Targeting Oxidant Stress as a Strategy for Preventing Vascular Complications of Diabetes and Metabolic Syndrome

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Abstract

Oxidant stress plays a central role in mediating the macro- and microvascular complications of diabetes and metabolic syndrome. Radicals antagonize protective nitric oxide (NO) bioactivity, through direct quenching of NO and uncoupling of NO synthase, while promoting inflammation and fibrosis via activation of NF-kappaB and TGF-beta, respectively. Oxidants are key mediators of insulin resistance in hypertrophied adipocytes – which gives rise to systemic insulin resistance – and may also promote beta cell dysfunction. In diabetics, a major effect of peroxynitrite is to trigger PARP-mediated inhibition of glyceraldehyde-3-phosphate dehydrogenase; glycolytic intermediates pile up behind this bottleneck, boosting the activity of 3 key pathways known to mediate complications: diacylglycerol synthesis (leading to protein kinase C activation), and the hexosamine and polyol pathways. The chief sources of excess oxidant stress in metabolic syndrome and diabetes are NADPH oxidase (activated by PKC, angiotensin II, and advanced glycation/lipoxidation endproducts), uncoupled NO synthase, and – in diabetics – mitochondria. Fortunately, it may be feasible to suppress the production and downstream effects of the radicals overproduced in these disorders, using safe nutraceuticals. Phycocyanobilin (PCB), a biliverdin-derived chromophore found in blue-green algae, has recently been shown to potently inhibit NADPH oxidase in a manner analogous to bilirubin; efforts to develop PCB-enriched algae extracts as antioxidant supplements are underway. High-dose folate can somehow "pinch-hit" for deficient tetrahydrobiopterin, effectively "recoupling" NO synthase and restoring its normal activity. Lipoic acid – as well as the drug metformin – boost the antioxidant defenses of endothelial mitochondria by activating AMP-activated kinase and PGC-1α; lipoic acid may also combat oxidant stress via phase II induction of glutathione and heme oxygenase-1. High doses of thiamine – or its better-absorbed analog benfotiamine - can increase transketolase activity, decreasing excess substrate in the upper glycolytic pathway by drawing it into the pentose phosphate pathway. Taurine has the potential to ameliorate the impact of myeloperoxidase-derived oxidants on diabetic complications, and suppresses development of neuropathy in diabetic rodents. The vitamer pyridoxamine can aid control of oxidative stress by inhibiting production of advanaced glycation/lipoxidation end products. Thus, a regimen of PCB, high-dose folate, lipoic acid, taurine, pyridoxamine, and benfotiamine may have considerable potential for preventing complications in patient who are diabetic and/or insulin resistant. Selenium, vitamin C, niacinamide, melatonin, and oligopeptide ACE inhibitors may also have some value in this regard. Clearly, there is considerable scope for the development of rational, well tolerated nutraceutical regimens which could substantially mitigate the health risks associated with metabolic syndrome and diabetes.

Oxidant Stress – Key Mediator of Diabetic Complications

Excessive production of superoxide – most notably in vascular endothelium – is believed to be a fundamental mediator of the macro- and micro-vascular complications of diabetes and metabolic syndrome.[1, 2] This increase in superoxide production reflects activation of NADPH oxidase, uncoupling of the endothelial isoform of nitric oxide synthase (eNOS), and – specifically in diabetes – short-circuiting of respiratory chain electron transport in mitochondria.[1-9]

The increase in NADPH activity is induced by activation of certain isoforms of PKC that can phosphorylate p47phox, promoting its translocation to the plasma membrane;[10] this increase in PKC activity is largely attributable to increased de novo synthesis of diacylglycerol (DAG), reflecting increased free fatty acid exposure and, particularly in diabetics, increased substrate in the upper portion of the glycolytic pathway within tissues such as endothelium that are highly glucose permeable.[1-3, 11] DAG synthesis is further boosted in tissues expressing aldose reductase owing to an increase in the cytosolic $NADH/NAD^+$ ratio that promotes generation of glycerol-3-phosphate. Up-regulation of the local renin-angiotensin system,[12-16] including a PKC-mediated increase in AT1 receptor expression,[17] further amplifies NADPH activity, in part by increasing the expression of NADPH subunits.[18-20] In diabetics, an additional increase in NADPH oxidase activity can be induced by advanced glycation and lipoxidation end-products (AGEs and ALEs), via interaction with the RAGE receptor.[21-23]

Uncoupling of eNOS is secondary to increased oxidant production by NADPH oxidase and/or mitochondria; peroxynitrite readily oxidizes tetrahydrobiopterin, and, in the absence of this key cofactor, eNOS catalyzes the transfer of electrons from NADPH to molecular oxygen, generating superoxide.[7] This uncoupling also entails decreased efficiency of nitric oxide production. There is considerable evidence for a functional deficiency of tetrahydrobiopterin in the vascular endothelium and renal mesangium of diabetic or insulin-resistant rats, associated with deficient eNOS activity;[24-32] furthermore, intra-arterial infusion of tetrahydrobiopterin in type 2 diabetic patients rapidly improves endothelium-dependent vasodilation.[33]

Increased production of superoxide by the endothelial mitochondria of diabetics presumably reflects increased substrate availability that leads to an excess of electrons in mitochondrial respiratory chains;[1] however, it is suspected that reduced efficiency of the distal portion of the respiratory chain, possibly secondary to oxidant stress, contributes to this phenomenon.[34]

Increased superoxide production may act in a number of interacting ways to promote vascular complications. Of key importance in this regard is the fact that superoxide antagonizes effective NO bioactivity – by directly quenching NO, by promoting

uncoupling of eNOS, and by impairing the efficiency of the insulin-PI3K-Akt pathway that induces an activating phosphorylation of eNOS.[35, 36] Moreover, the peroxynitrite generated when superoxide reacts with NO is a highly active oxidant that mediates much of the oxidant stress associated with diabetes and metabolic syndrome.[37, 38] In particular, peroxynitrite, as well as the hydrogen peroxide derived from superoxide dismutation, can promote activation of NF-kappaB, inducing an "activated" phenotype conducive to macrovascular disease and other inflammatory complications.[39-42] These oxidants can also promote the synthesis and activation of TGF-beta, thereby acting as mediators of the pro-fibrotic complications of diabetes, such as glomerulosclerosis.[43, 44] The utility of PARP inhibitors for mitigating endothelial dysfunction and related complications in diabetic rodents presumably reflects a key role for peroxynitrite-mediated DNA damage in the genesis of these complications.[45] Pericyte apoptosis, an early event in the evolution of diabetic neuropathy, appears to be triggered by oxidant stress.[46]

Recent studies employing the NADPH oxidase inhibitor apocynin point to a role for this enzyme complex in the early stages of diabetic neuropathy in rats,[47] and demonstrate that the oxidative stress generated by chronic activation of NADPH oxidase in the hypertrophied adipocytes of obese mice renders these adipocytes insulin resistant and 'inflamed", such that they secrete the adipokines characteristic of insulin resistance syndrome (e.g. IL-6, TNF-alpha, leptin).[48] In the pancreas of diabetics, activation of this enzyme complex via local up-regulation of angiotensin II activity promotes beta cell dysfunction and apoptosis.[49-51]

Brownlee and colleagues have demonstrated that oxidant stress works in several other ways to boost the activity of pathways thought to mediate diabetic vascular complications. In particular, peroxynitrite, by inducing DNA damage that activates PARP, leads to polyADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), diminishing the activity of this enzyme.[2] As a result, intermediates in the upper portion of the glycolytic chain build up – an effect that is of particular importance when hyperglycemia is promoting increased tissue glucose uptake. Thus, more substrate becomes available for de novo DAG synthesis, for aldose reductase, and for glutamine:fructose-6-phosphate amidotransferase, the first and rate-limiting enzyme for the hexosamine pathway that generates UDP-N-acetylglucosamine.[1, 2, 52] Studies with diabetic rodents have demonstrated that PKC activation, the polyol pathway, and the hexosamine pathway all contribute to the induction of diabetic complications.[53-55] Furthermore, oxidant stress can boost aldose reductase activity by blunting the tonic inhibitory impact of physiological levels of NO on this enzyme;[56-58] as a result, accelerated utilization of NADPH reduces the availability of this cofactor to eNOS, compounding the NO deficiency, while also impairing NADPH-dependent antioxidant mechanisms.

There is recent evidence that vascular-bound myeloperoxidase is dramatically increased in diabetic rats, and that its interaction with hydrogen peroxide diffusing from neighboring cells can give rise to hypochlorous acid (HOCl) and other chlorinating compounds that may be more detrimental to endothelial function than is hydrogen peroxide per se.[59] In vitro, the HOCl scavenger methionine alleviates the severe

impairment of endothelium-dependent vasodilation noted in rat aorta exposed to high glucose and myeloperoxidase – thus incriminating HOCl as the likely mediator of the endothelial toxicity associated with myeloperoxidase.[59]

In aggregate, these considerations suggest that measures which suppress superoxide production by NADPH oxidase, eNOS, and – in diabetics – mitochondria, may go a long way toward "getting to the root" of the macro- and micro-vascular complications associated with diabetes and metabolic syndrome. Practical nutraceutical strategies for blunting the production or downstream consequences of excessive superoxide would be of particular interest, as these might be more convenient, affordable, and safer than drug therapies. In this regard, a regimen comprised of phycobilins, high-dose folate, lipoic acid, taurine, pyridoxamine, and benfotiamine may have great potential.

Phycobilins – Phytonutrient Inhibitors of NADPH Oxidase

The vascular protection associated with relatively high plasma levels of unconjugated bilirubin,[60, 61] as well as that afforded by the antioxidant enzyme heme-oxygenase-1,[62-64] most likely reflects the ability of bilirubin to serve as an endogenous inhibitor of NADPH oxidase;[65, 66] although bilirubin has usually been characterized as an oxidant scavenger, its intracellular concentrations are in the low nanomolar range[67] and, moreover, it is a relatively inefficient scavenger of superoxide.[68] Thus, it is now suspected that the important antioxidant activity of bilirubin in tissues reflects, not scavenging activity, but rather direct inhibition of NAPDH oxidase.[66]

Bilirubin arises by reduction of biliverdin, a product of heme-oxygenase-mediated catabolism of heme; this reduction is mediated by the ubiquitously expressed enzyme biliverdin reductase.[67, 69] Plants and blue green algae generate chromophores known as "phycobilins" that are derivatives and close structural analogs of biliverdin; following conjugation to apoproteins, these chromophores function as light harvesters, much like chlorophyll.[70] These phycobilins are readily susceptible to reduction by biliverdin reductase, giving rise to "phycorubins" that are close analogs of bilirubin.[71] Recently, Inoguchi has demonstrated that phycocyanobilin (PCB), the chromophore responsible for the blue pigmentation of spirulina, is a potent inhibitor of NADPH oxidase activity in human endothelial, smooth muscle, and mesangial cell cultures; the effects are quite parallel to those of biliverdin in these cell lines, and are dose-dependent in the range of 1-20 μM (Toyoshi Inoguchi, personal communication). These findings may rationalize previous reports that oral administration of phycocyanin – the spirulina protein that includes PCB as a chromophore – exerts a wide range of anti-inflammatory effects in rodents.[72] Thus, it has been suggested that PCB-enriched spirulina extracts may have potential as antioxidant nutraceuticals that, in appropriate oral doses, could partially inhibit endogenous NADPH oxidase activity.[66] The implications of such a strategy for controlling the excess oxidant stress associated with diabetes or metabolic syndrome are clear.

Recently, Inoguchi and colleagues have examined risk for vascular complications in diabetics who have Gilbert syndrome, a genetic variant in which free unconjugated

bilirubin levels are 2-3-fold elevated owing to reduced hepatic expression of the isoform of UDP-glucuronosyl transferase (1A1) responsible for conjugation of bilirubin.[73] These researchers identified 96 diabetics with Gilbert syndrome, and compared them with 425 diabetics without this syndrome; all diabetics included in the analysis had been diabetic for at least five years. Risks for retinopathy, macroalbuminuria, and coronary disease were significantly lower in the former group. After multiple regression analyses adjusting for a number of relevant factors, including parameters related to insulin resistance and diabetic control (control tended to be somewhat better in the Gilbert group, possibly reflecting a role for NADPH oxidase activation in insulin resistance and beta cell failure[48, 49]), relative risk for retinopathy, macroalbuminuria, and coronary disease in the Gilbert group was calculated to be 0.216, 0.205, and 0.206, respectively. These findings suggest that the partial inhibition of NADPH oxidase activity associated with elevated unconjugated bilirubin in diabetics with Gilbert syndrome provides important protection from the vascular complications of diabetes. It is reasonable to anticipate that prompt administration of PCB, in a dosage schedule sufficient to replicate the degree of NADPH oxidase inhibition experienced by subjects with Gilbert syndrome, would likewise have an important effect on risk for major complications in diabetics.

While it is evident that excessive inhibition of NADPH oxidase could compromise immune defenses, it should be noted that subjects with Gilbert syndrome do not experience any evident increase in risk for infection or other disorders, and indeed appear to be at notably decreased risk for vascular disorders;[74-76] thus, it may be feasible to achieve moderate inhibition of NADPH oxidase without compromising health. Furthermore, if a serious infection developed during the course of PCB therapy, PCB administration could be temporarily discontinued to quickly restore full NADPH oxidase activity.

Pending the availability of PCB in supplemental form (extracted from spirulina, or synthesized), it may be feasible to use whole spirulina as a clinical source of PCB. A heaping tablespoon of spirulina (approximately 15g) provides about 100 mg PCB, and can be rendered palatable by inclusion in "smoothies" made with ingredients such as soy milk, fruit juices, or whole fruit. It is pertinent to note that inclusion of whole spirulina in the diet of rodents has been reported to exert potent anti-inflammatory effects.[77-82]

Vitamin E Has Not Fulfilled Expectations

An alternative approach to suppressing activation of NADPH oxidase in diabetics was proposed some years ago by King and colleagues. These researchers demonstrated that, in diabetic rodents, high-dose injections of vitamin E (d-alpha-tocopherol, 40 mg/kg i.p., every other day) prevented activation of PKC (and hence its downstream target NADPH oxidase) by lowering the elevated concentrations of diacylglycerol in tissues giving rise to diabetic complications (retina, glomeruli, aorta); as might be expected, this effect was associated with an amelioration of the early impacts of diabetes on retinal and glomerular structure and function.[83-89] In cell cultures exposed to high glucose, the impact of vitamin E on diacylglycerol levels was traced to an increase in diacylglycerol kinase activity, possibly indicating that oxidized lipids inhibit this enzyme.

Unfortunately, these benefits may have been contingent on exceptionally high tissue levels of vitamin E achieved through parenteral administration, as in clinical studies entailing oral administration of vitamin E, the benefits have been equivocal at best. In type 1 diabetics (average duration of diabetes 4 years), 1,800 IU d-alpha-tocopheryl acetate daily for 4 months was associated with a significant increase in retinal blood flow, reversing the trend toward decreased flow seen in unsupplemented diabetics.[90] Studies examining the impact of vitamin E supplementation on the depressed endothelium-dependent vasodilation of diabetics observed an improvement in type 1 subjects (1,000 IU daily for 3 months) but no change in type 2 subjects (1,600 IU daily for 8 weeks).[91, 92] More recently, the largest and longest study to evaluate high-dose vitamin E (1,800 IU daily for 1 year) in diabetics (of both types) failed to observe any improvement in endothelial function during vitamin E, and indeed trends in response were slightly but significantly better during placebo than vitamin E.[93] Significant rises, relative to placebo, were seen in systolic blood pressure and plasma endothelin during vitamin E – although C-reactive protein fell slightly in the vitamin E group. The authors concluded that "because vitamin E-treated patients had a worsening in some vascular reactivity measurements when compared to control subjects, the use of high dosages of vitamin E cannot be recommended". These disappointing results parallel those seen in randomized prevention trials with vitamin E in patients at high risk for coronary events; in particular, in the HOPE study, 400 IU vitamin E daily for an average of 4.5 years did not influence the subsequent incidence of coronary events or stroke in diabetic subjects, nor influence the onset of overt nephropathy.[94] While we cannot exclude the possibility that high-dose vitamin E might favorably influence some aspect of diabetic complications during some stage of the disease, the overall impression is disappointing compared to the clear-cut beneficial results seen in rats with recent-onset diabetes.

Re-Coupling eNOS with High-Dose Folate and Ascorbate

With respect to uncoupled eNOS, high-dose folate may offer a simple remedy. For reasons that are not yet clear, adequate concentrations of 5-methyltetrahydrofolate (the chief metabolite of folic acid circulating in plasma) can "pinch-hit" for the function of tetrahydrobiopterin in eNOS when concentrations of the latter are insufficient for coupled eNOS activity.[95-97] In other words, 5-methyltetrahydrofolate prevents uncoupled eNOS from generating superoxide, and restores its normal capacity to generate NO. Thus, acute infusions of 5-methyltetrahydrofolate have been shown to have a favorable impact on endothelium-dependent, NO-mediated vasodilation in various disorders associated with oxidant-mediated endothelial dysfunction[98] – including diabetes.[99] Of greater practical interest are studies demonstrating that relatively high daily oral intakes of folic acid – 5-10 mg per day have usually been used – can improve endothelium-dependent vasodilation in both type 1 and type 2 diabetics.[100, 101] It seems likely, however, that higher doses would produce greater benefit. Recently, Tawakol et al have shown that, in patients with ischemic heart disease, pre-administration of 30 mg folic acid (two 15 mg doses, 12 hours apart) produces a marked augmentation of adenosine-stimulated myocardial blood flow in ischemic regions of the heart;[102] this phenomenon is thought to reflect a normalization of shear-induced, NO-mediated vasodilation, attributable to re-

coupling of eNOS. Remarkably, over 30 years ago, Oster reported that daily mega-doses of folate – 40-80 mg per day – had a very favorable clinical impact on angina, intermittent claudication, and the healing of ischemic ulcers;[103, 104] most likely, these benefits reflected the fact that Oster inadvertently had repaired eNOS function in his patients.[105] (This phenomenon most likely is unrelated to modulation of homocysteine levels; in any case, recent evidence from prospective trials suggests that a moderate elevation of homocysteine is a marker for, rather than a mediator of, vascular risk.[106])

An alternative strategy for preserving tetrahydrobiopterin function in cells subjected to oxidant stress is to insure optimal intracellular concentrations of ascorbic acid. Exposure of cultured human endothelial cells to ascorbic acid increases their eNOS activity; this effect has been traced to ascorbate's ability to enhance intracellular levels of tetrahydrobiopterin, while decreasing the levels of oxidized forms of this cofactor.[107-110] Since ascorbate does not enhance tetrahydrobiopterin synthesis,[109] it seems to be protecting this cofactor from oxidation by peroxynitrite or other oxidants; presumably, it readily donates electrons to oxidized forms of this cofactor, restoring its proper tetrahydro structure. This phenomenon has also been demonstrated in vivo, in the aortas of ApoE-deficient mice treated with high-dose dietary ascorbate.[111]

However, there may be limited scope for the impact of ascorbate on eNOS activity in humans. Uptake of ascorbate by endothelial cells is saturated at plasma concentration of 100 μM,[107, 112] which is readily achieved and maintained by ascorbate intakes of 500 mg daily.[113] This suggests that supplemental ascorbate would only benefit endothelial function in subjects with mediocre baseline ascorbate status. Possibly, this accounts for the inconsistent findings of studies which have examined the impact of supplemental ascorbate on vascular endothelial function in subjects at increased coronary risk. (The favorable effects of intravenous vitamin C infusion on endothelial function in various studies, including those involving diabetics,[114, 115] are not likely to be germane, as the supraphysiological plasma concentrations of ascorbate achieved have superoxide scavenging activity that is insignificant at the lower physiological concentrations achievable through oral dosing.[116]) Several studies have found that supplemental ascorbate (at least 800 mg daily) does not benefit endothelium-dependent vasodilation or markers of endothelial inflammation in patients with diabetes.[117-119] One study reported a favorable effect of oral ascorbate on endothelial function in type 1 diabetics but not type 2;[120] another study found benefit only in diabetic patients concurrently afflicted with coronary disease.[121] A study evaluating the acute negative impact of a fatty meal on endothelial function in type 2 diabetics found that prior supplementation with ascorbate alleviated the endothelial dysfunction.[122]

The possible impact of ascorbate status on risk for diabetic microvascular complications, either in animal models or clinically, remains largely unexplored. There are several reports that plasma ascorbate levels tend to be lower in diabetics with microangiopathy than those without;[123-125] it is unclear whether ascorbate deficiency predisposes to microangiopathy, or whether these findings simply reflect the fact that high oxidative stress associated with a predilection to microangiopathy degrades ascorbate status.

In a recent analysis pooling 9 large prospective cohort studies (involving a total of over 293,000 subjects), Harvard researchers concluded that use of vitamin C supplementation (700 mg or more daily) was associated with a significant 25% reduction in risk for coronary heart disease.[126] Multiple regression analysis was employed to account for lifestyle factors associated with supplementation, and vitamin E supplementation was found to be without benefit in this analysis. In light of the facts that coronary disease is the chief cause of mortality in diabetes, and that adequate ascorbate status helps to preserve effective eNOS function, it would seem prudent to include a moderate dose of ascorbate in any nutraceutical program intended to ameliorate diabetic complications.

Flavanol-rich cocoa has recently attracted considerable attention for its cardiovascular protective potential. Epicatechin, richly supplied by unprocessed cocoa, is now known to provoke or up-regulate release of NO from vascular endothelium.[127, 128] Such an effect would presumably be more clinically beneficial in subjects with diabetes or insulin resistance syndrome if uncoupling of eNOS was concurrently corrected by administration of high-dose folate, in conjuction with other measures to limit endothelial superoxide production. Of particular interest is the fact that hypertension is essentially absent among the Kuna Indians of Panama, so long as they follow their traditional lifestyle that includes ingestion of 3-4 servings of unprocessed cocoa per day.[129] In clinical studies, cocoa administration has been shown to lower elevated blood pressure and to improve muscle insulin sensitivity.[130, 131] Its impact in clinical diabetes has not yet been studied.

Addressing Mitochondrial Superoxide – A Role for Lipoic Acid?

As noted, increased mitochondrial production of superoxide contributes to oxidant stress when endothelial cells are exposed to elevated glucose levels. The extent of this contribution remains in dispute. Brownlee and colleagues have reported that uncoupling agents and certain inhibitors of the mitochondrial respiratory chain can virtually normalize superoxide production in endothelial cells exposed to hyperglycemia.[132-134] Other researchers have presented data suggesting that NADPH oxidase has primacy as a superoxide source.[3, 135-138] More studies may be required to clarify this issue. One possible way to resolve this controversy would be to liken mitochondrial superoxide production to "kindling" that boosts DAG production, enabling NADPH oxidase to then provide the main "blaze"; once NADPH oxidase is active, the resulting superoxide production would be adequate to sustain inhibition of GAPDH and thus DAG production.

A strategy for blunting mitochondrial superoxide production in diabetic endothelium is suggested by the recent discovery that PPARgamma coactivator-1α (PGC-1α) boosts endothelial expression of a diverse group of antioxidant enzymes specific to mitochondria – including manganese-dependent superoxide dismutase, UCP2, several peroxiredoxins (which detoxify hydrogen peroxide and peroxynitrite), thioredoxin and a thioredoxin reductase – as well as catalase.[139] This effect of PGC-1α reflects its participation in the transcriptional complexes binding to the promoter regions of the affected genes. Overexpression of PGC-1α in endothelial cells reduced their production of oxidants by about 50% under both basal and high-glucose conditions.[139] This finding has practical significance in light of other recent work demonstrating that activators of

AMP-activated kinase (AMPK) – including AICAR and the anti-diabetic drug metformin – increase PGC-1α expression in endothelial cells.[140] Not surprisingly, these agents were shown to reduce oxidant production in endothelial cells exposed to high glucose – an effect abrogated by dominant negative AMPK. Moreover, there is other recent evidence that metformin (likely acting through AMPK) also can suppress superoxide production by NADPH oxidase in endothelial cells exposed to angiotensin II or high glucose.[141] Furthermore, by boosting fatty acid oxidation (via suppression of malonyl-coA production), AMPK can diminish the availability of substrate for DAG synthesis – a less direct way to diminish NADPH oxidase activation.[142-144] Thus, stimulation of endothelial AMPK may have great potential for control of oxidant stress in metabolic syndrome or diabetes. This may go a long way toward rationalizing the observation that metformin therapy in type 2 diabetics has a much more dramatic impact on macrovascular risk than do injectible insulin or sulphonylureas, despite comparable effects on glycemic control.[142, 145, 146] In this regard, recent evidence that the readily available and inexpensive natural agent berberine can activate AMPK in rat adipocytes and myotubes, as well as in human hepatocytes, is of particular interest.[147, 148] Berberine is used to treat diabetes in China, and is said to be without apparent side effects (unlike metformin) in doses up to 2 g daily.[149, 150]

The "metavitamin" lipoic acid, whether administered orally or parenterally, has recently been shown to activate AMPK in endothelial cells and skeletal muscle of rodents – whereas it inhibits this enzyme in the hypothalamus.[151-153] These effects are precisely parallel to those of the hormone leptin, but can be observed in the absence of leptin or its receptor - suggesting that lipoic acid may somehow be activating leptin's post-receptor signaling mechanism.[153] Remarkably, the inhibitory effect of lipoic acid on hypothalamic AMPK results in diminished appetite and weight loss (or decreased weight gain) in rodents; although no analogous effect has yet been reported in humans, the dose of lipoic acid used in rodent studies (usually 0.5% of diet) is high relative to the doses so far tested in humans. Lipoic acid has the further advantage that it acts as a phase II inducer – boosting cellular levels of reduced glutathione and inducing HO-1.[154-156] Thus, the versatile antioxidant activity of lipoic acid may reflect AMPK-mediated reductions in oxidant production by mitochondria and NADPH oxidase, complemented by an increase in intracellular glutathione and bilirubin levels.

Not surprisingly, lipoic acid has long been used for control of diabetes complications. In particular, its clinical utility in diabetic neuropathy is well established. Doses of 600-1800 mg lipoic acid daily have been shown to have a favorable impact on diabetic neuropathy in controlled clinical trials – albeit these trials included an initial phase in which lipoic acid was administered intravenously.[157-160] Recent open trials suggest that oral lipoic acid per se (600 mg daily) may indeed have efficacy in this regard.[161, 162] Lipoic acid may also have utility for prevention of diabetic nephropathy; in an open, non-randomized trial, therapy with lipoic acid (600 mg/day orally) was associated with a trend toward decreased urinary albumin over an 18 month follow-up, whereas urinary albumin increased significantly during this time in controls not receiving this agent.[163] Analogously, thrombomodulin – a serum protein often used as a marker for diabetic microangiopathy – rose in the control group, but fell in those receiving lipoic acid.

Rodent studies likewise suggest that lipoic acid can have a favorable impact on diabetic nephropathy.[164] And dietary lipoic acid was shown to prevent the formation of acellular capillaries and mitigate oxidant stress in the retinas of diabetic rats, leading the authors to suggest that "alpha-lipoic acid supplementation represents an achievable adjunct to help prevent vision loss in diabetic patients."[165]

Of course, improving glycemic control is the most definitive strategy for controlling mitochondrial superoxide production in diabetes. In this regard, PCB may have potential for improving insulin function in obesity-related metabolic syndrome and diabetes, since there is now evidence that excessive NADPH activation in hypertrophied adipocytes may be largely responsible for systemic insulin resistance;[166, 167] indeed, the NADPH-inhibitory drug apocynin has been shown to improve insulin sensitivity and glycemic control in obese diabetic mice.[167] Furthermore, there is evidence that lipoic acid can improve the insulin sensitivity of skeletal muscle in rats by activating AMPK.[152] There is an analogous report that oral lipoic acid can improve muscle insulin sensitivity in type 2 diabetics – albeit the fact that this effect did not show dose-dependency is puzzling, and lipoic acid has not had an evident impact on glycemic control in the clinical studies evaluating its efficacy in diabetic neuropathy.

In light of the fact that lipoic acid is known to be a phase II inducer, it is of interest to consider the possibility that other nutraceuticals or foods with such activity might have an impact in diabetes. Phase II induction is believed to mediate the anticarcinogenic activity of green tea flavonoids – most notably EGCG – in rodents.[168] While green tea or supplemental EGCG has indeed shown some favorable effects on glycemic control and various other parameters in diabetic rodents,[169-177] heavy consumption of green tea by human diabetics has not so far shown any evident benefit;[178, 179] conceivably, this reflects a proportionately lower intake of EGCG relative to the rodent studies. Although one recent Japanese epidemiological study noted a reduced risk for diabetes in regular users of green tea, no dose-dependency was observed, suggesting that green tea use might be serving as a marker for other lifestyle factors.[180]

Benfotiamine – Draining the Upper Glycolytic Pool

The build-up of glycolytic intermediates in the proximal portion of the glycolytic pathway quite clearly plays a role in the induction of diabetic complications. The enzyme transketolase has the ability to convert glyceraldehydes-3-phosphate and fructose-6-phosphate to pentose phosphates and other intermediates in the pentose phosphate cycle (a.k.a. hexose-monophosphate shunt). Furthermore, it is usually possible to enhance the activity of transketolase by boosting cellular levels of its cofactor thiamine diphosphate.[181] This increase in activity reflects not only greater cofactor binding, but also a feed-forward impact on transketolase expression.[182] Thus, greater thiamine availability, by boosting transketolase activity, has the potential to draw substrate from the proximal glycolytic pathway into the pentose phosphate pathway – an effect which would be expected to diminish production of DAG (thus blunting activation of PKC and NADPH oxidase), polyols, and hexosamines.[183, 184]

Unfortunately, the utility of high-dose thiamine therapy is limited by the intestine's low capacity for thiamine absorption – a carrier-mediated uptake mechanism is effectively saturated at modest physiological intakes of this vitamin.[185] For this reason, Japanese chemists over 40 years ago developed highly absorbable lipophilic analogs of thiamine that convert spontaneously to thiamine following absorption. The most effective of these is benfotiamine, which appears to have no more toxic risk than thiamine (indeed, its acute toxicity in rodents is less than that of thiamine), and is far better absorbed.[186-188] Benfotiamine has long been legally available as a nutritional supplement in Japan and the U.S. – and in Germany is currently prescribable as a clinically validated treatment for diabetic neuropathy.[189-191]

Indeed, oral benfotiamine has shown good efficacy for alleviating a range of diabetic complications in rodents, including neuropathy, nephropathy, and retinopathy.[192-198] Its clinical utility as a treatment for diabetic neuropathy – usually in divided doses providing 150-300 mg daily – is well documented, and there appear to be no discernible side effects at these doses.[189-191] Further clinical studies will be required to assess the long term impact of benfotiamine on progression of retinopathy or nephropathy in diabetics.

To date, benfotiamine has been tested in the context of diabetes – but might it be possible that this agent could also influence macrovascular risk in normoglycemic metabolic syndrome? In this circumstance, free fatty acid excess is primarily responsible for the enhanced endothelial generation of DAG that activates PKC;[3, 199-201] but benfotiamine administration could be expected to decrease levels of the glycerol-3-phosphate to which fatty acids are conjugated – albeit the magnitude of this effect would not be as great as in the context of hyperglycemia. Thus, the impact of benfotiamine on the endothelial dysfunction associated with metabolic syndrome merits investigation.

Taurine – Physiological Antidote to Hypochlorous Acid

Taurine is a "metavitamin" that has antioxidant and osmoregulatory properties, and also modulates transmembrane calcium flux. Taurine may have potential for controlling the oxidative damage attributable to myeloperoxidase (MPO) deposition in the vasculature. Recent evidence indicates that MPO, released by infiltrating phagocytes, accumulates in the arterial intima of rats with insulin resistance syndrome or diabetes.[202] This MPO can then make use of hydrogen peroxide released by inflamed endothelium or intimal macrophages to generate hypochlorous acid (HOCl), a highly reactive oxidant that can compromise endothelium-dependent vasodilation,[202] presumably by modifying the plasma membrane in a way that compromises activation of eNOS.[203] HOCl can also be generated and released by intimal foam cells. Taurine functions physiologically as a detoxicant of HOCl, reacting with it to generate taurine chloramine and innocuous hydroxyl ion. Taurine chloramine, while still an oxidant, is less promiscuously reactive than HOCl, and hence may be viewed as a detoxification product.[204-206] Moreover, taurine chloramine formed in phagocytes - which maintain high intracellular levels of taurine - down-regulates phagocyte activation by inhibiting activation of NF-kappaB.[207, 208] Taurine may thus have utility for alleviating the contribution of activated phagocytes to atherogenesis and ischemic syndromes. Indeed, oral taurine has shown efficacy in

rodent models of atherogenesis[209-211] (albeit in some studies this reflects, in part, a marked hypolipidemic activity not reported in humans), and improves endothelium-dependent vasodilation in rat aorta and in young human smokers.[212, 213] Forty years ago, many Italian clinicians reported that high daily doses of taurine could alleviate coronary angina and intermittent claudication – an effect which conceivably could reflect a decreased tendency of activated leukocytes to clog the microvasculature downstream from stenotic lesions.[214] Taurine supplementation has a safe positive inotropic effect on cardiac function in clinical congestive heart failure,[215, 216] has platelet-stabilizing activity complementary to that of aspirin,[217-219] and has antihypertensive properties that are well documented in rodent models[220-224] and supported by limited clinical evidence;[225-227] these latter effects likely reflect an impact of taurine on calcium flux.

In rodent models of both type 1 and type 2 diabetes, taurine alleviates development of diabetic neuropathy – improving nerve conduction velocity, blunting the decline in endoneurial perfusion, and preventing hyperalgesia. [228-231] No comparable clinical studies have been reported. Taurine also exerts antioxidant effects in the retina of diabetic rats, suggestive of a favorable impact on the course of diabetic retinopathy.[232-234] And taurine administration prolongs the survival of rats with streptozotocin-induced diabetes.[235] However, one year-long clinical study found that supplemental taurine (3 g daily) failed to improve albuminuria in diabetics[236] – not surprising in light of the limited utility of taurine in rodent models of diabetic nephropathy.[237] Since taurine may have some utility in atherogenesis, hypertension, platelet hyperaggregability, congestive heart failure, and ischemic syndromes – common complications of insulin resistance syndrome and diabetes – and also may have potential for moderating the course of diabetic neuropathy and retinopathy, its inclusion in nutraceutical programs for alleviating the complications of insulin resistance and diabetes appears warranted. Taurine is highly soluble, flavorless, inexpensive, and apparently without toxic risk even in high chronic doses – properties which make it an ideal ingredient for functional foods.

Protective Potential of Selenium

In light of taurine's potential to down-regulate NF-kappaB activation in phagocytes, it is germane to cite a recent French clinical study demonstrating that high-dose supplemental selenium (960 mcg daily) normalizes such activation in the monocytes of type 2 diabetics.[238] Since selenium is an essential component of key antioxidant enzymes – thioredoxin reductase, as well as several isoforms of glutathione peroxidase[239] – and selenium status is often suboptimal in regions with low soil selenium,[240] it stands to reason that insuring optimal selenium status through supplementation is appropriate in disorders such as diabetes in which oxidants play a prominent pathogenic role. An impact of selenium status on NF-kappaB activation conceivably could reflect the ability of glutathione peroxidase to degrade hydrogen peroxide as well as peroxynitrite,[241] each of which can promote NF-kappaB activation; moreover, selenium-dependent thioredoxin reductase helps to restore protein-bound cysteines to a proper reduced configuration, thereby reversing the impact of peroxides on signaling pathways.[242]

An anecdotal clinical report that high-dose supplemental selenium appears to slow progression of diabetic retinopathy[243, 244] has not been followed up. A report that high-dose selenium suppresses expression of VEGF in rat mammary carcinomas[245] may be relevant, since induction of VEGF expression in the hypoxic retina plays a key role in the induction of proliferative retinopathy. The effect of selenium on VEGF expression in cancer was mediated by methylselenol rather than selenium-dependent enzymes;[246] this suggests that the effect could be dose-dependent beyond the modest intakes of selenium (i.e. 100 mcg daily) required to optimize the expression of these enzymes.

Melatonin – Inducer of Antioxidant Enzymes

Physiological (low nanomolar) levels of the pineal hormone melatonin have been reported to increase the expression of a range of antioxidant enzymes in various tissues; these enzymes include melatonin include superoxide dismutase (types 1 and 2), catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and gamma-glutamylcysteine synthetase (rate-limiting for glutathione synthesis); an increase in the ratio of reduced to oxidized glutathione, and a decrease in tissue markers of oxidation (e.g. malondialdehyde) are also often demonstrated in these studies.[247-252] Melatonin regulates these enzymes at the transcriptional level, and membrane and/or nuclear receptors for melatonin appear likely to mediate this effect, although the precise mechanisms involved remain unclear. Melatonin's impact in this regard varies from tissue to tissue; in some studies, it does not influence baseline expression of these enzymes, but prevents pro-oxidant drugs from down-regulating them. Since melatonin is usually well tolerated when administered orally prior to bedtime (a schedule which presumably will not interfere with melatonin's chronotropic role), its use as an adjuvant in diabetes management can be contemplated.

Indeed, exogenous melatonin has been found to exert protective antioxidant effects in diabetic rodents, as well as in cultured cells exposed to hyperglycemia (albeit some of the in vitro studies must be viewed circumspectly in light of the micromolar concentrations of melatonin employed).[253-263] Most of the work in diabetic rodents has focused on renal structure and function; melatonin administration has been shown to help maintain normal kidney function, while also blunting the effects of diabetes on renal histology and protein expression. Other studies show that melatonin has a favorable impact on the endothelium-dependent vasodilation of diabetic rodents ex vivo.[258, 259] In a recent clinical study with type 2 diabetics (from the University of Baghdad!), nightly oral melatonin (10 mg, plus 50 mg zinc acetate) was found to decrease macroalbuminuria; favorable effects on glycemic control and serum lipid profile were also noted.[264, 265] Other clinical research concludes that oral melatonin has a favorable effect on nocturnal blood pressure in type 1 diabetics.[266, 267] These considerations suggest that melatonin deserves further evaluation as an adjuvant in diabetes management.

Inhibiting Formation of AGE/ALEs and Angiotensin II

Although accelerated de novo synthesis of diacylglycerol plays a crucial role in the over-activation of NADPH oxidase in diabetes, other factors contribute in this regard. As

noted above, advanced glycation and lipoxidation endproducts (AGEs and ALEs) trigger activation of NADPH oxidase via RAGE receptors expressed by many tissues, including key targets of diabetic damage such as vascular endothelium, retinal pericytes, and renal podocytes and mesangial cells.[268-271] Furthermore, up-regulation of the local renin-angiotensin system,[12-16] reflecting at least in part a PKC-mediated increase in AT1 expression,[17] also promotes activation of NADPH oxidase via AT1 receptors.[18-20] Fortunately, nutraceutical strategies are at hand for suppressing the production of AGEs and of angiotensin II.

The increased production of AGEs and ALEs noted in diabetic tissues presumably reflects the joint impact of hyperglycemia and oxidant stress. Major AGEs/ALEs, including carboxymethyl-lysine, carboxyethyl-lysine, and hydroimidazolones, act as potent agonists for RAGE receptor. The formation of AGEs/ALEs serves as an amplification mechanism for oxidant stress – oxidant stress catalyzes the production of these compounds, which in turn interact with RAGE to exacerbate the oxidant stress. Moreover, RAGE is oxidant inducible, reflecting the fact that NF-kappaB promotes transcription of the RAGE gene.[272-274]

Fortunately, certain compounds can block the formation of AGEs/ALEs by spontaneously forming adducts with highly reactive intermediates – such as alpha-dicarbonyls – that give rise to these toxins. In particular, the vitamer pyridoxamine has excellent efficacy in this regard, and is well tolerated. Studies with supplemental pyridoxamine in diabetic rodents confirm the efficacy of pyridoxamine as an antagonist of AGE/ALE formation in vivo, while providing further evidence that AGEs/ALEs play an important role in the induction of major diabetic complications such as nephropathy, retinopathy, neuropathy, cataracts, and atherosclerosis.[275-285] Pyridoxamine is effective in diabetic rats when administered in drinking water at 1g per liter. Phase III studies with pyridoxamine in diabetic nephropathy are currently in progress. Although pyridoxamine is being developed as a drug, it is sold as a nutritional supplement in the U.S., as it is a naturally occurring form of vitamin B6.

An agent which may have "sleeper" potential in this regard is the amino acid glycine. Glycine has the potential to inhibit glycation reactions, the earliest stage in the formation of AGEs. Diets highly enriched in glycine have been reported to lower glycated hemoglobin levels both in diabetic rats and diabetic humans, without influencing the underlying hyperglycemia; in the clinical study, glycine was administered in drinking water, 5g four times daily.[286, 287] These studies did not examine the impact of glycine on AGE production, but glycine supplementation in diabetic rats (1% in drinking water) has been reported to have favorable effects on kidney, lens, and retinal microvasculature.[288, 289] Glycine may have the potential to more directly inhibit NADPH oxidase activity in certain tissues, possibly because it exerts a hyperpolarizing effect inimical to this activity in cells that express glycine-activated chloride channels;[290-292] this effect may underlie the wide-ranging anti-inflammatory effects of high-glycine diets in rodents.[293] Although high intakes of glycine presumably would be required for clinical activity, this amino acid is highly soluble, has a pleasant sweet taste, and is quite inexpensive – ideal qualities for use in a functional food.

Local up-regulation of the renin-angiotensin system is believed to contribute to macrovascular, renal, and retinal complications of diabetes, and to promote the conversion of metabolic syndrome to overt diabetes; thus, even though the systemic RAS is typically not elevated in diabetics, drugs which suppress angiotensin II production or activity have been found to have a favorable impact on prognosis in diabetics, even in patients with "normal" blood pressure.[294-300] The utility of these agents for control of diabetic nephropathy is especially well documented, and their effects in this regard are greater than would be expected from reduction of blood pressure per se.[300] From the standpoint of developing nutraceuticals and functional foods for diabetics, it is intriguing to note that certain types of protein digests, derived from soy, fish, milk, and other foods, contain oligopeptides which can be absorbed intact and which exert moderate ACE-inhibitory activity in vivo. [301-309]

Nicotinamide for PARP Inhibition

A key role for oxidant-mediated activation of PARP [poly(ADP-ribose) polymerase] in the genesis of diabetic complications has been noted above. In addition to inhibition of glyceraldehyde-3-phosphate dehydrogenase,[2] PARP activation may contribute to cellular dysfunction by inducing NAD+ deficiency; this can compromise ATP generation while also decreasing the availability of NAD(P)H for eNOS and glutathione reductase (albeit for NADPH oxidase as well).[310] Furthermore, PARP serves as a promoter-specific co-activator for NF-kappaB in the transcription of certain pro-inflammatory genes, including iNOS and TNF-alpha; there is disagreement as to whether PARP activation is required for optimal co-activator activity.[311-313] Potent inhibitors of PARP have been shown to have a favorable impact on a range of diabetic complications in rodents, including endothelial dysfunction, neuropathy, nephropathy, and retinopathy.[310, 314-320]

Unfortunately, potent pharmaceutical PARP inhibitors are not yet available for clinical use. However, nicotinamide is a relatively weak PARP inhibitor that has the twin merits of low toxicity and ready availability.[321, 322] It also inhibits certain mono-ADP-ribosyltransferases, and presumably could aid reconstitution of the NAD+ pool when PARP is active. Very recently, high-dose nicotinamide administration (200-400 mg/kg/day, i.p.) has been reported to have a favorable dose-dependent impact on neuropathy in diabetic rats, improving neural perfusion while attenuating the decline in nerve conduction velocity and preventing hyperalgesia.[321] Whether these benefits were mediated by PARP inhibition is not clear. The authors concluded that "nicotinamide deserves consideration as an attractive, nontoxic therapy for diabetic peripheral neuropathy." This report is complemented by a much earlier study which found that niacinamide could slow the progression of nephropathy in diabetic rats.[323]

High-dose nicotinamide (usually 1.2g/m^2 daily, or 1g t.i.d.) has already been evaluated clinically for prevention of beta cell damage in first-degree relatives of patients with type 1 diabetes, as well as for treatment of osteoarthritis; induction of iNOS plays a key role in each of these disorders. Despite initial positive reports, results in diabetes prevention have been inconsistent and largely disappointing,[324-329] whereas limited data appear to

confirm its utility in osteoarthritis.[330, 331] It remains uncertain as to whether current clinical high-dose niacinamide therapy achieves tissue concentrations adequate for meaningful inhibition of PARP. The peak plasma concentrations seen with the 1.2g/m2 regimen is 100-120 μM; this appears meaningful relative to the IC50 for nicotinamide inhibition of PARP, which is around 30 μM with the purified enzyme.[332, 333] Nonetheless, low millimolar concentrations of nicotinamide are required to protect cells from PARP-activating stressors in vitro, and some authorities doubt that feasible clinical intakes of nicotinamide can achieve useful inhibition of PARP.[333, 334] Unfortunately, daily intakes in excess of 3g are prone to induce nausea, and thus are not practical for long term use.[333] A convenient way to assess the likely utility and dose-dependency of feasible intakes of niacinamide in human diabetics would be to evaluate its impact on diabetic endothelial dysfunction; to date, no such studies have been published. A dose schedule which proved effective in this regard would quite likely have a favorable impact on diabetic complications.

Why Nutraceticals?

These considerations suggest that a nutraceutical regimen comprised of PCB, high-dose folate, lipoic acid, taurine, pyridoxamine, and benfotiamine, in appropriate doses, would likely have a substantial favorable impact on the vascular complications of diabetes; PCB, high-dose folate, and taurine may also have potential for slowing the progression of atherosclerosis in patients with normoglycemic metabolic syndrome. Supplementation with vitamin C, selenium, niacinamide, melatonin, and oligopeptide ACE inhibitors might also be of some benefit in these syndromes – whereas there is little present evidence that vitamin E could be helpful. Naturally, these agents could be used in concert with other nutraceuticals that aid glycemic control, or that help to ameliorate major risk factors – e.g. hypertension, hyperlipidemia – that can greatly compound the risk associated with diabetes per se.

The particular merit of nutrients or phytonutrients (as opposed to drugs) for these purposes, is that it is feasible to combine a number of them together in a single nutraceutical product or functional food. Moreover, nutraceuticals tend to be cheaper than drugs, and do not entail the inconvenience and expense of requiring a physician's prescription. Finally, nutrients tend to be well tolerated – a consideration that is particularly trenchant when primary prevention is the aim. Thus, it would be highly appropriate to develop comprehensive nutraceutical regimens targeted to prevention of the macro- and microvascular complications of diabetes and metabolic syndrome.

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