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Metabolic Syndrome and Mitochondrial Function: Molecular Replacement and Antioxidant Supplements to Prevent Membrane Peroxidation and Restore Mitochondrial Function

Garth L. Nicolson*

Department of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, California 92647

Abstract Metabolic syndrome consists of a cluster of metabolic conditions, such as hypertriglyeridemia, hyper-low-density lipoproteins, hypo-high-density lipoproteins, insulin resistance, abnormal glucose tolerance and hypertension, that—in combination with genetic susceptibility and abdominal obesity—are risk factors for type 2 diabetes, vascular inflammation, atherosclerosis, and renal, liver and heart disease. One of the defects in metabolic syndrome and its associated diseases is excess cellular oxidative stress (mediated by reactive oxygen and nitrogen species, ROS/RNS) and oxidative damage to mitochondrial components, resulting in reduced efficiency of the electron transport chain. Recent evidence indicates that reduced mitochondrial function caused by ROS/RNS membrane oxidation is related to fatigue, a common complaint of MS patients. Lipid replacement therapy (LRT) administered as a nutritional supplement with antioxidants can prevent excess oxidative membrane damage, restore mitochondrial and other cellular membrane functions and reduce fatigue. Recent clinical trials have shown the benefit of LRT plus antioxidants in restoring mitochondrial electron transport function and reducing moderate to severe chronic fatigue. Thus LRT plus antioxidant supplements should be considered for metabolic syndrome patients who suffer to various degrees from fatigue. J. Cell. Biochem. 100: 1352–1369, 2007.

Key words: lipids; antioxidants; diabetes; atherosclerosis; vascular inflammation; heart disease; renal disease; liver disease; dietary supplement; mitochondria; fatigue

METABOLIC SYNDROME

Metabolic syndrome (MS) is a term that evolved from the 1920s observations of a Swedish physician [Kylin, 1923] as a cluster of metabolic risk factors for cardiovascular disease, type 2 diabetes and other disorders [Eckel et al., 2005]. MS is a complex syndrome made up of several interrelated disturbances of glucose and lipid homeostasis [Fonseca, 2005]. The major risk factors for MS are: abdominal obesity, elevated fasting plasma glucose, artherogenic dyslipidemia (increased triacylglyerols, increased levels of small and dense low-density lipoproteins and reduced levels of high-density lipoproteins), elevated blood pressure, and the presence of prothrombotic and proinflammatory states [Grundy, 2006]. MS has also been termed Syndrome X [Reaven, 1993] and insulin-resistance syndrome [Einhorn et al., 2003], and it is estimated that over 22% of the U.S. population have MS. In the age group over 60, over 40% have MS [Ford et al., 2002; Park et al., 2003]. Four separate organizations have validated the above risk factors in various diseases, such as coronary heart disease (CHD), type 2 diabetes, hypertension, among others, and each organization has its own approach to solving the problem of MS and its associated diseases [Cifkova et al., 2003; Einhorn et al., 2003; Pasternak, 2003; Whitworth, 2003; Grundy et al., 2004; Lagenfeld et al., 2004].

The most important interacting features of MS have been proposed by Grundy [2006] as

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*Correspondence to: Prof. Garth L. Nicolson, Office of the President, The Institute for Molecular Medicine, 16371 Gothard St. H, Huntington Beach, CA 92647.
E-mail: gnicolson@immed.org

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obesity plus endogenous (genetic) metabolic susceptibility, such as manifested by insulin resistance and other factors. Along with the multiple risk factors listed above, a diagnosis of MS can be made, although there is still some discussion as to the relative merits of using MS as a diagnosis in clinical practice, as opposed to a strictly biological concept [Reaven, 2006]. Grundy [2006] makes the case that genetic-based insulin resistance, increased abdominal fat, genetic factors, physical inactivity, advancing age and endocrine dysfunction establish the metabolic susceptibility of MS, which when combined with additional risk factors determined by laboratory tests, such as dyslipidemia, elevated blood glucose, etc., increases dramatically the propensity of contracting life-threatening diseases later in life.

In evaluating patients for MS by examining clusters of risk factors the goal is to identify patients who are at high lifetime risk for developing future CHD, type 2 diabetes, and other diseases. It is not done to simply identify MS patients [Reaven, 2006]. Thus when any of the MS risk factors are found, patients are usually examined for other risk factors in the cluster, and appropriate interventions can then be applied to prevent future life-threatening diseases. There are also additional risk factors for MS that have been added recently, such as elevated plasminogen activator inhibitor-1, and C-reactive protein (CRP) [Dandona et al., 2005].

This brief review will focus on the role that mitochondrial dysfunction plays in fatigue associated with certain clinical conditions, such as MS and associated diseases. I will also discuss ways that mitochondrial membrane damage can be prevented and, in some cases, even reversed by non-pharmacologic treatments. Thus this review is not intended to be a comprehensive discussion of the various molecular aspects of MS and associated diseases and their treatment. Some of this is dealt with in other reviews on the subject [Dandona et al., 2005; Fonseca, 2005; Houston and Egan, 2005]. Here the focus will be on preventing excess oxidative damage, increasing mitochondrial function, and reducing fatigue.

METABOLIC SYNDROME AND INSULIN RESISTANCE

Insulin resistance is a major worldwide clinical problem and one of the initial signs in the development of MS [Einhorn et al., 2003]. Insulin secreted by the pancreatic cells in response to increased circulating levels of glucose and amino acids is essential for development, growth, apoptosis, and maintenance of glucose homeostasis by regulating gene expression and carbohydrate, lipid and protein metabolism [Chakraborty, 2006]. For example, insulin regulates glucose homeostasis by reducing hepatic output and increasing the rate of glucose uptake in tissues as well as increasing lipid synthesis in liver and fat cells and reducing triglyceride breakdown in fat and muscle. When the circulating concentrations of insulin are insufficient to regulate the above processes, insulin resistance occurs. This, in turn, can lead to clinically diverse syndromes, such as type A syndrome, leprechaunism, Rabson–Mendenhall syndrome, and type 2 diabetes.

Insulin resistance has been identified in children long before the development of dyslipidemia, hypertension and hyperglycemia that occur much later in life [Kendall et al., 2003]. Insulin resistance and MS have been described as polygenetic disorders with lifestyle influences that determine their biochemical and clinical presentations [Houston and Egan, 2005]. Impaired size at birth has a bearing on insulin resistance and the future development of MS and type 2 diabetes [Simmons, 2006]. In one study men who had the smallest birth weights ($<$2.5 kg) and obese childhoods were nearly seven-times as likely to have impaired glucose tolerance, higher systolic blood pressure and triglyceride levels and eventual development of type 2 diabetes [Barker et al., 1993]. Fetal growth retardation is known to alter the development of adipose tissue, which is closely linked to the future development of insulin resistance [Jaquet et al., 2000], and when these are linked with overweight in childhood, there is a high risk of developing type 2 diabetes and CHD later in life [Bavdekar et al., 2004].

Insulin resistance in abdominally obese patients is often initially compensated for by pancreatic hyper-insulinemia and decreased hepatic insulin clearance. Over time, however, pancreatic beta cell exhaustion results in impaired glucose tolerance and eventually type 2 diabetes. During the early time period before noticeable pathologies are present a constellation of metabolic and biochemical changes occurs that are characteristic of MS. Houston and Egan [2005] have outlined several factors

that are involved in insulin resistance and MS: multiple genes (polygenetic disorder), epigenetic contributions (nutrition, low birth weight, etc.), visceral obesity, body-mass index, caloric and carbohydrate intake, sedentary lifestyle, age, ethnicity, gender, menopausal status, alcohol consumption and inflammation. Insulin resistance is one of the primary events in the development of MS, and it is thought to induce the biochemical, pathophysiological and clinical sequelae that we know as MS.

A primary link between insulin resistance and MS is thought to be abdominal obesity, the central risk factor for MS and its associated diseases (Fig. 1) [Fonseca, 2005]. In obese individuals free fatty acids levels remain elevated throughout the day, resulting in a wide range of metabolic effects in the liver, muscle, pancreas, and other tissues [Raz et al., 2005]. Importantly, increases in free fatty acids induce oxidative stress [Fonseca, 2005]. Free fatty acids can also decrease insulin sensitivity through inhibition of insulin-mediated glucose uptake transporters, for example the GLUT4 transporter in skeletal muscle, and by contributing to hyperinsulinemia [Vitarius, 2005]. This results in stimulation of adipose cell secretion of cytokines, such as tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6), which cause exacerbation of insulin resistance. In this state there is also an accumulation of acylglycerols and lipid intermediates in skeletal muscle. Increased adipose tissue stores, decreased non-esterified fatty acid uptake (resulting in increased circulating concentrations) and altered insulin regulated lipolysis promote insulin resistance and MS [Blaak, 2003].

Defects in the capacity to metabolize fatty acids and glucose are thought to play an important role in insulin resistance and MS [Schrauwen and Hesselink, 2004]. Accumulations of diacylglycerol (DAG), triacylglycerol,

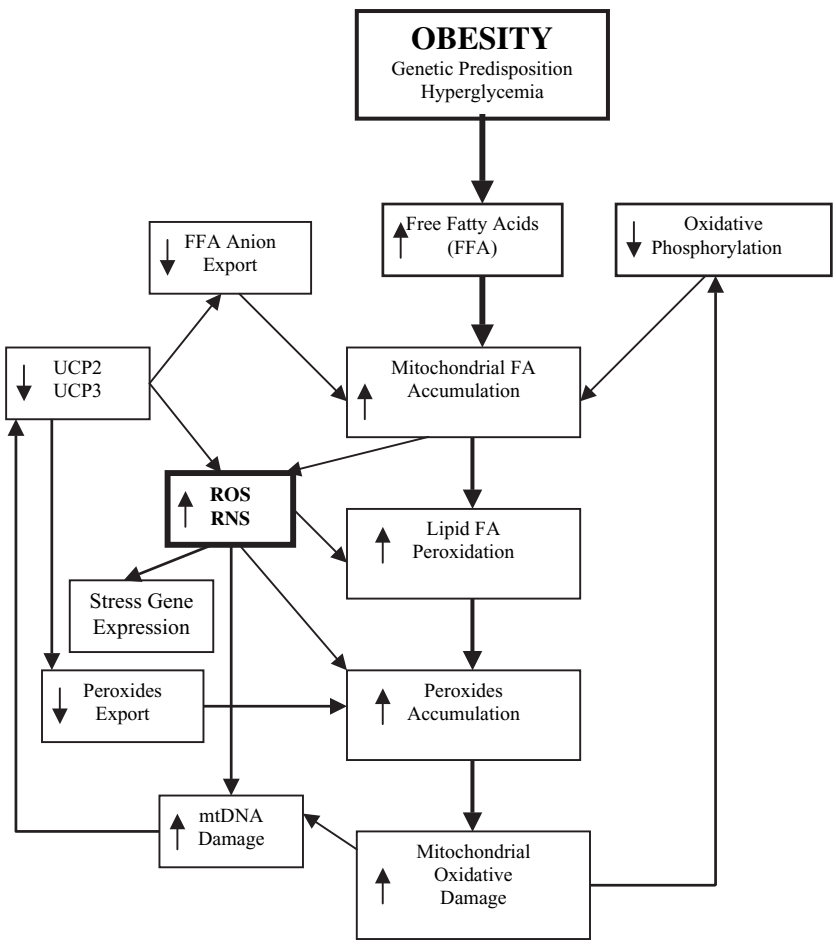

Fig. 1. Possible feedback mechanisms in the generation of excess ROS/RNS in MS, type 2 diabetes, and other MS-associated diseases. Modified from Ceriello [2003] and Schrauwen and Hesselink [2004].

and free fatty acids in non-adipose tissues correlate strongly with insulin resistance [Krssak et al., 1999; Perseghin et al., 1999; Itani et al., 2002]. Increases in free fatty acids may play a role in blocking insulin signal transduction [Dandona et al., 2005], and DAG, in particular, has been implicated in insulin resistance by activating distinct isoforms of protein kinase C, which in turn can directly modulate insulin signaling by phosphorylating and inhibiting the tyrosine kinase activity of the insulin receptor and activating genes responsible for fatty acid-induced impairment of insulin action [Itani et al., 2002; Shoelson et al., 2003]. Gene expression modifications in adipose tissue are thought to be responsible for enhanced secretion of MS-related factors, such as the proinflammatory cytokine TNFα and the tissue-specific protein adiponectin [Sonnenberg et al., 2004], and in muscle tissue decreased oxidative capacity and fat accumulation may also induce skeletal muscle insulin resistance and contribute to the development of type 2 diabetes [Schrauwen and Hesselink, 2004].

OXIDATIVE STRESS, MITOCHONDRIAL DAMAGE, AND TYPE 2 DIABETES

Various studies point to generalized mitochondrial dysfunction in MS and type 2 diabetes patients [Schrauwen and Hesselink, 2004]. For example, mitochondria of type 2 diabetes patients have been shown to possess reduced electron transport chain capacities and reduced citrate synthase activity [Kelly et al., 2002]. Mitochondrial dysfunction has been linked to chronic insulin resistance, which causes preferential metabolism of fatty acids, reducing glucose utilization [Perseghin et al., 2003]. This causes gradual pancreatic beta and other cell dysfunction due to fatty acid-stimulated changes in mitochondrial uncoupling proteins UCP2 and UCP3, resulting in an increase in uncoupling of mitochondrial respiration, reduced electron transport chain activity and ATP production and subsequent fatigue [Hagen and Vidal-Puig, 2002; Schrauwen, 2002].

No single lesion can account for MS or its associated diseases [St. Pierre et al., 2005; Flatt and Green, 2006]. However, gene expression studies have shown that there is a coordinate reduction in oxidative gene activities along with increased expression of several other genes in type 2 diabetes [Vechoor et al., 2002]. Using microarray assays to monitor gene expression clusters of oxidative genes were down-regulated. Since they could not find defects that pointed to a single gene or protein product involved in substrate oxidation in type 2 diabetics, the findings support the notion that more generalized mitochondrial dysfunction occurs in type 2 diabetes [Schrauwen and Hesselink, 2004]. This also correlated with reduced muscle electron transport chain activity [Kelly et al., 2002] and decreased whole body anaerobic capacity in type 2 diabetes patients [Schneider et al., 1984]. Kelly et al. [2002] found that mitochondria in type 2 diabetes patients were also smaller, correlating this finding with insulin sensitivity. In type 2 diabetics genetic polymorphisms have also been found that are involved in fatty acid oxidation and in factors that control transcriptional activities (reviewed in Houston and Egan [2005]).

It is well known that MS and type 2 diabetes patients show reduced fat oxidative capacities [Kelly and Simoneau, 1994]. In obese, pre-diabetic and diabetic patients free fatty acid levels are increased together with decreases in fat oxidative capacity, and in time this can result in accumulation of fatty acids and acylglycerols in beta cells and other tissues, which has been shown to correlate strongly with insulin resistance [Schrauwen and Hesselink, 2004; Touyz and Schiffrin, 2004; Fonseca, 2005].

When mitochondrial respiration functions properly, excess superoxide produced as a consequence of electron transport activity is effectively neutralized by endogenous antioxidants and antioxidant enzymes [Turrens, 2003]. Although continuous production of superoxide and peroxide are necessary for normal cellular functions [Linnane and Eastwood, 2006], in MS and associated diseases oxidants, mainly reactive oxygen species (ROS), are overproduced [Schrauwen, 2002; Schrauwen and Hesselink, 2004]. Mitochondria are one of but not the only cellular sources of ROS [Halliwell, 1999], and production of ROS is essential in cell signaling and gene regulation [Griendling et al., 2000; Touyz and Schiffrin, 2004; Goldstein et al., 2005]. Excess superoxide produced continually as a byproduct of normal mitochondrial respiration can directly damage iron sulfur center-containing enzymes, be converted to hydrogen peroxide (and ultimately to hydroxyl radical) and also react with nitrogen

oxide to produce peroxynitrite, a very reactive nitrogen species (RNS) [Beckman et al., 1990].

Fatty acids are particularly sensitive to ROS/RNS oxidation, resulting in the formation of lipid peroxides, which are cytotoxic and lead to free-radical damage to other lipids, proteins and DNA, especially in MS and type 2 diabetics [Green et al., 2004; Schrauwen and Hesselink, 2004]. In obese, insulin-resistant, pre-diabetic subjects higher amounts of free fatty acids and their peroxide derivatives have been found compared to endurance-trained subjects [Russell et al., 2003]. Free fatty acids accumulate, particularly in muscle cell mitochondria, where ROS/RNS damage can occur, and there they are thus prone to peroxidative events that result in damage to mitochondrial membranes, proteins, and DNA. Once mitochondrial membrane lipids have been modified, they are less likely to maintain the low levels of proton leakage and membrane fluidity required to maintain the proper mitochondrial membrane potential [Nicolson, 2003], an absolute requirement of oxidative phosphorylation [Mitchell, 1966].

Even before the diagnosis of MS or type 2 diabetes, the accumulation of oxidized fatty acids in mitochondria can result in progressive oxidative damage. For example, in elderly subjects who do not have MS or type 2 diabetes oxidized fatty acids accumulate in muscle mitochondria, and this is related to mitochondrial dysfunction [Peterson et al., 2003].

Normally the oxidative phosphorylation enzyme system that generates superoxide and ROS/NRS is tightly controlled. One of the control systems that regulates oxidative phosphorylation by regulating the flow of protons back across the inner mitochondrial membrane to maintain an appropriate membrane potential [Mitchell, 1966] is made up of uncoupling proteins (UCPs), such as UCP2 and UCP3. In addition to regulating electron transport chain activity, UCP3 and other UCPs also prevent build-up of excessive concentrations of ROS/RNS by limiting oxidative phosphorylation [Vidal-Puig et al., 2000]. Also, it has been suggested that UCP3 functions to remove fatty acid anions (formed by oxidative reactions) that can build-up during excess fatty acid partitioning into the mitochondria [Russell et al., 2003]. These fatty acid anions can cause reactions with other lipids, proteins, and DNA.

Under normal conditions UCP3 in skeletal muscle and brown adipose tissue is up-regulated during fasting, acute exercise, and high-fat intake when there is a high level of free fatty acids. In contrast, it is down-regulated when fatty acid oxidation is increased or plasma levels of free fatty acids are lowered (reviewed in Schrauwen and Hesselink [2004]). Type 2 diabetes patients have been found to have about one-half the normal levels of UCP3 in their skeletal muscles [Schrauwen et al., 2001], but the levels of UCP3 can be up-regulated, at least in pre-diabetic subjects, by exercise and lifestyle modifications [Mensink et al., 2003]. Pancreatic beta cells contain mainly UCP2, and while activation of UCPs can reduce ROS/RNS production in peripheral tissues, it may disrupt glucose-stimulated insulin secretion in beta cells [Green et al., 2004]. These results suggest that reduced levels of UCPs in MS and type 2 diabetic patients could indicate a defective feedback mechanism between ROS-lipid peroxides and mitochondrial protection against fat accumulation and could contribute to oxidative mitochondrial damage [Schrauwen and Hesselink, 2004].

The pathophysiology of type 2 diabetes is thought to occur as a consequence of persistent hyperglycemia [Green et al., 2004], which causes: (a) formation of advanced glycation end products (AGEs, the products of non-enzymatic glycation and oxidation), their oxidation and interactions with cell receptors and cellular accumulation; (b) activation of various isoforms of protein kinase C; (c) induction of the polyol pathway; and (d) increased hexosamine pathway flux [Brownlee, 2001; Rosen et al., 2001; Ceriello, 2003]. Most of these pathways are associated with elevated oxidative stress and overproduction of superoxide (and thus ROS/RNS) by the mitochondrial respiratory chain during hyperglycemia, but the link between hyperglycemia and increased mitochondrial superoxide production may not be mediated solely by the redox state of electron carriers [Green et al., 2004]. A proposal that arose from various studies (reviewed by Green et al. [2004]) is that an increase in mitochondrial ROS/RNS in response to hyperglycemia is the proximal defect that leads to most pathological consequences of hyperglycemia. In contrast to this proposal, hyperlipidemia as a consequence of obesity results in increased fatty acid oxidation products that stimulate insulin secretion, resulting in hyperinsulinemia. This, in turn, down-regulates insulin receptors, reducing

insulin action, and increasing blood glucose levels [Opara, 2004]. Whether or not this mechanism is essential to the formation of type 2 diabetes, beta cell mitochondria are essential to glucose-stimulated insulin secretion and are quite susceptible to ROS/RNS damage during hyperglycemia that suppresses glucose-stimulated insulin secretion. Thus mitochondrial excess oxidative stress likely contributes to disease progression by disrupting the ability of beta cells to respond to elevated blood glucose [Green et al., 2004].

In type 2 diabetes the pancreatic beta cells are quite susceptible to ROS/RNS-stimulated fibrosis and loss of tissue architecture, and compared to other cell types beta cells are unequipped to deal with excess ROS/RNS and are prone to ROS/RNS-mediated apoptosis [Hayden et al., 2005c, 2006]. In the oxidatively stressed beta cells there is an excessive demand for insulin secretion, which can result in biosynthetic overload ultimately resulting in deficient insulin secretion. Apoptosis stimulated by excess ROS/RNS results in loss of beta cells and reduced production of insulin [Butler et al., 2003]. Reduction in insulin production also occurs because of oxidative stress on the endoplasmic reticulum at the site of insulin synthesis. Excess ROS/RNS in the beta cell cytoplasm results in protein oxidation and misfolding, causing aberrant protein structures and polymer deposition in a process called conformation disease [Hayden et al., 2005c]. It has been proposed that beta cells modified by the build-up of aberrant, cytotoxic proteins, and polymers, such as islet amyloid polymers, in type 2 diabetics results in lethal cellular defects that further deplete beta cells [Kahn et al., 1999].

METABOLIC SYNDROME, INFLAMMATION, AND ENDOTHELIAL DYSFUNCTION

The vascular endothelium and surrounding tissue matrix, smooth muscle and other cells constitute the central tissue system involved in the pathogenesis of dyslipidemia, hypertension, and CHD. The endothelium regulates vascular tone through the release of vasodilating and vasoconstricting substances. One of the most important of the vasodilating substances is nitric oxide (NO), which is also a vascular protective substance that inhibits oxidation, inflammation and vascular smooth muscle cell migration and proliferation [Hsueh and Quiñones, 2003]. In addition to vasodilation, NO is considered antiatherosclerotic, anti-platelet, anti-growth, and antioxidant [Houston and Egan, 2005]. Damage to the endothelium causes endothelial dysfunction with impaired release of NO and loss of its antiatherogenic and other properties. At the early stages of insulin-resistance before the development of MS in obese young adults, reductions in vascular smooth muscle NO vasodilatory capacity have been seen using positron emission tomography [Hsueh and Quiñones, 2003]. Thus insulin resistance contributes to endothelial dysfunction by its linkage to NO-mediated vasodilation, and vascular dysfunction may be one of the initial steps in the development of hypertension, MS, and type 2 diabetes.

Insulin itself is a vasodilator and stimulates NO production as well as growth and motility in some cells. One of the signaling pathways for insulin action, the phosphatidylinositol 3-kinase pathway, is important in regulating insulin-mediated glucose uptake and insulin-dependent endothelial cell NO production [Shepherd et al., 1998; Zeng et al., 2000]. This pathway is negatively affected in obese subjects, MS and type 2 diabetes [Cusi et al., 2000]. In endothelial cells the impaired activation of the phosphatidylinositol 3-kinase pathway is also associated with enhanced activation of the other major insulin signaling pathway mediated by mitogen-activated protein kinase (MAPK) [Pessin and Saltiel, 2000]. NO formed in endothelial cells (eNO) by the action of endothelial nitric oxide synthase (eNOS) can be inactivated by superoxide anion radical to form the NRS peroxynitrite anion ($ONOO^-$), which can cause further oxidative damage and depress eNO endothelial-dependent, acetylcholine-induced arterial relaxation [Hsueh and Quiñones, 2003]. In MS many of the altered blood components, such as excess free fatty acids and small, dense low-density LDL, also decrease eNOS activity [Uittenbogaard et al., 2000]. A decrease in eNO and an excess of angiotensin-II synthesis or action causes vasoconstriction, growth promotion and pro-thrombotic, pro-inflammatory, pro-oxidant states [Houston and Egan, 2005]. This, in turn, is related to insulin resistance and dyslipidemia and eventually vascular inflammation, hypertension, MS and type 2 diabetes [Gotah and Mori, 2006].

Adipose tissue also plays a role in endothelial dysfunction by producing pro-inflammatory cytokines, such as IL-6 and TNFα, and other factors. Molecules like TNFα activate the important NF-κB transcription factor and can indirectly induce serine phosphorylation of the insulin receptor, thereby interfering with insulin receptor signaling pathways [Hotamisligil et al., 1996; Sonnenberg et al., 2004]. Activation of the NF-κB transcription pathway also increases production of NO [Collins, 1993]. Adipose cells express receptors for inflammatory cytokines. In addition, the infiltration of adipose tissue by inflammatory macrophages with their production of ROS is a common feature of obesity [Weisberg et al., 2003].

Hypertension is directly related to vascular dysfunction and MS, which can be preceded by insulin resistance for 10–20 years before becoming apparent in most patients [Hall et al., 2003; Sowers and Frohlich, 2004]. As indicated above, the primary abnormalities associated with hypertension include loss of eNOS and reduced eNO availability, up-regulation of the MAPK pathway, inflammation of the vascular endothelium and accumulation of ACEs, collagen overproduction and other factors [Bergandi et al., 2003; Wolk et al., 2003]. Linked to insulin resistance, excess oxidative stress mediated mainly by ROS/RNS causes changes in endothelial and smooth muscle cells that eventually result in vascular inflammation. Under normal conditions ROS/RNS are produced in a controlled manner where they function as signaling molecules regulating vascular smooth muscle cell contraction/relaxation and cellular growth [Irani, 2000; Touyz and Schiffrin, 2004]. However, at higher concentrations ROS/RNS can affect a variety of cellular targets and can initiate apoptosis and modify gene expression [Turrens, 2003].

METABOLIC SYNDROME, ATHEROSCLEROSIS, AND CORONARY HEART DISEASE

Atherosclerosis involves chronic inflammatory damage to blood vessels due to lipid accumulation, inflammatory response, vessel cell death and thrombosis, which can eventually result in the occlusion of heart and other tissue blood vessels. A main cause of CHD and stroke, atherosclerosis is characterized by a number of risk factors, including abnormalities in lipoprotein subclass distribution, increases in vascular acute phase response proteins, changes in vascular endothelial cell adhesion molecules and certain cytokines [Zambon et al., 2005]. In the cardiovascular system ROS/RNS play an essential physiological role in maintaining vascular integrity, and when they are in excess a pathological role in cardiovascular dysfunction by their association with hypertension, type 2 diabetes, atherosclerosis, ischemic heart disease, and congestive heart failure [Griendling et al., 2000; Touyz and Schiffrin, 2004].

The process of atherosclerosis is thought to begin with abnormalities in lipoprotein subclasses, such as triglyceride-rich lipoproteins, their remnants, and smaller, denser low-density lipoproteins, hallmarks of MS [Berliner and Watson, 2005]. In MS these proinflammatory lipoproteins and their remnants are susceptible to oxidation [Chait et al., 1993], and the presence of the oxidized lipoprotein subclasses is significantly associated with an abundance of macrophages in atherosclerotic lesions [Faggin et al., 2002]. As discussed above, macrophages are thought to be an important element of vascular inflammatory responses. A key event in leukocyte (mainly monocyte) adhesion to the vascular endothelium and migration into the intima is the expression of endothelial adhesion molecules, such as vascular adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and E-selection [Libby, 2003]. These endothelial cell adhesion molecules can be released into the circulation, and their presence and concentration in blood is associated with MS and degree of insulin resistance [Bonora et al., 2003]. Inflammatory cells in the intima release ROS as well as S100/calgranulins and amphoterin that stimulate a potent feedback loop for sustained oxidant stress.

When they interact with the blood vessel wall, the oxidized lipoprotein subclasses are proinflammatory and can induce endothelial adhesion molecules [Colome et al., 2000; Doi et al., 2000]. In vessel walls the expression of endothelial adhesion molecules attracts monocytes, and their adhesion to endothelial cells results in endothelial cell retraction and movement of adherent monocytes to subendothelial layers and their differentiation into inflammatory, ROS-producing macrophages that are abundant within thickened vessel walls and unstable atherosclerotic plaques that form slowly over time. The unstable plaques can break off and form thrombi that can occlude blood vessels and

interrupt blood flow, and when this occurs in the heart—myocardial infarction, ischemia, heart failure and sudden death can occur.

Another association between MS and chronic inflammation in the cardiovascular system is the elevation of CRP [Festa et al., 2000; Ford, 2003]. In MS the presence of CRP is one of the best predictors for future CHD and type 2 diabetes [Ridker, 2003; Ridker et al., 2004; Ndumele et al., 2006]. Synthesis and release of CRP, TNFα, IL-1, IL-6 and adhesion molecules (discussed above) begins in obese subjects before the onset of insulin resistance, type 2 diabetes and CHD [Heinrich et al., 1990; Visser et al., 1999], probably due to ROS/RNS stimulation of the NF-κB transcription pathway. CRP can also enhance the uptake of low-density lipoproteins by the endothelium and stimulate macrophages to express inflammatory cytokines [Pasceri et al., 2000; Zwaka et al., 2001].

Endothelial dysfunction and insulin resistance are thought to be among the most basic physiologic abnormalities in MS [Mikhail and Tuck, 2000; Hsueh and Quiñones, 2003]. Although the exact mechanism of endothelial dysfunction and insulin resistance and the contribution of dyslipidemia are not known, Hsueh and Quiñones [2003] have argued that endothelial dysfunction occurs early in the pathogenesis of insulin resistance, MS and related diseases, suggesting that vascular damage (associated with excess oxidation, inflammation, and thrombosis) is a primary event that may be critical to the development of effective measures to stop progression to CHD and other diseases.

METABOLIC SYNDROME AND OXIDATIVE DAMAGE TO OTHER ORGAN SYSTEMS

In addition to the pancreas, muscle, adipose, and cardiovascular system, MS affects many other tissues. Although type 2 diabetes, CHD and stroke are the best known diseases associated with MS, diseases of other organ systems are also at high risk in MS, such as diseases of the kidney, liver, brain, eye, among other sites, causing, nephropathy, intimopathy, neuropathy, retinopathy, and other pathologies [Hayden et al., 2005b, 2006]. A common feature among these diseases is extracellular matrix remodeling where degradation and modification of extracellular matrix components occurs [Hayden and Tyagi, 2003, 2005a]. Extracellular matrix collagens and elastins, in particular, are degraded by matrix metalloproteinases and elastinase, respectively, which are activated by excess ROS/RNS and released by endothelial cells, inflammatory cells such as macrophages, pericytes, and other cell types [Hayden et al., 2006]. Once matrix degradation has occurred and extracellular components are resynthesized, ACEs and other oxidized components play a role in cross-linking and changing the biochemical and mechanical properties of the newly synthesized extracellular matrix [Al Aly and Edwards, 2004; Hayden et al., 2005a]. This can also result in alterations of intracellular structures in what Hayden et al. [2005c] have described as conformational dysfunction where proteins undergo changes in shape and associations with other proteins and cellular components to generate abnormal intracellular polymer structures that alter cellular physiology and tissue architecture.

Approximately 11% of the U.S. population has some manifestation of chronic kidney disease (CKD). This is largely due to the prevalence of MS, hypertension, and type 2 diabetes. CKD also carries a high risk for subsequent CHD and other MS-associated diseases, and in fact, CHD accounts for the most prevalent cause of death in end stage renal disease [Hayden et al., 2005b]. Oxidative stress has been found in MS patients with renovascular hypertension, and it is thought that this and NO deficiency precede the development of renal disease. ROS/RNS can affect afferent arteriolar tone and enhance vascular smooth muscle cell reactivity as well as mediate extracellular matrix remodeling, resulting ultimately in alterations in blood flow and ion transport that precede CKD [Wilcox, 2005]. In patients with end stage renal disease natural antioxidant capacity is decreased, allowing excess ROS/RNS to cause severe organ damage [Manning et al., 2005].

In most patients with CKD and end stage renal disease insulin resistance, MS and type 2 diabetes are the predecessors. Patients that have type 2 diabetes and CKD as comorbid conditions generally have a worse outcome, and the management of the combined diseases is much more expensive than dialysis expenses alone. In addition, CHD is also frequently associated with CKD, and patients with CKD are likely to die of CHD before they develop fatal renal failure [Levey et al., 1998]. In such patients the presence of chronic intracellular

infections, inflammatory cytokines, dimethylarginine, vascular calcification and other factors also determine outcome [Al Aly and Edwards, 2004].

An important organ disease related to insulin resistance and MS is fatty liver disease unrelated to alcoholism. The majority of patients with non-alcoholic fatty liver disease are obese and have MS-associated metabolic risk factors, such as insulin resistance, dyslipidemia, cytokine production, among others [Bugianesi et al., 2005; Harrison, 2006]. Since the liver is intimately involved in lipid and glucose metabolism, it is clear that obesity-related increases in free fatty acids and altered glucose metabolites will affect the liver, and this can cause enhancement of oxidative stress and excess production of ROS/RNS. Eventually patients suffer from non-alcoholic hepatic steatosis, hepatocyte disarray, lobular and portal inflammation, fibrosis and eventually steatohepatitis [Pessayre and Fromenty, 2005; Harrison, 2006]. In such conditions increased lipid peroxidation and mitochondrial damage due to excess ROS/RNS results in hepatocyte apoptosis and stellate cell fibrosis and increased hepatic cancer risk [Pessayre et al., 2004].

Another organ at long-term risk in MS is the eye. Lipid peroxidation events caused by excess ROS/RNS produced in the macula, the underlying cell layer containing retinal pigment in the small area of the retina responsible for sight in the center of the field of vision, results in a build-up of abnormal material in lysosomes and deposition of lipofuscin compounds [Kopitz et al., 2004]. Eventually this can result in macular degeneration, the most common cause of visual loss and legal blindness in industrialized nations [Kopitz et al., 2004]. In diabetic retinopathy, oxidative stress or excess mitochondrial ROS/RNS causes lipid peroxidation and glycation (forming AGEs) in the retinal microvessels, resulting in vascular damage, infiltration of leukocytes and loss of vascular permeability properties. This eventually results in macular edema and damage and loss of vision [van Reyk et al., 2003].

In late-stage diabetes an important complication is the presence of diabetic neuropathies. The most common of these is diabetic sensory neuropathy associated with small fiber or unmyelinated fiber neuropathy, which results in progressive sensory loss. It can also result in pain, hyperestesia and parestesias. When this affects both somatic and autonomic peripheral nerves, it is termed diabetic polyneuropathy, and this can result in damage to sensory, motor and autonomic nerves [Sima, 2006]. Oxidative stress and mitochondrial dysfunction are thought to be the most overriding causes of diabetic neuropathies, but other factors, such as genetics, hyperglycemia and neurotrophin synthesis, are also important factors [Baynes and Thorpe, 1999]. Mitochondrial dysfunction also occurs following nerve injury and damage, and it is thought to be pivotal in neuronal cell survival and death following injury [Sullivan et al., 2004].

OXIDATIVE DAMAGE TO MITOCHONDRIAL MEMBRANES IN AGING AND FATIGUE

Fatigue or lack of energy occurs naturally during aging and is a common condition in many clinical diagnoses, including MS, type 2 diabetes, CHD, respiratory, musculoskeletal and bowel conditions as well as infections and cancer [Morrison, 1980; Kroenke et al., 1988; McDonald et al., 1993; Nicolson, 2003, 2005; von Roenn and Paice, 2005]. The phenomenon of fatigue has been defined as a multidimensional sensation, and recently attempts have been made to determine its extent and possible causes [Piper et al., 1987, 1988; McDonald et al., 1993]. Fatigue is related to reductions in the efficiency of mitochondrial energy systems, and ROS/RNS damage to mitochondrial components can impair oxidative phosphorylation and cause fatigue. This occurs naturally with aging [Huang and Manton, 2004] and in chronic illnesses where the overproduction of ROS/RNS can cause excess oxidative stress and oxidation of lipids, proteins, and DNA [Richter et al., 1988; Wei and Lee, 2002; Huang and Manton, 2004].

Mitochondria have been proposed to be critical elements in the process of aging and the regulators of cellular life span [Xu and Finkel, 2002]. It has also been proposed that during aging and fatigue antioxidant enzymes, low molecular weight antioxidants and enzyme repair mechanisms along with biosynthesis cannot restore or replace enough of the ROS/RNS-damaged molecules to maintain mitochondrial function [Harman, 1956; Richter et al., 1988; Chen et al., 2002; Huang and Manton, 2004]. Disease and infection can also result in

excess oxidative damage that exceeds the abilities of cellular systems to repair and replace damaged molecules [Richter et al., 1988; Halliwell, 2001; Opara, 2002].

In the case of fatigue and fatiguing illnesses there is good evidence that oxidative damage impairs mitochondrial function [Logan and Wong, 2001; Manuel y Keenoy et al., 2001]. For example, in chronic fatigue syndrome patients there is evidence of ROS/RNS-mediated damage to DNA and lipids as well as the presence of oxidized blood markers, such as methemoglobin, that are indicative of excess oxidative stress, and these occur in fatiguing illnesses more than in age matched control subjects [Richards et al., 2000; Manuel y Keenoy et al., 2001]. In addition, oxidative damage to DNA and membrane lipids has been found in muscle biopsy samples obtained from chronic fatigue syndrome patients as well as increases in antioxidant enzymes, such as glutathione peroxidase, suggesting an attempt to compensate for excess oxidative stress [Felle et al., 2000]. Chronic fatigue syndrome patients have sustained elevated levels of peroxynitrite, and this can result in lipid peroxidation, enzyme oxidation, and loss of mitochondrial function as well as changes in cytokine levels that exert a positive feedback on NO production [Castro et al., 1994; Radi et al., 1994; Pall, 2000]. Although there are small molecules that counteract the excess oxidative capacity of ROS/RNS, such as glutathione and cysteine, these have been found at lower levels in chronic fatigue syndrome patients [Manuel y Keenoy et al., 2000]. Thus similar to insulin resistance, MS, type 2 diabetes, and other MS-associated diseases aging and fatigue are linked to excess oxidative stress and overproduction of ROS/RNS, damage to mitochondrial electron transport systems and reduced oxidative phosphorylation capacities [Agadjanyan et al., 2003; Nicolson, 2003; Nicolson and Ellithrope, 2006].

USE OF ANTIOXIDANTS TO PREVENT EXCESS ROS/RNS AND MITOCHONDRIAL DAMAGE

Preventing damage to cellular and mitochondrial membranes is important in preventing loss of electron transport function and cellular energy in MS and other chronic conditions [Opara, 2002]. This can be accomplished, in part, by neutralizing excess ROS/RNS with various types of antioxidants or increasing free-radical scavenging systems [Machlin and Bendich, 1987; Chen et al., 2002; Opara, 2002, 2004; Green et al., 2004]. In MS and diseases caused or promoted by excess ROS/RNS and mitochondrial oxidative damage dietary supplementation with low molecular weight antioxidants, some accessory molecules, such as the metal ion cofactors zinc, manganese, copper, vanadium, chromium, and selenium necessary for antioxidant and other enzymes, and certain vitamins with some antioxidant properties (C, E, A, CoQ_{10}) are important in maintaining antioxidant levels and free-radical scavenging systems [Logan and Wong, 2001; Miquel, 2002; Opara, 2002; Green et al., 2004; Houston, 2005; Sheu et al., 2006]. In addition to trace metal ions and vitamins, there are at least 40 micronutrients required in the human diet [Ames, 1998], and aging increases the need to supplement these to prevent age-associated damage to mitochondria and other cellular components. Supplementation with low molecular weight antioxidants, enzyme and other cofactors and vitamins, however, may not be sufficient to maintain cellular components free of ROS/RNS damage [Granot and Kohen, 2003; Hsueh and Quiñones, 2003; Sheu et al., 2006], and antioxidants cannot replace damaged cellular components.

In animal studies dietary antioxidant administration has partially reversed the age-related declines in cellular antioxidants and mitochondrial enzyme activities and prevented or reduced the rate of decline of mitochondria from age-associated loss of function. For example, in rodents fed diets supplemented with antioxidants the antioxidants were found to inhibit the progression of certain age-associated changes in cerebral mitochondrial electron transport chain enzyme activities [Sugiyama et al., 1995; Sharman and Bondy, 2001]. The dietary use of antioxidants has also been shown to inhibit the age-associated decline in immune and other functions and prolong the lifespan of laboratory animals [De and Darad, 1991; Sugiyama et al., 1995; Arivazhagen et al., 2001; Sharman and Bondy, 2001]. In addition, antioxidant administration has certain neuroprotective effects, such as prevention of age-related hearing loss [Seidman et al., 2002]. Thus some animal studies have shown that antioxidants can partially prevent age-associated changes in

mitochondrial function, but it is not clear whether similar protection is afforded to humans [Granot and Kohen, 2004].

Dietary antioxidants along with trace metals and antioxidant vitamins may modify the pathogenic processes of certain human diseases [Heitzer et al., 1996; Ting et al., 1996; Logan and Wong, 2001; Opara, 2002; Houston, 2005; Sheu et al., 2006]. In support of this, patients with type 2 diabetes have been found to be deficient in certain antioxidant vitamins and minerals correlating with oxidative excess in type 2 diabetes [Strain, 1991; Preuss, 1998; Morris et al., 1999].

In MS-associated diseases dietary antioxidants, trace metal ions and vitamins have been proposed (separately or together) to alter the course of MS progression and inhibit the progression of MS-associated diseases [Opara, 2002; Green et al., 2004]. In most of these cases the effects of antioxidants and other supplements were measured by changes in blood or in the vascular system [Houston, 2005; Houston and Egan, 2005]. For example, vitamin C has been shown to improve endothelial-dependent vasodilation in MS and type 2 diabetes [Ting et al., 1996, 1997], and excess vitamin C in combination with vitamin E may reduce the overall risk of CHD [Hsueh and Quiñones, 2003]. However, despite the evidence for a link between excess oxidative stress in MS and associated diseases, a direct link between the intake of antioxidant nutrients, even in high concentrations, and the ability to prevent or delay MS disease progression has not been proven [Paolisso et al., 1999; Leppala et al., 2000; Granot and Kohen, 2004]. For example, in the antioxidant prevention of CHD or its complications only one-half (4/8) of the published clinical studies reviewed by Paolisso et al. [1999] showed positive results in terms of reducing markers associated with CHD. In type 2 diabetes patients antioxidant supplementation reduced blood glucose or other markers of diabetes in five of seven studies examined [Opara, 2004]. The variations in results were explained by differences in the design of the studies, differences in supplement dose(s) and duration of the trials as well as the criteria for beneficial results. Often randomized, controlled clinical trials failed to show any significant benefit of antioxidants, whereas initial cohort studies suggested otherwise [Ueda and Yasunari, 2006].

Mixtures of antioxidants, vitamins, trace minerals, and herbal extracts may be more effective in preventing early stage MS progression than in reversing late stage MS-associated disease states [Opara, 2002]. Even in late stage MS-associated diseases like type 2 diabetes, mixtures of antioxidants and minerals were useful in controlling some signs, such as blood pressure [Farvid et al., 2004; Houston, 2005]. Blinded, controlled studies on antioxidant-vitamin-mineral-herbal products like the Akesis supplement (Akesis Scientific, Inc.) have yet to be published, but preliminary open label studies indicate that such supplements may be beneficial in type 2 diabetes patients as measured by glycemic control or decreases in circulating oxidant markers [Opara, 2002]. A newer version of this antioxidant-vitamin-mineral supplement mixture (InResponse®, Response Micronutrients, Inc.) has shown good results in animal studies [Opara, 2004]. However, long-term studies will be necessary to see if nutritional antioxidant mixtures affect MS disease progression or the development of MS-associated diseases, such as type 2 diabetes, CHD, and other organ diseases.

Mitochondrial targeting of antioxidants is a promising new area that may overcome problems in bioavailability and tissue distribution of antioxidants [Green et al., 2004; Sheu et al., 2006; Szeto, 2006]. For example, mitochondria-targeted versions of CoQ_{10} and vitamin E may prove useful [Smith et al., 2003]. One of the problems in depending entirely on antioxidants, trace metal ions and vitamins is that although these components may have some protective effect, they have little direct effect on the removal and replacement of existing, oxidized cellular components.

REPLACEMENT OF DAMAGED MITOCHONDRIAL MEMBRANE COMPONENTS BY LIPID REPLACEMENT THERAPY

In MS and other diseases the critical targets of ROS/NRS damage are the genetic apparatus and cellular membranes, especially mitochondrial membrane lipids [Huang and Manton, 2004; Kanno et al., 2004]. Membrane oxidation modifies lipid structure and can affect lipid fluidity, permeability, and membrane function [Nicolson et al., 1977; Subczynski and Wisniewska, 2000]. In MS-associated diseases and fatiguing illnesses, such as chronic fatigue

syndrome, patients show increased susceptibility to oxidative stress, lipid peroxidation, and loss of electron transport function [Logan and Wong, 2001; Manuel y Keenoy et al., 2000, 2001].

Although lipid replacement therapy (LRT) plus antioxidants has been used to replace ROS/NRS damaged lipids and increase mitochondrial function in certain clinical disorders and conditions involving loss of mitochondrial function [Agadjanyan et al., 2003; Nicolson, 2003, 2005], LRT has not been used exclusively in MS patients. LRT should be useful in MS, however, because it results in replacement of damaged cellular lipids with undamaged lipids to ensure proper structure and function of critical components, including cellular and mitochondrial membranes [Nicolson, 2003, 2005]. Since damage to membrane lipids can impair membrane fluidity, electrical properties, enzymatic activities and transport functions, the LRT lipids must be protected from oxidative and other damage, and this is also necessary during storage as well as during ingestion, digestion, and absorption in vivo. To be effective LRT must result in delivery of high concentrations of unoxidized, undamaged membrane lipids in order to reverse the damage by replacement and restore function to cellular and organelle membranes. It should also be combined with antioxidants, vitamins, and minerals to provide additional antioxidant protection [Nicolson, 2003, 2005].

Combined with antioxidant supplements, LRT has proven to be an effective method to prevent and reverse ROS/RNS-associated changes in mitochondrial function [Agadjanyan et al., 2003; Nicolson, 2003, 2005]. LRT works because cellular lipids are in dynamic equilibrium in the body, and orally ingested lipids diffuse to the gut epithelium and are bound and eventually transported into the blood and lymph using specific carrier lipoproteins and also by non-specific partitioning and diffusion mechanisms. Within minutes, lipid molecules are transported from gut epithelial cells to endothelial cells, then excreted into and transported in the circulation bound to lipoproteins and blood cells where they are generally protected from oxidation [Hamilton, 1998; Fellmann et al., 2000; Hajri and Abumrad, 2002]. In the blood circulation, specific lipoprotein carriers and red blood cells protect lipids throughout their passage and eventual deposition onto specific cell membrane receptors where they can be taken into cells via endosomes and by diffusion [Conner and Schmid, 2003]. After binding to specific cell surface receptors that bring the lipids into cells, lipid transporters in the cytoplasm deliver specific lipids to cell organelles where they are taken in by specific transport proteins as well as by partitioning and diffusion. The concentration gradients that exist from the gut during the digestion of lipids to their absorption by gut epithelial cells and their transfer to blood and then tissues are important in driving the unoxidized lipids into cells and returning oxidized lipids to the intestinal track or to degradative enzymes [Mansbach and Dowell, 2000].

CLINICAL STUDIES USING LIPID REPLACEMENT ANTIOXIDANT THERAPY

As discussed above antioxidants alone may not completely eliminate or reverse ROS/RNS damage to mitochondrial and other membranes, and this is why LRT is an important addition to antioxidant dietary supplementation in order to replace damaged cellular and mitochondrial membrane phospholipids and other lipids that are essential structural and functional components of cells [Nicolson, 2003, 2005]. One such LRT dietary supplement is NTFactor® (Nutritional Therapeutics, Inc.), and this supplement has been used successfully in animal and clinical lipid replacement studies [Seidman et al., 2002; Agadjanyan et al., 2003; Ellithorpe et al., 2003; Nicolson and Ellithorpe, 2006]. NTFactor's encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without undue oxidative damage. NTFactor contains a variety of components, including phospholipids, glycophospholipids and other membrane lipids, nutrients, probiotics, antioxidants, vitamins, minerals, and plant extracts [Nicolson, 2003].

NTFactor has been used to reduce age-related mitochondrial damage in laboratory animals. In aged rodents, Seidman et al. [2002] found that NTFactor prevented hearing loss associated with aging and shifted the threshold hearing from 35 to 40 dB in control, aged animals to 13–17 dB. They also found that NTFactor preserved cochlear mitochondrial function. In addition, NTFactor prevented aging-related mitochondrial DNA deletions found in the cochlear

[Seidman et al., 2002]. Thus LRT was successful in preventing age-associated hearing loss and reducing mitochondrial damage in rodents.

In clinical studies LRT has been used to reduce fatigue and protect cellular and mitochondrial membranes from damage by ROS/RNS (Table I) [Agadjanyan et al., 2003; Nicolson and Ellithorpe, 2006]. Propax® (Nutritional Therapeutics, Inc.), a dietary supplement containing NTFactor, vitamins and minerals and other nutrients, has been used in a dietary LRT study with severely chronic fatigued patients, and it was found to reduce their fatigue approximately 40% within 8 weeks [Ellithorpe et al., 2003]. In more recent studies we examine the effects of NTFactor on fatigue in moderately and severely fatigued subjects and determined if their mitochondrial function and fatigue scores improved with administration of NTFactor. We found that after 12 weeks of oral supplement there was 35.5% reduction in fatigue ($P < 0.001$) [Agadjanyan et al., 2003]. In this clinical trial there was good correspondence between reductions in fatigue and gains in mitochondrial function. Within 8 weeks of LRT with NTFactor, mitochondrial function significantly improved ($P < 0.001$), and by 12 weeks of NTFactor supplementation, mitochondrial function was found to be similar to that found in young healthy adults. In contrast, after a 12-week washout period fatigue and mitochondrial function were intermediate between the initial starting values and those found after 8 or 12 weeks on supplement [Agadjanyan et al., 2003]. The results indicated that in moderately to severely fatigued subjects dietary LRT can significantly improve and even restore mitochondrial function and significantly improve fatigue scores. Similar findings have been observed in chronic fatigue syndrome and fibromyalgia syndrome patients on LRT plus antioxidants for 8 weeks [Nicolson and Ellithorpe, 2006]. In this case LRT with Propax containing NTFactor reduced moderate to severe fatigue by 43.1%. Such studies indicate that LRT plus antioxidants should also be useful as an adjuvant therapy in the management of MS and associated diseases where mitochondrial function is impaired due to excess ROS/RNS. The advantage of LRT plus antioxidants over antioxidant mixtures alone is that further oxidative damage is reduced *and* damaged lipid components (the main target of ROS/RNS) are gradually replaced by unoxidized lipids.

FINAL COMMENTS

MS is an emerging health problem that will require our immediate attention if we hope to reduce the incidence of CHD, type 2 diabetes, and other associated diseases that evolve from MS. Since excessive oxidative stress is one of the elements in the evolution of MS and associated diseases, attempts at reducing excess mitochondrial ROS/RNS by diet, exercise, and weight loss as well as by dietary supplementation and pharmacologic intervention should be undertaken in the rather large segment of the population that has MS characteristics. Attempts at reducing the progression of MS to more life threatening diseases, such as type 2 diabetes, heart, kidney and liver disease and stroke, should be a major focus of preventive medicine. Currently there is no one single therapeutic approach that has proven effective in managing MS progression [Houston and Egan, 2005; Collantes et al., 2006]. Thus future efforts should combine existing therapies with dietary supplements that reduce excess oxidative stress and replace molecules that are damaged by oxidative reactions.

TABLE I. Effects of NTFactor, a Dietary LRT Supplement, on Fatigue Scores in Patients With Chronic Fatigue, Chronic Fatigue Syndrome, or Fibromyalgia Syndrome[†]

Subjects/patients	Average age	Time on NTFactor	Piper Fatigue Scale fatigue reduction (%)	References
Chronic fatigue	50.3	8 weeks	40.5*	Ellithorpe et al. [2003]
Chronic fatigue	68.9	12 weeks	35.5**	Agadjanyan et al. [2003]
CFS/FMS[a]	44.8	8 weeks	43.1**	Nicolson and Ellithorpe [2006]

[†]From Nicolson [2005] with permission.
*$P < 0.0001$, compared to data without supplement. Data were collected using the Piper Fatigue Scale.
**$P < 0.001$, compared to data without supplement. Data were collected using the Piper Fatigue Scale.
[a]Chronic Fatigue Syndrome and/or Fibromyalgia Syndrome.

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