
25 The Interaction Between Nutrition and Inflammatory Stress Throughout the Life Cycle

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KEY POINTS

- Inflammation not only is an essential process for recovery from infection and injury, but plays an adverse role in chronic inflammatory diseases, heart disease, and diabetes mellitus.
- Inflammation is controlled by cytokines.
- Polymorphisms in cytokine genes influence the inherent level of cytokine production in individuals and have been linked with an increased propensity for the adverse effects of inflammation.
- Polymorphisms in cytokine genes influence longevity.
- Obesity and aging increase the level of inflammatory stress in the body.
- Dietary supplementation with antioxidants and *n*-3 polyunsaturated fatty acids suppress adverse aspects of cytokine biology.

1. INTRODUCTION: THE IMMUNE RESPONSE AS A PURPOSEFUL ACTIVITY

The human race inhabits a world in which it is surrounded by a myriad of different microorganisms—yeasts, bacteria, protozoa, and viruses. Most of these are benign, and some, such as the normal gut flora, play an important part in promoting health via the synthesis of vitamins and stimulation of normal function of gut epithelia. Approximately 0.1% of microbes in our environment have catastrophic effects if they penetrate the epithelial surfaces of the body (Bryson, 2003). History reveals many instances in which armies have been defeated and civilizations have collapsed because of encounters between humans and such microorganisms (Diamond, 1999).

Humans, like all mammals, have evolved with a complex immune system, which is present as specialized organs (spleen, thymus) or cell types (lymphocytes, macrophages, and mast cells) throughout the body. The system can detect and destroy any cell or particle that is not “self,” i.e., a normal component of the body. A complex series of events follows from contact between components of the immune system and microbes invading the body

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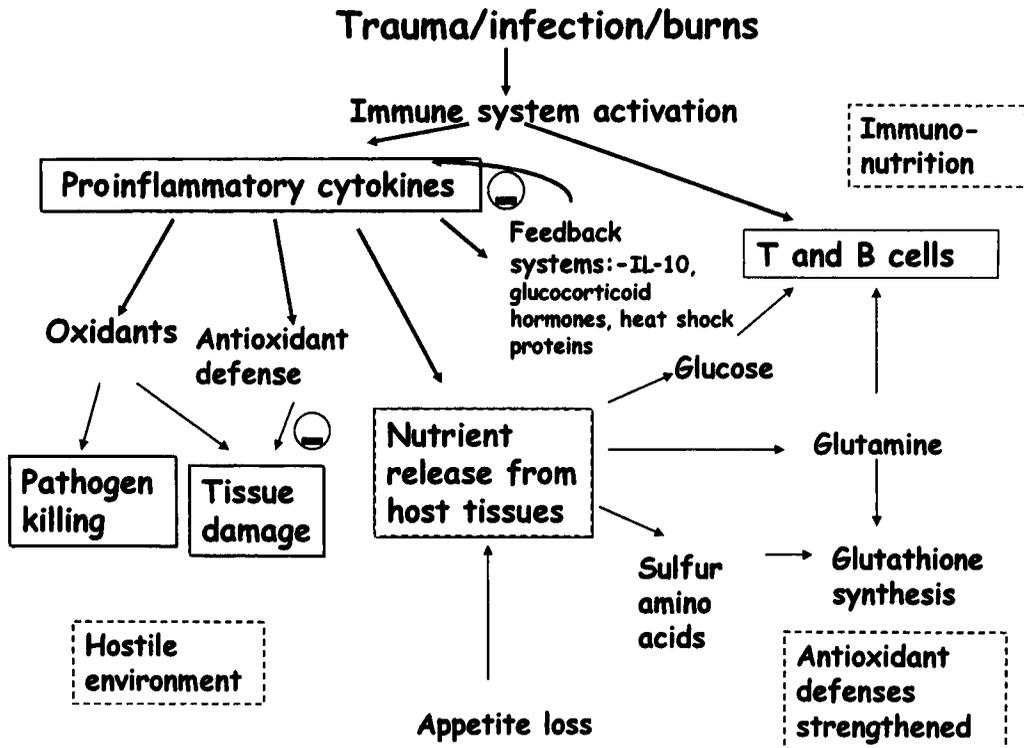


Fig. 1. Overview of the metabolic and immunological response to injury and infection. Pro-inflammatory cytokine production orchestrates the nonspecific inflammatory response; T and B cells carry out the specific immune response.

(Fig. 1). The response can be divided into two main categories. The first is the acquired immune response, in which the immune system recognizes specific chemical motifs on the invader and “remembers” the encounter so that a more rapid, specific, and intense response can be produced at any future meeting. The second category is the nonspecific response in which the response to each encounter is similar for all invaders of the body. The process of inflammation is a central part of the second category of response. The immune response is also activated by a wide range of adverse events, such as surgery, burns, and trauma.

The primary purposes of the response are to kill pathogens and initiate the curative processes that will restore body function to normal. The first purpose is achieved by creating a hostile tissue environment through production of oxidant molecules and activation of T and B lymphocytes. Part of the response ensures a supply of substrate, from endogenous sources, for supporting the activity of T and B lymphocytes and enhancement of antioxidant defenses. The latter event is important for protecting healthy tissue from the oxidants produced as part of the inflammatory response (Grimble, 2001a). The response exerts considerable biological demands and stress on the body. A central part of substrate provision is the release of amino acids into the blood from the breakdown of proteins in skeletal muscle, skin, and bone matrix, and fatty acids released from triglycerides stored in adipose tissue. Enhanced gluconeogenesis, catabolic hormone production, and decreased insulin sensitivity occurs to facilitate this redistribution of tissue

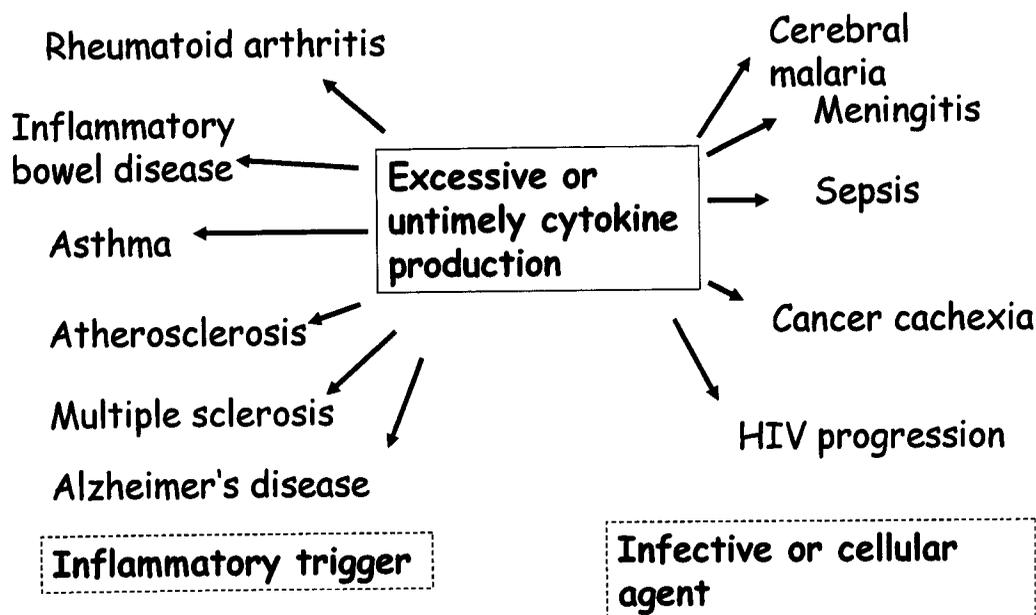


Fig. 2. Diseases and conditions in which inappropriate or excessive amounts of pro-inflammatory cytokines exert adverse or lethal effects on the host.

components (Fig. 1). The animal loses the desire to carry out many day-to-day activities. Physical weakness ensues, exploratory activity declines, appetite is decreased, and apathy and sleep may occur. The response thus exerts physiological and mental stress upon the body.

Inflammation comes under the control of signaling proteins (cytokines) that possess hormone-like actions. The pro-inflammatory cytokines interleukin (IL)- 1β , IL-6, and tumor necrosis factor (TNF)- α , are major activators and modulators of the events described above. To modulate the degree of stress imposed on the body, in achieving the essential functions of inflammation, the response comes under the control of powerful anti-inflammatory mechanisms. These will impose their biological effects with increasing vigor as the original stimulus for the inflammatory response (infection, injury) declines in intensity. Heat-shock proteins, endorphins, glucocorticoid hormones, and cytokine receptor antagonists are important components of this anti-inflammatory system. This system is essential for closing down the inflammatory response once it has achieved its primary purposes because of the high biological cost it imposes on the body (Grimble, 2001a).

1.1. Pathological Effects of the Inflammatory Response

Although cytokines play an important role in the response to infection and injury, they can exert damaging and lethal effects on the host. Many studies have shown that excessive or prolonged production of cytokines is associated with increased morbidity and mortality in a wide range of acute and chronic inflammatory conditions (Fig. 2). These include sepsis, adult respiratory distress syndrome, malaria, meningitis, cancer, cystic fibrosis, systemic lupus erythematosus, inflammatory bowel disease, rheumatoid arthritis, and asthma.

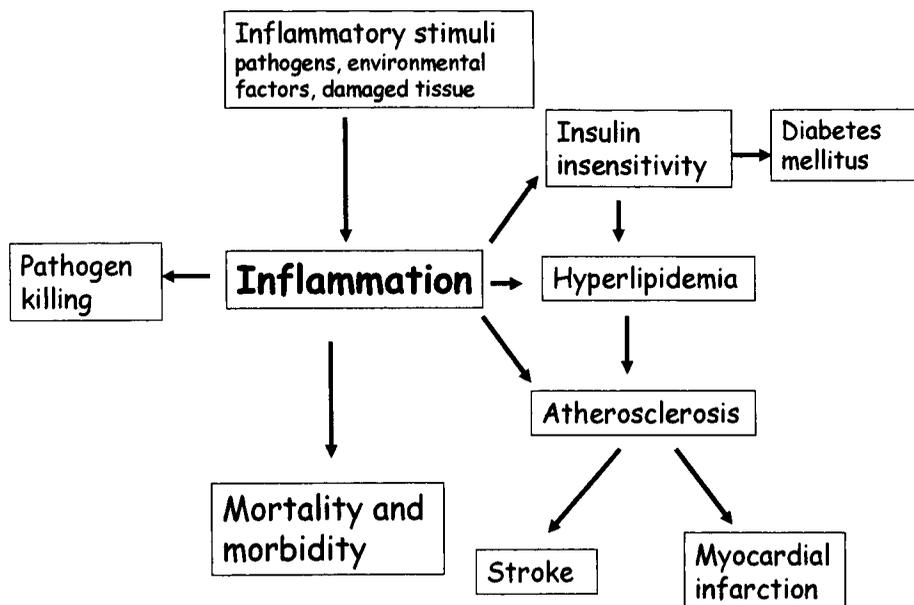


Fig. 3. Overview of the interrelationships between the inflammatory response to pathogens and diseases and pathology with a covert inflammatory basis.

Events similar to those seen in the inflammatory response to injury and infection can be observed during the course of overt inflammatory diseases such as rheumatoid arthritis and Crohn's disease and in diseases that have a covert inflammatory basis, for example, atherosclerosis and diabetes mellitus (Fig. 3). Clearly the inflammatory response in these situations does not have a purposeful nature and contribute to the disease process. Recent studies indicate that low-intensity inflammation occurs in elderly and obese individuals (Grimble 2002, 2003). Thus, the inflammatory response, which has evolved to allow humankind to survive infection and injury, is indiscriminate in both its triggers and targets. As a result, the process is a two-edged sword capable of both defending and damaging its bearer.

During the remainder of this chapter we will be exploring the biological and nutritional factors that determine the intensity of, and outcome from, the inflammatory process.

2. MAJOR FACTORS INFLUENCING THE STRENGTH AND OUTCOME OF THE INFLAMMATORY RESPONSE

2.1. *Interactions Between Components of the Inflammatory Response*

Various components of the inflammatory response interact to modulate its intensity. Predominant among these interactions are the relative amounts of pro- and anti-inflammatory cytokines produced during the response to microbes and injury and the effect of oxidant molecules on cytokine production.

2.1.1. THE BALANCE BETWEEN PRO- AND ANTI-INFLAMMATORY CYTOKINE PRODUCTION

Early work on cytokines and the response to infection linked excessive pro-inflammatory cytokine production with increased morbidity and mortality in a wide range of conditions, such as malaria, meningitis, and sepsis. However, research in the last 5 yr has

shown that the balance in production between pro- and anti-inflammatory cytokines has a more direct bearing on the outcome of infection and injury. For example, in sepsis, plasma IL-6 concentrations were higher and IL-10 concentrations were lower in patients who died than in those who survived (Arnalich et al., 2000; Taniguchi et al., 1999). A survey of over 400 patients admitted to hospital in the Netherlands with fever showed that, independently of how the patients were clinically classified (positive blood cultures, presence of endotoxin), those who subsequently died had a higher plasma IL-10:TNF- α ratio than patients who survived (Van Dissell, van Langervelde, Westendorp, Kwappenberg, & Frolich, 1998).

2.1.2. INTERACTION BETWEEN OXIDANT STRESS AND INFLAMMATION

Powerful oxidant molecules (e.g., superoxide, hydrogen peroxide, hypochlorous acid) are produced as part of the inflammatory response. Their biological purpose is to destroy invading microbes. However, these molecules also have the capacity to damage host tissues and to increase the intensity of the inflammatory response. Clearly both of these biological events can have adverse effects upon the host.

The oxidant molecules activate at least two important families of proteins in the host that are sensitive to changes in cellular redox state. The families are nuclear transcription factor κ B (NF- κ B) and activator protein 1 (AP1). These transcription factors act as “control switches” for biological processes, not all of which are of advantage to the individual. NF- κ B is present in the cytosol in an inactive form, by virtue of being bound to an inhibitory unit I- κ B. Phosphorylation and dissociation of I- κ B renders the remaining NF- κ B dimer active. The dissociated I- κ B is degraded, and the active NF- κ B is translocated to the nucleus, where it binds to response elements in the promoter regions of genes. A similar translocation of AP1, a transcription factor composed of the protooncogenes *c-fos* and *c-jun*, from cytosol to nucleus, also occurs in the presence of oxidant stress. Binding of the transcription factors is implicated in activation of a wide range of genes associated with inflammation and the immune response, including those encoding cytokines, cytokine receptors, cell adhesion molecules, acute-phase proteins, and growth factors (Schreck, Rieber, & Baeuerle, 1991) (Fig. 4). Activation of NF- κ B can be brought about by a wide range of stimuli including pro-inflammatory cytokines, hydrogen peroxide, mitogens, bacteria and viruses and their related products, and ultraviolet (UV) and ionizing radiations. The extent of activation of NF- κ B will depend in part upon the strength and efficiency of the antioxidant defenses of the body. These comprise endogenous components such as glutathione (GSH) and enzymatic components of antioxidant defenses, such as catalase, superoxide dismutase (SOD), and GSH peroxidase, and dietary components that have antioxidant properties (e.g., vitamins C and E and polyphenolic compounds). The influence of modulation of inflammation by these dietary factors are dealt with later.

An unfortunate side effect of activation of NF- κ B arises from the ability of the transcription factor to activate transcription of the genes of some viruses, such as human immunodeficiency virus (HIV) (Fig. 4). This sequence of events, in the case of HIV, accounts for the ability of minor infections to speed the progression of individuals who are infected with HIV towards acquired immunodeficiency syndrome (AIDS). Thus, if antioxidant defenses are poor, each encounter with general infections results in cytokine and oxidant production, NF- κ B activation, and an increase in HIV replication. It is thus

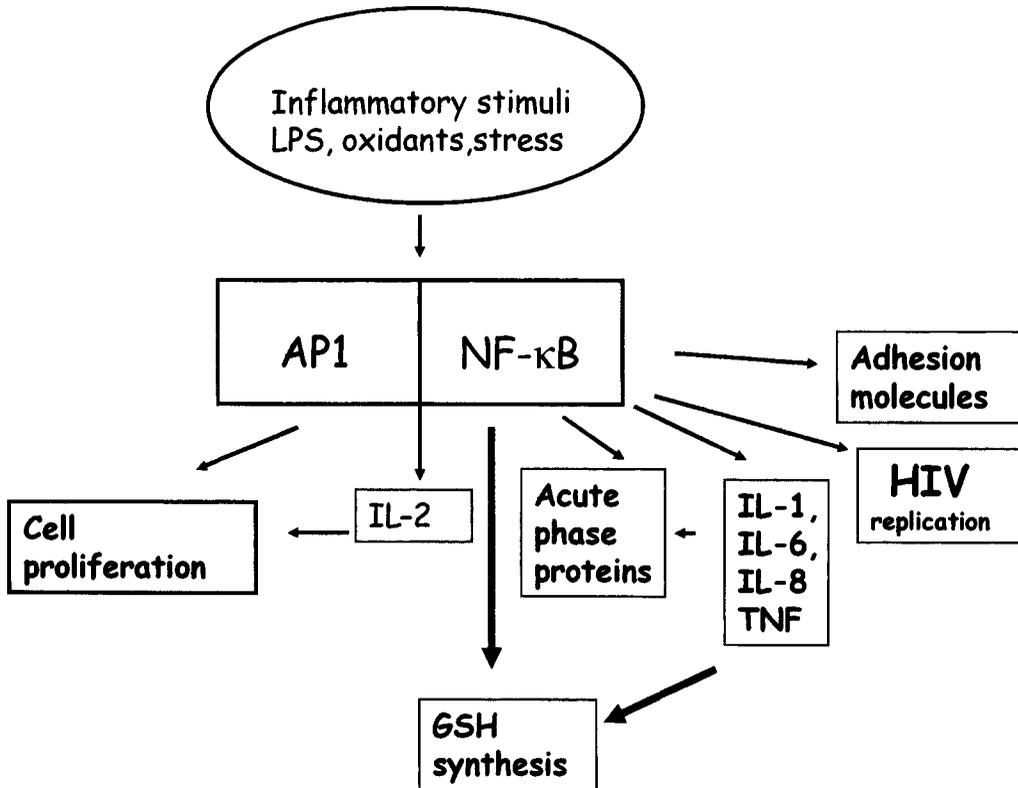


Fig. 4. Gene products whose synthesis is enhanced following activation of transcription factors by oxidant stress. NF- κ B, nuclear factor κ B; AP1, activator protein 1; IL-2, interleukin-2; HIV, human immunodeficiency virus.

unfortunate that reduced cellular concentrations of GSH are a common feature of infections, including that from the HIV (Staal, Ela, & Roederer, 1992).

Oxidant damage to cells will indirectly create a pro-inflammatory effect by the production of lipid peroxides. This situation may also lead to upregulation of NF- κ B activity.

As will be seen in later sections, genetic and dietary factors change the intensity of the inflammatory response. Thus, although the inflammatory response has evolved to ensure the survival of the human species, individuals may die as a result primarily of the response to invasion rather than from the invasive agent itself.

2.2. Genetic Influences on the Intensity of the Inflammatory Process

2.2.1. GENOMIC EFFECTS ON CYTOKINE PRODUCTION

It has recently become apparent that single base changes (single-nucleotide polymorphisms [SNPs]), usually in the promoter region of genes responsible for producing the molecules involved in the inflammatory process, exert a modulatory effect on the intensity of inflammation. In vitro production of TNF- α by peripheral blood mononuclear cells (PBMCs) from healthy and diseased subjects stimulated with inflammatory agents shows remarkable individual constancy in males and postmenopausal females (Jacob et al., 1990). This constancy suggests that genetic factors exert a strong influence. A number of studies have shown that SNPs in the promoter regions for the TNF- α and lymphotoxin

Table 1
Single Nucleotide Polymorphisms (SNPs) in Cytokine Genes Associated With Altered Levels of Cytokine Production^a

<i>Gene and location of polymorphism in promoter region</i>	<i>Genotype associated with raised cytokine production and/or altered clinical outcome to inflammation^b</i>
Pro-inflammatory cytokines	
TNF- α – 308	TNF- α – 308 A allele (TNF2)
LT- α + 252	LT- α + 252 AA (TNFB2:2)
IL-1 β – 511	CT or TT
IL-6 – 174	G allele
Anti-inflammatory cytokines	
IL-10 – 1082 ^c	GG
TGF-1 β +915 (arg-25-pro) ^c	GG

TNF, tumor necrosis factor; LT, lymphotoxin; IL, interleukin; TGF, transforming growth factor; C, cytosine; G, guanosine; T, thymidine, A, adenine.

^aThe location of the polymorphism is indicated by the nucleotide position in the promoter region.

^bPoor clinical outcome for pro-inflammatory cytokines.

^cImproved clinical outcome for anti-inflammatory cytokines.

(LT)- α genes are associated with differential TNF- α production (Allen, 1999; Messer et al., 1991; Wilson et al., 1993). The TNF2 (A) and TNFB2 (A) alleles (at -308 and +252 for the TNF- α and LT- α genes, respectively) are linked to high TNF production, particularly in homozygous individuals. The SNP in the LT- α gene (+252) is found in linkage disequilibrium with major histocompatibility molecules HLA-A1, B8, DR3 (Messer et al., 1991; Wilson et al., 1993). This genotype has also been reported to define a TNF “high expresser” haplotype (Warzocha et al., 1998), in addition to modifying expression of LT- α itself (Messer et al., 1991). A large body of research has indicated that SNPs occur in the upstream regulatory (promoter) regions of many cytokine genes (Bidwell et al., 2001). Many of these genetic variations influence the level of expression of genes and the outcome from the inflammatory response. Both pro- and anti-inflammatory cytokines are influenced by the differences in genotype (Allen 1999; Turner, Williams, & Sankaran, 1997). A number of SNPs that have been implicated in the outcome of inflammatory stress are shown in Table 1.

2.2.2. GENOMIC EFFECTS ON INDUCTION OF OXIDANT MOLECULES

NF- κ B is activated by oxidants and switches on many of the genes involved in the inflammatory response (cytokines, adhesion molecules, and acute-phase proteins). Enhancement of antioxidant defenses is important in protecting healthy tissues and in preventing excessive activation of NF- κ B by the oxidative cellular environment during inflammation (Schreck et al., 1991). NF- κ B upregulates cytokine and adhesion molecule expression, increasing the risk of host damage (Jersmann, Hii, Ferrante, & Ferrante, 2001).

Genetic factors also influence the propensity of individuals to produce oxidant molecules and thereby influence NF- κ B activation. Natural resistance-associated macrophage protein 1 (NRAMP1) has effects on macrophage functions, including TNF- α production and activation of inducible nitric oxide synthase (iNOS), which occurs by

cooperation between the NRAMP1, TNF- α , and LT- α genes (Ables et al., 2001). There are four variations in the NRAMP1 gene, resulting in different basal levels of activity and differential sensitivity to stimulation by inflammatory agents. Alleles 1, 2, and 4 are poor promoters, whereas allele 3 causes high gene expression. Hyperactivity of macrophages, associated with allele 3, is linked to autoimmune disease susceptibility and high resistance to infection, whereas allele 2 increases susceptibility to infection and protects against autoimmune disease (Searle & Blackwell, 1999).

As indicated earlier, a number of molecules suppress production of pro-inflammatory cytokines and exert an anti-inflammatory influence. These include antioxidant defenses and IL-10 (Chernoff et al., 1995; Espevik et al., 1987). Production is modulated by genetic factors. There are at least three polymorphic sites (-1082, -819, -592) in the IL-10 promoter that influence production (Perrey, Pravice, Sinnott, & Hutchinson, 1998). SNPs also occur in genes encoding enzymatic components of antioxidant defenses, such as catalase, SOD, and GSH peroxidase, which influence levels of activity (Chorazy, Schumacher, & Edlind, 1992; Forsberg, Lyrenas, de Faire, & Morgenstern, 2001; Mitrunen et al., 2001).

There is circumstantial evidence, that at an individual level, an inflammatory genotype exists that can adversely effect the host. In a study of inflammatory lung disease caused by exposure to coal dust, the TNF2 (LT- α +252 A) allele was almost twice as common in miners with the disease than in those who were healthy (Zhai, Jetten, Schins, Franssen, & Borm, 1998). Development of farmer's lung from exposure to hay dust was 80% greater in individuals with the TNF2 allele than in those without the allele (Schaaf, Seitzer, Pravica, Aries, & Zabel, 2001). The TNF2 allele was also twice as common in smokers who developed chronic obstructive pulmonary disease than in those who remained disease-free (Sakao et al., 2001). In addition to disease progression, genetic factors have important effects on mortality and morbidity in infectious and inflammatory disease. During malaria, children who were homozygous for TNF2 had a sevenfold greater risk of death or serious pathology than children who were homozygous for the TNF1 allele (McGuire, Hill, Allsopp, Greenwood, & Kwiatkowski, 1994). In intensive-care patients the occurrence of 1082*G high-producing allele for IL-10 was present in those who developed multiorgan failure with a frequency of one-fifth of that of the normal population (Reid, Hutchinson, Campbell, & Little, 1999). In sepsis, patients possessing the TNF2 allele had a 3.7-fold greater risk of death than those without the allele, and patients who were homozygous for the LT- α +252 A allele had twice the mortality rate and higher peak plasma TNF- α concentrations than heterozygotic individuals (Mira et al., 1999; Stuber, Peterson, Bokelmann, & Schade, 1996). The TNF2 allele also been found in increased frequencies in systemic lupus erythromatosus, dermatitis hepeticiformis, and insulin-dependent diabetes mellitus and noninsulin-dependent diabetes mellitus (NIDDM) (Jacob et al., 1990, Wilson, Clay, & Crane, 1995; Wilson, Gordon, & di Giovine, 1994).

Thus, it now appears that each individual possesses combinations of SNPs in their genes associated with inflammation corresponding to inflammatory drives of differing intensities when microbes or tissue injury are encountered. At an individual level this may express itself as differing degrees of morbidity and mortality (Fig. 5). The strength of the genomic influence on the inflammatory process may affect the chances of an individual developing inflammatory disease, particularly if their antioxidant defenses are poor. In addition to disease progression, genetic factors have important effects on mortality and morbidity in infectious and inflammatory disease and following injury (Paolini-Giacobino, Grimble, & Pichard, 2003).

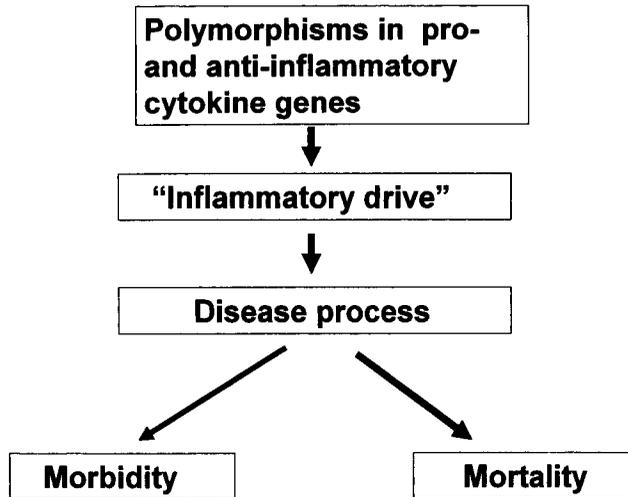


Fig. 5. Combined influences of single nucleotide polymorphisms in pro- and anti-inflammatory cytokine genes in modulating the inherent strength of the inflammatory response to injury and infection: the “inflammatory drive.”

There are sex-linked differences in the influence of genotype on the inflammatory processes. In general, males are more sensitive to the genomic influences on the strength of the inflammatory process than females. In a study on LT- α genotype and mortality from sepsis, it was found that men possessing a TNFB22 (LT- α +252 AA) genotype had a mortality of 72% compared with men who were TNFB11 (LT- α +252 GG), who had a 42% mortality rate. In female patients the mortalities for the two genotypes were 53% and 33%, respectively (Schroder, Kahlke, Book, & Stuber, 2000). In a study on patients undergoing surgery for gastrointestinal cancer, it was found that postoperative C-reactive protein (CRP) and IL-6 concentrations were higher in men than in women. Multivariate analysis showed that males possessing the TNF2 (TNF- α -308 A) allele had greater responses than men without it. The genomic influence was not seen in females (Table 2) (Grimble, Thorell, et al., 2003). Furthermore, possession of the IL-1-511 T allele was associated with a 48% greater length of stay in hospital in old men admitted for geriatric care (Table 3) (Grimble, Anderson, et al., 2003). Women were unaffected by these genetic influences.

Paradoxically, with improvements in hygiene and vaccination programs against infectious diseases, two major changes in public health and population characteristics have led to a general increase in inflammatory stress in populations of industrialized countries in the last half century. These are, respectively, an increase in the number of overweight and obese subjects and an increase in longevity. We will now examine the mechanisms underlying this phenomenon.

3. EVIDENCE FOR A LINK BETWEEN INFLAMMATION, OBESITY, INSULIN INSENSITIVITY, AND ATHEROSCLEROSIS FROM POPULATION STUDIES

It has been recognized for many years that there is a strong link between the “diseases of affluence”—obesity, insulin sensitivity, and atherosclerosis. However, it is only quite recently that the realization came that inflammation provided a link between the three

Table 2
Influence of TNF- α – 308 Polymorphism and Gender on the Inflammatory Response to Surgery in Gastrointestinal Cancer Patients

	<i>Males (n = 65)</i>	<i>Females (n = 56)</i>
Duration of operation (min)	214 \pm 125 (65)	172 \pm 76 (56)
Blood loss (mL)	473 \pm 521 (65)	258 \pm 348 (54)
Peak CRP concentration (mg/mL) ^a	150 \pm 81 (45)	126 \pm 48 (38)
TNF- α –308		
without A allele	132 \pm 46 (33)	128 \pm 57 (25)
with A allele	193 \pm 116 (12)*	121 \pm 37 (13)
Peak IL-6 concentration (pg/mL) ^b	467 \pm 411 (31)	342 \pm 310 (20)
TNF- α –308		
without A allele	439 \pm 402 (24)	362 \pm 376 (15)
with A allele	676 \pm 544 (7)*	315 \pm 147 (5)

TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein.

^a2 d postoperatively.

^b1 d postoperatively.

*Significantly different from females with same genotype by multivariate analysis allowing for longer operation time and greater blood loss; $p = 0.013$ and $p = 0.027$ for CRP and IL-6, respectively.

Means \pm SD, values in parentheses are the number of patients.

Table 3
Influence of Genotype and Gender on Hospital Length of Stay and Survival in Geriatric Care Patients (Mean Age 83 \pm 7 yr^a)

	<i>Males (n = 50)</i>	<i>Females (n = 39)</i>
Hospital length of stay (days)		
Patients with IL-1 β – 511 CC genotype	9 \pm 11 (9)	15 \pm 7 (26)
Patients with IL-1 β – 511 CT or TT genotype	14 \pm 6 (13)*	14 \pm 12 (28)
Survival posthospitalization (months)		
Patients with TNFB11 or 12 genotype	21 \pm 12 (11)	21 \pm 15 (19)
Patients with TNFB22 genotype	10 \pm 12 (10)*	22 \pm 15 (28)
Patients with IL-1 β – 511 CC genotype	27 \pm 13 (9)	19 \pm 15 (26)
Patients with IL-1 β – 511 CT or TT genotype	14 \pm 13 (16)*	25 \pm 14 (28)

TNF, tumor necrosis factor; IL, interleukin; C, cytosine; T, thymidine; A, adenine; G, guanine.

^aThe location of the polymorphism is indicated by the nucleotide position in the IL-1 β and LT- α genes, TNFB11 (GG), TNFB12(AG), TNFB22(AA) .

*Significantly different from value for same sex possessing the other genotype; $p < 0.05$ using Mann-Whitney Test.

Means \pm SD, values in parentheses are the number of patients.

biological phenomena (Fig. 3). Many studies have shown a clear link between obesity, oxidant stress, and inflammation (Grimble 2002). The link may lie in the ability of adipose tissue to produce pro-inflammatory cytokines, particularly TNF- α . There is a positive relationship between adiposity and TNF production. A positive correlation

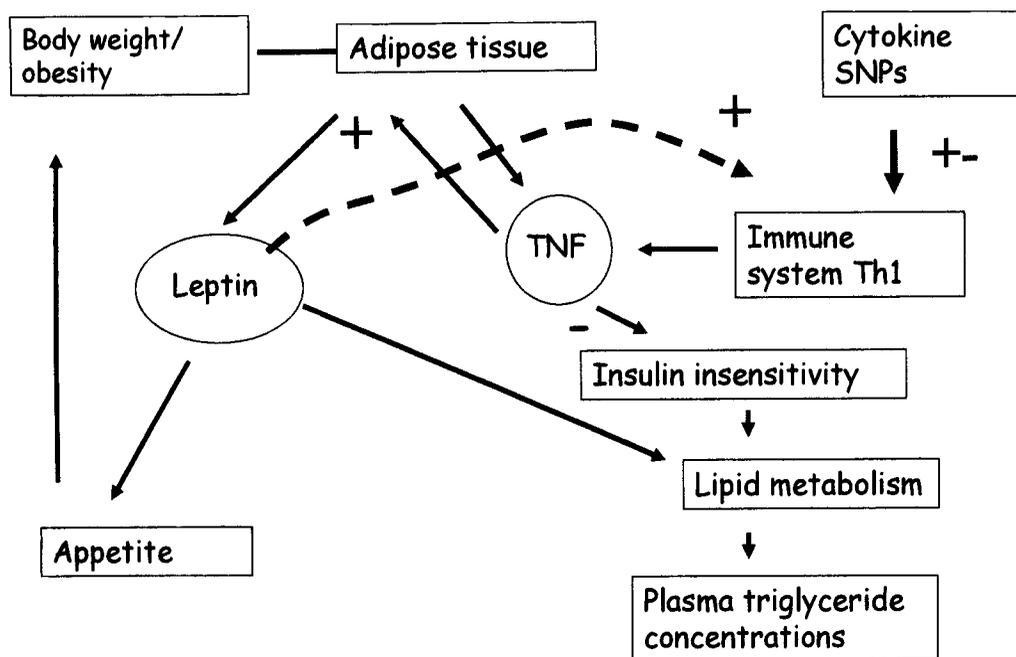


Fig. 6. Interaction between leptin and tumor necrosis factor (TNF) with adipose tissue mass, lipid metabolism, and inflammation. TNF and leptin stimulate the immune system and adipose tissue, respectively. Both also act on lipid metabolism and plasma triglyceride concentrations.

between serum TNF- α production and body mass index (BMI) has been noted in NIDDM patients and healthy women (Nilsson, Jovinge, Niemann, Reneland, & Lithell, 1998; Yaqoob, Newsholme, & Calder, 1999). Leptin has been shown to influence pro-inflammatory cytokine production (Fig. 6). Thus, plasma triglycerides, body fat mass, and inflammation may be loosely associated because of these endocrine relationships.

A number of population studies have been conducted to explore the extent and nature of the relationship of inflammation to these diseases of affluence. The studies have examined populations in which there is a high incidence of insulin insensitivity, such as Pima Indians and individuals with a South Asian background.

TNF- α is overexpressed in adipose and muscle tissues of obese subjects compared with tissues from lean individuals (Hotamisligl & Spiegelman, 1994). In a study of a group of nondiabetic Pima Indians, employing the hyperinsulinemic euglycemic clamp to assess insulin action, strong evidence of the links between inflammation, insulin insensitivity, and obesity emerged. Plasma IL-6 was found to be related positively to adiposity and negatively to insulin sensitivity. The investigators concluded that the relationship between IL-6 and insulin action appeared to be mediated through adiposity (Vozarova, Weyer, & Hanson, 2001).

A number of studies have looked at the extent of the interaction between insulin insensitivity and inflammation by studying the extreme form of diabetes, type 1 diabetes mellitus. A study assessed endothelial cell perturbation by measurement of von Willebrand factor and tissue-plasminogen activator (t-PA), in type 1 diabetics who had had the disease for <1 or >1 yr. Compared with normal subjects, children with diabetes

for <1year had the highest concentrations of von Willebrand factor, indicating that endothelial perturbation represents an early event in type 1 diabetes (Romano, Pomilio, & Vigneri, 2001).

Studies that have attempted either to remove the cause of inflammation, to lower plasma lipids, or improve insulin sensitivity have supported the hypothesis that inflammation, insulin sensitivity, and atherosclerosis are intimately interlinked. When a study of the potential anti-inflammatory effect of weight loss was conducted in obese women, it was found that a 1 yr weight-reduction program resulted in lowering of plasma IL-6, TNF- α , and adhesion molecule concentrations (Ziccardi, Nappo, & Giugliano, 2002).

3.1. Inflammation and Atherosclerosis

Since the 1990s large population studies have indicated that inflammation plays a key role in cardiovascular disease (CVD) (Grimble, 1990; Ross, 1993). Periodontal disease and other low-grade infections, such as *Chlamydia pneumoniae* infection, have been linked closely with atherosclerosis. Development of atherosclerotic plaques to which macrophages have already been recruited occurs by cytokines inducing hypertriglyceridemia and hypercholesterolemia (Kol, Sukhova, Lichtman, & Libby, 1998; Saldeen & Rand 1998). Chlamydial infection induces production of TNF- α . The cytokine inhibits the action of lipoprotein lipase, leading to changed lipid metabolism, elevation of serum triglycerides, and a decrease in serum high-density lipoprotein (HDL) cholesterol, thereby exerting an atherogenic influence (Armitage, 2000). In the Bruneck study, raised plasma bacterial lipopolysaccharide (LPS) was associated with an increased rate of thickening of the coronary artery intima. Smoking, a further inflammatory stress, exacerbated this effect (Williet & Kiechl, 2000). LPS binds in human serum to both low-density lipoprotein (LDL) and HDL cholesterol and makes LDL cholesterol immunogenic or toxic to endothelial cells. Thus, the link between specific infections and atherosclerosis may be a nonspecific effect of chronic inflammation on the atherosclerotic process.

It is well recognized that alterations in plasma protein concentrations invariably occur among the many metabolic changes that occur during inflammation. Proteins that increase during inflammation (positive acute-phase proteins, e.g., CRP and fibrinogen) and those that decrease (negative acute-phase proteins, e.g., serum albumin and retinol-binding protein) are used to diagnose inflammation in population nutritional surveys. The early indications that inflammation was involved in atherosclerosis came from the findings that there were links between concentrations of positive and negative acute-phase proteins in blood and CVD (Grimble, 1990). In the Bruneck study of a group of 826 40- to 79-yr-old Italians, it was found that impaired glucose tolerance and, to a greater extent, type 2 diabetes were strong independent predictors of advanced atherosclerosis (measured by high-resolution ultrasound of the carotid artery) (Bonora, Kiechl, & Oberhollenzer, 2000). A cross-sectional study of obesity, plasma CRP, fibrinogen, and carotid artery intima media thickness (an index of atherosclerosis) in more than 1500 multiethnic subjects showed a positive relationship between plasma CRP and body fat. Intima media thickness was related to CRP and fibrinogen in men. The relationship was attenuated by adjustment for BMI (Festa, D'Agostino, & Williams, 2001). A cross-sectional study of more than 1800 men and women examined the link between elevated plasma CRP concentrations and prevalent CVD, ankle/brachial blood pressure, and carotid artery intima media thickness. After adjustment for age and family type, there was a weak association between CRP and intima media thickness in both sexes and with prevalent heart disease

in women (Folsom, Pankow, & Tracy, 2001). A study on a Turkish population of 1046 individuals with low cholesterol concentration but a high prevalence of other risk factors for coronary heart disease investigated whether CRP acted as a predictor of coronary heart disease. Among the risk factors, only CRP and systolic blood pressure were independent risk factors for CVD (Onat, Sansoy, & Yildirim, 2001). In a study in which CRP concentrations and conventional risk factors for CVD in 500 healthy Indian Asians were compared with values in a similar number of healthy European white subjects, CRP and CVD risk factors were higher in the former group. However, differences were eliminated when adjustment was made for central obesity and insulin resistance score (Chambers, Eda, & Bassett, 2001). A study on a Brazilian population found that markers of inflammation correlated with components of the metabolic syndrome—cardiovascular and diabetes risk factors, insulin resistance, and central obesity (Duncan & Schmidt, 2001). In a study of 574 healthy elderly subjects in the Netherlands, acute-phase proteins, soluble adhesion molecules, IL-6, and insulin were measured and associated with cholesterol and obesity. The association between insulin, obesity, and cholesterol was as strong as between insulin, acute-phase proteins, and adhesion molecules (Hak, Pols, & Stehouwer, 2001).

3.2. Inflammation and Insulin Insensitivity

Insulin insensitivity occurs as part of the normal inflammatory response to pathogens. During inflammation the secretion of catabolic hormones, which enhances muscle protein breakdown and glutamine release, will oppose insulin action. Paradoxically, however, although insulin insensitivity may initially exert a beneficial effect during the response to infection and injury, it has an adverse influence in chronic disease processes.

Glucose and glutamine act as major fuels for immune cells during the normal response to infection. Large increases in glucose and glutamine utilization by immune cells occur during the response to infection and injury (Spitzer, Bagby, Meszaros, & Lang, 1988, 1989). Studies in rats given LPS and observations in patients with sepsis show that the flow of amino acids into the circulation increases and gluconeogenesis is enhanced under the influence of pro-inflammatory cytokines. An insulin-insensitive state will reduce glucose uptake by tissues in which the process is insulin dependent (muscle), thereby increasing availability to tissues in which the process is not insulin dependent (immune tissue).

A study in the United States investigated whether elevated plasma IL-6 and CRP was associated with the development of type 2 diabetes mellitus in more than 27,000 healthy women. In the 4-yr follow-up period, 188 women developed type 2 diabetes. For these women, baseline IL-6 and CRP were higher than in controls. The relative risk of future type 2 diabetes in women between the highest and lowest quartiles of these inflammatory markers was 7.5 for IL-6 and 15.7 for CRP (Pradhan, Manson, & Rifai, 2001). These data suggest a possible role for inflammation in diabetogenesis. Furthermore, data collected from the Third National Health and Nutrition Examination Survey (NHANES III) in the United States provide further evidence for a possible role of inflammation in insulin resistance and glucose intolerance. More than 2500 men and women were studied for associations between plasma CRP, fasting insulin, glucose, and glycosylated hemoglobin (HbA1c). Elevated CRP was associated with higher insulin and HbA1c in both sexes and with raised glucose in women (Wu, Dorn, & Donahue, 2002). A study on the link between CRP, central adiposity, and fasting glucose and insulin in more than 200 healthy

Italian women showed an independent relationship of adiposity to insulin resistance and CRP concentrations (Pannacciulli, Cantatore, & Minenna, 2001).

In a review, Nishimura and Murayama (2001) discussed the possibility that treatment of periodontal infection may improve insulin sensitivity. Their conclusions about the efficacious effect of an anti-infective approach are supported by a study in which 13 type 2 diabetic patients with periodontal disease were given antimicrobial treatment with minocycline. Blood TNF- α concentrations and glycosylated hemoglobin decreased (Iwamoto, Nishimura, & Nakagawa, 2001). Conversely, improvement in insulin sensitivity exerted an anti-inflammatory effect. A group of 18 hyperlipidemic patients and 20 normolipidemic controls who were insulin resistant and hypertriglyceridemic with low HDL cholesterol concentrations and raised TNF- α production and plasma IL-6 and fibrinogen concentrations were studied. All subjects were treated with the lipid-lowering drug bezafibrate. The drug normalized all parameters. The drug thus exerts an anti-inflammatory effect associated with its ability to normalize lipid metabolism and insulin sensitivity (Jonkers, Mohrschlatt, & Westendorp, 2002). In an in vitro study on a lung epithelial cell line, the oral hypoglycemic agent thiazolidinedione exerted an anti-inflammatory influence by suppressing production of monocyte chemoattractant protein (MCP-1) (Momi, Murao, & Imachi, 2001).

3.3. Mechanisms for the Link Between Inflammation and Insulin Insensitivity

It can be concluded from the studies reported in the above sections that inflammation is closely linked to obesity, CVD, insulin insensitivity, and diabetes mellitus. Although this interaction is a relatively novel concept, it has been known for many years that these diseases are interlinked and that insulin insensitivity is a common factor in their pathology. A number of recent studies have examined the mechanisms underlying the link between inflammation and the conditions and diseases in which insulin insensitivity plays a part (Fig. 3).

A difficult question to address is whether chronic inflammation leads to a condition of insulin insensitivity and associated diseases, or whether insulin insensitivity, which is associated with obesity, diabetes, and atherosclerosis, brings about a condition of chronic inflammatory stress. Many studies suggest that the former is more likely to be the case. Reviews have highlighted the risk of inflammation and infection of atherosclerotic plaques associated with poor diabetic control and the importance of elevated nonesterified fatty acid concentrations, glucocorticoids, and low-grade inflammation as causative agents in atherosclerosis and insulin insensitivity (Bell, 2000; Corry & Tuck, 2001). A study on type 2 diabetes mellitus patients determined the extent to which PBMCs contributed to oxidative stress and inflammation. A linear correlation between HbA1c and superoxide release was found. The authors concluded that type 2 diabetes mellitus is accompanied by priming of PBMCs and increased self-necrosis. The necrosis may start a chain of events that results ultimately in oxidant stress and endothelial dysfunction (Shurtz-Swirski, Sela, & Herskovitis, 2001). In a study on Pima Indians, who are characterized by high incidence of obesity and insulin resistance but not atherosclerotic disease, CRP, ICAM-1, and secretory phospholipase A₂ are correlated with body fat but not E-selectin and von Willebrand factor. In addition to showing that markers of inflammation increase with adiposity, the study showed that markers of endothelial dysfunction increase in proportion to insulin resistance and inflammation (Weyer, Yudkin, & Stehouwer, 2002). A further study on Pima Indians examined whether a raised white

blood cell count predicted a worsening of insulin action, insulin secretory function, and the development of type 2 diabetes. A high white blood cell count predicted the development of diabetes with a relative risk of 2.7 when adjusted for age and sex (Vozaarova, Weyer, & Lindsay, 2002).

TNF- α has been shown to play a key role in mediating insulin resistance as a result of obesity in patients and in numerous rodent models of obesity—diabetes syndromes (Hotamisligil & Spiegelman, 1994). Multiple mechanisms have been suggested to account for these metabolic effects of TNF- α . These include the downregulation of genes that are required for normal insulin action, direct effects on insulin signaling, induction of elevated plasma free fatty acids via stimulation of lipolysis, and negative regulation of PPAR- γ , an important insulin-sensitizing nuclear receptor (Moller, 2000). The induction of insulin resistance is mediated through its ability to produce serine phosphorylation of insulin receptor substrate (IRS)-1, decreasing the tyrosine kinase activity of the insulin receptor (Hotamisligil, Budavari, Murray, & Spiegelman, 1994). Neutralization of TNF- α in obese *fa/fa* rats by intravenous administration of a soluble TNF receptor immunoglobulin G chimeric protein substantially improved insulin sensitivity and restored the tyrosine kinase activity in fat and muscle (Hotamisligil et al., 1996). In an *in vitro* study using 3T3-L1 adipocytes, TNF- α was shown to induce sustained suppressor of cytokine signaling protein 3 (SOCS-3) production. SOCS-3 has been shown to decrease insulin-induced IRS1 tyrosine phosphorylation and its association with the p85 regulatory subunit of phosphatidylinositol-3 kinase (Emanuelli, Peraldi, & Filloux, 2001). These observations therefore suggest that SOCS-3 may be a key mediator in the development of insulin sensitivity during inflammation.

Obesity is associated with insulin resistance, particularly when body fat has a central distribution. While elevated plasma leptin concentrations are associated with obesity, some studies have suggested that insulin sensitivity is an additional determinant of circulating leptin concentrations. Leptin is produced by adipose tissue in proportion to adipose tissue mass and is a pleiotropic molecule. In addition to playing a role in appetite and adipose tissue regulation, leptin influences immune functions. Leptin concentrations increase acutely during inflammation and regulate T-cell responses, polarizing T-helper (Th) cells toward a Th1 phenotype. Thus, increased leptin production during obesity, may exert a pro-inflammatory influence (Faggioni, Feingold, & Grunfeld, 2001). Further complexity is added to the concept of a link between insulin sensitivity, inflammation, and obesity by the results of a study of 268 individuals selected from the Health Professionals follow-up study in the United States (Chu, Spiegelman, & Hotamisligil, 2001). In the study plasma insulin, leptin, and soluble TNF receptor (sTNF-R, an index of TNF- α production) concentrations were measured and correlated with BMI and the CVD risk factors insulin, triglyceride, t-PA antigen levels, and apolipoprotein (Apo)-A1. In a multivariate regression model controlling for age, smoking, alcohol intake, physical activity, and diet, BMI was inversely associated with HDL cholesterol and Apo-A1 and positively associated with triglyceride, Apo-B, and t-PA antigen levels. The associations between BMI and these CVD risk factors were only slightly changed after adjusting for leptin and/or sTNF-R, but were substantially attenuated after controlling for insulin levels. These data suggest that the association between obesity and biological predictors of CVD may be mediated through changes in plasma insulin, rather than leptin or sTNF-R levels, and that insulin may be exerting an anti-inflammatory effect. (Cnop, Landchild, & Vidal, 2002).

3.4. Genomic Influences on Interrelationships Between Inflammation, Insulin Sensitivity, and Obesity

Genetic factors may play a part in the interaction between inflammation, insulin insensitivity, and obesity. NF- κ B is an important mediator of inflammation by increasing transcription of a range of genes central to the inflammatory process (cytokines, adhesion molecules, acute-phase proteins). SNPs in the NF- κ B gene were investigated in a group of 217 type 1 diabetic patients and compared with gene frequencies in 111 normal controls. It was found that there was a higher frequency of allele 138 bp (A10) (high bioactivity) and a lower frequency of allele 146 bp (A14) (less bioactive) in diabetics than in controls. Genotype may thus contribute to inflammatory stress in diabetes mellitus (Hegazy, O'Reilly, & Yang, 2001). NF- κ B may also provide an important focus for PPAR- γ action, because it has been shown in a number of studies that PPAR- γ in combination with retinoid X receptor is able to inhibit NF- κ B activation (Wada, Nakajima, & Blumberg, 2001; Fruchart, Staels, & Duriez, 2001; Debril, Renaud, Fajas, & Auwerx, 2001). It is interesting to note that the *n*-3 polyunsaturated fatty acids (PUFAs), found in abundance in fish oil, are PPAR- γ agonists, raising the possibility that the oil may exert its anti-inflammatory effects partly via this mechanism.

In an investigation of cytokine production in 139 healthy males, the author found that in the study population as a whole there were no statistically significant relationships between BMI, plasma fasting triglycerides, and the ability of PBMCs to produce TNF- α . However, individuals with the LT- α +252 AA genotype (associated with raised TNF production) showed significant relationships between TNF production and BMI and fasting triglycerides (Fig. 7). Thus, despite the study population being comprised of healthy subjects, within that population were individuals with a genotype that resulted in an "aged" phenotype as far as plasma lipids, BMI, and inflammation were concerned (Paolini-Giacobino et al., 2003).

4. AGE-RELATED INCREASE IN OXIDATIVE AND INFLAMMATORY STRESS

4.1. Inflammation and Immune Function in the Elderly

It is well known that the incidence of diseases of affluence and recognizable inflammatory diseases, such as rheumatoid arthritis, increase with aging. Are these phenomena an unfortunate side effect of maturity, or is there a common mechanism that determines the appearance of these diseases patterns in the elderly?

Paradoxically, aging is associated with a decline in T-lymphocyte function and an increase in inflammatory stress. A number of elements of the chronic inflammatory response are apparent in otherwise healthy elderly subjects. The elements of the response include muscle protein loss, a rise in plasma acute-phase protein concentrations, and a decrease in plasma zinc. An age-related increase in IL-6 concentration has been found in serum, plasma, and supernatants of mononuclear blood cell cultures from apparently healthy elderly people and centenarians (Fagiolo et al., 1993; Baggio, Donnazzan, Monti, & Mari, 1998; Ershler & Kerller, 2000). Because IL-6 is a pleiotropic cytokine capable of regulating proliferation, differentiation, and activity of a variety of cell types (Ershler & Kerller, 2000) and plays a pivotal role in neuroendocrine and immune system homeostasis, it is not surprising that the rise in production of pro-inflammatory cytokines might have long-term pathological effects (Bethin, Vogt, & Muglia, 2000). Increases in serum

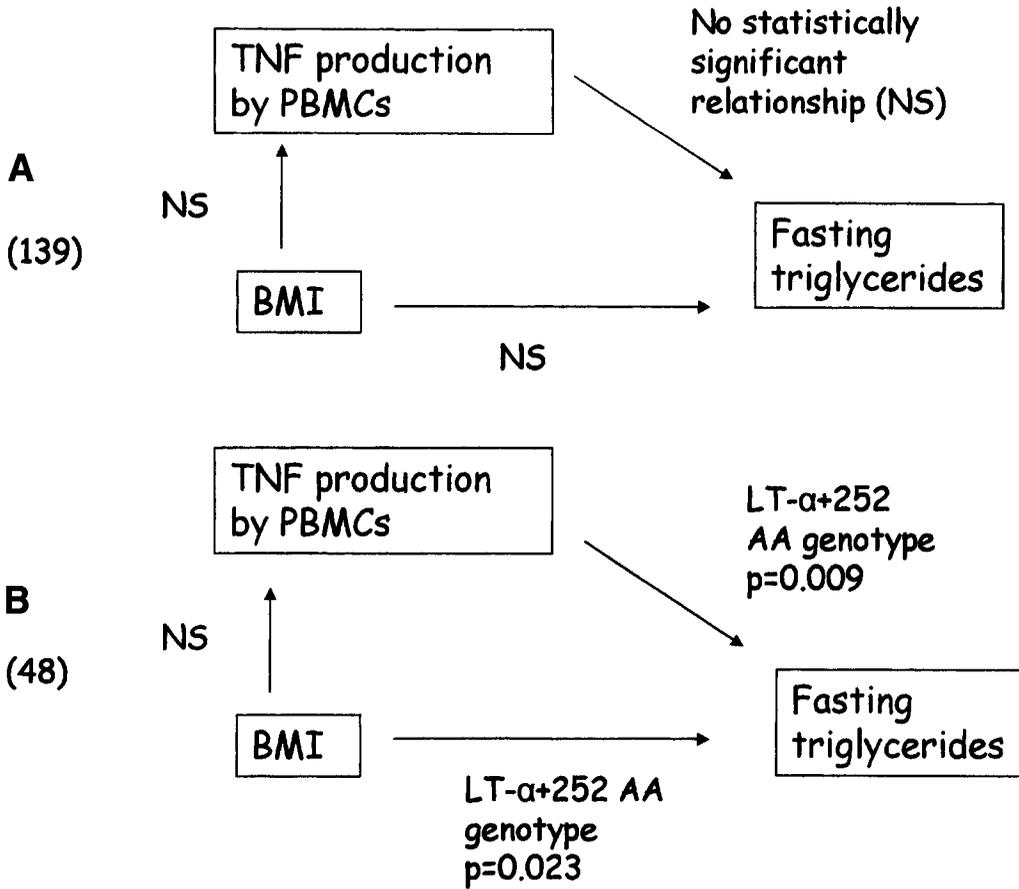


Fig. 7. Relationships between TNF production, BMI and fasting triglycerides, and the ability of PBMCs to produce TNF- α in the study populations: (A) all subjects irrespective of genotype, (B) individuals with the LT- α +252 AA genotype, associated with raised TNF production. NS, non-significant relationship; BMI, body mass index; PBMCs, peripheral blood mononuclear cells. The number of subjects is shown in parentheses; the correlations were examined by Spearman's rank correlation.

levels of this cytokine have also been found as early as at 30–40 yr of age (Mysliwska, Bryl, Foerster, & Mysliwski, 1998), particularly in men (Young, Skibinski, Mason, & James, 1999). Population studies have shown that the magnitude of increase in the concentration of IL-6 is a reliable marker for functional disability and a predictor of mortality in the elderly (Ferrucci, Harris, Guralnik, & Tracy, 1999; Harris, Ferrucci, Tracy, & Corti, 1999). Antioxidant status may decline with age (Nuttall, Dunne, Kendall, & Martin, 1999) and may thus be linked to increased TNF- α production (Kudoh, Katagai, Takazawa, & Matsuki, 2001; Rink, Cakman, & Kirchner, 1998).

An enhanced capacity for the release of pro-inflammatory cytokines by white blood cells may contribute to the pathogenesis of ischemic stroke. Grau et al. (2001) investigated the LPS-induced release of IL-1 β , IL-6, IL-8, and TNF- α in whole blood from patients with a history of ischemic stroke under the age of 50 and age- and sex-matched healthy control subjects. Release of IL-8 was significantly higher in young stroke patients than in control subjects (Grau et al., 2001).

The question of whether aging is associated with chronic elevation of cytokine production or whether an increased capacity for cytokine production following the normal inflammatory challenges of life develops during aging is an interesting one to consider. Insight into this issue can be gained from the response to surgery where an inflammatory stimulus is applied at a defined moment in time, making it easy to follow the subsequent response. Ono, Aosasa, Tsujimoto, Ueno, and Mochizuki (2001) investigated the age-related changes in the inflammatory response in patients with gastric cancer undergoing distal gastrectomy. Patients were divided into two groups: >75 yr of age (elderly group) and ≤75 years of age (young group). Serum IL-6 concentrations, TNF- α production and CD11b/CD18 expression by monocytes, and the postoperative clinical course were compared between the two groups to assess the inflammatory response to surgery. TNF- α production by LPS-stimulated monocytes and CD11b/CD18 expression on monocytes were significantly higher in the elderly than in the young group. Moreover, serum IL-6 concentrations on the first postoperative day in the elderly group were significantly higher than those in the young group.

Paradoxically, both loss of body weight and lean tissue and obesity are found in elderly populations. Is there, therefore, a link between this phenomenon and increased levels of inflammation?

4.2. Loss of Lean Tissue During Aging

The loss of muscle mass and strength that occurs with aging is described clinically as sarcopenia (Rosenberg, 1989; Roubenoff, 2001). It is an important contributor to the development of frailty and functional impairment during aging. It is well established that aging is associated with a significant decline in muscle strength that becomes functionally important by the seventh decade of life. The relationship between chronic inflammation owing to disease during aging and the prevalence of low body mass are well illustrated in rheumatoid arthritis. In a study on patients with rheumatoid arthritis, the loss of body mass was greater for lean tissue than fat, with over 50% of the rheumatoid group falling into the lowest 10th percentile of a reference population for skeletal muscle mass assessed from the upper arm muscle area. In female patients there was a significant correlation between reduced fat-free mass and two indicators of inflammatory stress—erythrocyte sedimentation rate and plasma CRP concentration (Munro & Capell, 1997). Clinical and animal studies show a relationship between raised plasma cytokine concentrations and low muscle mass. Visser et al. (2002) investigated whether markers of inflammation are associated with muscle mass and strength over a time course of several years in over 3000 healthy well-functioning black and white elderly persons (70–79 yr). Mid-thigh muscle cross-sectional area, appendicular muscle mass, and muscle strength were assessed. Plasma concentrations of IL-6 and TNF- α were also measured. Higher cytokine concentrations were associated with lower muscle mass and lower muscle strength. The most consistent relationship across the gender and race groups was observed for IL-6 and grip strength. When an overall indicator of elevated cytokine production was created by combining the concentrations of IL-6 and TNF- α , with the exception of white men, elderly persons having high concentrations of IL-6 (>1.80 pg/mL) as well as high levels of TNF- α (>3.20 pg/mL) had a smaller muscle area, less appendicular muscle mass, and lower muscle strength compared to those with low levels of both cytokines. Thus, raised plasma concentrations of IL-6 and TNF- α are associated with lower muscle mass and

lower muscle strength in well-functioning older men and women as well as those suffering frank inflammatory disease.

Nutrient intake is clearly another important determinant of lean body weight and fat mass and may play a part in the decline in lean tissue with age as well as an increase in inflammatory stress. A recent survey of 40,000 subjects in 88 communities in NHANES III in the United States also included a survey of about 5000 elderly people ranging in age from 60 to 69 yr, 70 to 79 yr, and 80+ yr (Marwick, 1997). The report indicated that the median intake of total energy was in general lower than the recommended 2300 kcal for men and 1900 kcal for women (Marwick, 1997).

4.3. The Link Between Obesity, Aging, and Inflammatory Stress

Chronic inflammation is either a causative agent or a closely associated process in the pathology of obesity, insulin insensitivity, and atherosclerosis. The incidence of these conditions increases with aging. A fundamental question is which precedes the other—the general increase in inflammation or the development of diseases with overt and covert inflammatory bases? This “chicken-and-egg” question is difficult to answer. However, examination of data from studies conducted in elderly populations may throw some light on the answer to this conundrum.

There are at least two potential mechanisms for the higher level of chronic inflammation observed in elderly than in younger subjects. The first of these is that the elderly are experiencing a higher level of asymptomatic urinary infection. This possibility was studied in 40 consecutive patients (70–91 yr) admitted to the hospital for functional disability. Patients were examined for the presence or absence of bacteria in the urine. Twenty subjects had a positive urine culture, and 20 sex- and age-matched subjects had a negative urine culture. Inclusion criteria were temperature $<37.8^{\circ}\text{C}$, no clinical signs of infection, and no current antibiotic treatment. Patients with asymptomatic bacteriuria had significantly increased levels of TNF receptors and a higher number of neutrophils in the blood compared to the group without bacteriuria. Thus, the study provides some support for the hypothesis that asymptomatic urinary infections are associated with low-grade inflammatory activity in frail, elderly subjects (Prio, Bruunsgaard, Roge, & Pedersen, 2002).

A second potential mechanism resides in endocrine changes during aging. In aging, dysregulation of secretion of hormones that come under the regulation of the hypothalamic–pituitary–adrenal axis may occur. This may have an effect on the regulation of cortisol secretion, as mentioned earlier. Cortisol is important as an anti-inflammatory agent. The effect of aging on glucocorticoid sensitivity of pro-inflammatory cytokine production was examined in elderly men, testosterone-treated elderly men, and young controls. Stress-induced increases in cortisol did not differ significantly between experimental groups, but glucocorticoid sensitivity increased significantly in young controls and testosterone-treated elderly men, whereas a decrease was found in untreated elderly men. As the increase in glucocorticoid sensitivity after stress serves to protect the individual from detrimental increases of pro-inflammatory cytokines, the disturbed mechanism in elderly men may result in an increase in inflammatory stress (Rohleder, Kudielka, Hellhammer, Wolf, & Kirschbaum, 2002).

There is now a large body of evidence suggesting that the decline in ovarian function with menopause is associated with spontaneous increases in pro-inflammatory cytokine production. As mentioned earlier, studies in men and postmenopausal women indicate a

remarkable individual constancy in the ability of PBMCs to produce TNF- α *ex vivo*, and genetic determinants underlie this constancy. However in premenopausal women production is highly variable at an individual level, indicating how ovarian hormones are able to override the influence of genotype (Jacob et al., 1990). The exact mechanisms by which estrogen interferes with cytokine activity are still incompletely known but may include interactions of the estrogen receptor with other transcription factors, modulation of nitric oxide activity, antioxidative effects, plasma membrane actions, and changes in immune cell function. Experimental and clinical studies also strongly support a link between the increased state of pro-inflammatory cytokine activity and postmenopausal bone loss (Pfeilschifter et al., 2002).

4.4. Influence of Genotype on Inflammation and Aging

Recent evidence indicates the presence of SNPs, associated with the strength of the inflammatory response, affects longevity. Human longevity may be directly correlated with optimal functioning of the immune system. Therefore, it is likely that one of the genetic determinants of longevity resides in polymorphisms for genes influencing the activity of the immune system.

It has been estimated that up to 7000 variations in the genome contribute to life span (Martin, 1997). Those contributing to loss of muscle and bone mass during aging are related to the inflammatory process and include pro- and anti-inflammatory cytokines and their receptors.

Studies in mice have shown that the genes controlling the major histocompatibility complex (MHC), known to control a variety of immune functions, are associated with differences in the life span of different strains of mice, but a major difference between observations in mice and humans is that the latter have a lifetime experience of exposure to pathogens, whereas for laboratory animals this exposure is kept to a minimum. Thus, although HLA studies in mice of different genotypes may be interpreted to support studies of MHC effects on longevity in humans, in mice the association may be by way of altered susceptibility to lymphomas, whereas in human beings the effect on longevity is likely to be via an altered response to pathogens and susceptibility to infectious disease.

A number of cross-sectional studies have examined the role of HLA genes on human longevity by comparing HLA antigen frequencies between groups of young and elderly persons. Conflicting findings have been obtained. When this topic was reviewed (Caruso et al., 2001), it was concluded that in humans there may be an association between longevity and some HLA-DR alleles or the HLA-B8,DR3 haplotype. These genotypes are involved in the antigen nonspecific control of immune response, in other words, the component of immune function associated with inflammation and cytokine biology.

Recent evidence indicates that presence of SNPs in certain pro- and anti-inflammatory cytokine genes influences life span. When 700 individuals between 60 and 110 yr of age were studied, it was noted that not only was plasma IL-6 concentration positively related to age but individuals with a SNP in the promoter region of the IL-6 gene, which predisposes to high levels of production of the cytokine (-174 GG), decreased in frequency with age. The effect was seen in men but not in women (Bonafè, Olivieri, & Cavallone, 2001). Although men with SNPs made up 58% of the 60- to 80-yr-old age group, the percentage fell to 38% in subjects <99 years of age. Conversely, one of three SNPs in the IL-10 gene (-1082 GG), which is closely linked to higher production of the anti-inflammatory cytokine IL-10 (Hutchinson, Pravica, Hajeer, & Sinnott, 1999; Turner et al., 1997), was

found in higher proportions in male centenarians than in younger controls (58 vs 34%). In females this genotype exerted no effect upon longevity (Lio et al., 2002). Thus, it would appear that genetic characteristics that might influence the balance between pro- and anti-inflammatory cytokines influence mortality in men but not in women (Franceschi et al., 2000). A study on SNPs that influence interferon (IFN)- γ production further reinforces the concept that possession of a genotype that predisposes to a raised pro-inflammatory status is not compatible with a long life span (Lio et al., 2002). In women, possession of the A allele, which is associated with low production of IFN- γ , significantly increased the possibility of reaching old age. It might be concluded that possession of high-producing alleles of the IL-10 is universally protective against morbidity as well as mortality. Possession of a genotype that results in low levels of IL-10 production (-1082 AA) increases the risk of developing inflammatory diseases (Hajeer, Lazanes, & Turner, 1998; Huizinga, Keijsers, & Yanni, 2002; Tagore, Gonsalkorale, & Pravica, 1999). However, as already mentioned, in a large survey of hospital admission in the Netherlands, patients with raised IL-10:IL-6 ratios had higher mortality rates (Van Dissel et al., 1998).

Not all studies implicate cytokine gene SNP in longevity. Cytokine gene polymorphisms at IL-1 α , IL-1 β , IL-1RA, IL-6, IL-10, and TNF- α were measured in 250 Finnish nonagenarians (52 men and 198 women) and in 400 healthy blood donors (18–60 yr) used as controls. No statistically significant differences were found in the distribution of genotype, allelic frequencies, and A2+ carrier status between nonagenarians and younger controls (Wang, Hurme, Jylha, & Hervonen, 2001).

In a review on the different impact of genetic factors on the probability of reaching old age, Franceschi et al. (2000) concluded from studies conducted in Italy that emerging evidence (regarding mtDNA haplogroups, tyrosine hydroxylase, and IL-6 genes) suggests that female longevity is less dependent on genetics than male longevity and that female centenarians are more likely to have had a healthier lifestyle and more favorable environmental conditions than males. However, a recent study conducted by our group suggests that although a pro-inflammatory genotype may be disadvantageous to elderly males, it may confer a survival benefit in females. Subsequent survival was studied in 79 elderly geriatric patients (87 ± 7 yr) after a period of hospitalization for a range of conditions necessitating geriatric care. Although women possessing a pro-inflammatory genotype (TNF- α -308 A allele or IL-6-174 GG) had improved 3 yr survival rates, men possessing pro-inflammatory genotypes (IL-1 β -511 T allele or LT- α +252 AA) had shortened survival rates (Grimble, Thorell, et al., 2003) (Table 3).

5. THE POTENTIAL FOR MODULATION OF THE INFLAMMATORY RESPONSE BY IMMUNONUTRITION

As outlined in the preceding sections, the inflammatory response, although essential for survival in the presence of pathogens, can exert deleterious effects on the host. The clear need to find ways of modulating cytokine production and other aspects of inflammation has fostered the research area of immunonutrition.

In a clinical context the purpose of immunonutrition is to find nutritional means of altering the patient's inflammatory response to infection and injury, from the detrimental to the beneficial side of the pivot on which an individual undergoing a response is positioned. While inflammation may be exerting deleterious effects most obviously in patients, people on the borderline of health and disease living in the general population

Table 4
Nutrients Commonly Used in Immunonutrient Supplements and Their Potential Mode of Action

-
- *n*-3 polyunsaturated fatty acids: act as anti-inflammatory agents and reverse immunosuppression
 - Sulfur amino acids and their precursors: enhance antioxidant status via GSH synthesis
 - Glutamine: nutrient for immune cells, improves gut barrier function, precursor for GSH
 - Arginine: stimulates nitric oxide and growth hormone production, improves helper T-cell numbers
 - Nucleotides: RNA and DNA precursors, improve T-cell function
-

may also require nutritional modulation of ongoing inflammatory processes. During the last 20 years the pace of evolution of immunomodulatory feeds and intravenous solutions has accelerated. These products contain combinations of a number of components to which various functional attributes are ascribed them (Table 4) (Grimble, 2001a).

Many studies have indicated that *n*-3 polyunsaturated fatty acids (PUFAs), glutamine, arginine, sulfur amino acids, and nucleotides are all potentially capable of shifting the balance from a disadvantageous to an advantageous response to infection and injury. The examples used here are illustrative rather than comprehensive. A number of studies indicate that improvement of antioxidant status is associated with an increase in cellular aspects of immune function. Meta-analyses have been conducted on the efficacy of immunonutrients that influence antioxidant status. In clinical trials, indices such as infection rates, mortality rates, and length of stay are often measured in the absence of functional and biochemical aspects of the response, such as T-cell function, cytokine production, and antioxidant status, and vice versa, giving a rather incomplete picture of the mechanisms of any observed effects of immunonutrition. However, Beale, Bryg, & Bihari (1999), in a meta-analysis of 12 studies containing more than 1400 patients receiving enteral immunonutrition, observed that although there was no effect upon mortality, there were significant reductions in infection rates, time spent on a ventilator, and length of hospital stay. While this finding indicates that immunonutrition may be useful in modulating the inflammatory process in patients experiencing severe inflammation, the consistency of the effects observed was disappointing.

There are at least three major reasons why it is difficult to demonstrate a consistent effect. First, patients used as the subjects of clinical trials of immunonutrients will constitute a diverse population—different ages, at different stages of a disease process, and undergoing complex clinical treatment in addition to nutrient therapy. Second, patients will have differing genetic backgrounds that will influence the intensity of the inflammatory and immune responses they are undergoing. This issue is dealt with below. Third, nutrients may exert paradoxical effects, as illustrated by the findings of the first observations of the effects of fish oil on cytokine production in healthy subjects. The findings of Endres et al. (1989) that a daily supplement of 18 g/d of fish oil given to nine young men for 6 wk was able to reduce *ex vivo* production of IL-1 and TNF- α by LPS-stimulated PBMCs aroused great interest in fish oil as an anti-inflammatory nutrient. This perception was supported by a large amount of animal data. However, Endres' data showed a wide variability in the effect of the fish oil supplements. The standard deviations of the mean for IL-1 β and TNF- α production were 59 and 51%, respectively. This indicates that cytokine production could have risen or fallen as a consequence of taking the supplement.

The effects of supplementing 116 healthy young men with 6 g/d of fish oil for 12 wk, on TNF- α production by PBMCs stimulated with endotoxin have been studied in the author's laboratory. It was found that 51% of subjects experienced a fall in production and 49% a rise. Although the ability of fish oil to increase TNF- α production is at first sight paradoxical, earlier work of Dinarello, Bishai, Rosenwasser, and Coceani (1984) and Kunkel, Remick, Spengler, and Chensue (1987) indicated that fish oil could potentially change cytokine production in either direction. What mechanisms could result in this divergent effect? Inflammation will result in activation of phospholipase A₂, which releases arachidonic acid (AA) (C20:4 *n*-6) from the cell membrane for prostaglandin E₂ (PGE₂) or leukotriene B₄ (LT B₄) synthesis. The *in vitro* studies (Kunkel et al., 1987) showed that PGE₂ suppressed TNF- α production, whereas LTB₄ had the opposite effect (Dinarello et al., 1984). Fish oil is rich in eicosapentaenoic acid (C20:5 *n*-3), which will replace AA in the cell membrane and results in the production of PGE₃ and LT B₅. PGE₃ and LT B₅ are considerably less potent than the corresponding compounds produced from AA, and thus dietary fish oil may lessen the inhibitory influence of PGE₂ or the stimulatory influence of LTB₄ on TNF- α production, resulting in a potential increase or decrease, respectively, in production of the cytokine. Fish oil could thus result in an inflammatory cytokine response, which could fall on either side of the pivot.

5.1. Immunomodulation by Enhancement of Antioxidant Defenses

The response to bacterial invasion of the body, or injury, contains a paradox. Although the inflammatory response and the T-cell response both play a part in defeating the invader, the inflammatory response may in some clinical circumstances exert an inhibitory influence on T-cell function. In severely infected or traumatized patients, an enhanced inflammatory state occurs, which is associated with immunosuppression. *In vitro* studies support this inverse relationship. PBMCs taken from healthy young subjects and incubated with GSH show decreased PGE₂ and LTB₄ production (reduced inflammation) and an increase in mitogenic index and IL-2 production (enhanced immune function) (Wu, Meydani, Sastre, Hayek, & Meydani, 1994).

Thus, enhancement of antioxidant defenses reduces the likelihood of the inflammatory response suppressing T-cell function (Grimble, 1997, 2001b). Although all antioxidants are important owing to the linked nature of antioxidant defense (Fig. 8), GSH plays a pivotal role as it acts directly as an antioxidant and maintains other components of defense in a reduced state through enzymic conversion between the oxidized and reduced states. Various compounds can be used to increase GSH synthesis (Fig. 9). *N*-Acetyl cysteine (NAC) and the GSH prodrug oxothiazalidine-4-carboxylate (procyteine) have been used in a number of clinical studies. Tissue GSH content is also influenced by protein and sulfur amino acid intake. Unfortunately, surgery, a wide range of diseases that have an inflammatory component, and aging and protein energy malnutrition decrease GSH concentration in blood and other tissues (Boya et al., 1999; Loguercio et al., 1999; Luo, Hammarqvist, Anderson, & Wernerman, 1996; Micke, Beeh, Schlaak, & Buhl, 2001; Nuttal et al., 1999; Reid et al., 2000) (Table 5). Within 24 h of elective abdominal surgery, muscle GSH content falls by >30%. Values return to normal 72 h postoperatively. A smaller perturbation in blood GSH occurs over a shorter time course.

Modification of the GSH content of liver, lung, spleen, and thymus in young rats by feeding diets containing a range of casein (a protein with a low sulfur amino acid content) concentrations changed immune cell numbers in lung (Hunter & Grimble, 1994). It was

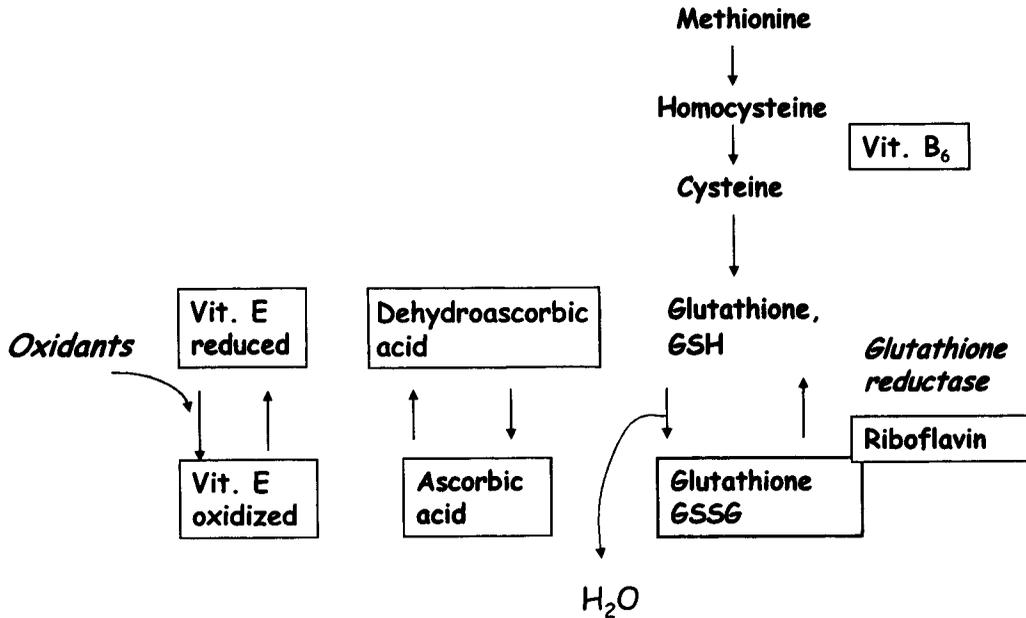


Fig. 8. The interlinked nature of the antioxidant defenses.

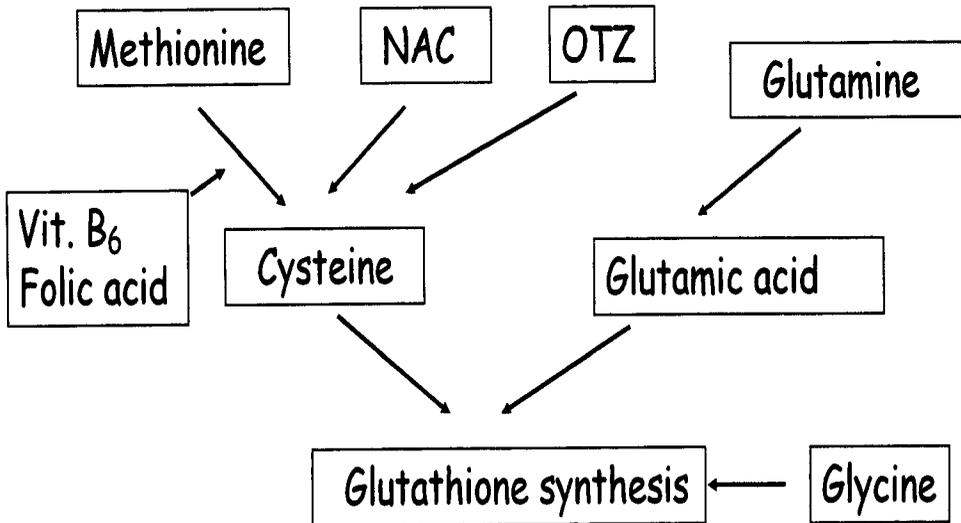


Fig. 9. Potential means of enhancing GSH synthesis by immunonutrients. NAC, *N*-acetyl cysteine; OTZ, 1-2-oxothiazolidine-4-carboxylate.

found that in unstressed animals the number of lung neutrophils decreased as dietary protein intake and tissue GSH content fell. However, in animals given an inflammatory challenge (endotoxin), liver and lung GSH concentrations increased directly in relation to dietary protein intake. Lung neutrophils, however, became related inversely with tissue GSH content. Addition of methionine to the protein-deficient diets normalized tissue GSH content and restored lung neutrophil numbers to those seen in unstressed animals fed a diet with adequate protein content (Fig. 10).

Table 5
Conditions Associated With Decreases in Glutathione Content of Tissues

Condition or disease	Tissue, fluid, or cell showing decrease in GSH content
Surgical stress	Skeletal muscle, plasma
HIV infection	PBMCs, lung lavage fluid
Sepsis	PBMCs, lung lavage fluid
Cirrhosis	Plasma, red blood cells
Ulcerative colitis	Colonic cells, red blood cells
Type 2 diabetes	Whole blood, red blood cells
Protein–energy malnutrition	Plasma, red blood cells
Old age	Plasma

GSH, glutathione; PBMCs, peripheral blood mononuclear cells; HIV, human immunodeficiency virus.

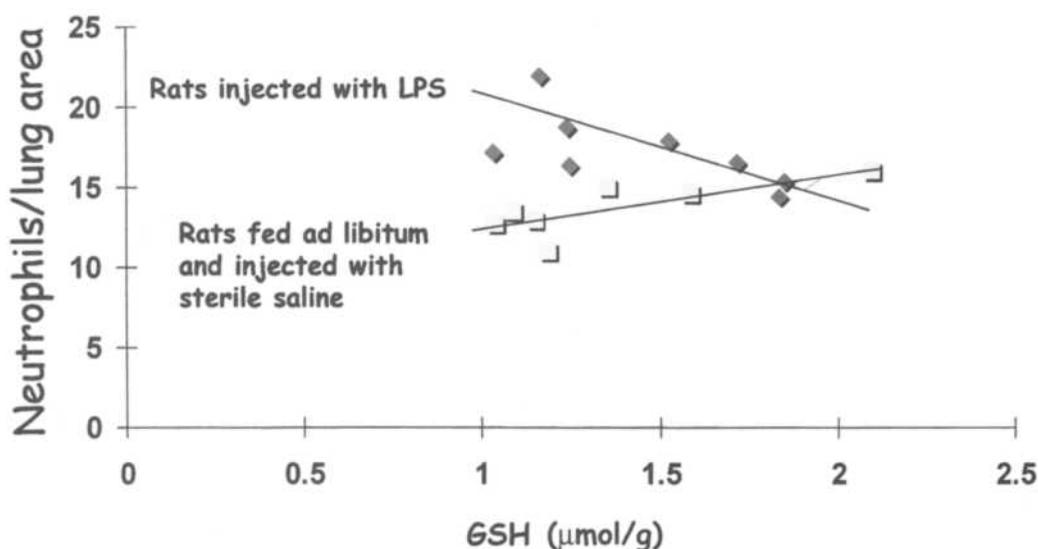


Fig. 10. The effect of dietary sulfur amino acid intake on lung neutrophil content in unstressed rats and stressed rats receiving an intraperitoneal injection of *E. coli* endotoxin.

Why does tissue GSH content have differing effects on immune cell populations depending on whether or not an inflammatory response is occurring? A partial explanation may come from an *in vitro* study using HeLa cells and cells from human embryonic kidney. In the study, both TNF- α and hydrogen peroxide resulted in activation of NF- κ B and AP1 (Wesselborg, Bauer, Vogt, Schmitz, & Schulze-Osthoff, 1997). Addition of the antioxidant sorbitol to the medium suppressed NF- κ B activation as expected, but unexpectedly activated AP1. Thus, the antioxidant environment of the cell might exert opposite effects upon transcription factors closely associated with inflammation (e.g., NF- κ B) and cellular proliferation (e.g., AP1). Evidence for this biphasic effect was seen when GSH was incubated with immune cells from young adults (Wu et al., 1994). A rise in cellular GSH content was accompanied by an increase in IL-2 production and lymphocyte proliferation (enhancement of T-cell function) and a decrease in production of the inflammatory mediators PGE2 and LTB4 (anti-inflammatory influence). Without doubt,

a decline in antioxidant status in the presence of oxidant stress will increase inflammatory stress. The interaction between oxidant stress and an impaired ability to synthesize GSH, a situation that stimulates inflammation, is clearly seen in cirrhosis, a disease that results in high levels of oxidative stress and an impaired ability to synthesize GSH (Pena, 1999). In Pena an inverse relationship between GSH concentration and the ability of monocytes to produce IL-1, IL-8, and TNF- α was observed. Treatment of cirrhotic patients with the procysteine increased monocyte GSH content and reduced IL-1, IL-8, and TNF- α production. Septic patients given an infusion of NAC (150 mg/kg bolus followed by infusion of 50 mg/kg over 4-h) showed a decrease in plasma IL-8 and soluble TNF receptor p55, had a reduced requirement for ventilator support, and spent 19 fewer days in intensive care than patients not receiving NAC (Spapen et al., 1998). De Rosa et al. (2000) showed that NAC was able to restore tissue GSH concentrations in individuals with HIV infection. In a study on HIV-positive patients, Brietkreutz et al. (2000) showed that a dose of 600 mg/d of NAC for 7 mo resulted in a decrease in plasma IL-6 (decreased inflammation), an increase in natural killer cell activity, and increased responsiveness of T lymphocytes to tetanus toxin stimulation (improved lymphocyte function).

Antioxidants might act to prevent NF- κ B activation by quenching oxidants. However, NF- κ B and AP1 may not respond to changes in cell redox state in the same way. When rats were subjected to depletion of effective tissue GSH pools by administration of diethyl maleate, there was a significant reduction in lymphocyte proliferation in spleen and mesenteric lymph nodes (Robinson et al., 1993). An increase in inflammatory stress would be expected in this study. Thus, it can be hypothesized that antioxidants exert an immunoenhancing effect by activating transcription factors that are strongly associated with cell proliferation (e.g., AP1) and an anti-inflammatory effect by preventing activation of NF- κ B by oxidants produced during the inflammatory response (Dröge et al., 1994). Thus, inclusion of antioxidants or substances that increase GSH synthesis in immunonutrient mixes would seem to be beneficial.

Improvement of antioxidant defenses is also possible by feeding other components of antioxidant defenses. Supplementation of the diet of healthy subjects and smokers with 600 IU/d α -tocopherol for 4 wk suppressed the ability of PBMCs to produce TNF- α (Mol, de Rijke, Demacher, & Stalenhoef, 1997). The same dose given to healthy elderly subjects for 235 d increased delayed-type hypersensitivity and raised antibody titers to hepatitis B (Meydani et al., 1997). An enteral feed enriched with vitamin E, vitamin C, and taurine given to intensive-care patients decreased total lymphocyte and neutrophil content in bronchioalveolar lavage fluid (decreased inflammation) and resulted in a reduction in organ failure rate, a reduced requirement for artificial ventilation, and a reduction of 5 d in intensive-care stay (Gadek et al., 1999).

5.2. Influence of Glutamine on Inflammation and Immune Function

A number of roles have been ascribed to glutamine as an immunonutrient: (a) as an essential nutrient for immune cells, (b) as an important modulator of gut barrier function, and (c) as a substrate for GSH synthesis. A number of reviews have been written about the first two of these roles (Newsholme, Crabtree, Salleh, & Ardawi, 1985; Elia, 1992); we will consider the last one here. Could glutamine be exerting an anti-inflammatory influence via an effect on GSH that enhances immune function? In a study in rats, glutamine supplementation resulted in an increased production of GSH by the gut (Cao, Feng, Hoos, & Klimberg, 1998), and total parenteral nutrition (TPN) with glutamine

raised plasma GSH concentrations in these animals (Denno, Rounds, Faris, Halejko, & Wilmore, 1996).

In randomized controlled trials the administration of glutamine, either as a dipeptide during TPN to surgical patients or as a glutamine-enriched enteral feed to trauma patients, resulted, respectively, in improved nitrogen retention (less tissue protein depletion) and a 6.2-d reduction in hospital stay, a concomitant suppression of the rise in plasma-soluble TNF receptors (reduced inflammation), and a lower incidence of bacteremia, pneumonia, and sepsis (improved immune function) (Houdijk et al., 1998; Morlion et al., 1998)

5.3. Dietary Intervention to Moderate Chronic Low-Grade Inflammation in the Elderly

In the previous section the influence of antioxidants on severe inflammation was considered. Do the general findings from this type of study also apply to modulation of low-grade chronic inflammation, such as has been observed in the elderly and obese?

Because aging is so closely associated with increased oxidative stress, which might both result from and contribute to a stimulation in the level of inflammation in the elderly, antioxidant therapy could produce beneficial effects. The effects would be seen in a decrease in oxidant damage, downregulation of inflammation, and, because of the inverse link between inflammation and immune function, an improvement in T-lymphocyte function. Meydani, Meydani, & Verdon (1986) reported that supplementation of aged mice (24 mo old) with dietary vitamin E (500 ppm) improved several indices of the immune system to levels comparable to those seen in young animals. Supplementation of aged mice with this vitamin also increased clearance of influenza virus from the lung to that observed in animals supplemented with other antioxidants such as melatonin, GSH, or strawberry extract, which contains a high level of flavonoids with antioxidant activity (Han et al., 2000). In a double-blind, placebo-controlled study, Meydani and colleagues (Meydani, Barklund, & Lui, 1990; Meydani et al., 1997) also reported that supplementation of elderly subjects with vitamin E for a short (1 mo) or long (4.5 mo) period of time also improved several *in vitro* and *in vivo* indices of immune response. The optimal immune response was observed with 200 IU of vitamin E per day in the long-term study. It is worth noting that this level of vitamin E has also been reported to be the optimal level for reducing plasma F₂-isoprostane, a reliable index of lipid peroxidation (Dillon, Vita, & Leeuwenburgh, 1998). Improving the immune response in the elderly may result in a lower incidence of infections, which are prevalent among the elderly, and thus may contribute to a longer and healthier life. Many observational and clinical trials have also indicated that a high intake or high plasma level of this vitamin is associated with a low risk of cardiovascular disease. The vitamin may be operating at two levels; first, by protecting LDL from peroxidation, thereby reducing its atherogenicity, and second, by lowering the level of chronic inflammation by downregulation of NF- κ B. A reduction in platelet aggregability may also arise out of this action (Huang et al., 2001; Tanus-Santos et al., 2002). Indeed, several lines of evidence indicated that supplements of vitamin E may prevent cardiovascular disease by reducing the susceptibility of LDLs to oxidation (Jailal, Fuller, & Huet, 1995), reducing the expression of chemokines, adhesion molecule expression, and monocyte adhesion (Wu, Koga, Martin, & Meydani, 1999), decreasing smooth muscle proliferation (Azzi, Boscoboinik, & Marilley, 1995), and decreasing platelet aggregation (Steiner 1999).

Another anti-inflammatory approach using nutrients would be to supplement diets of the elderly with *n*-3 PUFAs. Supplementation with *n*-3 PUFAs from fish oil, however, has been reported to suppress the immune response (Meydani, Endres, & Woods, 1991; Meydani, 1993), which hampers enthusiasm for the use of *n*-3 PUFAs for their benefits in CVD. However, the latter concern could be addressed by including a vitamin E supplement along with fish oil supplements. In a recent study it was found that supplementing elderly persons with (*n*-3) fatty acids of fish oil in combination with vitamin E while maintaining the anti-inflammatory properties of (*n*-3) PUFAs did not reduce immune indices in the elderly (Wu, Meydani, & Han, 2000).

6. INFLUENCE OF GENOTYPE ON RESPONSE TO NUTRIENTS

6.1. Fish Oil

Fish oil supplementation is not universally efficacious in the treatment of inflammatory disease (Grimble, 1998). Rheumatoid arthritis and inflammatory bowel disease have been the most successfully treated of all inflammatory diseases (Calder, 1997). The anti-inflammatory mechanism may be through suppression of TNF- α production. Endres et al. (1989) reported that large doses (15 g/d for 6 wk) of oil in nine healthy volunteers resulted in a small but statistically significant reduction in TNF- α and IL-1 β production from PBMCs. Subsequently, fewer than half of 11 similar small intervention studies were able to demonstrate a statistically significant reduction in cytokine production. To understand the differences in response more closely, the author's laboratory conducted a study on 111 young men fed 6 g fish oil daily for 12 wk and measured TNF- α production by PBMCs before and after supplementation in relation to the SNP at -308 in the TNF- α and at +252 in the LT- α genes. No significant effect of fish oil on cytokine production was noted in the group as a whole. However, when data were examined according to tertile of TNF- α production prior to supplementation, homozygosity for TNFB2 (LT- α +252 A) was 2.5 times more frequent in the highest than in the lowest tertile of production. The percentage of individuals in whom fish oil suppressed production was lowest (22%) in the lowest tertile and doubled with each ascending tertile. In the highest tertile, mean values were decreased by 43% ($p < 0.05$). In the lowest tertile, mean values were increased by 62% ($p < 0.05$). TNFB2 (LT- α +252 AA) homozygotes were strongly represented among unresponsive individuals in the lowest tertile of TNF- α production prior to supplementation. In this lowest tertile, only TNFB1/B2 (LT- α +252 GA) heterozygous subjects were responsive to the suppressive effects of fish oil. In the medium tertile, this genotype was six times more frequent than other LT- α genotypes among responsive individuals. No relationship between possession of TNF1 or 2 (TNF- α -308 G or A) alleles and responsiveness to fish oil was found. Clearly, although the level of inflammation determines whether fish oil will exert an anti-inflammatory influence or not and is influenced by the TNFB2 (LT- α +252 A) allele, the precise genomic mechanism for an anti-inflammatory effect is unclear at present (Grimble et al., 2002).

6.2. Vitamin E

Antioxidant intake also modifies cytokine production. In a study on healthy men and women and smokers, dietary supplementation with 600 IU/d α -tocopherol for 1 mo suppressed the ability of PBMCs to produce TNF- α . Production was reduced by 22 and

33% in nonsmokers and smokers, respectively (Mol et al., 1997). In a similar dietary intervention study on normolipidaemic and hypertriglyceridaemic subjects given 600 IU/d of α -tocopherol for 6 wk, reduced TNF- α , IL-1 β , and IL-8 production by LPS-stimulated blood mononuclear cells occurred (Mol et al., 1997; van Tits, Demacker, de Graaf, Hak-Lemmers, & Stalenhoef, 2000). A similar effect of α -tocopherol was noted in a study on normal subjects and type 2 diabetics (Devaraj & Jialal, 2000). However, there were large standard deviations in the data from these studies, indicating major intraindividual variability in the ability of vitamin E to suppress production of the cytokine. Although a number of studies have shown that α -tocopherol suppresses superoxide production, the situation with regard to nitric oxide is less clear (Mol et al., 1997; van Tits et al., 2000). The α -tocopherol derivative pentamethyl-hydroxychromane inhibited LPS-stimulated NF- κ B and iNOS activation in cultured J774 macrophages (Hattori, 1995). At present it is not known whether antioxidants interact differently with SNPs in the genes associated with oxidant stress and inflammation than they do with the other anti-inflammatory nutrient, *n*-3 PUFA. This topic is currently an area of active research at the author's laboratory.

Proteomic studies have shown that iNOS and SOD are both influenced by the NRAMP1 gene (Kovorova, Necasova, Porkertova, Radzoich, & Macela, 2001). The production of oxidant molecules enhancing pro-inflammatory cytokine production via high levels of NF- κ B activation may thus be under a genomic influence owing to the aforementioned variations in the NRAMP1 gene (Formica, Roach, & Blackwell, 1994). A better understanding of this interaction and of the interaction of *n*-3 PUFAs and antioxidants with genotype may allow the better design of nutrient products for the treatment of inflammatory disease.

7. CONCLUSIONS

It is clear from the current understanding about the purpose and functioning of the immune system throughout the life cycle that it is a powerful biological entity that profoundly alters body function while it is carrying out its prime purpose of defending the body against invasion by pathogens. However, within the response lie the seeds of disaster at an individual level, for the inflammatory component of the response can turn against the body, particularly as the body ages or becomes obese. The response, which can be devastating when directed against microbes entering the body, also sows the seeds of atherosclerosis, degradation of brain function, and insulin insensitivity and hastens the passage of HIV-infected individuals towards full-blown AIDS. Along with the insights arising from the unraveling of the human genome has come evidence that the inflammatory response is able to protect the human species from invasion by pathogens but not all individuals within the species from ill health. The differing ability of humans, particularly the male of the species, at an individual level to mount an inflammatory response of different levels of intensity owing to genotype can result in widely contrasting outcomes of invasion of the body by pathogens. On the one hand, individuals may effectively fight off invasion provided the immune response follows a normal pathway, whereas other individuals within the same community encountering the same pathogens will die from the strength and nature of the response rather than from the direct effects of the invader. Insights gained from the genomic influences on cytokine production and the response to malaria suggest that the retention of alleles in pro-inflammatory cytokine

genes that resulted in enhanced cytokine production within the human gene pool over generations could be a result of the heterozygotes' better capacity for fighting pathogens, whereas homozygotes of the high-producing genotype run an increased risk of a strong adverse inflammatory response. In the case of sickle cell anemia, where heterozygotic individuals reap an advantage in resistance to malaria by possession of only one copy of the anemia allele, homozygous individuals for the sickle cell trait pay the price for possession of two copies of the allele and die young. Because of the overall advantage of this situation to the species, the potentially disadvantageous allele will be retained within the human gene pool over generations.

With the twin discoveries that nutrients can modulate the inflammatory response and that cytokine genotype can modulate the effectiveness of nutrients in controlling inflammation, nutritional science sits at an exciting moment in its development. The mapping of how pro- and anti-inflammatory cytokine genotypes interact with responsiveness to immunonutrients at an individual level will allow tailor-made nutritional treatments of all diseases that have an underlying inflammatory basis. Furthermore, a better understanding of how nutritional therapies and genetics interact will allow the twin adverse biological factors increasing the level of inflammatory stress in populations—obesity and aging—to be tackled by targeted nutritional therapy.

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REFERENCES

- Ables, G. P., Takamatsu, D., Noma, H., El-Shazly, S., Jin, H. K., Taniguchi, T., Sekikawa, K., & Watanabe, T. (2001). The roles of Nramp1 and Tnfr α genes in nitric oxide production and their effect on the growth of *Salmonella typhimurium* in macrophages from Nramp1 congenic and tumor necrosis factor- α -/- mice. *Journal of Interferon Cytokine Research*, 21, 53–62.
- Allen, R. D. (1999). Polymorphism of the human TNF- α promoter—random variation or functional diversity? *Molecular Immunology*, 36, 1017–1027.
- Armitage, G. C. (2000). Periodontal infections and cardiovascular disease—how strong is the association? *Oral Disease*, 6, 335–350.
- Arnalich, F., Garcia-Palomero, E., Lopez, J., Jiminez, M., Madero, R., Renart, J., Vazquez, J. J., & Montiel, C. (2000). Predictive value of nuclear factor kappaB activity and plasma cytokine levels in patients with sepsis. *Infection and Immunity*, 68, 1942–1945.
- Azzi, A., Boscoboinik, D., & Marilley, D. (1995). Vitamin E: A sensor and an information transducer of the cell oxidation state. *American Journal of Clinical Nutrition*, 62 (6 Suppl.), 1337S–1346S.
- Baggio, G., Donnazzan, S., Monti, D., & Mari, D. (1998). Lipoprotein(a) and lipoprotein profile in healthy centenarians: A reappraisal of vascular risk factors. *FASEB Journal*, 12, 433–437.
- Beale, R. J., Bryg, D. J., & Bihari, D. J. (1999). Immunonutrition in the critically ill: A systematic review of clinical outcome. *Critical Care Medicine*, 27, 2799–2805.
- Bell, D. S. (2000). Inflammation, insulin resistance, infection, diabetes, and atherosclerosis. *Endocrinological Practice*, 6, 272–276.
- Bethin, K. E., Vogt, S. K., & Muglia, L. J. (2000). Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. *Proceedings of the National Academy of Science USA*, 97, 9317–9322.
- Bidwell, J., Keen, L., Gallagher, G., Kimberly, R., Huizinga, T., McDermott, M. F., Oksenberg, J., McNicholl, J., Pociot, F., Hardt, C., & D'Alfonso, S. (2001). Cytokine gene polymorphism in human disease: Online databases, supplement 1. *Genes and Immunology*, 2, 61–70.

- Bonafè, M., Olivieri, F., & Cavallone, L. (2001). A gender-dependent genetic predisposition to produce high levels of IL-6 is detrimental to longevity. *European Journal of Immunology*, *31*, 2357–2361.
- Bonora, E., Kiechl, S., & Oberhollenzer, E. (2000). Impaired glucose tolerance, type 2 diabetes mellitus and carotid atherosclerosis: Prospective results of the Bruneck Study. *Diabetologia*, *43*, 156–164.
- Boya, P., de la Pena, A., Beloqui, O., Larrea, E., Conchillo, M., Castelruiz, Y., Civeira, M. P., & Prieto, J. (1999). Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis C. *Journal of Hepatology*, *31*, 808–814.
- Breitkreutz, R., Pittack, N., Nebe, C. T., Schuster, D., Brust, J., Beichert, M., Hack, V., Daniel, V., Edler, L., & Droge, W. (2000). Improvement of immune functions in HIV infection by sulfur supplementation: Two randomized trials. *Journal of Molecular Medicine*, *78*, 55–62.
- Bryson, B. (2003). In *A short history of nearly everything*. New York: Doubleday.
- Calder, P. C. (1997). *n*-3 Polyunsaturated fatty acids and cytokine production in health and disease. *Annals of Nutrition and Metabolism*, *41*, 203–234.
- Cao, Y., Feng, Z., Hoos, A., & Klimberg, V. S. (1998). Glutamine enhances gut glutathione production. *Journal of Parenteral and Enteral Nutrition*, *22*, 224–227.
- Caruso, C., Candore, G., Romano, G. C., Lio, D., Bonafe, M., Valensin, S., & Franceschi, C. (2001). Immunogenetics of longevity. Is major histocompatibility complex polymorphism relevant to the control of human longevity? A review of literature data. *Mechanisms of Ageing and Development*, *122*, 445–462.
- Chambers, J. C., Eda, S., & Bassett, P. (2001). C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation*, *104*, 145–150.
- Chernoff, A. E., Granowitz, E. V., Shapiro, L., Vannier, E., Lonnemann, G., Angel, J. B., Kennedy, J. S., Rabson, A. R., Wolff, S. M., & Dinarello, C. A. (1995). A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. *Journal of Immunology*, *154*, 5492–5499.
- Chorazy, P. A., Schumacher, H. R., Jr., & Edlind, T. D. (1992). Glutathione peroxidase in rheumatoid arthritis: Analysis of enzyme activity and DNA polymorphism. *DNA Cell Biology*, *11*, 221–225.
- Choi, S. S., Gatanaga, M., Granger, G. A., & Gatanaga, T. (1996). Prostaglandin-E2 regulation of tumor necrosis factor receptor release in human monocytic THP-1 cells. *Cellular Immunology*, *170*, 178–184.
- Chu, N. F., Spiegelman, D., & Hotamisligil, G. S. (2001). Plasma insulin, leptin, and soluble TNF receptors levels in relation to obesity-related atherogenic and thrombogenic cardiovascular disease risk factors among men. *Atherosclerosis*, *157*, 495–503.
- Cnop, M., Landchild, M. J., & Vidal, J. (2002). The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: Distinct metabolic effects of two fat compartments. *Diabetes*, *51*, 1005–1015.
- Corry, D. B., & Tuck, M. L. (2001). Selective aspects of the insulin resistance syndrome. *Current Opinion in Nephrology and Hypertension*, *10*, 507–514.
- Debril, M. B., Renaud, J. P., Fajas, L., & Auwerx, J. (2001). The pleiotropic functions of peroxisome proliferator-activated receptor gamma. *Journal of Molecular Medicine*, *79*, 30–47.
- Denno, R., Rounds, J. D., Faris, R., Halejko, L. B., & Wilmore, D. W. (1996). Glutamine enriched TPN enhances plasma glutathione in resting state. *Journal of Surgical Research*, *61*, 35–38.
- De Rosa, S. C., Zaretsky, M. D., Dubs, J. D., Roederer, M., Anderson, M., Green, A., Mitra, D., Watanabe, N., Nakamura, H., Tjioe, I., Deresinski, S. C., Moore, W. A., Ela, S. W., Parks, D., Herzenberg, L. A., & Herzenberg, L. A. (2000). *N*-Acetylcysteine replenishes glutathione in HIV infection. *European Journal of Clinical Investigation*, *30*, 915–929.
- Devaraj, S., & Jialal, I. (2000). Low-density lipoprotein postsecretory modification, monocyte function, and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: The effect of alpha-tocopherol supplementation. *Circulation*, *102*, 191–196.
- Diamond J. (1999). In *Guns, germs and steel: The fate of human societies*. New York: W. W. Norton & Co.
- Dillon, G., Vita, J. A., & Leeuwenburgh, C. (1998). α -Tocopherol supplementation reduces systemic markers of oxidative damage in healthy adults. *Circulation*, *17S*, 6711.
- Dinarello, C. A., Bishai, I., Rosenwasser, L. J., & Coceani, F. (1984). The influence of lipoxygenase inhibitors on the in vitro production of human leukocytic pyrogen and lymphocyte activating factor (interleukin-1). *International Journal of Immunopharmacology*, *6*, 43–50.

- Dröge, W., Schulze-Osthoff, K., Mihm, S., Galter, D., Schenk, H., Eck, H. P., Roth, S., & Gmünder, H. (1994). Functions of glutathione and glutathione disulphide in immunology and immunopathology. *FASEB Journal*, 8, 1131–1138.
- Duncan, B. B., & Schmidt, M. I. (2001). Chronic activation of the innate immune system may underlie the metabolic syndrome. *Sao Paulo Medical Journal*, 119, 122–127.
- Elia, M. (1992). Glutamine in parenteral nutrition. *International Journal of Food Science and Nutrition*, 43, 47–49.
- Emanuelli, B., Peraldi, P., & Filloux, C. (2001). SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *Journal of Biological Chemistry*, 276, 47,944–47,949.
- Endres, S., Ghorbani, R., Kelley, V. E., Georgilis, K., Lonnemann, G., van der Meer, J. W. M., Cannon, J. G., Rogers, T. S., Klempner, M. S., Weber, P. C., Schaefer, E. J., Wolff, S. M., & Dinarello, C. A. (1989). The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *New England Journal of Medicine*, 320, 265–271.
- Ershler, W. B., & Kerler, E. T. (2000). Age-associated increased interleukin-6 gene expression, late diseases, and frailty. *Annual Reviews of Medicine*, 51, 245–270.
- Espevik, T., Figari, I. S., Shalaby, M. R., Lackides, G. A., Lewis, G. D., Shepard, H. M., & Palladino, M. A., Jr. (1987). Inhibition of cytokine production by cyclosporin A and transforming growth factor beta. *Journal of Experimental Medicine*, 166, 571–576.
- Faggioni, R., Feingold, K. R., & Grunfeld, C. (2001). Leptin regulation of the immune response and the immunodeficiency of malnutrition. *FASEB Journal*, 15, 2565–2571.
- Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C., & Paganelli, R. (1993). Increased cytokine production in mononuclear cells of healthy elderly people. *European Journal of Immunology*, 23, 2375–2378.
- Ferrucci, L., Harris, T. B., Guralnik, J. M., & Tracy, R. P. (1999). Serum IL-6 level and the development of disability in older persons. *Journal of the American Geriatric Society*, 47, 639–646.
- Festa, A., D'Agostino, R., Jr., & Williams, K. (2001). The relation of body fat mass and distribution to markers of chronic inflammation. *International Journal of Obesity and Related Metabolic Disorders*, 25, 1407–1415.
- Folsom, A. R., Pankow, J. S., & Tracy, R. P. (2001). The Investigators of the NHBLI Family Heart Study. Association of C-reactive protein with markers of prevalent atherosclerotic disease. *American Journal of Cardiology*, 88, 112–117.
- Formica, S., Roach, T. I., & Blackwell, J. M. (1994). Interaction with extracellular matrix proteins influences Lsh/Ity/Bcg (candidate Nramp) gene regulation of macrophage priming/activation for tumour necrosis factor-alpha and nitrite release. *Immunology*, 82, 42–50.
- Forsberg, L., Lyrenas, L., de Faire, U., & Morgenstern, R. (2001). A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radical Biology and Medicine*, 30, 500–505.
- Franceschi, C., Motta, L., Valensin, S., Rapisarda, R., Franzone, A., Berardelli, M., Motta, M., Monti, D., Bonafe, M., Ferrucci, L., Deiana, L., Pes, G. M., Carru, C., Desole, M. S., Barbi, C., Sartoni, G., Gemelli, C., Lescai, F., Olivieri, F., Marchegiani, F., et al. (2000). Do men and women follow different trajectories to reach extreme longevity? Italian Multicenter Study on Centenarians (IMUSCE). *Aging (Milano)*, 12, 77–84.
- Fruchart, J. C., Staels, B., & Duriez, P. (2001). PPARs, metabolic disease and atherosclerosis. *Pharmacological Research*, 44, 345–352.
- Gadek, J. E., De Michele, S. J., Karlstad, M. D., Pacht, E. R., Donahoe, M., Albertson, T. E., Van Hoozen, C., Wennberg, A. K., Nelson, J. L., Nourselehi, M., & The Enteral Nutrition in ARDS Study Group. (1999). Effect of enteral feeding with eicosapentaenoic acid, γ -linolenic acid, and antioxidants in patients with acute respiratory distress syndrome. *Critical Care Medicine*, 27, 1409–1420.
- Grau, A. J., Aulmann, M., Lichy, C., Meiser, H., Buggle, F., Brandt, T., & Grond-Ginsbach, C. (2001). Increased cytokine release by leucocytes in survivors of stroke at young age. *European Journal of Clinical Investigation*, 31, 999–1006.
- Grimble, R. (1990). Serum albumin and mortality. *Lancet*, 1, 348.

- Grimble, R. F. (1996). Theory and efficacy of antioxidant therapy. *Current Opinion in Critical Care*, 2, 260–266.
- Grimble, R. F. (1997). Effect of antioxidative vitamins on immune function with clinical applications. *International Journal of Vitamin Nutrition Research*, 67, 312–320.
- Grimble, R. F. (1998). Dietary lipids and the inflammatory response. *Proceedings of the Nutrition Society*, 57, 535–542.
- Grimble, R. F. (2001a). Nutritional modulation of immune function. *Proceedings of the Nutrition Society*, 60, 389–397.
- Grimble, R. (2001b). Stress proteins in disease: Metabolism on a knife edge. *Clinical Nutrition*, 20, 469–476.
- Grimble, R. F. (2002). Inflammatory status and insulin resistance. *Current Opinion in Clinical Nutrition and Metabolic Care*, 5, 551–559.
- Grimble, R. F. (2003). Inflammatory response in the elderly. *Current Opinion in Clinical Nutrition and Metabolic Care*, 6, 21–29.
- Grimble, R. F., Andersson, P., Madden, J., Palmblad, J., Persson, M., Vedin, I., & Cederholm, T. (2003). Gene:gene interactions influence the outcome in elderly patients. *Clinical Nutrition*, 22, S39.
- Grimble, R. F., Howell, W. M., O'Reilly, G., Turner, S. J., Markovic, O., Hirrell, S., East, J. M., & Calder, P. C. (2002). The ability of fish oil to suppress tumor necrosis factor-alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes which influence TNF-alpha production. *American Journal of Clinical Nutrition*, 76, 454–459.
- Grimble, R. F., Thorell, A., Nygren, J., Ljungqvist, O., Barber, N., Grant, S., & Madden, J. (2003). Cytokine genotype and gender influence the inflammatory response to surgery. *Clinical Nutrition*, 22, S45.
- Hajeer, A. H., Lazarus, M., & Turner, D. (1998). IL-10 gene promoter polymorphisms in rheumatoid arthritis. *Scandinavian Journal of Rheumatology*, 27, 142–145.
- Hak, A. E., Pols, H. A., & Stehouwer, C. D. (2001). Markers of inflammation and cellular adhesion molecules in relation to insulin resistance in nondiabetic elderly: The Rotterdam study. *Journal of Clinical Endocrinology and Metabolism*, 86, 4398–4405.
- Han, S. N., Meydani, M., Wu, D., Bender, B. S., Smith, D. E., Vina, J., Cao, G. P., & Meydani, S. N. (2000). Effect of long-term dietary antioxidant supplementation on influenza virus infection. *Journal of Gerontology Biological Sciences and Medical Sciences*, 55, B496–503.
- Harris, T. B., Ferrucci, T., Tracy, R. P., & Corti, M. (1999). Associations between elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *American Journal of Medicine*, 106, 506–512.
- Hattori, S., Hattori, Y., Banba, N., Kasai, K., & Shimoda S. (1995). Pentamethyl-hydroxychromane, vitamin E derivative, inhibits induction of nitric oxide synthase by bacterial lipopolysaccharide. *Biochemistry and Molecular Biology International*, 35, 177–183.
- Hegazy, D. M., O'Reilly, D. A., & Yang, B. M. (2001). NFkappaB polymorphisms and susceptibility to type 1 diabetes. *Genes and Immunology*, 2, 304–308.
- Hotamisligil, G. S., Budavari, A., Murray, D., & Spiegelman, B. M. I. (1994). Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. *Journal of Clinical Investigation*, 94, 1543–1549.
- Hotamisligil, G. S., Peraldi, P., Budavari, A., Ellis, R., White, M. F., & Spiegelman, B. M. I. (1996). IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science*, 271, 665–668.
- Hotamisligil, G. S., & Spiegelman, B. M. I. (1994). Tumor necrosis factor alpha: A key component of the obesity-diabetes link. *Diabetes*, 43, 1271–1278.
- Houdijk, A. P. J., Rijnsburger, E. R., Jansen, J., Wesdorp, R. I. C., Weiss, J. K., McCamish, M. A., Teerlink, T., Meuwissen, S. G. M., Haarman, H. J. ThM., Thijs, L. G., & Van Leeuwen, P. A. M. (1998). Randomised trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma. *Lancet*, 352, 772–776.
- Huang, Y. C., Guh, J. H., Cheng, Z. J., Chang, Y. L., Hwang, T. L., Liao, C. H., Tzeng, C. H., & Teng, C. M. (2001). Inhibition of the expression of inducible nitric oxide synthase and cyclooxygenase-2 in macrophages in macrophages by & HQ derivatives: Involvement of IkappaB-alpha stabilisation. *European Journal of Pharmacology*, 418, 133–139.
- Huizinga, T. W., Keijsers, V., & Yanni, G. (2002). Are differences in interleukin 10 production associated with joint damage? *Rheumatology*, 39, 1180–1188.
- Hunter, E. A. L., & Grimble, R. F. (1994). Cysteine and methionine supplementation modulate the effect of tumor necrosis factor alpha on protein synthesis, glutathione and zinc content of tissues in rats fed a low-protein diet. *Journal of Nutrition*, 124, 2319–2328.

- Hutchinson, I. V., Pravica, V., Hajeer, A., & Sinnott, P. J. (1999). Identification of high and low responders to allographs. *Review in Immunogenetics, 1*, 323–333.
- Iwamoto, Y., Nishimura, F., & Nakagawa, M. (2001). The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. *Journal of Periodontology, 72*, 774–778.
- Jacob, C. O., Franek, Z., Lewis, G. D., Koo, M., Hansen, J. A., & McDevitt, H. O. (1990). Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor- α : Relevance to genetic predisposition to systemic lupus erythematosus. *Proceedings of the National Academy of Science, 87*, 1233–1237.
- Jacob, R. A., Kelley, D. S., Pianalto, F. S., Swendseid, M. E., Henning, S. M., Zhang, J. Z., Ames, B. N., Fraga, C. G., & Peters, J. H. (1991). Immunocompetence and oxidant defense during ascorbate depletion of healthy men. *American Journal of Clinical Nutrition, 54*, 1302S–1309S.
- Jersmann, H. P., Hii, C. S., Ferrante, J. V., & Ferrante, A. (2001). Bacterial lipopolysaccharide and tumor necrosis factor alpha synergistically increase expression of human endothelial adhesion molecules through activation of NF-kappaB and p38 mitogen-activated protein kinase signaling pathways. *Infection and Immunology, 69*, 1273–1279.
- Jialal, I., Fuller, C. J., & Huet, B. A. (1995). The effect of alpha-tocopherol supplementation on LDL oxidation. *Arteriosclerosis, Thrombosis and Vascular Biology, 15*, 190–198.
- Jonkers, I. J., Mohrschladt, M. F., & Westendorp, R. G. (2002). Severe hypertriglyceridemia with insulin resistance is associated with systemic inflammation: Reversal with bezafibrate therapy in a randomized controlled trial. *American Journal of Medicine, 112*, 275–280.
- Kol, A., Sukhova, G. K., Lichtman, A. H., & Libby, P. (1998). Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor- α and matrix metalloproteinase expression. *Circulation, 98*, 300–307.
- Kovarova, H., Necasova, R., Porkertova, S., Radzioch, D., & Macela, A. (2001). A Natural resistance to intracellular pathogens: Modulation of macrophage signal transduction related to the expression of the Bcg locus. *Proteomics, 1*, 587–596.
- Kudoh, A., Katagai, H., Takazawa, T., & Matsuki, A. (2001). Plasma proinflammatory cytokine response to surgical stress in elderly patients. *Cytokine, 15*, 270–273.
- Kunkel, S. L., Remick, D. G., Spengler, M., & Chensue, S. W. (1987). Modulation of macrophage-derived interleukin-1 and tumour necrosis factor by prostaglandin E₂. *Advances in Prostaglandin, Leukotriene and Thromboxane Research, 17A*, 155–156.
- Lio, D., Scola, L., Crivello, A., Bonafe, M., Franceschi, C., Olivieri, F., Colonna Romano, G., Candore, G., & Caruso, C. (2002). Allele frequencies of +874TgA single nucleotide polymorphism at the first intron of interferon-gamma gene in a group of Italian centenarians. *Experimental Gerontology, 37*, 315–319.
- Loguercio, C., Blanco, F. D., De Girolamo, V., Disalvo, D., Nardi, G., Parente, A., & Blanco, C. D. (1999). Ethanol consumption, amino acid and glutathione blood levels in patients with and without chronic liver disease. *Alcohol Clinical and Experimental Research, 23*, 1780–1784.
- Luo, J. L., Hammarqvist, F., Andersson, K., & Wernerman, J. (1996). Skeletal muscle glutathione after surgical trauma. *Annals of Surgery, 223*, 420–427.
- Martin, G. M. (1997). Genetics and the pathobiology of ageing. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 352*, 1773–1780.
- Marwick, C. (1997). NHANES III health data relevant for aging nation. *Journal of the American Medical Association, 277*, 100–102.
- McGuire, W., Hill, A. V., Allsopp, C. E., Greenwood, B. M., & Kwiatkowski, D. (1994). Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature, 371*, 508–510.
- Messer, G., Spengler, U., Jung, M. C., Honold, G., Blomer, K., Pape, G. R., Riethmuller, G., & Weiss, E. H. (1991). Polymorphic structure of the tumour necrosis factor (TNF) locus: An Nco I polymorphism in the first intron of the human TNF- β gene correlates with a variant amino acid in position 26 and a reduced level of TNF α production. *Journal of Experimental Medicine, 173*, 209–219.
- Meydani, S. N., Barklund, P. M., & Liu, S. (1990). Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *American Journal of Clinical Nutrition, 52*, 557–563.
- Meydani, S. N., Endres, S., & Woods, M. N. (1991). Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: Comparison between young and older women. *Journal of Nutrition, 121*, 547–555.

- Meydani, S. N., Lichenstein, A. H., & Cornwall, S. (1993). Immunological effects of national cholesterol education panel (NCEP) step-2 diets with and without fish-derived (n-3) fatty acid enrichment. *Journal of Clinical Investigation*, *92*, 105–113.
- Meydani, S. N., Meydani, M., Blumberg, J. B., Leka, L. S., Silber, G., Loszewski, R., Thompson, C., Pedrosa, R. D., Diamond, D., & Stoller, D. (1997). Vitamin E supplementation and in vivo immune response in healthy subjects. A randomized controlled trial. *Journal of the American Medical Association*, *277*, 1370–1386.
- Meydani, S. N., Meydani, M., & Verdon, C. P. (1986). Vitamin E supplementation suppresses prostaglandin E2 synthesis and enhances the immune response of aged mice. *Mechanisms of Ageing and Development*, *34*, 191–201.
- Micke, P., Beeh, K. M., Schlaak, J. F., & Buhl, R. (2001). Oral supplementation with whey proteins increases plasma glutathione levels in HIV-infected patients. *European Journal of Clinical Investigation*, *31*, 171–178.
- Mira, J. P., Cariou, A., Grall, F., Delclaux, C., Losser, M. R., Heshmati, F., Cheval, C., Monchi, M., Teboul, J. L., Riche, F., Leleu, G., Arbibe, L., Mignon, A., Delpech, M., & Dhainaut, J. F. (1999). Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: A multicenter study. *Journal of the American Medical Association*, *282*, 561–568.
- Mitrunen, K., Sillanpaa, P., Kataja, V., Eskelinen, M., Kosma, V. M., Benhamou, S., Uusitupa, M., & Hirvonen, A. (2001). Association between manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk. *Carcinogenesis*, *22*, 827–829.
- Mol, J. T. M., de Rijke, Y. B., Demacher, P. M. N., & Stalenhoef, A. F. H. (1997). Plasma levels of lipid and cholesterol oxidation products and cytokines in diabetes mellitus and smokers: Effect of vitamin E treatment. *Atherosclerosis*, *129*, 169–176.
- Moller, D. E. (2000). Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinology Metabolism*, *11*, 212–217.
- Momoi, A., Murao, K., & Imachi, H. (2001). Inhibition of monocyte chemoattractant protein-1 expression in cytokine-treated human lung epithelial cells by thiazolidinedione. *Chest*, *120*, 1293–1300.
- Morlion, B. J., Stehle, P., Wachtler, P., Siedhoff, H-P., Koller, M., Konig, W., Furst, P., & Puchstein, C. (1998). Total parenteral nutrition with glutamine dipeptide after major surgery. A double blind controlled study. *Annals of Surgery*, *227*, 302–308.
- Munro, R., & Capell, H. (1997). Prevalence of low body mass in rheumatoid arthritis: Association with the acute phase response. *Annals of Rheumatic Disease*, *56*, 326–369.
- Mysliwska, J., Bryl, E., Foerster, A., & Mysliwski, A. (1998). Increase in IL-6 and decrease of interleukin 2 production during the ageing process are influenced by health status. *Mechanisms of Ageing and Development*, *100*, 313–328.
- Newsholme, E. A., Crabtree, B., Salleh, M., & Ardawi, M. (1985). Glutamine metabolism in lymphocytes. Its biochemistry, physiology and clinical importance. *Quarterly Journal of Experimental Physiology*, *70*, 473–489.
- Nilsson, J., Jovinge, S., Niemann, A., Reneland, R., & Lithell, H. (1998). Relation between plasma tumor necrosis factor-alpha and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. *Arteriosclerosis, Thrombosis and Vascular Biology*, *18*, 1199–1202.
- Nishimura, F., & Murayama, Y. (2001). Periodontal inflammation and insulin resistance—lessons from obesity. *Journal of Dental Research*, *80*, 1690–1694.
- Nuttall, S. L., Dunne, F., Kendall, M. J., & Martin, U. (1999). Age-dependent oxidative stress in elderly patients with non-insulin-dependent diabetes mellitus. *Quarterly Journal of Medicine*, *92*, 33–38.
- Onat, A., Sansoy, V., & Yildirim, B. (2001). C-reactive protein and coronary heart disease in western Turkey. *American Journal of Cardiology*, *88*, 601–607.
- Ono, S., Aosasa, S., Tsujimoto, H., Ueno, C., & Mochizuki, H. (2001). Increased monocyte activation in elderly patients after surgical stress. *European Journal of Surgical Research*, *33*, 33–38.
- Pannacciulli, N., Cantatore, F. P., & Minenna, A. (2001). C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. *International Journal of Obesity and Related Metabolic Disorders*, *25*, 1416–1420.
- Paolini-Giacobino, A. A., Grimble, R., & Pichard, C. (2003). Genomic interactions with disease and nutrition. *Clinical Nutrition*, *22*, 507–514.

- Pena, L. R., Hill, D. B., & McClain, C. J. (1999). Treatment with glutathione precursor decreases cytokine activity. *Journal of Parenteral and Enteral Nutrition*, 23, 1–6.
- Perrey, C., Pravica, V., Sinnott, P. J., & Hutchinson, I. V. (1998). Genotyping for polymorphisms in interferon-gamma, interleukin-10, transforming growth factor-beta 1 and tumour necrosis factor-alpha genes: a technical report. *Transplantation Immunology*, 6, 193–197.
- Pfeilschifter, J., Koditz, R., Pfohl, M., & Schatz, H. (2002). Changes in proinflammatory cytokine activity after menopause. *Endocrinological Reviews*, 23, 90–119.
- Pradhan, A. D., Manson, J. E., Rifai, N. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association*, 286, 327–334.
- Prio, T. K., Bruunsgaard, H., Roge, B., & Pedersen, B. K. (2002). Asymptomatic bacteriuria in elderly humans is associated with increased levels of circulating TNF receptors and elevated numbers of neutrophils. *Experimental Gerontology*, 37, 693–699.
- Reid, C. L., Hutchinson, I. V., Campbell, I. T., & Little, R. A. (1999). Genetic variation in cytokine production may be protective of ICU admission and may influence mortality. *Clinical Nutrition*, 18, 45.
- Reid, M., Badaloo, A., Forrester, T., Morlese, J. F., Frazer, M., Heird, W. C., & Jahoor, F. (2000). In vivo rates of erythrocyte glutathione synthesis in children with severe protein-energy malnutrition. *American Journal of Physiology*, 278, E405–412.
- Rink, L., Cakman, I., & Kirchner, H. (1998). Altered cytokine production in the elderly. *Mechanisms of Ageing and Development*, 102, 199–209.
- Robinson, M. K., Rodrick, M. L., Jacobs, D. O., Rounds, J. D., Collins, K. H., Saporoschetz, I. B., Mannick, J. A., & Wilmore, D. W. (1993). Glutathione depletion in rats impairs T-cell and macrophage immune function. *Archives of Surgery*, 128, 29–34.
- Rohleder, N., Kudielka, B. M., Hellhammer, D. H., Wolf, J. M., & Kirschbaum, C. (2002). Age and sex steroid-related changes in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Journal of Neuroimmunology*, 126, 69–77.
- Romano, M., Pomilio, M., & Vigneri, S. (2001). Endothelial perturbation in children and adolescents with type 1 diabetes: Association with markers of the inflammatory reaction. *Diabetes Care*, 24, 1674–1678.
- Rosenberg, I. H. (1989). Epidemiologic and methodologic problems in determining nutritional status of older persons. *American Journal of Clinical Nutrition*, 50 (Suppl.), 1231–1233.
- Ross, R. (1993). The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature*, 362, 801–809.
- Roubenoff, R. (2001). Origins and clinical relevance of sarcopenia. *Canadian Journal of Applied Physiology*, 26, 78–89.
- Sakao, S., Tatsumi, K., Igari, H., Shino, Y., Shirasawa, H., & Kuriyama, T. (2001). Association of tumor necrosis factor alpha gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, 163, 420–422.
- Saldeen, T. G., & Rand, K. (1998). Interactive role of infection, inflammation and traditional risk factors in atherosclerosis and coronary artery disease. *Journal of the American College of Cardiology*, 31, 1217–1225.
- Schreck, R., Rieber, P., & Baeuerle, P. A. (1991). Reactive oxygen intermediates as apparently widely used messengers in the activation of nuclear transcription factor- κ B and HIV-1. *EMBO Journal*, 10, 2247–2256.
- Schaaf, B. M., Seitzer, U., Pravica, V., Aries, S. P., & Zabel, P. (2001). Tumor necrosis factor-alpha –308 promoter gene polymorphism and increased tumor necrosis factor serum bioactivity in farmer's lung patients. *American Journal of Respiratory and Critical Care Medicine*, 163, 379–382.
- Schroder, J., Kahlke, V., Book, M., & Stuber, F. (2000). Gender differences in sepsis: Genetically determined? *Shock*, 14, 307–310.
- Searle, S., & Blackwell, J. M. (1999). Evidence for a functional repeat polymorphism in the promoter of the human NRAMPI gene that correlates with autoimmune versus infectious disease susceptibility. *Journal of Medical Genetics*, 36, 295–299.
- Shurtz-Swirski, R., Sela, S., & Herskovits, A. T. (2001). Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. *Diabetes Care*, 24, 104–110.
- Spapen, H., Zhang, H., Demanet, C., Vleminckx, W., Vincent, J.L., & Huyghens, L. (1998). Does N-acetyl cysteine influence cytokine response during early human septic shock? *Chest*, 113, 1616–1624.
- Spitzer, J. J., Bagby, G. J., Meszaros, K., & Lang, C. H. (1988). Alterations in lipid and carbohydrate metabolism in sepsis. *Journal of Parenteral and Enteral Nutrition*, 12 (Suppl.), 53S–58S.

- Spitzer, J. J., Bagby, G. J., Meszaros, K., & Lang, C. H. (1989). Altered control of carbohydrate metabolism in endotoxemia. *Progress in Clinical and Biological Research*, 286, 145–165.
- Staal, F. J. T., Ela, S. W., & Roederer, M. (1992). Glutathione deficiency in human immunodeficiency virus infection. *Lancet*, i, 909–912.
- Steiner, M. (1999). Vitamin E, a modifier of platelet function: Rationale and use in cardiovascular and cerebrovascular disease. *Nutrition Reviews*, 57, 306–309.
- Stuber, F., Petersen, M., Bokelmann, F., & Schade, U. (1996). A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor- α concentrations and outcome of patients with severe sepsis. *Critical Care Medicine*, 24, 381–384.
- Tagore, A., Gonsalkorale, W. W., & Pravica, V. (1999). Interleukin 10 (IL-10) genotypes in inflammatory bowel disease. *Tissues Antigens*, 54, 386–390.
- Taniguchi, T., Koido, Y., Aiboshi, J., Yamashita, T., Suzaki, S., & Kurokawa, A. (1999). Change in the ratio of interleukin-6 to interleukin-10 predicts a poor outcome in patients with systemic inflammatory response syndrome. *Critical Care Medicine*, 27, 1262–1264.
- Tanus-Santos, J. E., Desai, M., Deak, L. R., Pezzullo, J. C., Abernethy, D. R., Flockhart, D. A., & Freedman, J. E. (2002). Effects of endothelial nitric oxide synthase gene polymorphisms on platelet function, nitric oxide release, and interactions with estradiol. *Pharmacogenetics*, 12, 407–413.
- Turner, D. M., Williams, D. M., & Sankaran, D. (1997). An investigation of polymorphisms in the interleukin-10 gene promoter. *European Journal of Immunogenetics*, 24, 108–116.
- van Dissel, J. T., van Langevelde, P., Westendorp, R. G., Kwappenberg, K., & Frolich, M. (1998). Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet*, 351, 950–955.
- van Tits, L. J., Demacker, P. N., de Graaf, J., Hak-Lemmers, H. L., & Stalenhoef, A. F. (2000). Alpha-tocopherol supplementation decreases production of superoxide and cytokines by leukocytes ex vivo in both normolipidemic and hypertriglyceridemic individuals. *American Journal of Clinical Nutrition*, 71, 458–464.
- Visser, M., Pahor, M., Taaffe, D. R., Goodpaster, B. H., Simonsick, E. M., Newman, A. B., Nevitt, M., & Harris, T. B. (2002). Relationship of interleukin-6 and tumor necrosis factor- α with muscle mass and muscle strength in elderly men and women: The Health ABC Study. *Journal of Gerontology Biological Science and Medical Science*, 57, M326–332.
- Vozarova, B., Weyer, C., & Hanson, K. (2001). Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obesity Research*, 9, 414–417.
- Vozarova, B., Weyer, C., & Lindsay, R. S. (2002). High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes*, 51, 455–461.
- Wada, K., Nakajima, A., & Blumberg, R. S. (2001). PPAR- γ and inflammatory bowel disease: a new therapeutic target for ulcerative colitis and Crohn's disease. *Trends in Molecular Medicine*, 7, 329–331.
- Wang, X. Y., Hurme, M., Jylha, M., & Hervonen, A. (2001). Lack of association between human longevity and polymorphisms of IL-1 cluster, IL-6, IL-10 and TNF- α genes in Finnish nonagenarians. *Mechanisms of Ageing and Development*, 123, 29–38.
- Warzocha, K., Ribeiro, P., Bienvenu, J., Roy, P., Charlot, C., Rigal, D., Coiffier, B., & Salles, G. (1998). Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. *Blood*, 91, 3574–3581.
- Wesselborg, S., Bauer, M. K. A., Vogt, M., Schmitz, M. L., & Schulze-Osthoff, K. (1997). Activation of transcription factor NF- κ B and p38 mitogen-activated protein kinase is mediated by distinct and separate stress effector pathways. *Journal of Biological Chemistry*, 272, 12,422–12,429.
- Weyer, C., Yudkin, J. S., & Stehouwer, C. D. (2002). Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. *Atherosclerosis*, 161, 233–242.
- Willeit, J., & Kiechl, S. (2000). Biology of arterial atheroma. *Cerebrovascular Disease*, 10 (Suppl. 5), 1–8.
- Wilson, A. G., Clay, F. E., & Crane, A. M. (1995). Comparative genetic association of human leukocyte antigen class II and tumor necrosis factor- α with dermatitis herpetiformis. *Journal of Investigative Dermatology*, 104, 856–858.
- Wilson, A. G., de Vries, N., Pociot, F., di Giovine, F. S., van der Putte, L. B., & Duff, G. W. (1993). An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA-A1, B8, and DR3 alleles. *Journal of Experimental Medicine*, 177, 557–560.

- Wilson, A. G., Gordon, C., & di Giovine, F. S. (1994). A genetic association between systemic lupus erythematosus and tumor necrosis factor alpha. *European Journal of Immunology*, *24*, 191–195.
- Wu, D., Koga, T., Martin, K. R., & Meydani, M. (1999). Effect of vitamin E on human aortic endothelial cell production of chemokines and adhesion to monocytes. *Atherosclerosis*, *147*, 297–307.
- Wu, D., Meydani, S. N., & Han, S. N. (2000). Effect of dietary supplementation with fish oil in combination with different levels of vitamin E on immune response in healthy elderly human subjects. *FASEB Journal*, *14*, A238.
- Wu, D., Meydani, S. N., Sastre, J., Hayek, M., & Meydani, M. (1994). In vitro glutathione supplementation enhances interleukin-2 production and mitogenic responses in peripheral blood mononuclear cells from young and old subjects. *Journal of Nutrition*, *124*, 655–663.
- Wu, T., Dorn, J. P., & Donahue, R. P. (2002). Associations of serum C-reactive protein with fasting insulin, glucose, and glycosylated hemoglobin: The Third National Health and Nutrition Examination Survey, 1988–1994. *American Journal of Epidemiology*, *155*, 65–71.
- Yaqoob, P., Newsholme, E. A., & Calder, P.C. (1999). Production of tumour necrosis factor—alpha increases with age and BMI in healthy women. *Proceedings of the Nutrition Society*, *58*, 129A.
- Young, D. G., Skibinski, G., Mason, J. I., & James, K. (1999). The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-beta 1) levels in normal healthy blood donors. *Clinical and Experimental Immunology*, *117*, 476–481.
- Zhai, R., Jetten, M., Schins, R. P., Franssen, H., & Borm, P. J. (1998). Polymorphisms in the promoter of the tumor necrosis factor-alpha gene in coal miners. *American Journal of Industrial Medicine*, *34*, 318–324.
- Ziccardi, P., Nappo, F., & Giugliano, G. (2002). Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation*, *105*, 804–809.