Carbohydrate, Fat and Protein Metabolism in Obesity

Jose E. Galgani\textsuperscript{a,b,*}, Víctor Cortés\textsuperscript{a} and Fernando Carrasc\textsuperscript{o,d}

\textsuperscript{a}Departamento de Nutrición, Diabetes y Metabolismo. Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
\textsuperscript{b}UDA-Ciencias de la Salud, Carrera de Nutrición y Dietética. Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
\textsuperscript{c}Departamento de Nutrición. Facultad de Medicina, Universidad de Chile, Santiago, Chile
\textsuperscript{d}Department of Nutrition, Clínica Las Condes, Santiago, Chile

Abstract

Macronutrient metabolism is essential for transferring energy contained in food to usable forms of cellular energy. The balance between energy fuels flowing to cells and being released as cellular work will determine the body size. In the last decades, energy homeostasis has been challenged by an overwhelming macronutrient availability that imposes a need for further expansion of adipose mass. The capacity to handle such higher energy and macronutrient fluxes will determine metabolic disturbances (e.g., insulin resistance) at tissue and whole organism level. Herein, we reviewed carbohydrate, fat, and protein metabolism with special emphasis to the comparison between lean and obese individuals.

Keywords

Fuel oxidation; Energy balance; Fuel partitioning; Cellular work; Energy transfer

1 Introduction

All forms of life require exogenous energy supply and the biochemical machinery for transforming fuels to usable forms of cellular energy. This energy is required to sustain multiple processes that are pivotal for survival of living organisms, including the maintenance of electrochemical gradients, macromolecule synthesis and breakdown, and thermogenesis, among many others (Rolfe and Brown 1997). Energy resides in the carbon-hydrogen bonds of carbohydrate, fat, and protein and only can be used by cells after being transferred to suitable energy carriers. In the mitochondria, a sequence of complex reactions removes hydrogen atoms (i.e., dehydrogenation) from energy substrates. The energy contained in the resulting transmembrane proton (H\textsuperscript{+}) gradient is ultimately transferred to cellular energy carrier molecules (i.e., phosphorylated adenosine nucleosides).

Thermodynamically, obesity pathogenesis requires a chronic positive energy balance in which exogenous energy supply surpasses body energy expenditure. The most notorious biological consequence of this energy unbalance is the expansion of white adipose tissue (WAT). This adaptation allows massive amounts of metabolic energy accumulate as triacylglycerols (TAG). Every time that body energy balance is shifted, a new homeostatic level is set between exogenous and endogenous energy fluxes. Thus, obese subjects have increased energy turnover, which is due to higher body mass rather than to higher physical activity-dependent energy expenditure. These individuals often develop insulin resistance, a condition in

*Email: jgalgani@uc.cl
which insulin action is abnormal. Because of the pivotal roles of insulin on macronutrient metabolism regulation, insulin resistance may determine impaired carbohydrate, lipid, and protein metabolism. In turn, altered macronutrient metabolism may eventually lead to insulin resistance. Herein, we offer a condensed analysis of macronutrient metabolism with focus on aspects that appear to be especially relevant for the understanding of obesity-related metabolic disorders such as insulin resistance.

2 Role of Energy Homeostasis Regulation on Obesity Pathogenesis

The maintenance of stable body mass and composition requires that cumulative energy intake over a long period of time (days to weeks) matches cumulative energy demand, resulting in null energy balance. Upon these conditions, net macronutrient storage is also null, and thus, whole-body mass and composition are constant. Therefore, null energy balance necessarily derives from that the average proportion of macronutrient oxidized (respiratory quotient, RQ) equals the proportion of dietary macronutrients available for oxidation (food quotient, FQ) over a period (Hill et al. 1991; Westerterp 1993).

Nonetheless, long-term energy flux stability is the integrated result of the intra- and inter-day fluctuations in both energy/macronutrient intake and energy expenditure that lead to either slightly positive or negative energy/macronutrient balance. Such short-term variations in the energy balance are buffered by rapid adjustment of carbohydrate and protein oxidation and fat storage (Abbott et al. 1988). Thus, states of negative energy balance, in which the energy intake is lower than its demand, are mostly compensated by net fatty acid release from fat stores that cope energy deficit. Conversely, positive energy balance states, in which energy intake is greater than its demand, lead to net fatty acid storage in adipose tissue (Frayn 2002). Carbohydrate and protein oxidation follows their respective fluctuations in carbohydrate and protein dietary availability (Abbott et al. 1988). These aforementioned concepts have critical implications for understanding of obesity, where particular modifications in macronutrient balance without significant changes in energy balance have no impact on body weight.

A number of mechanisms have evolved to compensate restricted energy intake, including increased appetite and lower metabolic rate (Prentice et al. 1991). When negative energy balance extends for longer periods, metabolic adaptation includes increased energy efficiency in order to prevent further weight loss (Rosenbaum et al. 2005; Redman et al. 2009; Goldsmith et al. 2010). On the contrary, increased energy intake normally results in suppression of appetite and eventual reduction in the efficiency of energy production (Stock 1999). Interestingly, metabolic adaptation to energy availability appears to be more efficient in preventing energy depletion than in preventing body weight gain.

Thus, in individuals that gain weight, the finely tuned interplay of metabolic and behavioral adaptations aimed to ensure appropriate balance between energy supply, storage, and utilization is overwhelmed by constant macronutrient surplus. The nature and identity of external (e.g., food availability and composition, social and environmental cues) and internal (e.g., genetic and epigenetic background, physiological and pathological determinants) factors sustaining long-lasting positive energy balance are still puzzling the scientific community (Speakman 2013).

Obesogenic environment undoubtedly plays a role. For instance, Swinburn et al. estimated that most of the positive energy gap and excess body weight over the past three decades can be entirely accounted by the higher food energy availability (Swinburn et al. 2009). Decreased energy expenditure may also contribute to positive energy balance. Few decades ago, it was postulated that basal energy expenditure (i.e., the minimal energy needed for vital functions) was lower in obese versus lean individuals, suggesting that decreased energy utilization contributes to the obese phenotype. Nowadays, it is accepted that these findings must be interpreted under the concept that the relationship between body mass (in kg) and metabolic rate (kcal per day) is allometric (i.e., metabolic rate and body mass do not change in direct
As a consequence of this fact, metabolic rate (kcal·kg$^{-1}$·day$^{-1}$) of obese individuals will expectedly be lower than that of leaner individuals (Tschop et al. 2012; Speakman et al. 2013). Concordantly, proper analysis of lean versus obese individuals has consistently showed similar metabolic rates between them, which suggests that cellular energy homeostasis is not significantly influenced by obesity.

Nevertheless, most of the comparisons of the energy expenditure between lean and obese individuals have been reported in the literature taking into account their differential body mass and composition. It is known that organ mass, particularly of high metabolic rate organs (the liver, brain, and heart), significantly influences whole-body metabolic rate (Wang et al. 2001; Javed et al. 2010; Muller et al. 2013). In addition, individual organ size does not proportionally correlate with whole-body mass (Muller et al. 2011). This aspect can be particularly relevant in the well-described, but otherwise not well-understood, metabolic adaptation in response to weight loss (Bosy-Westphal et al. 2009). Therefore, analysis of metabolic rate by taking into account organ size between lean and obese as well as in response to changes in whole-body energy flux is required for a better understanding of the mechanisms linking chronic energy unbalance and obesity.

## 3 Carbohydrate Metabolism in Obesity

### 3.1 Overview of Glucose Metabolism

Carbohydrate is normally the main source of dietary energy for humans, and glucose is the major energy substrate for cells. Red blood cells lack mitochondria and thus depend exclusively on glucose for energy provision. Similarly, although due to other mechanisms, the brain and renal medulla also rely primarily on glucose as their energy source. Indeed, the sole brain, due to its high metabolic rate (~20% of whole-body basal metabolic rate (Rolfe and Brown 1997)), requires ~100 g per day of glucose (A Report of the Panel on Macronutrients et al. 2002).

Dietary carbohydrate and glucose intake fluctuate over 24 h, being null during the sleeping time and episodic over the awaking period. On the other hand, cells have continuous glucose requirements. This metabolic conundrum is compensated by a complex neuroendocrine regulatory system that provides constant glucose supply while prevents hyperglycemia after meals and hypoglycemia over the fasting periods (Mizgier et al. 2014).

After a standard glucose load in healthy humans, ~70% and ~20% of this glucose are taken up by peripheral (mainly skeletal muscle) and splanchnic (mainly liver) tissues, respectively (Ferrannini et al. 1985). This efficient glucose uptake buffers the massive increase in blood glucose levels that otherwise dietary glucose will impose. In fact, both insulin-dependent and insulin-independent glucose uptake (Baron et al. 1988) in coordination with increased glucose oxidation and glycogen synthesis prevents postprandial hyperglycemia. Thus, high blood glucose concentration is the main driver of hepatic glucose uptake (Ferrannini et al. 1985), while hyperinsulinemia is the main promotor of glucose uptake and utilization in the skeletal muscle (Ferrannini et al. 1985; Baron et al. 1988). Postprandial suppression of hepatic glucose production is also a major mechanism to prevent hyperglycemia and maintaining glycemia within a physiological range (Ferrannini et al. 1985; Bonuccelli et al. 2009).

In contrast, under conditions of null exogenous glucose supply, the concentration gradient of glucose between the extra- and intracellular compartments is sustained by the ability of the liver to release glucose into circulation. This process is accomplished through hydrolysis of hepatic glycogen and the conversion of specific metabolites (lactate, pyruvate, glycerol, and some amino acids) to glucose (Brosnan 1999). Concomitantly, other tissues such as skeletal muscle spares glucose by adapting its energy demand to alternative energy substrates (e.g., fatty acids) (Cahill 2006).
Finally, energy sufficiency at cellular, organ, and whole-body level is achieved after adapting fuel oxidation to fuel availability, a process known as metabolic flexibility (Galgani et al. 2008b). In this metabolic scenario, insulin plays a pivotal role in determining fuel partitioning, so dietary macronutrient availability matches their oxidation rate.

3.2 Glucose Uptake/Phosphorylation

Glucose uptake occurs through facilitated transport in a process involving 14 glucose transporter (GLUT) isoforms (Thorens and Mueckler 2010). GLUT1 is expressed ubiquitously and is constitutively located in plasma membrane. GLUT2 is present in the pancreatic beta (β) cells, hepatocytes, and basolateral membrane of intestinal and kidney epithelial cells. Its high capacity for glucose transport allows translocation of glucose between the extra- and intracellular compartments depending on glucose concentration gradient. GLUT2 also mediates the efflux of glucose from the liver into the circulation under conditions of limited exogenous glucose supply. GLUT3 is expressed in the brain and has high affinity for glucose. This feature allows it to provide a relatively constant glucose supply to the neurons, even upon low extracellular glucose concentration. GLUT4 is found in striated myocytes as well as in adipocytes and is largely responsible for insulin-stimulated glucose uptake in those cells. In addition, GLUT4 translocation from the cytosol to the plasma membrane is also stimulated by muscle contraction, and this seems to be driven by a decrease in cellular oxygen concentration (Egan and Zierath 2013).

To prevent the outflow of newly incorporated glucose, this sugar is rapidly phosphorylated. This is an ATP-dependent reaction catalyzed by hexokinases. The isoform found in the liver (type 4 hexokinase, glucokinase) has relatively low affinity for glucose, with a $K_m$ that doubles the fasting blood glucose concentration (~5 mM). This kinetic feature allows hepatocytes to phosphorylate glucose that massively comes from intestine after meals. Once glucose is converted to glucose-6-phosphate (G6P), it has two major metabolic fates: (i) glycolytic oxidation to pyruvate and further conversion to lactate (anaerobic condition) or oxidation to acetyl-CoA (aerobic condition) and (ii) conversion to glucose-1-phosphate (G1P), the precursor of glycogen synthesis.

Under non-insulin-stimulated conditions (e.g., overnight fasting), circulating glucose is mostly taken up by nonskeletal muscle tissues (e.g., central nervous system), with about 20 % being cleared up by the skeletal muscle (Baron et al. 1988). Interestingly, both lean and obese individuals have similar glucose clearance rates (Kelley et al. 1999a), which is consistent with the observation that most of the glucose uptake in fasting conditions relies on insulin-independent mechanisms. Concordantly, the transport of a non-metabolizable glucose analog (3-O-methylglucose) (Dohm et al. 1988) and the content of G6P (Allenberg et al. 1988) were similar in muscle biopsies from lean and obese donors.

Under insulin-stimulated conditions, Bonadonna et al. compared glucose uptake at different insulin doses (4–400 mU·m$^{-2}$·min$^{-1}$) in lean and obese volunteers by the glucose clamp procedure (Bonadonna et al. 1990). They found that insulin dose-response curve was right shifted in obese when compared with lean individuals indicating impaired insulin action. Laakso et al. (Laakso et al. 1990) reported that the most important contributor to the decreased whole-body glucose uptake in obese patients was the diminished insulin-stimulated glucose clearance by skeletal muscle. At the molecular level, insulin-stimulated GLUT4 translocation is quantitatively lower in insulin-resistant obese individuals versus their normal counterparts, which is consistent with the impaired insulin-stimulated glucose transport detected in muscle biopsies of obese versus lean subjects (Dohm et al. 1988; Goodyear et al. 1995).

In response to a dietary challenge (i.e., ingestion of a fixed glucose dose or a mixed meal) and in contrast to the glucose clamp method, on which a fixed insulin dose is infused and the administered glucose is continuously adjusted so euglycemia is maintained, whole-body glucose uptake mostly
depends on the capacity of pancreatic beta (\(\beta\)) cells to release as much insulin as required to compensate any eventual defect on insulin action in tissues. Thus, in obese individuals with normal beta (\(\beta\))-cell function, hyperinsulinemia might well be sufficient to compensate the defect in peripheral insulin action and maintain normal glucose uptake (Fig. 1). However, contrary to this prediction, (Baron et al. 1990) found that both whole-body and skeletal muscle glucose uptake were both reduced after an oral glucose dose (1 g per kg body weight) in obese when compared with lean individuals (Laakso et al. 1990).

3.3 Glycolysis and Oxidation

The glycolytic processing of one mole of G6P yields two moles of pyruvate and two moles of ATP (net production). In turn, pyruvate can be converted to lactate through the action of lactate dehydrogenase or oxidized to acetyl-CoA through the action of the mitochondrial pyruvate dehydrogenase complex (PDH). In the mitochondria, acetyl-CoA is integrated in the tricarboxylic acid (TCA) cycle, and its oxidation results in the release of CO\(_2\) and the generation of reducing equivalents (NADH and FADH\(_2\)). The energy contained in these molecules is used to build an H\(^+\) gradient across the internal membrane of the mitochondria and ultimately drive mitochondrial ATP synthesis.

There is a plethora of studies aimed to assess whole-body and skeletal muscle glucose oxidation as well as the ratio between glucose-to-fat oxidation in obese patients in comparison with lean individuals (Galgani et al. 2008b). Most of these studies have been conducted in fasted individuals, which is hardly distinguishable whether the reported differences among obese and lean subjects correspond to intrinsic
metabolic alterations of obesity or are merely result from inaccurate control of previous dietary and metabolic conditions. Interestingly, when macronutrient intake and energy balance were carefully controlled, fasting respiratory quotient, measured in a respiratory chamber for 48 h, was similar in obese and lean subjects (Weyer et al. 2001).

Regarding postprandial conditions, we assessed whole-body glycolysis and glucose oxidation rates in insulin-sensitive and insulin-resistant individuals, defined by the glucose clamp technique (Galgani and Ravussin 2012). In this study, the insulin-resistant group also resulted to be heavier than the insulin-sensitive group (29 ± 4 [SD] vs. 25 ± 4 kg·m⁻², respectively). Despite these contrasting characteristics, whole-body glycolysis and glucose oxidation over 4 h of ingesting a standard oral glucose dose were similar between groups. In line with this finding, no differences in the oxidative disposal of glucose and other macronutrients in obese compared to lean subjects were observed over an 8-h feeding protocol (Owen et al. 1992). Using a more prolonged feeding paradigm (96 h), McDevitt et al. (McDevitt et al. 2000) delivered a hypercaloric diet (50 % excess energy over individuals’ energy requirements) to lean and obese volunteers and monitor them in a whole-body metabolic chamber. Interestingly, both groups showed a similar capacity to handle energy excess, with macronutrient oxidative disposal remaining similar between groups.

Whole-body glucose oxidation has also been assessed during euglycemic-hyperinsulinemic clamp conditions, where the extent at which RQ increases upon insulin stimulation is used as a marker of metabolic flexibility (Galgani et al. 2008b). It has been reported that insulin resistance is accompanied by an impaired ability to increase whole-body and muscle-specific glucose oxidation in the clamping, indicating metabolic inflexibility (Kelley et al. 1999b; Galgani et al. 2008a) (Fig. 1). On the other hand, improvement of insulin sensitivity, for example, after weight loss, is paralleled by enhanced metabolic flexibility (Kelley et al. 1999a; Galgani et al. 2008a). Traditionally, these findings have been considered a feature of intrinsic cellular defects, mostly at the mitochondrial level, owing to reduced ability to switch off lipid oxidation and simultaneously switch on glucose oxidation in the transition from fasting to insulin-stimulated conditions (Muio 2014).

An alternative explanation for the impaired capacity to raise glucose oxidation over lipid oxidation that insulin-resistant individuals exhibit in the glucose clamping is that this phenomenon results from the lower intracellular glucose availability of these individuals in comparison with insulin-sensitive subjects (Galgani et al. 2008a) (Fig. 1). Indeed, when insulin-stimulated glucose disposal rate was taken into account, the increase in the RQ remained equivalent in obese, nondiabetic vs. obese, type 2 diabetic patients. In addition, similar metabolic flexibility after correcting for insulin-stimulated glucose disposal rate was also observed when obese, type 2 diabetic patients were studied before and after a one-year weight loss intervention (Galgani et al. 2008a).

Another aspect deserving further analysis is the observation that obese vs. lean individuals show relatively elevated blood lactate levels (Lovejoy et al. 1990; Lovejoy et al. 1992). In fact, obesity and insulin resistance are both independently associated with increased lactacidemia (Galgani et al. 2013; Adeva-Andany et al. 2014). Furthermore, direct assessment of lactate turnover showed increased conversion of lactate to glucose and from glucose to lactate in obese vs. lean children (Stunff and Bougneres 1996). The pathophysiological relevance of this finding and its mechanistic basis remain unclear. At the molecular level, the ability to convert pyruvate to lactate or acetyl-CoA is pivotal for cellular metabolic flexibility. In this regard, an animal model having defective PDH activity (by hyperacetylation of PDH E1 alpha (α) subunit) has impaired metabolic flexibility, reduced glucose oxidation, enhanced lactate production, and higher fatty acid oxidation even in the fed state (Jing et al. 2013). Future studies should focus in investigating the molecular basis of metabolic inflexibility as well as its pathophysiological meaning.
3.4 Glucose Storage

Conversion of G6P to G1P is mediated through phosphoglucomutase. G1P is then converted to uridine diphosphate (UDP) glucose and finally bonded to a growing glycogen polymer. Insulin stimulates glycogen synthesis by relieving the inhibition that glycogen synthase kinase 3 exerts on glycogen synthase. Also, insulin-mediated GLUT4 translocation to plasma membrane results in higher glucose flux and thus higher availability of the substrates for glycogen synthase action (Yki-Jarvinen et al. 1987). Glycogen is synthesized by both the liver and skeletal muscle. The former has a higher content per gram of wet tissue, whereas the latter has a greater contribution to total body glycogen because of its larger contribution to the body mass (~50 % of fat-free mass in adult individuals).

Hepatic glycogen has a systemic role because it contributes to sustain hepatic glucose production and normoglycemia during periods of fasting. In contrast, skeletal muscle glycogen mostly sustains local ATP production during contraction. Under conditions of limited exogenous glucose supply, glycogen is hydrolyzed in order to increase G6P supply, which is then converted to glucose by action of glucose-6-phosphatase in the liver. G6P cannot be converted to glucose in skeletal muscle because it lacks glucose-6-phosphatase, so G6P is metabolized to pyruvate. It has been reported that obese individuals have decreased glycogen synthase activity under fasting conditions, although muscle glycogen content remained unaltered (Allenberg et al. 1988).

Under glucose clamp conditions, non-oxidative glucose disposal, mostly dependent on glycogen synthesis, is decreased in obese when compared with lean individuals (Young et al. 1988), possibly owed to decreased intracellular glucose availability and lower insulin-dependent glycogen synthase activity (Cline et al. 1999; Hojlund et al. 2009). However, under feeding conditions, whole-body non-oxidative glucose disposal was similar in lean and obese females studied for 96 h in a whole-body metabolic chamber (McDevitt et al. 2000).

3.5 Hepatic Glucose Production

Hepatic cells can produce glucose out of two different mechanisms: (i) glycogenolysis, i.e., hydrolysis of stored glycogen, and (ii) gluconeogenesis, i.e., de novo glucose production out of nonsugar precursors. Conditions of limited exogenous glucose supply are characterized by low blood insulin-to-glucagon ratio. This hormonal milieu promotes glycogenolysis as well as gluconeogenesis. Biochemically, gluconeogenesis follows the reverse glycolytic flux, although some reactions are exclusive for glycolysis (i.e., glucose phosphorylation and the synthesis of fructose-6-phosphate and phosphoenolpyruvate). Thus, gluconeogenesis requires specific energy-demanding enzymes to convert precursors such as pyruvate, alanine, lactate, and glycerol to glucose.

Increased basal (fasting) hepatic glucose production as well as impaired ability of insulin to suppress this process is observed in obese individuals (Bonadonna et al. 1990). Epidemiological studies have consistently found a direct correlation between abdominal obesity and insulin resistance and its systemic consequences, the so-called metabolic syndrome. However, at the tissue level, intrahepatic rather visceral fat associates with impaired hepatic glucose control (Fabbrini et al. 2009). These findings suggest that the metabolic dysfunction of the liver, more than any other intra-abdominal organ, is central in the pathogenesis of insulin resistance. In concordance with this hypothesis, the surgical removal of visceral adipose tissue appeared to have little impact on insulin sensitivity in humans (Fabbrini et al. 2010; Dunn et al. 2012; Lima et al. 2013). The fact that less than 20 % of portal vein free fatty acids (FFA) comes from visceral fat in lean and obese humans while ~10 % of the total FFA found in peripheral blood circulation is derived from visceral fat challenges any causative role of visceral fat on metabolic disturbances (Nielsen et al. 2004).
3.6 De Novo Lipogenesis

Although the main metabolic fates of glucose are oxidation or glycogen synthesis, under special metabolic circumstances, glucose can also be converted into palmitate, the main product of endogenous fatty acid synthesis pathway. Oxidation of acetyl-CoA in the mitochondria originates citrate, a TCA intermediary. Under conditions of excess glucose supply, citrate leaves the mitochondria and is converted to acetyl-CoA and oxaloacetate by the action of citrate lyase in the cytosol. Acetyl-CoA is then carboxylated to malonyl-CoA in a reaction stimulated by insulin and catalyzed by acetyl-CoA carboxylase. Malonyl-CoA is the substrate of fatty acid synthase that generates palmitate in a multistep sequence of NADPH-dependent reactions. Therefore, de novo lipogenesis (DNL) only occurs when cellular energy status is high (e.g., it requires of NADPH) and excess glucose is largely available.

Decades ago, it was postulated that DNL was partially responsible for increased adiposity of obese patients, by converting carbohydrate excess in fat, particularly in those individuals eating high-carbohydrate diets. Acheson et al. (Acheson et al. 1988) evaluated the RQ of individuals consuming large amounts of glucose (500 g per day) and found that RQ values above 1.00 were transitorily observed, indicating that net DNL was minimal after short-term carbohydrate overfeeding.

Later studies based on the stable isotopic labeling of metabolic substrates aimed to quantify in vivo hepatic very low-density lipoprotein (VLDL) secretion as a marker of hepatic DNL. Using this approach, McDevitt et al. (2001) evaluated hepatic DNL after 4 days of overfeeding in lean and obese females in response to 50 % surplus of energy as glucose or sucrose. They found that hepatic DNL was stimulated at a similar extent in lean and obese individuals regardless of carbohydrate type. Total hepatic DNL ranged between 0.7 and 4.5 g·day⁻¹, equivalent to less than 3 % of the carbohydrate energy supply and less than 2 % of the total energy balance. Additional studies performed over shorter periods of time concluded that hepatic DNL is a process of minor metabolic relevance in humans (Hellerstein et al. 1996) and highlighted the potential role of adipose tissue in this process (Aarsland and Wolfe 1998). Thus, adipose tissue DNL was approached by deuterium incorporation in fatty acids and gene expression analysis of lipogenic enzymes in lean and obese individuals (Guo et al. 2000; Minehira et al. 2004). Overall, those studies showed that carbohydrate feeding did not stimulate adipose tissue DNL or expression of lipogenic enzymes at a greater extent in obese when compared with lean participants.

These findings can be interpreted under the consideration that fatty acids are largely available in human diets, and then there is no need for lipid synthesis from an alternative precursor. In addition, when carbohydrate is provided at a level below total energy needs, DNL does not play a metabolically relevant role. By contrast, the contribution of hepatic DNL to the total fatty acid pool in subjects with nonalcoholic fatty liver disease, a frequent condition in obese subjects with insulin resistance, appears significant as discussed below.

3.7 Fructose Metabolism and Obesity

Fructose is also a hexose abundant in human diet, although its presence in foods is mostly restricted to sucrose, honey, and fruits. Lately, with the introduction of a corn-derived product (high-fructose corn syrup) to many processed foods, fructose consumption has been drastically increased, especially in societies with elevated consumption of industrialized food stuffs (Bray and Popkin 2014). This situation has renewed the interest in fructose metabolism, in particular, its effect on human metabolic disease.

Fructose metabolism is unique in many aspects. For instance, fructose is primarily metabolized in the liver; therefore, its blood concentration is minimally increased after ingestion. Once inside hepatic cells, fructose is phosphorylated to fructose-1-phosphate through fructokinase, and two metabolites are generated: (i) dihydroxyacetone phosphate, which is a glycolytic intermediate, and (ii) glyceraldehyde, which can be converted to glycolytic intermediates. Because fructokinase is not subjected to allosteric control by cellular energy status, dihydroxyacetone and glyceraldehyde production will proceed...
according to fructose availability. Thus, fructose is quickly oxidized and spares glucose and fatty acids as energy fuels. In addition, it provides precursors for TAG synthesis.

In line with these particular metabolic properties, elevated blood TAG concentration and exacerbated visceral and ectopic fat accumulation were detected in humans fed with large doses of fructose versus glucose for several weeks (>100 g per day) (Stanhope and Havel 2009; Stanhope et al. 2009). However, fructose can also speed up hepatic glucose metabolism because fructose-1-phosphate prevents the inhibition of hexokinase, which leads to enhanced glycolytic disposal and hepatic insulin sensitivity (Hawkins et al. 2002). Indeed, some authors have reported that small doses of fructose consumed in a meal (~20 g) may have beneficial impact on glycemic control (Sievenpiper et al. 2012).

The role of fructose on human metabolic regulation and disease remains highly controversial, with some authors proposing that dietary fructose is intrinsically harmful for humans (Bray and Popkin 2014), whereas others postulate that energy overconsumption is the major factor leading to metabolic disturbances, regardless of the energy source (Kahn and Sievenpiper 2014).

4 Fat Metabolism in Obesity

4.1 Overview of Fat Metabolism

Fats are integral components of all cellular systems and fulfill energetic, structural, and regulatory roles. Fatty acids and cholesterol are the most abundant dietary lipids. Dietary fatty acids are mostly found as TAG in WAT, which is able to store a vast amount of energy (~7,000 kcal/kg). Because of its ability to buffer short- and long-term fluctuations in calorie intake, WAT is a major evolutionary adaptation against starvation in vertebrates. WAT also secretes a variety of endocrine factors, called adipokines, which integrate whole-body energy balance, feeding behavior, basal metabolic rate, insulin sensitivity, and vascular function. WAT also regulates fertility, mating selection, offspring growth, immune function, and even bone accrual (Norgan 1997; Trujillo and Scherer 2006).

Fatty acid oxidation fulfills 25–45 % of daily energy needs in humans, which in an average healthy adult represents about 60–110 g of fat per day. Unlike fatty acids, cholesterol cannot be oxidized for energy production and can only be converted to derivative sterols, steroids, and biliary acids for disposal. Abnormal lipid accumulation in non-adipose tissues, such as blood, muscle, and the liver, is a frequent abnormality in obesity, and it is possibly connected with the insulin resistance present in these individuals (McGarry 2002).

Dietary TAG is hydrolyzed in the lumen of small intestine by pancreatic lipase, and the released fatty acids are absorbed and reesterified by enterocytes into TAG. Small intestine incorporates this TAG and other lipid nutrients and vitamins in chylomicrons, which ultimately reach systemic circulation. Upon extracellular hydrolysis mediated by lipoprotein lipase (LPL), chylomicrons deliver their lipid load mostly to the WAT, skeletal muscle, and heart. Finally, chylomicron remnants are cleared by the liver. In turn, hepatocytes incorporate TAG into secreted VLDL, which can then be hydrolyzed by LPL and the released FFA taken up by WAT and muscle.

LPL is located on the endothelial surface of WAT capillaries and is potently regulated by insulin. Circulating as well as LPL-released FFA are internalized by a number of binding proteins present in the plasma membrane of adipocytes, including the scavenger receptor FAT/CD36 and members of fatty acid transport protein (FATP) family (Hajri and Abumrad 2002). Importantly, whereas FAT/CD36 is abundant in adipose tissue and skeletal muscle, it is expressed at very low levels in the adult liver. Inside the cell, fatty acids are rapidly esterified with coenzyme A (CoA) by the action of acyl-CoA synthetase. Acyl-CoAs are then esterified to glycerol-3-phosphate backbone for glycerolipid and glycerophospholipid synthesis in a series of reactions catalyzed by acyltransferases and phosphatases. In the muscle
and liver, acyl-CoAs are mainly destined to mitochondrial beta (β)-oxidation for ATP synthesis. In the brown adipose tissue, acyl-CoAs are burnt out for heat generation upon cold and/or adrenergic stimulation (Ravussin and Galgani 2011). Fatty acids can also be esterified to sphingosine to form ceramide. Some of these lipids (e.g., diacylglycerol, DAG, and ceramide) are well-characterized second messengers in signaling pathways and have been consistently implicated in the pathogenesis of insulin resistance (Coen and Goodpaster 2012).

In fasted individuals, circulating FFA are the main source for the synthesis of hepatic TAG, and they constitute the bulk of fatty acids incorporated in TAG of secreted VLDL particles (Parks et al. 1999). In addition, low insulin-to-counter-regulatory hormone ratio triggers intracellular lipolysis of TAG in adipocyte lipid droplets. FFA are released to the extracellular space and circulate bound to plasma proteins, mostly albumin. Then, FFA are taken up in non-adipose tissues for reesterification, oxidation, or hepatic conversion to ketone bodies.

At the transcriptional level, endogenous fatty acid and TAG synthesis are mostly regulated by sterol regulatory element-binding protein (SREBP) 1c, carbohydrate-responsive element-binding protein (ChREBP), and peroxisomal proliferator-activated receptor (PPAR) gamma (γ). Although SREBP1c, ChREBP, and PPAR gamma (γ) have extensively overlapped control of gene expression of enzymes involved in lipogenesis, they diverge on their primary regulatory stimuli, suggesting cooperative rather than redundant physiological roles. In fact, while SREBP1c is regulated by insulin, ChREBP is responsive to glucose, and PPAR gamma (γ) appears to be directly regulated by fatty acids.

PPAR gamma (γ) is the only lipogenic transcriptional regulator that is currently targeted by drugs approved for clinical use. In fact, thiazolidinediones (pioglitazone and rosiglitazone) are effective insulin sensitizers used in type 2 diabetic patients. Ironically, PPAR gamma (γ) endogenous ligand still remains unknown. It is possible that some of proposed lipid ligands identified in in vitro assays (polyunsaturated fatty acids and prostanoids) correspond to physiological agonist/antagonist of this nuclear receptor; however, the support for this assertion is circumstantial. By contrast, 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine was recently identified as the endogenous ligand of PPAR alpha (α) isoform in the liver of mouse (Chakravarthy et al. 2009).

Whereas all these three transcriptional regulators are expressed in the human adipose tissue, only SREBP1c and ChREBP are present in the normal liver. By contrast, upon steatotic conditions, hepatic PPAR gamma (γ) is increased at the mRNA and protein level (Browning and Horton 2004). It is plausible that PPAR gamma (γ) ectopic expression further contributes to the excessive TAG accumulation and abnormal gene expression observed in nonalcoholic fatty liver disease (Gavrilova et al. 2003; Matsusue et al. 2003). The extent at which these metabolic pathways proceed will determine tissue lipid balance and insulin action on critical tissues such as the liver and skeletal muscle.

4.2 Fatty Acid Uptake

After extracellular LPL-mediated hydrolysis of TAG, resulting FFA are taken up by tissues through FATPs that facilitate fatty acid influx from extra- to intracellular compartment (Bonen et al. 2007). Then, fatty acids bind to cytosolic fatty acid-binding proteins for intracellular transport and utilization (Glatz et al. 2010). At the physiological level, both LPL and FAT/CD36 are critical determinants of fatty acid uptake. Mice with specific overexpression of LPL in the skeletal muscle show reduced levels of circulating TAG along with increased muscle fatty acid uptake and augmented peroxisomal and mitochondrial proliferation. Importantly, these metabolic changes were accompanied by progressive myopathy (Levak-Frank et al. 1995), indicating cellular toxicity triggered by excessive tissue lipid accumulation. Although FFA (Levak-Frank et al. 1995) and TAG (Hoefler et al. 1997) levels were increased in skeletal muscle of these mice, the effect of muscle LPL overexpression on insulin resistance remains uncertain as animals with different genetic backgrounds have divergent phenotypes.
In addition, specific liver or muscle LPL overexpression led to elevated intrahepatic or intramuscular TAG contents as well as accumulation of long-chain acyl-CoAs, DAG, and ceramides. In addition, these three lipid species were directly correlated with tissue-specific insulin resistance (Kim et al. 2001).

FAT/CD36 may also determine tissue lipid load as suggested from a mouse knockout model (Hajri and Abumrad 2002), which shows reduced VLDL clearance and muscle fatty acid uptake while increased plasma TAG levels. Noteworthy, FAT/CD36 deficiency determined reduced muscle TAG content and increased DAG-to-TAG ratio (Coburn et al. 2000; Goudriaan et al. 2005). Such changes were associated with improved skeletal muscle insulin sensitivity but, unexpectedly, impaired hepatic insulin sensitivity (Goudriaan et al. 2003). Conversely, skeletal muscle-specific FAT/CD36 overexpression elevated plasma glucose and insulin concentrations, which suggests impaired insulin-dependent glucose homeostasis (Ibrahimi et al. 1999).

In humans, the assessment of tissue fatty acid uptake has been restricted to skeletal muscle and adipose tissues. In vitro determinations performed in giant sarcolemmal vesicles have suggested that obese and type 2 diabetic patients have increased fatty acid uptake as well as increased membrane-associated FAT/CD36 and intramuscular TAG content (Bonen et al. 2004). However, gene expression analysis of muscle FATP has showed inconsistent results in both lean and obese individuals (Simoneau et al. 1999; Bonen et al. 2004; Pelsers et al. 2007). On the other hand, in vivo studies found similar skeletal muscle fatty acid uptake rate in fasted and insulin-stimulated lean and obese subjects (Kelley et al. 1999a).

### 4.3 Fatty Acid Oxidation

Fatty acids are the main metabolic fuel for oxidation in the transition from fed to fasted condition. Fatty acid oxidation is regulated at three enzyme-mediated steps: (i) fatty acid activation to acyl-CoA in cytosol, (ii) acyl-carnitine translocation to the mitochondrial matrix (catalyzed by carnitine palmitoyltransferase [CPT] 1), and (iii) mitochondrial beta (β)-oxidation through four sequential enzymatic reactions.

Impaired fatty acid oxidation attributed to mitochondrial abnormalities has been postulated as a major driver of muscle and hepatic fat accumulation leading to insulin resistance (Kelley and Mandarino 2000; Shulman 2014). In line with this hypothesis, Kim et al. found reduced palmitate (CPT1-dependent) and palmitoyl-carnitine (CPT1-independent) oxidation as well as lower CPT1 activity in muscle biopsies from obese versus lean donors (Kim et al. 2000). In vivo human studies have only partially corroborated this finding (Galgani et al. 2008b). On the other hand, experimental inhibition of in vivo fatty acid oxidation through administration of etomoxir (a drug that decreases CPT1 activity) led to expectedly higher glucose-to-fat oxidation ratio, which was accompanied by higher sarcolemmal GLUT4 content and lower circulating glucose, indicative of enhanced insulin sensitivity. In turn, etomoxir-treated mice had increased muscle TAG and DAG content in parallel with improved insulin-stimulated GLUT4 translocation (Timmers et al. 2012). Taken together, these findings suggest that reduced fat oxidation does not necessarily impair insulin sensitivity by itself.

### 4.4 Fatty Acid Turnover

Fatty acids are stored as TAG in lipid droplets, which are dynamic structures that appear to regulate intracellular fatty acid trafficking (Walther and Farese 2012). Thus, the signaling cascade mediating lipolysis converges in the elevation of intracellular cAMP and activation of protein kinase A. This enzyme phosphorylates lipid droplet-associated protein perilipin to allow adipose TAG lipase (ATGL, also known as patatin-like phospholipase domain-containing protein 2 and desnutrin) to physically interact with the lipid droplet surface and hydrolyze TAG in the sn-1 position (Zimmermann et al. 2004). Resulting sn-2,3 DAG is subsequently hydrolyzed by hormone-sensitive lipase and monoacylglycerol lipase (Walther and Farese 2012; Badin et al. 2013).
The balance between glycerolipid synthesis and intracellular lipolysis ultimately determines tissue lipid balance as well as the synthesis of lipid intermediates (Badin et al. 2013). Thus, the increase in lipolytic rates led to higher fatty acid availability as well as de novo ceramide synthesis in a muscle cell line overexpressing ATGL (Liu et al. 2007). Alternatively, incomplete TAG hydrolysis might also favor DAG accumulation (Badin et al. 2011).

The relevance of fatty acid turnover is highlighted by studies showing that whole-body adiposity associates directly with intramyocellular lipid content, but not with muscle content of DAG or ceramide (Moro et al. 2009). Thus, muscle-specific lipid metabolism is a determinant of muscle fatty acid turnover that is independent of total body adiposity level. In this regard, the DAG-to-TAG hydrolase activity ratio (an index of incomplete TAG hydrolysis) seems to be lower in obese individuals, which is accompanied by increased muscle ceramide and DAG content as well as impaired insulin sensitivity (Itani et al. 2002; Moro et al. 2009).

4.5 Consequences of Altered Tissue Lipid Balance

Obesity is characterized by increased WAT mass at the subcutaneous and intra-abdominal level. As mentioned above, obese people commonly have augmented tissue lipid accumulation in the liver, skeletal muscle, and heart (Shulman 2014). It appears that elevated fat content in ectopic versus eutopic (i.e., WAT) location is more deleterious for whole-body and tissue metabolic homeostasis. In fact, clinical and experimental observations suggest that excessive fat accumulation in non-adipose cells is causative of insulin resistance in obese individuals (Krassak et al. 1999; McGarry 2002; Virtue and Vidal-Puig 2008; Moro et al. 2009). Upon this hypothesis, chronic caloric overload results in a series of pathologic changes in the WAT, including exaggerated hypertrophy of adipocytes, activated immune cells infiltration, abnormal vascular supply, and fibrotic extracellular matrix (Rutkowski et al. 2015). This pathologically remodeled adipose tissue lacks the ability to fully expand and thus leaks fatty acids toward cells and tissues that are not adapted to store massive amounts of these molecules (Rutkowski et al. 2015).

In support of this hypothesis, type 2 diabetic patients have increased intramyocellular TAG content (Anastasiou et al. 2009; Nielsen et al. 2010) as well as postprandial hepatic and skeletal muscle fat storage (Ravikumar et al. 2005). These findings are in line with the observation that intrahepatic fat correlates with impaired glucose tolerance, systemic insulin resistance, and increased circulating levels of enlarged VLDL particles (Despres 1998; Adiels et al. 2006; Fabbrini et al. 2009).

Interventions that decrease ectopic tissue lipid load are usually associated with improved insulin sensitivity, further supporting the role of excessive lipid levels in insulin resistance pathogenesis. For instance, thiazolidinediones reduce plasma FFA concentration and liver TAG content while enhancing insulin-stimulated glucose disposal rate in type 2 diabetic subjects (Mayerson et al. 2002; Promrat et al. 2004). Importantly, the role of exercise, a well-established insulin-sensitizing tool, on intramuscular TAG remains controversial. Some studies have shown that physical training decreases intramyocellular TAG (Bergman et al. 1999), whereas others found the opposite result (Hoppeler et al. 1985). What seems to be consistent is that the increased intramyocellular lipid content normally observed in endurance-trained athletes does not associate with impaired muscle insulin sensitivity, and this phenomenon has been referred as the athlete’s paradox (Goodpaster et al. 2001).

Ectopic fat accumulation is also instrumental to explain why lipodystrophic patients, who have severe paucity of adipose tissue mass, show severe insulin resistance. These individuals are characterized by substantial accumulation of lipids in the liver and skeletal muscle (Gan et al. 2002; Simha et al. 2003). Remarkably, leptin, the most potent insulin sensitizer for patients with generalized lipodystrophy, also decreases lipid overload in the liver and skeletal muscle (Oral et al. 2002; Simha et al. 2003).
5 Protein Metabolism in Obesity

5.1 Overview of Protein Metabolism
Proteins are heterogeneous macromolecules with a broad range of molecular mass, structure, and functions. All the biological properties of proteins are determined by their unique sequence of amino acids. Amino acids are organic structures containing at least one atom of nitrogen. Essential amino acids, i.e., those that cannot be synthesized in human cells, and nitrogen must be obtained from diet to match amino acid requirement for protein synthesis.

Dietary amino acids as well as those derived from endogenous protein hydrolysis are significant energy substrates for humans, normally corresponding to 10–20 % (70–100 g per day) of total energy needs. As a by-product of amino acid oxidation, nitrogen is lost in urine, mainly in the form of urea, implying that amino acids undergoing oxidation must be replaced by dietary amino acids. Thus, the balance between protein degradation (mainly assessed by nitrogen loss in urine), synthesis, and intake is critical for preserving whole-body lean mass.

Dietary amino acids reach the liver via portal vein, and a significant proportion is retained by hepatic tissue. Interestingly, branched-chain amino acids (BCAA), i.e., valine, leucine, and isoleucine, are poorly metabolized by hepatocytes and are preferentially channeled to skeletal muscle for energy production as well as conversion into alanine and glutamine. These two latter amino acids are then released from muscle and taken up by the liver and other tissues for further utilization.

Amino acid turnover is dependent on the level of energy sufficiency that determines the extent at which amino acids are spared as energy source including the balance between protein synthesis and degradation. Insulin is a key regulator of this balance, although its effect depends on circulating insulin concentration. Thus, low circulating insulin concentration (similar to observed in insulin-sensitive fasted individuals) in the presence of elevated amino acid supply stimulates muscle protein synthesis without affecting skeletal muscle protein breakdown (Greenhaff et al. 2008). However, increasing blood insulin concentration does not further enhance protein synthesis, while it strongly suppresses protein degradation (Greenhaff et al. 2008).

At the molecular level, insulin increases AKT (also referred as PKB) activity, which then relieves the inhibition over mammalian target of rapamycin (mTOR). As a consequence, the activity of eukaryotic initiation factor-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase (p70S6K) increases leading ultimately to higher protein synthesis. Insulin also decreases protein degradation by inhibiting proteasome activity (Chondrogianni et al. 2014).

5.2 Protein Turnover in Obesity
Theoretically, obesity-related hyperinsulinemia should promote protein accretion unless defective insulin action also extends to amino acid utilization. However, empirical demonstration of this proposition has resulted inconclusive. In fact, many studies have been carried out to compare whole-body and tissue-specific amino acid metabolism between lean and obese individuals. Some of these studies found that, in fasting condition, obese patients have increased protein degradation in comparison with lean individuals (Nair et al. 1983; Bruce et al. 1990; Welle et al. 1992; Chevalier et al. 2005); however, several other studies did not confirm that finding (Luzi et al. 1996; Solini et al. 1997; Guillet et al. 2009). Upon insulin stimulation, obese subjects have either impaired (Jensen and Haymond 1991; Luzi et al. 1996) or unchanged (Caballero and Wurtman 1991; Welle et al. 1994; Solini et al. 1997; Chevalier et al. 2005) suppression of protein degradation.

Regarding protein synthesis, similar controversial results also exist (Luzi et al. 1996; Solini et al. 1997; Chevalier et al. 2005; Chevalier et al. 2006; Guillet et al. 2009). Therefore, it is uncertain what role plays insulin resistance in amino acid and protein metabolism. Differences in study design (e.g., adjustment in
protein kinetic by body size, relative versus absolute expression, insulin dose, duration, etc.) as well as in subject characteristics including body fat distribution (Jensen and Haymond 1991; Solini et al. 1997) can partly explain the lack of consistency across studies. Alternatively, insulin regulation of glucose and amino acid metabolism may not lie on the same molecular pathways or insulin dose-response kinetic.

5.3 Branched-Chain Amino Acids (BCAA) and Obesity
For over 50 years, it has been known that circulating BCAA concentration is elevated in human obesity (Newgard 2012). Even more, there is evidence suggesting that increased blood BCAA is an independent risk factor for insulin resistance (McCormack et al. 2013) and type 2 diabetes (Wang et al. 2011); however, a mechanistic explanation of these findings is elusive (Lynch and Adams 2014). Importantly, Everman et al. recently challenged the notion that BCAA may actually be a causal determinant of insulin resistance. Thus, they found that a short-term infusion of BCAA in healthy individuals did not change insulin sensitivity (Everman et al. 2015). Still the question why blood BCAA is increased in obesity and what is its pathophysiological relevance remain unsolved. One possible explanation comes from the fact that most of dietary BCAA reach peripheral circulation, which prompts the idea that increased protein intake in obese individuals may lead to higher circulating BCAA. Indeed, there is a tight direct correlation between BCAA intake and blood BCAA concentration (Meguid et al. 1986a, b).

Impaired tissue clearance of circulating BCAA might also play a role. In this regard, decreased content of enzymes involved in the oxidation of BCAA in skeletal muscle biopsies of obese donors has been reported at the protein (Lefort et al. 2010) and the mRNA levels (Lackey et al. 2013). Furthermore, the content of mitochondrial BCAA aminotransferase and branched-chain keto acid dehydrogenase subunit E1 (two important catabolic enzymes of BCAA) was increased after gastric bypass-induced weight loss in the WAT of obese individuals, and this was paralleled by a reduction in circulating BCAA concentration (She et al. 2007). Although the possible contribution of WAT to whole-body BCAA metabolism seems minor (Lackey et al. 2013), these studies suggest that high blood BCAA concentration in obesity may not just be a consequence of higher food/protein intake.

6 Concluding Remarks
Obesity is the result of a chronic mismatch between energy intake and expenditure. This unbalance challenges the capacity of the body to properly handle and dispose glucose and lipid macronutrients. Over the time, positive energy balance leads to a new steady state, set at a higher energy flux levels, in which macronutrient overflow matches macronutrient oxidation. Why when individuals reach this new steady state cannot resolve the metabolic disturbance associated with excessive adiposity remains unknown.

It is possible that abnormally high steady-state energy flux, attributed to increased body size rather than to elevated physical activity, might itself determine metabolic stress. On the other hand, tissue-specific metabolic disturbances may be undetectable when a whole-body approach is utilized. On this regard, the fact that whole-body macronutrient oxidative and non-oxidative disposal under physiological conditions (e.g., in the transition from fasting to feeding conditions or over a 24-h period) is fairly similar in lean and obese individuals may underscore subtle tissue-specific macronutrient unbalances.

It is very likely that our common notion of obesity as a single metabolic entity may be wrong. In fact, it has lately been described two types of obese individuals: the metabolically healthy and unhealthy obese (Samocha-Bonet et al. 2014). The identification of tissue, cellular, and molecular determinants of metabolic adaptation to high-energy fluxes will require the expansion of our capabilities to study in vivo tissue metabolic dynamics. It will be critical to identify the key biological features that promote metabolic stress during overfeeding and weight gain and understand the mechanisms underlying...
interindividu variation in the adaptation to overfeeding. Answering these questions should accelerate our comprehension of obesity-related metabolic disorders.

References


