Nutrient Involvement in Preeclampsia[1]

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ABSTRACT Preeclampsia is a pregnancy-specific condition that increases maternal and infant mortality and morbidity. It is diagnosed by new-onset increased blood pressure and proteinuria during gestation; for many years these markers were the sole targets for study. More recently, increased attention to the multisystemic nature of the syndrome with involvement of almost all organs, activation of coagulation and increased sensitivity to pressor agents has expanded understanding of the disorder. The epidemiology of preeclampsia, being more common in poor women, long ago suggested that nutrients might be involved in the disorder. Numerous conflicting hypotheses were advanced but the testing of these hypotheses has either been done poorly or not at all. Review of the available data indicates very few studies that provide useful insights. In many studies the syndrome is poorly defined and in most studies nutritional data (questionnaires or biomarkers) are obtained on women with the clinical syndrome. In overtly preeclamptic women it is impossible to decipher cause from effect. Nonetheless, current concepts of the genesis of preeclampsia that include endothelial dysfunction, inflammatory activation, oxidative stress and predisposing maternal factors provide targets for well-designed nutritional investigation. In this review the current concepts of the pathogenesis of preeclampsia are reviewed and available data are assessed in light of these concepts. Targets for nutritional investigation based on the current knowledge of pathophysiology are suggested. J. Nutr. 133: 1684S–1692S, 2003.

KEY WORDS: • *pregnancy* • *preeclampsia* • *nutrients* • *micronutrients* • *pathophysiology*

Preeclampsia is a disease with worldwide significance to mothers and infants (1). Its greatest impact is in developing countries, where it accounts for 20–80% of the strikingly increased maternal mortality. However, even in developed countries there is a major effect, primarily on the fetus. Application of appropriate prenatal care and management (consisting largely of the delivery of women with preeclampsia before the disease progresses to become life threatening) has largely eliminated maternal mortality, frequently at the cost of preterm delivery. Ten percent of cases occur at a stage of gestation where delivery exchanges a sick fetus in utero for a sick premature infant in the nursery. In developed countries perinatal mortality of infants of preeclamptic mothers is fivefold greater than for nonpreeclamptic women, and indicated preterm deliveries for preeclampsia account for 15% of preterm births (2).

The disorder was first recognized almost 2000 years ago. Celsus described pregnant women with seizures that abated with delivery. This disorder was termed eclampsia and for 2000 years was considered a pregnancy-specific seizure disorder. In the late 1800s the association of, initially, proteinuria and later increased blood pressure with eclampsia was recognized. It was also noted that increased blood pressure and urinary protein antedated the seizures. From this came the term preeclampsia (3). Even in the absence of seizures, maternal and infant risk was increased. Interestingly, despite the recognition by care providers that blood pressure was not usually the major problem for mother or baby but was rather a marker of a multisystemic syndrome, blood pressure was the focus of preeclampsia research for nearly 100 y. Not surprisingly, progress was slow. In the past 10–15 y thinking about the disorder has changed and all aspects of the syndrome are considered, with an attendant increase in understanding (4).

For many years diet has been suggested to play a role in preeclampsia. The hypotheses have been diverse and often mutually exclusive. Thus, increased and reduced dietary sodium, protein, fats or carbohydrates were proposed as possible etiological factors (3). Rarely were these hypotheses appropriately tested in trials. Not surprisingly, many care providers became disenchanted with these hypotheses and the role of nutrition has not been extensively studied in recent years. In this presentation we suggest that in light of new tools and concepts, the question be readdressed. We assess the minimal information available on nutrition and specifically on micro-

[1] Manuscript prepared for the USAID-Wellcome Trust workshop on "Nutrition as a preventive strategy against adverse pregnancy outcomes," held at Merton College, Oxford, July 18–19, 2002. The proceedings of this workshop are published as a supplement to *The Journal of Nutrition*. The workshop was sponsored by the United States Agency for International Development and The Wellcome Trust, UK. USAID's support came through the cooperative agreement managed by the International Life Sciences Institute Research Foundation. Supplement guest editors were Zulfiqar A. Bhutta, Aga Khan University, Pakistan, Alan Jackson (Chair), University of Southampton, England, and Pisake Lumbiganon, Khon Kaen University, Thailand.

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nutrients in preeclampsia on the basis of strength of evidence and when possible test relevance in relationship to current thinking. We also ask whether current concepts of preeclampsia suggest nutrient or micronutrient deficiencies that might be reasonable targets for study.

To achieve this goal we review current thinking about preeclampsia and current classification of the disorder, pathological changes and pathophysiological changes. We pay special attention to attempts to subclassify preeclampsia with the idea that nutrition may affect only certain subsets.

Current concept of preeclampsia

Classification. The following definitions are from National High Blood Pressure Education Program classification (5).

Preeclampsia is defined as a pregnancy-specific syndrome observed after the 20th week of pregnancy with systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg accompanied by significant proteinuria (i.e., urinary excretion of ≥0.3 g protein in a 24-h specimen). In women with preeclampsia, blood pressure usually returns to baseline within days to weeks after delivery.

Eclampsia is the occurrence, in a woman with preeclampsia, of seizures that cannot be attributed to other causes.

Gestational hypertension is defined as a blood pressure elevation detected for the first time after midpregnancy and is distinguished from preeclampsia by the absence of proteinuria. Gestational hypertension is a working diagnosis only during pregnancy. If proteinuria develops and the hypertension resolves after the pregnancy, the diagnosis is changed to preeclampsia. If elevated blood pressure persists, chronic hypertension is diagnosed. In the absence of other factors, the diagnosis is termed transient hypertension of pregnancy.

Chronic hypertension refers to an elevated blood pressure in the mother that predated the pregnancy. It can also be diagnosed in retrospect when preeclampsia or gestational hypertension fails to normalize after delivery. Thus, hypertension that has not normalized by 12 wk postpartum is considered to be chronic hypertension.

Further subclassification. Although considering preeclampsia as distinct from preexisting hypertension is vitally important, further subdivisions could also be useful. In the National High Blood Pressure Education Program classification, transient hypertension of pregnancy is defined as gestational hypertension without proteinuria and is presented as a separate entity from preeclampsia (5). This is quite important because laboratory assessment (6,7) and epidemiological follow-up suggest that gestational hypertension with proteinuria and gestational hypertension alone are different disorders. In the past, many studies of nutrition did not rigorously separate the disorders. In the past 10 y, most investigations of preeclampsia have required proteinuria as part of the definition, which has aided understanding of the disorder. Nonetheless, clinical findings suggest that even hypertension with proteinuria during pregnancy defines a heterogeneous group of women.

Further subclassification of preeclampsia would be useful to understand what might be very different pathophysiological features for the different subtypes. It might be, for example, that selected nutrients are important in some but not all subsets. Perhaps the most obvious further division is preeclampsia with onset in early pregnancy versus preeclampsia occurring in late pregnancy. Women with preeclampsia occurring before 34 wk have a greater risk of recurrent preeclampsia (40%) than women with preeclampsia occurring in later pregnancy (10%) (8). In addition, women who deliver preeclampsia preterm have a striking increase in later-life cardiovascular morbidity that is not present in women with preeclampsia later in pregnancy (9). The association of preeclampsia with fetal growth restriction is limited to pregnancies with early onset disease; later-pregnancy preeclampsia is associated with an excessive number of large infants (10).

Current concepts of the pathogenesis of preeclampsia. Preeclampsia is pregnancy-specific condition that resolves with delivery. The placenta appears to be the pregnancy component that leads to the disorder. Uterine distention had once been considered important but preeclampsia can occur with abdominal ectopic pregnancies without increased uterine size, eliminating this possibility. Likewise, preeclampsia is actually more common in pregnancies without a fetus (hydatidiform moles). Thus, the fetus is not the contributor. The placenta is necessary for preeclampsia but all pregnant women have placentas and only 5% become preeclamptic. Many years ago Page (11) suggested that the important placental feature in preeclampsia was poor perfusion. This is supported by the abnormal implantation with subsequent reduced vascularization of the placental site characteristic of preeclampsia (12). Similarly, medical conditions such as hypertension and collagen vascular disease that are associated with microvascular disease, which might be expected to reduce uterine perfusion, also predispose to preeclampsia. In addition, obstetrical conditions associated with large placentas such as hydatidiform moles or multiple gestations all predispose to preeclampsia. It is posited that with the large placenta the normal vasculature of the placental site is inadequate to perfuse the very large organ and relative placental hypoperfusion is present (1).

Current thinking about preeclampsia characterizes the reduction in perfusion as stage 1 of preeclampsia, which is proposed to be a two-stage disease. Stage 2 is the maternal syndrome (13). Although recognized by hypertension and proteinuria, preeclampsia is much more than these two changes. A predominant pathophysiological feature is reduced perfusion of virtually all organs that is due to vasoconstriction, microthrombi formation and reduced circulating plasma volume. The vasoconstriction is secondary to an increased sensitivity of the vasculature to any pressor agent. Activation of the coagulation cascade produces microthrombi. The reduced plasma volume, reflecting an endothelial leak with fluid loss from the intravascular compartment, further compromises perfusion. These abnormalities precede clinically evident disease by weeks to months and have led to the suggestion that a primary target in preeclampsia is the vascular endothelium (14). This hypothesis has been extensively supported by both old and new data. Abundant data from the past 10 y not only indicate endothelial dysfunction in preeclampsia but also demonstrate that alterations in function antedate clinically evident preeclampsia. This supports the concept that endothelial dysfunction may be causally important in the disorder (15).

What is the linkage between reduced placental perfusion and the maternal syndrome? It is evident that reduced placental perfusion alone is insufficient to explain preeclampsia. Conditions such as intrauterine growth restriction (16) and preterm birth (17) are associated with the same implantation abnormality as preeclampsia. They are associated with reduced placental perfusion yet manifest none of the maternal signs and symptoms found in preeclampsia. This suggests that preeclampsia involves an interaction of reduced perfusion with maternal factors. These risk factors for preeclampsia include obesity, black race, insulin resistance, thrombophilias and hyperhomocysteinemia, among others (13). These factors are also risk factors for atherosclerosis, and other similarities exist between preeclampsia and atherosclerosis. In both preeclampsia and atherosclerosis, endothelial cells are important targets

and the dyslipidemia predisposing to atherosclerosis occurs in preeclamptic pregnancies (18). Women with preeclampsia have reversible increases of triacylglycerols and LDL cholesterol and reduced HDL cholesterol. Quite importantly, small dense LDLs, the most predictive lipoprotein anomaly for atherosclerosis, are also increased in women with preeclampsia (19).

A major question concerns how reduced perfusion can interact with the maternal constitutional factors to result in the maternal syndrome of preeclampsia. Recent thinking has extended the similarities between preeclampsia and atherosclerosis to suggest that pathophysiological changes important in atherosclerosis may also have a role in preeclampsia. Oxidative stress, interacting with the dyslipidemia of atherosclerosis, has been hypothesized to be important in the altered endothelial function leading to atherosclerosis (20). It is posited that small dense LDLs contribute to the generation of oxidative stress. These lipoproteins preferentially enter the subendothelial space, where they are protected from circulating antioxidants. Small dense LDLs are also more sensitive to oxidation and this combination leads to the generation of oxidized LDLs, which are quite toxic. In addition to nonspecific effects to damage proteins and DNA and injure endothelium, oxidized LDLs activate cell surface antigens to recruit monocytes to the endothelial surface. The monocytes release free radicals to further injure endothelium and generate oxidized LDLs and also scavenge the oxidized LDLs. These actions result in formation of the fatty streak and atheromatous plaque characteristic of atherosclerosis.

On the basis of the similarities of atherosclerosis and preeclampsia, oxidative stress has been proposed as the linkage of the two stages of preeclampsia (21). It is posited that reduced placental perfusion generates free radicals that in the appropriate maternal environment generate systemic oxidative stress. This hypothesis is supported by evidence of oxidative stress in the circulation and tissues of women with preeclampsia (18). Perhaps the most compelling evidence comes from a small trial of antioxidant therapy in pregnancy (22). Women were identified as at high risk for preeclampsia by history of prior preeclampsia or preexisting hypertension or by abnormal uterine artery Doppler determinations at 20 and 24 wk gestation; 283 women were randomly assigned to receive either 1000 mg ascorbic acid or 400 IU α-tocopherol. The design of the study led to many exclusions, resulting in only 79 women taking drugs throughout pregnancy. Even so, therapy was effective when assessed as intention to treat or when only the women actually taking the drug were considered. Perhaps more importantly, the study had been designed and powered to test a 30% reduction in evidence of oxidative stress in the antioxidant treated group and this was demonstrated. Whether the effectiveness of therapy can be reproduced and whether treatment is safe for the fetus awaits results of larger trials now in progress. Nonetheless, this study provides strong evidence for the role of oxidative stress in the genesis of preeclampsia.

Another important question about preeclampsia concerns why, if risk factors persist for endothelial activation, the syndrome abates with delivery and the renewal of abnormal endothelial dysfunction as atherosclerosis does not appear for many years. Endothelial sensitivity appears to be increased in preeclampsia. Recent evidence indicates marked activation of inflammatory response during pregnancy. The Oxford group (23) demonstrated that in normal pregnancy inflammatory markers are increased to levels seen in sepsis. Evidence of inflammation was further increased in women with manifest preeclampsia. These findings are supported by studies indicating increased inflammatory markers before clinically evident disease (24). Activation of the inflammatory response could explain the increased sensitivity of endothelium during pregnancy and is an important target for future research (25).

Previous studies of the role of nutrients in preeclampsia

In this section we attempt to analyze previous studies in terms of the concepts and classifications of preeclampsia presented. Very few studies lend themselves to such analysis. The studies we present are almost all of preeclampsia rather than simply all cases of increased blood pressure in pregnancy. However, the ability to subclassify preeclampsia by gestational age of onset, severity or growth restriction of the fetus is rarely possible. Either the data are not available or the numbers are inadequate to make this subdivision. Thus, we give a general overview of information that is available, with comments regarding the quality of the studies. The review is selective with the emphasis on studies with clear definitions, reasonable experimental design and adequate numbers of subjects. A major problem is that virtually all studies are retrospective or they study biomarkers in women with the disease.

Energy intake and composition of the diet. Over the years preeclampsia has variably been proposed to occur secondary to over- or undernutrition with no meaningful data to support either hypothesis (3). In 1976, when undernutrition was just beginning to be considered as more important than overnutrition, Davies et al. (26) published a large study of women in Jerusalem that used dietary recall with 180 women who had preeclampsia at the time of dietary assessment. The preeclampsia diagnosis was not rigorous because it included women with hypertension and proteinuria or edema. These women were compared with 360 concurrent control women. Data indicated that women with preeclampsia had a lower intake of energy, protein and fats than did control women. However, further interrogation indicated that these differences occurred after the woman became ill and were considered to be secondary to the disease rather than causal. This important observation is of obvious relevance to several other studies of women studied while ill with preeclampsia. More recently, Morris et al. (27) evaluated a 24-h dietary recall at 13–21 wk gestation in 4157 women in the United States who had been enrolled in a randomized controlled trial of calcium supplementation to prevent preeclampsia. Because the calcium was not effective, treated and placebo patients were combined, and both preeclampsia and transient hypertension of pregnancy were analyzed as outcomes. The investigators found no difference in energy intake (or intake of any of 28 nutrients compared in cases and controls). The study is limited by assessment at 1 d in pregnancy and the wide spread of the days that were evaluated. In another recent study, Clausen et al. (28) examined dietary intake by food frequency questionnaire in 3771 Norwegian women. The questionnaire was administered at 17–19 wk gestation. Preeclampsia was the outcome and cases were separated into women with the diagnosis who delivered before (early onset) or after 37 wk gestation. Energy intake was higher in women with preeclampsia and highest in early onset preeclampsia. Differences persisted when adjusted for age, smoking and body mass index. The main difference between cases and controls was an increased intake of sucrose-containing soft drinks, which again was greatest in the women delivering before 37 wk gestation. This study is one of the few to look at early-onset preeclampsia separately from late-onset preeclampsia and supports differences. A study in Zimbabwe examined 180 women with well-defined preeclampsia (29). A crude food frequency questionnaire administered to women

with preeclampsia and the researchers could not identify gross nutritional differences.

It is difficult to reach conclusions from these studies of diverse populations. The best designed study is perhaps the Clausen et al. (28) study because the dates of administration were fairly consistent and the food frequency questionnaire was validated. Its results conflict with the results of the other studies; whether increased energy and carbohydrate intake observed in women later developing preeclampsia in this study compared with others is due to population differences, the time in pregnancy at which diet was assessed or the quality of the study cannot be determined. In both the Clausen et al. (28) and Morris et al. (27) studies women developing preeclampsia were heavier before pregnancy, suggesting dietary differences before gestation. No information on prepregnancy weight was given in the Davies et al. (26) or Zimbabwean (29) study. The increased weight before pregnancy, well established to be associated with preeclampsia, might point toward continued overnutrition in the women who develop preeclampsia.

Despite the belief that low protein intake is associated with an increased risk of preeclampsia (30), none of these studies indicated reduced protein intake in women with or destined to develop preeclampsia. This is supported by trials of protein supplementation that did not reduce the incidence of preeclampsia (31,32).

Lipids. The similarities between preeclampsia and atherosclerosis suggest that dietary lipids deserve close attention. There have been numerous studies of biomarkers, some of dietary assessment and some randomized controlled trials. Several studies documented the dyslipidemia of preeclampsia. Reduced HDL (33, 34) and increased triacylglycerols (18), LDL cholesterol (35,36) and small dense LDL (19,37) were consistently demonstrated. One study indicates that the differences in fatty acids and triacylglycerols were present before 20 wk gestation, long antedating clinical preeclampsia (38). There is of course no evidence that these differences are related to dietary intake.

Based on the effects of fatty acids on vascular and endothelial function (detailed below), considerable attention has been directed to fatty acids in preeclampsia. Al et al. (39) performed a prospective assessment of fatty acid intake, fatty acids in maternal and fetal blood and umbilical cord fatty acid composition. Maternal blood was sampled in a large cohort of women in the Netherlands at <16 and 22–32 wk gestation and within 24 h after delivery. A subset of women had diet assessed in each trimester. Fifty-two women developed gestational hypertension with or without proteinuria and were compared with 156 normal pregnant women. There were no differences in nutrient intake and no difference in maternal fatty acids at 16 and 32 wk gestation. All differences in maternal samples were in the samples collected 24 h after delivery. Fatty acids (n-6 and n-3) were lower but long-chain polyunsaturated fatty acids were higher in the hypertensive women. Cord blood values were minimally different and no differences were found in the lipid composition of umbilical cord vessels. The authors suggested that differences in fatty acids only in the postpartum samples indicate changes are likely caused by preeclampsia rather than themselves being causal. This elegant study unfortunately has the major flaw of poor diagnostic criteria that combined women with and without proteinuria. Clausen et al. (28) in their study with more appropriate diagnostic criteria found an increased intake of polyunsaturated fatty acids in women who later developed preeclampsia.

Omega-3 (n-3) fatty acids, as found in marine fats, have been suggested to be important in the prevention of preeclampsia. These materials are not only essential for fetal tissue formation (40) but also would theoretically alter prostanoids in favor of vasodilators eicosanoids. The possibility of the beneficial effect of these fatty acids was suggested by differences in rates of preeclampsia in population ingesting large quantities of fish oil. Studies of (n-3) fatty acids demonstrated that these were lower in erythrocytes of women with preeclampsia (41). Further support came from a 1938–39 trial carried out by the People's Health League in London (42). This was a high-quality study that has largely been ignored or misinterpreted. Over 5000 women were alternately assigned to receive or not receive a dietary supplement from 20 wk gestation. The supplement included iron, calcium, iodine, manganese, copper, vitamin B complex, vitamin C and halibut liver oil. The halibut liver oil dose contained about 0.1 g/d of (n-3) fatty acids. There was a statistically significant ($p < 0.005$) 31% reduction in the incidence of preeclampsia. However, attempts to duplicate this in more recent well-designed, randomized controlled clinical trials have not demonstrated such efficacy with fish oils alone (43) or fish oil and another source of (n-3) fatty acids, primrose oil (44).

Although increased polyunsaturated and total free fatty acids and other lipids and reduced (n-3) fatty acids can be identified in women with preeclampsia, the studies as designed do not separate cause from effect. In the prospective assessment of fatty acids by Al et al. (39), changes were not present before overt disease. All of the biomarkers studies are subject to the same reservation because biomarkers were assessed during overt disease. Only the study of Clausen et al. (28) found increased polyunsaturated fatty acid intake in women before preeclampsia, and their measurements of blood lipids indicate differences before overt disease. As attractive as a role for dietary lipids in preeclampsia may be, the results are tenuous as to whether increasing or decreasing specific fatty acids is useful. Only for the (n-3) fatty acids does the information seem to be convincing and this indicates no benefit. It may, nonetheless, be important that these supplement studies were begun at or beyond 16 wk gestation. Periconceptional supplementation has not been assessed.

Micronutrients. *Calcium.* Calcium is the micronutrient that has been best studied in relationship to preeclampsia. Several epidemiological studies in developing nations indicate an association between reduced calcium intake and preeclampsia (45,46). These observations led to the hypothesis that the incidence of preeclampsia can be reduced in populations of low calcium intake by calcium supplementation (47).

Several randomized controlled trials supplemented pregnant women with calcium in comparison with placebo. The Cochrane review of 11 studies performed in 6894 women (48) indicated a 32% reduction of the incidence of preeclampsia with calcium supplementation. This effect was most evident in groups with low baseline calcium intake. Most studies were small trials with potential publication bias. The largest trial to date, however, showed no effect of calcium supplementation on preeclampsia. This trial included women with adequate dietary calcium intake and, in addition, all women in both groups received low-dose calcium supplementation as part of routine prenatal supplementation (49). Data from this trial may not be applicable to the care of women with low calcium intake who may be more likely to benefit from calcium supplementation.

A unique feature of calcium trials is the acquisition of follow-up information on offspring. Children whose mothers received calcium in a randomized trial had a lower risk of increased systolic pressure at age 7 y (50).

Sodium. Sodium retention is a feature of preeclampsia.(1) Because of this, sodium intake has long been a target for dietary intervention in preeclampsia. A Cochrane review indicated

that manipulating sodium intake does not affect the frequency of preeclampsia (51). In addition, a recent study also demonstrates the dietary implications of attempting to restrict sodium. Dietary histories of 68 women assigned to either a low-sodium or normal-sodium diet indicated that sodium restriction was associated with a reduced intake of protein, calcium and energy (52). Sodium restriction or supplementation has no place in the management of preeclampsia.

Zinc. An association between zinc and preeclampsia has been suggested by reduced zinc concentrations in women with preeclampsia (53–56). This was not confirmed in a Chinese study (57). By contrast leukocyte concentrations of zinc are increased in women with preeclampsia (58). Attempts to modify the frequency of preeclampsia with zinc supplementation has not been successful (53,54).

Magnesium. The success of magnesium therapy as a treatment for eclamptic seizures and the known effect of magnesium on vascular responses in vitro (59) suggested that magnesium might be deficient in women with preeclampsia. This hypothesis was supported by the observation of reduced magnesium concentrations in serum, intracellularly and in erythrocyte membranes in some (60–62) but not all studies (63–67). In a prospective assessment by food frequency questionnaire and dietary interview at 30 wk gestation, there was no difference in magnesium intake in Danish women who developed preeclampsia (68). This finding is supported by Cochrane review of magnesium supplementation trials that found no evidence of benefit (69). The data available suggest if magnesium deficiency does occur in preeclampsia, it is a result rather than a cause of the disorder and that supplementation is unlikely to be beneficial.

Iron. Iron and markers of iron status have been reported as abnormal in preeclampsia. Entman et al. (70) reported increased free iron in preeclampsia. Several studies suggested an association with anemia (71), and ferritin is increased (72) and transferrin is decreased (73) in women with preeclampsia. In addition to the problems with measuring a marker in overt preeclampsia, there are several other cautions regarding interpreting data on iron biomarkers in preeclampsia. Increased free iron may represent hemolysis, known to be a feature of preeclampsia. Anemia is a marker for many forms of nutritional deficiency (71). Increased ferritin is not only a marker of reduced iron stores but also an inflammatory marker as is also the case with reduced transferrin (73,74). Because inflammatory responses are increased in preeclampsia, these results as they relate to iron homeostasis must be interpreted with caution.

Folate. Periconceptional folate is recommended for the prevention of neural tube defects (75). Folate is an important methyl donor and thus is crucial for protein and DNA synthesis. Another role of methyl donation is the conversion of homocysteine to methionine (76). Reduced folate intake or genetic abnormalities of folate metabolism are associated with increased serum homocysteine concentration (77). Homocysteine is increased in preeclampsia (76,78–80) and is an independent risk factor for cardiovascular disease (81). There are little data on the relationship of folate to preeclampsia. However, whether periconceptional folate reduces the risk of preeclampsia will soon be answered as preeclampsia rates are observed after the supplementation of foods with folic acid, as is now being done in the United States and other countries.

Potentially relevant micronutrients based on current concepts of preeclampsia

Current concepts of preeclampsia suggest the potential relevance of certain classes of nutrients. The quality of studies leaves the potential role of many of these candidate nutrients an open question. Information gathered largely from studies outside of pregnancy suggests nutrients that could be involved in several important steps in the currently proposed pathogenesis of preeclampsia. Specific nutrients suggested are largely implicated in what has been termed stage 2 of preeclampsia, the maternal syndrome. Equally important would be nutrients that might contribute to stage 1 or abnormal implantation. Unfortunately, our understanding of this step has not reached the stage where specific nutrients can be proposed as important. Nonetheless, the importance of nutrients in early pregnancy and the periconceptional period has been clearly established by the recognition of the importance of folic acid intake periconceptionally (82). Nutrition has not been assessed during the periconceptional period or during early pregnancy in preeclampsia. This gap needs to be filled.

Current hypotheses discussed above suggest that future studies should address nutrients that could contribute to endothelial activation, maternal constitutional factors predisposing to preeclampsia (e.g., dyslipidemia and insulin resistance) and pregnancy-specific changes such as the activated inflammatory response.

Nutrients potentially affecting endothelial function. Endothelial activation can be secondary to a variety of stimuli including oxidative stress and inflammatory mediators. These two phenomena will be considered in some detail. In addition, elevated lipids and especially increased fatty acids may directly affect endothelial function. These effects may be beneficial or detrimental. Oleic acid, for example, can be demonstrated in vitro to reduce the endothelial expression of V-CAM, an endothelial signal for leukocyte recruitment, in response to endotoxin (83). Conversely, the failure to suppress nonesterified fatty acids in insulin resistance was associated with reduced endothelium-mediated vasodilatation (84). No information is available on the influence of diet modifications that might alter specific fatty acids in preeclampsia.

Arginine as the precursor of the key determinant of endothelial function, nitric oxide, has attracted considerable attention as a modifier of endothelial function (85). Besides being a precursor for synthesis of nitric oxide, arginine also has vascular actions independent of nitric oxide. Arginine increases smooth muscle cell relaxation and endothelial cell proliferation, and decreases endothelin-1 release, leukocyte adhesion, platelet aggregation, superoxide production, expression of cell adhesion molecules, expression of monocytes chemotactic peptides, proliferation of smooth muscle cells and endothelial cell apoptosis. Other nitric oxide–independent vascular actions include increases in polarization of endothelial cell membranes, release of insulin and growth hormone and plasmin generation and fibrinogenolysis. Decreases in leukocyte adhesion to nonendothelial cell matrix, blood viscosity, angiotensin-converting enzyme activity, superoxide release and lipid peroxidation and decreases in formation of thromboxane, fibrin and platelet-fibrin have also been noted (86). Many of these functions are relevant to current concepts of the pathogenesis of preeclampsia. Arginine has been reported to be decreased (87) or unchanged (88) in preeclampsia but has never been studied before disease onset.

Nutrients or nutrient deficiencies contributing to oxidative stress. Oxidative stress is proposed as the linkage of the two stages of preeclampsia (21). Nutrients can affect oxidative stress by increasing or decreasing free radicals or antioxidants or by providing substrate for the formation of free radicals. Lipids are extensively involved in the generations of free radicals (20). Thus, diets high in fat, especially unsaturated fats, may be atherogenic whereas polyunsaturated fats are substrate for

oxidation to lipid peroxides (89) and have been reported to be increased in the diet of women developing preeclampsia (28). *Trans* fatty acids have recently gained attention because they raise plasma LDL cholesterol and serum triacylglycerol levels (90). Although epidemiological evidence indicates an association between consumption of *trans* fatty acids and coronary heart disease as well as preeclampsia (91), there are no controlled clinical trials addressing this in either setting (92).

Ascorbate is the linchpin antioxidant in humans whereas vitamin E is the major lipid-soluble antioxidant. As such they have attracted most attention as antioxidants important in human diseases including preeclampsia. Ascorbate is located in the aqueous phase but replenishes reduced lipid soluble vitamin E at the lipid aqueous interface (93). In studies of antioxidant depletion, no antioxidants are reduced until ascorbate is depleted (94). Thus, because ascorbate is not synthesized in humans, adequate dietary intake appears to be mandatory to prevent oxidative stress (95). Vitamin E seems likely to be ideally situated to prevent the formation of oxidized lipid products. Vitamin E defines a family of tocopherols. The tocopherols are found in lipoprotein particles and increase with increased lipids. Ascorbate is decreased in women with preeclampsia (96–100). Vitamin E has been reported to be reduced in some (96,100,101) but not all studies (102–105). It is most consistently reduced in severe cases (99,106). Failure to find reduced vitamin E in some studies may reflect the failure to take into account the increased lipids present in preeclampsia. In addition, in these ill women it is not possible to discriminate cause and effect.

Epidemiological studies of ascorbate and vitamin E intake tend to support a role in the reduction of atherosclerosis but are not totally consistent (107). Recent large randomized controlled trials have not demonstrated beneficial effects of antioxidants on cardiovascular disease (108). Several objections have been raised to the results of these studies including questions about antioxidant choice, dose and patient selection (108). Whether even the negative results of the studies are relevant to preeclampsia is open to question. Although preeclampsia and atherosclerosis share many pathophysiological features, the unique feature of preeclampsia is its rapid appearance and disappearance. In atherosclerosis, which has been present for many years before the onset of antioxidant therapy, it is quite possible that irreversible (perhaps structural) changes are present. This is much less likely in preeclampsia. In the small trial of antioxidants to prevent preeclampsia, whether vitamin C or vitamin E was acting as a replacement for inadequate dietary therapy or as a pharmacological antioxidant cannot be determined, although very few women in this population were receiving prenatal vitamins (22).

Other antioxidants also need to be considered. Carotenoids, such as α-carotene, β-carotene, lycopene and canthaxanthin, are potent antioxidants. In addition, soy reduces LDL cholesterol's susceptibility to oxidation (109) and in cruciferous vegetables, indole-3-carbinol, protects against lipid peroxidation (110). Nonetheless, carotenoids have not been successful in randomized controlled trials to prevent heart disease (111). Antioxidants as food may have different effects than antioxidants as supplements (112,113).

Certain substrates and cofactors are necessary for the adequate functioning of antioxidant enzymes. Glutathione is substrate for enzymes catalyzing reduction of reactive oxygen species and free radicals. At least three regulatory points, influenced by diet and nutritional status, are involved in controlling tissue glutathione concentration: *1*) the availability of glutathione supplied by the diet, *2*) nutritional effects on glutathione synthetic enzymes and *3*) nutritional influences on the uptake and efflux mechanisms for glutathione all control glutathione availability. Sulfur amino acid content of the diet regulates tissue glutathione concentration. Glutathione-synthesizing enzymes are maintained even in starvation, but protein status affects response of glutathione concentration to sulfur amino acid supplementation. In rats fed a normal-protein diet, supplementation with sulfur amino acids did not change glutathione whereas in those fed a low-protein diet, supplementation with sulfur amino acid increased hepatic glutathione concentration and sustained it at a higher concentration than in those fed the normal-protein diet (114). Thus, the protein status of a subject will affect glutathione concentration, which could then affect antioxidant effects. Studies of women with overt preeclampsia (with all the questions of cause and effect) indicate lower glutathione in their blood (115).

The suggested importance of the deficiencies of trace elements in preeclampsia relates to the fact that they are present in metallothionein (zinc), ceruloplasmin (copper), superoxide dismutases (copper, selenium, zinc) and glutathione peroxidase (selenium). Conversely, copper as a transition metal can catalyze the formation of free radicals. Several studies of zinc in preeclampsia are reviewed above, but little information is available on the other trace elements. Biomarker information on copper (116–119) and selenium is conflicting (58,120–123). There are no studies of supplementation with preeclampsia as endpoint.

Contribution of nutrients to the inflammatory response. Recent evidence indicates an increased inflammatory response in normal pregnancy that is further augmented in preeclampsia (25). There are suggestions that nutrients or micronutrients may modify the inflammatory response. Compelling preliminary evidence suggests that vitamin E and other antioxidants decrease the production of monocyte tumor necrosis factor-α, which likely plays a role in atherogenic events and as a potent endothelial-activating factor (124). Vitamin E deficiency sensitizes animals to mild inflammatory stimuli encountered during daily activities and a similar phenomenon may occur in human subjects (124). Iron status also influences cytokine production, with iron deficiency suppressing interleukin-1 production in rats (125).

Fatty acids may also affect inflammatory responses. Animal studies indicate that a range of fats can modulate pro-inflammatory cytokine production and actions. Fats rich in (n-6) polyunsaturated fatty acids enhance interleukin-1 production and tissue responsiveness to cytokines, fats rich in (n-3) polyunsaturated fatty acids have the opposite effect, monounsaturated fatty acids decrease tissue responsiveness to cytokines and interleukin-6 production is enhanced by total unsaturated fatty acid intake (126). These data are supported by in vitro studies indicating that exposure of human umbilical vein endothelial cells to polyunsaturated fatty acids results in activation of genes involved in the inflammatory response (127).

Role of nutrients in insulin resistance. Certain nutrients and trace elements are suggested to contribute to insulin resistance. Diets high in saturated, monounsaturated and polyunsaturated fats from the (n-6) family generate pronounced hepatic and peripheral insulin resistance in rats. Substituting the (n-3) fatty acids from fish oil or linseed oil prevent this development. In men, improved insulin action was associated with an increasing percentage of long-chain highly unsaturated fatty acids in skeletal muscle phospholipids (128). Chromium supplementation was also suggested as improving impaired glucose tolerance (129). However, as pointed out by Catalano et al. (130), it is likely that an individual's nutritional exposure in utero is far more relevant. Exposure to low nutrient

availability in utero programs the thrifty phenotype, with subsequent insulin resistance (131).

Summary

Our knowledge of preeclampsia has increased dramatically in the past 10 y. The role of diet and the potential for micronutrient supplements or therapy has not been adequately studied in light of this knowledge. A review of current information indicates a number of problems with studies of nutrition and preeclampsia, all of them surmountable. A major difficulty is studying women during the overt disease. Likewise, assessing biomarkers in this setting cannot resolve cause and effect. With a few rare exceptions, most of the nutritional and dietary studies have been underpowered and poorly designed. Almost no studies attempted to look at early- and late-onset preeclampsia separately. Dietary assessments have not usually been validated for pregnancy or the stage of pregnancy.

Very few nutritional questions about nutrition in preeclampsia have been answered definitively. Sodium restriction is not useful. Administering calcium or (n-3) fatty acids in unselected women from midgestation is not effective therapy for reducing the risk of preeclampsia. It also seems unlikely that zinc or magnesium supplements with the same strategy are useful. Antioxidant therapy with vitamins C and E is promising but must be tested in larger studies. It is unclear based on the study performed whether the administered vitamins were pharmacological treatment or replacement of inadequate nutritional intake.

This question of whether administration of a micronutrient and diet manipulation replace inadequate nutrients or are therapy has not been adequately addressed. The results of the calcium trials, apparently effective when there is low calcium intake but not when there is adequate calcium, suggest replacement rather than supplementation. With this in mind, an important strategy should be selective nutrient administration to deficient populations before a potential therapy is discarded.

Recommendations

- Reexplore the role of nutrition in preeclampsia with state-of-the-art techniques and guided by current concepts. Studies should be done before preeclampsia is clinically evident.
- Dietary assessment should be done at different stages of pregnancy with tools validated for that stage of pregnancy.
- Special attention should be directed at periconceptual nutrition in light of the importance of abnormal implantation in the pathogenesis of preeclampsia.
- Biomarker studies guided by credible hypotheses must be performed before preeclampsia is clinically evident.

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