Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 491 – 504
Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis☆

Ignazio Grattagliano*, Vincenzo O. Palmieri, Piero Portincasa, Antonio Moschetta, Giuseppe Palasciano

Department of Internal Medicine and Public Medicine, University Medical School of Bari, Clinica Medica "A. Murri," 70124 Bari, Italy

Received 12 April 2007; received in revised form 5 June 2007; accepted 18 June 2007

Abstract

Although the biochemical steps linking insulin resistance with the metabolic syndrome have not been completely clarified, mounting experimental and clinical evidence indicate oxidative stress as an attractive candidate for a central pathogenic role since it potentially explains the appearance of all risk factors and supports the clinical manifestations. In fact, metabolic syndrome patients exhibit activation of biochemical pathways leading to increased delivery of reactive oxygen species, decreased antioxidant protection and increased lipid peroxidation. The described associations between increased abdominal fat storage, liver steatosis and systemic oxidative stress, the diminished concentration of nitric oxide derivatives and antioxidant vitamins and the endothelial oxidative damages observed in subjects with the metabolic syndrome definitively support oxidative stress as the common second-level event in a unifying pathogenic view. Moreover, it has been observed that oxidative stress regulates the expression of genes governing lipid and glucose metabolism through activation or inhibition of intracellular sensors. Diet constituents can modulate redox reactions and the oxidative stress extent, thus also acting on nuclear gene expression. As a consequence of the food–gene interaction, metabolic syndrome patients may express different disease features and extents according to the different pathways activated by oxidative stress-modulated effectors. This view could also explain family differences and interethnic variations in determining risk factor appearance. This review mechanistically focused on oxidative stress events leading to individual disease factor appearance in metabolic syndrome patients and their setting for a more helpful clinical approach.

Keywords: Diabetes; Endothelial dysfunction; *HFE*, hemochromatosis gene; Insulin resistance; Nonalcoholic fatty liver disease; Reactive oxygen species; Visceral obesity

1. Introduction

The metabolic syndrome is a multifactorial condition leading to accelerated atherosclerosis and increased risk for diabetes. It is associated with major cardiovascular events and a high mortality rate [1,2]. The metabolic syndrome is characterized by different combinations of three or more of the following features: abdominal obesity, blood hypertension, hyperglycemia and serum dyslipidemia as defined by the criteria of the Third Report of the National Cholesterol Education Program Adult Treatment Panel III [3] or by the updated criteria of the International Diabetes Federation [4].

Whichever definition is used, epidemiological surveys show that the metabolic syndrome is extremely common.

Abbreviations: ADMA, asymmetric dimethylarginine; AGE, advanced glycation end product; FFA, free fatty acid; LDL, low-density lipoprotein; MTP, microsomal triglyceride transfer protein; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NO, nitric oxide; Nox, NADPH-dependent oxidases; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha.

☆ This study was supported by research grants from the University of Bari "Progetti di ricerca Ateneo 2004" to G.P.

* Corresponding author. Section of Internal Medicine, Department of Internal Medicine and Public Medicine, University Medical School of Bari, 70124 Bari, Italy. Tel.: +39 080 5478233; fax: +39 080 5478232.
E-mail address: i.grattagliano@semeiotica.uniba.it (I. Grattagliano).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.06.011

The 1999–2002 National Health and Nutrition Examination Survey estimated the age-adjusted prevalence of the metabolic syndrome in U.S. adults over 20 years to be between 34.6% and 39.1% and to be even higher if considering adults over 60 years [4]. It is a little more common in men, and there exist ethnic differences. Overall, the prevalence of the metabolic syndrome parallels the increasing aging population and "epidemic" obesity [5].

The link between individual metabolic syndrome components is unknown. Recent studies support the notion that these metabolic abnormalities do indeed cluster beyond the effect of chance [6] and that a single factor may underlie the association [7]. The fact that insulin resistance and abdominal obesity are also associated with perturbations in plasma adipokine levels, altered fatty acid metabolism, endothelial dysfunction, procoagulant state and systemic inflammation underscores the breadth and complexity of the pathophysiology of this clustering [8].

Indeed, this systemic disorder may be considered as the result of the imbalance of some dynamic mechanisms supporting the robustness and the adaptive response toward both intrinsic and extrinsic factors (i.e., nutrients, energy consumption, metabolic rate...) [9]. However, major problems in approaching a patient with the metabolic syndrome are on how to manage his or her individual risk factors and whether it is better to focus on single components by a reductionist method or to encompass interactions and dynamics with a systems method. Reductionism is less helpful to study complex symptoms in which the interactions among components amplify and dominate the final expression of the disease. In this context, the interaction of multiple factors needs to be incorporated in some meaningful way to be responsible for disease evolution [10,11]. These considerations are important in chronic conditions such as the metabolic syndrome in which individualized treatments and multidimensional uses of medications have to be promoted. On the other hand, setting a complex disease by considering only the overall behavior inevitably fails in keeping under control all the single parameters responsible for the interindividual variability and the spontaneous fluctuation of the disease features [12].

Therefore, the identification of common basic mechanisms driving to a unifying pathogenic hypothesis for the metabolic syndrome would be helpful in explaining the clinical manifestations and in approaching patients. As this review shows, oxidative stress could explain most of the second-level events resulting in risk factor appearance and may lead to a unitary pathogenic view of this chronic disorder.

2. Oxidative stress and the metabolic syndrome constituent factors

Although it is generally accepted that the main pathogenic mechanism underlying the first level of metabolic changes in patients with the metabolic syndrome relies on insulin resistance, an abundance of evidence demonstrating a close link among the metabolic syndrome, a state of chronic low-level inflammation and oxidative stress as second-level abnormalities had emerged [13]. In fact, oxidative stress plays an important role in the pathogenesis of vascular alterations by either triggering or exacerbating the biochemical processes accompanying the metabolic syndrome [14]. In addition, experimental and clinical observations indicate oxidative stress as an important mechanism in obesity-associated metabolic syndrome, in the development of diabetes and its complications and in "satellite" conditions such as nonalcoholic steatohepatitis (NASH) [15–18].

Defined as an impaired balance between free radical production and antioxidant capacity resulting in accumulation of oxidative products, oxidative stress is a well-recognized mechanism playing important roles in many pathological conditions [19], and several human diseases have been closely related to oxidative stress [20]. A number of cell functions appear to be regulated by free radical molecules, which may also act as intracellular and intercellular signals [21,22]. Also, the protein redox state is implicated in the regulation of several cellular activities, including cell differentiation and activation of specific metabolic pathways [23,24].

Oxidative stress has been associated with all the individual components and with the onset of cardiovascular complications in subjects with the metabolic syndrome [13,15,25,26]. In a recent study [27], the role of oxidative stress in the pathophysiologic interactions among the constituent factors of the metabolic syndrome has been remarked. However, although some of the constituent characteristics of the metabolic syndrome are known to share common pathogenic mechanisms of damage, the impact of hereditary predisposition and the regulation of gene expression as well as the role of environment and dietary habit in determining inflammatory process-triggered oxidation are still unclear. These aspects of the problem deserve special attention since it is hypothesized that in patients with the metabolic syndrome, oxidative stress may be amplified by a concomitant antioxidant deficiency that may favor the propagation of oxidative alterations from intra- to extracellular spaces and from confined to distant sites, thus realizing a systemic oxidative stress state [28,29].

Altogether, these considerations would suggest a unifying hypothesis to explain the mechanisms underlying the onset and development of metabolic syndrome-associated risk factors. As the following subsections report, excessive free radical production and oxidative damages are supported by several experimental demonstrations and human observations. Therefore, oxidative stress appears to possess, at least in part, the credentials to mechanistically explain the perpetuation of insulin resistance, the altered energy production, the endothelial dysfunction and the appearance of vascular complications in this condition.

2.1. Oxidative alterations, visceral obesity and liver steatosis

A number of clinical studies have reported the importance of visceral fat accumulation in the development of metabolic disorders, including reduced glucose tolerance, hyperlipidemia and cardiovascular diseases. Visceral fat accumulation causes dysregulation of adipocyte functions, including oversecretion of leptin and tumor necrosis factor-alpha (TNF-α), and diminished secretion of adiponectin, which results in the development of a variety of metabolic and circulatory disorders, including quantitative and qualitative changes in serum lipids and lipoproteins such as small dense low-density lipoprotein (LDL) [30]. Visceral adiposity represents an independent determinant of all the metabolic syndrome components [31]. In humans, ultrasonographically measured mesenteric fat has been independently associated with body mass index and metabolic risk factors better as compared with measured waist circumference [27,32]. Abdominal adiposity is also critically important as a source of free fatty acids (FFAs) and inflammatory factors such that the International Diabetes Federation gives it a central role in the diagnosis of the metabolic syndrome [4]. Visceral obesity represents per se a low-grade systemic inflammation as reflected by elevated serum markers, such as C-reactive protein and TNF-α [33]. Genetic manipulation and overnutrition studies have convincingly shown that insulin resistance is regulated by cytokines and mediators released from mesenteric adipocytes [34].

Because of its location and function in fat and sugar metabolisms, the liver is damaged early in the metabolic syndrome. In fact, nonalcoholic fatty liver disease (NAFLD), a chronic potentially progressive liver disease resembling the alcoholic form but occurring in subjects who do not abuse alcohol, develops as a companion of the metabolic syndrome [18]. It is generally accepted that the sequence of events leading to hepatocyte fatty degeneration begins with insulin resistance, which precedes fat accumulation [35]. Excess intracellular fatty acids, oxidative stress, energy depletion and mitochondrial dysfunction then cause cellular injury [24,36,37]. NASH, the inflammatory form of NAFLD, is thus viewed as the result of "two hits," in which the first hit is fat accumulation [38]. Lipid retention within hepatocytes triggers oxidative stress (the "second hit") generating reactive oxygen species (ROS) at different intracellular levels and cytokine release. In particular, the alteration of intracellular fatty acid trafficking and mitochondrial β-oxidation, consequent to differential expressions of perilipin and adipophilin [39] and hepatic refractoriness to adipokines [34,40], contributes to the impairment of hepatic lipid turnover and leads to lipid accumulation. Lipid accumulation and insulin resistance activate different sources of ROS: (a) the cytochrome *P*450 2E1, which generates ROS during the metabolism of endogenous ketones and dietary constituents [41]; (b) mitochondria, which continuously generate ROS, being damaged themselves if the production of ROS is increased [42]; and (c) peroxisomes, which generate H_2O_2 and are activated when mitochondrial β-oxidation is saturated or impaired [43].

Enhanced hepatic lipid peroxidation causes changes in physical and chemical membrane properties, with fluidity and permeability alteration [17] affecting signal transduction and ion exchange properties [44]. Changes in lipid composition and characteristics induce membrane remodeling [45]. Most lipid peroxides are volatile molecules that may reach sites distant from those of generation and cause damages and fibroblastic cell activation in the presence of inflammation [46]. In this respect, it has been observed that subjects with NASH show high hepatic and systemic levels of lipid peroxidation products. This phenomenon is associated with an increased risk for cardiovascular disease [47].

Recently, impaired serum redox balance with decreased antioxidant capacity and increased lipid peroxidation has been observed in patients with fatty liver, visceral obesity and metabolic syndrome (Fig. 1). In the study by Suthanthiran et al. [27], the amount of visceral fat and systemic oxidative alterations were significantly related, thus indicating that excess visceral fat is an important and independent determining factor of the observed serum

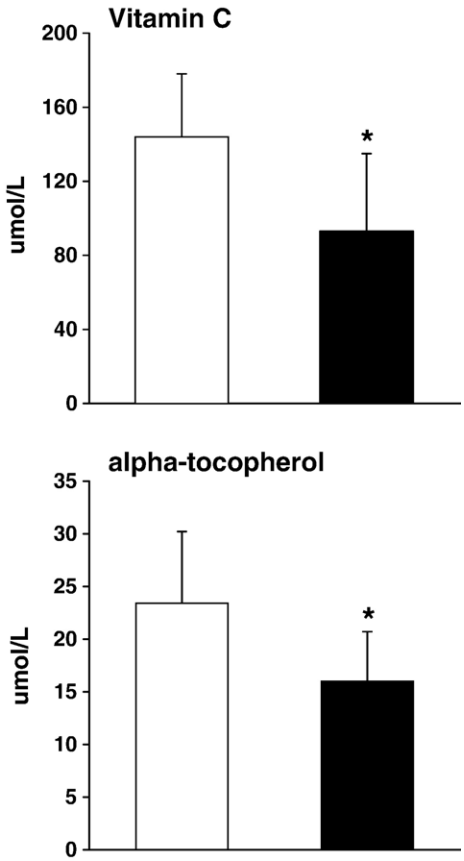

Fig. 1. Serum concentrations of vitamin C and α-tocopherol in 23 healthy individuals (open bar) and 41 patients with the metabolic syndrome (filled bar). Data are reported as mean±S.D. $^{*}P<.001$ versus healthy controls.

oxidative changes. Moreover, in these patients, the presence of the metabolic syndrome was predicted from a linear combination of variables, including liver steatosis, visceral fat and serum oxidative changes [28,29,47]. The participation of the liver both as a damaged organ and a contributory source for systemic oxidative alterations in patients with the metabolic syndrome and visceral adiposity is therefore unequivocally suggested [48]. This role is further supported by the coexistence of fatty liver with blood hypertension and metabolic syndrome in nonobese patients [49,50] and by the observation that NAFLD is associated with the metabolic syndrome to a higher extent than excess adipose tissue in obese subjects [51].

Another clinically relevant aspect is the increased vulnerability of fatty livers toward stress events [52] especially as they occur in transplantation surgery. These mainly depend on the fact that hepatic steatosis sensitizes hepatocytes to injury and inflammation through enhanced fatty acid synthase expression and increased fatty acid synthase-mediated apoptosis [53].

Finally, another potentially damaging factor in NAFLD is intestinal bacteria. The contribution of small bowel bacteria overgrowth to liver inflammatory processes may in fact be realized through an increased intestinal permeability that allows entry of gut-derived toxins with consequent portal inflammation, Kupffer cell activation and liver injury [54].

In conclusion, accumulation of fat in the abdominal region and that in the liver in particular induce increases in systemic lipid peroxidation and damages through excess FFAs, lipoprotein-bound lipids, cytokines and vasoactive peptides. These considerations are consistent with abdominal fat and liver steatosis as the initial alterations responsible for the subsequent appearance of other metabolic syndrome factors, representing the trigger for systemic oxidative alterations and revealing a purported incapacity of patients to correct excess oxidation.

2.2. Systemic and tissue oxidative changes in hyperglycemic states and diabetes

Hyperglycemia is the fundamental abnormality underlying the mechanisms causing endothelial dysfunction in diabetes. In fact, increases in blood glucose linearly depress endothelium-dependent vasodilatation [55]. The early and high incidence of atherosclerosis and cardiovascular events in patients with diabetes and postprandial high blood glucose levels has been associated, at least in part, with oxidative stress [56]. There are several observations demonstrating that diabetic patients have increased tissue accumulation of protein and lipid oxidative products [16], enhanced levels of circulating oxidative stress markers and reduced antioxidant defenses [57]. This imbalance is thought to participate in the onset of diabetic complications [58].

Hyperglycemia can induce oxidative stress by several mechanisms independently associated [59,60] and including glucose auto-oxidation, advanced glycation end product (AGE) formation, abnormal arachidonic acid metabolism and its coupling to cyclooxygenase catalysis, protein kinase C activation, increase in the activity of nitric oxide (NO) synthase and activation of the aldose reductase pathway [61–63]. The increased intracellular metabolism of glucose in the hyperglycemic state leads to nicotinamide adenine dinucleotide (NADH) and flavin adenosine dinucleotide overproduction with consequent stimulation of adenosine triphosphate generation by the electron transport chain [64]. Excess NADH causes increases in the mitochondrial proton gradient, and, as a consequence, electrons are transferred to oxygen, producing superoxide at the NADH dehydrogenase of complex I and at the interface between ubiquinone and complex III [59,65,66]. Mitochondrial-derived superoxide causes increased diacylglycerol synthesis and protein kinase C activation [64].

The mechanism leading to the formation of AGEs begins with nonenzymatic covalent binding of ketone or aldehyde groups of reducing sugar to the free amino groups of proteins and other molecules; next, series of rearrangements and reactions occur to irreversibly produce AGEs [67]. An AGE, at its turn, induces ROS production, probably via activation of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases (Nox) and after interacting with receptor/binding proteins [68]. It has been proposed that the vascular toxicity of AGEs is realized by binding to and activating specific receptors on vascular cells, by modifying the extracellular matrix and circulating lipoproteins, finally leading to atherosclerosis.

Two enzymes of the polyol pathway contribute to ROS generation. The first, aldose reductase, uses NADPH for the reduction of glucose to sorbitol, which is a minor reaction under normal conditions but reaches up to 30–35% of the metabolic glucose pathway in hyperglycemic states [69]. When this occurs, the availability of NADPH is critically reduced because of competition with glutathione reductase. This results, in turn, in reduced regeneration of glutathione, thus contributing to the generation of oxidative stress [70,71]. The second enzyme, sorbitol dehydrogenase, oxidizes sorbitol to fructose with concomitant NADH production. Increased NADH is used, in turn, by Nox to produce superoxide [72].

Other sources of oxidative stress in diabetic subjects include FFAs, which are generally elevated in such patients [73], LDL oxidation and accumulation of asymmetric dimethylarginine (ADMA). Excess FFAs enter the citric acid cycle and generate acetyl–CoA with increased NADH production. ADMA is an endogenous competitive inhibitor of endothelial NO synthase and is derived from the catabolism of ubiquitous proteins containing methylated arginine residues. One metabolic pathway for ADMA clearance is by enzymatic degradation. High serum levels of homocysteine, as it may occur in patients with the metabolic syndrome, result in direct inhibition of the ADMA-degrading enzyme, likely due to the attack of homocysteine on a critical sulfhydryl group of the enzyme [74].

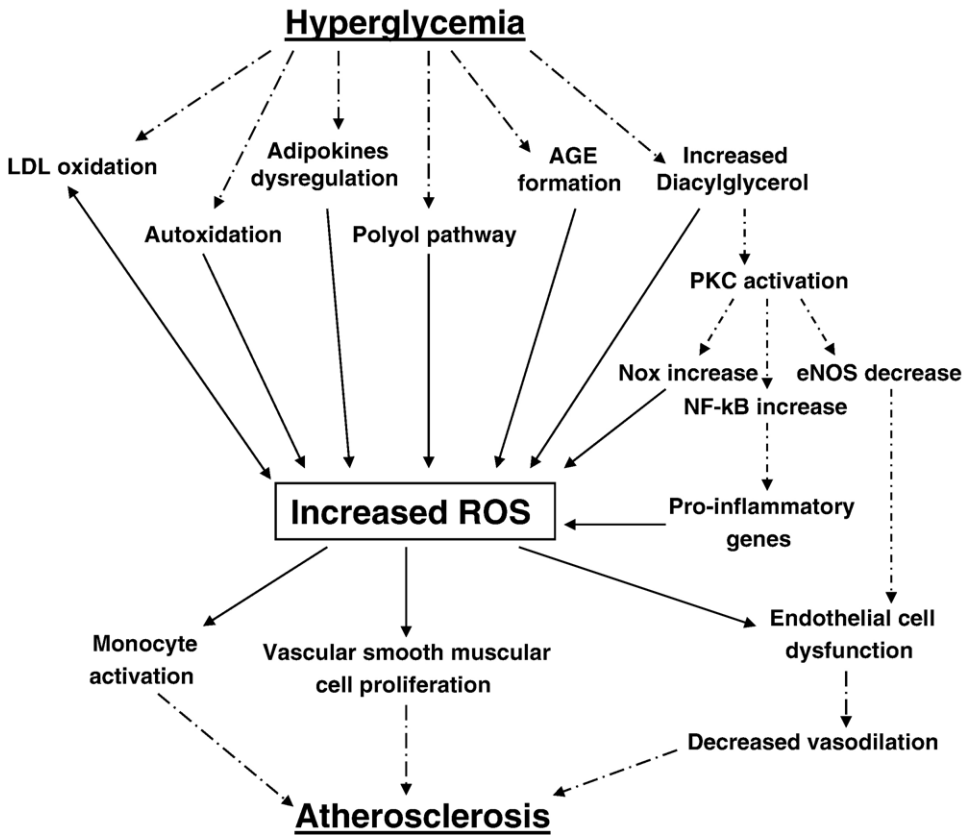

Fig. 2. Mechanisms of hyperglycemia-triggered vascular oxidative stress events. eNOS=endothelial NO synthase; NF-κB=nuclear factor kappa B; PKC=protein kinase C.

In addition, hyperglycemia-induced ROS formation may successively lead to endothelial dysfunction by decreasing NO and prostacyclin bioavailability and by increasing the synthesis of vasoconstrictor prostanoids and endothelin [75]. Hyperglycemia-induced microvascular permeability is responsible, in turn, for the expression of endothelial growth factors, angiogenesis promoters and neovascularization, finally leading to diabetic microangiopathy. The mechanistic role of hyperglycemia-induced oxidative stress in adventitial inflammation and neovascularization also provides new pathogenic links between hyperglycemia and atherosclerosis (Fig. 2).

2.3. Endothelial oxidative dysfunction and blood hypertension

The normal endothelium has anticoagulant and anti-inflammatory properties and promotes vasodilatation by releasing NO, prostacyclin and other molecules. In various chronic diseases, the endothelium becomes dysfunctional and promotes inflammation and thrombosis. Loss of NO bioavailability is a key feature of endothelial dysfunction. Although incomplete knowledge of ROS and oxidants involved in vascular damage exists, there is no doubt that oxidative events and vascular injury are causally linked, and excessive ROS production is actually seen as the major cause of reduced vascular NO levels. Among acknowledged mechanisms, the reaction between superoxide and NO to form peroxynitrite deserves special attention [76].

Oxidative stress has been closely related to atherosclerotic processes [14,77] and is believed to be an important secondary consequence of inflammation associated with the atherosclerotic process and its cardiovascular complications [78]. Indeed, the inflammatory process is modulated by the activity of several families of enzymes, including cyclooxygenases, lipoxygenases, Nox, NO synthases and peroxidases — all possessing the capacity to produce ROS and NO species.

The participation of arterial hypertension in the generation of systemic oxidative stress associated with the metabolic syndrome is suggested by a number of observations on the role of insulin resistance and the sympathoadrenal system [79], NO metabolism changes and the low circulating levels of vitamin C in patients with high-grade hypertension [27] and the improvement of systemic oxidative stress with antihypertensive treatment [26]. These considerations have an even higher impact when associated with endothelium activation and dysfunction as characterized by increased levels of circulating oxidized LDL, intercellular and vascular adhesion molecules and C-reactive protein [28] and with the evidence that vascular complications are also associated with oxidative stress events [80].

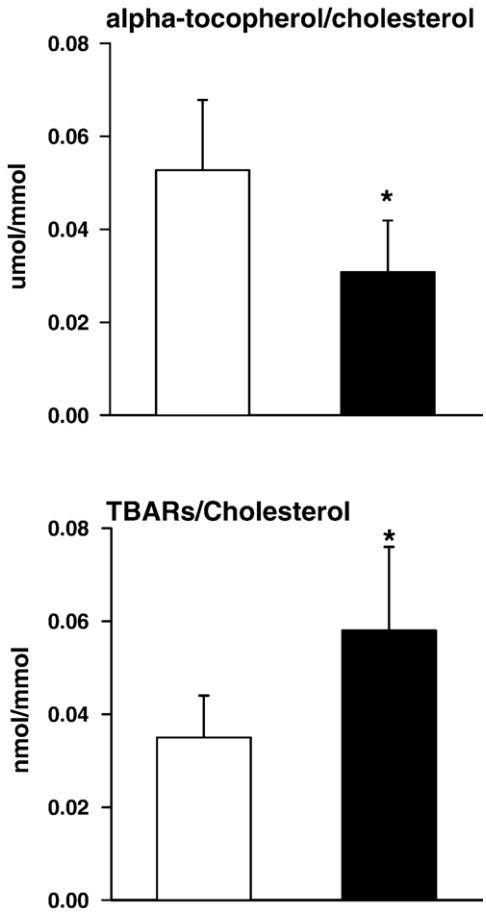

Fig. 3. Serum α-tocopherol/total cholesterol ratio and thiobarbituric acid-reactive substances (TBARs)/total cholesterol ratio in 23 healthy individuals (open bar) and 41 patients with the metabolic syndrome (filled bar). Data are reported as mean±S.D. *P<.001 versus healthy controls.

In particular, small dense LDL particles are able to filtrate through the endothelium of blood vessels. Oxidized LDL activates endothelial cells with the promotion of an immune response leading to the formation of lipid-laden macrophages. Also, in response to inflammatory stimuli, endothelial cells produce adhesion molecules that will further facilitate macrophage migration from the blood into the intima, thus generating an endothelial damage. These events are favored by insulin resistance and obesity [81,82].

Other factors have a role in the modulation of these pathophysiologic mechanisms. Among them, vitamin E is a fat-soluble vitamin that is sequestered in the hydrophobic interior of membranes where it acts as an antioxidant, quenching lipid peroxidation. Under normal conditions, the reduced state of LDL is maintained by vitamin E [83], which also acts by regulating inflammatory reactions and metabolic pathways, including platelet aggregation [84]. In combination with tocopherols, vitamin C counters free radicals and regulates vitamin E metabolism by recycling oxidized tocopherols. The synergic action of these two vitamins is also modulated by the intervention of glutathione, which maintains vitamin C in the reduced form [85]. Both vitamins, E and C, appear to be important for the prevention of cardiovascular events. In fact, consumption of vitamin E has been associated with a lower risk for coronary heart disease [86] and with reduced LDL oxidation [87]. Also, the connection between serum concentrations of vitamin E and lipid peroxidation products in relation to cholesterol level and abdominal obesity has been recently studied in patients with the metabolic syndrome (Fig. 3). An inverse relation between the serum cholesterol-adjusted vitamin E concentrations and the grade of hepatic steatosis and a linear relation between the extent of visceral fat and the lipid peroxide/cholesterol ratio have been observed [27]. In other studies, supplementation of vitamin E was able to prevent the onset of type 2 diabetes [88] and improve NAFLD in obese children [89].

Concerning the specific role of vitamin C in oxidative stress-associated arterial hypertension, mounting evidence suggest the importance of this vitamin in regulating endothelial function and vasodilation. In fact, vitamin C is known to improve elastic artery [90] by contrasting endothelial cell oxidation [91] and by stimulating both endothelium-dependent and endothelium-independent arterial vasodilation [92]. In addition, vitamin C administration was able to restore endothelium-dependent vasodilation in hyperglycemic patients [93].

Relatively new and interesting pathways of oxidative stress-induced vascular damages include enzymes such as Nox and homocysteine [94,95]. Membrane-bound Nox are major sources of ROS in preatherosclerotic conditions and have been found in human peripheral and coronary arteries [96,97]. A direct spatial relationship between Nox-generated ROS and LDL oxidation was demonstrated in carotid plaques and in lesions associated with unstable angina [98]. Enhanced expression and activity of Nox enzymes have also been detected in new accumulated adipose tissue of obese mice and have been related to impaired antioxidant defense and adipocytokine dysregulation [15]. By increasing oxidative stress, activation of Nox in vascular cells has been reported to be an important mechanism in the pathogenesis of hypertension and atherosclerosis [99]. Angiotensin II is one of the most potent stimuli activating vascular Nox. This property clearly links ROS production with activation of the renin–angiotensin system in hypertension [100]. As a consequence, drugs acting on the renin–angiotensin system reduce Nox activity, thus rendering this enzyme a specific drug target (Fig. 4).

Disorders of the folate-dependent methionine metabolism have been described in experimental models and human conditions associated with a high cardiovascular risk [101,102]. These metabolic abnormalities result in high levels of homocysteine, a molecule belonging to the group of thiols. Differently from glutathione and cysteine, which exert protective effects against ROS, homocysteine is considered to be a “bad thiol” because of its association with a variety of chronic disease conditions [95]. Homocysteine is formed

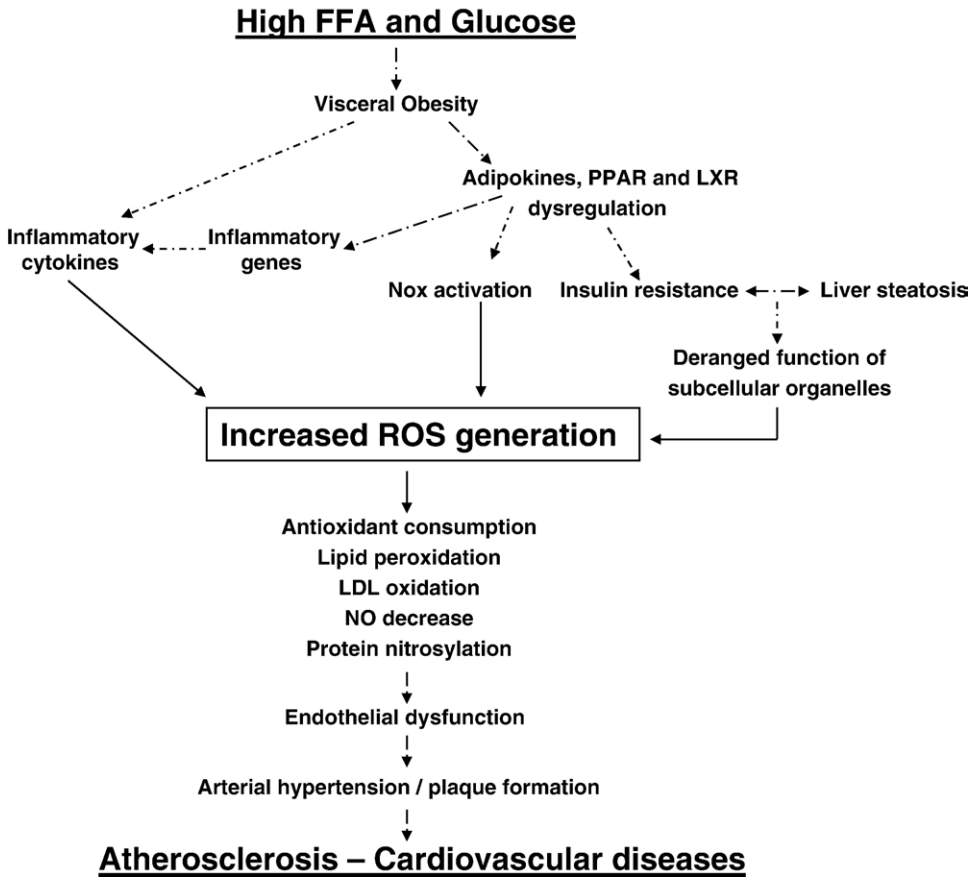

Fig. 4. Conceptual view of increased ROS generation and consequent oxidative stress as a "second-level" event causing metabolic syndrome-associated cardiovascular risk factor manifestation. LXR=liver X receptor.

in the transsulfuration and remethylation pathways that convert homocysteine to methionine with folate and betaine intervention. As a consequence of the impairment of the methionine metabolism, the increased level of circulating homocysteine has been associated with endothelial dysfunction, both directly and via NO interaction [103]. Although it is known that homocysteine can be toxic per se by acting as an *N*-methyl-D-aspartate agonist, thus decreasing the availability of NO and impairing arterial vasodilation capacity [104], the specific molecular mechanisms of damage involved are still unclear. Three mechanisms have been proposed, namely, oxidative stress, endoplasmic reticulum stress and activation of pro-inflammatory factors [105]. Hyperhomocysteinemia has been associated with oxidative stress in liver steatosis, a hypothesis favored by recent observations [106]. Several in vitro studies have also demonstrated the ability of homocysteine to enhance the production of ROS and to impair endothelial NO availability [107]. An additional mechanism that may be involved in homocysteine-mediated vascular alteration in patients with the metabolic syndrome is the indirect connection between high homocysteine levels and low nitrosothiol levels. At this concern, it is known that circulating nitrosothiols act as free NO donors for vascular tone modulation [108]. In addition, by representing a storage form of thiols and glutathione in particular, extracellular nitrosothiols exert antioxidant functions and favor removal of toxic products [109], contributing to the role of the extracellular microenvironment in the regulation of the redox status and function of cell surface proteins [23]. The above-reported interference of homocysteine with circulating NO availability could explain, at least in part, the low levels of nitrosothiols and glutathione found in conditions associated with hyperhomocysteinemia [27,95]. Therefore, the equilibrium between "good" and "bad" thiols may determine outcomes in studies of tissue degeneration and inflammation (i.e., NASH and the metabolic syndrome).

3. Intracellular sensors of oxidative stress and lipid metabolism and genetic susceptibility to the metabolic syndrome

Patients with the metabolic syndrome show altered mitochondrial metabolism, lipid storage and high levels of circulating FFAs — all favoring the imbalance between increased free radical release at different levels and reduced antioxidant defense and pointing attention to the regulation

of cellular function in patients with the metabolic syndrome. This view has stimulated the quest for molecular integration between lipid and glucose metabolism.

Nuclear receptors are transcription factors that regulate the gene expression of some proteins involved in different cell processes. These receptors are activated by several specific ligands that must be lipophilic to cross the cellular membranes. Nuclear receptors actually represent the meeting point between the regulation of genetic transcription and cell physiology, including acquisition, storage and disposal of dietary nutrients. Some of the nuclear receptors act as sensors for dietary lipids and answer to the intracellular lipid levels, causing the transcription of specific genes codifying for proteins and protecting the cells from the increased lipid concentration itself. The discovery of these proteins is important in explaining the intracellular feedback mechanisms for cholesterol and fatty acid homeostasis. Members of this protein family are also fatty acid receptors [peroxisome proliferator-activated receptor (PPAR)], oxysterol receptors (liver X receptor), xenobiotic receptors (steroid–xenobiotic receptor) and bile salt receptors (farnesoid X receptor) [110,111]. In particular, the PPAR family includes three members that are conserved for 60–80% of their DNA- and ligand-binding domains and are activated mainly by FFAs and eicosanoids. Lipid-sensing nuclear receptors dispose of a fast track to rearrange cell dynamics upon metabolic needs, thus allowing accurate feedforward or feedback responses. The genetic regulation cascades controlled by nuclear receptors appear to be essential in studying the effect of different diet regimens in animal models; complete understanding of their physiology may contribute to disclosing the complexity of the metabolic syndrome. In fact, metabolic syndrome patients may express different features of the disease according to the variability of some altered homeostatic mechanisms.

Family studies and interethnic variations in susceptibility suggest that genetic factors are important in determining disease risk appearance in patients with the metabolic syndrome. With respect to the associated condition of NAFLD, genetic risk factors for advanced forms include factors influencing the severity of oxidative stress events. Evidence for genetic susceptibility to NAFLD is suggested by recent reports of family clustering. Struben et al. [112] reported the coexistence of NASH in kindreds studied, while Willner et al. [113] found that 18% of NASH patients had an affected first-degree relative. Preliminary data suggest that polymorphisms in the genes encoding microsomal triglyceride transfer protein (MTP), antioxidant enzyme superoxide dismutase 2, TNF-α and angiotensinogen may be associated with oxidative stress-mediated injury and appearance of NASH [114]. Polymorphisms in genes involved in the synthesis, storage and export of hepatic triglyceride will clearly influence the magnitude of steatosis; however, thus far, the only gene studied in this respect is the gene encoding MTP. Evidence that patients with NAFLD homozygous for a low-activity promoter polymorphism in the MTP gene have a higher grade of steatosis as compared with heterozygous patients has recently been presented [115]. Clearly, studies of polymorphisms of other genes encoding proteins involved in hepatic lipid metabolism, as susceptibility factors for oxidative stress and progressive NAFLD, are needed.

The principal class of genes that influence oxidative stress in patients with obesity, insulin resistance and the metabolic syndrome is that of genes encoding proteins involved in fatty acid oxidation. There exists a connection between fatty acid metabolism and oxidative stress. In fact, while appropriate fat oxidation is required to prevent fat accumulation, excessive fatty acid oxidation leads to oxidative stress [116]. With respect to peroxisomal and microsomal fat oxidation, as both are capable of generating ROS, it might be predicted that “gain-of-function” polymorphisms in genes encoding proteins involved in these processes would predispose to NASH. However, these pathways play a role in limiting mitochondrial overload by excessive FFA supply, and it may therefore be the case that “loss-of-function” polymorphisms affecting these pathways would predispose to NASH. An intriguing hypothesis also emerges from preliminary observations reporting that a mutation (*PPARA*3*) in the gene encoding PPARα is associated with NASH [117]. PPARα regulates the transcription of a variety of genes encoding enzymes involved in mitochondrial oxidation, peroxisomal β-oxidation and microsomal ω-oxidation of fatty acids [118,119]. Studies of PPARα knockout mice [120] and the fact that adiponectin activates PPARα and protects against steatosis [121] suggest that any *PPARA* mutation associated with NASH and metabolic syndrome should be associated with either loss of function or reduced gene expression. Moreover, in experimental models of obesity, polyunsaturated fatty acids reduce steatosis and improve insulin sensitivity by down-regulation of sterol regulatory elements and activation of PPARα [122].

As in other chronic conditions, other genes may influence the magnitude and effect of oxidative stress in NAFLD and metabolic syndrome by activating biochemical pathways leading to lipid and protein oxidation (e.g., the Fenton reaction, which leads to protein carbonyl formation by hydroxyl radical release after conversion of $Fe2^{+}$ to $Fe3^{+}$ or vice versa in the presence of hydrogen peroxide). These include the *HFE* gene and the gene encoding superoxide dismutase 2. With respect to *HFE*, an Australian study showed that 31% of NASH patients possessed at least one copy of the *C282Y HFE* mutation, as compared with only 13% of controls [123]. However, an Italian study of NAFLD patients denied these results [124].

4. Nutritional approach to modulate oxidative stress

The idea that life extension may be obtained by delaying pathophysiologic processes associated with aging has extended this research to conditions characterized by high risk for cardiovascular events and atherosclerosis, including

the metabolic syndrome. Many studies have evaluated the associations between nutrients and chronic diseases, and a consensus about the importance of nutritional factors in the etiology of these conditions has definitively emerged [125]. As a consequence, targeting individual subjects with tailored nutritional programs is one major clinical objective today. This approach is based on the different individual expressions of metabolic features and on knowledge of the effect of single nutrients as emerged from studies of nutrigenomics. This is a body of science that studies how nutrients alter the expression of an individual's genetic information and why individuals differ in metabolism of food at molecular levels [126]. Common variations in gene sequences may produce differences in complex traits such as weight potential, food metabolism, food–gene interactions and disease susceptibilities. Therefore, identifying genes that are regulated by diet and that cause or contribute to chronic diseases could result in the development of diagnostic tools, individualized interventions and, eventually, health-maintaining strategies [127]. More recently, metabolite network experiments (metabolomics) aim to quantify all metabolites in a cellular system under defined states and at different time points so that the dynamics of any genetic perturbation can be accurately assessed [128].

Many observational and experimental studies have considered that caloric restriction may be associated with life prolongation [129] possibly through an improvement of the cell redox balance [130]. Also, increased generation of mitochondrial ROS and oxidative damages seem to be differently induced by nutritional perturbation and state [131]. In animal experiments, hypocaloric diet and antioxidant supplementation were associated with improvement of some tissue functions and redox states that, conversely, were oxidatively depressed in aged control animals [132,133]. A key event associated with diet restriction is the activation of a class of genes belonging to the Sirt family, which is involved in cell maturation and apoptotic processes [134]. Recently, Howitz et al. [135] showed that resveratrol, an antioxidant poliphenol of red wine, was able to activate these genes by mimicking the effect of diet restriction. Successively, Baur et al. [136] showed that high-dose resveratrol was able to contrast the development of cardiovascular diseases and diabetes in mice that underwent a hyperlipidic diet, suggesting a role for oxidative stress in systemic inflammation and damages in conditions simulating the metabolic syndrome.

On the other hand, nutrients appear to play different roles in the expression of metabolic diseases. In fact, consumption of refined sugar, especially fructose in soft drinks, has been suspected to be a key factor in the development of insulin resistance and metabolic syndrome [137], and the increased intake of saturated fats is undoubtedly associated with the worldwide diffusion of obesity [138]. In addition, a diet rich in saturated fatty acids and sugar increases blood cholesterol, triglycerides and the risk for coronary disease. It is consequent that lower intake of fatty acids and refined carbohydrates would be the best choice. It is also known that the risk for coronary disease is lower in the Mediterranean area, where the consumption of lipids is mostly of vegetal origin and at a low saturated fatty acid content [139,140]. The Mediterranean diet contains a high rate of olive oil, fish, vegetable and low consumption of alcohol, thus spreading a wide antioxidant capacity. The Mediterranean diet has also been associated with a reduced incidence of blood hypertension, suggesting that a diet regimen well balanced in carbohydrates and fats could be indicated to correct metabolic abnormalities in metabolic syndrome patients. In a recent controlled crossover trial [141], lower plasma oxidized LDL and lipid peroxide levels and higher glutathione peroxidase activity were observed after an olive oil intervention, suggesting that consumption of olive oil, rich in phenolic antioxidant compounds, could provide beneficial effects in patients with cardiovascular risk factors. In this respect, it is also known that dietary fats can accomplish regulation of hepatic lipid metabolism through modification of gene transcription [142]. This is achieved by long-chain polyunsaturated fatty acids that are able to direct (a) fatty acids away from triglyceride storage by enhancing their oxidation and (b) glucose away from fatty acid synthesis by increasing its flux to glycogen [45,143].

Increased consumption of fruits and vegetables has also been shown to be associated with a reduced risk for stroke in most epidemiological studies [144]. In a recent meta-analysis of prospective cohort studies, He et al. [145] demonstrated that intake of fruits and vegetables higher than the average of three servings per day was associated with a lower risk for stroke, thus providing strong support for the use of antioxidant vitamin-rich food in the diet of patients with cardiovascular risk factors. However, experimental and human studies of the addition of antioxidants to diets and other treatments in patients with NASH and metabolic syndrome yielded controversial results. In particular, although a vitamin E-deficient diet elevated the lipid peroxidation levels in the rat liver, both ubiquinol and glutathione seem to protect mitochondria from lipid peroxidation more than vitamin E [146]. In humans, whereas addition of vitamin E to ursodeoxycholate in the treatment of NASH patients improved laboratory test and hepatic histology findings in a small number of metabolic syndrome patients [147], a combined vitamin E and vitamin C treatment did not improve necro-inflammatory activity or alanine aminotransferase and was not superior to weight loss in reducing biochemical indexes in two different studies of NASH patients [148,149].

Another natural food compound with protective properties is betaine. Betaine is distributed widely in plants (wheat germ, bran and spinach), and rich dietary sources include seafood, especially marine invertebrates. The principal physiologic role of betaine is the methyl donor (transmethylation) in the methionine cycle. Inadequate dietary intake of betaine leads to disturbed hepatic methionine metabolism resulting in elevated plasma homocysteine concentrations

and to inadequate hepatic fat metabolism leading to steatosis and subsequent increased serum lipid levels. These metabolic alterations may contribute to coronary, cerebral, hepatic and vascular diseases. Betaine has been shown to protect internal organs, improve vascular risk factors and enhance performance [150].

Finally, natural elements appear to have a role in the regulation of serum glycemia and associated metabolic dysfunctions. In particular, some observations have suggested that excess intake of refined carbohydrates is associated with decreased levels of serum chromium [151] and that this element has potential benefits on hyperglycemia, diabetes and elevated serum lipids [152,153]. It has been suggested that chromium explicates its action by improving some insulin effects, including the glucose transport within mitochondria, and improving the energetic demand.

5. Conclusions and perspectives

The metabolic syndrome is common, and the associated risk burdens of diabetes and cardiovascular disease are a major public health problem. The hypothesis that the main constituent parameters of the metabolic syndrome share common pathophysiologic mechanisms of damage provides a new conceptual framework for future research, although clinical trials will be necessary to confirm that the results from animal studies are applicable to humans.

Beyond the well-documented beneficial effects of exercise and body weight reduction in the prevention of insulin resistance and in the amelioration of diseases associated with the metabolic syndrome [154,155], the paradigm discussed in this review suggests that interrupting intracellular and extracellular ROS overproduction would contribute to normalizing the activation of metabolic pathways leading to the onset of diabetes and its complications and contrast the appearance of endothelial dysfunction leading to cardiovascular complications. This view supports the hypothesis that oxidative stress, mechanistically explaining the perpetuation of insulin resistance, the altered energy production and the endothelial dysfunction, may contribute to the appearance of cardiovascular complications in patients with the metabolic syndrome.

On the other hand, therapeutically, it might be difficult to contrast the development of cardiovascular complications by using conventional antioxidants as these ROS scavengers used in a stoichiometric manner have failed in reaching therapeutic goals [156]. The most interesting point concerns the genetic determinants of susceptibility to oxidative stress-induced appearance of risk factors for cardiovascular complications. Their role in this condition is supported by familial clustering, and this would stimulate prospective studies dealing with the changes in oxidative parameters and appearance of metabolic abnormalities in family clusters. It is clear that future studies examining susceptibility to the metabolic syndrome need to be considerably larger than those performed thus far if we are to come up with nutritional and genetic associations that are robust enough to guide targeted treatment and prevention strategies. These studies are critically dependent on the collection of large numbers of well-phenotyped cases and controls. In the future, the choice of candidate genes is likely to be further extended by genome and proteome expression studies of tissue from patients with various disease features, whole-genome single nucleotide polymorphism scans of cases and controls and, of course, mouse mutagenesis studies.

It is also time for large-scale multicenter studies to address important unanswered questions in the metabolic syndrome, as clearly underscored in this discussion. For example, future research should help further define the potential role of antioxidant supplementation to diet and exercise. Indeed, for many years, interest has focused on strategies that enhance removal of ROS using either antioxidants or drugs that enhance endogenous antioxidant defense [157,158]. Although those strategies have been effective in laboratory experiments, several trials have shown that they do not reduce cardiovascular events and in some cases have actually worsened the outcome [159]. An intriguing alternative approach to reduce oxidative stress is inhibiting ROS production by blocking enzymes involved in its synthesis. This hypothesis opens testing novel molecules that could interfere with the production of free radicals and may result in reversing, or even retarding, diseases caused by oxidative and inflammatory processes, such as the metabolic syndrome.

References

[1] Isomaa B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683–9.
[2] McNeill AM, Rosamond WD, Girman CJ, et al. The metabolic syndrome and 11-year risk of incident cardiovascular disease in the Atherosclerosis Risk in Communities Study. Diabetes Care 2005;28:385–90.
[3] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 2002;287:356–9.
[4] Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes Care 2005;28:2745–9.
[5] Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among U.S. adults. Diabetes Care 2004;27:2444–9.
[6] Aizawa Y, Watanabe H, Ramadan MM, et al. Clustering trend of components of metabolic syndrome. Int J Cardiol 2006.
[7] Pladevall M, Singal B, Williams LK, et al. A single factor underlies the metabolic syndrome: a confirmatory factor analysis. Diabetes Care 2006;29:113–22.
[8] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:2735–52.
[9] Kitano H, Oda K, Kimura T, et al. Metabolic syndrome and robustness tradeoffs. Diabetes 2004;53:S6–S15.
[10] Ahn AC, Tewari M, Poon CS, Phillips RS. The clinical applications of a systems approach. PLoS Med 2006;3:e209.

[11] Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004;25:4–7.
[12] Aguilar-Salinas CA, Rojas R, Gonzalez-Villalpando C, et al. Design and validation of a population-based definition of the metabolic syndrome. Diabetes Care 2006;29:2420–6.
[13] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005;115:1111–9.
[14] Stocker R, Keaney Jr JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004;84:1381–478.
[15] Furukawa S, Fujita T, Shimabukuro M, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–61.
[16] Grattagliano I, Vendemiale G, Boscia F, et al. Oxidative retinal products and ocular damages in diabetic patients. Free Radic Biol Med 1998;25:369–72.
[17] Grattagliano I, Vendemiale G, Caraceni P, et al. Starvation impairs antioxidant defense in fatty livers of rats fed a choline-deficient diet. J Nutr 2000;130:2131–6.
[18] Portincasa P, Grattagliano I, Palmieri VO, Palasciano G. Nonalcoholic steatohepatitis: recent advances from experimental models to clinical management. Clin Biochem 2005;38:203–17.
[19] Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997;82:291–5.
[20] Vendemiale G, Grattagliano I, Altomare E. An update on the role of free radicals and antioxidant defense in human disease. Int J Clin Lab Res 1999;29:49–55.
[21] Knopp RH, Paramsothy P. Oxidized LDL and abdominal obesity: a key to understanding the metabolic syndrome. Am J Clin Nutr 2006;83(1):1–2.
[22] Saez GT, Tormos C, Giner V, et al. Factors related to the impact of antihypertensive treatment in antioxidant activities and oxidative stress by-products in human hypertension. Am J Hypertens 2004;17: 809–16.
[23] Palmieri VO, Grattagliano I, Portincasa P, Palasciano G. Systemic oxidative alterations are associated with visceral adiposity and liver steatosis in patients with metabolic syndrome. J Nutr 2006;136: 3022–6.
[24] Couillard C, Ruel G, Archer WR, et al. Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. J Clin Endocrinol Metab 2005;90:6454–9.
[25] Keaney Jr JF, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003;23:434–9.
[26] Pinzani M, Marra F, Carloni V. Signal transduction in hepatic stellate cells. Liver 1998;18:2–13.
[27] Suthanthiran M, Anderson ME, Sharma VK, Meister A. Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. Proc Natl Acad Sci U S A 1990;87:3343–7.
[28] Sahaf B, Heydari K, Herzenberg LA, Herzenberg LA. The extracellular microenvironment plays a key role in regulating the redox status of cell surface proteins in HIV-infected subjects. Arch Biochem Biophys 2005;434:26–32.
[29] Vendemiale G, Guerrieri F, Grattagliano I, et al. Mitochondrial oxidative phosphorylation and intracellular glutathione compartmentation during rat liver regeneration. Hepatology 1995;21:1450–4.
[30] Matsuzawa Y. The metabolic syndrome and adipocytokines. FEBS Lett 2006;580:2917–21.
[31] Liu KH, Chan YL, Chan WB, et al. Mesenteric fat thickness is an independent determinant of metabolic syndrome and identifies subjects with increased carotid intima-media thickness. Diabetes Care 2006;29:379–84.
[32] Stolk RP, Meijer R, Mali WP, Grobbee DE, van der GY. Ultrasound measurements of intraabdominal fat estimate the metabolic syndrome better than do measurements of waist circumference. Am J Clin Nutr 2003;77:857–60.
[33] Diehl AM. Tumor necrosis factor and its potential role in insulin resistance and nonalcoholic fatty liver disease. Clin Liver Dis 2004;8: 619–38.
[34] Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: Cytokine–adipokine interplay and regulation of insulin resistance. Gastroenterology 2006;131:934–45.
[35] Vigano M, Vergani A, Trombini P, et al. Insulin resistance influences iron metabolism and hepatic steatosis in type II diabetes. Gastroenterology 2000;118:986–7.
[36] Grattagliano I, Caraceni P, Portincasa P, et al. Adaptation of subcellular glutathione detoxification system to stress conditions in choline-deficient diet induced rat fatty liver. Cell Biol Toxicol 2003; 19:355–66.
[37] Portincasa P, Grattagliano I, Lauterburg BH, et al. Liver breath tests non-invasively predict higher stages of non-alcoholic steatohepatitis. Clin Sci (Lond) 2006;111:135–43.
[38] James OF, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol 1998;29:495–501.
[39] Dalen KT, Ulven SM, Arntsen BM, et al. PPARalpha activators and fasting induce the expression of adipose differentiation-related protein in liver. J Lipid Res 2006;47:931–43.
[40] Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 2006;101:2629–40.
[41] Robertson G, Leclercq I, Farrell GC. Nonalcoholic steatosis and steatohepatitis: II. Cytochrome *P*-450 enzymes and oxidative stress. Am J Physiol Gastrointest Liver Physiol 2001;281:G1135–9.
[42] Caldwell SH, Swerdlow RH, Khan EM, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 1999;31: 430–4.
[43] Hashimoto T, Fujita T, Usuda N, et al. Peroxisomal and mitochondrial fatty acid beta-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl–CoA oxidase. Genotype correlation with fatty liver phenotype. J Biol Chem 1999;274:19228–36.
[44] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.
[45] Videla LA, Rodrigo R, Araya J, Poniachik J. Oxidative stress and depletion of hepatic long-chain polyunsaturated fatty acids may contribute to nonalcoholic fatty liver disease. Free Radic Biol Med 2004;37:1499–507.
[46] Casini A, Ceni E, Salzano R, et al. Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: role of nitric oxide. Hepatology 1997;25:361–7.
[47] Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004;99: 1497–502.
[48] Kim HC, Choi SH, Shin HW, et al. Severity of ultrasonographic liver steatosis and metabolic syndrome in Korean men and women. World J Gastroenterol 2005;11:5314–21.
[49] Diehl AM. Fatty liver, hypertension, and the metabolic syndrome. Gut 2004;53:923–4.
[50] Donati G, Stagni B, Piscaglia F, et al. Increased prevalence of fatty liver in arterial hypertensive patients with normal liver enzymes: role of insulin resistance. Gut 2004;53:1020–3.
[51] Gholam PM, Flancbaum L, Machan JT, et al. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol 2007;102: 399–408.
[52] Caraceni P, Bianchi C, Domenicali M, et al. Impairment of mitochondrial oxidative phosphorylation in rat fatty liver exposed to preservation–reperfusion injury. J Hepatol 2004;41:82–8.
[53] Feldstein AE, Canbay A, Guicciardi ME, et al. Diet associated hepatic steatosis sensitizes to Fas mediated liver injury in mice. J Hepatol 2003;39:978–83.

[54] Solga SF, Diehl AM. Non-alcoholic fatty liver disease: lumen–liver interactions and possible role for probiotics. J Hepatol 2003;38: 681–7.
[55] Kawano H, Motoyama T, Hirashima O, et al. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. J Am Coll Cardiol 1999;34:146–54.
[56] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.
[57] Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med 2006;40:183–92.
[58] Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405–12.
[59] Davi G, Ciabattoni G, Consoli A, et al. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation 1999;99:224–9.
[60] Keaney Jr JF, Loscalzo J. Diabetes, oxidative stress, and platelet activation. Circulation 1999;99:189–91.
[61] Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997;96: 25–8.
[62] Nishio E, Watanabe Y. Glucose-induced down-regulation of NO production and inducible NOS expression in cultured rat aortic vascular smooth muscle cells: role of protein kinase C. Biochem Biophys Res Commun 1996;229:857–63.
[63] Tesfamariam B. Free radicals in diabetic endothelial cell dysfunction. Free Radic Biol Med 1994;16:383–91.
[64] Bonnefont-Rousselot D. Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care 2002;5:561–8.
[65] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24:816–23.
[66] Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787–90.
[67] Basta G, Schmidt AM, de CR. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004;63:582–92.
[68] Yan SD, Schmidt AM, Anderson GM, et al. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 1994;269: 9889–97.
[69] Ramana KV, Chandra D, Srivastava S, et al. Nitric oxide regulates the polyol pathway of glucose metabolism in vascular smooth muscle cells. FASEB J 2003;17:417–25.
[70] Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and aging. Biochem J 1988;256:205–12.
[71] Grattagliano I, Russmann S, Palmieri VO, et al. Low membrane protein sulfhydrils but not G6PD deficiency predict ribavirin-induced hemolysis in hepatitis C. Hepatology 2004;39:1248–55.
[72] Morre DM, Lenaz G, Morre DJ. Surface oxidase and oxidative stress propagation in aging. J Exp Biol 2000;203:1513–21.
[73] Steinberg HO, Baron AD. Vascular function, insulin resistance and fatty acids. Diabetologia 2002;45:623–34.
[74] Stuhlinger MC, Tsao PS, Her JH, et al. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 2001;104:2569–75.
[75] Moreno PR, Fuster V. New aspects in the pathogenesis of diabetic atherothrombosis. J Am Coll Cardiol 2004;44:2293–300.
[76] Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–8.
[77] Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. Hypertension 1995;25:155–61.
[78] Nathan C. Points of control in inflammation. Nature 2002;420: 846–52.
[79] Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities — the role of insulin resistance and the sympathoadrenal system. N Engl J Med 1996;334:374–81.
[80] Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. Free Radic Biol Med 2005;39(7): 841–52.
[81] Lim SC, Caballero AE, Smakowski P, et al. Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. Diabetes Care 1999; 22:1865–70.
[82] Weyer C, Yudkin JS, Stehouwer CD, et al. Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. Atherosclerosis 2002;161: 233–42.
[83] Jessup W, Dean RT, de Whalley CV, et al. The role of oxidative modification and antioxidants in LDL metabolism and atherosclerosis. Adv Exp Med Biol 1990;264:139–42.
[84] Farvid MS, Jalali M, Siassi F, Hosseini M. Comparison of the effects of vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. Diabetes Care 2005;28: 2458–64.
[85] Martensson J, Meister A. Glutathione deficiency decreases tissue ascorbate levels in newborn rats: ascorbate spares glutathione and protects. Proc Natl Acad Sci U S A 1991;88:4656–60.
[86] Rimm EB, Stampfer MJ, Ascherio A, et al. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993; 328:1450–6.
[87] Jialal I, Grundy SM. Effect of dietary supplementation with alpha-tocopherol on the oxidative modification of low density lipoprotein. J Lipid Res 1992;33:899–906.
[88] Montonen J, Knekt P, Jarvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 2004;27:362–6.
[89] Lavine JE. Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. J Pediatr 2000;136:734–8.
[90] Moreau KL, Gavin KM, Plum AE, Seals DR. Ascorbic acid selectively improves large elastic artery compliance in postmenopausal women. Hypertension 2005;45:1107–12.
[91] May JM, Qu ZC. Transport and intracellular accumulation of vitamin C in endothelial cells: relevance to collagen synthesis. Arch Biochem Biophys 2005;434:178–86.
[92] Schneider MP, Delles C, Schmidt BM, et al. Superoxide scavenging effects of *N*-acetylcysteine and vitamin C in subjects with essential hypertension. Am J Hypertens 2005;18:1111–7.
[93] Beckman JA, Goldfine AB, Gordon MB, Creager MA. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 2001;103:1618–23.
[94] Guzik TJ, Harrison DG. Vascular NADPH oxidases as drug targets for novel antioxidant strategies. Drug Discov Today 2006;11:524–33.
[95] Mato JM, Lu SC. Homocysteine, the bad thiol. Hepatology 2005;41: 976–9.
[96] Guzik TJ, Sadowski J, Kapelak B, et al. Systemic regulation of vascular NAD(P)H oxidase activity and nox isoform expression in human arteries and veins. Arterioscler Thromb Vasc Biol 2004;24: 1614–20.
[97] Spiekermann S, Landmesser U, Dikalov S, et al. Electron spin resonance characterization of vascular xanthine and NAD(P)H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. Circulation 2003;107:1383–9.
[98] Azumi H, Inoue N, Ohashi Y, et al. Superoxide generation in directional coronary atherectomy specimens of patients with angina

pectoris: important role of NAD(P)H oxidase. Arterioscler Thromb Vasc Biol 2002;22:1838–44.
[99] Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86:494–501.
[100] Warnholtz A, Nickenig G, Schulz E, et al. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin–angiotensin system. Circulation 1999;99:2027–33.
[101] Namekata K, Enokido Y, Ishii I, et al. Abnormal lipid metabolism in cystathionine beta-synthase-deficient mice, an animal model for hyperhomocysteinemia. J Biol Chem 2004;279:52961–9.
[102] Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998;49:31–62.
[103] Hayden MR, Tyagi SC. Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: the pleiotropic effects of folate supplementation. Nutr J 2004;3:4.
[104] Lipton SA, Choi YB, Pan ZH, et al. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 1993;364:626–32.
[105] Ji C, Kaplowitz N. Hyperhomocysteinemia, endoplasmic reticulum stress, and alcoholic liver injury. World J Gastroenterol 2004;10: 1699–708.
[106] Adinolfi LE, Ingrosso D, Cesaro G, et al. Hyperhomocysteinemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients. Hepatology 2005;41:995–1003.
[107] Upchurch Jr GR, Welch GN, Fabian AJ, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. J Biol Chem 1997;272:17012–7.
[108] Myers PR, Minor Jr RL, Guerra Jr R. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble *S*-nitrosocysteine than nitric oxide. Nature 1990;345:161–3.
[109] Quintana A, Rodriguez JV, Scandizzi A, Guibert EE. Effect of *S*-nitrosoglutathione (GSNO) added to the University of Wisconsin solution (UW): I. Morphological alteration during cold preservation/reperfusion of rat liver. Int J Surg Investig 2001;2:401–11.
[110] Modica S, Moschetta A. Nuclear bile acid receptor FXR as pharmacological target: are we there yet? FEBS Lett 2006;580: 5492–9.
[111] Shulman AI, Mangelsdorf DJ. Retinoid X receptor heterodimers in the metabolic syndrome. N Engl J Med 2005;353:604–15.
[112] Struben VM, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. Am J Med 2000; 108:9–13.
[113] Willner IR, Waters B, Patil SR, et al. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. Am J Gastroenterol 2001;96:2957–61.
[114] Day CP. From fat to inflammation. Gastroenterology 2006;130: 207–10.
[115] Bernard S, Touzet S, Personne I, et al. Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients with type II diabetes. Diabetologia 2000;43:995–9.
[116] Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–92.
[117] Merriman RB, Aouizerat BE, Bass NM. Genetic influences in nonalcoholic fatty liver disease. J Clin Gastroenterol 2006;40:S30–3.
[118] Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med 2002;53:409–35.
[119] Sapone A, Peters JM, Sakai S, et al. The human peroxisome proliferator-activated receptor alpha gene: identification and functional characterization of two natural allelic variants. Pharmacogenetics 2000;10:321–33.
[120] Ip E, Farrell GC, Robertson G, et al. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology 2003;38:123–32.
[121] Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288–95.
[122] Sekiya M, Yahagi N, Matsuzaka T, et al. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. Hepatology 2003;38:1529–39.
[123] George DK, Goldwurm S, MacDonald GA, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 1998;114:311–8.
[124] Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, *HFE* mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology 2004;39:179–87.
[125] Trichopoulos D, Lagiou P. Dietary patterns and mortality. Br J Nutr 2001;85:133–4.
[126] Kaput J. Nutrigenomics — 2006 update. Clin Chem Lab Med 2007; 45:279–87.
[127] Kaput J, Noble J, Hatipoglu B, et al. Application of nutrigenomic concepts to type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2007;17:89–103.
[128] Griffin JL, Nicholls AW. Metabolomics as a functional genomic tool for understanding lipid dysfunction in diabetes, obesity and related disorders. Pharmacogenomics 2006;7:1095–107.
[129] Weindruch R, Sohal RS. Seminars in Medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. N Engl J Med 1997;337:986–94.
[130] Rebrin I, Kamzalov S, Sohal RS. Effects of age and caloric restriction on glutathione redox state in mice. Free Radic Biol Med 2003;35: 626–35.
[131] Domenicali M, Vendemiale G, Serviddio G, et al. Oxidative injury in rat fatty liver exposed to ischemia–reperfusion is modulated by nutritional status. Dig Liver Dis 2005;37:689–97.
[132] Cocco T, Sgobbo P, Clemente M, et al. Tissue-specific changes of mitochondrial functions in aged rats: effect of a long-term dietary treatment with *N*-acetylcysteine. Free Radic Biol Med 2005;38: 796–805.
[133] Grattagliano I, Portincasa P, Cocco T, et al. Effect of dietary restriction and *N*-acetylcysteine supplementation on intestinal mucosa and liver mitochondrial redox status and function in aged rats. Exp Gerontol 2004;39:1323–32.
[134] Das UN. A defect in the activity of Delta6 and Delta5 desaturases may be a factor predisposing to the development of insulin resistance syndrome. Prostaglandins Leukot Essent Fatty Acids 2005;72: 343–50.
[135] Howitz KT, Bitterman KJ, Cohen HY, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. Nature 2003; 425:191–6.
[136] Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006;444:337–42.
[137] Sorensen LB, Raben A, Stender S, Astrup A. Effect of sucrose on inflammatory markers in overweight humans. Am J Clin Nutr 2005;82:421–7.
[138] Fernandez de la Puebla RA, Fuentes F, Perez-Martinez P, et al. A reduction in dietary saturated fat decreases body fat content in overweight, hypercholesterolemic males. Nutr Metab Cardiovasc Dis 2003;13:273–7.
[139] Pitsavos C, Panagiotakos DB, Chrysohoou C, et al. The effect of Mediterranean diet on the risk of the development of acute coronary syndromes in hypercholesterolemic people: a case–control study (CARDIO2000). Coron Artery Dis 2002;13:295–300.
[140] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599–608.
[141] Fito M, Cladellas M, de la TR, et al. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. Atherosclerosis 2005;181:149–58.
[142] Sessler AM, Ntambi JM. Polyunsaturated fatty acid regulation of gene expression. J Nutr 1998;128:923–6.

[143] Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. J Nutr 2001;131:1129–32.
[144] Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. Int J Epidemiol 1997;26:1–13.
[145] He FJ, Nowson CA, Macgregor GA. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. Lancet 2006;367: 320–6.
[146] Rafique R, Schapira AH, Cooper JM. Sensitivity of respiratory chain activities to lipid peroxidation: effect of vitamin E deficiency. Biochem J 2001;357:887–92.
[147] Dufour JF, Oneta CM, Gonvers JJ, et al. Randomized placebo-controlled trial of ursodeoxycholic acid with vitamin E in nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2006;4:1537–43.
[148] Bugianesi E, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol 2005;100:1082–90.
[149] Harrison SA, Torgerson S, Hayashi P, et al. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2003;98:2485–90.
[150] Craig SA. Betaine in human nutrition. Am J Clin Nutr 2004;80: 539–49.
[151] Vincent JB. Recent advances in the nutritional biochemistry of trivalent chromium. Proc Nutr Soc 2004;63:41–7.
[152] Anderson RA. Nutritional factors influencing the glucose/insulin system: chromium. J Am Coll Nutr 1997;16:404–10.
[153] Ghosh D, Bhattacharya B, Mukherjee B, et al. Role of chromium supplementation in Indians with type 2 diabetes mellitus. J Nutr Biochem 2002;13:690–7.
[154] Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. Hepatology 2004;39:647–1654.
[155] Ivy JL. Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. Sports Med 1997;24:321–36.
[156] McCord JM. Therapeutic control of free radicals. Drug Discov Today 2004;9:781–2.
[157] Day BJ. Catalytic antioxidants: a radical approach to new therapeutics. Drug Discov Today 2004;9:557–66.
[158] Grattagliano I, Wieland P, Schranz C, Lauterburg BH. Disposition of glutathione monoethyl ester in the rat: glutathione ester is a slow release form of extracellular glutathione. J Pharmacol Exp Ther 1995;272:484–8.
[159] Guallar E, Hanley III DF. An editorial update: annus horribilis for vitamin E. Ann Intern Med 2005;143:143–5.