A Potential Role for Spirulina in the Prevention and Management of Preeclampsia

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Abstract

Preeclampsia (PE) arises when an improperly formed, chronically hypoxic placenta secretes into the maternal circulation anti-angiogenic, pro-inflammatory factors that impair nitric oxide production and boost oxidative stress in the maternal vasculature, giving rise to hypertension, proteinuria, and potentially additional symptoms. PE is characterized by oxidative stress both in the placenta and the maternal vasculature, and this essay argues that oxidative stress may play a mediating role with respect to virtually all phases of the preeclamptic syndrome – promoting placental malformation by suppressing the capacity of trophoblast to remodel the uterine spiral arteries; boosting the secretion of anti-angiogenic factors by the hypoxic placenta; and in some measure mediating the pathogenic impact of these factors on the mother. Oxidative activation of hypoxia-inducible factor-1 is proposed to play a key role in this regard. NADPH oxidase complexes are likely to be a primary source of oxidative stress in PE. Recent evidence indicates that the phycocyanobilin richly supplied by spirulina can mimic the ability of bilirubin to inhibit certain NADPH oxidase complexes – likely explaining the profound anti-inflammatory effects of orally administered spirulina in numerous rodent studies. It is therefore proposed that a sufficiently high intake of spirulina may have potential for prevention and control of PE – a proposition that could be readily tested in rodent models of this disorder. Since spirulina is a safe whole food – and has shown anti-teratogenic potential in rodent studies – it may be highly appropriate for administration during pregnancy, as contrasted with many drugs. If spirulina can be shown to be useful in PE, its utility may be complemented by ancillary measures which increase the subnormal production of vascular nitric oxide in PE; citrulline, cocoa flavanols, and quercetin have potential in this regard. Hence, a functional food featuring spirulina, citrulline, and flavanol-rich cocoa powder can be envisioned for PE prevention and control.

Overview of the Pathogenesis of Preeclampsia

Although preeclampsia (PE) has long been considered an enigmatic disorder, a broad outline of its pathogenesis is beginning to emerge.[1-4] Of central importance is impaired placentation associated with a subnormal uteroplacental blood flow. In a healthy pregnancy, the extravillous trophoblast (EVT) cells that anchor the fetus in the maternal deciduum have the further role of remodeling the uterine spinal arteries, eroding away their medial smooth muscle so that they become low-resistance conduits for high-volume blood flow.[5] In preeclamptic pregnancies, this remodeling is inadequate, preserving a high-resistance vasculature that compromises oxygen delivery to the placenta, and that may result in capriciously fluctuating blood flow, setting the stage for ischemia-reperfusion damage.[6] A number of studies have established that placental oxidative stress is markedly elevated in PE;[7-11] ischemia-reperfusion may be one mechanism which triggers this. Reduced trophoblast expression of superoxide dismutase (SOD1) and other antioxidant enzymes, as well as increased expression or activity of NADPH oxidase complexes may also contribute to this phenomenon.[10, 12-17]

Syncytiotrophoblast secretes a number bioactive factors into the maternal circulation; in pregnancies destined for PE, the hypoxic/oxidative stress imposed by improper placentation perturbs the quantities of these factors that are secreted.[17] In particular, in preeclamptic pregnancies, the syncytiotrophoblast releases increased amounts of two factors which oppose angiogenesis, a soluble receptor for VEGF – sFlt-1 – and a soluble receptor for TGF-beta, sEndoglin (sEng).[4, 18-21] Both VEGF and TGF-beta act on vascular endothelium to boost expression and activation of eNOS, enable angiogenesis and to promote healthful endothelial function; sFlt-1 and sEng act as false receptors for these hormones, tying them up so that they are less capable of interacting with their receptor on endothelium.[22, 23] Blood levels or sFlt-1 and or sEng are dramatically elevated in PE, in proportion to the severity of the disorder; this elevation precedes the onset of symptoms by a number of weeks. Elevated systemic levels of these agents are also seen in rodent models of placental underperfusion.[24, 25] Moreover, joint infusion of these agents in pregnant rats leads to a spectrum of maternal pathologies that include not only the hypertension and proteinuria that constitute the definition of PE (when they arise de novo after 20 weeks of pregnancy), but also the components of the HELLP syndrome – hemolysis, elevated liver enzymes, low platelets – often seen in severer cases of PE; infusion of sFlt-1 alone can induce hypertension and proteinuria.[23] Most notably, a recent pilot clinical study demonstrated that repeated sessions of apheresis could remove sFlt-1 from the maternal circulation, and that this was associated with reduced proteinuria and stabilized blood pressure.[26] The preclamptic placenta is also characterized by a decreased release of placenta growth factor, a hormone which mimics the bioactivity of VEGF.[27, 28] Hence, it is now widely accepted that the maternal symptoms of PE are traceable to impaired access of the maternal endothelium to trophic factors which support endothelial health. Consistent with this view, anti-angiogenic cancer drugs which suppress VEGF signaling can produce side effects – hypertension and proteinuria – that typify PE.[29]

Nonetheless, placental secretion of a number of pro-inflammatory bioactive factors is increased in PE – including activin-A, inhibin-A, corticotrophin-releasing hormone, leptin, and trophoblast microparticles – and it seems likely that some of these contribute to an up-regulation of systemic inflammation that is seen in PE.[1, 3] Mild systemic inflammation is characteristic of healthy pregnancies; PE intensifies this phenomenon. Plasma levels of TNF-alpha and of IL-6 and IL-8 are elevated, as are markers of endothelial activation; leukocytosis is common, and leukocytes, platelets, and clotting factors tend to be in a more active state. Presumably, these effects reflect the impact of pro-inflammatory factors released from the traumatized placenta.

Placental underperfusion also tends to evoke increased maternal production of agonistic antibodies targeting the angiotensin type 1 receptor (AT1R), which evidently can contribute to the hypertension, oxidative stress and inflammation seen in PE.[30-33] Additionally, a couple of studies have reported increased plasma levels of the bufadienolide hormone marinobufagenin (MBG), which originates in the adrenal cortex.[34, 35] MBG is capable of inhibiting isoforms of the "sodium pump" (Na^+,K^+-ATPase) expressing the alpha-1 subunit; at lower concentrations, its interaction with these pumps can transmit intracellular signals with pro-inflammatory and pro-oxidant impacts.[36-38] MBG may be a key mediator of the hypertension provoked by high-salt diets in salt-sensitive individuals; hence, it could conceivably play a role in the hypertension associated with PE.[39]

The compromised endothelial function associated with sFlt-1 and sEng excess somehow provokes an increase in endothelial production of endothelin-1 (ET-1); remarkably, antagonists of the ET-A receptor have been shown to prevent the hypertension and proteinuria observed in pregnant rats continually

infused with sFlt-1, or in rats with simulated PE induced by surgical constriction of uterine blood flow (the so-called "reduced uterine perfusion pressure" – RUPP – model).[40-44] Proinflammatory agents elevated in the maternal circulation during PE, such as tumor necrosis factor-alpha and AR-1 antibodies, also have potential to provoke increases endothelial production of ET-1.[40, 45, 46] Hence, excessive stimulation of ET-A by overproduced ET-1 may be a final common mediator of the hypertension and proteinuria that characterize PE.[44]

Oxidative Stress May Mediate Preeclampsia at Multiple Levels

There is considerable evidence that PE is associated with increased oxidative stress, both in the placenta and the mother's vasculature. [7-11, 47-49] It has long been suspected that this oxidative stress may play a mediating role in the pathogenesis of PE, at multiple levels.[1, 50, 51] There is some reason to suspect that oxidative stress within EVT cells may limit their ability to effectively remodel the spiral arteries, giving rise to the placental underperfusion that evidently drives the PE syndrome. Once placental malformation has occurred, oxidative stress within the syncytiotrophoblast may be a key reason for the perturbed secretion of bioactive factors such as sFlt-1 and sEng that evokes maternal pathology. And, within the mother's vasculature and kidneys, oxidative stress may mediate many of the pathogenic effects of these placenta-derived factors – including the increase in vascular endothelin production that appears to be key importance. Moreover, endothelin itself boosts the production of oxidants via the ET-A receptor.

As noted, there is considerable evidence for elevated placental oxidative stress in PE. A study comparing trophoblast cells from preeclamptic and healthy placentas found that superoxide production was roughly four times as high, and superoxide dismutase only about half as active, in the preeclamptic trophoblast.[49] While this phenomenon likely reflects placental malformation – it is observed in the RUPP model of PE[49] – it is reasonable to suspect that oxidative stress might also afflict trophoblast cells early in the process of placentation, and impair their capacity to remodel the spiral arteries appropriately, hence contributing to the placental malformation that is at the root of PE. In the genetic BPH/5 mouse model of spontaneous PE, placental activity of the cytoplasmic SOD is about half as high in normal pregnancies, and the remodeling of spiral arteries is inadequate, resulting in a syndrome analogous to human PE.[52, 53] Notably, treatment of the pregnant BPH/5 mice throughout gestation with the superoxide dismutase-mimetic drug tempol tended to normalize placentation, improve growth and survival of the fetuses, and prevent hypertension and proteinuria in the mother.[54]

Furthermore, in mice heterozygous for knockout of the key antioxidant enzyme heme oxygenase-1 (HO-1), placentation and remodeling of the spiral arteries likewise is abnormal, and a syndrome comparable to PE is seen.[55] *In vitro*, inhibition of HO-1 impairs the invasiveness of human cytotrophoblast cells.[56] HO-1activity gives rise to bilirubin, which serves to inhibit NADPH oxidase activity[57-59] – a key source of superoxide in the placenta.[17] In vitro, exposure of trophoblast cells to hormones which exert pro-oxidative effects – angiotensin II, TNF-alpha, and MBG – reduces their invasiveness and migratory capacity, and hence would be expected to impair their ability to remodel spiral arteries.[60-63] (These observations are particularly intriguing in light of the elevated levels of MBG, TNF-alpha, and ATR1 antibodies observed in human PE – albeit there is no proof that these agents are elevated early in the placentation process.) The ability of these hormones to inhibit EVT invasiveness has been traced to induction of plasminogen activator-inhibitor-1 (PAI-1), the expression of which is indeed quite elevated in preeclamptic trophoblast.[59, 60, 64] In trophoblast cells, the transcription of PAI-1 is induced by hypoxia-

inducible factor-1 (HIF-1), a transcription factor which is boosted not only by hypoxia, but by oxidant stress as well.[65, 66] This effect may reflect oxidant-mediated activation of NF-kappaB, which promotes transcription of HIF-1alpha;[67] moreover, oxidant stress can boost both the half-life and transcriptional activity of HIF-1 by inhibiting prolyl and asparaginyl hydroxylases that target HIF-1alpha.[68-72] Additionally, oxidative stress may promote PAI-1 transcription via activation of the JNK and p38 MAP kinases.[73, 74] Hence, oxidative stress in EVT cells could be expected to impair their invasiveness by stimulating PAI-1 expression.

These results are evidently consistent with the intriguing *possibility* that trophoblastic oxidative stress is a key mediator of the impaired placentation that underlies human PE, a hypothesis that is particularly attractive in light of the high oxidative stress observed in human preeclamptic placentas; nonetheless, whether trophoblast cells are under oxidative stress early in the process of placentation in PE patients is not yet clear, and it is not unlikely that other types of cellular dysfunction could contribute to impaired trophoblast performance during the genesis of PE.

Rodent models provide suggestive evidence that, once placental underperfusion has been established, trophoblastic oxidative stress is a key mediator of the perturbed placental secretion of bioactive factors that triggers the maternal disorder – likely because increased HIF-1 activity is largely responsible for this disruption of placental secretory activity. In the RUPP model of PE, induction of the antioxidant enzyme HO-1 via administration of cobalt protoporphyrin normalizes maternal plasma levels of sFlt-1, suppresses the elevation of superoxide and NADPH oxidase activity in placenta, and markedly blunts the increase in blood pressure.[75] In endothelial cells, adenoviral overexpression of HO-1 antagonizes the hormonally-mediated induction of both sFlt-1 and sEng; conversely, inhibition of HO-1 activity placental villous explants obtained from preeclamptic pregnancies boosts the release of both of these factors.[76] Although there is evidence that the carbon monoxide evolved by HO-1 activity is partially responsible for these effects, there is reason to believe that the antioxidant activity of HO-1-generated bilirubin also contributes in this regard. Rat placental explants secrete increased amounts of sFlt-1 when incubated under hypoxic conditions; addition of either free bilirubin or of a CO-releasing drug to the incubation blunts this increase.[77] Hypoxia-inducible factor-1 (HIF-1) binds to the promoter of sFlt-1 and promotes its transcription; as noted, oxidative stress can boost the level of this transcription factor.[78-80] Analogously, oxidative stress has the potential to boost expression of sEng, which is also HIF-1 inducible.[79, 81] Hence, it is reasonable to suspect that increased placental oxidative stress is at least partially responsible for the increased secretion of sFlt-1 and sEng by underperfused preeclamptic placentas.

There is also evidence that oxidative stress is a primary mediator of the adverse impact of sFlt-1 and other placentally-derived bioactive factors. The superoxide dismutase-mimetic tempol attenuates the rise in blood pressure observed in RUPP pregnancies, whereas it has no effect on blood pressure in normal pregnancies.[82] To clarify whether tempol was modulating sFlt-1's pathogenic effects (as opposed to inihibiting its secretion by the placenta), s-Flt-1 was administered continuously to pregnant rats via intraperitoneal osmotic pump. In these rats, mean arterial pressure increased notably – whereas concurrent administration of either tempol or the HO-1 inducer cobalt protoporphyrin completely prevented this rise in blood pressure, and blunted the increase in vascular superoxide provoked by sFlt-1.[83, 84]

As noted, induced expression of ET-1 in the maternal circulation has been shows to be the predominant mediator of the increased blood pressure seen in pregnant rats subjected to placental underperfusion or infused continuously with sFlt-1; a drug which blocks ET-A receptors prevents this increase in blood pressure, without influencing the blood pressure of healthy pregnant rats.[41-43] It is not yet entirely clear how antagonism of VEGF activity (via sFlt-1 in PE, or VEGF-antagonist drugs in anti-angiogenic cancer therapy) evokes increased endothelial production of ET-1; however, an increase in endothelial oxidative stress, coupled with a reduction in endothelial nitric oxide (NO) production and bioactivity, may collaborate to mediate this effect.

VEGF and TGF-beta act in tandem to boost both the expression and the activation of eNOS in endothelial cells; VEGF activates eNOS via Akt-mediated phosphorylation of Ser1177, whereas TGF-beta promotes this activity by suppressing eNOS phosphorylation at Thr495.[23, 85] Hence, by diminishing the effective activity of VEGF and TGF-beta, respectively, sFlt-1 and sEng can notably impair endothelial NO synthesis; this presumably helps to explain recent reports that plasma nitrite levels are significantly lower in PE than in healthy pregnancy.[86, 87] NO suppresses the expression of endothelin-converting enzyme-1 by destabilizing its mRNA, and thereby inhibits ET-1 production; hence, loss of NO activity would be expected to increase ET-1 activation.[88] To the extent that decreased endothelial production of NO contributes to the pathogenesis of PE, measures which minimize endothelial superoxide production and hence prevent NO superoxide-mediated quenching should optimize the bioactivity of the NO that is produced. Moreover, such measures should also prevent peroxynitrite-mediated uncoupling of eNOS.[89]

Conversely, oxidative stress can markedly boosts transcription of the preproendothelin-1 gene, an effect which conceivably is mediated by HIF-1.[90-93] NO does not appear to influence transcription of this gene.[90] sFlt-1 infusion is known to increase oxidative stress in the vascular wall, an effect which would be expected to boost ET-1 production;[83] nonetheless, it is conceivable sFlt-1's impact on vascular oxidative stress is a downstream consequence of its impact on ET-1 activity. In any case, it remains to be established whether effective antioxidant measures will lessen the increase in ET-1 expression evoked by withdrawal of VEGF's trophic effect on endothelia.

Other bioactive factors typically increased in PE, including AT1R antibodies and TNF-alpha, may contribute to increased endothelial production of ET-1 in PE; these agents clearly can boost endothelial oxidative stress. Indeed, the ability of serum from RUPP rats to induce ET-1 production by human umbilical vein endothelial cells was largely abrogated by pre-incubating this serum with a soluble receptor for TNF-alpha (etanerecept).[46] Administration of etanerecept also lowered elevated blood pressure in RUPP rats.[46] The origin of the two-fold increase of plasma TNF-alpha in PE requires further clarification; some of it may stem from the hypoxic placenta, and sFlt-1 may increase this placental production of TNF-alpha.[94]

It is known that, acting via the ET-A receptor, ET-1 increases vascular NADPH activity both by promoting phosphorylation of p47phox, and by increasing the expression of this subunit.[95-97] Whether antioxidant measures can antagonize the increase in blood pressure evoked by ET-1 remains a controversial matter. In rats fed a high-salt diet, administration of either tempol or the NADPH oxidase inhibitor apocynin controlled the oxidative stress provoked by continuous infusion of ET-1, but did not influence the ET-1-induced rise in blood pressure.[98] On the other hand, in rats fed a diet of normal sodium content, tempol administration completely prevented the rise in blood pressure induced by

continuous ET-1 infusion.[98] ET-1 is capable of acting directly on vascular smooth muscle to induce calcium influx and vasoconstriction, an effect that is not obviously dependent on oxidative stress; however, this effect is more significant in the context of a high-salt diet.[99] In any case, the extent to which antioxidants can blunt the pro-hypertensive effect of ET-1 in the context of PE remains to be evaluated.

As noted, AT1R antibodies and MBG may play some role in the clinical course of PE. As each of these has the potential to induce vascular oxidative stress, it is not unlikely that effective antioxidant measures could favorably influence whatever contribution they make to the evolution of PE.

In summation, there is reason to suspect that measures which effectively control oxidative stress might intervene favorably in the pathogenesis of PE at a number of levels: promoting effective function of trophoblast cells, so that placental underperfusion is avoided; blunting the underperfused placenta's release of the bioactive factors (sFlt-1, sEng, etc.) that mediate maternal pathology in PE; limiting the impact of these factors on the maternal vasculature and kidneys; suppressing the production and pathogenic impact of ET-1; and limiting the activity of certain other factors (e.g. AT1R antibodies, TNF-alpha, MBG) that may play a role in PE.

Potential of Spirulina for Prevention and Control of Preeclampsia

Clinical trials of supplementation with vitamin E or vitamin C in PE have not demonstrated useful activity; in light of the fact that there is no evidence that these agents are useful in rodent models of PE, this is not too surprising.[100-102] The antioxidants cited above which *have* proven useful in PE models either suppress the production of superoxide (HO-1, bilirubin, apocynin), or promote its rapid dismutation (tempol, tiron). Superoxide exerts effects on cellular signaling mechanisms – reflecting scavenging of NO and peroxynitrite generation, hydrogen peroxide-mediated cysteine oxidation in proteins, and poorly understood effects not mediated by either peroxynitrite or hydrogen peroxide[103, 104] – that are not antagonized by most scavenging antioxidants, including vitamins C and E. Moreover, oral supplementation with vitamin C generally fails to increase intracellular ascorbate levels except in patients with poor vitamin C nutrition, and can only modestly raise ascorbate serum levels.[105, 106]

It appears likely that a high proportion of the pathogenic oxidative stress in PE stems from overactivated NADPH oxidase. There is now evidence that phycocyanobilin (PhyCB), which accounts for about 0.6% of the dry weight of spirulina, can act as a potent inhibitor of NADPH oxidase;[107] its activity in this regard appears to mimic the physiological antioxidant role of free bilirubin.[108-111] Moreover, this compound likely has good bioavailability, as feeding of sprirulina (or of phycocyanin, the spirulina protein which includes PCB as a chromophore) has shown profound and versatile anti-inflammatory, cytoprotective, and antiatherogenic activities in rodent studies[107, 112-122] – effects which could be readily explained by partial inhibition of NADPH oxidase.[107, 111] Although little clinical evaluation of spirulina of significance has been accomplished to date, a recent open clinical study reported that feeding 4.5 g spirulina daily to healthy volunteers was associated with hypolipidemic effects, as well as significant reductions in blood pressure (averaging 11 points systolic, 6 points diastolic).[123] The impact on blood pressure could well reflect inhibition of NADPH oxidase, thus suggesting that PhyCB may have good bioavailability in humans as it does in rodents. Moreover, this effect is substantial, when one considers that only a minority of the subjects in this study were considered hypertensive. In a more recent study, ingestion of 19 g spirulina daily was associated with a tripling of insulin sensitivity in insulin-resistant HIV patients

receiving protease inhibitor therapy.[105] These findings suggest that, in a sufficiently large intake – possibly 15-30 g daily[107] - spirulina has the potential to achieve in humans the potent antioxidant and anti-inflammatory effects documented in numerous rodent studies.

Spirulina was used as a traditional food by the Aztecs, and is still used for that purpose by Africans living near Lake Chad.[124, 125] Within the last 30 years, spirulina has been popularized as supplemental food among "healthfood" consumers, consumed as a powder or in tablets. Toxicological studies in rodents have failed to note any adverse effects when spirulina is fed in substantial quantities;[126] in particular, no teratogenicity or other adverse effects have been noted when spirulina is fed to pregnant mice or rats.[127-129] Indeed, concurrent administration of spirulina has been shown to antagonize the teratogenicity of cadmium and of hydroxyurea in mice – suggesting that maternal ingestion of spirulina can provide important antioxidant protection to the fetus.[107, 130] This observation is particular interest in light of evidence that oxidative stress may be the common pathway whereby a number of teratogens induce teratogenicity; the antioxidant defenses of developing embryos are relatively weak.[131]

In light of the understandable reluctance to prescribe drugs during pregnancy, the possibility of using a healthful whole food in the prevention and management of PE is particularly appealing. An evaluation of oral spirulina in the RUPP and other rodent models of PE therefore can be recommended. If spirulina proves useful in these models, two types of controlled clinical trials would be warranted. Spirulina could be tested in pregnant women who are showing the first clinical signs of PE, to determine whether spirulina could ameliorate clinical symptoms and minimize infant prematurity. In a more ambitious trial, women judged to be at high risk for PE could be recruited as soon as pregnancy is diagnosed, and spirulina administration commenced immediately, to determine whether spirulina could influence *risk* for PE by promoting better trophoblast function.

Toward A Functional Food for Prevention/Control of Preeclampsia

If spirulina proves to have value for control, and possibly prevention, of PE, it would be reasonable to envision the development of a spirulina-centered functional food for this purpose. Such a food might be designed to concurrently provide support for physiological NO production.

The negative impact of the anti-angiogenic factors sFlt-1 and sEng on endothelial function reflects in large part a suppression of eNOS activity. In pregnant rats, chronic inhibition of eNOS with the drug L-NAME produces a syndrome similar to PE, characterized by hypertension and proteinuria; it is therefore seems likely that loss of NO activity contributes importantly to the clinical course of PE.[132, 133] Intriguingly, this L-NAME-induced syndrome is associated with a marked increase in maternal levels of sFlt-1, albeit sEng levels are not enhanced; this suggests that placental NO activity contributes to the control of sFlt-1 production.[134] Indeed, in another study, treating human syncytiotrophoblast explants with glyceryl trinitrate was found to reduce expression of HIF-1alpha and decrease the hypoxia-evoked secretion of sFlt-1.[80]

As noted, sFlt-1 and sEng collaborate to reduce the expression and activation state of eNOS in endothelia. However, other factors linked to oxidative stress can further impair NO bioactivity in PE. These include superoxide-mediated quenching of NO – generating toxic peroxynitrite in the process; uncoupling of eNOS , stemming from peroxynitrite-mediated oxidation of eNOS' essential cofactor tetrahydrobiopterin, and from oxidant-mediated reduction in the expression of GMP cyclohydrolase, required for synthesis of

this cofactor;[89, 135] and oxidant-mediated inhibition of dimethylarginine dimethylaminohydrolase (DDAH), which catabolizes the physiological eNOS inhibitor asymmetric dimethylarginine (ADMA).[136]

Inasmuch as ADMA competes with eNOS' substrate arginine for access to the enzyme, the ratio of arginine to ADMA is an important determinant of the effective activity of eNOS.[136] A number of reports – though not all[137-139] – indicate that plasma ADMA levels are elevated in preeclamptic mothers; indeed, this elevation may precede the onset of symptoms.[140-148] The placenta may be a major source of ADMA during pregnancy,[138] and there is one recent report that DDAH levels are barely detectible in preeclamptic placenta.[148] Also, some studies find that maternal arginine levels are decreased in PE, possibly reflecting increased expression of arginase II, which converts arginine to ornithine.[149-151] Hence the maternal arginine/ADMA ratio tends to be decreased in PE, and this likely exacerbates the deficit of NO bioactivity in this syndrome. This ratio may also be low in preeclamptic placenta. Evidently, supplemental arginine has the potential to rectify this situation.

Indeed, several studies – in clinical PE, or rodent models of this syndrome – have evaluated the impact of arginine supplementation. 2% arginine in the drinking water notably lowered blood pressure in RUPP rats, and also reduced blood pressure and renal cortical preproendothelin expression in pregnant rats chronically infused with sFlt-1.[152, 153] Clinically, several controlled trials have found that 3-4 g supplemental arginine daily lowered systolic and diastolic pressure while prolonging pregnancy in PE; improved fetal outcomes were reported in one study.[154-156] One such study however failed to find benefit.[157] One recent controlled study, pregnant women judged to be at increased risk for PE (owing to a previous preeclamptic pregnancy or a first-degree relative with PE) were randomized to receive 2 food bars daily containing either arginine plus antioxidant vitamins, antioxidant vitamins alone, or no additives.[158] Those randomized to arginine received 3.3 g arginine twice daily; supplementation began around the 14th week of gestation. Subsequent incidence of PE was 30% in those receiving the placebo bar, and 13% ($p<.001$) in the arginine group. The arginine group also benefited significantly with respect to pre-term birth (20% vs. 11%), mean gestational age at delivery, and Apgar scores. The arginine group also performed better than the antioxidants alone group with respect to all of these parameters. This study is of particularly interest, as it suggests that optimal nutraceutical strategies may indeed be useful for PE prevention.

There is reason to suspect, however, that supplemental citrulline may be more effective than supplemental arginine, inasmuch as previous clinical studies have found that a given supplemental intake of the former does a better job of raising serum arginine levels than a comparable intake of the latter.[159-161] The superiority of citrulline as a delivery form for arginine reflects the fact that supplementary arginine is largely degraded by arginase activity in the GI tract and intestinal mucosa before it can reach the circulation. Orally administered citrulline escapes this degradation, and, once absorbed, is gradually converted by the kidneys to arginine, thereby boosting plasma arginine levels. Clinical trials with oral citrulline suggest that it is well tolerated, and may have a favorable impact on sickle cell disease, erectile dysfunction, exercise performance, and arterial compliance.[159, 162-165] It can be supplied either as a synthetic chemical, or as a watermelon extract (watermelon being unusually rich in this amino acid).[166]

Alternatively, eNOS activity can be boosted by ingestion of certain flavanols or flavonols that can act directly on vascular endothelium to provoke NO release. The epicatechin in cocoa flavanols has this effect, and the common flavonol quercetin may be even more potent in this regard.[167-169] Intriguingly,

there are two epidemiological studies which have concluded that women who consume chocolate regularly are less prone to PE than those who don't.[170, 171] The possible utility of cocoa flavanols for PE prevention has been suggested by Hollenberg.[167] Moreover, it has been noted that cocoa powder can be useful for masking the somewhat disagreeable flavor of spirulina.[172]

A recent epidemiological study in Norway found that, among over 23,000 nulliparous pregnant women, a dietary pattern rich in fruits and vegetables was associated with a reduction in risk for PE of about 28%.[173] An extremely low incidence of PE – only one mother in 775 – was reported at a vegan commune in Tennessee where the mothers were routinely supplemented with vitamin B12, iron, and calcium.[174] Whole plant foods contain a wide range of phytochemicals, some of which have the potential to boost expression of various antioxidant enzymes (including HO-1) via phase 2 induction, and some of which can provoke endothelial NO release. Hence, a diet rich in whole plant-based foods may be recommendable during pregnancy, and further studies in rodent models of PE should evaluate nutraceuticals which have phase 2-inducing or eNOS-activating potential. Moreover, consumption of green leafy vegetables or beet juice, rich in nitrate, has been shown to boost plasma levels of nitrite, which in turn can give rise to NO independently of NOS activity;[175] this "spontaneous" generation of NO is most efficient in hypoxic tissues, and thus might constitute a strategy for supplying NO to the hypoxic placenta. It would be of interest to see whether dietary nitrate could be beneficial in rodent models of PE.

An additional agent worthy of study in rodent PE models is high-dose folic acid. Many tissues concentrate folic acid against a gradient, accumulating it intracellularly in reduced tetrahydro forms that have outstanding oxidant-scavenging potential. In particular, tetrahydrofolates are potent scavengers of peroxynitrite-derived radicals;[176] this may account for their ability to prevent the peroxynitrite-mediated uncoupling of eNOS that occurs in many clinical endotheliopathies.[177, 178] Preadminstration of high-dose folate has been shown to be outstandingly effective in a rat model of ischemia-reperfusion cardiac damage.[179] Dr. Kurt Oster long maintained that 40-80 mg folic acid daily was very useful in the management of angina[180] – a claim that finds some support in recent clinical studies.[181, 182] Endotheliopathy is of course a central feature of PE, and peroxynitrite-mediated oxidant damage has been documented in this syndrome.[183-190]

In summation, oxidative stress may play a pathogenic role in PE at multiple levels – suppressing trophoblast invasion, boosting the secretion of key bioactive mediators from the hypoxic placenta, and to some extent mediating the impact of these bioactive factors on the maternal vasculature and kidneys. NADPH oxidase is likely to be a major source of this oxidative stress, and adequate intakes of spirulina may be able to quell oxidative stress during pregnancy by partial inhibition of NADPH oxidase complexes. Spirulina should therefore be evaluated in rodent models of PE, and positive results in such studies could set the stage for appropriate clinical trials in at-risk pregnancies. Presuming that spirulina proves to have efficacy in this regard, ancillary agents which can boost eNOS activity in preeclamptic pregnancies are likely to complement its utility. Functional foods featuring spirulina, citrulline, and cocoa flavanols and/or quercetin can be envisioned in this regard.

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