Why there is an Increased Risk of Cardiac Failure, Widening of Pulse Pressure and Hemorrhagic Stroke in Type 2 Diabetics Over Age 60: Roles of Unrecognized Hypomagnesemia and Epigenetics Coupled with Increased Levels of Ceramides, Cytokines, ROS, 4-HNE and Platelet-Activating Factor

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Abstract

Despite more than 80 years of intensive and outstanding research, it remains to be determined how Type 2 (non-insulin dependent) Diabetes (T2D) results in extensive angiopathy and elevated Pulse Pressure (PP) in patients over 60 years of age, with large risks for heart attack or stroke. We present below a summary of recent findings from our laboratories which may give us clues to a better understanding of these very dangerous pathologies. Our studies present cogent reasons for why T2D is a multivariate syndrome with numerous pitfalls. T2D appears to be due, in origin, to numerous pathological pathways working in concert which lead to elevated PP (in the elderly), heart failure and/or stroke. Our observations on isolated animal tissues and cells as well as T2D subjects support a major role for Mg deficiency as a prime trigger leading to the production and release of ceramides (and possibly other sphingolipids), certain phospholipids (i.e., PKC isozymes), membrane peroxidation, activation of NADPH oxidase, Platelet-Activating Factor (PAF), reactive oxygen and nitrogen species, and release/generation of cytokines and adhesion molecules, all of which can be ameliorated by increased dietary intake and supplemental Mg. Based on extensive biochemical and biophysical-molecular assays, performed in our labs (reviewed herein), we are convinced that long-term Mg deficiency produces genotoxic effects which cause epigenetic alterations (e.g., angiogenetic/atherogenic) in cell phenotypes resulting in micro- and microvascular changes in T2D patients.

Keywords: Pulse pressure; Diabetes; Heart failure; Mg deficiency; Ceramides; Platelet-activating factor

Introduction

Ever since diabetic angiopathy, characterized by abnormal angiogenesis, was identified as a clinical entity, approximately 80 years ago, a growing awareness seems to indicate that this major complication of both type 1 (T1D) and type 2 (T2D) diabetes must be caused by either morphologic changes in the microvasculature, or by a primary functional metabolic disturbance(s) which is followed by structural vascular lesions. In addition, as T2D patients age (i.e., >60), their Pulse Pressure (PP) widens significantly (e.g., see Table 1) [1,2].

However, despite more than 80 years of intensive research, it remains to be determined how this angiopathy and widened PP develop. The lack of highly sophisticated *in-vivo*, non-invasive biophysical and biochemical techniques which are needed to diagnose, early, the microvascular structural and hemorheological alterations in the heart and brain, unfortunately, has often missed the potential underlying changes which can lead to cardiac failure and/or stroke in people over 60 years of age. About seven in 10 people over 65 with diabetes will die of heart disease and one in six from a stroke.

Table 1: Progressive widening of pulse pressure and reduction in RBC Mg^{2+} with age in T2D subjects.

Age (years)	Systolic BP	Diastolic BP	RBC Mg^{2+}
25-38	118 ± 14	82 ± 8	252 ± 18
51-65	128 ± 12	76 ± 8	228 ± 10
66-76	134 ± 10	69 ± 9	206 ± 12
77-86	138 ± 8	65+/10	198 ± 8

All values are means ± S.E.M. All values within a group are different from one another (P<0.05, ANOVA). All subjects were white males. RBC Mg^{2+} was measured using 31P-NMRS. N=18-52/group.

Despite lifestyle modification, weight reduction and medication, T2D diabetes leads to high risk for cardiomyopathy and hemorrhagic stroke

Although the clinician often diagnoses T2D on the basis of elevated serum glucose and HbA1c in the elderly, other factors must be excluded such as pancreatic disease, injury or rare syndromic or genetic forms of diabetes. To these factors, the clinician usually adds age, family history, ethnicity, mental health, medications, biochemical profile, lifestyle and body weight, often making a T2D diagnosis problematical. Early diagnosis of T2D will often prevent the sequelae of events leading to uncontrolled T2D. However, in people over 60 years of age this often becomes difficult and can result in early cardiac failure and/or hemorrhagic stroke; the greater the duration of T2D, the higher the risk for intracerebral hemorrhage [3]. Poor control of HbA1c appears to be a cardinal sign for T2D-induced intracerebral hemorrhage [4,5]. But, why are many of these patients obese and have underlying kidney diseases is not clear? What is the connection to T2D?

For the approximately 450 million people living with diabetes, about 90% suffer from T2D. These patients present with beta-cell failure and resistance to insulin. Although diet is believed to play an important (maybe critical) role in early development of T2D, it is not clear as to why [6].

Diabetics exhibit low cellular levels of ionized Mg coupled to elevated levels of calcium

Over the past several years, a number of epidemiological/met-analyses studies have appeared, in the literature, to indicate that dietary magnesium intake is inversely associated with the incidence of T2D around the globe [3,7-10].

Approximately 40 years ago, two of us suggested that alterations in the calcium and magnesium contents (and their membrane transport mechanisms) could be major factors in development of diabetic angiopathy which lead to cardiac failure [11-13]. More recently, our laboratories helped to develop new Mg^{2+} ion selective electrodes which can rapidly, and accurately, measure the biologically-active Mg^{2+} as well as Ca^{2+} in whole blood, serum, plasma and cerebral spinal fluid in less than two minutes [14-19]. Using this new tool coupled with 31P-Nuclear Magnetic Resonance Spectroscopy (31P-NMRS) we have been able to accurately measure free Mg^{2+} levels in whole blood, serum, plasma and CSF, as well as cellular levels of free Mg^{2+} and Ca^{2+} in both T1D and T2D patients as well as in women with gestational diabetes [19-34]. Our results clearly demonstrate that both T1D and T2D diabetic patients exhibit significant deficits in cellular, cardiac and brain

Mg^{2+}; the longer the patient had either T1D or T2D diabetes, and the older the subject, the lower was the serum, cellular, cardiac, and brain Mg^{2+} ([19-34], unpublished findings). These alterations were inversely proportional to the elevation in free Ca^{2+} levels in the cells and tissues. Interestingly, we also noted that racial factors were observed; i.e., the deficits in Mg^{2+} and elevations in Ca^{2+} were significantly (P<0.01) greater in the black compared to white population matched for age and sex [31]. To us, this is not surprising, as the black population is known to have greater genetic risks for heart attacks, diabetic-induced nephropathy and strokes, particularly in the T2D diabetic population. But, why should low Mg^{2+} in both tissues and cells coupled to elevated levels of Ca^{2+} provoke microvascular angiopathy and high-risk for cardiac failure, renal failure and strokes in T2D diabetics?

Low cellular levels of Mg^{2+} coupled to elevated cellular levels of Ca^{2+} results in central and peripheral vasoconstriction and vasospasm of most all types of microvessels, arteries and veins leading to decreased microvascular blood flows, coronary arterial ischemia and hypertension

Approximately 50 years ago, two of us found that lowering the extracellular level of Mg^{2+} surrounding both large and small blood vessels from the periphery, kidneys, heart and brain (from a variety of mammals (including humans) resulted in intense vasospasm; the lower the external Mg^{2+} level, the greater the degree of vasospasm [21-23,35-57]. Careful measurement of the blood vessel Ca^{2+}, as predicted by us, revealed increased cellular levels of Ca^{2+} [21,26,30,35-37,41,42,44,45,48,50,52,55,56,58-61]. Using intact rats and dogs, and studying the intact microcirculation with TV quantitative microscopy (at magnifications >6,000x), we found identical results to match the findings we obtained on the isolated blood vessels ([21,37,41,42,55-57], unpublished findings). Collectively, such results would explain how, over a period of years, as the microvascular vessels became exposed to low dietary intake of Mg and continual T2D-induced depletion of Mg, along with elevated intracellular Ca^{2+}, this would, most likely, result in stiffening of blood vessels, hypertension, and ischemia in coronary, renal blood vessels and cerebral blood vessels, as well as a widening of PP, thus resulting in Ischemic Heart Disease (IHD), cardiac failure, renal failure and/or stroke. Our laboratories were the first to report that dietary Mg deficiency in experimental animals, under carefully controlled conditions, would lead to elevations in both systolic and diastolic blood pressure [21,22,47]. Several years after our latter publication, others confirmed several of our findings [62,63]. There is now an overwhelming amount of clinical evidence to show that hypomagnesemia is, indeed, linked to heart disease, IHD, diabetic-linked heart disease (and stroke) and sudden-cardiac death in infants, children and young adults (see [1-3,7,12,13,15,27,28,30,40,54-57,64-73], among many other studies).

Low extracellular levels of Mg^{2+} lead to synthesis and release of sphingolipids and phospholipids in vascular smooth muscle cells and cardiac myocytes

In the early 1990's, working with 31P-NMRS and Proton-NMRS (1H-NMRS), and isolated vascular smooth muscle cells, we noted that low $[Mg^{2+}]_0$ induced formation of a variety of sphingolipids and phospholipids [74,75]. We found that several of the sphingolipids, particularly ceramides, exert potent contractile effects on arterial and arteriolar blood vessels, including those in the heart, brain and periphery [76-78]. In addition, in the rat brain, using direct *in-vivo* quantitative video microscopy (at magnifications > 6,000x), we found that several of the ceramides induced potent venular vasoconstriction

followed by adhesion (sticking) of leukocytes, macrophages and monocytes to the postcapillary venular walls leading to petechial hemorrhages and transudation of blood- formed elements into the brain perivascular tissues, similar to a hemorrhagic stroke [76,78].

These observations became quite intriguing to us and we, therefore, pursued them and noted that low Mg^{2+} induced/stimulated a variety of cellular signaling pathways, including up-regulation of diverse PKC isozymes and other phospholipids (e.g., DAG) [79,80] which we believe underlie many reasons for why and how T2D diabetes can result in high blood pressure, inflammations, ischemia, elevated PP, cardiac failure, renal failure and/or stroke. Several years after our Mg-sphingolipid studies were published, other investigators reported indications that synthesis/releases of diverse sphingolipids may be instrumental in the origin of T2D [81-84].

Low Mg^{2+} induces formation and release of ceramides and Platelet-Activating Factor (PAF) in vascular muscle cells: Potential relationship to diabetic angiopathy, cardiac failure and strokes

Mg^{2+} is a co-factor for more than 500 enzymes in the body. All energy-generating pathways as well as most carbohydrate, lipid, nucleotide, and protein synthetic pathways require Mg^{2+} [85]. Membrane transport of cations require Mg^{2+} [20,48,85]. Examination of vascular and cardiac muscle exposed to low Mg^{2+} revealed, to us, for the first time that all enzymes involved in the synthesis and release of ceramides, surprisingly, were up-regulated both in *in-vitro* and *in-vivo* [78,79,86-90]. These Mg-deficient environments also resulted in increased levels of sphingosine, sphingosine-1-phosphate, Diacylglycerol (DAG), and diverse PKC isozymes [54,55,57,78,79,91].

Our labs, using intact animals and isolated blood vessels reported that, depending upon vessel type, and vascular tone, the sphingolipids resulted in contraction (and reduced blood flows) or sometimes vasodilation [57,75-78,91-94]. Collectively, these studies showed that coronary, renal and cerebral blood vessels underwent contraction and reduced blood flows when stimulated with a variety of ceramides. In addition, we found that a number of the ceramides and other sphingolipids resulted in increased membrane transport of Mg^{2+}, increased membrane permeability, and inflammatory responses in cerebral, intestinal and skeletal muscle microvascular beds ([78,95], unpublished findings). When all these physiological actions of the ceramides, sphingolipids and phospholipids are taken into consideration, one must conclude that low dietary Mg intake and/or errors in Mg metabolism could underlie the observed detrimental cardiac, renal and cerebral effects of T2D.

However, on closer examination of our early 31P-NMRS and proton-NMRS data, we noted a synthesis and release of PAF and PAF-related lipids when vascular smooth muscle cells were exposed to low Mg^{2+} [71,74,75,78]. Further examination of responses of intact and isolated blood vessels revealed that these PAF molecules produced different degrees of contraction and increased permeability in the intestinal, skeletal muscle, and cerebral microvasculatures ([71,78], unpublished findings).

In 1992, Nathan and colleagues reported that bloods of T1D patients exhibited elevated levels of PAF [95]. Subsequently, other workers have reported similar findings [96]. Examination of the sera of several of our elderly T2D patients also revealed increased levels of both ceramides and PAF; the greater the duration of the

T2D, the higher the measured serum levels of both ceramides and PAF (P<0.01) [97]. The higher the serum levels of ceramides and PAF, the lower the serum levels of ionized Mg in our T2D patients (P<0.01) [97]. Given our findings, we must conclude that low Mg^{2+} levels in the T2D patients must perforce lead to synthesis and release of ceramides, DAG and PAF molecules.

Experiments done in our labs have confirmed that rat vascular smooth muscle and cardiac muscle exposed to low Mg lead to synthesis and release of ceramides, DAG, diverse PKC isozymes, and PAF [54,55,57,71-76,78,79]. Whether human-type 2 diabetic vascular smooth muscle and cardiac myocytes produce ceramides, DAG, PKC isozymes and PAF when exposed to low Mg in culture remains to be tested.

But are the synthesis and release of ceramides, the other phospholipids and PAF-related molecules totally responsible for the fibroses and angiogenic alterations observed in T2D patients or are they working in concert with cytokines like transforming growth factor-beta (TGF-beta)? It has been suggested that TGF-beta and adhesion molecules play important roles in T2D-induced nephropathy via inflammatory and fibrotic responses [98-101].

Low Mg^{2+} levels are associated with elevated levels of TGF-beta and other cytokines in vascular and cardiac muscle and sera of T2D subjects

Over the past several years, working with intact animals exposed to dietary deficiency of Mg (21 days) and isolated vascular smooth muscle cell, in primary cell cultures, exposed to low levels of Mg^{2+}, we found, as predicted, that low levels of Mg^{2+} resulted in a synthesis and release of TGF-beta, IL-2, IL-6, TNF-alpha and the adhesion molecule VCAM-1 ([21,55,57,78,79,88], unpublished findings). Examination of the sera of several T2D patients, over 60 years of age, indicated that all these patients exhibited elevated levels of these cytokines and VCAM-1 [102]. Having this data, in view of the above sphingolipid and PAF findings, we are tempted to believe this presents a strong basis for how and why T2D patients over 60 years of age exhibit considerable inflammatory responses, elevated PP, hypertension, cardiomyopathy, renal diseases and a risk for intracerebral hemorrhagic stroke. However, how does one account for T2D patients over 60 years old presenting with accelerated atherogenesis?

Low serum Mg^{2+} levels in T2D subjects are associated with elevated levels of cholesterol, triglycerides and LDL in both animal and clinical studies: Relationship to atherogenesis and platelet aggregation

Approximately 30 years ago, two of us reported that dietary deficiency of Mg in rabbits coupled with elevated dietary levels of cholesterol induced very pronounced arterial plaques invested with elevated levels of cytokines, macrophagesand monocytes ([103], unpublished findings). Sera from these atherosclerotic animals demonstrated low levels of ionized Mg coupled to elevated levels of cholesterol, triglycerides and LDL, very similar to what has been found in many elderly T2D patients [104] as well as in our hospitals (e.g., see Table 2). More than 30 years ago, several workers reported increased platelet aggregation in the sera of T2D subjects [105,106]. We have found that the higher the degree of platelet aggregation, the higher the serum levels of both PAF and ceramides and the lower the serum level of ionized Mg in elderly TD2 patients [102]. All of these physiological alterations would produce atherogenesis.

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Table 2: Comparison of serum cholesterol, triglyceride, LDL and ionized Mg levels in young normal *vs.* elderly male T2D subjects.

Age (years)	Chol (mmol/L)	Trigly (mmol/L)	LDL (mmol/L)	Mg^{2+} (mmol/L)
25-38	3.8 ± 0.8	1.6 ± 0.4	2.5 ± 0.6	0.68 ± 0.08
77-86	5.5 ± 1.0	2.6 ± 0.6	3.8 ± 0.8	0.58 ± 0.06

All values are ± S.E. M. All values within a group are different from one another (P<0.05, ANOVA). All subjects are same as table 1.

Table 3: Progressive serum elevation of MDA concomitant with reduction in SOD and GSH in T2D subjects on aging.

Age (years)	MDA(U/g Hb)	SOD(U/g Hb)	GSH(U/g Hb)
23-38	1.28 ± 0.24	5.96 ± 1.04	3.75 ± 0.68
66-76	3.76 ± 0.42	3.46 ± 0.56	2.22 ± 0.14
77-86	4.24 ± 0.48	2.98 ± 0.38	1.88 ± 0.12

All values are means ± S.E.M. All values within a group are different from one another (P<0.05). All subjects within a group are the same as in table 1.

Roles of Mg in membrane peroxidation and generation of reactive oxygen and nitrogen species in T2D: Relation to angiogenesis and cell death

In the late 1980's, our laboratories noted that dietary deficiency of Mg^{2+}, both *in-situ* and on isolated cardiovascular tissues and cells, as well as sera, resulted in membrane peroxidation of diverse cardiac and vascular muscle tissues, and generation of a number of ROS and Reactive Nitrogen Species (RNS) [54,55,57,71,74,107-110]. Very recently, we found that dietary deficiency of Mg (21 days) in rats resulted in an up-regulation of NADPH oxidase in all cardiovascular tissues and cells examined (i.e., 4-7-fold) ([78], unpublished findings). Activation of NADPH oxidase is known to produce superoxide radicals which lead to hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot$OH) and peroxynitrite radicals ($ONOO^-$) (for review, see [111]).

It has been reported that oxidative stress in T2D patients is also associated with elevated serum and leukocyte levels of myeloperoxidase (MPO) [111-114]. Using some of our elderly T2D subjects, we have found a strong inverse relationship between RBC Mg^{2+} and MPO content (P<0.01) (unpublished findings). MPO is one of the most aggressive oxidants of ROS which usually results in elevated serum levels of hypochlorite (OCl^-) which are found in T2D patients [112-118]. We have reported that OCl^-, just like the other ROS, can promote constriction of blood vessels [119-124]. Studying the intact microcirculation and isolated vascular smooth muscle cells, we noted that every single ROS found in T2D patients (i.e., $\cdot$OH, H_2O_2, OCl^-) or RNS ($ONOO^-$) investigated, would promote constriction/contraction of cerebral and peripheral blood vessels and increased vascular reactivity [78,123-124]. Many other investigators have reported similar pharmacological actions [e.g., 125,126]. Such actions clearly must perforce result in tissue ischemia, leading to hypoxic areas of no or little capillary/nutritional blood flow, stiffening of blood vessel walls, elevated PP and diverse inflammatory lesions.

In addition, as others have reported [116-118], we found progressive peroxidation in the T2D subjects, as exemplified by elevated serum levels of Malondialdehyde (MDA) (e.g., Table 3). Interestingly, but not surprising, as the MDA levels rose with age, the levels of two major anti-oxidants, Superoxide Dismutase (SOD) and Glutathione (GSH), declined with age (Table 3). The concentrations of the latter two moieties clearly parallel the RBC Mg^{2+}, while the concentrations of MDA is inversely proportional to the RBC Mg^{2+} of the aged T2D subjects (Compare Table 1 with Table 3).

Lipid peroxidation by-product 4-Hydroxy-2-Nonenal (4-HNE) found in sera of T2D patients and in vascular muscle cells exposed to low Mg

Approximately five years ago, our laboratories reported that when aortic, cerebral and neonatal piglet coronary vascular smooth muscle cells were exposed to low concentrations of $[Mg^{2+}]_0$, in primary cultures, the cells generated 4-HNE [71]. 4-HNE, a major aldehyde product of lipid peroxidation in membranes, is known to exert numerous cytotoxic, genotoxic, biological and signaling actions [71,127-132]. 4-HNE is a forerunner of hydrogen peroxide production [130-132]. As little as 1.0 uM of 4-HNE can produce chromosomal abnormalities and result in DNA fragmentation. Thus, low dietary Mg intake, over a period of years, could be expected to produce increased cellular and blood levels of 4-HNE, in T2D subjects over 60 years of age, as our investigation has now found (e.g., see Table 4). These new findings could be very important to the evolvement of the progressive microvascular structural-wall alterations, reduced nutritive-capillary blood flow, atherogenic, and inflammatory conditions observed in T2D patients. At about the time, we initiated our studies on 4-HNE, others reported evidence for 4-HNE induction of insulin-resistance in T2D subjects [133] and elevated 4-HNE adducts in sera of T2D patients with chronic periodontitis [134].

Table 4: Serum elevation of 4-HNE-His adducts in elderly *vs.* young T2D subjects.

Age (years)	Serum 4-HNE-His (µmol)
23-38	0.22 ± 0.04
77-86	0.73 ± 0.06

All values are means ± S.E.M. Mean values for ages 77-86 years are sig. diff. from 28-38 yr olds (P<0.001, t-test). 4-HNE-His analytes were determined with ELISA assays.

Interestingly, it has been reported that DNA damage and the DNA-damage response has been identified in human atherosclerosis [135,136]. As we demonstrated approximately 30 years ago, in a rabbit model, suboptimal dietary intake of Mg (similar to that which 65-75% of the North Americans ingest daily) results in rapid atherosclerosis with plaques over more than 60% of the aortic and coronary arterial surfaces [103]. Recent human studies, using serial angiography coupled with postmortem studies, suggest that many plaques appear to invade coronary arterial walls before myocardial infarctions and in the absence of blood clots [136,137]. As indicated above, our earlier previous studies indicated that low $[Mg^{2+}]_0$ environments lead to coronary arterial vasospasm and ischemia prior to plaque formation [13,23,30,54,55,57,71,78,138]. Depending upon the diverse types of DNA-modifications, cells in T2D patients (i.e., vascular, cardiac and endothelial cells) would exact different repair processes *in-situ* in order to attempt to remove such damage. Thus, it would be important to keep in mind that both DNA damage and synthesis could be expected in diverse tissues, *in vivo*, depending upon time and circulating/cellular levels of free ionized Mg. Using this hypothesis, we have recently posited how hypomagnesemia may underlie an "epigenetic" basis for disturbances leading to cardiovascular tissue and disease states [78,91,138-141].

ROS, RNS and 4-HNE can lead to various forms of cell death

All of the ROS, RNS and 4-HNE molecules, mentioned above, have been found to lead to angiogenesis/atherogenesis [78,116-118] and various forms of programmed cell death (i.e., apoptosis, necroptosis), which are hallmarks of atherogenesis. We have found, using scanning EM, that low Mg diets or primary cultured VSMC exposed to low Mg^{2+} lead to several forms of programmed cell death (i.e., apoptosis, necroptosis, ferroptosis, and pyroptosis) [78,110,142-144].

We are convinced that these associations are more than coincidental. However, the hypotheses in this paper must remain hypothetical until more rigorous studies are completed.

But it is, indeed, of considerable interest, to note here, that treatment of T2D as well as T1D subjects with Mg, by different groups [6,145-153], including ours [32-34], appears to stabilize these patients metabolically and provide a better life style than just diabetic drugs, alone, which often have very dangerous side effects.

Daily intake of bioavailable Mg in drinking water should go a long-way to the amelioration/prevention of vascular and cardiac damage in T2D patients

We believe, at the very least, that the evidence presented here in, adds considerable support to the hypothesis suggested more than two decades ago [154,155] that water intake (from tap waters, well waters, bottled waters, and beverages using tap/well/spring waters) in humans varying between one and two liters/day with Mg^{2+} intakes varying from 20 to >100 mg/l , may, as we have suggested recently [54,79,86,87,90,138], represent an excellent way to overcome and control marginal intakes of Mg obtained with most Western diets (with shortfalls of between 250-350 mg Mg/day). Moreover, in view of our findings and those of others [6,146-153], it is probably propitious to suggest that all desalinated-purified recovered/recycled waters, harvested rain waters, well waters, and all bottled waters given to humans to drink should be supplemented with bioavailable Mg^{2+} to ameliorate the induction of cardiovascular risk factors, disease processes, and the progression of diabetic disease processes worldwide.

Conclusions and Future Thoughts

Herein, we present a summary of recent findings from our laboratories which reveal a new hypothesis for why people over the age of 60 often develop a T2D disease which has numerous microvascular and macrovascular manifestations that are difficult to diagnose and treat. Our studies also present cogent reasons for why T2D is a multivariate syndrome with numerous pitfalls. T2D appears to be due, in origin, to numerous pathophysiological pathways which lead to heart failure, elevated PP and strokes. We also present observations and investigations on isolated tissues and cells as well as T2D patients which we believe support major roles for Mg deficiency as a prime trigger leading to the production and release of ceramides (and possibly other sphingolipids), phospholipids, membrane peroxidation, 4-HNE adducts, PAF molecules, ROS, and RNS together with cytokines and adhesion molecules, all of which can be ameliorated with Mg supplementation. It is clear that our hypothesis can, and should, be tested, particularly as there are inhibitors available which can block the synthesis of both ceramides and PAF.

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