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## **Metabolic Syndrome, Cardiovascular Disease and Mitochondrial Function: Molecular Replacement and Antioxidant Supplements to Prevent Membrane Oxidation and Restore Mitochondrial Function**

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### **Summary**

One of the central defects in metabolic syndrome (MS) and its associated diseases (type-2 diabetes, vascular inflammation, atherosclerosis, among other diseases) as well as fatiguing illnesses is excess cellular oxidative stress. Oxidative stress affects many organ systems, including pancreatic beta cells, nerve cells, immune cells, and it generally affects the vascular system. Oxidative damage to membranes results in reduced efficiency of the mitochondrial electron transport chain, the main source of energy in our cells. Recent evidence indicates that reduced mitochondrial function caused by membrane oxidation is related to fatigue, a complaint in MS and cardiovascular disease (CVD) and the major complaint in fatiguing illnesses. Lipid Replacement Therapy administered as a nutritional supplement with antioxidants can prevent excess oxidative membrane damage, restore mitochondrial membrane function and reduce fatigue in a variety of clinical conditions.

### **Introduction—Metabolic Syndrome**

Metabolic syndrome (MS), estimated in over 22% of the U.S. population, is made up of several interrelated disturbances of glucose and lipid metabolism.<sup>1</sup> The major risk factors for MS are: abdominal obesity, hyperglycemia, lipid dysfunction (increased triacylglycerols, 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.<sup>2,3</sup>

Insulin resistance is one of the initial signs in the development of MS.<sup>4</sup> Insulin secreted by the pancreatic  $\beta$  cells in response to increased circulating levels of glucose and amino acids is essential for development, growth and maintenance of glucose homeostasis, and it acts by regulating gene expression and cellular metabolism.<sup>5</sup> When the circulating concentrations of insulin are insufficient to regulate the above processes, insulin resistance occurs. Insulin resistance is one of the primary events in the development of MS, and it is thought to induce the clinical sequelae that we know as MS.<sup>1</sup>

Defects in the capacity to metabolize fatty acids and glucose are thought to play an important role in insulin resistance and MS.<sup>6</sup> Accumulations of glycerols and free fatty acids in non-adipose tissues correlate strongly with insulin resistance.<sup>7-9</sup> Gene expression modifications in adipose tissue are thought to be responsible for enhanced secretion of MS-related factors, and in muscle tissue decreased oxidative capacity and fat accumulation may also induce skeletal muscle insulin resistance.<sup>6</sup>

## **Mitochondrial Damage and Type 2 Diabetes**

Various studies point to generalized mitochondrial dysfunction in MS and reduction in electron transport chain activity in type 2 diabetes patients along with fatigue.<sup>6,10,11</sup> Mitochondrial dysfunction has been linked to chronic insulin resistance, which results in preferential metabolism of fatty acids, reducing glucose utilization.<sup>12</sup> This causes gradual pancreatic beta and other cell dysfunction due to fatty acid-stimulated changes in mitochondrial proteins, resulting in an uncoupling of mitochondrial respiration, reduced electron transport chain activity and energy production and increased production of Reactive Oxygen Species (ROS).<sup>6,13-15</sup> This results in membrane damage and fatigue.<sup>13</sup>

When mitochondria function properly, the amount of superoxide produced as a consequence of electron transport activity is effectively neutralized by endogenous antioxidants and antioxidant enzymes.<sup>16</sup> In MS and associated diseases excess ROS is produced.<sup>6,14,15</sup> Fatty acids are particularly sensitive to ROS oxidation, resulting in the formation of lipid peroxides, which are toxic and lead to free-radical damage, especially in MS and type 2 diabetics.<sup>6,18</sup> In obese, insulin-resistant, pre-diabetic subjects higher amounts of free fatty acids (and their peroxide derivatives) have been found.<sup>19</sup> These are subject to peroxidative events that result in damage to mitochondrial components. Even before the diagnosis MS or type 2 diabetes, the accumulation of oxidized fatty acids in mitochondria can result in progressive damage. For example, in elderly subjects oxidized fatty acids accumulate in muscle mitochondria, and this is related to mitochondrial dysfunction.<sup>20</sup>

Type 2 diabetes is thought to occur as a consequence of persistent hyperglycemia,<sup>18</sup> which causes: (a) formation of advanced glycation end products (AGEs, the products of nonenzymatic glycation and oxidation), their oxidation and interactions with cell receptors and cellular accumulation; (b) activation of various forms of protein kinase C; (c) induction of the polyol pathway; and (d) increased hexosamine pathway flux.<sup>24-26</sup> These pathways are associated with elevated oxidative stress and over-production of superoxide (and thus ROS/RNS), but the link between hyperglycemia and increased mitochondrial superoxide production may not be mediated solely by the redox state of electron carriers.<sup>18</sup>

## **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 CVD 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.<sup>24</sup> In the cardiovascular

system ROS play an essential physiological role in maintaining vascular integrity, and when they are in excess they play a pathological role in cardiovascular dysfunction.<sup>25,26</sup>

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.<sup>27</sup> In MS these proinflammatory lipoproteins and their remnants are susceptible to oxidation,<sup>28</sup> and the presence of the oxidized lipoprotein subclasses is significantly associated with an abundance of macrophages in atherosclerotic lesions.<sup>29</sup>

When they interact with the blood vessel wall, the oxidized lipoprotein subclasses are proinflammatory and can induce endothelial adhesion molecules, which attract monocytes.<sup>30,31</sup> The adhesion and movement of adherent monocytes to subendothelial layers and their differentiation into inflammatory, ROS-producing macrophages is associated with atherosclerotic plaques. The unstable plaques can break off and form thrombi that can occlude blood vessels in the heart, resulting in myocardial infarction, ischemia and heart failure.

Another association between MS and chronic inflammation in the cardiovascular system is the elevation of C-reactive protein (CRP).<sup>32,33</sup> In MS the presence of C-reactive protein is one of the best predictors for future CVD and type 2 diabetes.<sup>34,35</sup>

## **Mitochondria 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, CVD, respiratory, musculoskeletal and bowel conditions as well as infections and cancer.<sup>36</sup> The phenomenon of fatigue has been defined as a multidimensional sensation, and recently attempts have been made to determine its extent and possible causes.<sup>37</sup> Fatigue is related to reductions in the efficiency of mitochondrial energy systems, and oxidative damage to mitochondrial components can impair oxidative phosphorylation and cause fatigue.<sup>36</sup> Mitochondria are critical elements in the process of aging, and they have been proposed to be the regulators of cellular life span.<sup>38</sup> 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-damaged molecules to maintain mitochondrial function.<sup>39-40</sup> Disease and infection can also result in excess oxidative damage that exceeds the abilities of cellular systems to repair and replace damaged molecules.<sup>39-41</sup>

## **Use of Antioxidants to Prevent Excess Oxidative 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.<sup>42</sup> This can be accomplished, in part, by neutralizing excess ROS with various types of antioxidants or increasing free-radical scavenging systems.<sup>18,42,43</sup> In MS and associated diseases dietary supplementation has been used with low molecular weight antioxidants, some accessory molecules, such as the metal ion cofactors zinc, manganese, copper, vanadium, chromium and selenium, and certain vitamins with some antioxidant properties (C, E, A, CoQ<sub>10</sub>).<sup>18,36,44-46</sup> In addition to trace metal ions and vitamins, there are at least 40 micronutrients required in the human diet,<sup>47</sup> and aging increases the need to supplement these to prevent age-associated damage. Such supplementation, however, may not be sufficient to maintain cellular components free of oxidative damage,<sup>36,48,49</sup> and antioxidants alone cannot replace damaged cellular components.<sup>36,50</sup>

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.<sup>18,43</sup> In most of these cases the effects of antioxidants and other supplements were measured by changes in blood markers.<sup>2,46</sup> For example, vitamin C has been shown to improve endothelial-dependent vasodilation in MS and type 2 diabetes,<sup>51,52</sup> and excess vitamin C in combination with vitamin E may reduce the overall risk of CVD.<sup>53</sup> 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.<sup>48,54,55</sup> For example, in the antioxidant prevention of CVD or its complications only one-half (4/8) of the published clinical studies reviewed by Paolisso et al.<sup>53</sup> showed positive results in terms of reducing markers associated with heart disease. In type 2 diabetes patients antioxidant supplementation reduced blood glucose or other markers of diabetes in five of seven studies examined.<sup>55</sup> 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 follow-on randomized, controlled clinical trials failed to show any significant benefit of antioxidants.<sup>56</sup>

Mixtures of antioxidants, vitamins, trace minerals, and herbal extracts may be more effective in preventing early stage progression of MS.<sup>42</sup> Even in late stage diseases like type 2 diabetes and CHD, mixtures of antioxidants and minerals were useful in controlling some signs, such as blood pressure.<sup>46,57</sup> Blinded, controlled studies on antioxidant-vitamin-mineral-herbal products like the Akesis supplement (Akesis Scientific Inc.) have yet to be published, but preliminary studies indicate that such supplements may be beneficial in type 2 diabetes patients as measured by glycemic control or decreases in circulating oxidant markers.<sup>42</sup> A newer version of this supplement mixture (InResponse®, Response Micronutrients Inc.) has shown good results in animal studies.<sup>55</sup> However, long-term studies will be necessary to see if nutritional antioxidant mixtures affect MS disease progression and the development of MS-associated diseases.

## **Replacement of Damaged Mitochondrial Membrane Components by Lipid Replacement Therapy**

Lipid Replacement Therapy (LRT) plus antioxidants has been used to reverse oxidative damage and increase mitochondrial function in certain clinical disorders involving loss of mitochondrial function.<sup>50,58,59</sup> LRT should be useful for MS patients, because it replaces damaged lipids with undamaged lipids to ensure proper structure and function of cellular and mitochondrial membranes. It is usually combined with antioxidants, vitamins and minerals to provide additional antioxidant protection.<sup>58,59</sup> LRT plus antioxidants has proven to be an effective method to prevent ROS-associated changes in mitochondrial function.<sup>50,58,59</sup> As discussed above, antioxidants alone may not completely eliminate or reverse ROS damage, and this is why LRT is an important addition to antioxidant dietary supplementation.<sup>42,43</sup> One LRT supplement, NTFactor® (Nutritional Therapeutics Inc.), has been used successfully in animal and clinical studies.<sup>50,58-60</sup> NTFactor's encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without significant oxidative damage.<sup>50,58</sup>

In clinical studies LRT has been used to reduce fatigue and protect mitochondrial membranes.<sup>36,58-60</sup> Propax (Nutritional Therapeutics Inc.), a dietary supplement containing

NTFactor along with vitamins, minerals and other nutrients, has been used in severely chronic fatigued patients, and it was found to reduce their fatigue approximately 40% within 8 weeks.<sup>59</sup> NTFactor in moderately and severely fatigued subjects was found to increase mitochondrial function and improve fatigue scores. For example, in patients with chronic fatigue there was a 35.5% reduction in fatigue ( $P < 0.001$ ) with a proportionate increase in mitochondrial function.<sup>59,60</sup> The results indicated that in moderately to severely fatigued subjects dietary LRT plus antioxidants can significantly improve and even restore mitochondrial function and significantly improve fatigue scores. Similar findings with LRT and antioxidants have been observed in CFS and fibromyalgia syndrome patients.<sup>60</sup> The advantage of LRT plus antioxidants over antioxidant mixtures alone is that further oxidative damage is reduced *and* damaged (oxidized) lipid components are gradually replaced, restoring function to cellular membranes.

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