ensures fasting during a prolonged duration until hunger primes the eating decision.

A diet enriched in fat or simple carbohydrates does not afford a strong signal of satiety. Upon repletion, elevated cerebral signals launched by insulin and leptin increase sensitivity to satiation signals.

Endogenous satiation factors secreted in response to food ingestion activates specific receptors that cause cessation of eating (Tables 5.47 and 5.48). Some satiation signals are released from enteroendocrine cells in the mucosa of the gastrointestinal tract in response to food intake. Local sensory nerves expressing receptors for these gut peptides signal to the brain.

5.6.7.1 Amylin

Amylin, which is secreted by pancreatic β cells with insulin, inhibits gastric acid secretion and lowers glucagon concentration and food intake [1014].

Amylin signals via the calcitonin receptor modified by receptor activity-modifying proteins. It may act as a hormone and directly stimulate neurons in the area postrema in the hindbrain rather than visceral afferent nerves. Its anorexigenic action is potentiated by insulin action and strengthens the effects of cholecystokinin and gastrin-releasing peptide [1014].

Table 5.48 Hormones of entero-endocrine cells of the large bowel (Source: [1075]; ↑ increase, ↓ decrease)

Peptide	Effects
Glicentin	Stimulation of enterocyte proliferation
	Inhibition of gastric acid secretion
	Stimulation of insulin secretion
	Regulation of gut mobility
Glucagon-like peptide-1	Incretin effect (↑ insulin and ↓ glucagon secretion)
	Gastrointestinal motility (delays gastric emptying)
	Postprandial satiety
Glucagon-like peptide-2	Stimulation of enterocyte proliferation
	Inhibition of enterocyte and crypt cell apoptosis
	Increased nutrient uptake through transporters
	Reduced intestinal trans- and paracellular permeability; anti-inflammatory effect
	Inhibition of gastric acid secretion
	Stimulation of intestinal blood flow via NOS3
	Relaxation of murine gastric smooth myocytes
Oxyntomodulin	Inhibition of gastric emptying
Peptide-YY	Inhibits gastric emptying, intestinal motility, gastric acid secretion, pancreatic exocrine function
	Suppresses appetite
	Stimulates enterocyte proliferation
Serotonin	Stimulates intestinal motility and secretion
Somatostatin	Inhibits digestive endo- and exocrine function
	Stimulates colonic peristalsis

5.6.7.2 Apolipoprotein-A4

Apolipoprotein-A4 is synthesized by intestinal mucosal cells during the packaging of digested lipids into chylomicrons. It is also produced in the ArcN[1014].

The ArcN is endowed with a relatively leaky blood–brain barrier. It integrates diverse hormonal and neural signals and controls energy homeostasis. Two main categories of neurons include POMC+ and AgRP+ NPy+ neurons[283]

Apolipoprotein-A4 attenuates food intake in rats. It may interact with cholecystokinin. Both intestinal and hypothalamic ApoA4 are regulated by absorption of lipids, but not carbohydrates.

[283] AgRP: agouti-related peptide, an antagonist of the MC_3 and MC_4 receptors. Neuropeptide-Y stimulates food intake. AgRP+ NPy+ neurons have an anabolic effect.

5.6.7.3 Bombesin Analogs

Members of the BN (bombesin) family of peptides, which includes the amphibian peptide bombesin and its mammalian analogs, *gastrin-releasing peptide* (GRP) and *neuromedin-B*, reduce food intake in humans [1014].

5.6.7.4 Cholecystokinin

The prototypical satiation signal is the duodenal peptide cholecystokinin (Cck) secreted by duodenal I cells in response to dietary lipids or proteins. It influences gut motility and primes gallbladder contraction, and secretion of pancreatic enzymes and gastric acid. Moreover, it activates its receptors on duodenal sensory nerves [1014]. The Cck_1 receptor on nearby branches of vagal sensory nerves decreases the size of the meal once it has begun, reducing hunger and increasing the fullness sensation.

Neurons of various subnuclei of the *NTS*[284] receive vagal information from the cardiovascular and respiratory apparatuses and from the gastrointestinal tract. Cholecystokinin activates ^{NTS}PPG+ neurons[285] via adrenergic and glutamatergic neurons [1083]. ^{NTS}PPG+ neurons integrate signals related to long-term energy balance and short-term nutritional status to produce an output to feeding and autonomic circuits.

During meals, cholecystokinin, glucagon-like peptide GLP1, and distension of the stomach and intestine trigger nerve impulses in sensory nerves afferent to the hindbrain in communication with neurons in the NTS.

[284] This visceral sensory relay station receives inputs from the cardiovascular, respiratory, and gastrointestinal apparatuses. Its neighboring structures include the wall of the fourth ventricle, area postrema, subnucleus commisuralis, fasciculi of nuclei gracilis and cuneatus, spinal nucleus of the trigeminal nerve, DMNV nerve, hypoglossal nucleus parasolitary nucleus, vestibular and cochlear nuclei, and parvocellular reticular formation.

The *solitary tract* consists of fibers from the inferior ganglion of the vagus nerve, glossopharyngeal nerve, and geniculate ganglion of the facial nerve.

Sensory afferent fibers from chemo- and mechanoreceptors of the gastrointestinal tract, which use GABA and glutamate as inhibitory and excitatory neurotransmitters, relay signals of satiety to the NTS during feeding. The NTS is involved in generating and synchronizing peristalsis in the upper gastrointestinal tract during swallowing via the nucleus ambiguus in the medulla oblongata, which supplies motor fibers to the vagus, glossopharyngeal, and accessory nerves innervating the pharynx, larynx, and esophagus in addition to parasympathetic fibers to the vagus.

[285] PPG: pre-proglucagon. Neuronal circuits in the hypothalamus and hindbrain are implicated in the control of food intake and energy expenditure, and hence AT mass. Glucagon-like peptide GLP1 is produced in the CNS by PPG+ neurons of the NTS in the hindbrain that project to various regions of the brain such as the hypothalamus.

5.6.7.5 Enterostatin

The pentapeptide enterostatin is linked to the intestinal processing of lipids. It is cleaved in the intestinal lumen by colipase secreted by the exocrine pancreas. It then enters the blood circulation. It reduces food intake in rats fed with lipids (but not with carbohydrates or proteins) [1014].

5.6.7.6 Ghrelin

Ghrelin nutritionally produced in endocrine cells of the gastric fundus and duodenum is the most potent circulating orexigen. Its concentration increases during food deprivation and peaks before meals. It stimulates eagerness for ingesting palatable food and drink.

Ghrelin differs from all other peptide hormones by its octanoylation, which enables its binding to its receptor [1085]. Ghrelin targets growth hormone secretagogue receptor,[286] which possesses two subtypes (GHSR1a–GHSR1b) and localizes to vagal sensory nerves in addition to the hypothalamus, especially the lateral hypothalamus, which produces the orexigenic hypocretin, and ventromedial and arcuate nuclei.

Ghrelin thus acts on the vagus nerve. Furthermore, it directly stimulates neurons in the ArcN [1014]. In the ArcN, NPy+ AgRP+ neurons possess ghrelin receptor; ghrelin induces immediate early gene expression [1011]. Ghrelin also targets GHRS in the NTS in the hindbrain,[287] ventral tegmental area (VTA),[288] nucleus accumbens, and ventral hippocampus [1084].

Ghrelin acts in the LHA in a sex-specific manner. In male and female rats, ghrelin delivered to the LHA favors food intake and appetite for sucrose [1084]. However, it motivates food search and raises body weight only in females, which have a slightly higher GHSR concentration in the LHA than males. Acute GHSR blockade in the LHA reduces food intake, body weight, and sucrose-motivated behavior in female, but not male rats. In $Ghsr^{-/-}$ female rats, the reward-driven behavior is abolished, but does not affect basal food intake [1084].

Ghrelin regulates GH release, appetite, metabolism, neurotransmission, behavior, stress response, and immune function via the Gq/11-coupled GHSR1a receptor. When its constitutive activity is unusually high, this ghrelin receptor stimulates CamK4, PLC, and PKC, hence leading to phosphorylation of cAMP-responsive element-binding protein (CREB), activation of Ca^{2+} channels, and inhibition of

[286] A.k.a. ghrelin receptor and growth hormone-releasing peptide receptor (GHRP). Growth hormone secretagogues (GHSs), or GH-releasing peptides (GHRPs), elicit GH secretion, not via GH-releasing hormone receptor, but via GHS receptor (GHSR), which tethers to ghrelin.

[287] The hindbrain, also called the rhombencephalon, is the lower part of the brainstem. It comprises the cerebellum, pons, and medulla oblongata.

[288] The ventral tegmental area in the midbrain sends dopaminergic neural projections to the limbic and cortical areas.

K^+ channels [1085]. On the other hand, Gi/o-coupled GHSR1a activated by ghrelin launches the β Arr–ERK1/2 and Src–PKB pathways. The constitutive internalization of GHSR1a relies on the sequential activation of Rab5 and Rab11 linked to early endosomes and endosomal recycling compartments, respectively. The agonist-independent activity of GHSR1a may modulate food intake and body weight.

GHSR1a signals in a ligand-independent manner basally or when it is heterodimerized with other GPCRs. Liganded GHSR1a heterodimerizes with receptors of serotonin $5HT_{2C}$ (control of food intake), dopamine D_1 (enhanced dopamine signaling), somatostatin Sst_5 (regulation of insulin release), and melanocortin MC_3 (body weight regulation). It also heterodimerizes with its truncated form (GHSR1b; without the distal domain of its C-terminus, which is used for receptor internalization, β-arrestin recruitment, and signal termination), which has a dominant negative effect on ghrelin receptor function. GHSR1b is primarily located in the endoplasmic reticulum, where it tethers to GHSR1a, hence lowering plasmalemmal GHSR1a amount, as the GHSR1a–GHSR1b heterodimers accumulate at the ER [1086].

Ghrelin is associated with the anticipation of meal ingestion, as its concentration peaks shortly before scheduled meals. This orexigenic peptide may dampen the effects of GLP1 and $PYY_{(3–36)}$ on gastric emptying and food intake.

In rats, cells with a truncated GHSR1a form respond more intensively to ghrelin than cells with the full-length receptor, thereby potentiating the fattening effect of ghrelin, without affecting appetite [1087]. This GHSR1a mutant form ($Ghsr^{Q343X}$) preserves plasmalemmal GHSR concentration. Rats have a more stable body weight under caloric restriction, whereas under standard conditions, body weight and adiposity increase and glucose tolerance lowers.

Ghrelin restores the NO–ET1 balance, hence improving endothelial function [720]. Ghrelin stimulates NO production in ECs using the PI3K–PKB–NOS3 pathway. Its concentration decays in obese subjects.

5.6.7.7 Glicentin

Glicentin inhibits gastric acid secretion, but, at least in rats, does not affect food intake [1014]. Glicentin and oxyntomodulin are also detected in the CNS, principally in the hypothalamus and brainstem.

5.6.7.8 Glucagon-Like Peptide-1

The circulating hormone glucagon-like peptide, GLP1, is produced by intestinal L cells, especially in the ileum and colon, and α cells in the pancreas from cleavage by PCSK1 or PSCK3 of proglucagon [1088]. The transcription factor Pax6 in enteroendocrine cells activates proglucagon synthesis [1057]. β-Catenin also stimulates proglucagon expression in the intestine, but not in the pancreas, via the

transcription factor bHLHb19 (TCF4).[289] Increased pancreatic GLP1 production may result from impaired β-cell function linked to a defective inhibition by insulin of GLP1 production.

The neuropeptide GLP1 is also synthesized by PPGcg+ (pre-proglucagon) neurons primarily in the brainstem by the NTS, which projects to the appetite centers in the hypothalamus (ArcN and PVN) activated by leptin and the vagus nerve (Sect. 5.6.2.3). This anorexigen can activate the area postrema and neural circuits that mediate vagal satiety signaling.

High glucose concentrations upregulate the formation of PCSK1 (but not that of PCSK2). Its secretion is elicited by nutrients. Lipids cause GLP1 secretion. GLP1 diffuses across the basement membrane and lamina propria to be taken up by a capillary.

In rats, glucose-dependent insulinotropic peptide (GIP) stimulates GLP1 secretion via the vagus nerve, but not in pigs and humans [1057]. A local paracrine control is exerted by neighboring somatostatin+ D cells. Gastrin-releasing peptide in addition to its C-terminal decapeptide neuromedin-C are powerful stimulants of GLP1 secretion [1057].

A neural pathway stimulates GLP1 secretion. Both muscarinic receptors M_1 and M_2 may be involved in the control of GLP1 release. Catecholamines may also be implicated via β-adrenoceptors. In fact, the sympathetic innervation (NAd transmitter) to the gut inhibits GLP1 secretion, whereas the extrinsic vagal innervation (cholinergic activity) is not implicated or may play only a minor role. Nevertheless, vagal stimulation provokes GRP release.

Glucagon-like peptide-1 is rapidly catabolized (inactivated) in the blood circulation by dipeptidyl peptidase DPP4 on the luminal surface of ECs (half-life <2 mn) [1014]. Only 25% of the secreted amount reaches the portal circulation. In the liver, 40–50% of the remaining quantity is destroyed, so that only 10–15% enters the systemic circulation and can reach the pancreas and brain. Its metabolite is cleared rapidly, mainly in the kidney (half-life 4–5 mn) [1057]. GLP1 is also a substrate for neprilysin.[290]

Three GPCRs, the glucose-dependent insulinotropic receptor GPR119, ω-3 fatty acid receptor ω3FAR1 (GPR120), and G-protein-coupled bile acid receptor, GPBAR1, are implicated in the release of GLP1 from intestinal L cells and pancreatic α cells.[291] GPBAR1 responds to fatty acids such as linolenic acid [1088]. In fact, GLP1 secretion is stimulated by activated ω3FAR1 and GPBAR1, but not by GPR119. In enteroendocrine cells, ω3FAR1 is stimulated by α-linolenic and palmitoleic acids.

[289] A single nucleotide polymorphism that engenders TCF7L2 is linked to the T2DM development.

[290] A.k.a. neutral endopeptidase 24.11, atriopeptidase, and enkephalinase.

[291] Bile acids, cannabinoids, FFAs, leukotrienes, lysophosphatidic acid, prostanoids, platelet-activating factor, and sphingosine 1-phosphate are lipidic ligands of class-A GPCRs.

Glucagon-like peptide-1 can cross the blood–brain barrier. However, its action is mainly transmitted via sensory neurons of the intestine. It activates sensory afferent neurons originating in the nodose ganglion that stimulate neurons of the NTS.

Parasympathetic (vagal) signaling, especially that from the posterior gastric branch of the vagus nerve, yields cardioprotection upon remote (e.g., inferior limb) ischemic conditioning (pre- or perconditioning, i.e., before or during myocardial ischemia) via cardiac M3 muscarinic ACh receptor, reducing myocardial ischemia–reperfusion injury and hence infarct size [1089]. This signaling comprises GLP1 and paracrine activation of its receptor GLP1R lodging on visceral vagal afferents (vagal sensory fibers) and subsequent phosphorylation of the prosurvival kinase, PKB. On the other hand, release of GLP1 by intestinal L cells is modulated by vagal cholinergic efferent (motor) fibers that also innervate the myocardium. Several other humoral factors of remote ischemic conditioning include ApoA1, CXCL12α, EglN1, IL10, miR144, and NO, but cardioprotection cannot be fully explained by any of these factors acting alone [1089]. The gastrointestinal tract thus represent a major source of cardioprotective factors.

The GLP1 (GLP1R or GLP_1), GIPR, and glucagon receptors (GcgR), belong to the family of Gs-coupled receptors [1057]. However, GLP1R and GcgR play opposing roles in insulin release and glucose homeostasis [1090]. The T2DM treatment thus requires activation of GLP1R linked to inhibition of glucagon secretion and stimulation of insulin secretion in a glucose-dependent manner.

The GLP1 receptor resides in the gastrointestinal tract and pancreatic islets in addition to the brain, heart (myocardium and coronary vessels), kidney, lung, and liver. This receptor also resides on neurons of the peripheral and central nervous system, in particular in hypothalamic regions involved in the regulation of food intake. It launches the AC–cAMP–PKA–RapGEF4 pathway, closes the K_{ATP} and K_V channels, thereby favoring cell depolarization, and causes Ca^{2+} influx from the intracellular store through IP_3R and RyR, which triggers exocytosis.

Glucagon-like peptide-1 stimulates insulin secretion and inhibits glucagon release, thereby limiting postprandial glycemia excursion. It upregulates transcription of the genes involved in insulin secretion and action, such as those encoding glucokinase and GluT2 [1057].

The inhibitory effect of GLP1 on glucagon secretion in vivo is only observed for glycemia above fasting levels. GLP1 stimulates the pancreatic secretion of somatostatin, which precludes glucagon release in a paracrine manner. Insulin also hampers glucagon secretion in a paracrine fashion.

In the digestive tract, GLP1 impedes gastrointestinal motility and secretion, hence acting as an inhibitory *enterogastrone*,[292] a component of the *ileal brake* associated with a longer emptying time of the stomach and small intestine after a meal high in lipids, thereby slowing digestion and adapting to meal duration, size, and content.

[292] Enterogastrone represents any hormone secreted by the mucosa of the duodenum in response to dietary lipids that inhibit the forward motion of the chyme (liquid substance corresponding to partly digested food), that is, cholecystokinin, GLP1, glucose-dependent insulinotropic peptide (formerly known as gastric inhibitory peptide), peptide-YY, and secretin.

In the pancreas, GLP1 can promote β-cell regeneration [1088]. It stimulates not only proliferation of β cells but also their differentiation from progenitors in the pancreatic duct epithelium. The transcription factor pancreas and duodenum homeobox gene product PDx1, which regulates pancreatic islet growth and insulin gene transcription, is involved in the glucoregulatory, proliferative, and cytoprotective effects of GLP1 [1057].

In the heart, GLP1 may inhibit myocardial contractility in the basal state, but after cardiac injury, it improves the left ventricular performance in patients via its combined effects of enhanced insulin secretion and action, in addition to direct effects [1057].

Glucagon-like peptide-1, $GLP1_{(1–36)}$, and $GLP1_{(7–36)}$ operate on human dermal and retinal microvascular ECs and those of rat mesenteric arteries that synthesize GLP1R [1091]. They impede VEGFa-mediated endothelium-dependent gradual vasodilation, as they reduce VEGFa-induced phosphorylation of PLCγ, subsequent Ca^{2+} signaling, and NOS3 activity. Hence, after food intake, GLP1 abolishes relaxation primed by VEGFa in resistance arteries. In addition, GLP1 provokes phosphorylation of Src, ERK1, and ERK2, and subsequent cell proliferation and affects vessel permeability [1091].

Decreased release of GLP1 may contribute to the development of obesity. The diurnal GLP1 secretion from L cells can decrease and does not increase during the postprandial period in obese subjects; its secretion improves after weight loss [1057].

Patients with T2DM have fasting hyperglucagonemia in addition to an exaggerated glucagon response to meal ingestion; hyperglucagonemia may contribute to the hyperglycemia.

Type-2 diabetes mellitus is characterized by a highly reduced or absent incretin effect associated with an almost complete loss of GIP insulinotropic action, its secretion remaining normal or quasi-normal, and a marked decline in meal-induced GLP1 secretion, but its insulinotropic effect is maintained [1057].

5.6.7.9 Glucagon-Like Peptide-2

Glucagon-like peptide GLP2 co-secreted with GLP1 from enteroendocrine cells in the small and large intestines binds to the receptor GLP2R (or GLP_2) and stimulates small and large intestinal mucosal growth [1014].

It induces the transcription of IGF1 that mediates the intestinotropic action of GLP2 [1092]. Insulin-like growth factor, IGF1, and its receptor, IGF1R, are involved in GLP2-induced proliferation of crypt cells. Growth of the intestinal mucosa in response to IGF1 and/or R-spondin-1 is normal in mice after full depletion in the Igf1 or Igf2 gene [1093]. Intestinotrophic action of IGF1 depends on IGF-binding proteins IGFBP3 and IGFBP5, but not that of GLP2 [1094]. However, GLP2 also exert effects independently of IGF1 or FGF7 via members of the HER family [1095].

Furthermore, GLP2 prevents apoptosis via GLP2R, as it suppresses BAD proapoptotic activity, thereby enhancing cell survival independently of the PI3K–PKB axis, but via the Gs–AC–cAMP–PKA pathway, PKA phosphorylating (inactivating) GSK3α (Ser21) and GSK3β (Ser9) [1096]. Moreover, the GLP2R-Gβγ–Ras–ERK1/2 pathway also prevents apoptosis [1097].

In the gastrointestinal tract, GLP2 influences digestion, nutrient absorption (increased nutrient transporter production), chyme mobility, and intestinal blood flow:

- It reduces gastric acid secretion, but does not influence gastric emptying [1098]. However, in the mouse stomach, GLP2 causes gastric fundus relaxation via VIP release by prejunctional neurons [1099]. GLP2R resides on subepithelial myofibroblasts and enteric neurons of the muscularis layer.
- It stimulates glucagon secretion, hence counteracting the glucagonostatic effect of GLP1.
- It acts as an anti-inflammatory agent, as it activates enteric VIP+ neurons of the submucosal plexus, stimulating neuronal VIP release in the intestinal submucosa and mucosa and reducing intestinal mucosal inflammatory cytokine production, crypt cell proliferation, and apoptosis [1100].
- It enhances intestinal blood flow using NO and vasoactive neurotransmitters [1101, 1102]. GLP2R localizes to serotonin+ enteroendocrine cells and NOS3+ VIP+ enteric neurons.
- It lowers food intake in rats (absorption of enteral lipids in addition to hexose transport), but not in humans [1014].

Populations of enteric neurons and gliocytes possess GLP2R [1103]. GLP2 rapidly (in 1–4 h) activates PI3Kγ and PKB, thereby leading to phosphorylation of PKB, ERK1, ERK2, and S6K, expression of VIP and growth factors (IGF1, EGF, HBEGF, Areg [amphiregulin], Ereg (epiregulin], btC [β-cellulin], and Nrgs [neuroregulins]) and their receptors (IGF1R and HERs [EGFR]), and stimulating neuronal differentiation via the TOR and ERK pathways.

5.6.7.10 Glucagon

Glucagon is secreted from pancreatic α cells and probably also in small amounts from the distal intestine. Glucagon is also detected in the brain (Sect. 5.6.2.3).

Glucagon increases hepatic glucose production, as it stimulates glycogenolysis and gluconeogenesis. The heart possesses the glucagon receptor; glucagon has inotropic and chronotropic effects [1057].

Exogenous glucagon reduces meal size [1014]. However, in rats, endogenous glucagon raises food intake. In healthy humans, glucagon does not influence food intake.

5.6.7.11 Growth and Differentiation Factor

Dimeric growth and differentiation factor GDF15 signals via GDNF family receptor-α-like receptor (GFRα L) in the brainstem to control appetite and hence food uptake and body weight [1104].[293] The receptor GFRα L, which is encoded by the GFRAL gene, lodges exclusively in neurons of only two regions of the brainstem in mice, GFRα L+ neurons in the area postrema and NTS. It regulates a neuronal circuit involving the parabrachial nucleus and central amygdala that constitutes a component of the *emergency circuit*, which adjusts feeding to stress conditions, mediating weight loss during cellular stress, tissue damage, and disease [1104]. The GDF15–GFRα L complex binds to and activates the ReT receptor.

In mice, GDF15 overproduction reduces food intake and body weight with respect to WT mice; these mice do not develop obesity and insulin resistance [1013]. On the other hand, adiposity increases in GDF15-deficient SD-fed mice. Plasmatic GDF15 concentration in humans rises in CVD, chronic kidney disease, and cancer; it correlates with body mass in people with advanced cancer and in healthy human twins.

5.6.7.12 Oxyntomodulin

Oxyntomodulin signals via both the GcgR and GLP1R with weak affinity. Oxyntomodulin may cross the blood–brain barrier and stimulate GLP1R+ neurons in the ArcN. It exerts an anorexigenic effect via GLP1R [1014].

In humans, oxyntomodulin acts as an incretin and enterogastrone [1105]. In rodents, it inhibits meal-stimulated gastric acid secretion and gut motility, as do GLP1 and GLP2. It modulates energy and glucose metabolism in various organs (e.g., the brain, liver, and pancreas).

In the liver, it can reset transcription rhythms, as it activates the hepatocytic circadian clock genes, PER1 and PER2, and hence carbohydrate metabolism regulators [1106].

[293] Most messengers of the TGFβ superfamily activate conventional transmembrane receptor protein Ser/Thr kinases. However, dimeric gliocyte-derived neurotrophic factors (GDNFs) bind to the GDNF family receptor-α attached to the cell membrane via glycolipid linkage; the resulting GDNF–GFRα complex (e.g., GDNF–GFRα1 and artemin–GFRα3) connects to and activates the transmembrane protein Tyr kinase receptor rearranged during transfection ReT, which stimulates PKB, PLCγ, and the MAPK module, thereby regulating various neuronal populations in the peripheral and central nervous systems [1013].

In addition, GDF15 protects dopaminergic neurons in animal models of Parkinson's disease using another pathway. The extracellular domain of GFRα1 can be cleaved off the receptor and then circulate in the bloodstream [1013]. Alternatively, GDNF–GFRα1 can signal independently of ReT. GDF15 may use similar signaling strategies.

5.6.7.13 Peptide-YY

Peptide tyrosine–tyrosine (PYY) forms a family of homologous peptides with pancreatic polypeptide and NPy. It is synthesized and secreted by enteroendocrine L cells in the distal ileum and colon upon the presence of nutrients within the ileum. It is released as $PYY_{(1–36)}$ and cleaved into $PYY_{(3–36)}$ by DPP4 [1014]. It inhibits NPy+ AgRP+ neurons in the ArcN via Y_2 receptors [1011].

It affects gastrointestinal motility. It reduces food intake via the family of NPy receptors (Y_1–Y_2 and Y_4–Y_5), $PYY_{(3–36)}$ linking preferentially to Y_2. PYY crosses the blood–brain barrier and influences food intake via the Y_2 receptor. Fasting and postprandial PYY concentrations are lower in obese adolescents and adults than in lean controls [1014].

5.6.7.14 Leptin

Leptin produced by adipocytes serves as a neuroendocrine messenger. It acts in the hypothalamus to signal the status of body energy stores and satiety, thereby regulating the body weight. Leptin can also improve insulin sensitivity and circulating lipoprotein concentrations in subjects with metabolic anomalies. In addition, this predominant adipocytic circulating hormone participates in the control of angiogenesis, immunity, and fertility.

Leptin receptors[294] include a long form ($LepR_L$) and several short forms ($LepR_S$), one of which has 34 cytoplasmic residues, is widespread, and mediates transport or clearance of leptin [1108].[295] The long LepR form is detected in several brain regions, the highest concentration being observed in the ventral basal hypothalamus, especially the arcuate, ventromedial, dorsomedial, and ventral premammillary nuclei [1011]. Neurons that have high LepR concentrations congregate around the median eminence, the floor of the third ventricle, which lacks a blood–brain barrier; they are thus easily excited by circulating leptin. Leptin receptor also resides in several other sites such as the brainstem, albeit at a lower concentration.

[294]The single membrane-spanning leptin receptor is also named Ob receptor, as leptin was previously dubbed the obese protein. Parabiosis of an ob/ob mouse and a lean control normalizes at least partly body weight in the ob/ob mutant mouse that is deficient in a circulating factor. In 1994, the mutation responsible for obesity was identified in the ob/ob mouse, the Ob gene encoding a satiety hormone, called leptin, which is synthesized and secreted by the WAT [1107].

Genetic obesity is studied using |two types of mutant mice with distinct single gene mutations on mouse chromosomes 6 (ob) and 4 (db), ob/ob and db/db mice. In the homozygous db/db mouse, a model for diabetic dyslipidemia, obesity, and hence accelerated atherosclerosis, the gene encoding the leptin receptor is mutated.

[295]The predominant short form in most organs is a transmembrane protein with a short cytoplasmic domain of 34-amino acid residues. In the hypothalamus, the long form has an extracellular domain identical to that of the short form, but with a 302-residue-long cytoplasmic domain. The long form LepRb is a member of the IL6 receptor family of class-*I* cytokine receptors, which activates the Jak–STAT3 pathway.

The leptin receptor variants (LepRa–LepRf) are generated by alternative splicing and proteolytic cleavage [1109].[296] Both LepRa and LepRb transduce signals via IRS and the MAPK module, but only LepRb also activates the JaK–STAT pathway. The subtype LepRb lodges predominantly in the hypothalamus but also in peripheral organs, such as the pancreas, liver, and intestine. Deletion of LepRs in either POMC+ or AgRP+ intestinal cells reduces microsomal TG transfer protein and controls lipid absorption across enterocytes [1109]. In intestinal epitheliocytes, LepRb intervenes in the onset of DIO.

Upon leptin exposure, $LepR_L$, but not $LepRs_S$, undergoes phosphorylation, $Tyr985^{Ph}$ controlling the Tyr phosphorylation of PTPn11 and $Tyr1138^{Ph}$ of STAT3 activation [1108]. These two $LepR_L$ phosphorylated Tyr residues mediate distinct signaling pathways; PTPn11 binding to Tyr985 stimulates the ERK–Fos pathway and STAT3 binding to Tyr1138 inhibits the SOCS3 pathway. In cells coexpressing LepR and JaK2, all forms of LepRs trigger leptin-dependent Tyr phosphorylation of JaK2 [1110]. Leptin also induces Tyr phosphorylation of IRS1, whatever the LepR form. In addition, $LepR_L$ increases ERK1 activity, the effect being potentiated by coexpression of JaK1 or JaK2, in addition to STAT activation, $LepR_L$ having a weaker effect.

In the ArcN, leptin inhibits orexinergic NPy+ AgRP+ neurons and activates anorexinergic POMC+ neurons, which then produce Fos, a marker of neuronal activation, and SOCS3.[297] Once it is bound to its receptor, leptin activates STAT3, which regulates expression of its target genes, such as POMC and SOCS3. However, the STAT3-binding site in the POMC promoter overlaps with a FoxO1-binding element. FoxO1 inhibits POMC transcription. Whereas PDK1 and PI3K promote POMC formation, PTen hinders it in POMC+ neurons. Both SOCS3 and PTPn11 can hamper the leptin signaling cascade. Overexpression of SOCS3 precludes not only leptin action but also insulin signaling [1112].

Concentration of leptin increases in obese patients due to *leptin resistance*. HFD induces elevated hypothalamic STAT3 phosphorylation and abrogates the effect of STAT3 in POMC+ neurons. In mice expressing a constitutively active STAT3 form selectively in POMC+ neurons, leptin and insulin resistance are associated with hypothalamic SOCS3 expression.

[296] Alternate splicing of the leptin receptor creates multiple isoforms that contain a common extracellular domain. The isoform LepRe lacks a transmembrane domain; LepRa, LepRc, LepRd, and LepRf have short (30–40 amino acids) cytoplasmic extensions.

[297] SOCS3 impedes JAK activation or favors ubiquitination and subsequent proteasomal degradation of the cytokine receptor. On the other hand, NPy+ AgRP+ neurons express SOCS3, but not Fos upon leptin exposure (inhibition).

Inactivation of SOCS3 only in LepR+ cells protects against HFD-induced leptin resistance, but does not prevent DIO [1111]. However, SOCS3 inhibition in LepR+ cells prevents diet-induced insulin resistance, as it increases the hypothalamic formation of K_{ATP} channel subunits and Fos in POMC+ neurons.

Table 5.49 Insulin and leptin effects ($\ominus \longrightarrow$ inhibition)

Stimulus	Pathway
Insulin	IR–IRS–PI3K–PDK1–PKB$\ominus \longrightarrow$ FoxO1
	FoxO1$\ominus \longrightarrow$ POMC synthesis
Leptin	LepR–JaK2–STAT3–SOCS3
	SOCS3$\ominus \longrightarrow$ PI3K, JaK2

5.6.7.15 Insulin

Co-ordinated action of leptin and insulin in POMC+ neurons contributes to the regulation of energy homeostasis. Leptin and insulin signal the body's lipidic content to the brain. They both cross the relatively permeable blood–brain barrier at the ArcN and interact with POMC+ or AgRP+ NPy+ neurons, which project to other hypothalamic regions, such as the LHA and PVN [1014]. The PVN enhances the potency of satiation signals in the hindbrain. On the other hand, the lateral hypothalamus represses satiation signals.

Insulin and leptin receptors exist in many regions of the brain. The insulin receptor triggers the IRS–PI3K–PDK1–PKB pathway, which inhibits FoxO1, which inhibits POMC synthesis (Table 5.49). Therefore, activated PI3K relieves FoxO1-mediated repression of POMC creation [1112]. The leptin receptor launches the JaK2–STAT3 axis, which stimulates SOCS3 production. SOCS3 is implicated in a negative feedback loop, as it inhibits PI3K and JaK2 and hence insulin and leptin signaling.

Insulin and leptin suppress the activity of NPy+ AgRP+ neurons in the ArcN. Obesity is associated with insulin and leptin resistance; the brain is unable to efficiently respond to excessive lipid amounts.

5.6.8 Feeding Signals

Selective activation of hypothalamic NPy+ AgRP+ neurons primes feeding behavior in mice [1113]. On the other hand, ablation of hypothalamic NPy+ AgRP+ neurons lowers body weight, but genetic depletion of either NPy or agouti gene-related peptide does not markedly affect the body weight and feeding behavior. Other mediators thus contribute to the regulation of feeding and body weight regulation.

5.6.8.1 PCSK1n and Its Products

Peptides that derive from PCSK1n (or SAAS) are among the most abundant neuropeptides targeting cognate receptors in the cerebral feeding centers in the

hypothalamus to regulate the body weight and metabolism, at least in mice, especially in the NPy+ AgRP+ neurons of the ArcN [1113]. Two proPCSK1n-derived peptides, AVDQDLGPEVPPENVLGALLRV (PEN) and LENSSPQAPARRLLPP (bigLEN [bLEN]), released from the hypothalamus, provoke feeding.

ProPCSK1n is widely formed in the brain, with its highest levels in the ArcN, where it co-localizes with NPy.

Furin (or PCSK3) and carboxypeptidase-D cleaved proPCSK1n into the intermediate peptide PCSK1n and the PEN–LEN neuropeptide. Furin can also cleave PEN–LEN into PEN and LEN_L.

The neuroendocrine secretory protein, PCSK1n, and its derived neuropeptide PEN–LEN, which both contain the C-terminal inhibitory domain, but not the further processed peptides, reduce the activity of the 84-kDa form of PCSK1 in the ER and GB, but not the autocatalytically derived 66-kDa form of PCSK1. PCSK1n also slows down processing by convertase of POMC and pro-enkephalin. In addition, it acts as an amyloid anti-aggregant chaperone in Alzheimer's disease. It also plays a role in the circadian rhythm.

Within secretory vesicles, $PCSK1n_L$ is cleaved into KEP and $PCSK1n_S$, and the middle portion of proPCSK1n into GAV and another peptide, PEN–LEN into PEN and LEN_L, and LEN_L into LEN_S and a four-residue peptide, LLPP, primarily by PCSK1 and carboxypeptidase, CPe. In addition to these major forms of the proPCSK1n-derived peptides, smaller forms of $PCSK1n_S$, GAV, and PEN also reside in the mouse brain, but to a lesser extent.

In the mouse hypothalamus, PCSK1n, PEN–LEN, and LEN exist in large ($PCSK1n_L$, PEN–LEN_L, and LEN_L) and small forms ($PCSK1n_S$, PEN–LEN_S, and LEN_S) [1114].

The neuropeptides PEN and LEN are implicated in the regulation of feeding and hence body weight. PEN and LEN_L co-localize with NPy, whereas PCSK1n and LEN_S do not lodge in cell bodies of NPy+ neurons. Peptides that co-localize with NPy, $PCSK1n_L$ and PEN, affect feeding, whereas peptides that do not co-localize with NPy, LEN_S and PCSK1n, do not influence feeding.

The neuropeptide LEN_L, but not the C-terminally truncated peptide LEN_S, binds to the Gi/o-coupled receptor GPR171 in the mouse hypothalamus [1113]. Hence, the LEN_L–GPR171 couple regulates food intake in mice. Other selective GPR171 ligands such as MS0015203 stimulate appetite and food intake and hence increase body weight [1115].

The four C-terminal amino acids of LEN_L suffice to tether to and activate GPR171 [1113]. The LEN_L–GPR171 complex primes acute feeding during food deprivation in mice. The peptide PEN is abundant in the brain and activates GPR83 (or GPR72) in the mouse hypothalamus [1116]. The receptor GPR83 is also involved in behavior, learning, and metabolic regulation. In some brain regions, GPR83 co-localizes with GPR171. Coexpression of these two receptors alters the signaling properties of each receptor, suggesting a mutual inhibition. GPR83 also co-resides and interacts with the ghrelin receptor. Therefore, two ligand–receptor couples, LEN_L–GPR171 and PEN–GPR83, can be functionally coupled in the regulation of feeding.

5.6.8.2 Hypocretin (Orexin)

Hypocretins (Hcrt1–Hcrt2), or orexins (Ox1/OxA–Ox2/OxB), are hypothalamic neuropeptides that are produced from a common precursor, the hypocretin precursor encoded by the HCRT gene, or prepro-orexin.

They regulate sleep, arousal, wakefulness, vigilance, attention, learning, memory, reward, thermogenesis, nociception, and appetite, in addition to regulation of analgesia and neuroendocrine, gastrointestinal, and cardiovascular systems [1117, 1118].

Hypocretins act primarily as excitatory neurotransmitters. They facilitate neurotransmission mediated by acetylcholine, dopamine, γ-aminobutyric acid, glutamate, histamine, and serotonin [1117]. Insulin engenders hypoglycemia and then activates up to one third of Hcrt+ neurons.

Orexin co-localizes with dynorphin, galanin, prolactin, neuronal activity-regulated pentraxin, and glutamate [1119]. Many Hcrt+ neurons express vesicular glutamate transporters, but not glutamate decarboxylase, GAD1; they are glutamatergic, but not GABAergic.

Hypocretins lower plasmatic concentrations of prolactin and GH and raise that of corticotropin, cortisol, insulin, and luteinizing hormone. Central Hcrt administration increases water consumption and gut motility and stimulates gastric acid secretion. They can also elevate mean arterial pressure and cardiac frequency [1117]. They may control both sympathetic and parasympathetic activities.

They target their cognate GPCRs (HcrtR1–HcrtR2 [Ox_1–Ox_2]). The HcrtR1 receptor has a much higher affinity for Hcrt1 than for Hcrt2 by one order of magnitude [1119]. On the other hand, HcrtR2 has similar affinities for both neuropeptides. OX_1 is coupled to the Gq/11 subclass, thereby activating PLC. OX_2 is coupled to both Gq/11 and Gi proteins when expressed in cell lines.

The receptors Hcrt1R and HcrtR2 have partly overlapping, but largely distinct and complementary distribution patterns. The former resides in many brain regions, such as the pre-frontal and infralimbic cortex, hippocampus, amygdala, bed nucleus of the stria terminalis, paraventricular thalamic nucleus, anterior hypothalamus, dorsal raphe, ventral tegmentum, locus ceruleus, and laterodorsal tegmental and pedunculopontine nuclei [1119]. Hcrt1R is also observed in the kidney, adrenal gland, thyroid, jejunum, testis, and ovaries. HcrtR2 lodges in the amygdala, the bed nucleus of the stria terminalis, paraventricular thalamic nucleus, dorsal raphe, ventral tegmentum, and laterodorsal tegmental and pedunculopontine nuclei. It abounds in the ArcN, tuberomammillary nucleus, DMH nucleus, PVN, lateral hypothalamus, hippocampus, and medial septal nucleus [1119]. It is also detected in the lung and adrenal and pituitary glands.

Hcrt+ soma are specific to the hypothalamus, especially the feeding center. They have widespread projections within the CNS of the rat. They project with the highest density to the noradrenergic locus ceruleus, with less density to the basal ganglia, thalamus, medullary reticular formation, and NTS, and with low density to the cortex, central and anterior amygdaloid nuclei, and the olfactory bulb [1117]. In humans, Hcrt+ soma are restricted to the dorsolateral hypothalamus, with extensive

Table 5.50 Role of Hcrt+ neurons of the lateral hypothalamic area (Source: [1118]; *5HT* serotonin, *ACh* acetylcholine, *DA* dopamine, *GABA* γ-aminobutyric acid, *HA* histamine, *NAd* noradrenalin, *NPy* neuropeptide-Y, *POMC* pro-opiomelanocortin, *ArcN* arcuate nucleus, *BAT* brown adipose tissue, *BNST* bed nucleus of the stria terminalis, *DRN* dorsal raphe nucleus, *LC* locus ceruleus, *LDTN* laterodorsal tegmental nucleus, *LHA* lateral hypothalamic area, *NAcc* nucleus accumbens, *POA* pre-optic area, *PPTN* pedunculopontine tegmental nucleus, *SCN* suprachiasmatic nucleus, *TMN* tuberomammillary nucleus, *VTA* ventral tegmental area)

Input	Involved structures (neurotransmitter)	Output	Involved structures (neurotransmitters)
Emotion	Amygdala, BNST, NAcc	Arousal	Cortex; LDTN/PPTN (ACh); DRN (5HT), TMN (HA), LC (NAd)
Light	SCN	Feeding	DR (5HT), TMN (HA), LC (NAd); ArcN (NPy, POMC)
Sleep	POA (GABA)	Reward	ArcN (NPy, POMC); VTA (DA)
		Thermogenesis	BAT

Hcrt+ neurons receive inputs from the hypothalamus, including the suprachiasmatic nucleus and lateral region, limbic system, and pre-optic area, especially the ventrolateral pre-optic nucleus. Hcrt+ neurons are also regulated by humoral factors (extracellular concentration of glucose [activation] and amino acids, ghrelin [activation], and leptin [inhibition])

projections to the locus ceruleus, dorsal raphe nuclei, amygdala, suprachiasmatic nucleus, basal forebrain, cholinergic brainstem, and spinal cord.

The body energy level influences the activity of Hcrt+ neurons to coordinate arousal and energy homeostasis (Table 5.50) [1118].

In the ArcN, POMC+ neurons, the master subset of hypothalamic anorexinergic neurons, and the POMC-derived peptide α-melanocyte-stimulating hormone promote satiety. In the cerebrospinal fluid of obese mice and in the plasma of obese humans, hypocretin-1 concentration correlates inversely with α MSH concentration. Hypocretin-1 (or orexin-A) synthesized in the neurons of the lateral hypothalamus elicits wakefulness, including that for food search; it is thus involved in energy homeostasis. In the hypothalamus, hypocretin contributes to the regulation of sleep, arousal, and motivation.

Neurons of POMC+ possess both hypocretin-1 receptor $Hcrt_1$ (HcrtR1 or Ox1R) and cannabinoid receptor CB_1 [1120]. In $Hcrt_1$+ neurons, Hcrt1 primes synthesis of the endocannabinoid 2-arachidonoylglycerol catalyzed by diacylglycerol lipase-α, hence exerting a hyperphagic action via the hypothalamic CB_1 receptor. In hypothalamic neurons, 2AG activity is prevented by leptin. On the other hand, the Hcrt1–PLC–DAGL–2AG–CB_1 pathway launches autocrine regulation in $Hcrt_1$+ CB_1+ POMC+ neurons that represses α MSH production via the CB_1–ERK1/2–STAT3 pathway, hence triggering appetite and causing hyperphagia and weight gain, as phosphorylated STAT3 (Ser727) impedes the transcription of the Pomc gene.

The transcription factor STAT3 binds to the transcription factor SP1, itself tethered to the Pomc promoter, thereby eliciting POMC formation. Stimulation of STAT3 by leptin in POMC+ neurons of the hypothalamus participates in the maintenance of energy homeostasis. On the other hand, in obesity, the STAT3 activation level is elevated. Expression of SOCS3 is primed by STAT3, hence inhibiting both leptin and insulin signaling [1112]. Activation of STAT3 does not suffice to promote POMC expression, as it requires simultaneous repression by the PI3K–PKB axis of FoxO1 activity. The transcription factor FoxO1, which is upregulated during the early stages of diet-induced leptin resistance, interacts with STAT3 and prevents STAT3 to link to SP1–Pomc complex, thereby precluding the leptin–STAT3 signaling cascade [1121].

Therefore, in lean animals, Hcrt1 launches the 2AG–CB_1–STAT3 axis, which inhibits both α MSH and POMC, thereby priming alertness accompanied by food seeking. On the other hand, in obesity, aberrant activation of the Hcrt1–2AG cascade in POMC neurons due to defective leptin signaling creates a vicious circle with hyperphagia and hepatosteatosis [1120].

5.6.8.3 Uridine

The nucleoside uridine is used for nucleic acid synthesis. The base of uridine, uracil, is a building block of RNA, where it pairs with adenosine. Uridine monophosphate is enriched in mother's milk and may act as a nutrient-derived messenger (*nutrikine*) [1122]. The liver breaks down dietary uridine. Uridine from the digestive tract is rapidly excreted into bile; biliary clearance of uridine follows food intake and gut uptake into the portal venous circuit [1123]. Nucleoside carriers on both canalicular and sinusoidal membranes of hepatocytes enable excretion of uridine into bile canaliculi from hepatocytes.

Adipose tissue is a major source of uridine synthesis and secretion into blood in response to fasting; uridine can then serve as a hunger signal [1123]. Therefore, circulating uridine concentration depends on nutrient availability. Elevated uridinemia increases hypothalamic UDP concentration, which then promotes food intake via $P2Y_2$-mediated activation of AgRP+ neurons.[298]

Fasting via adipocyte-derived uridine also elicits a hypothalamic response, which slightly lowers the metabolic rate and hence body temperature. Leptin deficiency amplifies a fasting-induced decline in the body's temperature.

On the other hand, uridinemia drops rapidly in the postprandial period owing to a reduction of uridine synthesis in adipocytes and biliary clearance of uridine via Kupffer cells. In addition, insulin sensitivity is enhanced in a leptin-dependent manner. Uridine interacts with leptin and modulates leptin signaling. Conversely, leptin hinders hunger and can mediate some of the effects of uridine. Feeding-

[298] Uridine diphosphate activates $P2Y_6$ receptor on hypothalamic neurons, which then release AgRP, an appetite stimulator [1122].

induced biliary excretion of uridine reduces body temperature during fasting, and improves glucose metabolism and insulin sensitivity via leptin [1123]. Leptin also mediates plasmatic uridine clearance.

Uridine released from bile assists intestinal nutrient absorption, especially increasing glucose assimilation. In addition, uridine triphosphate activates glycogen synthesis. Uridine is required not only for glycogen storage but also for protein and lipid glycosylation, matrix synthesis, and detoxification of xenobiotics.