

Role of oxidative stress in pathogenesis of metabolic syndrome

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Abstract

The metabolic syndrome (MS) recognized as a major cause of type 2 diabetes and cardiovascular diseases, has become one of the major public health challenges worldwide. The pathogenesis of the metabolic syndrome is multiple and still poorly understood. No single factor has yet been identified as an underlying causal factor. There is a growing belief, however, that obesity, specially visceral obesity, may play an important role in the development of the syndrome. Visceral adiposity seems to be an independent predictor of insulin sensitivity, impaired glucose tolerance, dyslipidaemia and elevated blood pressure. An increasing number of studies confirm that oxidative stress, chronic inflammation and angiogenesis all play important roles in the pathogenesis of MS. Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes. Oxidative stress occurs in a cellular system when the production of free radical moieties exceeds the antioxidant capacity of that system. If cellular antioxidants do not remove free radicals, radicals attack and damage proteins, lipids, and nucleic acids. The oxidized or nitrosylated products of free radical attack have decreased biological activity, leading to loss of energy metabolism, cell signaling, transport, and other major functions. These altered products are also targeted for proteasome degradation, further decreasing cellular function. Accumulation of such injury ultimately leads a cell to die through necrotic or apoptotic mechanisms. In conclusion, a puzzle of many pieces of evidence suggests that free radical overgeneration may be considered the key in the generation of insulin resistance, diabetes, and cardiovascular disease.

Key words: Oxidative stress, Metabolic syndrome, Pathogenesis, Diabetes, Cardiovascular diseases.

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Oxygen is vital for most organisms, but, paradoxically might be the source of molecules that can damage key biological sites. Reactive oxygen and nitrogen species (RONS) are continuously produced in the body as by-products of the reaction leading to energy production through the mitochondrial and microsomal electron-transport chains. Other endogenous sources of RONS *in vivo* are oxidative bursts in phagocytes and enzyme systems such as xanthine oxidase and cytochrome P-450 oxidase. Exogenous sources are represented by cigarette smoking, pollution, physical exercise etc. Physiological levels of RONS are crucial for a proper cell function (i.e. intracellular signalling, inflammation and immune function); problems arise when RONS levels largely exceed their physiological concentration leading to oxidativestress. Oxidative stress, the shift of the redox balance through oxidative potentials, may damage biological molecules altering cell function and leading to cell death. Antioxidants, molecules that inhibit or prevent oxidation of a substrate, evolved to protect biological systems against damage induced by RONS. An increasing number of studies confirm that oxidative stress play important roles in the pathogenesis of different diseases.

The mechanisms of oxidative stress in animal models and status of oxidative stress in different diseases were investigated by Mahjoub et al. in the population of Babol, North of Iran (1-12). Also, the prevalence and risk factors of metabolic syndrome in the population of Babol were investigated by Mahjoub and colleagues (13, 14).

The metabolic syndrome (MS), a cluster of several risk factors for diabetes and cardiovascular disease, major causes of morbidity and death, is a highly prevalent condition in the world. Etiopathogenesis of MS is highly complex and participating factors multiple and highly varied. Despite the availability of diverse treatment tools and methodologies, the problem persists in its increasing tendency. This fact shows the necessity to improve the knowledge on MS, both in its overall complexity and in its biochemical mechanisms. An increasing number of studies confirm that oxidative stress, chronic inflammation and angiogenesis all play important roles in the pathogenesis of the MS. Although many growth factors and cytokines have been reported to interfere somehow in those entities, the precise interplay of those effectors among them and towards the MS is not yet clear. Most of the published studies focus on the epidemiology, clinical symptoms, association between features of MS, or the respective prevention/treatment strategies (14-17).

In the present review, the knowledge gathered on MS as a whole, as well as on the implication of mechanisms of oxidative stress, chronic inflammation and angiogenesis in its development and progression is critically reviewed and discussed. It, thus, allows an integrated view of the condition, favoring a holistic approach towards preventive and therapeutic possibilities.

Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes (18, 19). Diabetes is an epidemic in developed countries. In the United States, 16 million individuals are diabetic, and the number is increasing at a rate of 5% per year. The major form of diabetes in the population is type 2, which accounts for up to 95% of diabetes cases in the United States (20). Among children, type 1 diabetes poses a greater risk, although this may change in the future because the rate of type 2 diabetes in children and adolescents is increasing (21). The microvascular complications of diabetes carry a high morbidity and, when coupled with macrovascular complications, high mortality results (22).

Several free radical species are normally produced in the body to perform specific functions. Superoxide (O_2^-),

hydrogen peroxide (H_2O_2), and nitric oxide (NO) are three free radical reactive oxygen species (ROS) that are essential for normal physiology, but are also believed to accelerate the process of aging and to mediate cellular degeneration in disease states. These agents together produce highly active singlet oxygen, hydroxyl radicals, and peroxyxynitrite that can attack proteins, lipids, and DNA.

A. Superoxide O_2^-

Superoxide O_2^- is generated by the mitochondrial electron transfer chain during the oxidation of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD^+) and also as a by-product of many enzymes that act as oxidases. Approximately 4% of electrons that enter the respiratory chain lead to the formation of O_2^- (23). The beneficial effects of O_2^- include regulation of vascular function, cell division (24), inflammation (25), apoptosis (26), and bactericidal activity of neutrophils (27). Decreased levels of O_2^- can lead to an increased susceptibility to bacterial infections, as illustrated in Down's syndrome patients with elevated cytoplasmic superoxide dismutase (SOD)1 (28). Thus, cellular levels of O_2^- are under tight regulation. Excess O_2^- is removed through the activity of a family of SOD enzymes that convert O_2^- to H_2O_2 and oxygen. O_2^- overproduction occurs in complication-prone tissues when cellular metabolism is perturbed by excess glucose.

ATP synthase is inhibited, and electron transfer slows. This can cause overproduction of O_2^- in two ways. First, the half-life of highly reactive quinone intermediates is prolonged, increasing the release of electrons to combine with molecular oxygen and form O_2^- . Second, when electron transfer no longer can regenerate NAD^+ , the enzyme NADH oxidase is activated and generates O_2^- as a byproduct.

B. Hydrogen peroxide (H_2O_2)

H_2O_2 is produced after the spontaneous or SOD-catalyzed dismutation of O_2^- as well as many other enzymatic reactions. Unlike O_2^- , which remains at the site of production, H_2O_2 can diffuse across membranes and through the cytosol (29). This ROS is another component of leukocyte-mediated defense against bacteria. Because H_2O_2 is a powerful oxidizing agent, cells express abundant catalase, glutathione (GSH), and thioredoxin (Trx) that convert H_2O_2 to water. When H_2O_2 reacts with free Fe^{2+} , the iron is oxidized and hydroxyl radicals are produced. There are many severe consequences of hydroxyl radical

production, including loss of vasodilation that can lead to endothelial injury and tissue hypoxia (30).

Hyperglycemia activates many signaling mechanisms in cells. Four major pathways that can lead to cell injury downstream of hyperglycemia are illustrated. 1) Excess glucose shunts to the polyol pathway that depletes cytosolic NADPH and subsequently GSH. 2) Excess glucose also undergoes auto oxidation to produce AGEs that impair protein function and also activate RAGEs that use ROS as

second messengers. 3) PKC activation both further increases hyperglycemia and also exacerbates tissue hypoxia. 4) Overload and slowing of the electron transfer chain leads to escape of reactive intermediates to produce O_2^- as well as activation of NADH oxidase that also produces O_2^- . A unifying mechanism of injury in each case is the production of ROS that impair protein and gene function. TCA, Trichloroacetic acid; PAI-1, plasminogen activator inhibitor-1 (figure 1) (31).

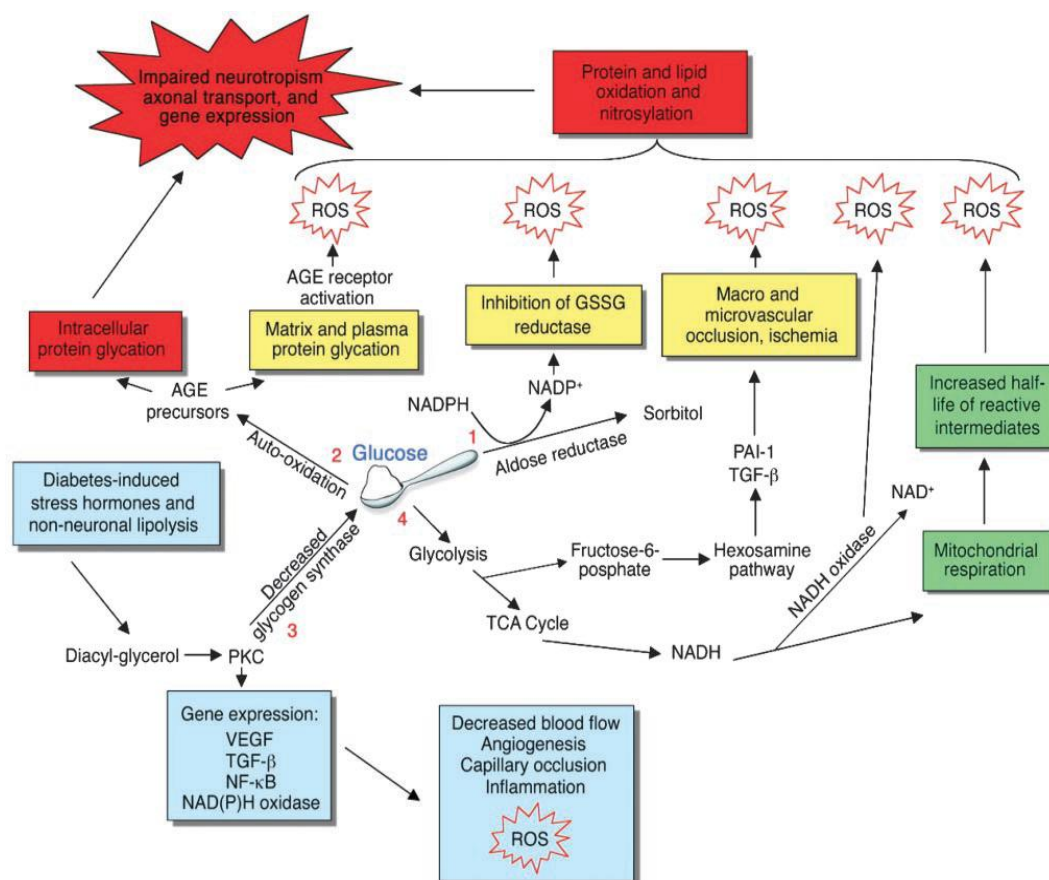


Figure 1. Four major pathways that hyperglycemia can lead to cell injury via oxidative stress (31).

C. Nitric oxide (NO)

NO is generated through the activity of a cytosolic enzyme known as NO synthase (NOS). There are both constitutively expressed calcium-dependent isoforms of NOS and an inducible isoform that is associated with inflammation and cell activation (32). NO plays a major role in regulating vascular tone by activating soluble guanylate cyclases that regulate ion channels. In addition, NO modulates cellular respiration through direct inhibition of cytochrome oxidase by competitively occupying the oxygen-

binding site (33). The inducible form of NOS is increased in the arteries of diabetic rats (34). Damaged neurons recover more slowly in the presence of NO, and conversely, NOS inhibitors promote neuronal recovery from injury (35). NO is also believed to act as a neurotransmitter (36). The dual role of NO as both beneficial and detrimental is illustrated in stroke models. Under ischemic insult, endothelial NO produces vasodilation that can improve blood flow, but neuronal NO is produced down stream of calcium dysregulation and can prevent energy generation in the

mitochondria (37). More importantly, NO acts as an antioxidant in certain environments and prevents lipid peroxidation (38). However, when $O_2^{\cdot-}$ increases, NO reacts with the $O_2^{\cdot-}$ to form peroxynitrite and becomes a prooxidant.

Cellular Injury through Excess ROS Production

The production of ROS is under tight control in healthy cells, but overproduction during metabolic dysfunction leads to cellular injury. Although both $O_2^{\cdot-}$ and NO are relatively inert, when they combine they form the highly reactive peroxynitrite that attacks and inhibits proteins and lipids. In addition, both $O_2^{\cdot-}$ and NO can attack iron-sulfur centers of enzymes and other proteins to release iron atoms and consequently inhibit enzyme/protein activities. There are many important proteins that are exquisitely sensitive to this type of inhibition including complexes I–III of the electron transfer chain, aconitase of the trichloroacetic acid cycle, and biotin synthase (39, 40). The formation of lipid, protein, and nucleic acid adducts involves a complex chain reaction using a range of biological substrates that contain reactive methylene groups. Intermediates in the chain reaction can have extremely high oxidative ability and so cellular damage can be extensive. The chemistry of these reactions has been reviewed previously (41, 42). Lipids present in plasma, mitochondrial, and endoplasmic reticulum membranes are major targets of ROS attack and peroxidation. End products of lipid peroxidation, known as lipid peroxides, can be toxic to a cell and require removal by GSH as described below. Similarly, proteins and nucleic acids can be subject to peroxidation and nitrosylation. Although these end products are not usually directly toxic to the cell, accumulation of inactive proteins can overload the ability of a cell to recycle them, and damage of DNA is known to activate the mechanisms of apoptosis. In addition, accumulation of modified proteins decreases their function, leading to severe loss of normal activity. Axonal transport can be slowed, leading to decreased delivery of growth factors and intermediates from the synapse to the cell body and resulting in induction of apoptosis (43). Oxidative modification of transcription factors not only leads to decreased expression of many proteins such as apoptosis inhibitory factor, complex I, and Bcl-2, but also results in increased expression of stress proteins that may be proapoptotic, including cyclooxygenase 2, poly-ADP ribose polymerase, and Jun kinase (JNK) (44–47). Production of ROS in all cells not only results in deleterious events but also can play a role in

differentiation and development. Redox status can have profound effects on gene expression, so that oxidative stress increases growth factors, stress response elements, and apoptosis pathways (48). In contrast, certain proteins including cytokines, cytochrome c oxidase, and enzymes involved in glucose respiration are repressed by oxidative stress signaling (49). Understanding of gene regulation by reactive oxygen intermediates is rapidly expanding. Once the mechanisms are more fully understood, the ability of a cell to respond to stress by changing gene expression may provide an important therapeutic target. The most significant consequence of oxidative stress in dividing cells may be DNA modifications that produce genomic instability and mutations (50). Nondividing neurons may suffer less from oxidative damage of DNA. Yet, mitochondrial DNA is particularly sensitive to oxidative damage (51), which would impair energy regulation and thus would be critically important in high energy-requiring neurons. Oxidative stress-mediated neuronal degeneration is implicated in several types of neurodegenerative disease (52–54). In nondividing cells like neurons, damage to proteins and lipids may be more injurious than DNA damage, because this may render proteins unable to perform axonal transport and signaling (43). For example, synaptosomal membranes as well as cytosolic proteins become oxidized, and these changes can be correlated to alterations in brain function (55). Loss of function in neurons rapidly promotes necrotic or apoptotic mechanisms (53, 56).

In the past few decades, type 2 diabetes mellitus (T2DM) has rapidly increased in the world. It has been estimated that the number of diabetic patients will become more than double within 15 years (57). Moreover, although T2DM was previously considered a slow-onset disease of middle-aged and older subjects, an emerging issue is the recent increase in diagnoses of T2DM and prediabetic conditions in children (58). T2DM is mainly characterized by the development of increased morbidity and mortality for cardiovascular disease (CVD) (59), so that it is suggested that diabetes may be considered a cardiovascular disease (60). However, CVD risk is elevated long before the development of diabetes (61). The close relationship between T2DM and CVD has led to the “common soil” hypothesis (62), postulating that T2DM and CVD share common genetic and environmental antecedents. One of the most important roles of these possible antecedents is considered insulin resistance. In genetically predisposed subjects, the combination of excess

caloric intake and relatively scarce physical activity, with the likely consequence of obesity, can induce a state of resistance to the action of insulin (63). Insulin resistance is an important component of the metabolic syndrome, first described as a clinical syndrome in which the clustering of factors such as obesity, dyslipidemia, and hypertension leads to a substantial increase in CVD risk (64). Insulin resistance is also a crucially important metabolic abnormality in T2DM, and overt diabetes is thought to be preceded by a long period of insulin resistance, during which blood glucose is maintained near normal levels by compensatory hyperinsulinemia (63). When cells are no longer able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance (IGT) appears. This condition is characterized by an excessive blood glucose concentration in the postprandial phase, with fasting normal range. Persistence of imbalance between caloric intake and expenditure eventually leads to overt diabetes, characterized by high glycemia in any condition whether fasting or postprandial (63).

Because evidence suggests that overnutrition, insulin resistance, IGT, diabetes, and CVD share in common the presence of an oxidative stress (65-67), in this review article, oxidative stress generation is proposed as the common persistent pathogenic factor mediating the appearance of insulin resistance as well as the passage from insulin resistance to overt diabetes, via IGT, while producing the increased cardiovascular risk condition typical of prediabetic and diabetic subjects by favouring atherosclerotic complications. This hypothesis may help us understand why diverse therapeutic interventions, which have in common the ability to reduce oxidative stress, can impede or delay the onset of diabetes and CVD.

Production of ROS in Diabetes

One unifying mechanism of injury in diabetes lies in the ability of both metabolic and vascular insults to increase cellular oxidative stress and impair the function of mitochondria (68, 69). Recent studies have supported this hypothesis, including *in vivo* and *in vitro* measurement of oxidative stress in sensory neurons as well as neuronal protection by antioxidants. *In vitro*, application of 10–20 mm glucose to dorsal root ganglia neurons leads to production of O₂⁻ and H₂O₂ that leads to lipid oxidation and neuronal death. This glucose-induced death is prevented by IGF-I, in part through decreased ROS production (70). Further evidence comes from feeding mice with a high-

glucose diet. In this case, the mice experience hyperglycemia that leads to free radical production and oxidative stress (71). There is a close correlation between oxidative stress in diabetes and the development of complications. In type 1 diabetic patients, oxidative stress is evident within a few years of diagnosis before the onset of complications. As the disease progresses, antioxidant potential decreases, and plasma lipid peroxidation products increase depending upon the level of glycemic control (72). Type 2 diabetic patients have increased lipid peroxidation compared with age matched control subjects, as well as decreased plasma GSH and GSH-metabolizing enzymes and antioxidant potential, all of which relate directly to the rate of development of complications (73, 74). Similarly, oxidative stress is linked to preclinical features of disease, such as vascular endothelial activation that can lead to atherosclerosis (75). The lowered total antioxidant capacity may impair the protection against ROS and RNS causing O&NS and damage to fatty acids, proteins, DNA and mitochondria. The O&NS modified epitopes may mount an autoimmune response against these neopeptides, which further may aggravate the initial inflammatory response. These pathways in turn may induce depression and the (neuro) degenerative processes that accompany depression (figure 2) (76).

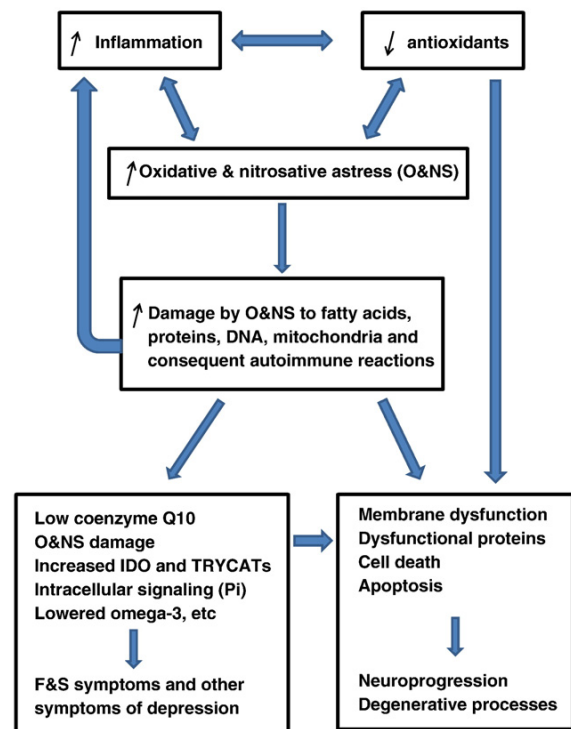


Figure 2. Oxidative and nitrosative stress (O&NS) (76).

Biomarkers of Oxidative Stress

Measuring biomarkers of oxidative stress is an essential step toward better a understanding of the pathogenesis and developing treatments for diabetic neuropathy. There are several approaches that may be adopted, including measurements of the depletion of antioxidant reserves, changes in the activities of antioxidant enzymes, free radical production, and presence of protein, lipid, and DNA free radical adducts. For the purposes of clinical assessment, measurements of end products of free radical attack may be the most reliable determination of the occurrence of oxidative stress because enzyme activities and cellular antioxidants are likely to display transient changes. Yet, the other measures also have utility depending on the nature of the study. The presence of oxidative stress in biological fluids can be simply assessed by examination of spontaneous visible luminescence. This phenomenon is the result of oxidized biomolecules with long half-life luminescent intermediates (77).

Measures of spontaneous luminescence increased in the urine of patients with known oxidative stress such as hyperthyroid and muscular dystrophy patients or smokers compared with healthy controls (78). At present, this method is not routinely used in diabetes studies, because more specific end points are selected. These analyses can be performed not only on tissue but also on plasma, urine and saliva. Urine analysis can reveal nitrosylated proteins (79), lipid oxidation products such as 8-isoprostanes (80), and the DNA adduct 8-hydroxy-2-deoxyguanosine (8-OH-2dG) (81). These three indicators, along with other lipid adducts, *i.e.*, malondialdehyde and 4-hydroxynonenyl and carbonyl derivatives of protein side chains, constitute the most common markers of oxidative stress in biological systems. Generally, measures of antioxidants or oxidized end products are more consistently performed in plasma than urine (1-12, 82). The excretion of 8-OH-2dG in urine may be misleading, because this parameter is more strongly influenced by the degree of oxygen consumption and activity of xenobioticmetabolizing enzymes (83).

Blood cell 8-OH-2dG is increased in both type 1 and type 2 diabetic patients (84).

Carbonylated proteins and peptides are also inactivated by oxidative stress (85). Measurements of protein carbonyls are highly sensitive, and they can be detected in the plasma of both type 1 and type 2 diabetic patients even without complications (86, 87).

Role of Oxidative Stress in Insulin Resistance

The most important tissues involved in the pathogenesis of insulin resistance are muscle and adipose tissue. When caloric intake exceeds the energy expenditure, the substrate-induced increase in citric acid cycle activity generates an excess of mitochondrial NADH (mNADH) and reactive oxygen species (ROS) (88). To protect themselves against harmful effects of ROS, cells may reduce the formation of ROS and/or enhance ROS removal. Prevention of ROS formation is accomplished by preventing the build-up of mNADH by inhibiting insulin stimulated nutrient uptake and preventing the entrance of energetic substrates (pyruvate, fatty acids) into the mitochondria. Controversy exists as to whether free fatty acid (FFA) or glucose is the primary fuel source in the overnourished muscle and adipose tissue. In either case, an influx of substrates into the citric acid cycle generates mitochondrial acetyl-CoA and NADH (88). Acetyl-CoA, derived either from glucose through pyruvate or from beta-oxidation of FFA, combines with oxaloacetate to form citrate, which enters the citric acid cycle and is converted to isocitrate. NAD-dependent isocitrate dehydrogenase generates NADH. When excessive NADH cannot be dissipated by oxidative phosphorylation (or other mechanisms), the mitochondrial proton gradient increases and single electrons are transferred to oxygen, leading to the formation of free radicals, particularly superoxide anion (89, 90). The generation of excessive NADH may be prevented in several ways, one of which is the inhibition of FFA oxidation (32). An increase in intracellular FFA, in turn, leads to reduced GLUT4 translocation to the plasma membrane, resulting in resistance to insulin stimulated glucose uptake in muscle and adipose tissue. In this setting, insulin resistance may be considered a compensatory mechanism that protects the cells against further insulin stimulated glucose and fatty acid uptake and therefore oxidative damage (91, 92). Many studies support this hypothesis: *in vitro* studies and in animal models, antioxidants have been shown to improve insulin sensitivity (90, 93).

Role of Oxidative Stress in Dysfunction of Beta and Endothelial Cells

It is a reasonable hypothesis that what happens in muscle and fat cells may also occur in other cells, particularly in beta-cells and endothelial cells. Moreover, these cell types may be particularly affected by overfeeding. These cells are notably not dependent on insulin for glucose uptake, which

here is via facilitative diffusion instead of insulin-regulated glucose transporters. Therefore, if overfed, they cannot down-regulate the influx of nutrients by means of insulin resistance, and must allow intracellular concentrations to increase further. Many studies have suggested that beta-cell dysfunction results from prolonged exposure to high glucose, elevated FFA levels, or a combination of both (93). Beta-Cells are particularly sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as catalase, glutathione per-oxidase, and superoxide dismutase (94). Therefore, the ability of oxidative stress to damage mitochondria and markedly blunt insulin secretion is not surprising (95).

Recent studies have suggested that beta-cell lipotoxicity is enhanced by concurrent hyperglycemia and that oxidative stress may be the mediator (96, 97). The response-to-injury hypothesis of atherosclerosis states that the initial damage affects the arterial endothelium in terms of endothelial dysfunction (98). Notably, today's evidence confirms that endothelial dysfunction, associated with oxidative stress, predicts cardiovascular disease (99, 100).

Role of Oxidative Stress in Inflammation, Insulin Resistance, Diabetes, and CVD

Although the concept of atherosclerosis as an inflammatory disease is now well established, line of evidence suggests that chronic inflammation may be involved in the pathogenesis of insulin resistance and T2DM. This lead to the hypothesis that inflammatory changes may be considered a common pathogenic step in all of these conditions (10, 11, 101).

The concept that oxidative stress is the common factor underlying insulin resistance, T2DM, and CVD, and may explain the presence of inflammation in all these conditions (2, 4, 9, 102, 103). It is well recognized that inflammation is one manifestation of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress (104).

Conclusion

In conclusion, a puzzle of many pieces of evidence suggests that free radical overgeneration may be considered the key in the generation of insulin resistance, diabetes, and cardiovascular disease.

References

1. Mahjoub S, Hasanjani Roshan MR, Gholami M. Evaluation of oxidative stress before and after treatment of patients with acute brucellosis. 12th Iranian Congress of Biochemistry and 4th International Congress of Biochemistry and Molecular Biology. 2011 Sep 6-9; Mashhad, Iran. Elsevier; Clinical Biochemistry 2011; 44: S365.
2. Mahjoub S, Jalali F, Seyyedi A. Status of Plasma Lipid Peroxidation in Patients with congestive Heart Failure. Euro Prevent Congress; 2009 may 6-9. Stockholm, Sweden. Eur J Cardiovasc Prev Rehabil 2009; 16: S 58.
3. Mahjoub S, Tamadoni A, Gorji R. Lipid and Protein Peroxidation in Patients with beta- Thalassemia Major and Intermedia. 3rd International Congress on Biochemistry and Molecular Biology (3rd ICBMB); 2009 Nov 16-19; Tehran Iran. J Iranian Chem Society, 2009; 6: 40.
4. Mahjoub S, Jalali F, Askari M. Plasma Total Antioxidant Capacity in Patients with Congestive Heart Failure. 16th Congress of Iranian Heart Association in collaboration with American College of Cardiology; 2008 Nov 18-21; Tehran Iran. Iranian Heart J 2008; Suppl 6: S55.
5. Mahjoub S, Tamadoni A, Nikoo M. Iron overload and oxidative stress in beta-Thalassemia patients in north of Iran. 11th Asian Pacific Congress of Clinical Biochemistry; 2007 Oct 14-16; Beijing, China. Chinese Med J 2007; 120: 187.
6. Mahjoub S, Tamadoni A, Nikoo M, Moghadamnia AA. Oral supplement of vitamin E and beta carotene reduce lipid & protein peroxidation of erythrocytes in beta-Thalassemia major patients. 9th Iranian Congress of Biochemistry and The Second International Congress of Biochemistry & Molecular Biology; 2007 Oct 29-Nov1; Shiraz, Iran. Arch Iran Med 2007; 10: S19.
7. Mahjoub S, Tamadoni M, Nikoo M, Moghadamnia AA. The effects of beta carotene and vitamin E on erythrocytes lipid peroxidation in beta-thalassemia. J Res Med Sci, 2007; 12: 301-7.
8. Sarkisian V, Dabidi Roshan V, Mahjoub S, Shahandeh S, Hosseinzadeh S. The effect of acute exercises on BDNF levels in rats' hippocampuse exposed by lead acetate. BECTHHK 2011; 16: 50-7.
9. Asali M, Dabidi Roshan V, Hosseinzadeh S, Mahjoub S, Hajizadeh Moghaddam A. The Role of Exercising and

- Curcumin on the Treatment of lead-induced Cardiotoxicity in Rats. *Iran J Health Phys Act* 2011; 2: 1-5.
10. Hosseinzadeh M, Dabidi-Roshan V, Hoseinzadeh S, Jolazadeh T, Mahjoub S. Lead Acetate, and Oxidant/Antioxidant, Inflammatory and Neurothrophic-Markers: Neuro and Cardio-protective Effects of Exercise training and Curcumin. 16th Annual Congress of the European College of Sport Science; 2011 July 6-9; Liverpool John Moores University, Liverpool, Uk.
 11. Azizi G, Mohamadi F, Hajizadeh A, Dabidi roshan V, Mahjoub S. The effect of continuous training on homocysteine-induced lipid peroxidation in the dorsal hippocampus of the male rats. *The First National Congress in Physiology of Exercise*; 2009 March 14-16 Kermansha, Iran. 2009.
 12. Gholami M, Hasanjani Roushan MR, Mahjoub S. How is total antioxidant status in plasma of Patients with Brucellosis? *Caspian J Intern Med* 2012. [In Press]
 13. Mahjoub S, Haji Ahmadi M, Faramarzi M, Jalali F, Moazzezi Z, Sajjadi P. How is Lipid Profile and Morbidity Risk in Smokers and Nonsmokers? *Casp J Intern Med* 2010; 1: 128-33..
 14. Moazzezi Z, Haji Ahmadi M, Mahjoub S, Ghorbani H, Faramarzi M Prevalence of the Metabolic Syndrome Defined Iranian National Committee of Obesity and ATP III Criteria among Adults in the Babol, Iran. *Casp J Intern Med* 2012. [In press]
 15. Balkau B, Valensi P, Eschwège E, Slama G. A review of the metabolic syndrome. *Diabetes Metab* 2007; 33: 405-13.
 16. Handelsman Y. Metabolic Syndrome Pathophysiology and Clinical Presentation *Toxicol Pathol* 2009; 37: 18-20.
 17. Moller DE, Kaufman KD. Metabolic Syndrome: A Clinical and Molecular Perspective. *Annu Rev Med* 2005; 56: 45-62.
 18. Rosen P, Nawroth PP, King G, et al. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 2001; 17:189-212.
 19. Greene DA, Sima AA, Stevens MJ, Feldman EL, Lattimer SA 1992Complications: neuropathy, pathogenetic considerations. *Diabetes Care* 1992; 15: 1902-25.
 20. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 2003; 290:1884-90.
 21. Fagot-Campagna A, Pettitt DJ, Engelgau MM. Type 2 diabetes among North American childrenand adolescents: an epidemiologic review and a public healthperspective. *J Pediatr* 2000; 136:664-72.
 22. Windebank AJ, Feldman EL Diabetes and the nervous system. In: Aminoff MJ *Neurology and general medicine*. 3 rd ed. London:Churchill Livingstone; 2001; pp: 341-64.
 23. Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 1995; 64: 97-112.
 24. Uemura S, Matsushita H, Li W, et al. 2001 Diabetes mellitus enhances vascularmatrix metalloproteinase activity: role of oxidative stress. *Circ Res* 2001; 88:1291-8.
 25. Khodr B, Khalil Z. Modulation of inflammation by reactiveoxygen species: implications for aging and tissue repair. *Free Radic Biol Med* 2001; 30:1-8.
 26. Lee MH, Hyun DH, Jenner P, Halliwell B. Effect of proteasomeinhibition on cellular oxidative damage, antioxidant defences and nitric oxide production. *J Neurochem* 2001; 78: 32-41.
 27. Vazifeh D, Abdelghaffar H, Labro MT. Effect of telithromycin (HMR 3647) on polymorphonuclear neutrophil killing of *Staphylococcus aureus* in comparison with roxithromycin. *Antimicrob Agents Chemother* 2001; 46: 1364-74.
 28. Sawa A. Alteration of gene expression in Down's syndrome (DS) brains: its significance in neurodegeneration. *J Neural Transm Suppl* 2001; 61: 361-1.
 29. Antunes F, Cadenas E. Estimation of H₂O₂ gradients across biomembranes. *FEBS Lett* 2000; 475: 121-6.
 30. Pieper GM, Langenstroer P, Gross GJ. Hydroxyl radicalsmediate injury to endothelium-dependent relaxation in diabeticrat. *Mol Cell Biochem* 1993; 122: 139-45.
 31. Feldman EL. Oxidative stress and diabetic neuropathy: a newunderstanding of an old problem. *J Clin Invest* 2003; 111:431-3.
 32. Liu B, Gao HM, Wang JY, et al. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann NY Acad Sci* 2002; 962: 318-1.

33. Ghafourifar P, Bringold U, Klein SD, Richter C. Mitochondrial nitric oxide synthase, oxidative stress and apoptosis. *Biol Signals Recept* 2001; 10:57–65.
34. Bardell AL, Macleod KM. Evidence for inducible nitric-oxidesynthase expression and activity in vascular smooth muscle of streptozotocin-diabetic rats. *J Pharmacol Exp Ther* 2001; 296:252–9.
35. Suzuki T, Tatsuoka H, Chiba T, et al. Beneficial effects of nitric oxide synthase inhibition on the recovery of neurological function after spinal cord injury in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2001; 363:94–100.
36. Bruhwiler J, Chleide E, Liegeois JF, Carreer F. Nitric oxide: a new messenger in the brain. *Neurosci Biobehav Rev* 1993; 17: 373–84.
37. Huang PL, Lo EH. Genetic analysis of NOS isoforms using nNOS and eNOS knockout animals. *Prog Brain Res* 1998; 118: 13–25.
38. Violi F, Marino R, Milite MT, Loffredo L. Nitric oxide and its role in lipid peroxidation. *Diabetes Metab Res Rev* 1999; 15: 283–8.
39. Brown GC, Borutaite V. Nitric oxide, cytochrome c and mitochondria. *Biochem Soc Symp* 1999; 66: 17–25.
40. Andersson U, Leighton B, Young ME, Blomstrand E, Newsholme EA. Inactivation of aconitase and oxoglutarate dehydrogenase in skeletal muscle in vitro by superoxide anions and/or nitric oxide. *Biochem Biophys Res Commun* 1998; 249: 512–6.
41. Beckman KB, Ames BN. Endogenous oxidative damage of mtDNA. *Mutat Res* 1999; 424: 51–8.
42. Requena JR, Fu MX, Ahmed M et al. Lipoxidation products as biomarkers of oxidative damage to proteins during lipid peroxidation reactions. *Nephrol Dial Transplant* 1996; 11: 48–53.
43. Metodiewa D, Koska C. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. *Neurotox Res* 2000; 1: 197–233.
44. De La Monte SM, Ganju N, Feroz N, et al. Oxygen free radical injury is sufficient to cause some Alzheimer-type molecular abnormalities in human CNS neuronal cells. *J Alzheimers Dis* 2000; 2: 261–1.
45. Conn KJ, Ullman MD, Eisenhauer PB, Fine RE, Wells JM. Decreased expression of the NADH: ubiquinone oxidoreductase (complex I) subunit 4 in 1-methyl-4-phenylpyridinium-treated human neuroblastoma SH-SY5Y cells. *Neurosci Lett* 2001; 306: 145–8.
46. Paschen W, Mengesdorf T, Althausen S, Hotop S. Peroxidative stress selectively down-regulates the neuronal stress response activated under conditions of endoplasmic reticulum dysfunction. *J Neurochem* 2001; 76: 1916–24.
47. Pugazhenti S, Nesterova A, Jambal P, et al. Oxidative stress-mediated down-regulation of bcl-2 promoter in hippocampal neurons. *J Neurochem* 2003; 84: 982–96.
48. Allen RG, Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med* 2000; 28: 463–99.
49. Morel Y, Barouki R. Repression of gene expression by oxidative stress. *Biochem J* 1999; 342: 481–96.
50. Bohr VA, Dianov GL. Oxidative DNA damage processing in nuclear and mitochondrial DNA. *Biochimie* 1999; 81: 155–60.
51. Nagley P, Zhang C, Lim ML, Merhi M, Needham BE, Khalil Z. Mitochondrial DNA deletions parallel age-linked decline in rat sensory nerve function. *Neurobiol Aging* 2001; 22: 635–43.
52. Kikuchi H, Furuta A, Nishioka K, Suzuki SO, Nakabeppu Y, Iwaki T. Impairment of mitochondrial DNA repair enzymes against accumulation of 8-oxoguanine in the spinal motor neurons of amyotrophic lateral sclerosis. *Acta Neuropathol (Berl)* 2002; 103:408–14.
53. Deng G, Su JH, Ivins KJ, Van Houten B, Cotman CW. Bcl-2 facilitates recovery from DNA damage after oxidative stress. *Exp Neurol* 1999; 159: 309–18.
54. Ozawa T, Hayakawa M, Katsumata K, Yoneda M, Ikebe S, Mizuno Y. Fragile mitochondrial DNA: the missing link in the apoptotic neuronal cell death in Parkinson's disease. *Biochem Biophys Res Commun* 1997; 235: 158–61.
55. Butterfield DA, Koppal T, Howard B, et al. Structural and functional changes in proteins induced by free radical-mediated oxidative stress and protective action of the antioxidants N-tert-butyl-alpha-phenyl nitron and vitamin E. *Ann N Y Acad Sci* 1998; 854: 448–62.
56. Aksenova MV, Aksenov MY, Payne RM. Oxidation of cytosolic proteins and expression of creatine kinase BB in frontal lobe in different neurodegenerative disorders. *Dement Geriatr Cogn Disord* 1999; 10: 158–65.

57. Amos A, McCarthy D, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 1997; 14: S1–85.
58. Rosebloom AL, Joe JR, Young RS, Winter WE. Emerging epidemic of type 2 diabetes in youth. *Diabetes Care* 1999; 22: 345–54.
59. Kannel WB, McGee DL. Diabetes and cardiovascular diseases. The Framingham Study. *JAMA* 1979; 241: 2035–8.
60. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285: 2486–97.
61. Hu FB, Stampfer MJ, Haffner SM. Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care* 2002; 25: 1129–34.
62. Stern MP. Diabetes and cardiovascular disease. The “common soil” hypothesis. *Diabetes*. 1995; 44:369–74.
63. Kahan SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia* 2003; 46: 3–19.
64. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA* 1990; 263: 2893–8.
65. Heilbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. *Am J Clin Nutr* 2003; 78: 361–9.
66. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and Beta cell dysfunction? *Diabetes* 2003; 52: 1–8.
67. Griending KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation* 2003; 108: 1912–6.
68. Vinik AI. Diabetic neuropathy: pathogenesis and therapy. *Am J Med* 1999; 107: 17S–26.
69. Russell JW, Golovoy D, Vincent AM, et al. High glucose induced oxidative stress and mitochondrial dysfunction in neurons. *FASEB J* 2002; 16: 1738–48.
70. Russell JW, Sullivan KA, Windebank AJ, Herrmann DN, Feldman EL. Neurons undergo apoptosis in animal and cell culture models of diabetes. *Neurobiol Dis* 1999; 6: 347–63.
71. Folmer V, Soares JC, Rocha JB. Oxidative stress in mice is dependent on the free glucose content of the diet. *Int J Biochem Cell Biol* 2002; 34: 1279–85.
72. Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes* 1994; 43: 1010–4.
73. Altomare E, Vendemiale G, Chicco D, Procacci V, Cirelli F. Increased lipid peroxidation in type 2 poorly controlled diabetic patients. *Diabete Metab* 1992; 18: 264–71.
74. Sundaram RK, Bhaskar A, Vijayalingam S, et al. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clin Sci (Lond)* 1996; 90: 255–60.
75. Elhadd TA, Kennedy G, Hill A, et al. Abnormal markers of endothelial cell activation and oxidative stress in children, adolescents and young adults with type 1 diabetes with no clinical vascular disease. *Diabetes Metab Res Rev* 1999; 15: 405–11.
76. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2011; 35: 676–92.
77. Lissi EA, Salim-Hanna M, Sir T, Videla LA. Is spontaneous urinary visible chemiluminescence a reflection of in vivo oxidative stress? *Free Radic Biol Med* 1992; 12: 317–22.
78. Lissi EA, Salim-Hanna M, Videla LA. Spontaneous urinary visible luminescence: characteristics and modification by oxidative stress-related clinical conditions. *Braz J Med Biol Res* 1994; 27: 1491–500.
79. Oldreive C, Bradley N, Bruckdorfer R, Rice-Evans C. Lack of influence of dietary nitrate/nitrite on plasma nitrotyrosine levels measured using a competitive inhibition of binding ELISA assay. *Free Radic Res* 2001; 35: 377–86.
80. Devaraj S, Hirany SV, Burk RF, Jialal I. Divergence between LDL oxidative susceptibility and urinary F(2)-

- isoprostanes measures of oxidative stress in type 2 diabetes. *Clin Chem* 2001; 47: 1974–79.
81. Leinonen J, Lehtimäki T, Toyokuni S, et al. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett* 1997; 417:150–2.
82. Gopaul NK, Anggard EE, Mallet AI, et al. Plasma 8-epi-PGF₂ levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Lett* 1995; 368: 225–9.
83. Poulsen HE, Loft S, Prieme H, Oxidative DNA damage in vivo: relationship to age, plasma antioxidants, drug metabolism, glutathione-S-transferase activity and urinary creatinine excretion. *Free Radic Res* 1998; 29: 565–71.
84. Dandona P, Thushu K, Cook S, et al. Oxidative damage to DNA in diabetes mellitus. *Lancet* 1996; 347: 444–5.
85. Moskovitz J, Yim MB, Chock PB. Free radicals and disease. *Arch Biochem Biophys* 2002; 397: 354–9.
86. Telci A, Cakatay U, Kayali R, et al. Oxidative protein damage in plasma of type 2 diabetic patients. *Horm Metab Res* 2000; 32: 40–3.
87. Telci A, Cakatay U, Salman S, Satman I, Sivas A. Oxidative protein damage in early stage type 1 diabetic patients. *Diabetes Res Clin Pract* 2000; 50: 213–23.
88. Maddux BA, See W, Lawrence JC Jr, et al. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of α -lipoic acid. *Diabetes* 2001; 50: 404–10.
89. Maechler P, Jornot L, Wolheim CB. Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. *J Biol Chem* 1999; 274:27905–13.
90. Qujeq D, Davary S, Moazzi Z, Mahjoub S. Effect of *Urtica dioica* leaf extract on activities of nucleoside diphosphate kinase and acetyl coenzyme A carboxylase, in normal and hyperglycemic rats. *Afr J Pharm Pharmacol* 2011; 5: 792–6.
91. Rudich A, Tirosh A, Potashnik R, et al. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes* 1998; 47: 1562–9.
92. Talior I, Yarkoni M, Bashan N, Eldar-Fielman H. Increased glucose uptake promotes oxidative stress and PKC delta activation in adipocytes of obese, insulin-resistant mice. *Am J Physiol* 2003; 285: E295–E302.
93. Ceriello A. Oxidative stress and glycemic regulation. *Metabolism* 2000; 49: 27–9.
94. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin producing cells. *Diabetes* 1997; 46: 1733–42.
95. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in Beta - cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*. 2003; 52: 581–7.
96. El-Assad W, Buteau J, Peyot ML, et al. Saturated fatty acids synergize with elevated glucose to cause pancreatic beta-cell death. *Endocrinology* 2003; 144: 4154–63.
97. Piro S, Anello M, Di Pietro C, et al. Chronic exposure to free fatty acids or high glucose induces apoptosis in rat pancreatic islets: possible role of oxidative stress. *Metabolism* 2002; 51: 1340–47.
98. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993; 326: 801–9.
99. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001; 104: 2673–78.
100. Mahjoub S, Taghikhani M, Amir Rasouli H, Noohi F. Lipids and lipoproteins status in normal subjects and in acute and stable phases of myocardial infarction. *JBUMS* 2001; 3: 30–6.
101. Hu FB, Stampfer MJ. Is type 2 diabetes mellitus a vascular condition? *Arterioscler Thromb Vasc Biol* 2003; 23: 1715–16.
102. 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.
103. Vincent AM, Russell GW, Low P, Feldman EL. Oxidative Stress in the Pathogenesis of Diabetic Neuropathy. *Endocr Rev* 2004; 25: 612–28.
104. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB. *Int J Mol Med*. 1999; 4: 223–30.