Université de Montréal

Maternal Nutrition and The Risk of Preeclampsia

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RÉSUMÉ

La prééclampsie est responsable du quart des mortalités maternelles et est la deuxième cause de décès maternels associés à la grossesse au Canada et dans le monde. L'identification d'une stratégie efficace pour la prévention de la prééclampsie est une priorité et un défi primordial dans les milieux de recherche en obstétrique. Le rôle des éléments nutritifs dans le développement de la prééclampsie a récemment reçu davantage d'attention. Plusieurs études cliniques et épidémiologiques ont été menées pour déterminer les facteurs de risque alimentaires potentiels et examiner les effets d'une supplémentation nutritive dans le développement de troubles hypertensifs de la grossesse.

Pour déterminer les effets de suppléments antioxydants pris pendant la grossesse sur le risque d'hypertension gestationnelle (HG) et de prééclampsie, un essai multicentrique contrôlé à double insu a été mené au Canada et au Mexique (An International Trial of Antioxidants in the Prevention of Preeclampsia – INTAPP). Les femmes, stratifiées par risque, étaient assignées au traitement expérimental quotidien (1 gramme de vitamine C et 400 UI de vitamine E) ou au placebo. En raison des effets secondaires potentiels, le recrutement pour l'essai a été arrêté avant que l'échantillon complet ait été constitué. Au total, 2640 femmes éligibles ont accepté d'être recrutées, dont 2363 (89.5%) furent incluses dans les analyses finales. Nous n'avons retrouvé aucune évidence qu'une supplémentation prénatale de vitamines C et E réduisait le risque d'HG et de ses effets secondaires (RR 0,99; IC 95% 0,78-1,26), HG (RR 1,04; IC 95% 0,89-1,22) et prééclampsie (RR 1,04; IC 95% 0,75-1,44). Toutefois, une analyse

secondaire a révélé que les vitamines C et E augmentaient le risque de « perte fœtale ou de décès périnatal » (une mesure non spécifiée au préalable) ainsi qu'une rupture prématurée des membranes avant terme.

Nous avons mené une étude de cohorte prospective chez les femmes enceintes recrutées dans l'INTAPP afin d'évaluer les relations entre le régime alimentaire maternel en début et fin de grossesse et le risque de prééclampsie et d'HG. Un questionnaire de fréquence alimentaire validé était administré deux fois pendant la grossesse (12-18 semaines, 32-34 semaines). Les analyses furent faites séparément pour les 1537 Canadiennes et les 799 Mexicaines en raison de l'hétérogénéité des régimes alimentaires des deux pays. Parmi les canadiennes, après ajustement pour l'indice de masse corporelle (IMC) précédant la grossesse, le groupe de traitement, le niveau de risque (élevé versus faible) et les autres facteurs de base, nous avons constaté une association significative entre un faible apport alimentaire (quartile inférieur) de potassium (OR 1,79; IC 95% 1,03-3,11) et de zinc (OR 1,90; IC 95% 1,07-3,39) et un risque augmenté de prééclampsie. Toujours chez les Canadiennes, le quartile inférieur de consommation d'acides gras polyinsaturés était associé à un risque augmenté d'HG (OR 1,49; IC 95% 1,09-2,02). Aucun des nutriments analysés n'affectait les risques d'HG ou de prééclampsie chez les Mexicaines.

Nous avons entrepris une étude cas-témoins à l'intérieur de la cohorte de l'INTAPP pour établir le lien entre la concentration sérique de vitamines antioxydantes et le risque de prééclampsie. Un total de 115 cas de prééclampsie et 229 témoins ont été inclus. Les concentrations de vitamine E ont été mesurées

de façon longitudinale à 12-18 semaines (avant la prise de suppléments), à 24-26 semaines et à 32-34 semaines de grossesse en utilisant la chromatographie liquide de haute performance. Lorsqu'examinée en tant que variable continue et après ajustement multivarié, une concentration de base élevée de γ-tocophérol était associée à un risque augmenté de prééclampsie (quartile supérieur vs quartile inférieur à 24-26 semaines : OR 2,99, IC 95% 1,13-7,89; à 32-34 semaines : OR 4,37, IC 95% 1,35-14,15). Nous n'avons pas trouvé de lien entre les concentrations de α-tocophérol et le risque de prééclampsie.

En résumé, nous n'avons pas trouvé d'effets de la supplémentation en vitamines C et E sur le risque de prééclampsie dans l'INTAPP. Nous avons toutefois trouvé, dans la cohorte canadienne, qu'une faible prise de potassium et de zinc, tel qu'estimée par les questionnaires de fréquence alimentaire, était associée à un risque augmenté de prééclampsie. Aussi, une plus grande concentration sérique de γ-tocophérol pendant la grossesse était associée à un risque augmenté de prééclampsie.

Mots-clés: Prééclampsie, Hypertension gestationnelle, Vitamines C et E, Alimentation maternelle, Tocophérol, Étude clinique, Étude de cohorte, Étude cas-témoins

ABSTRACT

Preeclampsia (PE) accounts for about one-quarter of cases of maternal mortality, and ranks second among the causes of pregnancy-associated maternal deaths in Canada and worldwide. The identification of an effective strategy to prevent PE is a priority and fundamental challenge in obstetrics research. The role of nutritional factors in the etiology of PE has recently received increased attention. Many clinical and epidemiological studies have been conducted to investigate potential dietary risk factors for PE and to examine the effects of nutritional supplementation on the development of hypertensive disorders of pregnancy.

To investigate the effects of prenatal antioxidant supplementation on the risk of gestational hypertension (GH) and PE, a double blind, multicenter trial (The International Trial of Antioxidants for the Prevention of Preeclampsia – the INTAPP trial) was conducted in Canada and in Mexico. Women were stratified by their risk status and assigned to daily experimental treatment (1 gram vitamin C and 400 IU vitamin E) or to placebo. Due to concerns about potential adverse effects, recruitment for the trial was stopped before the full sample had been achieved. A total of 2640 consenting eligible women had been recruited at that point with 2363 women (89.5%) included in the final analysis. We found no evidence that prenatal supplementation of vitamins C and E reduced the risk of GH and its adverse conditions (RR: 0.99, 95% CI 0.78-1.26), GH (RR 1.04, 95% CI 0.89-1.22), and PE (RR 1.04, 95% CI 0.75-1.44). However, in a secondary

analysis, we found that vitamins C and E increased the risk of 'fetal loss or perinatal death' (a non-pre-specified outcome) as well as preterm premature rupture of membranes (PPROM).

We conducted a prospective cohort study on pregnant women enrolled in the INTAPP trial to investigate the associations between maternal diet in early and late pregnancy and the risk of PE and GH. A validated food frequency questionnaire (FFQ) was administered twice during pregnancy (12-18 weeks, 32-34 weeks). Analyses were conducted separately for 1537 Canadian and 799 Mexican women as there were significant heterogeneities in various nutrient intakes between the two countries. Among Canadian women, after adjusting for pre-pregnancy body mass index (BMI), treatment group, risk stratum (high versus low) and other baseline risk factors, we found that the lowest quartiles of potassium (OR 1.79, 95% CI 1.03-3.11) and zinc (OR 1.90, 95% CI 1.07-3.39) intake were significantly associated with an increased risk of PE. Also in Canadian women, the lowest quartile of polyunsaturated fatty acids was associated with an increased risk of GH (OR 1.49, 95% CI 1.09-2.02). None of the nutrients analyzed were found to be associated with PE and GH risk among Mexican women.

We further conducted a case control study ancillary to the INTAPP trial to assess the relationship between plasma concentration of antioxidant vitamins and the risk of PE. A total of 115 PE cases and 229 matched controls were included. Vitamin E concentrations were measured longitudinally at 12-18 weeks (prior to supplementation), 24-26 weeks, and 32-34 weeks of gestation using high-

performance liquid chromatography (HPLC). When examined as a continuous variable, and after multivariate adjustment, elevated baseline γ-tocopherol concentrations were associated with an increased risk of PE (OR 1.35, 95% CI 1.02-1.78). Analyses of repeated measurements indicated that elevated γ-tocopherol levels were associated with an increased risk of PE (highest vs. lowest quartile at 24-26 weeks: OR 2.99, 95% CI 1.13-7.89; at 32-34 weeks: OR 4.37, 95% CI 1.35-14.15). We found no associations between α-tocopherol concentrations and the risk of PE.

In summary, we found no effects of vitamins C and E supplementation on the risk of PE in the INTAPP trial. However, in the Canadian cohort we found that lower intakes of potassium and zinc as estimated by the FFQ were associated with an increased risk of PE. Moreover, higher plasma concentration of γ-tocopherol during pregnancy was associated with an increased risk of PE.

Key words: Preeclampsia, Gestational Hypertension, Vitamins C and E, Maternal Nutrition, Tocopherol, Clinical Trial, Cohort study, Case Control study

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LISTE DES ABRÉVIATIONS

APOB: Apolipoprotein B

BMI: Body Mass Index

CIHR: Canadian Institute of Health Research

CPEP: Calcium for Preeclampsia Prevention

CRF: Case Report Form

FFQ: Food Frequency Questionnaire

GH: Gestational Hypertension

GSH-Px: Glutathione Peroxidase

HLA: Human Leukocyte Antigen

HPLC: High-Performance Liquid Chromatography

IFN-γ : Interferon-gamma

IMMS: Instituto Mexicano del Seguro Social

INTAPP: International Trial of Antioxidants in the Prevention of Preeclampsia

IQ: Intelligence quotient

IUGR: Intrauterine Growth Restriction

LCPUFA: Long Chain Polyunsaturated Fatty Acids

LDL-C: Low-Density Lipoprotein Cholesterol

NAD(P)H: Nicotinamide Adenine Dinucleotide Phosphate-Oxidase

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

OR : Odds Ratio

PAI: Plasminogen Activator Inhibitor

PE: Preeclampsia

PPROM：Preterm Premature Rupture Of Membranes

PROM: Premature Rupture Of Membranes

PUFA: Polyunsaturated Fatty Acids

RR : Relative Risk

sEng: soluble Endoglin

sFlt1: Soluble fms-Like Tyrosine Kinase 1

TCC: Trial Coordinating Center

VCAM-1: Vascular Cell Adhesion Molecule 1

VEGF: Vascular Endothelial Growth Factor

VLDL: Very-Low-Density Lipoprotein

95% CI : 95% Confidence Interval

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INTRODUCTION

Preeclampsia (PE), defined as pregnancy-induced hypertension and proteinuria, is a syndrome that is unique to human pregnancy, affecting between 2% and 8% of pregnancies.(1-3) It accounts for 10%-15% of direct maternal deaths in low- and middle income countries as well as in high income countries.(4, 5) Gestational hypertension (GH), especially PE, is a frequent cause of low birth weight (<2500g) in infants and thereby perinatal deaths through both preterm delivery and intrauterine growth restriction (IUGR).(6-10) Since delivery is the only known cure, PE is a leading cause of indicated premature delivery(11) and accounts for about 15% of infants with growth restriction.(12) As many as 60% of extremely low birth weight (< 800g) infants suffer learning disabilities and low IQ(13), increasing the hidden costs of the disease. The study of the etiology, prevention and outcomes of PE and other hypertensive disorders of pregnancy remains a research priority. Effective prevention of PE would have major health benefits and result in considerable savings to health care budgets.

It has long been suggested that diet may play a role in PE. Much of the clinical and basic research into the nutritional causes of the hypertensive disorders of pregnancy has paralleled research on the etiology of hypertension, focusing on individual nutrients such as calcium, sodium, magnesium, and fatty acids. Until now, the effects of diet and specific nutrients on the hypertensive disorders of pregnancy have rarely been studied in a prospective cohort. Dietary assessment methods have not often been validated for use among pregnant women. The present research represents one of the very few studies to date that

have comparatively assessed the role of diet in the etiology of PE among women living in different geographic settings– in this case, in Canada and in Mexico - both in early pregnancy and in late pregnancy. In addition, our goal was to assess the role of diet on the development of PE at different stages of gestation. We believe that this study makes a novel contribution to understanding of the role of maternal nutrient intakes and supplementation in early and late pregnancy on the risk of PE.

STUDENT'S CONTRIBUTION

The studies described in this thesis were conducted in the context of a research program that involved a number of researchers. As a PhD student in this program, I played a key role in all of the studies described. With respect to the INTAPP trial, I played a leading role in preparation and modification of Case Report Forms and Standard Operation Procedures, the data management and adjudication of the primary outcomes (Gestational hypertension and Preeclampsia), and the planning and execution of data analysis. I played a key role in the Data Safety and Monitoring Committee, making significant contributions to the work of that committee including: 1) preparation of the report of potential adverse events associated with vitamins C and E in the literature; and 2) conducting the interim analysis regarding the adverse events observed in the INTAPP trial. With respect to the preparation of the tools for the nutritional surveys, I worked closely with nutritional experts in the INTAPP team and made significant contributions to FFQ validations study in Canada

including preparing of the statistical analysis plan and conducting data analysis. Regarding the ancillary study of nutrient intakes during pregnancy and risk of hypertensive disorders, I played a leading role in the conceptualization of the study, data management and quality assessment of the FFQ data, preparation of study analysis plan and conduct of the study analysis.

I worked closely with our colleagues at Québec Lipid Research Center and played a leading role in the conceptualization and implementation of the case control study of plasma tocopherol concentrations in relation to PE risk. The main specific responsibilities for this study were: 1) study design and preparation of study protocol; 2) implementation of the study including identifying cases and controls for laboratory measurements; and 3) preparing the statistical analysis plan and conducting data analysis using appropriate statistical models.

I drafted all manuscripts listed in the present dissertation and was the primary author for each of the manuscripts.

CHAPTER 1 LITERATURE REVIEW

1.1 Causal mechanisms of PE

PE is a multisystem disorder that is specific to human pregnancy and only can be resolved by delivery. Generally, the etiology of PE can be conceptualized in two broad categories: PE of placental origin and PE of maternal origin.(14) PE of placental origin arises from a hypoxic placenta and progresses in two stages described as pre-clinical (poor placentation) and clinical features.(14) PE of maternal origin arises from the interaction between a normal placenta and maternal constitutional factors such as microvascular disease, chronic hypertension, obesity, inflammation or diabete that predispose the woman to the condition. Thus pregnancy may represent a metabolic and vascular 'stress test' that unmasks latent cardiovascular risk.(15) However, involvement of both placental and maternal constitutional factors is very common in the development of PE and these broad categories are likely not mutually exclusive. Most patients are somewhere on a continuum between these two etiologic pathways.

Several etiologic theories of PE have been proposed and extensively investigated. (14), (16-21) During normal pregnancy, cytotrophoblasts invade the maternal decidua and spiral arteries and completely remodel the maternal spiral arteries into large capacitance vessels with low resistance. In preeclampatic pregnancies, shallow endovascular cytotrophoblast invasion of the spiral arteries results in a hypoxic and dysfunctional placenta, and the release of factors such as cytokines, growth factors and certain chemicals into the maternal

circulation.(14-26) These maternal circulating factors mediate endothelial dysfunction, leading to the clinical signs of PE. Increased levels of factor VIII-related antigen, total and cellular fibronectin, thrombomodulin, endothelin, and disturbances of the prostacyclin to thromboxane A2 ratio all support the hypothesis that systemic endothelial dysfunction plays a central role in the pathogenesis of PE.(27-36) Several lines of evidence support the hypothesis that the abnormal placentation may play a role in inducing an alteration in the balance of circulating levels of angiogenic/antiangiogenic factors such as vascular endothelial growth factor (VEGF), free placental growth factor (PlGF), soluble fms-like tyrosine kinase (sFlt1) and soluble endoglin (sEng), which contributes to endothelial cell dysfunction in the maternal vasculature.(19, 37-40) Recent studies suggest that women with clinically established PE have significantly lower levels of PlGF and VEGF compared with gestational age-matched normotensive controls.(16, 41-46) Circulating sFlt1, a receptor binding VEGF and PlGF, is significantly increased before the onset of PE.(46-48) However, it remains unclear whether impaired placental perfusion initiates symptoms such as hypertension, endothelial dysfunction, and increased sFlt1 expression, or whether inadequate placental development occurs initially and is followed by a pathological rise in sFlt1 expression and secretion.(49)

The role of oxidative stress in the pathogenesis of PE is also increasingly recognized.(21, 50, 51) Oxidative stress is an imbalance between pro-oxidant and antioxidant forces, resulting in an accumulation of free radicals or reactive oxygen or reactive nitrogen species. Deleterious effects of free radicals include

lipid peroxidation, oxidative damage to bimolecules, and cellular dysfunction. It has been hypothesized that hypoxia stimulates the activity of xanthine or nicotinamide adenine dinucleotide phosphate-oxidase (NAD (P)H) in placenta, which leads to superoxide generation. Oxidative stress likely contributes to maternal endothelial cell activation, enhanced apoptosis of trophoblast, and is believed to underlie the intense vasoconstriction and procoagulant state of PE.(14) (16-21) Markers of oxidative stress, such as isoprostanes and malondialdehyde, are increased in plasma,(52, 53) small arteries(54) and decidua basalis(55) of women with PE.

Experimental and epidemiological data support the role of maternal-fetal immune maladaptation in the etiology of PE.(56-59) There are reports of altered immune status in PE.(60-62) A significantly lower proportion of T-helper cells was demonstrated in women who later developed PE.(60) Deposition of immunoglobulin (IgM), complement (C3), and fibrin has been observed in the walls of spiral arteries in women who develop PE.(61, 63) Studies have shown that mothers lacking most or all activated killer cell immunoglobulin-like receptors (KIRs, AA genotype) when the fetus had HLA-C (human leukocyte antigens) were at a substantial risk of PE.(63) These findings are supported by epidemiological studies investigating the relationship between parity, paternity and the risk of PE.(64, 65) It has been demonstrated that multiparity is associated with a reduced risk of PE, which suggests an immune tolerance phenomenon. Interestingly, some studies suggest that the protective role of primiparity is lost

with the change of partner, suggesting that primpaternity, rather than primparity, is related to the risk of PE. (64, 65)

PE is associated with an increase in systematic inflammatory responses. The causes of these responses remain unknown. One attractive concept is that placental ischemia and reperfusion with oxidative stress may induce the higher proliferation of cytotrophoblasts and increase the deportation of syncytiotrophoblasts.(14, 20) Thus, the altered balance between proliferation and apoptosis of trophoblasts may cause aponecrotic or even necrotic release of trophoblasts, accentuating maternal inflammatory burdens. It has been reported that there are increased amounts of trophoblast debris, comprised of syncytiotrophoblast membrane microparticles, cytokeratin fragments, and soluble fetal proteins in maternal circulation in women with PE. (14), (16-21) Enhanced activation of cytokine mediators of apoptosis (especially interferon, tumour necrosis factor) have been found in PE. (14), (16-21) It is well known that severe PE and eclampsia have a familial tendency. Nilsson and colleagues reported a heritability of 31% for PE and 20% for GH.(66) Chesley et al. reported a 26% incidence of PE in daughters of women with PE compared to only an 8% incidence in the daughters-in-law.(67) It seems that a number of maternal susceptibility genes or perhaps fetal genes may contribute to the pathogenesis of PE by interacting with the maternal cardiovascular or hemostatic systems, or by regulating endothelial activation and inflammatory responses.(68-71)

In summary, there are numerous theories of the pathogenesis of PE. (Appendix: Figure1) These different underlying mechanisms are not mutually exclusive, but rather likely interactive. A vast array of initiating agents and multiple pathogenic mechanisms have been implicated in the development of PE, including increased systematic vascular resistance, enhanced platelet aggregation, activation of coagulation systems and endothelial dysfunction.

1.2 Involvement of nutritional factors in the pathogenesis of PE

The role of maternal diet in the etiology of PE has recently received increased attention. Information largely derived from studies external to pregnancy indicates that certain nutrients may be involved in several important steps in the current proposed concepts of the pathogenesis of PE.(71-74) Several nutrients, in particular, omega-3 (n-3) fatty acids, antioxidants, folic acid, and L-arginine have important roles in modulating endothelial function.(71, 72) Higher intake or supplementation of these nutrients is associated with the decreased expression of endothelium adhesion molecules (VCAM-1), but increased levels of endothelium dependent vasodilation and nitric-oxide production.(75-80) The influence of these nutrients on endothelial function is multiple and complex, including inhibition of monocyte adhesion and platelet activation, and improvement of vasodilation and blockage of lipid oxidation.(71, 72, 74)

Nutrients can affect oxidative stress by increasing or decreasing free radicals or antioxidants, by providing substrates for the formation of free radicals, or by modulating functions of antioxidant enzymes. For instance, lipids are extensively involved in the generation of free radicals.(81) Antioxidants (vitamin C, E, alpha

or beta-carotene, copper, selenium, zinc, etc.) can directly or indirectly scavenge free radicals or function as essential substrates or cofactors for the adequate functioning of antioxidant enzymes. Therefore, adequate dietary antioxidant intake is crucial for maintaining pro-oxidant and antioxidant balance as some nutrients are not synthesized in humans.

Compelling evidence suggests that nutrients may modify certain inflammatory responses.(82-85) For example, nutrients can affect the production of monocyte tumor necrosis factor-α (e.g. antioxidants and fatty acids), modulate pro-inflammatory cytokine production and actions (e.g. iron, fatty acids), or activate genes involved in the inflammatory responses (e.g. polyunsaturated fatty acids). (82-85)These mediators are essentially implicated in the pathogenesis of PE such as trophoblast apoptosis, inflammatory response and endothelial activation.

It has also been suggested that nutrients such as trace elements, fatty acids and folic acid can contribute to insulin resistance, a risk factor for PE.(86-89) Both experimental and epidemiological studies have indicated that n-3 fatty acids can improve glucose tolerance and prevent insulin resistance.(90, 91)

1.3 Macronutrients and risk of PE

1.3.1 Energy and diet composition

It is proposed that high-energy diets can affect endothelial function and inflammatory responses by activating oxidative stress-responsive transcription factors, inflammatory cytokine production and the expression of adhesion molecules.(92, 93) Abnormal lipid metabolism can be present in women with

mild or severe PE: these anomalies are characterized by increased levels of triglycerides, low-density lipoprotein cholesterol (LDL-C), LDL-III (small dense lipoprotein) and apolipoprotein A-I.(94, 95)

A large case-control study was conducted in Jerusalem, involving 180 women with PE and 360 healthy controls who were matched for country of origin, parity, month of delivery, age, year of immigration and years of schooling. A dietary history was obtained at the time of delivery. Results indicated that preeclamptic women had significantly lower intakes of protein, fat and energy. However, further investigations suggested that these differences might be secondary to the disease rather than causal.(96) Atkinson et al. carried out a case-control study in Zimbabwe using a crude (simple/qualitative/non-quantitative) food frequency questionnaire (FFQ) and found no significant differences between 180 women with PE and 194 normtensive controls.(97) Only a few prospective population-based studies have examined the relationship between energy intake and the risk of PE, and they have yielded inconsistent findings.(98, 99) A US study evaluated diet using a 24-hour dietary recall at 13-21 weeks gestation in 4157 women who had been enrolled in a randomized controlled trial of calcium supplementation in the prevention of PE.(99) There was no evidence of an increased risk of PE in women with a higher intake of energy. Moreover, there was no difference between cases and controls in the intake of any of the 28 nutrients that were studied.(99) A Norwegian team administered a semi-quantitative FFQ to 3771 women at 17-19 weeks of gestation.(98) The risk of PE was increased among women with a high energy intake (adjusted OR: 5.4, 95%

CI: 2.3 –12.4, for the 4th quartile) and a high intake of polyunsaturated fatty acids (adjusted OR: 2.3, 95% CI: 1.1-4.6). Differences persisted even after adjusting for age, smoking and body mass index (BMI). Moreover, the authors observed a stronger association for early onset PE. The discrepancy between these studies may be partially explained by the methods used to estimate dietary intake, the time in pregnancy at which diet is assessed, different definitions of PE and GH, or population differences (i.e. lifestyle, heterogeneity in nutrient intake, socio-demographic factors). It is worth pointing out that in both the Norwegian(98) and American Studies, (99) women who later developed PE had a higher pre-pregnancy body weight, suggesting the potential role of energy balance before pregnancy in the development of PE.

1.3.2 Fiber

Evidence derived from randomized controlled trials indicates that dietary fiber may have beneficial effects on plasma lipid and lipoprotein profiles, postprandial glucose metabolism, insulin sensitivity and blood pressure.(100, 101)The clinical data on the role of fiber in pregnancy are however quite limited.

In 1991, Skajaa et al. found no differences in mean daily fiber intake during the third trimester between PE cases and controls.(102) Frederick et al. conducted a case-control study of 172 preeclamptic women and 339 normotensive controls to explore the relation between PE risk and maternal intake of dietary fiber, potassium, magnesium and calcium. They reported that fiber intake was inversely associated with the risk of PE. (103) They found that women with fiber intake in the highest quartile (>24.3g/day) had a reduction in

the risk of PE (OR 0.46, 95% CI 0.23-0.92) compared to the lowest quartile (<13.1g/day). In this study, the FFQs were administered at the end of pregnancy, therefore the possibility of recall bias can be not excluded.(103) More recently, Qiu et al. carried out a prospective cohort study of 1,538 pregnant women in Washington State, in which a 121-item FFQ was administered at a mean gestational age of 13.1 weeks. The adjusted relative risk of PE for women in the highest (>21.2 g/day) vs. the lowest quartile (<11.9 g/day) was 0.28 (95% CI 0.11-0.75). (104) The authors observed similar magnitudes of associations for the highest vs. the lowest quartiles of water-soluble fiber (RR 0.30; 95% CI 0.11-0.86) and insoluble fiber (RR 0.35; 95% CI 0.14-0.87).(104) Furthermore, mean triglyceride concentrations were significantly lower and high-density lipoprotein cholesterol concentrations were nonsignificantly higher for women in the highest quartile compared to those in the lowest quartile.(104) Additional well designed cohort studies and clinical trials are needed to further explore the role of fiber as well as of obesity, insulin resistance, and dyslipidemia in the development of PE.

1.3.3 Protein intake

It has been suggested that certain amino acids such as arginine, citrulline, glycine, taurine, and histidine, as well as small peptides that directly scavenge oxygen free radicals are essential for normal endothelial vasomotion.(71, 72) However, epidemiological studies have not yielded compelling evidence to support an association between protein deficiency and the increased risk of PE.(96-99) Furthermore, trials of protein supplementation have failed to demonstrate a reduction in the risk of PE.(105, 106) The effects of high protein

supplementation (protein/energy supplementation in which the protein content of the supplement provided >25% of its total energy content) on pregnancy outcome were assessed in a Cochrane systematic review. No significant benefits of protein supplementation were observed.(107) Another systematic review to assess the effects of the balanced protein-energy supplementation on pregnancy outcomes (protein content less than 25% of total energy content) showed no effects on pregnancy outcomes including the risk of PE.(108) It should be noted that the trials included in these systematic reviews had methodological flaws. Alternate treatment allocation rather than a solid randomization method was used, and a large proportion of women were lost to follow up for the primary outcome.

On the other hand, it has been hypothesized that high protein diets may increase the risk of PE by contributing to oxidative stress via increased homocysteine production and increased whole-body nitric oxide (NO) production from nitric oxide synthase (NOS) induction.(109) However, a published meta analysis showed that, in three trials involving 384 women, energy/protein restriction had no effect on pregnancy-induced hypertension or PE, despite the fact that women who were overweight or who exhibited high weight gain significantly reduced weekly maternal weight gain and mean birth weight.(110)

1.3.4 Lipid intake

Several studies have documented dyslipidemia in women with PE. Reduced HDL (111, 112) and increased triacylglycerols (113), LDL cholesterol (114, 115) and small dense LDL (116) were demonstrated in women with PE. Increases in

serum triglycerides and free fatty acids among women who later developed PE were evident before 20 weeks of gestation.(117)

Increased levels of polyunsaturated and total free fatty acids, and other lipids and reduced (n-3) fatty acids have been observed in women with PE.(118, 119) One study prospectively assessed dietary fatty acid intake and fatty acid composition in maternal, fetal and umbilical blood.(120) Maternal blood was sampled in a large cohort of women at less than 16 and at 22-32 weeks of gestation, and within 24 hours of delivery. A subset of women underwent dietary assessment in each trimester. The results showed that there were no differences between groups (GH with or without proteinuria vs normotensive women) in maternal fatty acid and nutrient intake at 16 and 32 weeks of gestation. After delivery, levels of essential fatty acids, including 18:2 (n-6) Linoleic acid and 18:3 (n-3) α-Linoleic acid were significantly lower, whereas the sum of (n-6) long-chain polyenes (polyunsaturated fatty acids with 20 or more carbon atoms and three or more double bonds) were significantly higher in hypertensive women compared to controls.(120) In another prospective study, an increased intake of polyunsaturated fatty acids was demonstrated in women who later developed PE.(98)

Omega-3 (n-3) fatty acids have been suggested to have a preventive effect on early delivery and hypertensive disorders of pregnancy.(121, 122) Omega-3 (n-3) fatty acids are known to reduce fasting and postprandial triglycerides and to decrease platelet and leukocyte reactivity. It has been suggested that high-dose n–3 fatty acid intake could reduce maternal thromboxane A2 synthesis and

enhance maternal refractoriness to angiotensin II, which may reduce the risk of PE.(123) Low erythrocyte levels of omega-3 fatty acids and high levels of omega-6 fatty acids, particularly arachidonic acid, appear to be associated with an increased risk of PE.(124) Wang et al. observed a significantly lower level of total n–3 and n–6 polyunsaturated fatty acids in women with PE.(125) However, recent clinical trials failed to detect any significant effect of fish oil supplementation on PE risk in women at high risk of GH.(126-129) Interestingly, a recent study reported that dietary intake in polyunsaturated fatty acids (PUFAs: n-3 and n-6) was positively correlated with glutathione peroxidise (GSH-Px) activity in healthy pregnant women. (130) The author suggested that increased GPx activity may be a response to the increased oxidative stress generated by the relatively higher concentrations of PUFAs.(130) Moreover, a recent prospective study indicated that the odds ratio for hypertensive disorders presented a U-shaped curve across different intake levels of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA). (131) The authors concluded that excessive consumption in early pregnancy of n-3 LCPUFA or other nutrients (e.g. vitamin A, D, E) found in liquid cod-liver oil may increase the risk of developing hypertensive disorders in pregnancy.(131) However, Horvath et al. carried out a meta analysis of randomized controlled trials to evaluate the LCPUFAs on pregnancy outcomes.(132) There was no evidence of an effect of LCPUFAs on the rate of pregnancy-induced hypertension or PE.

1.4 Micronutrient and risk of PE

1.4.1 Calcium

Calcium is the micronutrient that has been most extensively studied in relation to PE. Numerous studies have demonstrated reduced levels of serum or urinary calcium in PE.(133-136) Several epidemiological studies indicate an association between low dietary intake of calcium and increased risk of PE.(103, 137)

Encouraged by the results of observational studies, a number of controlled trials have been conducted to confirm the beneficial effects of calcium supplementation, but with conflicting results.(138-140) A recent large trial investigated whether calcium supplementation of pregnant women with low calcium intake reduced PE and preterm delivery.(141) Calcium supplementation was associated with a small reduction in the incidence of PE and/or eclampsia (4.1% versus 4.5%; RR 0.91, 95% CI 0.69-1.19), early onset PE and/or eclampsia (RR 0.77; 95% CI 0.54-1.11) and GH (RR 0.96, 95% CI 0.86-1.06), however, null effects were not excluded. A life table analysis indicated that effects on PE and/or eclampsia were evident by 35 weeks of gestation (1.2% in calcium group versus 2.8% in placebo group, P = .04). Furthermore, calcium supplementation was associated with a reduced risk of eclampsia (RR 0.68, 95% CI, 0.48-0.97) and severe GH (RR 0.71, 95% CI 0.61-0.82).(141) Overall, there was a statistically significant reduction in the severe preeclamptic complications index including any of the following: severe PE, early onset PE, eclampsia, placental abruption, HELLP syndrome (hemolysis, elevated liver enzymes, and

low platelet count), or severe GH (RR 0.76, 95% CI 0.66-0.89, life-table analysis, log rank test P =.04).(141) Hofmeyr et al. recently conducted a meta analysis of 12 randomized controlled trials, including 15,528 women, in which 66% women had a low dietary calcium intake and 96% women were at low risk for GH or PE.(142) The dose of calcium administered varied from 1.5 to 2.0 grams per day. Calcium supplementation significantly reduced the risk of high blood pressure (11 trials, 14,946 women: relative risk 0.70, 95% CI 0.57-0.86), PE (12 trials, 15,206 women: RR 0.48, 95% CI 0.33-0.69), and maternal death or serious morbidity was reduced (four trials, 9732 women: RR 0.80, 95% CI 0.65-0.97). The effect was greatest for women at high risk for hypertensive disorders of pregnancy (five trials, 587 women: RR 0.22, 95% CI 0.12-0.42) and for those with low baseline calcium intake (seven trials, 10,154 women: RR 0.36, 95% CI 0.18-0.70).(142) However, HELLP syndrome was increased in the calcium supplementation group compared to the placebo group (two trials, 12,901 women: RR 2.67; 95% CI 1.05-6.82). There were no differences in neonatal outcomes such as preterm birth or stillbirth or death before discharge from hospital.(142)

1.4.2 Sodium

A Cochrane review indicated that manipulating sodium intake does not affect the frequency of PE.(143) In addition, a study in Japan indicated that a low-salt diet is not only ineffective for the prevention of PE, but also accelerates volume depletion in PE.(144) A reduction in sodium intake may cause a significant reduction in the intake of energy, protein, carbohydrates, fat, calcium and other

nutrients.(145) Therefore, based on recent evidence, salt restriction is not recommended in pregnancy.

1.4.3 Vitamins C and E

Vitamins C and E are two essential nutrients that can scavenge free radicals and constitute a strong line of defence in delaying or preventing ROS-induced cellular damage. Vitamin C (ascorbic acid) is an essential water-soluble vitamin, and serves as a non-enzymatic antioxidant by delivering a hydrogen atom with a single electron to a reactive oxygen molecule. Adequate dietary intake is required to prevent oxidative stress. Vitamin E is the major peroxyl radical scavenger in biological lipid phases, such as membranes or LDL. Its antioxidant action has been ascribed to its ability to chemically act as a lipid-based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation and oxLDL formation.(146) Vitamins C and E also play a key role in the modulation of enzymes involved in the vascular endothelial damage known to contribute to the pathophysiological mechanisms of the clinical expression of PE.(147) In vitro and in vivo studies demonstrate a synergistic effect between the two vitamins.(148)

Numerous studies have reported low levels of vitamin C in women with PE.(149-151) A case- control study (99 women with PE compared with 99 controls) found that women with both elevated oxidized LDL and low vitamin C concentration had a 9.8 fold risk of PE (95% CI 3.0-32.2).(151) Sagols et al. reported that plasma levels of ascorbic acid and serum antioxidant activities were significantly decreased in mild and severe PE compared to normtensive

controls.(152) Serum alpha-tocopherol levels were significantly decreased only in severe PE.(152) Furthermore, a case control study using a semi-quantitative FFQ found that women who consumed <85mg of vitamin C daily, as compared with others, experienced a two-fold risk of PE. Women with plasma ascorbic acid less than 34.6 micromol/liter had a 3.8-fold increased risk of PE, compared with those in the highest quartile. Analyses were adjusted for maternal age, parity, pre-pregnancy BMI, and energy intake.(153)

A reduced level of vitamin E in association with PE has been reported in some,(154-156) but not in all studies.(149, 150, 157-160) Reduced levels of vitamin E have been most consistently demonstrated in severe cases of PE.(152, 154, 161, 162) The variation across studies may be explained by the fact that concentrations of lipid soluble vitamin E had not been adjusted for lipid concentrations although the elevation of total cholesterol and triglycerides is one of characteristics of PE.(163) Moreover, the measurement of plasma vitamin E is a far from satisfactory estimate of the focal site of vitamin E activity, namely the cell membrane. To date, no study has measured vitamin E concentrations in red cell membrane or those in any other tissue in women with PE. It is striking that the diversion from normal values correlated with severity of disease in the reports describing either lowered or elevated plasma vitamin E concentration in women with PE.(152, 154, 156, 164)

The first clinical trial to investigate the effects of vitamin C and E on the risk of PE was conducted by a UK research group.(165) Patients were included in the study if they were at increased risk of PE, as defined by abnormal uterine artery

Doppler waveform or by past history of the disease. Among women who were at risk, the investigators reported a reduction in PE in the group with supplementation of vitamin C (1000 mg/day) and vitamin E (400 IU alpha-tocopherol/day) for (RR 0.39; 95% CI 0.17-0.90). The ratio of PAI-1 (plasminogen activator inhibitor-1, a marker of endothelial cell activation) to PAI-2 (a marker of placental function) was significantly decreased in the vitamin-treated group. High-risk women who developed PE in the placebo group had lower plasma vitamin C concentrations ($p<0.002$) compared with normal pregnant controls and these returned to normal levels on supplementation.(166) Plasma concentrations of the isoprostane, 8-epi-prostaglandin F2alpha, a marker of lipid peroxidation were raised in the high-risk placebo group but fell to concentrations comparable to low risk subjects after vitamin C and E supplementation.(166) Another small trial of women who were considered as being at high risk on the basis of their clinical history found no evidence of benefits with the same antioxidants.(167)

1.4.4 Vitamin A

The role of vitamin A and β-carotene (pro-vitamin A) in pregnancy induced hypertension and PE is also a subject of controversy. Many clinical studies have found significantly lower levels of vitamin A and β-carotene in preeclamptic women than in healthy women.(168-172) However, the decreased levels of retinol and β-carotene might be secondary to disease as a part of an acute phase reaction rather than the results of a causal relationship. Further studies are needed to determine the temporal relationship between carotenoids and the risk

of adverse pregnancy outcomes. High dose of vitamin A could be toxic and there is concern about its teratogenicity. (173-177) Given the fact that it is unlikely that a safety threshold of vitamin A consumption in early pregnancy will be established over the next few years, it will therefore be ethically difficult to conduct human trials to assess the effects of vitamin A supplementation in early pregnancy on the risk of PE.

1.4.5 Folate (Folic acid)

Folate is the generic term for this water-soluble B-complex vitamin. It functions as a coenzyme in single-carbon transfers in the metabolism of amino acids and nucleic acids, and is therefore required by all cells for growth. Folic acid (pteroylmonoglutamic acid, or PGA), which is the common form used in vitamin supplements and fortified food products, is the most oxidized and stable form of folate. Most naturally occurring folates, called food folate, are pteroylmonoglutates, which contain one to six additional glutamate molecules joined in a peptide linkage to the γ-carboxyl of glutamate.

The importance of adequate folate supply during pregnancy and lactation is increasingly recognized. It has been suggested that folic acid from food intake and routine supplementation may be sufficient during the periconception period, but larger doses may be required in early gestation, in particular for women with higher risk of adverse pregnancy outcomes (e.g. PE). Folate may reduce the risk of developing PE by improving endothelial function at both the placental and systemic levels,(178) or by lowering homocysteine, a risk factor for PE.(179)

Epidemiologic studies have found that supplementation of multivitamins containing folic acid was associated with reduced risk of PE.(180),194) Bodnar et al. examined the association between regular use of multivitamins containing folic acid at <16 weeks' gestation and the risk of PE in 1,835 women in Pittsburgh, Pennsylvania between 1997 and 2001.(180) They found that regular use of multivitamins containing folic acid was associated with a 45% reduction in PE risk compared with nonusers (OR 0.55; 95% CI 0.32-0.95). Hernandez-Diaz et al. also observed a significant reduction of risk for GH after supplementation of multivitamins containing folic acid (adjusted OR 0.55; 95% CI 0.39- 0.79).(181) Wen et al. carried out a prospective cohort study of 2951 women in Ottawa and Kingston, Canada. They found that supplementation with multivitamins containing folic acid in the early second trimester was associated with increased serum folate, lowered plasma homocysteine, and reduced risk of PE (adjusted odds ratio 0.37; 95% CI 0.18-0.75).(182) Catov et al. examined the associations between supplementation of multivitamin containing folate or folate only during a 12-week periconceptional period, using data from the Danish National Birth Cohort.(183) They found that regular use of periconceptional multivitamin containing folate use was associated with a 20% reduction of the risk of PE among normal-weight women. However, such a reduction was not observed for folate only supplements.(183) Furthermore, Ray and Mamdani found that there is a small reduction in the rates of PE in Canada after folic acid food fortification in 1998 (prevalence ratio 0.96; 95% CI 0.94- 0.98).(184)

Evidence from trials assessing the effects of folate supplements on the risk of PE, is very limited. Taylor et al. conducted a randomized trial to assess the effects of supplementation of elemental iron (65 mg/day) and folic acid (350 μg/day) on adverse pregnancy outcomes in 48 healthy pregnant women. They found no effect of iron-folic acid supplementation on the risk of PE.(185) Charles et al. re-analysed data from a large randomised controlled trial performed between 1966 and 1967 and found that the risk of PE was lower in groups receiving the supplementation of folic acid 200μg/day and 5mg/day compared to the placebo group.(186)

1.4.6 Vitamin D

The immunomodulatory properties of the hormonal vitamin D system could potentially have beneficial effects for successful maintenance of pregnancy.(187) Impaired vitamin D metabolism is demonstrated in preeclamptic pregnancy.(187, 188) Therefore, ensuring adequate vitamin D status/intake could potentially contribute to the prevention of PE.(189)

Studies exploring the role of maternal vitamin D status in adverse pregnancy outcomes are scarce. Bodnar et al. conducted a nested case-control study of pregnant women followed from less than 16 wk gestation to delivery (1997-2001) to assess the association of maternal serum 25(OH) D levels with the risk of PE.(190) Their results indicated that serum 25(OH) D concentrations in early pregnancy were lower in women who subsequently developed PE compared with controls. There was a monotonic dose-response relationship between serum 25(OH) D concentration at <22 week of gestation and the risk of developing PE.

A 50-nmol/liter decline in 25(OH)D concentration doubled the risk of developing PE (adjusted OR 2.4, 95% CI 1.1-5.4). Newborns of preeclamptic women were twice as likely as control newborns to have 25(OH)D less than 37.5 nmol/liter (adjusted OR 2.2, 95% CI 1.2-4.1).(190) A recently published study by Haugen et al. examined the association between vitamin D intake during pregnancy and the risk of PE in 23,423 nulliparous pregnant women taking part in the Norwegian Mother and Child Cohort Study.(191) They found that the odds ratio of PE for women with a total vitamin D intake of 15-20 [mu]g/d was 0.76 (95% CI 0.60-0.95) compared those with less than 5 [mu]g/d. Moreover, they reported a 27% reduction in the risk of PE (OR 0.73; 95% CI 0.58-0.92) for women taking 10-15 [mu]g/d vitamin D supplements as compared with no supplements. However, no association was found between vitamin intake from the diet alone and the risk of PE. (191) There may be a correlation between vitamin D supplementation and intake of other nutrients (e.g. calcium, Omega-3 fatty acid).(191) Further studies with data on other nutrients intake and vitamin D status will be necessary to further disentangle the effect of each on PE risk.

1.4.7 Magnesium

Magnesium is an essential mineral needed by humans in relatively large amounts. It is crucial for regulating temperature and protein synthesis and maintaining electrical potential in nerves and muscle membranes. A prospective observational study used a FFQ to assess diet at 30 weeks of gestational age and found no difference in magnesium intake in Danish women who developed PE compared to controls.(102) Observational studies suggest that supplementation

with magnesium is associated with a reduced risk of PE.(192) However, a Cochrane systematic review of randomized trials found no evidence of a benefit of magnesium supplementation on the risk of PE.(193) The methodological quality of trials included in the review was poor.

1.4.8 Other micronutrients

Certain trace elements are essential co-factors for adequate activation of antioxidant enzymes. These trace elements (e.g., copper, iron and selenium) are directly implicated in oxidative/anti-oxidative balance - a key pathogenic process in PE, and are highly dependent on dietary habits and supplements.(194-196) Serum concentrations of magnesium, copper and zinc have been reported to be significantly lower in PE compared with controls.(136, 197)

Epidemiological studies have suggested that deficiencies of zinc, iron, and selenium are associated with an increased risk of PE. However, randomized trials have failed to demonstrate a beneficial effect of trace elements supplementation in the prevention or management of PE.(198-200) Little information is available with respect to the specific roles of these trace elements in early pregnancy for PE susceptibility.

1.5 Obesity, weight gain and risk of PE

Obesity is an independent risk factor for PE.(17) The link between obesity and PE is complex. The metabolic changes in obese women, such as increased lipid availability, higher cholesterol and triglyceride levels, or insulin resistance may lead to a derangement of the VLDL/toxicity-preventing activity balance and

enhance cytokine-mediated oxidative stress, subsequently leading to endothelial cell dysfunction.(201-203) Moreover, elevated cardiac output with compensatory vasodilation in women with obesity may also lead to endothelial cell dysfunction. Most observational studies consistently demonstrate that maternal obesity or a higher prepregnancy BMI is associated with an increased risk of PE or GH.(204-207)

Bodnar et al. reported that pre-pregnancy adiposity is a strong independent risk factor for PE.(204) The authors further explored the dose-dependent relationship between pre-pregnancy BMI and the risk of PE. The results indicated that PE risk rises through most of the BMI distribution. Compared with women with a BMI of 21, the risk of PE doubled for women at BMI of 26, and nearly tripled risk for those at a BMI of 30.(204) A systematic review, identifying three cohort studies in 1.4 million women (from US, Sweden, the Netherlands, Latin America, the Caribbean, Taiwan, and the UK), demonstrated that the risk of PE typically doubled with each 5-7 kg/m^2 increase in pre-pregnancy body mass index.(205)

The prevalence of obesity is rapidly increasing worldwide and the epidemic is especially pronounced in women of child bearing age.(208, 209) It has been reported that the prevalence of pre-pregnancy obesity increased by 69% over a 10-year period, from 13% in 1993–1994 to 22% in 2002–2003.(209) As obesity confers a significant risk for PE, the epidemic of obesity therefore will undoubtedly increase the incidence of PE. Wallis et al. analyzed public-use data from the National Hospital Discharge Survey and reported that rates of PE and

GH had increased by 25% and 184% respectively from 1987 to 2004.(210) Thus, public health programs to promote the reduction of overweight or obesity as well as further research to evaluate their effectiveness are needed to suppress the epidemic of obesity and thereby the significant rise in PE.

Over the past 25 years, several authors have demonstrated a significant association between excessive weight gain and hypertensive disorders of pregnancy.(206, 207, 211-213) Brennand et al. showed that obese women with excessive weight gain had a higher prevalence of PE (14.9%) than obese women with low (3.7%) or acceptable (6.3%) weight gain.(214, 215) Saftlas et al. did not find an association between excessive weight gain and the risk of PE although the risk of transient hypertension was increased more than twofold among women in the highest quartile of the weight gain index (OR 2.55; 95% CI 1.66-3.92).(215) A prospective population-based cohort study by Cedergren of 245,526 singleton term pregnancies showed that obese women with low gestational weight gain had a decreased risk for PE (OR 0.52; 95% CI 0.42-0.62) compared to those with excessive weight gain. There was a 2-fold increased risk for PE among average and overweight women with excessive weight gain.(216) Kiel et al. carried out a population-based cohort study of 120,251 pregnant obese women to examine the associations between gestational weight change and adverse outcomes.(217) The authors reported that, among overweight or obese pregnant women, gestational weight gain of less than the currently recommended 15 lb was associated with a significantly lower risk of PE. The authors concluded that limited or no weight gain in obese pregnant women has favourable

pregnancy outcomes. Langford et al. conducted a population-based cohort study to examine the association between gestational weight gain and adverse outcomes among overweight women (BMI 26.0-29.0 kg/m^2).(218) Compared to women who gained 15-25 lbs, women who gained <15 lbs were 0.8 (95% CI 0.6-1.0) times as likely to have PE, but women who gained >25 lbs were 1.7 (95% CI 1.5-1.9) times as likely to have PE.(218) The Institute of Medicine (IOM) has recently revised guidelines for healthy ranges of weight gain in pregnancy for overweight or obese women: 15-25 lb of weight gain for overweight (BMI 25-29.9 kg/m^2), and 11-20 lb of weight gain for obese women (>30kg/m^2).(219) Continued research and changes in health policy should promote the implementation of the new guidelines and determine its impact on health care.

1.6 Other risk factors of PE

Other risk factors including life style factors, environmental factors, genetic factors, psychosocial factors, and pregnancy related factors were reviewed in the following sections.

1.6.1 Genetic and epigenetic factors and risk of PE

Studies have suggested that a family history of PE nearly tripled the risk of PE. (220) Some ethnic groups, like African-American and Hispanic women in the US, have a higher incidence of hypertensive disorders of pregnancy compared to white women.(221) Various candidate genes implicated in thrombophilia, haemodynamics, cytokines, oxidative stress, lipid metabolism,

angiogenesis, and invasion were identified. (222) Epigenetic features are also implicated in the pathogenesis of PE. It has been described that medically assisted procreation increased the risk of PE. (58) For non-imprinted genes, epigenetic alterations are also possible in PE. For instance, methylation alterations of the SER-PINB5 and SERPINA3 promoters have been demonstrated recently in PE.(222)

1.6.2 Life style factors and risk of PE

1.6.2.1 Smoking, alcohol use and PE

Smoking is associated with a variety of adverse pregnancy outcomes, but paradoxically it has a protective role against hypertensive disorders of pregnancy. Previous studies suggest that women who smoke during pregnancy have a reduced risk of PE compared to non-smokers, even when confounders are carefully controlled.(99),(223-225) Ioka et al. conducted a retrospective cohort study and did not find evidence of a protective effect of cigarette smoking on the risk of PE.(226) Lain et al. found that smoking during pregnancy is associated with reduced cellular fibronectin and increased intracellular adhesion molecule-1.(227) The authors further suggested that the negative association of smoking and PE may be mediated, in part, by the interaction of changes in endothelial activation that are the results of pregnancy and changes that are the result of smoking. Smoking may result in a decrease in basal endothelial activation and a stronger perturbation may be required among smokers to achieve the endothelial activation that is present in preeclamptic women. Results from previous studies on whether or not smoking before pregnancy may also reduce the risk of PE are

conflicting.(228-230) A secondary data analysis from a large trial of Calcium for Preeclampsia Prevention (CPEP) indicated that women who smoked at enrolment had a reduced risk of GH (RR 0.8, 95% CI 0.6-0.9). Women who quit smoking before their LMP did not demonstrate a reduced risk (RR 1.1, 95% CI 0.9-1.3). The author adjusted for maternal age, race, BMI, type of health insurance, and clinical centre. Results were similar when GH and PE were considered separately.(230)

The prevalence of smoking during pregnancy in developed countries ranges from 15% to 50%.(231, 232) It has been reported that smoking or alcohol use during pregnancy may increase maternal micronutrient requirements. Numerous studies indicated that serum concentrations of certain micronutrients (e.g. vitamin C, vitamin B12, ß-carotene, folate, iron, etc.) appear to be lower in smokers than in non smokers.(233, 234) Current evidence suggest that smoking or alcohol use could interact with micronutrient deficiencies to affect pregnancy outcome.(234) It is possible that smoking or alcohol use may decrease appetite and therefore may decrease the amount of nutrients consumed by pregnant women. Smoking or alcohol use may decrease the absorption of nutrients and affect their metabolism. It is also possible, however, that micronutrient deficiency or excess may increase the risk of adverse pregnancy outcomes in women who smoke or drink alcohol. Smoking or alcohol use may also be associated with drug use and other unhealthy lifestyle.

Therefore, studies evaluating the associations between maternal nutrient intake during pregnancy and PE and other adverse pregnancy outcomes should consider the joint effects of smoking or alcohol use and nutrient intakes.

1.6.2.2 Physical activity and PE

The results of epidemiological studies of the association between physical activity and risk of PE have been conflicting.(235-238) In a prospective cohort of 1,043 predominantly Puerto Rican prenatal care patients conducted from 2000-2004 in Western Massachusetts, there was a statistically significant trend of decreasing risk of hypertensive disorders with increasing sports/exercise in early pregnancy (ptrend=0.04). High levels of early pregnancy active living activity (OR: 0.4, 95% CI: 0.1-1.1, ptrend=0.07) and household/caregiving activity (OR: 0.4, 95% CI: 0.1-1.3, ptrend=0.07) were associated with a 60% reduction in risk of hypertensive disorders relative to low levels. However, these associations were of marginal statistical significance.(235) In another hospital-based and longitudinal study conducted in Congo, physical activity during pregnancy (RR=0.63 CI 95% 0.33 to 0.94) was found to be significantly associated with the reduced risk of PE.(236) Østerdal et al. conducted a prospective cohort of 85,139 pregnant Danish women to assess the associations between leisure time physical activity in first trimester and the risk of PE.(237) The authors reported that the two highest physical activity levels were associated with increased risk of severe PE compared with the nonexercising group, with adjusted ORs of 1.65 (95% CI: 1.11-2.43) and 1.78 (95% CI: 1.07-2.95) respectively. They found no statistically significant association between more

moderate levels of physical activity (1-270 minutes/week) and the risk of PE.(237) Tyldum et al. conducted a population based prospective cohort study of 3,656 pregnant women and found no link between pre-pregnancy physical activity and PE. Only among the women physically active for 120 min/week or more, a tendency for reduced risk was found (adjusted OR: 0.6, 95% CI 0.3-1.2).(238)

1.6.3 Pregnancy related factors and risk of PE

Research indicated that nulliparity triples the risk for PE. (239, 240) Case control studies suggested that women with PE are twice as likely to be nulliparous as women without PE.(241, 242)

Duckitt and Harrington conducted a systematic review of controlled studies published between 1966 and 2002 to determine the risk of PE associated with factors that may be present at antenatal booking.(220) Five cohort studies show that women who have PE in a first pregnancy have seven times the risk of PE in a second pregnancy relative to those with uncomplicated first pregncies (RR 7.19, 95%CI 5.85 - 8.83).(220) Six case control studies indicate that women with PE in their second pregnancy are also more than seven times more likely to have a history of PE in their first pregnancy than women who did not develop PE in their second pregnancy (OR 2.35, 95%CI 1.80-3.06).(220)

Numerous epidemiological studies indicate that twin pregnancy nearly triples the risk for PE.(220) Neither the chorionicity nor zygosity of the pregnancies alters this increased risk.(243, 244) Moreover, compared with twin pregnancy, a triplet pregnancy nearly triples the risk of PE.(245)

In a Norwegian population based study, the time interval between pregnancies significantly increased the risk of PE. (246) The association was more significant compared to the association between change of partner and the risk of PE. The risk of PE was increased by 1.12 for each year increase in interval after adjusting for change of partner, maternal age, and year of delivery (OR 1.12, 95%CI 1.11-1.13). After an interval of at least 10 years following the 1st pregnancy, the absolute risk of PE was about the same as that in nulliparous women. (246) Another Danish cohort study found that a long interval between pregnancies was significantly associated with the increased risk of PE in a second pregnancy when PE had not been present in the first pregnancy and paternity had not changed.(247) A cross sectional study reported that time intervals of more than 59 months had significantly increased risks of PE compared to 18-23 months between pregnancies (OR 1.83, 95%CI 1.72-1.94).(248)

1.6.4 Psychosocial factors and risk of PE

Several epidemiological studies have demonstrated that maternal stress played a role in the development of PE. In a cohort study of 2,601 pregnant women by Qiu et al., a positive history of maternal mood or anxiety disorder was associated with a 2.12-fold increased risk of PE (95%CI 1.02-4.45). The risk of PE appeared to be more strongly related with maternal mood or anxiety disorders first diagnosed during the index pregnancy (adjusted RR = 3.64; 95% CI 1.13-11.68). The corresponding RR for maternal mood and anxiety disorders diagnosed before pregnancy was 1.73 (95% CI 0.71-4.20).(249) In another

prospective population-based study, depression and anxiety were significantly associated with the risk of PE with the reported ORs of 2.5 (95%CI 1.1-5.4) and 3.2 (95% CI 1.4-7.4) respectively. (250)

1.6.5 Pre-existing medical conditions

A population based case control study found that the frequency of chronic hypertension was higher in women who developed PE than women who did not. (251) Studies indicated that a diastolic blood pressure before 20 weeks of either >110 mmHg or >100 mmHg was most predictive of the development of superimposed PE. (252)

Women with hypertensive disorders in pregnancy were more likely to have gestational diabetes and pre-existing diabetes compared with normotensive women (2.3% and 0.3%, respectively). (220)

Davies et al. also reported the higher prevalence of renal disease in women who developed PE compared to those that did not (5.3% vs 1.8%).(251) Martinell et al. compared women with renal disease due to a history of urinary tract infection with a prospective control population matched for age, parity, smoking and date of delivery. A total of 6.7% of women with renal disease (scarred kidneys) developed PE compared with 2.6% of women in the control group. (253)

A recently reported systematic review of 49 published observational studies found that the risk of PE was significantly increased in pregnant women in the presence of urinary tract infection (pooled OR 1.57, 95%CI 1.45-1.70) and periodontal disease (pooled OR 1.76, 95%CI 1.43-2.18). However, no

associations between PE and presence of antibodies to Chlamydia pneumonia, Helicobacter pylori, and cytomegalovirus, HIV infection, and malaria were observed in the pooled analysis.(254)

1.6.6 Environmental chemicals and risk of PE

There is a small but accumulating body of evidence that suggests that exposure to heavy metals such as lead may play a role in the etiology of GH and PE. A study of 705 women found that maternal blood lead concentrations were significantly related to hypertension in pregnancy.(255) In a case-control study, amniotic fluid from women with PE showed significant differences in levels of lead compared to women with normal pregnancies. (256) Cadmium has been hypothesized to play a role in the etiology of eclampsia,(257) and PE. (258, 259) One study has reported that hypertension in pregnant women smokers is related to significantly higher blood cadmium concentrations.(260) An adverse association between mercury exposure at background levels and systolic blood pressure has been observed among non-fish-consuming young and middle-aged women in the US, which suggests that mercury may also impact on hypertension risk in pregnant women.(261)

1.7 Dietary measurements

1.7.1 Field methods for assessing dietary measurements

Dietary intake measurements only provide estimates of the amounts of energy and nutrients available for metabolism. Several methods have been developed to measure dietary intake in the field, which can be characterized into

two broad categories: prospective records (record intake as it occurs) and retrospective recalls (recall intake after it has occurred). (262)

Dietary records used in the field can be generally categorized as two types: estimated records, and weighed records. Estimated records require the respondent (or representative) to record all food consumed during a specified period, generally between 1 and 7 days. They provide sufficient details of food consumed to allow the investigator to select an appropriate food from tables of food composition or for laboratory analysis. The amounts of food consumed are provided, either by means of the measures used in the household (jugs, cups, bowls, and spoons) or by a set of standard measures. The principal advantage of estimated records using household measures is that they involve less disruption to normal eating patterns than the weighed records. However, precision is lost with estimating rather than weighing the food consumed. Weighed records can be either a record of food consumed (weighed inventory) or a much detailed record of the weights of ingredients, final cooked weights of prepared foods, the weights of food eaten and any plate waste (precise weighing method). The former approach is generally kept by the respondents for only 1-4 days. Weighed records provide the most accurate description of the types and amounts of food consumed over a specified period. However, keeping weighed record is time consuming and it may only reflect actual intake during the record-keeping period rather than habitual intake. It may also cause the respondent to change his/her diet to facilitate record keeping.

Dietary recalls can include the 24-hour diet recall, diet history, and food frequency questionnaire (FFQ). A 24-hour recall is usually obtained during an interview where the respondent is asked to provide a recall of all food consumed, most often, over the previous 24 hours. Recalls can be conducted by face-to-face interview, telephone interview, and computer assisted interview, often using aids such as food photographs or models to assist with quantity. The 24-hour recall generally has a higher response rate than food records. It can provide detailed information on food intake and it is suitable for use in face-to-face, telephone, and computer assisted interviews. The principal disadvantage of this method is that it cannot provide information on habitual intake, unless it is repeated on multiple occasions. It may not be suitable for certain groups who have difficulties in describing foods eaten from memory.

The diet history, as first proposed by Burke in the 1940s, seeks to obtain a semi-quantitative picture of typical or habitual consumption as reflected by intake in the immediate past. It is often considered more appropriate to categorize diet history data (e.g. as high, medium, low) than provide absolute intake. The main advantage of this method is that it can provide an estimate of habitual intake for individuals if successfully carried out. The principal disadvantages are that the data is dependent on the time and skills of both respondents and interviewers and the nature of data obtained is semi-quantitative.

Another often-used recall method is the FFQ, which is basically a list of foods considered relevant for the target population and the research question with a selection of options for reporting how often each food is consumed. A

semi-quantitative FFQ includes an additional variable for indicating usual serving size. Respondents need to indicate the most appropriate frequency option for each of the foods on the list by marking the appropriate column in the questionnaire. FFQs are designed to collect long-term dietary intake data from large numbers of respondents and provide a practical, cost-effective way of collecting information from a large number of respondents. The main disadvantages include the limitations to the food list and lack of details obtained for composite foods and cooking methods, the semi-quantitative nature of the data, and the large random errors.

1.7.2 Selection of methods for dietary measurement

The choice of the dietary measurement to be used is dependent on the purpose of the study. To describe the diet of a group for comparison with another group or groups, either short-term methods such as the 24-hour diet recall or record, or long-term methods such as food records obtained on multiple occasions, a diet history or a FFQ can be used. In studies or situations where information on the usual pattern of food intake rather than precise quantitative information is required, the diet history can be appropriate for this purpose. In order to assess relationships between nutrient intake and health status (e.g. diet in relation to the risk of cardiovascular disease) individuals' long-term dietary intake data needs to be estimated.

In general, the food intake of individuals is not a static quantity. It varies in both types and amounts from day to day, from week to week, and from year to year. In order to assess the adequacy of energy or nutrient intake in relation to

requirements, it is important that short-term measurements are always adjusted for within-person variation in intake. A single 24-hour dietary recall or dietary record is considered as ‘non representative’ of individual usual intake. The numbers of days needed to measure dietary intake reliably vary among subjects and for different nutrients, and depend on the level of precision. For most nutrients, an average of three or more 24-hour recalls or dietary records on non-consecutive days is considered sufficient to produce a reasonably accurate estimate of intake for an individual. It should also include weekdays and weekend days to reduce bias.(263)

1.7.3 FFQs in epidemiological studies

The dietary measurement instrument used most often in large-scale epidemiological studies, particularly prospective cohort studies, is the FFQ. Two major FFQs, the National Cancer Institute/Block’s Questionnaire and the Harvard University/Willett’s questionnaire as well as their modified versions have been used in numerous studies. (264-270) The measurements of subjects’ dietary intake and the related methodological considerations have long been at the centre of discussion in the field of nutritional epidemiology. The major obstacle in epidemiological studies using questionnaire assessment is lack of accuracy of estimations of subjects’ habitual dietary intakes. Random errors and uncorrelated measurement errors can cause attenuations of risk estimates and reduce the statistical power.(271) This has prompted researchers to incorporate validation sub-studies that include more intensive, presumably more accurate

'reference' methods, typically multiple-day food records or multiple 24-hour dietary recalls.(272)

1.7.4 Validation of FFQs

'Validation' refers to the evaluation of whether a measuring instrument truely measures what it is actually intended to. A major practical problem in nutritional studies is that no 'gold standard' methods exist that can provide perfectly accurate measurements of the habitual intake levels. Thus, the relative validity of questionnaire is generally measured by evaluating the 'test' questionnaire assessment methods against other more 'accurate' dietary methods. The issues related to design and analysis of dietary validity studies have been extensively discussed in the literature.(272-274) It has been concluded that the estimation of the validity coefficient of dietary questionnaire measurements requires a comparison with at least two additional measurements per person.

In practice, the commonest approach for a dietary validity study is to obtain a number of replicate measurements of the actual daily food intakes at regular intervals during one-year period, using 24-hour dietary recalls or diet records. Validity studies generally include about 100-200 subjects. The relative validity of FFQs can be assessed using a variety of statistical approaches.(272) Most validation studies have commonly assessed the agreement between questionnaire measurements and the individuals' averages of k daily intake records, using either Pearson's correlation or Spearman's ranked correlation analysis (Pearson's correlation for normally distributed data, Spearman's for non-normally distributed data). (272) This approach requires random errors to be independent

not only between questionnaire and daily intake records, but also between replicate measurements using same method on the same individuals. Violation of these assumptions will lead to either overestimation or underestimation of the validity coefficients. One can also calculate cross-classification or joint classification of nutrient intake estimated from the FFQ and the average of the non-consecutive food records or recalls, by dividing each nutrient intake into quartiles or quintiles of distribution. This method is very useful if the data are divided into quartiles or quintiles and compared to the likelihood of an association with a disease outcome, as is commonly done in large nutritional epidemiological studies. (272) Another approach is called as 'Bland-Atman analysis', which assesses the agreement between two dietary measurement methods across the range of intakes, referred as Bland-Altman limits of agreement (LOA: mean± two standard deviations of the difference).(274) Other statistical methods such as structural equation models have been extensively discussed in the literature as well.(271, 273)

It is important to note that the results of validation studies are context specific. The results of one validation study are not necessarily transferable to another population, or even other nutrients in the same population. The performance of a FFQ depends on both the characteristics of instruments and the heterogeneity of intake in the population. Moreover, certain sociodemographic, lifestyle, and other characteristics of the population may influence the reliability and accuracy of diet measurements.

As described by Beaton, there is not, and probably never will be, a method that can estimate dietary intake without error.(275) This does not mean that dietary data with measurement errors should not be collected. The point is to understand, estimate, and make the use of error structure in the analysis and to consider these issues in the interpretation of nutritional data.

1.8 Summary

Many initiating agents and multiple pathogenic mechanisms have been implicated in the development of PE. Increasing evidence suggests the hypothesis that oxidative stress plays an essential role in the development of PE. In response to the Chappell et al. trial,(165) we designed the International Trial of Antioxidants In the Prevention of Preeclampsia (INTAPP Trial) to evaluate the effects of prenatal antioxidant supplementation during pregnancy on the risk of GH and its adverse conditions.

A vast array of risk factors have been associated with the risk of PE, including extremes of maternal age, primiparity, black race, maternal or pregnancy related risk factors, previous history or family history of hypertensive disorders, multiple pregnancies, obesity, and chronic medical conditions such as long-term hypertension, diabetes, renal diseases.(17, 220) We believe that the interactions between economic, nutritional, psychosocial, environmental and genetic factors may lead to the common biological alterations: endothelial dysfunction and inflammatory responses, and finally to the clinical manifestation of PE. Nutritional factors, as modifiable risk factors, may play a crucial role in the development of PE. However, nutritional intervention studies have not yet

provided unequivocal evidence in favour of an association between maternal nutrient intakes during pregnancy, in particular micronutrients (e.g. folate, vitamins) and the risk of PE. (Appendix: Table A)

The measurements of subjects' dietary intake and the related methodological considerations remain at the centre of discussion in the nutritional field. The major obstacle in epidemiological studies using questionnaire assessment is lack of accuracy of estimations of subjects' habitual dietary intakes. Random errors and uncorrelated measurement errors can cause attenuations of risk estimates and reduce the statistical power.(271) Major challenges connected with assessment of diet in pregnancy are the large intra-individual variations due to pregnancy complications that may influence eating habits, e.g. nausea, vomiting, constipation and bed rest. Furthermore, the time periods of interest may vary, i.e. preconceptional, by trimester or by critical windows for fetal organ/tissue development. It is critically important to decide the optimal time point for administering the FFQ and what time period should be addressed by the diet questions (pre-pregnancy versus in pregnancy).

Validation of a FFQ should always be performed in the same target group in which it will be used. Compared to the large number of validation studies in non-pregnant populations, few validation studies on the use of FFQs in pregnant populations have been published. Until recently, there has been little knowledge about potential dietary changes over the course of a pregnancy or whether a woman changes her diet at all, and whether changes affect the pregnancy

outcomes. We therefore designed a prospective cohort study to assess diet among pregnant women.

CHAPTER 2 OBJECTIVES AND HYPOTHESES

2.1 Objectives

2.1.1 General objective:

The general objective of this research project is to assess the relationship between maternal dietary intake or supplementation during pregnancy and the risk of GH and PE.

2.2.2 Specific objectives:

The specific objectives are: 1) to investigate the effects of prenatal antioxidant supplementation (vitamins C and E) on the risk of GH and PE; 2) to prospectively examine whether maternal nutrient intake during pregnancy is associated with the risk of PE and GH; and 3) to longitudinally assess the relationship between plasma concentration of antioxidant vitamins and the risk of PE.

2.2 Hypotheses

Maternal nutrient intake or supplementation during pregnancy is associated with the risk of GH and PE. (Appendix: Figure 1-Hypothetical framework of pathogenesis of preeclampsia)

2.2.1 Hypothesis I:

Oral prenatal supplementation of antioxidants (vitamins C and E) significantly reduces the risk of GH and its adverse conditions in 1) nulliparous

women without additional identified major risk factors and 2) nulliparous or multiparous women with risk factors.

2.2.2 Hypothesis II:

Maternal nutrient intakes during pregnancy, in particular in early pregnancy, are associated with the risk of GH and PE.

2.2.3 Hypothesis III:

Maternal plasma concentrations of α- and γ-tocopherols (measured at 12-18, 24-26, and 32-34 weeks of gestational age) are associated with the risk of PE.

CHAPTER 3 METHODOLOGY

3.1 Study design

3.1.1 Objective I

To investigate the effects of prenatal antioxidant supplementation on the risk of GH and PE, a double blinded, multicenter trial (An International Trial of Antioxidants in the Prevention of Preeclampsia –INTAPP trial) was conducted in Canada and Mexico. Randomization was stratified by center and by risk status according to pre-specified clinical risk criteria. Women were at high risk if they were nulliparous or multiparous with pre-pregnancy chronic hypertension (or diastolic blood pressure $\geq$ 90 mmHg before 20 gestational weeks or use of antihypertensive medication for hypertension), pre-pregnancy diabetes (insulin-dependent or hypoglycemic agents), multiple pregnancy, or a history of PE in the previous pregnancy. Women were stratified into the low risk stratum if they were nulliparous without any identified clinical risk factors. Women were assigned either to the antioxidant supplementation group (1g of vitamin C and 400 IU of vitamin E) or to the placebo group. (Details provided in Chapter 4-Article I)

Women were eligible for the INTAPP trial if they were between 12 and 18 completed weeks of pregnancy on the basis of last menstrual period and confirmed by early ultrasound examination. The exclusion criteria were: 1)

women who regularly consumed supplements greater than 200 mg/day for vitamin C and/or 50 IU/day for vitamin E; 2) women who took warfarin; 3) women who had known fetal abnormalities (e.g. hydatidiform mole), or known fetal chromosomal or major malformations in the current pregnancy; 4) women who had a history of medical complications including endocrine disorders (e.g., thyroid disease), renal disease with altered renal function, epilepsy, any collagen vascular disease (e.g., systemic lupus erythromatosus and scleroderma), active and chronic liver disease (e.g., hepatitis), heart disease, serious pulmonary disease, cancer, or hematologic disorder (e.g., anaemia or thrombophilia); 5) women with recurrent spontaneous abortion (women with a history of bleeding in the first trimester were included if the site documented a viable fetus at the time of recruitment); and 6) women who used illicit drugs during the current pregnancy.

We planned to recruit 5,000 patients per group in Stratum I (low risk) for a total of 10, 000 patients and 1,250 women per group in Stratum II (high risk) for a total of 2, 500 patients in order to detect 30% reduction of PE, with a power of 90% and alpha error of 5%. After reviewing the evidence from the trials conducted by the UK research group (Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia-VIP trial)(276) and the Australian Collaborative Trial of Supplements (ACTS) Study group(277) as well as our internal data on serious adverse events, and in accordance with the recommendations of the Data Safety & Monitoring Committee, the Trial Steering Committee decided to stop

recruitment in March 2006. A total of 2640 consenting eligible women had been recruited at that point.

3.1.2 Objective II

A nested ancillary prospective cohort study was conducted to evaluate whether the maternal nutrient intake in early and late pregnancy was associated with the risk of PE and GH. A validated FFQ was administered twice during pregnancy (12-18 weeks, 32-34 weeks) to assess dietary nutrient intakes during pregnancy. All patients recruited in the INTAPP trial were included for this prospective cohort study.

3.1.3 Objective III

A case control study ancillary to the INTAPP trial was conducted to assess the relationship between plasma concentration of antioxidant vitamins and the risk of PE. All cases identified in the INTAPP trial with available baseline plasma samples were included in the study. Controls were normotensive women from the INTAPP trial, randomly selected at a ratio of 2:1 by matching for country (Canada, Mexico), maternal age (within 3 years), parity (primiparous: yes/no), and multiple pregnancy (yes/no). Blood specimens were collected at each of the four INTAPP study visits (12-18 weeks, 24-26 weeks, 32-34 weeks, and delivery/postpartum) and stored in the trial's central laboratory (Quebec Lipid Research Centre).

3.2 Outcomes

The main study outcomes are GH and PE. The patient phenotype with regards to hypertensive disorders of pregnancy was carefully documented in the INTAPP trial Case Report Forms. GH was defined as at least two readings of diastolic blood pressure ≥90 mmHg taken 4 hours apart, but within 72 hours, occurring after 20 weeks of gestation.(1, 278) Severe GH was defined as two or more readings of diastolic blood pressure systolic⩾110 mmHg or systolic blood pressure⩾160 mmHg at least four hours apart. (1, 278) Proteinuria was defined as the urinary excretion of ≥0.3g/24 hours, or ⩾2+ on diagnostic strips. PE was defined as GH or severe GH with proteinuria.(1, 278) For women with pre-existing hypertension, PE was classified as the new or worsening proteinuria as defined above. For women with pre-existing proteinuria (e.g. diabetes with renal involvement), the diagnosis of PE was made on clinical or biochemical grounds by identifying at least one additional adverse condition (e.g. abnormal liver enzymes, low platelets and eclampsia).(1, 278)

3.3 Independent variables

3.3.1 Treatment allocation

Women in the INTAPP trial were assigned either to the antioxidant supplementation group (1g of vitamin C and 400 IU of vitamin E) or to the placebo group and provided with the vitamins C and E or placebo, respectively.

3.3.2 Nutritional variables

Information on dietary intakes of nutrients (lipids, vitamins C, E, A, calcium, zinc, iron, selenium etc.) was obtained from the FFQ administered at trial entry (12-18 weeks of gestation) and repeated at 32-34 weeks of gestation. Information on perinatal vitamin or mineral supplements was obtained from the INTAPP case report forms (CRFs).

3.3.3 Food Frequency Questionnaire (FFQ)

The Canadian sites of the INTAPP trial used a self-administered semi-quantitative 78-item FFQ developed by Shatenstein et al. and validated in several adult populations in both French and English.(279) It was modified for INTAPP to reflect the previous three months' usual food consumption rather than the standard 12 month period and two seasonal foods in the food list were fused in consideration of the shortened reference timeframe. It was further validated among 107 pregnant women from a subset of the Canadian INTAPP cohort. (280)

In the validation study, the research nurse aided respondents in the completion of their FFQ at their first INTAPP visit and provided validation study materials to willing recruits with instructions for completion and dates for completing the food records (FRs) at home. The research protocol stipulated that the three non-consecutive FR (two weekdays and one weekend day) were to be completed within the month following administration of the FFQ, and returned to the INTAPP study centre along with the FFQ at the participant's second INTAPP visit. Participants were asked to sign and date their FFQ to confirm the recorded information prior to nutrient analysis. The Trial Coordination Centre

(TCC) sent copies of the completed FFQs and FRs to the nutritionist for data entry and nutrient analysis. Upon verification, the nutritionist forwarded queries as needed to the research nurses if participants' instruments (FFQs and FR) were incomplete or showed inconsistencies. The research nurses then contacted respondents to clarify information, as required, and this was used to complete the FFQ and FR data.

Relative validity was assessed by evaluating agreement between crude nutrient intakes (energy and 24 nutrients) estimated from the FFQ and the average of three non-consecutive FRs (3D-FR). The results indicated that Spearman correlation coefficients between FFQ and FR nutrients were positive (r_S ranged from 0.17 for iron to 0.49 for folate) and generally statistically significant ($0.05<p<0.01$). (Appendix: Table B-D) Moreover, cross-classification of energy and 24 nutrients from the FFQ and means of the 3D-FRs placed 35% of them into identical quartiles, 75% into identical and contiguous quartiles, and only 6% were frankly misclassified. (Appendix: Table D) These results suggest that the FFQ is a relatively valid instrument for determining usual diet in pregnant women. However, variability in food intake during the course of pregnancy complicates assessment of its accuracy, and differences in gestational stage at the time of completion of the FFQ and FRs must be considered when assessing results to avoid misinterpretation of the accuracy of nutrient intake estimates. (280)

3.3.4 FFQ validation in Mexico

In the Mexican sites, the Canadian FFQ was modified to reflect local foods and dietary habits and information from the second Mexican National Nutrition Survey published data.(281). The FFQ was developed and tested in Spanish and it was validated against three non-consecutive 24-hour food recalls. (282) Participants of the FFQ validation study in Mexico were selected from women in any of the three trimesters of pregnancy who attended the Mexican Social Security Institute for a prenatal visit. Participants were interviewed by a trained nutritionist regarding the information collected in the FFQ and 3 non-consecutive 24 hour food recalls. Among 164 participants, a total of 85 pregnant women who completed the whole set of dietary interviews (1 FFQ and 3 non-consective recalls) were included in the validation analysis. Relative validity of the FFQ in relation to the three 24-hour recalls was assessed by correlation analysis and regression models. (Appendix: Table E-G) On average, approximately 50% of FFQ participants were classified into the identical tertile of the 3 non-consecutive food recalls. (Appendix: Table G) The results of the validation study suggested that the Mexican FFQ could adequately measure habitual nutritional intakes in Mexican pregnant women and capture enough variability in the population studied.(282)

3.3.5. Plasma concentration of Vitamin E

Plasma concentrations of vitamin E (α-and γ-tocopherols) were assessed using High-Performance Liquid Chromatography (HPLC) with coulometric electrochemical detection.

3.3.6 Covariates

Information on a wide range of maternal and pregnancy characteristics was collected and recorded in the INTAPP Case Report Forms (CRFs). Variables collected included maternal age, education, marital status, income, pre-pregnancy BMI, ethnicity, previous history of hypertensive disorder during pregnancy, family history of hypertensive disorder during pregnancy (mother or sister), current medical problem (chronic hypertension, diabetes), and lifestyle variables such as smoking and drinking.

3.4 Data management and quality assessment

Data were collected on standardized forms (CRFs) on which nearly all responses were pre-coded. All data were entered through an Electronic Data Management Platform and reviewed by the Trial Coordinating Center (TCC). Any discrepancies or questions concerning the data were sent to the investigator's site for corrections or clarification.

3.4.1 Nutrient intake data

Standard procedures for completing the self-administered FFQ and information on potential problems and strategies to resolve them were provided to research nurses. In the Canadian arm, an experienced research nutritionist was responsible for training research nurses working at each site. Participants were aided in the completion of the FFQ by the INTAPP research nurse at each site. The FFQ data was entered using a customized data entry interface using Microsoft Access software. Energy and nutrient values were then calculated

from the instrument food list, frequency options and portion sizes. Preliminary statistical analyses were conducted by the nutrition teams to detect outliers and assess the plausibility of the FFQ data. In Mexico, a similar approach was developed with respect to data entry, collection and quality assessment.

Nutrient intakes were also adjusted for energy.(283) Nutrient intake values were replaced with their respective residuals from a regression model with each nutrient intake as the dependent variable and the total energy intake as the independent variable. A constant, the predicted nutrient intake at the mean total energy intake, was added to the residual for each nutrient.

3.5 Statistical analysis

Exploratory analyses were conducted to assess the distribution of all continuous study variables. Means and standard deviations (for continuous variables) and frequencies and proportions (for discrete variables) were used to describe study variables. Analyses of variance (t test) and nonparametric rank tests (i.e. Wilcoxon Rank-Sum (Mann-Whitney) Test, if the distribution was skewed), were used to assess differences in continuous variables. Chi-square or Fisher's exact test, if appropriate, were used to compare the differences in rates between groups.

Data analyses were conducted in accordance with the research questions. Regression analyses were performed and the Odds Ratios and their 95% confidence interval (95% CI) derived from regression models were used to quantify the associations between exposure variables and study outcomes. Variables found to be significantly associated with study outcomes at the $P<0.15$

level in the univariate analysis were considered as candidates for inclusion in the multivariate regression models. Interaction terms were examined based on the likelihood ratio test (p value<0.05) using simple logistic regression. All analyses were performed using SAS version 9.2 and significance was set at two tailed p<0.05.

3.5.1 Objective I

The analysis was carried out on an 'intention-to-treat' basis. The baseline prognostic variables were compared between intervention and placebo groups. If there was no difference in these baseline variables, RRs and their 95% CIs were calculated to express the effects of the intervention. Otherwise, the Mantel-Haenszel RRs were calculated using stratified analysis or odds ratios (ORs) were obtained by multivariable logistic regression adjusting for potential confounding variables. For the primary outcome, the effect of vitamins C and E were also estimated separately for the two risk strata: 1) nulliparous women without additional risk factors, 2) women with additional risk factors. For secondary outcomes, binary variables (e.g. PE, preterm birth) were analyzed using Cochran-Mantel-Haenszel analysis. Continuous outcome measures (e.g., birth weight, gestational age) were analyzed by using T-test, ANOVA or multiple linear regression (if necessary) for adjustment of other covariates. Stratified analysis and multivariable logistic regression were also performed to assess whether the effect estimates differed according to country, ethnic group and socio-economic status, smoking, maternal age (<20, >35), commercial and dietary vitamin C and E consumption (estimated by FFQ) at the time of

randomization and at 26 weeks of gestation, or patient compliance that was calculated as the proportion of tablets not returned in the bottles over the total number of tablets given to each woman and defined as compliant to treatment if >80% of tablets were used .

3.5.2 Objective II

Women were classified into 3 groups as follows: (1) normotensive pregnancy, (2) GH, and (3) PE. The data from the two trial arms (treatment and placebo) were pooled as there was no difference in the rates of hypertensive disorders of pregnancy.(284)

Models for analyzing repeated dietary measurements

Our primary exposures of interest were nutrient intakes that were categorized into quartiles. Models for each of the nutrient variables contained an additional term to adjust for the confounding effect of total energy intake. (283) Energy requirements depend on body size, physical activity, and metabolic efficiency of each individual, which may confound absolute total intake in relation to disease risk. Adjustments for energy intake may reduce such confounding effects. (283) Nutritional variables were also examined as continuous variables. A sequence of nested regression models (e.g. linear, quadratic, and quadratic –spline models) was also used to explore dose-response and trend patterns between nutritional variables and the risk of disease outcomes (GH and PE).(285)

The statistical model used for the analysis of dietary intakes was logistic regression. The critical time window for the development of PE may be pre- or peri-conception period or early pregnancy. Therefore, nutrient intakes before or

in early pregnancy may be more important for the development of PE as opposed to nutrient intakes in late pregnancy, which are important for fetal growth. Therefore, the following approaches were used for handling repeated dietary measurement: 1) baseline diet (nutrient intakes estimated from FFQ collected at visit 1) only, in which PE or GH risks were related to baseline diet only; 2) diet in late pregnancy only (nutrient intakes estimated from FFQ collected at 32-34 weeks of gestation), in which PE or GH risks were related to nutrient intakes in late pregnancy only; and 3) diet intakes both in early and late pregnancy models, in which PE or GH risks were related to both baseline nutrient intakes and changes of nutrient intake from early to late pregnancy (standardized as Z-scores) (main exposure variables).

Non-dietary covariates

Non-dietary covariates were grouped into blocks with same nature. Each block contained the variables that were found to be statistically significant in the previous univariate analyses ($p<0.15$). Five blocks were successively entered into the multiple logistic regression models. Each block of variables were examined the collinearity between variables before proceeding mutilvariate analysis. Potential interactions between variables, including smoking status, alcohol use, and obesity (pre-pregnancy BMI), and nutritional variables were explored using stratified analyses. The interaction terms were included in the final multivariate models if the interactions were observed in the previous analyses. Terms representing random assignment (risk stratum and treatment group) were treated as force-entry in each step. The fit of multiple regression

models were ascertained by examination of residuals. (Appendix: Figure 2. Multivariate analysis framework for nutrient intakes during pregnancy in association with GH and PE)

3.5.3 Objective III

Maternal characteristics in cases and controls were compared using chi-square test, Fisher exact test or student t test where appropriate. To evaluate the differences in continuous variables (i.e. plasma tocopherols) between cases and controls, Student's t-test was used if the distribution was normal, and Wilcoxon test was applied if the distribution was skewed. Chi-square or Fisher's exact tests were used to compare the differences in categorical variables. Plasma concentrations of tocopherols were examined as both continuous variables-standardized Z-scores - and as categorical variables by quartiles. Odds ratios and 95% confidence intervals were estimated from logistic regression to quantify the associations between plasma concentrations of tocopherols and the risk of PE.

The Mantel extension test was used to assess linear trends in the levels of plasma tocopherols and the risk of PE. Multivariate conditional logistic regression was used to assess the independent effects of plasma concentrations of tocopherols on the risk of PE. A covariate was retained in the model if it changed the estimates by >10%. Interactions were assessed by evaluating stratum-specific ORs, and including multiplicative interaction terms in the multivariable models, and assessing their statistical significance using likelihood ratio statistics.

We estimated the associations between baseline plasma concentrations of tocopherols and the risk of PE. Intervention status may significantly change the post-baseline measurements of vitamin E concentrations and therefore may have influenced the risk of PE. For this reason, analyses were conducted in the total study population as well as in the treatment and placebo groups separately. Analyses were repeated for plasma concentrations of tocopherols at visit 2: 24-26 weeks, visit 3: 32-34 weeks of gestation, as well as the mean of three measurements at three gestational age windows. We also evaluated the patterns of changes in the plasma concentrations across gestational age and their effects on the risk of PE. (Appendix: Figure 3. Analytical framework for the case control study of plasma tocopherol concentrations in relation to PE risk)

3.6. ETHICAL CONSIDERATIONS

The Sainte Justine Hospital Research Ethics Committee (Montreal, Canada; number 1863, date: 01/12/2003) and the Instituto Mexicano del Seguro Social (IMMS) Ethics Board provided ethics approval for the INTAPP trial. Ethics approvals from each participating center were also obtained. All participants gave their written consent. These ethics approvals also covered the ancillary cohort and nested case control studies.

CHAPTER 4 ARTICLE I

An international trial of antioxidants in the prevention of Preeclampsia (INTAPP trial)

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An international trial of antioxidants in the prevention of Preeclampsia (INTAPP trial)

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16. CHUM, Hôpital St-Luc, Montréal, Canada
17. Ottawa Civic Hospital, Ottawa, Canada
18. Mount Sinaï Hospital, Toronto, Canada
19. Royal University Hospital Saskatoon, Canada
20. St. Joseph's Health Centre, London, Canada
21. Jewish General Hospital Montreal, Canada
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Condensation

Vitamin C and E supplementation during pregnancy failed to reduce the risk of preeclampsia (PE) or gestational hypertension (GH), but did increase the risk of the composite outcome 'fetal loss or perinatal death' as well as the risks of prelabour rupture of membranes (PROM) and preterm prelabour rupture of membranes (PPROM).

ABSTRACT

Objective: We sought to investigate whether prenatal vitamins C and E supplementation reduces the incidence of gestational hypertension (GH) and its adverse conditions among high-and low-risk women. **Study design**: In a multicenter randomized controlled trial, women were stratified by the risk status and assigned to daily treatment (1g vitamin C and 400 IU vitamin E) or placebo. The primary outcome was GH and its adverse conditions. **Results:** Of the 2647 women randomized, 2363 were included in the analysis. There was no difference in the risk of GH and its adverse conditions between groups (Relative Risk: 0.99, 95% Confidence Interval 0.78-1.26). However, vitamins C and E increased the risk of fetal loss or perinatal death (nonprespecified) as well as preterm prelabor rupture of membranes (PPROM). **Conclusion**: Vitamin C and E supplementation did not reduce the risk of preeclampsia or GH, but increased the risk of fetal loss or perinatal death and preterm prelabor rupture of membranes.

Key words: preeclampsia, randomized controlled trial, vitamins C and E

Introduction

Preeclampsia (PE), defined as gestational hypertension (GH) and proteinuria, is a syndrome unique to, and that complicates 2%- 8% of human pregnancies.[1-3] It accounts for about 10%-15% of direct maternal deaths in low-, middle- and high- income countries and is associated with low birth weight (<2500g) infants and thereby perinatal deaths through both preterm birth and intrauterine growth restriction (IUGR).[4-10]

Several lines of evidence support the hypothesis that oxidative stress, an imbalance between pro-oxidant and antioxidant forces, plays an essential role in the development of hypertensive disorders of pregnancy.[11-15] Markers of oxidative stress, such as isoprostanes and malondialdehyde, are increased in plasma,[16, 17]small arteries[18] and decidua basalis[19] of women with PE. In response to these findings, several clinical studies have been conducted that attempt to improve the antioxidant capability of pregnant women and thereby reduce the risk of PE.[20-25] A pilot randomized trial by Chappell et al.[20] reported a 54% reduction in PE in the group that was supplemented with vitamins C and E [relative risk (RR) 0.39; 95% confidence interval (CI) 0.17-0.90)] compared with the placebo group. Most women included in the trial were at an increased risk for PE as defined by abnormal uterine artery Doppler waveform or by past history of the disease.[20]

In response to the Chappell et al. trial,[20] we designed the International Trial of Antioxidants In the Prevention of Preeclampsia (INTAPP Trial) to assess

whether or not vitamins C and E supplementation during pregnancy reduces the risk of developing gestational hypertension and its adverse conditions in 1) nulliparous women without additional identified major risk factors and 2) nulliparous and multiparous women having those risk factors.

Methods

Between 2004 January and 2006 March, we conducted a double blinded, multicenter trial in Canada (17 centers) and Mexico (10 centers). Women were eligible for the trial if they were between 12 and 18 completed weeks of pregnancy on the basis of last menstrual period and confirmed by early ultrasound examination. The exclusion criteria were: 1) women who regularly consumed supplements greater than 200 mg/day for vitamin C and/or 50 IU/day for vitamin E; 2) women who took warfarin; 3) women who had known fetal abnormalities (e.g. hydatidiform mole), or known fetal chromosomal or major malformations in the current pregnancy; 4) women who had a history of medical complications including endocrine disease (e.g., thyroid disease), renal disease with altered renal function, epilepsy, any collagen vascular disease (e.g., systemic lupus erythromatosus and scleroderma), active and chronic liver disease (e.g., hepatitis), heart disease, serious pulmonary disease, cancer, or hematologic disorder (e.g., anaemia or thrombophilia); 5) women with repeated spontaneous abortion (women with a previous bleeding in the first trimester were included if the site documented a viable fetus at the time of recruitment); and 6) women who used illicit drug during the current pregnancy.

Randomization was performed through an Electronic Data Management Platform, which enabled randomization and data entry over the internet through a secured and restricted access internet web site and stores the data in a centralized database. Randomization was stratified by center and by risk status according to pre-specified clinical risk criteria. Women were at high risk if they were nulliparous or multiparous with prepregnancy chronic hypertension (or diastolic blood pressure $\geq$ 90 mmHg before 20 gestational weeks or use of antihypertensive medication for hypertension), prepregnancy diabetes (insulin-dependent or hypoglycemic agents), multiple pregnancy, or a history of PE in the previous pregnancy. Women were stratified into the low risk stratum if they were nulliparous without any identified clinical risk factors. They were randomly allocated at a ratio of 1:1 to antioxidant supplementation (vitamins C and E) group or to placebo group through an Electronic Data Management Platform. None of the trial staff or any other person involved in the trial knew the treatment allocation for any women until after completion of the trial analysis. The Sainte Justine Hospital Ethics Research Committee (Montreal, Canada; number 1863, date: 01/12/2003) and the Instituto Mexicano del Seguro Sosial (IMMS) Ethics board provided ethics approval, and we acquired ethics approval from each participating center. All participants gave written consent form.

Women were provided either with the vitamins C and E or placebo (Carlson Laboratories Inc. USA). Women assigned to the vitamin group were advised to take two soft gel capsules, each containing 500 mg vitamin C (ascorbic acid) and 200 IU of vitamin E (100 IU d-alpha-tocopherol, 100 IU d-alpha-tocopheryl

acetate). The total daily dose of vitamin C was 1000 mg, and that of vitamin E was 400 IU. Women in the placebo group were advised to take capsules that were identical appearance to the active treatment capsules. Women were asked to swallow the capsules whole without crushing or chewing them and were advised not to take other antioxidant supplements.

Women and their infants received care according to standard practice in each center, with surveillance for hypertension using standardized measurements of blood pressure. Systolic and diastolic blood pressure were measured by the clinical staff at each visit using a sphygmomanometer and were assessed in a sitting position, with the cuffed arm resting on a desk at the level of the heart. Korotkoff phase V was used to measure diastolic blood pressure and Korotkoff phase IV was utilized when a phase V was absent.

The composite primary outcome was defined as gestational hypertension and its adverse conditions. Our choice of the primary outcome for the trial relied on definitions proposed in the the Canadian Consensus Statement of 1997.[1] The goal was to assess the impact of antioxidants on clinically significant hypertensive disorders of pregnancy, whether or not proteinuria was present. GH was defined as at least two readings of diastolic blood pressure ≥90 mmHg taken 4 hours apart but within 72 hours occurring after 20 weeks of gestation.[1, 26] Severe GH was defined as two or more readings of diastolic blood pressure systolic⩾110 mmHg or systolic blood pressure⩾160 mmHg at least four hours apart. [1, 26] Proteinuria was defined as the urinary excretion of ≥0.3g/24 hours, or ⩾2+ on diagnostic strips. PE was defined as GH or severe GH with

proteinuria.[1, 26] For women with pre-existing hypertension, PE is classified as the new or worsening proteinuria as defined above. For women with pre-existing proteinuria (e.g. diabetes with renal involvement), the diagnosis of PE was made on clinical or biochemical grounds by identifying at least one additional adverse condition (e.g. abnormal liver enzymes, low platelets and eclampsia).[1, 26] All cases of GH and PE were further adjudicated by two independent investigators working in the Trial Coordinating Centre with failure to achieved consensus resolved by a third independent investigator.

Adverse conditions were defined as one or more of the following selected medical conditions: 1) diastolic pressure ≥ 110 mmHg or systolic pressure ≥ 160 mmHg; 2) proteinuria ≥ 300 mg/ 24 hours urine collection or ⩾2+ on diagnostic strips ; 3) convulsion (eclampsia); 4) thrombocytopenia (platelet count < 100,000 × 109/L); 5) elevated liver enzyme levels (AST or ALT >70 U/L); 6) hematocrit < 24% or blood transfusion; 7) IUGR (i.e., birth weight<3rd centile for gestational age using Canadian population based birth weight for gestational age as reference);[27] and 8) perinatal death (fetal death after 20 weeks or neonatal death within 7 days). Other maternal outcomes included maternal death, severe GH, severe PE, prelabor rupture of membranes, preterm prelabor rupture of membranes (PPROM), and hospitalization prior to giving birth. Severe PE was defined as PE and the presence of at least one of following adverse conditions: 1) diastolic pressure ≥ 110 mmHg or systolic pressure ≥ 160 mmHg; 2) proteinuria ⩾ 5g/ 24 hours urine collection or dipstick⩾3+; 3) convulsion; 4) thrombocytopenia; 5) elevated liver enzyme levels; 6) hematocrit < 24% or

blood transfusion; 7) IUGR; 8) perinatal death; or 9) preterm delivery (less than 34 weeks of gestational age).[1, 26],[28] PROM was defined as spontaneous rupture of the membranes at or after 37 weeks of gestation and before onset of the labor. PPROM was defined as spontaneous rupture of the membranes before 37 weeks of gestation and before onset of the labor.

A composite outcome – 'fetal loss or perinatal death', was defined as any fetal loss at less than 20 weeks, stillbirth or neonatal death. Other fetal or neonatal outcomes included: 1) preterm birth before 37 weeks of gestational age (gestational age corrected by early ultrasound scan); 2) preterm birth before 34 weeks of gestational age; 3) small for gestational age (defined as < 5th, or 10th centile) ; 4) perinatal mortality; 5) spontaneous abortion; and 6) neonatal morbidity indicators such as Apgar score <4 at 5 minute, retinopathy of prematurity, periventricular leukomalacia, thrombocytopenia, neutropenia, sepsis, necrotizing enterocolitis, hypotonia, intraventricular hemorrhage, convulsion, sepsis, respiratory distress requiring oxygen therapy and/or assisted ventilation for more than 24 hours, and the need for intensive care for more than 4 days.

Based on published data from the Trial of calcium to prevent preeclampsia (CPEP) in low risk women[29] and one-year delivery records from two collaborating tertiary obstetric centers: the Royal Alexandra Hospital, Edmonton (RAH, 1996) and St-Francois d'Assise Hospital, Quebec (HSFA, 1999), we estimated 4% and 15% incidences of the primary outcome in the low and high risk strata, respectively. We planned to recruit 5,000 patients per group in Stratum I (low risk) for a total of 10, 000 patients and 1,250 women per group in

Stratum II (high risk) for a total of 2, 500 patients in order to detect 30% reduction of PE, with a power of 90% and alpha error of 5%. After reviewing the evidence from the trials conducted by the UK research group (Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia-VIP trial)[22] and the Australian Collaborative Trial of Supplements (ACTS) Study group[23] as well as our internal data on serious adverse events, and in accordance with the recommendations of the Data Safety & Monitoring Committee, the Trial Steering Committee decided to terminate the trial. A total of 2640 consenting eligible women were recruited. The last woman was recruited on March 30th 2006 and the last infant was born on September 1st 2006.

The analysis was carried out on an 'intention-to-treat' basis. We used Student's t tests to compare continuous variables and the Chi-squared test or Fisher's exact test for categorical variables, as appropriate. The effects of the intervention were expressed as RR (95% CI). Participants with missing outcomes due to withdrawal or loss to follow-up were excluded from the analysis of outcomes. We assessed twin and triplet infants as if cluster randomized (the cluster being the mother). Neonatal outcomes were analyzed by adjusting for the multiplicity of the pregnancy as the main neonatal outcomes were strongly affected by multiple births except for the outcome of preterm birth. Stratified analysis and multivariable logistic regression were performed to assess whether the effect estimates differed according to country, ethnic group and socio-economic status, smoking, maternal age (<20, >35), commercial and dietary vitamin C and E consumption (estimated by FFQ) at the time of

randomization and at 26 weeks of gestation, or patient compliance that was calculated as the proportion of tablets not returned in the bottles over the total number of tablets given to each woman and defined as compliant to treatment if >80% of tablets were used .

The study was registered as an International Standard Randomised Controlled Trial, number ISRCTN 85024310.

Results

Figure 1 displays the trial profile. Of the 2647 eligible women who were randomized, a total of 2640 women were validly randomized (randomization error in 3 women, recruitment halted at randomization visit in two women, two women non eligible after randomization). Of these, 1315 (49.81%) women were assigned to vitamins C and E group and 1325 women (50.19%) were allocated to the placebo group. Patients who were lost to follow up were excluded from the analyses and a total of 2363 women and their 2536 infants (vitamin group: 1167 women and 1243 infants, placebo group: 1196 women and 1293 infants) were included in the final analyses.

At study entry, maternal baseline characteristics were similar in two groups, except that there was a slightly higher proportion of multiple pregnancies in the placebo group (Table1) There was no significant difference in patient compliance between the vitamin and the placebo groups (85.5% vs 86.5%, p=0.3640).

There was no statistically significant difference in the risk of the primary outcome, GH and its adverse conditions in the treatment group and the placebo

group (10.11% and 10.20% respectively; RR: 0.99, 95% CI 0.78-1.26). The incidence of PE was similar between the two groups (5.95% versus 5.71%; RR 1.04, 95% CI 0.75-1.44) and there was no statistically significant difference in the risk of GH between the two groups (21.68% vs 20.82%; RR 1.04, 95% CI 0.89-1.22). Furthermore, there were no significant differences for any individual outcomes included in the primary composite outcome. (Table 2)

Table 3 provides the results of the primary outcome, GH, and PE stratified by specific risk factor at enrolment. Vitamins C and E did not reduce the risk of GH and its adverse conditions, GH or PE, irrespective of risk at enrolment. (Table 3)

Compared with the placebo group, women in the vitamin supplementation group had statistically significantly higher rates of PROM (10.17 % in the vitamin group versus 6.15% in placebo group; RR 1.65, 95% CI 1.23-2.22) and PPROM (5.97% in the vitamin group versus 3.03 in placebo group; RR 1.97, 95%CI 1.31-2.98). There were no differences in the rates of severe GH and severe PE. There was no reported maternal death in the study. There were no differences in other maternal adverse outcomes in the vitamin group compared with the placebo group. (Table 4)

The rate of total death of the composite outcome ‘fetal loss or perinatal death’ was significantly higher in the vitamin supplemented group (1.69% versus 0.78; RR 2.20, 95%CI 1.02-4.73) (Table 5). The rates of spontaneous abortion, stillbirth and neonatal death before discharge were higher in the supplementation group, however these differences were not statistically significant. There were no statistically significant differences in the rates of preterm birth, IUGR (<3rd

centile), or small for gestational age (<5th or 10th centile) in two groups. There were no differences between the vitamin supplementation and placebo groups in neonatal morbidity indicators including respiratory distress requiring supplemental oxygen therapy, assisted ventilation for more than 24 hours, the need for intensive care for more than 4 days, Apgar score <4 at 5 minute, convulsion, sepsis, intraventricular hemorrhage, necrotizing enterocolitis, hypotonia, hypertonia, retinopathy of prematurity, leukomalacia, or neutropenia.

Stratification analysis by risk status for PE (low versus high) and country (Canada and Mexico) indicated no evidence of heterogeneity between countries. We further assessed the effects of vitamin supplementation on the risk of PE adjusted by pre-selected covariates (i.e., smoking, maternal age (<20, >35), vitamin C and E intake at the time of randomization, and the proportion of patients compliant with treatment). The effect estimates remained similar.

Discussion

Taking into account risk profile, the rate of PE in our study was similar to that reported in previous trials.[22, 23] We did not find that supplementation with vitamins C and E reduced the risk of gestational hypertension and its adverse conditions among patients at high risk and low risk for PE. The results were consistent with those of other recently reported RCTs,[20-25] with the exception of the first small trial by Chappell.[20] Poston et al. reported no reduction of PE risk associated with vitamins supplementation (RR 0.97; 95% CI 0.80-1.17) in 2410 women identified at increased risk of PE.[22] Rumbold et al. found no differences between the vitamin and placebo groups in the risk of PE (RR 1.20; 95% CI

0.82-1.75), and other adverse birth outcomes (e.g. perinatal death, small for gestational age) in 1877 nulliparous women recruited between 14 and 22 weeks of gestation.[23] The World Health Organization recently completed a multicenter trial and indicated that supplementation of vitamins C and E did not reduce the risk of PE, eclampsia, and gestational hypertension among pregnant women with low socio-economic status and low nutritional status in developing countries.[25]

In addition to the trials of combined vitamins C and E supplementation , Rivas et al. conducted a trial to assess the effects of aspirin, vitamin C, E and fish oil supplements.[30] They found that such supplements significantly reduced the risk of PE (RR 0.07, 95%CI 0.01-0.54). However, it is hard to infer whether such an effect was due to vitamins C and E, fish oil or aspirin, or the effects of their interaction. Steyn et al. reported no effects of vitamin C supplementation on the risk of PE (RR 1.00, 95%CI 0.21-4.84). The study was stopped early because of an increase in spontaneous preterm labor in the treatment group.[31]

Women in the group receiving vitamin supplementation had higher rates of PROM and PPROM than did the placebo group. The treatment differences remained significant after covariate adjustment for maternal age, smoking, body mass index, the patient compliance, and the presence of medical risk factors (e.g. chronic hypertension, multiple pregnancy, history of PE, history of diabetes). Of the previously reported large RCTs of vitamins C and E supplementation in the prevention of PE risk, only two trials reported and examined the effects of vitamins C and E supplementation and the risk of PPROM.[23, 24] Rumbold et al. reported a slight increase of PPROM risk in the supplemented group (3.2%

versus 2.4%; RR 1.31, 95%CI 0.77-2.25).[23] However the difference was not statistically significant. Spinnato et al. observed a significant increased risk of PPROM in the antioxidant group (10.6% versus 5.5%; adjusted RR 1.89, 95% CI 1.11-3.23).[24] Casanueva et al. conducted a randomized trial, in which 109 women were randomly assigned at 20 weeks of gestation either to 100 mg vitamin C or placebo.[32] Despite the fact that there was no measured difference in plasma vitamin C concentration between two groups, there was a significantly lower rate of PROM in the supplemented group compared with the placebo group.[32]

We did note an increase risk of the composite outcome 'fetal loss or perinatal death' (1.69% vs 0.78%; RR 2.20, 95%CI 1.02-4.73). While this was not a pre-specified outcome in the study protocol, it was used as an outcome for the monitoring of morbidity by the Data Safety Monitoring Board, and contributed to the decision to stop the trial early. We noted that there were more perinatal deaths in the antioxidant group than the placebo group, but the effect did not reach statistical significance. We did not find evidence of differences between groups in the rates of low birth weight, preterm birth, small for gestational age, or low Apgar score. Poston et al. found vitamin supplements to be positively associated with the risk of low birth weight compared with controls. This effect was particularly strong among women with prepregnancy diabetes who were in the Vitamin supplement group (RR: 1.15; 95%CI: 1.02- 1.30). [22]

The daily doses of 1000 mg vitamin C and 400 IU vitamin E (RRR-αlpha-tocopherol) are certainly below the maximum recommended intake in pregnant

women. We do not know why supplementation of vitamins C and E at these doses does not reduce the risk of PE, or GH, but increased the rates of PROM and PPROM in our study. The dose of vitamin E that is required to suppress isoprostane levels (a marker of oxidative stress) has been documented in adult males,[33] but not in pregnant women. Exogenous vitamin E may prevent an immunologic switch (Th1 to Th2) that is considered as crucial for early to late transition in normal pregnancy and it could be a potential interferon-gamma (IFN- γ) mimic, facilitating pro-inflammatory responses at the maternal-fetal interface.[34] It is possible that vitamin E exerts both potentially beneficial and detrimental effects. Therefore, vitamin E treatment might have undesirable side-effects and may partially explain the unexpected results of the increased risks of PROM and PPROM. There is some evidence that high doses of alpha tocopherol (primary form of vitamin E in supplementation) could deplete plasma and tissue gamma tocopherol (major form in plant seeds and in the North American diet).[35-38] Therefore, the efficacy of vitamin E supplementation (alpha tocopherol) may be offset by deleterious changes in the levels of other nutrients. In fact, our preliminary data analyses using the INTAPP population found that women in the supplementation group had significant higher level of alpha-tocopherol at 32 weeks of gestation (58%; $p<0.001$) and lower ratio of gamma-/alpha-tocopherol (-58%; $p=0.001$), compared with the baseline visit ('visit 1': week 12-16 gestation). While levels of alpha-tocopherol were significantly increased compared to baseline (34%; $p<0.001$) among women in the placebo group, the ratios of gamma-/alpha-tocopherol were not affected.[39] The

ineffectiveness of vitamin C and E in the prevention of PE and the potentially harmful effects emphasize the need for a better understanding of the underlying mechanisms and metabolism of both vitamins C and E in the human body.

Witztum et al. hypothesized that only individuals under oxidative stress are likely to benefit from antioxidant supplementation.[40] Meagher et al. proposed that only people deficient in vitamin E may benefit from vitamin E supplementation.[41] To date, a series of trials have been conducted, including the present study, involving both low and high risk patients (e.g. presence of chronic hypertension, history of PE, multiple gestation, diabetes, low socio-economic status and low nutritional status). It is clear that irrespective of study population (i.e. risk profile and nutritional status), supplementation with vitamins C and E during pregnancy is unlikely to prevent PE, gestational hypertension, preterm birth or low birth weight.

The definitions for GH and PE retained for this trial are those of the Canadian Consensus Statement on Hypertensive Disorders of Pregnancy.[1] This definition focuses on the diastolic blood pressure value for diagnosis. There is no universal consensus regarding the definition of GH or PE, nor studies are there comparing the relative validity of the different definitions. However, it is unlikely that the choice of an alternative definition would have modified the results of the trial.

The trial was prematurely stopped with a total of 2640 eligible pregnant women included in the final analysis. This resulted in a significant decrease in power relative to the initially planned sample size. Nevertheless, in the light of

our results and those of other investigators, it is unlikely that further recruitment would have identified a difference in treatment group. Furthermore, given the increased risk of certain adverse outcomes ('fetal loss/perinatal deaths and PPROM), we considered it unethical to continue the study. We also acknowledge the fact that an approximate 20% of lost to follow up occurred in Mexican centres. This is because a high proportion of Mexican women beginning prenatal care in the IMSS Centres change health care provider in the course of prenatal care and deliver in non-IMSS hospitals where data could not be accessed. However, the proportion of lost to follow up was balanced between treatment and placebo groups and stratification analysis by country did not result in any difference for effect estimates.

Despite the fact that the underlying mechanisms remain largely unclear, there is increasing concern that supplementation of vitamins C and E at the doses studied [i.e. 1000 mg vitamin C and 400 IU vitamin E (RRR α tocopherol)] may increase the risk of other adverse pregnancy outcomes such as low birth weight[22] and PPROM. Therefore, based on our present knowledge, vitamins C and E supplementation at the above doses cannot be recommended for pregnant women to prevent adverse pregnancy outcomes including PE.

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Figure 1. Trial profile

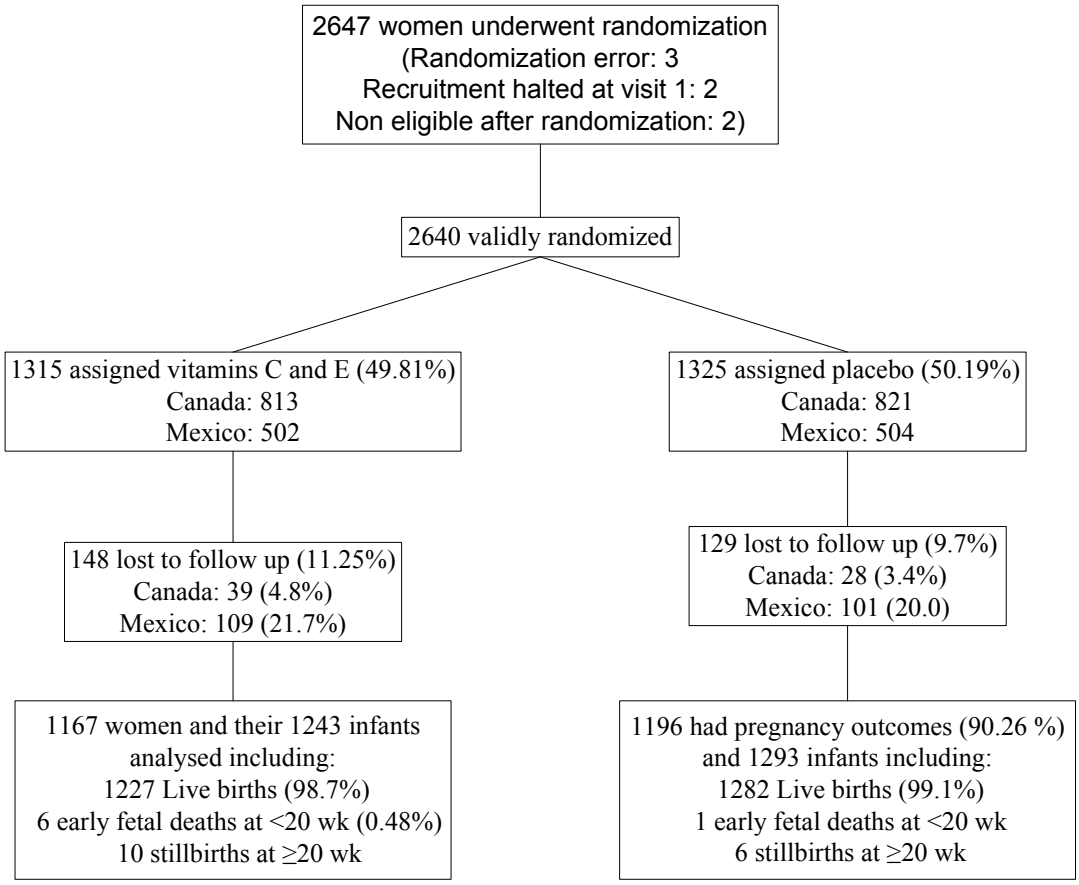

Table 1 Women's baseline demographic and obstetric characteristics by treatment group[a]

Characteristic	Vitamins C and E N=1167	Placebo N=1196
Maternal age, y	28.66 (5.57)	28.68 (5.44)
Maternal education (years)	14.48 (3.47)	14.53 (3.62)
Maternal pre-pregnancy BMI[b]	25.45 (5.69)	25.47 (2.09)
Maternal visit1 BMI[b]	26.69 (5.81)	26.75 (6.21)
Ethnic origin		
Asian	26 (2.23)	12 (1.01)
South Asian	6 (0.51)	4 (0.34)
Caucasian	364 (31.22)	406 (34.00)
French Canadian	285 (24.44)	279 (23.37)
African	10 (0.86)	16 (1.34)
Hispanic	403 (34.56)	416 (34.84)
First Nation	7 (0.60)	5 (0.42)
Other	65 (5.57)	56 (4.69)
Gestational age, wk	15.19 (2.10)	15.28 (2.09)
Gravidity	1.65 (1.02)	1.67 (1.10)
Nulliparous	934 (80.03)	957 (80.02)
Employed	890 (76.33)	911 (76.36)
Smoking before pregnancy	340 (29.16)	330 (27.64)
Current smoker	76 (6.56)	88 (7.43)
Current drinker	14 (1.20)	23 (1.93)
Blood pressure		
Systolic	108.92 (13.13)	109.27 (13.57)
Diastolic	67.50 (9.03)	67.33 (9.07)
Dipstick proteinuria		
Normal or trace	1109 (96.43)	1137 (96.85)
1+	40 (3.48)	33 (2.81)
2+	1 (0.09)	4 (0.34)
High risk group (stratum)	338 (28.96)	346 (28.93)
Chronic hypertension	78 (6.68)	70 (5.85)
Diabetes	82 (7.03)	76 (6.35)
Multiple pregnancy [c]	67 (5.74)	93 (7.78)c
History of preeclampsia	147 (12.60)	148 (12.37)
Multiple risk factors	33 (2.83)	38 (3.18)

Table 1 Women's baseline demographic and obstetric characteristics by treatment group[a] (*continued*)

Characteristic	Vitamins C and E N= 1167	Placebo N= 1196
Use of supplements		
Multivitamins	711 (61.29)	756 (63.26)
Vitamin C	20 (1.72)	10 (0.84)
Vitamin E	2 (0.17)	1 (0.08)
Folate	527 (45.35)	519 (43.54)
Calcium	164 (14.11)	163 (13.67)
Iron	272 (23.41)	271 (22.75)
Family history of PE, eclampsia or GH	156 (13.37)	143 (11.96)
Family history of PE	95 (8.14)	84 (7.02)
Family History of Eclampsia	16 (1.37)	10 (0.84)
Family History of GH	89 (7.63)	82 (6.86)
Obstetrical history		
History of abortion	316 (27.10)	315 (26.36)
History of stillbirth	17 (1.46)	11 (0.92)
History of preterm birth	97 (8.32)	84 (7.03)
History of low birth weight	60 (5.15)	48 (4.02)

BMI, body mass index; *GH*, gestational hypertension; *PE*, preeclampsia
a: Data are presented as mean (S.D.) or n (%)
b: Maternal BMI = weight (kilo)/height^2 (m^2)
c: $p<0.05$

Table 2. Primary outcomes

Characteristic	Vitamins C and E N=1167	Placebo N= 1196	RR(95%CI)	P
GH and its adverse conditions [a]	118 (10.11)	122 (10.20)	0.99 (0.78-1.26)	.94
GH	253 (21.68)	249 (20.82)	1.04 (0.89-1.22)	.61
Preeclampsia	69 (5.95)	68 (5.71)	1.04 (0.75-1.44)	.81
Eclampsia	1 (0.10)	0	--	.50
Diastolic pressure ≥110 mm Hg	32 (2.74)	27 (2.26)	1.21 (0.73-2.01)	.45
Systolic pressure≥160 mm Hg	53 (4.54)	68 (5.69)	0.80 (0.56-1.13)	.21
Hematocrit <24%	3 (0.26)	5 (0.42)	0.61 (0.15-2.57)	.50
Blood transfusion	3 (0.26)	6 (0.50)	0.51 (0.13-2.04)	.33
Thrombocytopenia	7 (0.60)	7 (0.59)	1.02 (0.36-2.91)	.96
Elevated liver enzymes levels (AST or ALT>70 U/L)	9 (0.77)	7 (0.59)	1.32 (0.49-3.53)	.58
IUGR (<3 rd percentile)[b]	18 (1.54)	15 (1.25)	1.23 (0.62-2.43)	.55
Perinatal death[c]	5 (0.43)	1 (0.08)	5.12 (0.60-43.79)	.10

ALT, alanine amniotransferase; *AST*, aspartate amniotransferase; *CI*, confidence interval; *GH*, gestational hypertension; *IUGR*, intrauterine growth restriction; *RR*, relative risk

Data are presented as mean (S.D.) or n (%)

a. GH and ≥ 1 of the following: 1) diastolic pressure ≥ 110 mmHg or systolic pressure ≥ 160 mmHg; 2) proteinuria ≥ 300mg/24 hours urine collection or dipstick≥2+; 3) convulsion (eclampsia); 4) thrombocytopenia (platelet count < 100,000 × 109/L); 5) elevated liver enzyme levels (AST or ALT >70 U/L); 6) hematocrit < 24% and blood transfusion; 7) intrauterine growth restriction <3rd centile; and 8) perinatal death(fetal death after 20 weeks or neonatal death within 7 days) ; b only singleton pregnancy was considered in the primary composite outcome; c Counted as the number of pregnancies (mothers)

Table 3. Primary outcome, gestational hypertension, and preeclampsia stratified by risk at enrolment

Characteristic, n(%)	Vitamins C and E	Placebo	RR (95%CI)
GH and its adverse conditions			
High-risk stratum	68 (20.12)	70 (20.23)	0.99 (0.74-1.34)
Chronic hypertension	28 (35.90)	27 (38.75)	0.93 (0.61-1.41)
Diabetes	11 (13.41)	15 (19.74)	0.68 (0.33-1.39)
Multiple pregnancy	9 (13.43)	13 (13.98)	0.96 (0.44-2.12)
History of PE	33 (22.45)	29 (19.59)	1.15 (0.74-1.79)
Multiple risk factors	11 (33.33)	13 (34.21)	0.97 (0.51-1.87)
Low risk stratum	50 (6.03)	52 (6.12)	0.99 (0.68-1.44)
GH			
High risk stratum	114 (33.73)	119 (34.39)	0.98 (0.80-1.21)
Chronic hypertension	44 (56.41)	39 (55.71)	1.01 (0.76-1.35)
Diabetes	20 (24.39)	23 (30.26)	0.81 (0.48-1.34)
Multiple pregnancy	13 (19.40)	15 (16.13)	1.20 (0.61-2.36)
History of PE	57 (38.78)	57 (38.51)	1.01 (0.76-1.34)
Multiple risk factors	18 (54.55)	14 (36.84)	1.48 (0.88-2.49)
Low risk stratum	139 (16.77)	130 (15.29)	1.10 (0.88-1.37)
PE			
High risk stratum	41 (12.17)	38 (11.05)	1.10 (0.73-1.67)
Chronic hypertension	16 (20.51)	11 (15.71)	1.31 (0.65-2.62)
Diabetes	6 (7.32)	11 (14.47)	0.51 (0.20-1.30)
Multiple pregnancy	4 (6.06)	6 (6.52)	0.93 (0.27-3.16)
History of PE	24 (16.33)	16 (10.88)	1.50 (0.83-2.71)
Multiple risk factors	7 (21.21)	5 (13.16)	1.61 (0.56-4.60)
Low risk stratum	28 (3.40)	30 (3.55)	0.96 (0.58-1.59)

CI, confidence interval; *GH*, gestational hypertension; *PE*, preeclampsia; *RR*, relative risk.

Table 4. Secondary maternal outcomes

Characteristic, n (%)	Vitamins C and E N=1167	Placebo N= 1196	RR(95%CI)
Severe GH[a]	70 (6.0)	78 (6.52)	0.92 (0.67-1.26)
Severe PE[b]	33 (2.83)	39 (3.26)	0.87 (0.55-1.37)
PROM[c]	109 (10.17)	67 (6.15)	1.65 (1.23-2.22)[e]
PPROM[d]	64 (5.97)	33 (3.03)	1.97 (1.31-2.98)[e]
Maternal infection	20 (1.73)	29 (2.46)	0.71 (0.40-1.24)
Delivery method			
Spontaneous delivery	526 (45.11)	551 (46.15)	
Instrumental delivery	120 (10.29)	126 (10.55)	--
Caesarean	520 (44.60)	517 (43.30)	
Antepartum hemorrhage	4 (0.34)	2 (0.17)	2.05 (0.38-11.17)
ICU admission	16 (1.37)	18 (1.51)	0.91 (0.47-1.78)
Predelivery hospitalization	333 (28.63)	341 (28.66)	0.99 (0.88-1.14)

CI, confidence interval; *GH*, gestational hypertension; *PE*, preeclampsia; *PROM*, prelabor rupture of membranes; *PPROM*, preterm prelabor rupture of membranes; *RR*, relative risk.

a. Defined as $\geq$ 2 readings of diastolic blood pressure systolic $\geqslant$ 110 mmHg or systolic blood pressure $\geqslant$ 160 mmHg at least four hours apart;
b. Defined as PE and $\geq$1 of following adverse conditions: 1) diastolic pressure ≥ 110 mmHg or systolic pressure ≥ 160 mmHg; 2) proteinuria $\geqslant$ 5g/ 24 hours urine collection or dipstick $\geqslant$ 3+; 3) convulsion (eclampsia); 4) thrombocytopenia (platelet count < 100,000 × 10^9/L); 5) elevated liver enzyme levels (AST or ALT >70 U/L); 6) hematocrit < 24% or blood transfusion; 7) IUGR (i.e., birth weight<3rd centile for gestational age); 8) perinatal death (fetal death after 20 weeks or neonatal death within 7 days); or 9) preterm delivery less than 34 weeks of gestation.
c. Defined as spontaneous rupture of the membranes at or after 37 weeks of gestation and before onset of the labor
d. Defined as spontaneous rupture of the membranes before 37 weeks of gestation and before onset of the labor
e. P<0.05

Table 5. Secondary Neonatal outcomes

Characteristic, n(%)	Vitamins C and E N= 1243	Placebo N= 1293	RR(95%CI)
Fetal loss or death of infants[a,b]	21 (1.69)	10 (0.78)	2.20 (1.02-4.73)
Spontaneous abortion	6 (0.48)	1 (0.08)	6.25 (0.72-54.52)
Stillbirth	10 (0.80)	6 (0.47)	1.73 (0.63-4.78)
Neonatal death before discharge	5 (0.40)	3 (0.23)	1.73 (0.41-7.25)
IUGR (<3rd percentile)	60 (4.87)	60 (4.67)	1.05 (0.72-1.51)
Preterm birth[c]			
<37 weeks	193 (16.57)	184 (15.48)	1.07 (0.89-1.29)
<34 weeks	67 (5.75)	65 (5.47)	1.05 (0.76-1.47)
Small for gestational age			
<5th percentile	91 (7.38)	102 (7.93)	0.93 (0.69-1.25)
<10th percentile	173 (14.03)	194 (15.09)	0.92 (0.73-1.15)
Convulsion	5 (1.07)	2 (0.42)	2.55 (0.49-13.19)
Respiratory distress requiring oxygen	267 (21.87)	281 (22.02)	0.99 (0.81-1.21)
Assisted ventilation≥24 hours	55 (4.51)	54 (4.25)	1.07 (0.67-1.69)
NICU care >4 d	46 (4.08)	48 (4.09)	1.00 (0.62-1.61)
Congenital anomalies	37 (3.03)	30 (2.35)	1.30 (0.78-2.19)
Sepsis	13 (1.07)	6 (0.47)	2.28 (0.77-6.80)
Intraventricular hemorrhage	11 (0.90)	8 (0.63)	1.44 (0.53-3.94)
Necrotizing enterocolitis[b]	1 (0.08)	9 (0.71)	0.12 (0.01-0.91)
Hypertonia	6	0	-
Hypotonia	7 (0.57)	6 (0.47)	1.22 (0.41-3.63)
Retinopathy of prematurity	4 (0.33)	3 (0.24)	1.39 (0.16-12.46)
Leukomalacia	1 (0.08)	0	-
Neutropenia	6 (0.50)	3 (0.24)	2.09 (0.52-8.38)
Apgar score <4 at 5 min	9 (0.73)	6 (0.47)	1.57 (0.56-4.44)

CI, confidence interval; *IUGR*, intrauterine growth restriction; *NICU*, neonatal intensive care unit; *RR*, relative risk

a. Defined as spontaneous abortion, stillbirth or neonatal death

b. P<0.05

c. Counted as number of pregnancies

CHAPTER 5 ARTICLE II

Maternal nutrient intake and the risk of hypertensive disorders in pregnancy

Maternal nutrient intake and the risk of hypertensive disorders in pregnancy

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Abstract

Objective: To assess effects of perinatal nutrient intakes on the risk of hypertensive disorders (gestational hypertension and preeclampsia) of pregnancy in Canada and Mexico.

Study design: We analyzed nutrient intakes of women enrolled in a randomized trial of antioxidants for the prevention of preeclampsia (PE) conducted in 17 centres in Canada (n=1537) and 10 centres in Mexico (n=799). Validated Food Frequency Questionnaires (FFQs) were administered in the 1st and 3rd trimesters of pregnancy to assess usual dietary intakes over the previous three months.

Results: Of 1537 Canadian and 799 Mexican women included in the final analysis, 498 (21.3%) developed gestational hypertension (GH), and 136 (5.82%) developed preeclampsia (PE). There were significant heterogeneities in various nutrient intakes between Canadian and Mexican women. Therefore, risk models were developed separately for the two populations. After adjusting for pre-pregnancy body mass index, treatment, risk stratum (high versus low) and other baseline risk factors, we found that the lowest quartiles of potassium (adjusted OR 1.79, 95% CI 1.03-3.11) and zinc (adjusted OR 1.90, 95% CI 1.07-3.39) intakes were significantly associated with an increased risk of PE among Canadian women. The lowest quartile of polyunsaturated fatty acids was associated with the risk of GH (adjusted OR 1.49, 95% CI 1.09-2.02). None of

the nutrients analyzed were found to be associated with PE and GH risk among Mexican women.

Conclusion: Lower intakes of potassium and zinc were moderately associated with the risk of PE in Canadian women. There was significant heterogeneity in nutrient intakes between Canada and Mexico.

Key words: Preeclampsia, FFQ, Nutrient Intake

Introduction

Hypertensive disorders of pregnancy including preeclampsia (PE) and gestational hypertension (GH) are associated with significantly increased risks of perinatal morbidity and mortality [1-4] as well as an increased risk for subsequent chronic hypertension or cardiovascular disease for mothers in the long term.[5, 6]

PE is a multisystem disorder that is specific to human pregnancy and its etiology remains largely unknown. Although there is little evidence to support routine prenatal dietary intervention or supplementation, it is generally believed that maternal diet may be important in reducing the risk of adverse pregnancy outcomes. Certain nutrients, such as vitamins C and E, calcium and omega-3 fatty acids, have been proposed to decrease the risk of PE.[7-15] The International Trial of Antioxidant supplementation in the Prevention of Preeclampsia (INTAPP),[16] and other similar studies[14, 15] failed to provide evidence of an effect of prenatal vitamins C and E supplementation on the incidence and severity of GH or PE. However, the INTAPP cohort offered a

unique opportunity to assess the impact of nutrient intakes on well-defined hypertensive disorders of pregnancy in a prospective cohort (early and late pregnancy) as well as in two different ecological settings (Canada and Mexico). The aim of the study was to investigate maternal nutrient intakes in early and late pregnancy in relation to the risk of hypertensive disorders of pregnancy in Canada and Mexico.

Methods

We analyzed nutrient intake data from a prospective pregnancy cohort of women enrolled in the INTAPP study - a randomized controlled trial that investigated the effects of vitamins C and E supplementation in the prevention of PE.[16] The trial was conducted in Canada (17 centers) and Mexico (10 centers) between January 2004 and March 2006. The design of the trial has been described in detail elsewhere.[16] Briefly, women at 12-18 completed weeks of gestation were randomly assigned either to the antioxidant treatment (1g vitamin C and 400 IU vitamin E daily) or the placebo group. Randomization was stratified by center and by risk status according to pre-specified clinical risk criteria. Women were at high risk if they were nulliparous or multiparous with pre-pregnancy chronic hypertension (diastolic blood pressure $\geq$ 90 mmHg before 20 gestational weeks or use of antihypertensive medication for hypertension), pre-pregnancy diabetes (insulin-dependent or hypoglycemic agents), multiple pregnancy, or a history of PE in the previous pregnancy. Women were stratified into the low risk stratum if they were nulliparous without any identified clinical risk factors. Women and their babies received care according to standard practice

in each center, with surveillance for hypertension using standardized measurements of blood pressure.

Assessment of nutrient intake

Nutrient intake among women in the INTAPP trial was assessed by information gathered through self-administered food frequency questionnaires (FFQs) that were country-specific and that were administered at trial entry (12-18 weeks of gestation) and repeated at 32-34 weeks of gestation to ensure capture of pre-pregnancy diet as well as changes during pregnancy. The data from the FFQ furnished estimates of absolute nutrient values and food group consumption, and permitted ranking of individual intakes. Similar approaches were used in both Mexico and Canada to ensure accuracy in data collection, data entry, and FFQ quality assessment and to ensure comparability between the Canadian and Mexican dietary data.

In the Canadian sites, a semi-quantitative 78-item FFQ developed by Shatenstein et al. and validated in several adult populations in both French and English,[17] was modified for the INTAPP trial to include all food sources of vitamins C and E, and to reflect usual intakes over the previous 3-months rather than the standard 12-month time period. It was validated for use in a subset of 107 pregnant women from the Canadian INTAPP cohort.[18] Participants' estimation of consumption frequency and portions was aided by detailed instructions for completion and food-specific photos of sample portion sizes.

In the Mexican sites, the Canadian FFQ was modified to reflect current local foods and diets using information from the second Mexican National Nutrition

Survey published data [19] and a survey conducted in 4 clinics and 2 gynecological hospitals from the Mexican Social Security Institute. The FFQ was developed and tested in Spanish. It was validated against three non-consecutive 24-hour food recalls administered to 85 pregnant women. [20]

Standard procedures for completing the self-administered FFQ and information on potential problems and strategies to resolve them were provided to research nurses. An experienced research nutritionist was responsible for training research nurses working at each site. After completion, the FFQ was signed and dated by the participant to confirm the accuracy of the recorded information. In the Canadian arm of the study, the FFQs were entered using Microsoft Access software for customized data entry, and analysis was based on the algorithms developed to compute energy and nutrient values from the instrument food list, frequency option and portion size. Data-entry took approximately 10 minutes per FFQ, with double entry done systematically to verify accuracy. Nutrient values were calculated from the reference food nutrient composition values (Canadian Nutrient File-CNF2001b, Health & Welfare Canada, 1982) incorporated into the FFQ data entry utility. In the Mexican arm, nutrient values were calculated based on the United States Department of Agriculture (USDA) food composition tables using a previously validated and patented computerized system.[21-23] Preliminary analyses were conducted by the nutrition teams in both countries to detect outliers, and the quality of FFQ data was assessed by the trained nutritionists using a score, ranging from ‘1’ indicating ‘good quality’ to ‘4’ indicating ‘ poor quality’.

To collect information on perinatal vitamin or mineral supplements, women were asked the following question at trial entry (gestational age of 12-18 weeks) and at 32-34 weeks of gestation: “In the past 3 months, have you taken any multivitamins or prenatal vitamins regularly?”. Women were classified as users or non- users of vitamin or mineral supplements. The majority of patients reported daily supplement use and the composition of the most commonly used multivitamin supplements was reported to be similar. However, we had no information on product name or formulation in most cases.

Study outcomes

The main study outcomes were GH and PE. GH was defined as at least two readings of diastolic blood pressure ≥90 mmHg taken 4 hours apart but within 72 hours occurring ≥ 20 weeks of gestation.[24, 25] PE was defined as GH with proteinuria.[24, 25] Proteinuria was defined as the urinary excretion of ≥0.3g/24 hours, or ⩾2+ on diagnostic strips. For women with pre-existing hypertension, PE was classified as new or worsening proteinuria as defined above. For women with pre-existing proteinuria (e.g. diabetes with renal involvement), the diagnosis of PE was based on clinical or biochemical grounds by identifying at least one additional adverse condition (e.g. hypertension, abnormal liver enzymes, low platelets and eclampsia).[24, 25] All cases of GH and PE were further adjudicated by two independent investigators in the Trial Coordinating Centre. Failures to achieve consensus were resolved by a third independent investigator.

Statistical analysis

Data on socio-demographic and clinical characteristics were obtained from the INTAPP trial. Nutrient intakes were categorized by quartiles. Exploratory analyses were conducted to assess the distribution of all continuous variables. Means and standard deviations (for continuous variables) and frequencies and proportions (for discrete variables) were used to describe study variables. Analyses of variance and nonparametric rank tests (Wilcoxon test if the distribution was skewed) were used to assess differences in continuous variables. Chi-square tests were used to compare the differences in rates between groups. The data from the two trial arms (treatment and placebo) were pooled as there was no difference in the rates of hypertensive disorders of pregnancy.[16] Univariate logistic regression analyses were conducted to test for the effect of a single maternal characteristic or nutritional factor on the outcomes (PE, GH). Nutrients included in the models were adjusted for the potential confounding effect of total energy intake.[26] Tests for trend across ordered categories for nutrient intakes were conducted by modeling variables of nutrient intakes as continuous variables. Dichotomous variables were used for each nutrient intake (exposed -lowest intake quartile vs. non-exposed - other quartiles) in final logistic regression models since there were no significant risk gradients among the three higher quartiles in most nutrients. The statistical significance was assessed by the likelihood ratio test statistic. The odds ratios (OR) and 95% confidence intervals (95%CI) were obtained from logistic regression models to quantify the associations. Socio-demographic and clinical variables (at trial entry)

and nutritional variables found to be significantly associated with PE at the P<0.15 level in the univariate analysis were considered as candidates for inclusion in a parsimonious multivariate model identified using forward selection as well as stepwise selection procedures. Terms representing the treatment assignment and total energy intake as well as the quality score of FFQ data were forced into the model at every step. Analyses were repeated for the following models: 1) baseline diet (nutrient intakes estimated from FFQ collected at visit 1) only , in which PE or GH risks were related to baseline diet only (analysis based on women with plausible FFQ at trial entry, n=2336) ; 2) diet in late pregnancy only (nutrient intakes estimated from FFQ collected at 32-34 weeks of gestation), in which PE or GH risks were related to nutrient intakes in late pregnancy only (analysis based on women with plausible FFQ in the third trimester, n=1887) ; and 3) diet intakes both in early and late pregnancy models, in which PE or GH risks were related to both baseline nutrient intakes and changes of nutrient intake from early to late pregnancy (standardized as Z-scores) (n=1887). Analyses were performed using SAS version 9.2 and significance was set at two tailed $p<0.05$.

Results

Of the 2640 women randomized, 277 women were lost to follow up and 4 women terminated their pregnancies at less than 20 weeks of gestation, and were excluded from the analysis. Among the remaining 2352 patients, a total of 2336 patients - including 1537 from Canada and 799 from Mexico - with complete and plausible FFQ data at the first study visit were included in the final analysis.

Among these, GH occurred in 284 (18.5%) women from Canada and 214 (26.8%) women from Mexico, PE occurred in 68 women (4.4%) in Canada and 68 (8.5%) women in Mexico.

There was significant heterogeneity in most nutrient intakes between Canadian and Mexican women (Table 1). The proportion of regular users of mineral or vitamin supplements (i.e. multivitamin, folate, iron, calcium, etc) was significantly higher in Canadian women than in Mexican women. Regarding any specific supplement, the proportions of regular users of multivitamin and calcium supplements were significantly higher in Canadian women than Mexican women. However, the frequencies of regular users of folate and iron supplements were significantly higher in Mexican women. Therefore, all analyses were conducted separately for the two study populations.

Table 2 shows the socio-demographic and clinical characteristics (at trial entry) of the whole cohort, women with GH and PE, and women with normal blood pressure in the two cohorts. Women with GH or PE were more likely to be nulliparous or with higher pre-pregnancy body mass index (BMI), higher mean baseline diastolic and systolic blood pressure. There were no differences in ethnicity, marital status, employment status, socioeconomic status and lifestyle factors (drinking and smoking) between hypertensive and normotensive patients. Similar distributions of baseline characteristics between hypertensive and normotensive patients were observed in Canada and Mexico.

As expected, the proportions of hypertensive disorders were comparable between antioxidant and placebo groups (Table 3). The proportion of the

presence of risk factors (i.e. high risk stratum) was significantly higher in women with GH and PE compared to overall cohort and normotensive women. The proportions of regular users of mineral or vitamin supplements (i.e. multivitamin, folate, iron, calcium, etc) were similar between hypertensive and normotensive patients. Among Canadian women, the proportion of regular baseline users of multivitamin, vitamins C and E was slightly lower in women with PE and GH.

In Canadian women, univariate analysis showed that quartile distributions of dietary intakes of fiber, maganesium, potassium, sodium, zinc, vitamins A, C, E, thiamine, and folate were associated with PE at pre-defined $p<0.15$ level. There were significant trends toward an increased risk of PE with decreasing quartiles of fiber, zinc, maganesium, potassium, vitamins A, C, E, thiamine, and folate ($p<0.05$). The quartile distributions of dietary intakes of protein, pantothenic acid, polyunsaturated fatty acid were associated with GH at $p<0.15$ level among Canadian women. In the Mexican pregnant cohort, univariate analysis indicated that no quartiles of any nutrient intake were associated with GH or PE at the level of $p<0.15$. (Appendix: Table I- The risk of GH or PE according to quartile distributions of nutrient intakes estimated from FFQ administered at 12-18 weeks of gestational age; Table J: Unadjusted Odds ratios of dietary nutrients intake in association with preeclampsia (PE) and gestational hypertension (GH) in Canadian and Mexican pregnancy cohorts)

As there were no significant risk gradients among the three higher quartiles in most nutrients, logistic regression models were therefore used to assess dichotomous exposure variables (lowest quartile versus the three higher quartile

groups) of nutrient intakes in association with the risks of PE and GH. For Canadian women, univariate analysis found that compared to the higher three quartiles, the lowest quartile of intake of magnesium, potassium, vitamin A, vitamin C, and folate were significantly associated with PE ($p<0.05$). The lowest quartiles of intakes of calcium, zinc, vitamin E, and vitamin B6 were found to be associated with PE at the preselected $p<0.15$ level. The lowest quartiles of protein and polyunsaturated fatty acid were found to be associated with GH at $p<0.05$ level. For Mexican women, there was no association between the lowest quartile of any nutrient intake and the risk of PE at $p<0.05$ level. The lowest quartiles of carbohydrate, calcium and magnesium were associated with PE at $p<0.15$ level. Only the lowest quartile of vitamin B12 was found to be related to the risk of GH among Mexican women (OR 0.58, 95%CI 0.39-0.86). (Table 4)

A total of 1217 women in Canada and 670 women in Mexico had plausible FFQ data that were collected at the third trimester to reflect nutrient intake during pregnancy. (Table 5) Quartile distributions of lipoprotein, monounsaturated fatty acids, and vitamin A were significantly associated with PE ($p<0.05$) among Canadian women. (Appendix: Table K) Only protein quartiles were found to be significantly associated with risk of GH in Canadian women. In the Mexican pregnant cohort, quartile distributions of vitamin B6, pantothenic acid, monounsaturated fatty acids, and thiamine were associated with PE. Also, only thiamine quartiles were associated with GH. (Appendix: Table K- The risk of GH or PE according to quartile distributions of nutrient intakes estimated from FFQ administered at 32-34 weeks of gestational age) We

further calculated changes of nutrients intake from early to late pregnancy, which were standardized as Z-scores. However, we found no associations between changes of intakes of any specific nutrient and the risk of PE and GH. Thus these variables were not included in the final models. (Appendix: Table L- Unadjusted Odds ratios of changes in nutrient intakes (standardized as Z score) in association with preeclampsia (PE) and gestational hypertension (GH) in Canadian and Mexican cohorts)

Multivariate logistic regression models for the outcomes of PE and overall GH were constructed from baseline risk factors and dichotomous variables of nutrient intakes, for the variables found to be significant at $p<0.15$ in the univariate analysis. After adjusting for pre-pregnancy BMI, the presence of clinical risk factors (i.e. chronic hypertension, diabetes, history of PE, multiple pregnancy), family history of PE or GH, nulliparity, quality score of FFQ, and treatment group (antioxidants versus placebo), the lowest quartiles of potassium (adjusted OR 1.79, 95%CI 1.03-3.11) and zinc (adjusted OR 1.90, 95%CI 1.07-3.39) intakes were significantly associated with an increased risk of PE among Canadian women. Furthermore, the lowest quartile of polyunsaturated fatty acids was associated with an increased risk of GH (adjusted OR 1.49, 95%CI 1.09-2.02). Among Mexican women, we found no associations between any nutrient intake and the risk of PE and GH using multivariate regression models.

Discussion

The present prospective cohort study assessed relationships between perinatal nutrient intakes and the risk of hypertensive disorders in pregnancy. We

observed an increased risk of PE associated with low intake of potassium, zinc, and polyunsaturated fatty acids among Canadian women. However, we found no associations between any nutrient intake and the risk of PE and GH among Mexican women.

Potassium is an essential dietary mineral and electrolyte. The richest sources of potassium are fruits and vegetables (e.g. potato, tomato, carrot, prune, etc.) as well as protein foods (e.g. beans and tuna).[27] Potassium intake has been reported to be inversely associated with blood pressure or risk of hypertension among non pregnant populations in observational studies, and clinical trials have tended to find that potassium had the strongest hypotensive effects.[28, 29] However, the role of potassium intake on pregnancy outcomes including PE remains is not well established. A prospective cohort study found that low plasma potassium level during the first half pregnancy is associated with the reduced risk for PE. The authors suggested that low potassium level may be an indicator for appropriate high insulin concentration, increased glomerular filtration ratio and systematic vasodilatation.[30] In the present study, we found that an inverse association between potassium intake and the risk of PE. The result is consistent with the previous published case control study of 172 preeclamptic women and 339 normotensive controls.[31] The authors found that, compared to the lowest quartile (< 2.4 g/d), the top quartile of potassium intake (> 4.1 g/d) was associated with the reduced risk for PE (adjusted OR 0.49, 95% CI 0.24-0.99).[31]

Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement. There are a variety food that contain zinc, including oysters, red meat, poultry, beans, nuts, certain types of seafood (such as crab and lobster), whole grains, fortified breakfast cereals, and dairy products.[27] We found the low intake of zinc was significantly associated with PE (adjusted OR 1.90, 95%CI 1.07-3.39). It has been suggested that alterations in zinc homeostasis might have a devastating effect on pregnancy outcome.[32] Several clinical studies have reported an inverse relationship between serum zinc concentrations and the risk of PE.[33, 34]

We noted significant differences in nutrient intakes between Canadian and Mexican pregnant women. These could be explained by population differences in dietary habits between the two countries or among pregnant women in Canada and Mexico. For instance, Mexico is a country with a rich variety of locally-grown fruits and vegetables. Compared to Canadian women, Mexican pregnant women eat more yellow fruits (e.g. papaya, melon, mango, etc.), which provide excellent sources of vitamin A and other vitamins and may explain significant higher levels of vitamin A among Mexican pregnant women. The FFQs used in the Canadian and Mexican cohorts may differ in their ability to accurately capture nutrient intakes. It is very likely that a differential reporting bias exists between two countries. For example, women in Mexico may tend to report higher milk intake as they are counseled to drink a lot of milk during pregnancy.

Although the underlying mechanisms of PE remain largely unknown, it has been generally hypothesized as a 'two stage' disease described as pre-clinical

(poor placentation) and clinical features. The disease process may have occurred in a very early stage, even before placentation. Therefore, assessment of nutrient intakes at an early stage may be the optimal approach for unravelling the actual causal associations between nutrient intakes and the risk of hypertensive disorders of pregnancy.

Dietary assessment in pregnancy is challenging as the diet of pregnant women is likely to be highly variable compared to usual pre-pregnant patterns, and may fluctuate according to their state of comfort and well-being along with changes in food preferences during the course of pregnancy. In the present study, the FFQ was administered twice, once in the first trimester and again in the third to capture nutrient intakes at early pregnancy as well as changes from early to late pregnancy. The FFQ is designed to assess long term usual intakes rather than intakes on a few specific days, and is thus better able to capture day to day variability in food intakes than quantitative methods such as food records or 24 hour diet recalls. It also permits ranking of respondents by their usual intakes. Although the FFQ in the present study was pre-tested and validated in several adult populations in both French and English,[17] and the results of our validation studies indicated that the FFQ is a relatively valid instrument for determining usual diet in pregnant women,[18] it is also possible that some nondifferential misclassification of nutrient intakes from the FFQ may have occurred and therefore have underestimated the true effects. However, nutrient intakes were grouped into quartiles, and results are therefore less likely to be affected by errors in intake estimates.

It is notable that, both in Canada and Mexico, the majority of participating women took vitamin or mineral supplements regularly early in gestation or even before pregnancy. In Canada, it is recommended that vitamin supplementation begin early in gestation, optimally before conception. As shown in the present study, approximately 80% of women from the Canadian cohort were regular users of multivitamin supplements, while the proportions of using folate and calcium supplements were 30% and 16% respectively. In the Mexican cohort, approximately 70% of women took folate supplements and 60% women took iron supplements. It is possible that we did not detect an association between dietary micronutrient intakes and GH or PE since most women had generally adequate micronutrient intakes through supplements.

We also obtained detailed information on a number of other maternal factors that have been shown to be important risk factors for GH and PE (i.e. pre-pregnancy BMI). After adjustment for these potential factors, only lower intakes of potassium and zinc were found to be associated with PE risk and lower intake of polyunsaturated fatty acid was associated with GH risk among Canadian women. We found no association between nutrient intakes and the risk of GH and PE among Mexican women. It should be noted that the present study outcomes were also the primary outcomes for the INTAPP trial, in which rigorous research criteria for definition of GH and PE were applied based on the published Canadian consensus statement,[24] and the cases of GH and PE were further adjudicated independently by a team of clinicians specialized in the area.

Given the fact that there were significant differences in dietary habits between Canada and Mexico and that the ecological settings of the two countries were significantly different, the data were not pooled together and parallel analyses were performed. Thus we might have had insufficient power to detect moderate associations. The powers for the current sample size (n=1537) for an odds ratio of 1.79 for potassium and 1.90 for zinc in association with PE risk among Canadian women were approximately 70% and 76% respectively to allow a two sided alpha error of 5%. On the other hand, parallel analyses conducted in two countries provided a unique opportunity to assess and compare the effects of nutrient intakes on the risks of hypertensive disorders in two ecologically different settings. It is worth pointing out that the study populations in the present study were patients enrolled from a clinical trial with specific inclusion and exclusion criteria. Therefore one should be prudent in generalizing the findings to other populations.

It has been suggested that diet plays a role in the risk of PE. Much of the clinical and basic research into nutritional causes of hypertensive disorders of pregnancy has paralleled research conducted on hypertension focused on nutrients such as calcium, sodium, magnesium, and fatty acids. The results derived from previous studies were inconsistent, which may be partially explained by the methods used to estimate dietary intake, the time in pregnancy at which diet is assessed, inconsistent definition of PE and GH, or population differences (i.e. lifestyle, heterogeneity in nutrient intake, socio-demographic factors).[35-37] Our recent review of published dietary intervention trials found

no evidence that increasing or restricting energy or protein intake, sodium restriction, or supplementation of magnesium, zinc, iron, vitamins C and E, or fish oil reduces the risk of PE or GH.[37]

In summary, we found that, among Canadian women, lower intakes of potassium and zinc were moderately associated with the risk of PE. Among Mexican women, we found no nutrient intakes during pregnancy in relation to the risk of GH and PE. There was significant heterogeneity in nutrient intakes between Canada and Mexico.

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Table1. Maternal Dietary intake from Food Frequency Questionnaire (FFQ) administered at trial entry (12-18 weeks of gestational age) and in the third trimester (32-34 weeks of gestational age) in Canada and Mexico

Dietary Intake	Canada			Mexico		
	FFQ1[1,3]	FFQ2[1, 4]	Change[5,6]	FFQ1[1,3]	FFQ2[1, 4]	Change[5,6]
Energy (Kcal)[2]	1962.6±811.0	1962.5±733.3	7.6(-0.04)	2667.1±1065.5	2496.5±923.3	-79.6(0.08)
Protein (g)[2]	88.5± 38.6	89.4±33.4	0.8(-0.02)	78.6±32.4	76.9±31.3	-0.35(0.04)
Total carbohydrate (g)[2]	246.5±110.9	244.2±106.5	0(-0.02)	374.0±164.3	350.1±146.8	-15.2(0.05)
Total lipid (g)[2]	73.4±32.2	74.1±29.1	2.6(0.02)	102.4±46.5	94.2±38.9	-5.7(0.05)
Total fiber (g)[2]	16.2±7.4	15.7±7.0	-0.2(0.01)	27.2±13.2	25.5±12.7	-1.8(0.01)
Total cholesterol (mg)[2]	261.5±136.4	267.5±123.4	7.2(-0.01)	242.2±138.4	244.4±131.5	3.5(0.02)
Calcium (mg)[2]	1111.6±553.1	1187.0±538.0	61.5(-0.04)	1309.9±748.7	1350.7±776.5	33.3(-0.01)
Iron (mg)[2]	11.12±4.79	10.7±4.2	-0.2(0.02)	18.92±12.77	17.1±10.7	-1.1(0.06)
Magnesium (mg)[2]	289.9±119.4	295.6±114.0	2.1(-0.05)	381.3±169.5	366.7±161.9	-3.6(0.07)
Potassium (mg)[2]	3135.5±1328.1	3169.7±1254.1	65.4(0.01)	3425.3± 1421.6	3371.3±1399	17.1(0.04)
Sodium (mg)[2]	3011.3±1618.4	2848.0±1224.7	-77.4(0.03)	2212.3±1032.4	2115.0±945.9	-31.2(0.06)
Zinc (mg)[2]	10.7±4.7	10.8±4.2	0.2(0.004)	10.3±4.3	9.9±4.1	-0.2(0.04)
Vitamin A (mcg)[2]	2582.5±1707	8525.2±5597.6	-4.8(0.01)	6563.1±4309.6	6546.9±3728	187.3(0.04)
Vitamin C (mg)[2]	192.6±121.7	184.2±121.1	-4.6(0.02)	261.4±152.1	255.4±162.8	-4.0(0.01)
Vitamin E (mg)[2]	2.1±1.6	2.1±1.5	-0.03(0.03)	17.1±10.2	15.3±8.5	-1.0(0.09)
Vitamin D (mcg)[2]	4.9±3.6	5.6±3.6	0.4(-0.09)	36.9±62.6	40.8±75.3	0.2(-0.03)

Table 1 (*Continued*)

Dietary Intake	Canada			Mexico		
	FFQ1[1,3]	FFQ2[1, 4]	Change[5,6]	FFQ1[1,3]	FFQ2[1, 4]	Change[5,6]
Vitamin B6 (mg)[2]	2.1±1.0	2.2±1.0	0.1(-0.02)	2.5±1.4	2.4±1.3	-0.005(0.06)
Vitamin B12 (mcg)[2]	4.4±2.5	4.7±2.5	0.2(-0.03)	5.8±4.8	6.2±4.7	0.37(-0.004)
Folate (mcg)[2]	342.0±158.0	328.9±150.1	-9.5(0.004)	582.3±312.6	549.7±285.0	-18.8(0.06)
Thiamine (mg)[2]	1.5±0.6	1.5±0.6	0(0.001)	1.7±1.0	1.6±0.9	-0.03(0.06)
Riboflavin (mg)[2]	1.9±0.8	2.1±0.8	0.08(-0.005)	2.2±1.32	2.2±1.2	0.01(0.01)
Niacin (mg)[2]	18.2±8.0	17.9±7.0	-0.07(0.03)	22.7±12.8	21.1±11.1	-0.9(0.06)
Pantotenic acid (mg)[2]	5.2±2.2	5.3±2.1	0.16(-0.01)	5.5±2.4	5.5±2.3	0.1(0.02)
Saturated fatty acid (g)[2]	24.9±12.0	25.8±11.0	1.07(-0.03)	31.8±18.1	32.1±18.5	0.08(-0.03)
Monounsaturated fatty acid (g)	28.7±12.6	29.0±11.8	0.72(0.004)	28.3±14.6	25.7±11.8	-2.2(0.02)
Polyunsaturated fatty acid (g)[2]	13.4±6.9	12.9±6.2	-0.17(0.02)	28.3±18.0	24.6±14.8	-2.2(0.10)

1. Data presented as Mean±Standard Deviation; 2. $p<0.05$; 3. FFQ estimated at 12-18 weeks of gestational age; 4. FFQ estimated at 32-34 weeks of gestational age; 5. Change=FFQ2-FFQ1; 6. Data presented as median in change from FFQ1 to FFQ3 (median in standardized Z score of change from FFQ1 to FFQ3)

Table 2. Socio-demographic and clinical characteristics of total cohort, women with hypertensive disorders, and women with normal blood pressure in Canada and Mexico[1]

	Canada				Mexico			
Characteristic	**Total N=1537**	**Normal BP N=1253**	**GH N=284**	**PE N=68**	**Total N=799**	**Normal BP N=585**	**GH N=214**	**PE N=68**
Maternal age (yrs)	30.0 (5.2)	29 (5.1)	30.0 (5.2)	31.0 (5.2)	26.0 (5.2)	25.6 (5.0)	27.2 (5.9)[2]	27.1 (4.8)
Maternal education (yrs)	15.9 (3.0)	16.0 (3.1)	15.6 (2.7)	15.3 (2.6)	11.8 (2.8)	11.9 (2.8)	11.5 (3.0)	11.6 (2.7)
Maternal pre-pregnancy BMI	25.5 (6.3)	24.8 (5.8)	28.9 (7.4)[2]	28.8 (7.3)[2]	25.3 (4.8)	24.5 (4.1)	28.2 (5.6)[2]	27.5 (6.0)[2]
Ethnic origin								
Caucasian	1315 (85.7)	1071 (85.6)	244 (85.9)	57 (83.8)	0	0	0	0
Hispanic	20 (1.3)	15 (1.2)	5 (1.8)	2 (2.9)	796 (99.6)	583 (99.7)	213 (99.5)	68
Other	200 (13.0)	165 (13.2)	35 (12.3)	9 (13.2)	3 (0.4)	2 (0.3)	1 (0.5)	0
Marital status								
Married/common law	1417 (93.0)	1154 (93.0)	263 (93.3)	61 (89.7)	618 (77.4)	442 (75.6)	176 (82.6)	57 (83.8)
S*ingle*	106 (7.0)	87 (7.0)	19 (6.7)	7 (10.3)	181 (22.6)	143 (24.4)	37 (17.4)[2]	11 (16.2)
Employed	1270 (82.7)	1029 (82.2)	241 (84.9)	56 (82.4)	511 (64.0)	383 (65.5)	128 (60.1)	44 (64.7)

Table 2 ***(continued)***

Characteristic	Canada				Mexico			
	Total N=1537	**Normal BP N=1253**	**GH N=284**	**PE N=68**	**Total N=799**	**Normal BP N=585**	**GH N=214**	**PE N=68**
Annual household income								
<20000	62 (4.6)	53 (4.8)	9 (3.6)	4 (6.2)	195 (26.9)	141 (26.7)	54 (27.6)	20 (31.3)
20-34999	123 (9.1)	100 (9.2)	23 (9.1)	8 (12.3)	317 (43.8)	235 (44.5)	82 (41.8)	23 (35.9)
35-49999	167 (12.4)	129 (11.8)	38 (15.1)	12 (18.5)	121 (16.7)	89 (16.9)	32 (16.3)	9 (14.1)
50-74999	335 (24.9)	271 (24.8)	64 (25.4)	16 (24.6)	46 (6.4)	31 (5.9)	15 (7.7)	6 (9.4)
>75000	658 (49.0)	540 (49.4)	118 (46.8)	25 (38.5)	45(6.2)	32 (6.1)	13 (6.6)	6 (9.4)
Smoking before pregnancy	367 (24.0)	303 (24.2)	64 (22.5)	13 (19.1)	297 (37.2)	222 (38.0)	75 (35.2)	21 (30.9)
Current smoker	149 (9.8)	126 (10.1)	23 (8.2)	7 (10.3)	13 (1.6)	8 (1.4)	5 (2.4)	2 (3.0)
Current drinker	32 (2.1)	28 (2.2)	4 (1.4)	1 (1.5)	4 (0.5)	2 (0.3)	2 (0.9)	2 (2.9)
Gestational age (wks)	15.1 (2.1)	15.2 (2.1)	15.0 (2.1)	15.1 (2.0)	15.4 (2.1)	15.3 (2.1)	15.7 (2.1)	15.5 (2.0)
Nulliparous	1227 (79.8)	1036 (82.7)	191 (67.3)[2]	36 (52.9)[2]	644 (80.6)	500 (85.2)	146 (68.2)	45 (66.2)
Baseline systolic BP	113.1 (12.8)	111.0 (11.9)	122.2 (12.0)[2]	123.3 (13.4)[2]	101.3 (10.6)	99.4 (9.9)	106.4 (10.7)[2]	107.1 (11.6)[2]
Baseline diastolic BP	68.5 (9.2)	67.0 (8.5)	75.1 (9.3)[2]	75.0 (8.7)[2]	65.2 (8.4)	63.9 (8.1)	69.0 (7.8)[2]	68.9 (8.8)[2]
Family history of PE or GH	172 (11.2)	115 (9.2)	57 (20.1)[2]	14 (20.6)[2]	122 (15.3)	88 (15.0)	34 (15.9)	10 (14.7)

1. Data presented as mean (SD) or N(%)
2. $P<0.05$

Table 3. Treatment allocation, risk status at trial entry, and vitamins or mineral supplementation of total cohort, women with hypertensive disorders, and women with normal blood pressure [1]

	Canada				Mexico			
Characteristic	**Total N=1537**	**Normal BP N=1253**	**GH N=284**	**PE N=68**	**Total N=799**	**Normal BP N=585**	**GH N=214**	**PE N=68**
Treatment								
Antioxidant group	762 (49.6)	616 (49.2)	146 (51.4)	33 (48.5)	394 (49.3)	288 (49.2)	106 (49.5)	35 (51.5)
Placebo group	775 (50.4)	637(50.8)	138 (48.6)	35 (51.5)	405 (50.7)	297 (50.8)	108 (50.5)	33 (48.5)
High risk group (stratum)	489 (31.8)	346 (27.6)	143 (50.4)[2]	47 (69.1)[2]	185 (23.2)	97 (16.6)	88 (41.2)[2]	31 (45.6)[2]
Chronic hypertension	97 (6.3)	44 (3.5)	53 (18.7)[2]	15 (22.1)[2]	47 (5.9)	18 (3.1)	29 (13.6)[2]	11 (16.2)[2]
Diabetes	132 (8.6)	100 (8.0)	32 (11.3)	13 (19.1)[2]	26 (3.3)	15 (2.6)	11 (5.1)	4 (5.9)
Multiple pregnancy	144 (9.4)	122 (9.7)	22 (7.8)	8 (11.8)	13 (1.6)	7 (1.2)	6 (2.8)	2 (3.0)
History of PE	181 (11.8)	114 (9.1)	67 (24.0)[2]	24 (35.3)[2]	110 (13.8)	64 (10.9)	46 (21.5)[2]	16 (23.5)[2]
Multiple risk factors	59 (3.8)	31 (2.5)	28 (9.9)[2]	10 (14.7)[2]	11 (1.4)	7 (1.2)	4 (1.9)	2 (2.9)

1. Data presented as mean (SD) or N (%);
` 2. P<0.05

Table 3 (continued) [1]

Characteristic	Canada				Mexico			
	Total N=1537	Normal BP N=1253	GH N=284	PE N=68	Total N=799	Normal BP N=585	GH N=214	PE N=68
Use of vitamin or mineral supplements	1458 (95.1)	1188 (95.0)	270 (95.4)	65 (95.6)	708 (88.6)	519 (88.7)	189 (88.3)	60 (88.2)
Multivitamin	1280 (83.7)	1052 (84.4)	228 (80.6)	52 (76.5)	166 (20.8)	128 (21.9)	38 (17.8)	13 (19.1)
Vitamin C	22 (1.4)	22 (1.8)	0[2]	0[2]	7 (0.9)	4 (0.7)	3 (1.4)	2 (2.9)
Vitamin E	2 (0.1)	2 (0.2)	0[2]	0[2]	1 (0.1)	1 (0.2)	0	0
Folate	468 (30.6)	370 (29.7)	98 (34.6)	29 (42.7)[2]	574 (71.8)	420 (71.8)	154 (72.0)	49 (72.1)
Calcium	249 (16.3)	185 (14.8)	64 (22.6)[2]	14 (20.6)	77 (9.6)	54 (9.2)	23 (10.8)	9 (13.2)
Iron	57 (3.7)	47 (3.8)	9 (3.2)	2 (2.9)	484 (60.7)	343 (58.6)	141 (66.2)	42 (61.8)
Vitamin A	1 (0.1)	1	0	0	1 (0.1)	1 (0.2)	0	0
Other supplement	90 (5.9)	74 (6.0)	16 (5.7)	4 (5.9)	50 (6.3)	35 (6.0)	15 (7.0)	6 (8.8)

1. Data presented as mean (SD) or N (%);
2. $P<0.05$

Table 4. Unadjusted Odds ratios of dietary nutrients intake (lowest quartile vs other quartiles, 12-18 weeks of gestational age) in association with preeclampsia (PE) and gestational hypertension (GH) in Canadian and Mexican pregnancy cohorts

	Canada		Mexico	
Characteristic	**PE**	**GH**	**PE**	**GH**
Protein	0.78(0.43-1.43)	0.73(0.53-1.00)[1]	1.24(0.72-2.14)	0.94 (0.65-1.36)
Lipoprotein	0.76(0.42-1.38)	1.12(0.84-1.50)	1.29(0.75-2.22)	0.91 (0.64-1.31)
Carbohydrate	1.01(0.58-1.77)	1.08(0.81-1.45)	0.57(0.29-1.12)[2]	0.93 (0.65-1.34)
Fiber	1.44(0.86-1.77)	0.96(0.71-1.30)	1.44(0.84-2.48)	1.08 (0.75-1.56)
Total cholesterol	0.91(0.51-1.61)	0.86(0.63-1.16)	1.21(0.70-2.10)	1.02 (0.71-1.46)
Saturated fatty acids	0.93(0.52-1.64)	0.88(0.65-1.19)	1.18(0.68-2.04)	0.92 (0.64-1.32)
Pantothenic acid	1.45(0.86-2.44)	0.99(0.73-1.33)	1.09(0.62-1.89)	1.05 (0.73-1.50)
Monounsaturated fatty acid	0.99(0.56-1.73)	0.98(0.73-1.33)	1.42(0.83-2.43)	0.80 (0.55-1.16)
Polyunsaturated fatty acid	0.99(0.57-1.74)	1.38(1.04-1.83)[1]	0.96(0.54-1.71)	1.12 (0.78-1.59)
Calcium	1.47(0.87-2.47)[2]	0.98(0.73-1.32)	0.56(0.29-1.09)[2]	0.93 (0.65-1.35)
Iron	1.25(0.73-2.13)	1.24(0.93-1.65)	1.16(0.66-2.05)	1.18 (0.82-1.69)
Magnesium	2.16(1.32-3.56)[1]	1.11(0.83-1.48)	0.56(0.29-1.09)[2]	0.90 (0.62-1.31)

1. $p<0.05$
2. $p\leq0.15$

Table 4. (Continued)

	Canada		Mexico	
Characteristic	**PE**	**GH**	**PE**	**GH**
Potassium	2.48(1.51-4.05)[1]	1.02(0.76-1.37)	1.19(0.69-2.05)	1.05 (0.73-1.51)
Sodium	0.91(0.52-1.62)	1.09(0.81-1.46)	1.40(0.81-2.41)	1.10 (0.77-1.57)
Zinc	1.60 (0.96-2.69)[2]	1.00(0.74-1.35)	0.91(0.51-1.63)	0.93 (0.64-1.34)
Vitamin A	2.00(1.21-3.29)[1]	1.06(0.79-1.43)	0.57(0.29-1.11)[2]	0.89 (0.62-1.29)
Vitamin C	1.93(1.17-3.20)[1]	1.03(0.77-1.39)	1.10(0.63-1.94)	1.09 (0.76-1.56)
Vitamin E	1.48(0.88-2.49)[2]	1.06(0.79-1.43)	1.33(0.77-2.28)	1.13 (0.79-1.61)
Vitamin D	1.07 (0.58-1.78)	1.00(0.74-1.34)	1.39(0.81-2.39)	0.93 (0.65-1.34)
Vitamin B6	1.47(0.87-2.47)[2]	0.85(0.63-1.16)	1.03(0.58-1.83)	1.08 (0.76-1.56)
Vitamin B12	1.38(0.81-2.33)	0.86(0.63-1.17)	1.14(0.66-1.99)	0.58 (0.39-0.86)[1]
Folate	1.95(1.18-3.22)[1]	1.25(0.93-1.66)[2]	0.74(0.39-1.38)	1.10 (0.77-1.59)
Thiamine	1.18(0.69-2.03)	0.92(0.68-1.24)	1.19 (0.68-2.07)	1.13 (0.79-1.62)
Riboflavin	1.28(0.75-2.18)	0.90(0.67-1.22)	0.88 (0.49-1.57)	1.14 (0.79-1.64)
Niacin	1.08(0.62-1.88)	0.93(0.69-1.26)	0.96 (0.54-1.73)	1.06 (0.73-1.52)

1. $p<0.05$
2. $p\leq0.15$

CHAPTER 6 ARTICLE III

Case Control study of Plasma concentration of Tocopherols in relation to the risk of preeclampsia

Case control study of Plasma concentration of Tocopherols in relation to the risk of preeclampsia

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Abstract

Objective: To investigate the levels of maternal plasma concentrations of vitamin E (α- and γ-tocopherol) during pregnancy in relation to the risk of preeclampsia (PE).

Design: A nested case control study using the pregnancy cohort from a trial of antioxidant supplementation for the prevention of PE. Vitamin E concentrations were measured longitudinally at 12-18 weeks (prior to supplementation), 24-26 weeks, and 32-34 weeks of gestation using high-performance liquid chromatography (HPLC) with coulometric electrochemical detection**.** A total of 115 women with PE and 229 matched controls were included.

Result: After multivariate adjustment, we observed a direct association between the baseline γ-tocopherol concentrations, when examined as a continuous variable, and the risk of PE (OR 1.35, 95%CI 1.02-1.78). Analyses of repeated measurements indicated that elevated γ-tocopherols were associated with an increased risk of PE when examined as categorical variables [highest vs. lowest quartile at 24-26 weeks: OR 2.99 (95% CI 1.13-7.89); at 32-34 weeks: 4.37 (1.35-14.15)]. We found no associations between α-tocopherol concentration and the risk of PE.

Conclusion: The present study found that higher γ-tocopherol concentrations during pregnancy were associated with a greater risk of PE, contradicting the presumed protective effects of γ-tocopherol in some studies.

Key words: Preeclampsia, Tocopherol, Case Control

Introduction

Preeclampsia (PE), a syndrome unique to human pregnancy, remains a significant cause of maternal and perinatal morbidity and mortality.[1-6] The etiology of PE is multifactorial and has not been clearly defined. It has been proposed that the pathophysiology involves a combination of immunologic, environmental, and genetic factors that result in shallow endovascular cytotrophoblast invasion and impaired remodelling of spiral arteries. These in turn cause a reduction in uteroplacental perfusion pressure and placenta ischemia/hypoxia. [7-9] Placental hypoxia then stimulates the activity of xanthine or nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H) in placenta, which leads to superoxide generation, and contributes to maternal endothelial cell activation, enhanced apoptosis of trophoblast, and an increased inflammatory response.[7-9] All of these are believed to eventually leading to endothelial and vascular dysfunction associated with PE.

Vitamin E is a lipid soluble antioxidant of dietary origin. Its antioxidant property has been ascribed to its ability to chemically act as a lipid-based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation and oxLDL formation.[10] Of the 8 isomers of vitamin E that occur naturally, α-tocopherol is the most abundant in plasma, cell membranes and other human tissues. It is the major isomer in micronutrient supplements which have been examined in clinical trials, whereas γ-tocopherol is the primary form of the nutrient in the human diet. Recently, several clinical trials have been conducted

to assess the potential benefits of antioxidant supplementation in the reduction of adverse pregnancy outcomes including PE.[11-17] With the exception of the first small trial by Chappell et al, [11] the results of other clinical trials found no effect of vitamins E and C supplementation on the prevention of PE. However, concerns have been raised concerning the potential harmful effects of supplementation with these nutrients associated with the increased risk of low birth weight, small for gestational age and preterm premature ruptures of membranes.[12, 14, 17, 18] Previous studies suggested that α-tocopherol may present pro-oxidant propensity depending on oxidative conditions and presence of other co-antioxidants.[19, 20] It has been proposed that high doses of α-tocopherol could deplete plasma and tissue γ-tocopherol which is considered an important antioxidant, and hence may actually increase oxidation.[21-24] However, there is a lack of information on longitudinal measures of plasma concentrations of α-and γ-tocopherols in relation to the risk of PE. We carried out a longitudinal analysis of plasma concentrations of α- and γ-tocopherols (at 12-18, 24-26, and 32-34 weeks of gestational age) in association with the risk of PE.[17]

Methods

Study design and population

This is a case control study ancillary to a randomized, placebo-controlled trial of antioxidants supplementation (vitamins C and E) for the prevention of PE, which was conducted in Canada (17 centers) and Mexico (10 centers) between January 2004 and March 2006. The design and methods of the trial have been

described in details elsewhere. [17] Briefly, women at 12-18 completed weeks of gestation were randomly assigned either to antioxidant treatment (1 g vitamin C and 400 IU vitamin E daily) or placebo group. Randomization was stratified by center and by risk status according to pre-specified clinical risk criteria. Women were at high risk if they were nulliparous or multiparous with pre-pregnancy chronic hypertension (diastolic blood pressure $\geq$ 90 mmHg before 20 gestational weeks or use of antihypertensive medication for hypertension), pre-pregnancy diabetes, multiple pregnancy, or a history of PE in the previous pregnancy. Women were stratified into the low risk stratum if they were nulliparous without any identified clinical risk factors.Women and their infants received care according to standard practice in each center, with surveillance for hypertension using standardized measurements of blood pressure. Women were excluded from the trial if they were taking a multivitamin preparation at a daily dose of > 200 mg vitamin C or >50 IU vitamin E at the time of enrollment.

The cases of PE were defined as gestational hypertension (*de novo* hypertension occurring at $\geqslant$20 weeks of gestation) with proteinuria.[25, 26] Proteinuria was defined as the urinary excretion of $\geqslant$0.3g in 24-hours urine collection, or $\geqslant$2+ on urine dipstick test. For women with pre-existing hypertension, PE was diagnosed on the basis of new or worsening proteinuria as defined above. For women with pre-existing proteinuria (e.g. diabetes with renal involvement), the diagnosis of PE was made on clinical or biochemical grounds by identifying at least one additional adverse condition (e.g. abnormal liver enzymes, low platelets and eclampsia). [25, 26] All cases of PE were adjudicated

by two independent investigators in the Trial Coordinating Centre. In the case of disagreement, a third independent investigator was consulted. A total of 115 PE cases (63 in Canada and 52 in Mexico) with baseline plasma samples available were identified. Normotensive controls were randomly selected at a ratio of 2:1 by matching for country (Canada, Mexico), maternal age (within 3 years), parity (primiparous: yes/no), and multiple pregnancy (yes/no). A total of 229 controls were selected as only one eligible control could be identified for a Mexican case.

Specimen collection and tocopherols assays

Blood specimens were collected prior to randomization (12^{+0}-18^{+6} weeks of gestation), at 24-26 weeks of gestation, at 32-34 weeks of gestation and after delivery. Venous blood was drawn into EDTA tubes and plasma samples were immediately separated by centrifugation at 500 g for 10 minutes at 4°C. Plasma samples were rapidly frozen at -80°C for analyses. Simultaneous monitoring of ubiquinols-9 and -10 was carried out using High-Performance Liquid Chromatography (HPLC) with coulometric electrochemical detection. Plasma samples were extracted using a method adapted from Menke *et al*.[27] The HPLC protocol has been described in details elsewhere.[28, 29] Briefly, after the addition of internal standards (4 ng of γ-tocotrienol and 5 ng of ubiquinol-9) for post-HPLC quantification purpose, 300 μL plasma sample was thawed at 4°C in the dark and processed immediately by addition of 2 ml of methanol/ethanol (1:1 mixture) and vigorous shaking, then followed by addition of 10 ml of hexane. The solvent was evaporated under a nitrogen stream, and the dry sample was re-dissolved in 700 μL of ethanol and injected (10 μL) in a Gold HPLC system

(Beckman Coulter Canada, Mississauga, Canada) with an autosampler connected to a Prontosil column (4.0 mm X 150 mm, 3 μm particle size; Bischoff Chromatography, Atlanta, GA). The HPLC mobile phase contained sonicated methanol/ethanol/isopropanol (88/24/10 v/v/v) and 15 mmol of lithium perchlorate at a flow of 1mL/min. The α- and γ- tocopherols were detected by the coulometric electrochemical detector (Coulochem III, ESA, Bedford, MA). The concentrations of lipophilic antioxidants were determined by use of calibration standard curves. No oxidation of the ubiquinol-9 standard was detected after plasma extraction and HPLC analysis.

Statistical analysis

Maternal characteristics in cases and controls were compared using Chi-square test, Fisher exact test or Student's *t* test where appropriate. To evaluate the differences in continuous variables (i.e. plasma tocopherols) between cases and controls, *Student*'s t-test was used if the distribution was normal, and *Wilcoxon* test was applied if the distribution was skewed. Chi-square or Fisher exact tests were used to compare the differences in categorical variables. Plasma concentrations of tocopherols were examined as both continuous variables-standardized Z-scores - and as categorical variables by quartiles. Odds ratios and 95% confidence intervals were estimated from logistic regression to quantify the associations between plasma concentrations of tocopherols and the risk of PE.

The *Mantel* extension test was used to assess linear trends in the levels of plasma tocopherols and the risk of PE. Multivariate conditional logistic

regression was used to assess the independent effects of plasma concentrations of tocopherols on the risk of PE. A covariate was retained in the model if it changed the estimates by >10%. Interactions were assessed by evaluating stratum-specific ORs, and including multiplicative interaction terms in the multivariable models, and assessing their statistical significance using likelihood ratio statistics.

We estimated the associations between baseline plasma concentrations of tocopherols and the risk of PE. Intervention status may significantly change the post-baseline measurements of vitamin E concentrations and therefore may have influenced the risk of PE. For this reason, analyses were conducted in the total study population as well as in the treatment and placebo groups separately. Analyses were repeated for plasma concentrations of tocopherols at visit 2: 24-26 weeks, visit 3: 32-34 weeks of gestation, as well as the mean of three measurements at three gestational age windows. We also evaluated the patterns of changes in the plasma concentrations across gestational age and their effects on the risk of PE. All analyses were performed using SAS software, version 9.2 (SAS Institute, Cary, NC).

Results

There were no differences between cases and controls in years of schooling, annual household income, and family history of PE or GH, periconceptional vitamin or mineral supplementation, lifestyle factors (i.e. smoking or drinking) or ethnic origin (Table 1). However, cases tended to have higher pre-pregnancy body mass index (BMI), and higher mean systolic and diastolic blood pressure at

trial entry compared to normotensive controls. The proportion of patients with pre-existing chronic hypertension or history of PE was significantly higher in cases than in controls.

There were no significant differences in plasma concentrations of total tocopherols (α- plus γ-) and α-tocopherol between cases and controls across all the three gestational age windows (table 2). However, the plasma concentrations of γ-tocopherol as well as the ratios of γ-/α-tocopherol were significantly higher in women with PE compared to normotensive controls throughout the three gestational age periods. There were progressive and significant increases in total plasma vitamin tocopherols and α-tocopherols from baseline (12-18 weeks) to visit 3 (32-34 weeks) in both cases and controls, while no such significant changes were observed for γ-tocopherol.

Table 3 displays the plasma concentrations of tocopherols for cases and controls stratified by intervention status, e.g. vitamin supplementation versus placebo. Regardless of the intervention or case control status, plasma levels of α-tocopherol increased significantly from baseline visit 1 at 12-18 weeks to visit 3 at 32-34 weeks of gestational age. It is interesting to note that a significant decrease in plasma concentrations of γ-tocopherol was found in women in the supplementation group. In contrast, for women in the placebo group, plasma concentrations of γ-tocopherol increased. Ratios of γ-/ α-tocopherol showed no significant changes across gestational age in the placebo group, but were significantly decreased in the supplementation group.

After adjustment for smoking, the presence of pre-selected clinical risk factors (i.e. chronic hypertension, history of preeclampsia, diabetes), regular prenatal use of vitamins or mineral supplementation, intervention status, gestational age and baseline BMI, we found no associations between the quartile distributions of α-and γ-tocopherols at trial entry and the risk of PE. However, when examined as a continuous variable, after adjusting for the same covariates, baseline plasma concentration of γ-tocopherol (standardized as Z-score) showed a significant positive linear relationship to the risk of PE (adjusted odds ratio 1.35, 95%CI 1.02-1.78). (Table 4)

Concentrations of α-tocopherol at all the three gestational age windows were not associated with the risk of PE. (Table 4, 5) Compared to the reference lowest quartile, the highest quartile of the average of all three measurements of γ-tocopherol was associated with a significant increased risk of PE (adjusted OR 4.02; 95% CI 1.63-9.93). The multivariate analyses indicated that the highest quartiles of γ-tocopherol measured at both 24-46 weeks and 32-34 weeks of gestation were associated with an increased risk of PE (highest vs. lowest quartiles at 24-26 weeks: adjusted OR 2.99, 95%CI 1.13-7.89; at 32-34 weeks: adjusted OR 4.37; 95%CI 1.35-14.15). When γ-tocopherol levels were examined as a continuous variable, elevated plasma concentrations of γ-tocopherol (z-score) were significantly associated with an increased risk for PE (average measurement: adjusted OR 1.47, 95% CI 1.11-1.95; at 24-26 weeks: adjusted 1.69, 95% CI 1.14-2.51; at 32-34 weeks: adjusted OR 1.94, 95%CI 1.23-3.06).

We also observed positive associations between the incremental changes across gestation (from baseline to visit 2 and visit 3) in plasma γ-tocopherol concentrations and the risk of PE (from baseline to visit 2: adjusted OR 1.62, 95%CI 1.06-2.46; from baseline to visit 3: adjusted OR 2.02, 95%CI 1.24-3.31). The result is consistent with the finding that plasma concentration of γ-tocopherol at visit 3 has the strongest association with PE risk.

Discussion

The principal findings are: 1) no association between α-tocopherol levels in pregnancy and the risk of PE; 2) high plasma γ-tocopherol level was associated with an increased risk of PE; 3) plasma concentrations of α-tocopherol increased progressively across gestational age regardless of the treatment; 4) plasma γ-tocopherol concentrations decreased during pregnancy in the supplemented group but increased in the placebo group.

All previous studies of the association of serum or plasma concentrations of tocopherols with PE risk used a single measurement only (mostly at baseline) and have shown mixed results.[30-35] Several studies have reported that, compared to normotensive controls, preeclamptic women had lower α-tocopherol concentrations.[31, 33, 35, 36] Other investigators, however, have suggested that women with PE have higher mean α-tocopherol levels compared to normotensive controls.[30, 37, 38] Some studies also report no association between α-tocopherol and the PE risk.[39-41] The variability in results across studies may be explained by differences in study design, population

characteristics (e.g. ethnicity, dietary habits, use of prenatal multivitamin supplements, smoking, etc.), technical differences in assay protocols, and statistical power. We found no association between α-tocopherol and the risk of PE. These results are consistent with the results of our and other reported clinical trials. [12, 14, 16-18] The observed progressive increase in both α- and γ-tocopherols across gestational ages, in particular in the placebo group, could perhaps reflect a natural compensatory mechanism against oxidative stress in human pregnancy or that women could have taken undocumented micronutrient supplements during pregnancy in anticipation of potential benefits, despite reports to the contrary. Our study confirmed the reduction of γ-tocopherol after oral supplementation of α-tocopherol in pregnancy women, which has been consistently reported in previous studies.[42, 43]

Compared to the α-tocopherol form of the vitamin, γ-tocopherol has received less attention although it is estimated that approximately 70% of the food source vitamin E intake in the American diet is in the form of γ-tocopherol. This is due to the high intake in the American diet of soybean and other vegetable oils rich in γ-tocopherol, such as canola oil.[44] It has been suggested that γ-tocopherol could be a more potent antioxidant than α-tocopherol [45] as it has been shown that γ-tocopherol supplementation alone or in combination with α-tocopherol significantly reduces biomarkers of oxidative stress and inflammation.[45] To our knowledge, unlike α-tocopherol which is the major form of nutritional supplements, no intervention studies with clinical disease endpoints have been conducted on γ-tocopherol. Only a few observational studies have examined the

relationship between γ-tocopherol and the risk of PE or gestational hypertension. There was no clear pattern observed between the serum or plasma concentrations of γ-tocopherol and the risk of PE in previous studies. [34, 37, 46] Paradoxically, we found that the plasma γ-tocopherol was associated with an increased risk of PE at all the three gestational age windows, contradicting its presumed potential protective effects in some studies. There are a number of possible explanations for these findings. A recent study found that γ-tocopherol was associated with an increased risk of myocardial infarction.[47] The authors noted that dietary intake of γ-tocopherol is associated with the consumption of trans-fatty acids, which are known to promote atherosclerosis. The authors suggested that since tocopherols were strongly associated with lipoproteins, residual confounding by elevated lipids could contribute to the observed positive association despite statistical adjustment.[47] A study by Kabat et al. reported that, after multivariate adjustment, an increased risk of breast cancer was associated with elevated serum γ-tocopherol levels.[48] Another recent study observed an association between elevated plasma γ-tocopherol levels and an increased risk of spontaneous preterm birth, but no similar association was seen with trans-fatty acids.[49] Other investigators also raised questions concerning the anti-inflammatory capacity of γ-tocopherol. [50] [51] An animal study by Berdnikovs et al. demonstrated the opposing function of D-γ-tocopherol compared to the D-α-tocopherol isoforms in experimental asthma. The study reported that D-γ-tocopherol not only elevates inflammation but also ablates the anti-inflammatory benefit of D-α-tocopherol isoform.[51] The authors pointed out that there was

little benefit of α-tocopherol for inflammation in the presence of elevated plasma γ-tocopherol. [51] Thus, a possible explanation for our findings is that elevated plasma γ-tocopherol levels could be a marker of trans fat intake which has in turn been shown to represent a risk factor for PE.[7] Furthermore, plasma concentrations of tocopherols are influenced by the plasma lipoproteins that act as transport molecules of the antioxidants. Unfortunately, plasma lipoprotein was not measured in the present study. It is also possible that there is a real association between γ-tocopherol and PE risk. Our data provide a relative strong case: the average concentration of γ-tocopherol showed a progressively stronger association with PE risk over advancing gestational age, as the repeated measurements over the follow up period may improve exposure classification and precision, and be an indicator of accumulated exposure in later measures.

This study has several strengths. The disease status itself is unlikely to have been influenced by the measured plasma tocopherol concentrations as all samples were collected before disease onset. We included repeated measurements of plasma concentration of both α-and γ-tocopherols in multiple gestational age windows. The baseline, follow up measurements, and average measurements analyses provided a relatively complete picture of the influence of tocopherols on the risk of PE. The sample size is relatively large. Plasma concentrations of tocopherols were assayed by staff blinded to pregnancy outcomes.

Potential limitations of our study include the possibility of residual confounding. It has been suggested that the plasma concentrations of

tocopherols are influenced by plasma lipoprotein that are transport molecules of the antioxidant. Plasma lipoprotein was not measured and therefore was not adjusted in analyses.

In summary, this is the first report indicating that elevated γ-tocopherol levels may be associated with an increased risk of PE. Further epidemiologic and intervention studies are needed to better understand the potential role of γ-tocopherol in the etiology of PE.

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Table 1 Socio-demographic and clinical characteristics of PE cases and normotensive controls at trial entry (12-18 weeks of gestational age)[1]

Characteristics	PE	Control	
	N=115	N=229	P
Maternal age (years)	29.19 (5.70)	29.00 (5.64)	NS
Maternal education (years)	13.42 (3.24)	14.05 (3.76)	NS
Maternal pre-pregnancy BMI	27.97 (6.46)	25.76 (6.57)	<0.05
Maternal visit1 BMI[b]	30.02 (7.88)	26.87 (6.51)	<0.05
Ethnic origin			
Caucasian	52 (45.22)	105 (45.85)	
Hispanic	53 (46.09)	104 (45.41)	NS
Other	10 (8.70)	20 (8.73)	
Married	98 (85.22)	205 (90.31)	NS
Employed	86 (74.78)	156 (68.12)	NS
Smoking before pregnancy	29 (25.22)	61 (26.64)	NS
Current smoker	8 (6.96)	12 (5.24)	NS
Current drinker	2 (1.74)	3 (1.31)	NS
Gestational age (weeks) at trial entry	15.32 (1.97)	15.35 (2.11)	NS
Antioxidant treatment	58 (50.43)	117 (51.09)	NS
High risk group (stratum)	67 (58.26)	102 (44.54)	<0.05
Mean systolic BP at trial entry	116.0 (14.66)	108.5 (13.63)	<0.05
Mean diastolic BP at trial entry	72.20 (8.91)	67.31 (9.17)	<0.05
Family history of GH, PE	20 (17.39)	30 (13.10)	NS
Prenatal vitamin or mineral supplementation	105 (91.30)	207 (90.39)	NS

1. Data presented as Mean (SD) or N (%)

Table 2. Plasma concentrations of antioxidant vitamins among preeclamptic women and normotensive controls[1]

		V1 (12- 18 weeks) [4]	V2 (24-26 weeks)[5]	V3 (32-34 weeks)[6]	P[3]	P[3]
Characteristics	Group	N=344	N=308	N=275	(1 vs 2)	(1 vs 3)
Total tocopherols	Case	31.66±9.51(29.63)	43.19±13.68(40.50)	47.90±16.26(45.34)	*<0.05*	*<0.05*
	Control	30.81±10.20(29.39)	43.98±16.08(41.44)	49.12±17.72(46.61)	*<0.05*	*<0.05*
	P[2]	*NS*	*NS*	*NS*		
α-tocopherol	Case	28.84±8.99(26.82)	40.34±13.61(38.55)	44.79±15.97(41.84)	*<0.05*	*<0.05*
	Control	28.40±9.52(27.05)	41.91±16.19(38.47)	46.72±17.83(43.54)	*<0.05*	*<0.05*
	P[2]	*NS*	*NS*	*NS*		
γ-tocopherol	Case	2.83±1.52(2.29)	2.85±2.43(2.19)	3.10±2.35(2.60)	*NS*	*NS*
	Control	2.40±1.22(2.13)	2.07±1.30(1.74)	2.40±1.49(2.03)	*<0.05*	*NS*
	P[2]	*<0.05*	*<0.05*	*<0.05*		
γ-/α-tocopherol	Case	0.10±0.06(0.09)	0.08±0.06(0.07)	0.08±0.05(0.07)	*<0.05*	*<0.05*
	Control	0.09±0.03(0.08)	0.06±0.04(0.05)	0.06±0.04(0.05)	*<0.05*	*<0.05*
	P[2]	*<0.05*	*<0.05*	*<0.05*		

1. Data present as mean±SD (median); 2. *Wilcoxon-Mann Whitney* test; 3. *Wilcoxon* signed ranks test
4. 115 cases and 229 controls; 5. 100 cases and 208 controls; 6. 88 cases and 187 controls

Table 3. Plasma concentrations of antioxidant vitamins among preeclamptic women and normtensive controls stratified by treatment group[1]

Characteristics	Group	V1 (12- 18 weeks)[4] N=344	V2 (24-26 weeks)[5] N=308	V3 (32-34 weeks)[6] N=275	P[3] (1 vs 2)	P[3] (1 vs 3)
Total tocopherols						
Supplemented	Case	31.48±10.07(29.24)	47.54±15.25(45.79)	51.58±18.28(49.47)	<0.05	<0.05
	Control	30.62±10.38(28.53)	50.51±17.24(49.55)	56.75±18.30(55.48)	<0.05	<0.05
	p[2]	*NS*	*NS*	*<0.05*		
Placebo	Case	31.85±8.99(29.89)	39.01±10.51(36.98)	44.53±13.50(45.07)	<0.05	<0.05
	Control	31.00±10.06(29.81)	37.20±11.39(36.09)	41.57±13.46(41.19)	<0.05	<0.05
	p[2]	*NS*	*NS*	*NS*		
α-tocopherol						
Supplemented	Case	28.75±9.51(27.50)	45.35±15.59(44.20)	49.12±18.44(45.67)	<0.05	<0.05
	Control	28.25±9.68(26.72)	49.02±17.27(48.08)	55.13±18.32(54.32)	<0.05	<0.05
	P[2]	*NS*	*NS*	*<0.05*		
Placebo	Case	28.92±8.51(26.67)	35.33±9.01(34.15)	40.84±12.25(40.17)	<0.05	<0.05
	Control	28.56±9.39(27.59)	34.52±10.91(33.54)	38.40±12.76(37.82)	<0.05	<0.05
	P[2]	*NS*	*NS*	*NS*		

1. Data present as mean±SD (median); 2. *Wilcoxon-Mann Whitney* test; 3. *Wilcoxon* signed ranks test ; 4. 115 cases and 229 controls; 5. 100 cases and 208 controls; 6. 88 cases and 187 controls

Table 3. (Continued) [1]

Characteristics	Group	V1 (12- 18 weeks)[4] N=344	V2 (24-26 weeks)[5] N=308	V3 (32-34 weeks)[6] N=275	P[3] (1 vs 2)	P[3] (1 vs 3)
γ-tocopherol						
Supplemented	Case	2.73±1.47(2.26)	1.99±1.87(1.41)	2.45±2.39(1.65)	<0.05	<0.05
	Control	2.37±1.16(2.08)	1.49±1.13(1.16)	1.61±1.18(1.34)	<0.05	<0.05
	P[2]	*NS*	*NS*	*NS*		
Placebo	Case	2.93±1.58(2.46)	3.68±2.63(3.04)	3.69±2.17(3.09)	<0.05	<0.05
	Control	2.44±1.29(2.23)	2.68±1.19(2.46)	3.17±1.35(2.94)	<0.05	<0.05
	P[2]	*NS*	*<0.05*	*NS*		

1. Data present as mean±SD (median); 2. *Wilcoxon-Mann Whitney* test; 3. *Wilcoxon* signed ranks test ; 4. 115 cases and 229 controls; 5. 100 cases and 208 controls; 6. 88 cases and 187 controls

Table 4. Baseline plasma concentrations of tocopherols in relation to the risk of preeclampsia [1,2,3]

Baseline analyte	COR (95%CI)	AOR(95%CI)
Total tocopherols		
Q1	1.00	1.00
Q2	1.30(0.67-2.52)	1.60(0.78-3.27)
Q3	1.13(0.54-2.37)	1.17(0.54-2.53)
Q4	1.45(0.65-3.23)	1.34(0.58-3.08)
P trend	NS	NS
Z-score	1.11(0.85-1.46)	1.11 (0.83-1.49)
α-tocopherol		
Q1	1.00	1.00
Q2	0.96(0.50-1.83)	1.07(0.54-2.11)
Q3	0.90(0.44-1.85)	1.01(0.48-2.14)
Q4	1.06(0.49-2.32)	1.00(0.44-2.24)
P trend	NS	NS
Z-score	1.05(0.81-1.37)	1.06 (0.79-1.42)
γ-tocopherol		
Q1	1.00	1.00
Q2	1.34(0.65-2.76)	1.24(0.58-2.64)
Q3	1.02(0.49-2.11)	1.00(0.46-2.10)
Q4	2.00(0.95-4.23)	1.63(0.75-3.57)
P trend	<0.05	<0.05
Z-score	1.48 (1.13-1.92)[4]	1.35(1.02-1.78)[4]
γ-/α-tocopherol ratio	-	-
Q1	1.00	1.00
Q2	1.10(0.58-2.09)	1.08(0.56-2.10)
Q3	0.80(0.41-1.59)	0.80(0.39-1.67)
Q4	1.88(0.94-3.76)	1.49(0.71-3.10)
P trend	<0.05	<0.05
Z-score	1.52 (1.16-2.00)[4]	1.43(1.08-1.90)[4]

1. COR: Crude odds ratio; 2. AOR: Adjusted odds ratio;
3. Adjusted variables: smoking, the presence of pre-selected clinical risk condition (i.e. chronic hypertension, history of preeclampsia, diabetes), prenatal regular using of vitamins or mineral supplementation, intervention status (vitamins supplementation vs placebo), gestational age and baseline BMI; 4. $p<0.05$.

Table 5. Repeated measurements of concentrations of tocopherols in the relation to the risk of preeclampsia[1,2,3,4]

	Average[5]		Visit 2(24-26 wks of gestation)		Visit 3(32-34 wks of gestation)	
Aanalyte	COR (95%CI)	AOR(95%CI)	COR (95%CI)	AOR(95%CI)	COR (95%CI)	AOR(95%CI)
Total tocopherols						
Q1	1.00	1.00	1.00	1.00	1.00	1.00
Q2	1.65(0.86-3.15)	1.69(0.85-3.33)	1.41(0.65-3.07)	1.46(0.62-3.43)	1.52(0.67-3.45)	1.58(0.67-3.76)
Q3	1.23(0.62-2.45)	1.23(0.59-2.57)	1.14(0.50-2.56)	1.19(0.46-3.07)	1.42(0.60-3.37)	1.46(0.58-3.70)
Q4	1.11(0.52-2.35)	1.14(0.48-2.67)	1.15(0.48-2.72)	1.28(0.45-3.68)	0.90(0.37-2.21)	0.78(0.26-2.31)
P trend	NS	NS	NS	NS	NS	NS
Z-score	0.93(0.71-1.22)	1.02(0.78-1.33)	0.98(0.73-1.31)	1.00(0.70-1.43)	0.91(0.67-1.24)	0.88(0.60-1.29)
α-tocopherol						
Q1	1.00	1.00	1.00	1.00	1.00	1.00
Q2	1.25(0.65-2.41)	1.23(0.62-2.43)	1.34(0.61-2.94)	1.60(0.69-3.74)	1.75(0.77-3.98)	1.76(0.74-4.21)
Q3	1.14(0.58-2.24)	1.01(0.49-2.10)	1.24(0.57-2.71)	1.21(0.49-2.98)	1.16(0.49-2.76)	1.18(0.46-3.00)
Q4	0.81(0.39-1.67)	0.83(0.37-1.87)	1.11(0.48-2.58)	1.33(0.48-3.69)	0.91(0.38-2.16)	0.80(0.28-2.29)
P trend	NS	NS	NS	NS	NS	NS
Z-score	0.87(0.67-1.14)	0.94(0.72-1.21)	0.91(0.68-1.22)	0.94(0.65-1.34)	0.85(0.63-1.16)	0.82(0.55-1.21)

1. COR: Crude odds ratio; 2. AOR: Adjusted odds ratio; 3. Regression models were repeatedly conducted, in which plasma concentrations at each visit or average measurements as main independent variables; 4. Adjusted variables: smoking, the presence of pre-selected clinical risk condition (i.e. chronic hypertension, history of preeclampsia, diabetes), prenatal regular using of vitamins or mineral supplementation, intervention status vitamins supplementation vs placebo), gestational age and baseline BMI; 5. Average of three measurements at baseline, visit2 and visit 3; 6. $P<0.05$

Table 5. (Continued)

	Average[5]		Visit 2(24-26 wks of gestation)		Visit 3(32-34 wks of gestation)	
Aanalyte	COR (95%CI)	AOR(95%CI)	COR (95%CI)	AOR(95%CI)	COR (95%CI)	AOR(95%CI)
γ-tocopherol						
Q1	1.00	1.00	1.00	1.00	1.00	1.00
Q2	1.72(0.83-3.56)	1.93(0.89-4.19)	1.19(0.54-2.62)	1.19(0.51-2.77)	1.17(0.49-2.78)	1.27(0.48-3.32)
Q3	1.47(0.72-3.02)	1.76(0.79-3.92)	1.71(0.78-3.74)	2.04(0.79-5.24)	2.07(0.94-4.56)	2.59(0.91-7.40)
Q4	3.41(1.58-7.37)[6]	4.02(1.63-9.93)[6]	2.42(1.07-5.47)[6]	2.99(1.13-7.89)[6]	3.07(1.22-7.73)[6]	4.37(1.35-14.15)[6]
P trend	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Z-score	1.79(1.33-2.40)[6]	1.47(1.11-1.95)[6]	1.73(1.23-2.42)[6]	1.69(1.14-2.51)[6]	1.86(1.29-2.69)[6]	1.94(1.23-3.06)[6]
γ-/α-tocopherol ratio	-	-	-	-	-	-
Q1	1.00	1.00	1.00	1.00	1.00	1.00
Q2	1.44(0.70-2.94)	1.50(0.72-3.15)	1.02(0.47-2.20)	1.20(0.52-2.76)	1.41(0.59-3.37)	1.99(0.73-5.45)
Q3	1.28(0.64-2.58)	1.31(0.60-2.85)	1.25(0.57-2.73)	1.40(0.55-3.61)	2.82(1.22-6.51)[6]	8.00(2.33-27.49)[6]
Q4	2.56(1.23-5.31)[6]	2.40(1.04-5.55)[6]	2.58(1.17-5.71)[6]	2.72(1.03-7.18)[6]	2.93(1.17-7.33)[6]	5.68(1.54-20.90)[6]
P trend	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Z-score	1.71(1.29-2.28)[6]	1.28(0.98-1.68)	1.59(1.19-2.14)[6]	1.58(1.12-2.22)[6]	1.66(1.19-2.31)[6]	1.82(1.18-2.82)[6]

1. COR: Crude odds ratio; 2. AOR: Adjusted odds ratio; 3. Regression models were repeatedly conducted, in which plasma concentrations at each visit or average measurements as main independent variables; 4. Adjusted variables: smoking, the presence of pre-selected clinical risk condition (i.e. chronic hypertension, history of preeclampsia, diabetes), prenatal regular using of vitamins or mineral supplementation, intervention status vitamins supplementation vs placebo), gestational age, and baseline BMI; 5. Average of three measurements at baseline, visit2 and visit 3; 6. P<0.05

CHAPTER 7 DISCUSSION

7.1 Nutrition and PE

Preeclampsia (PE) is a multisystem disorder that is specific to pregnancy and only can be resolved by delivery. The underlying mechanisms are complex and remain poorly understood. A causal network of socioeconomic, genetic, maternal health and nutritional factors likely contributes to the etiology of PE. It has been suggested that maternal nutritional imbalance may lead to altered gene methylation and expression, altered homocysteine metabolism, inflammatory responses and oxidative stress, all of which may lead to adverse pregnancy outcomes including PE.(286)

Encouraged by the results of Chappell *et al.* (165) that reported a 54% reduction in PE in the group supplemented with vitamins C and E compared with the placebo group [RR 0.39; 95% CI 0.17-0.90)], we conducted the International Trial of Antioxidants in the Prevention of Preeclampsia to assess the effects of antioxidants (vitamins C and E) in the reduction of PE among high and low risk populations. We found no evidence that supplementation with vitamins C and E reduced the risk of GH and its adverse conditions among patients at high risk and low risk for PE. The results are consistent with those of other recently reported RCTs.(165, 167, 276, 277, 287, 288) We also observed unexpected increased risk of PROM and PPROM. It is not clear why the prenatal supplementation with vitamins C and E was unsuccessful in the reduction of risk of GH and PE in our study or in other studies. It is possible that although oxidative stress is present in PE, it may not be fundamental to the pathophysiology of the condition. It is

possible that oxidative stress may be relevant to the pathogenesis of a subgroup of patients. It has been hypothesized that only individuals under oxidative stress are likely to benefit from antioxidant supplementation.(289) In our study, a significant proportion of the population was taking prenatal multivitamin at the time of randomization. The dose of vitamin E that is required to suppress oxidative stress has not been well determined in pregnant women. It is also possible that vitamin E (pharmaceutical form: α-tocopherol) may have a pro-oxidant propensity, depending on oxidative conditions and presence of other co-antioxidants (290, 291).

It is important to note that recruitment for Chappell *et al.*'s trial was stopped early due to the significant main treatment effect observed at interim analysis.(165) The primary endpoint in the trial was the ratio of plasminogen-activator inhibitor 1 (PAI-1) and placental dysfunction (PAI-2).Therefore, the observed effect in Chappell *et al.*'s trial may be higher than the true treatment effect and the results may suffer from the type I error.(292) The majority of women recruited in the trial had an abnormal two-stage uterine-artery doppler screening, which indicating inadequate uteroplacental blood flow and probable defective placentation.(165) The results from the Chappell *et al*.'s trial can be only generalized to their specific study population.

Several large trials designed to evaluate the effects of vitamin E and C in the prevention of PE have been also conducted. These trials are from several countries including the United Kingdom, the United States, as well as from developing countries. The UK study (VIP trial) enrolled 2410 women identified

at increased risk of PE.(276) Women were randomly assigned either to the experimental group (1000mg vitamin C and 400 IU RRR alpha tocopherol) or to a matched placebo group. The incidence of PE was similar in treatment and control groups (15% versus 16%; RR 0.97; 95% CI 0.80, 1.17). However, the incidence of low birth weight was higher in women with antioxidant treatment than in controls (28% versus 24%; RR: 1.15; 95% CI: 1.02, 1.30). Plasma concentrations of vitamin C and E did not differ between groups. In the placebo group, plasma concentrations of vitamin C were lower throughout the gestational period studied in women who developed PE than those who did not. Furthermore, the highest quartile of baseline vitamin C intake was associated with a reduced risk of small for gestational age (OR 0.42, 95% CI 0.26-0.67), low birth weight (OR 0.39, 95% CI 0.24-0.63), and PE (OR 0.59, 95% CI 0.38-0.93) after adjusting for risk group, degree of education, housing status, and smoking status.

An Austrian multicentre trial was conducted among 1877 nulliparous women recruited between 14 and 22 weeks of gestation.(277) The results indicated that there were no significant differences between the vitamin and placebo groups in the risk of PE (6 vs 5%; RR 1.20; 95% CI 0.82-1.75), death or serious infant outcomes (9.5% vs 12.1%; RR 0.79; 95% CI 0.61-1.02), or small for gestational age (8.7% vs 9.9%; RR 0.87; 95% CI 0.66-1.16). Women in the vitamin group were more likely than those in the placebo group to be admitted antenatally with hypertension and to be treated with antihypertensive drugs. This finding may be due to chance. However, research has suggested that antioxidants may promote DNA oxidation by interacting with metal ions.(293) Spinnato II reported the

results of an antioxidant (vitamins C and E) trial in women recruited between 12 and 19 weeks of gestation and diagnosed as having chronic hypertension or a prior history of PE.(287) There was no evidence of a reduction in the risk of PE in the supplementation group compared to placebo (adjusted RR 0.87, 95% CI 0.61-1.25). There were no differences in mean gestational age and rates of perinatal mortality, abruptio placentae, preterm delivery, and small for gestational age (SGA) or low birth weight infants.(287) The World Health Organisation recently completed a multicentre trial that was conducted among pregnant women with low socio-economic status and low nutritional status from developing countries (e.g. India, Peru, South Africa, and Viet Nam).(288) The trial followed the research protocol that was used in the VIP trial(276) with only minor adaptation to local resources. The result showed that supplementation of vitamins C and E did not reduce the risk of PE (RR: 1.0; 95% CI: 0.9-1.3),eclampsia (RR: 1.5; 95% CI: 0.3-8.9), and GH(RR: 1.2; 95% CI: 0.9-1.7).(288)

In addition to the trials of combined vitamins C and E supplementation, Steyn et al. reported no effects of vitamin C supplementation alone on the risk of PE (RR 1.00, 95% CI 0.21-4.84). (294) Rivas et al. conducted a trial, in which supplements of aspirin, vitamin C, E and fish oil were used, and found that such supplements significantly reduced the risk of PE (RR 0.07, 95% CI 0.01-0.54). However, it is hard to infer whether such an effect was due to vitamins C and E, fish oil or aspirin, or the effects of their interaction.(295) Our group has recently updated the meta analysis by Polyzos et al.(296), involving eight trials, of which

one trial assessed the effect of vitamin C alone(294), six trials evaluated the combined effect of vitamins C and E supplementation,(165, 167, 276, 277, 287, 288) and one trial examined the benefits of vitamin C and E, combined with fish oil.(295) Overall, we were unable to detect any benefits of vitamin C alone, or vitamin C combined with vitamin E, or vitamin C and E with other supplements in reducing the risk for PE (random effects model, RR 0.93, 95% CI 0.76-1.13).

The literature suggests that vitamin E may exert both beneficial and detrimental effects. The ineffectiveness of vitamin C and E in the prevention of PE emphasizes the need for understanding the underlying mechanisms and metabolism of both vitamins C and E in the human body. It is noteworthy that vitamin E also has non-antioxidant pleiotropic effects in addition to its antioxidant capacity.(297) Exogenous vitamin E may prevent an immunologic switch (Th1 to Th2) that is considered as crucial for early to late transition in normal pregnancy and it could be a potential interferon-gamma (IFN- γ) mimic, facilitating pro-inflammatory responses at the maternal-fetal interface. Therefore, vitamin E treatment might have undesirable side-effects and may partially explain the conflicting results of previously published trials.(165, 167, 276, 277) There is some evidence that high doses of alpha-tocopherol (primary form of vitamin E in supplementation) could deplete plasma and tissue gamma-tocopherol (major forms in plant seeds and in the North American diet).(298-301) Therefore, efficacy of vitamin E supplementation (alpha-tocopherol) may be offset by deleterious changes of other nutrients.

Although the INTAPP trial failed to provide evidence of a beneficial effect

of prenatal vitamins C and E supplementation on the risk of PE, it offered a unique opportunity to investigate the role of maternal dietary factors in relation to the risk of PE and GH. In our study, the FFQ was administered both in early pregnancy and late pregnancy to assess usual dietary intake prior to the three month period. There was significant heterogeneity in nutrient intake between participants in Canada and Mexico. Among Canadian women, we found that the lowest quartiles of potassium (adjusted OR 1.79, 95%CI 1.03-3.11) and zinc (adjusted OR 1.90, 95%CI 1.07-3.39) intakes were significantly associated with an increased risk of PE among Canadian women. The lowest quartile of polyunsaturated fatty acids was associated with an increased risk of GH (adjusted OR 1.49, 95%CI 1.09-2.02). None of the nutrients analyzed were found to be associated with PE or GH risk among Mexican women.

Several studies have been conducted to investigate the nutritional risk factors for hypertensive disorders of pregnancy, and have yielded inconsistent results. For instance, Morris *et al.* conducted a prospective cohort study of 4157 women at 13-21 weeks gestation that had been enrolled in a randomized controlled trial of calcium supplementation in the prevention of PE.(99) A 24-hour dietary recall was administered to assess nutrient intake at the time of random assignment (at 13-21 of weeks of gestation).. After adjustment for baseline risk factors, none of the 28 nutritional factors analyzed were significantly related to either PE or pregnancy-associated hypertension.(99) A Norwegian team conducted a prospective, population based study of 3771 women pregnancy women to investigate maternal nutrient intake in relation to the risk of PE. A semi-

quantitative FFQ was administered to women in the secondary trimester to assess dietary intake.(98) The risk of PE was increased among women with a high energy intake (adjusted OR: 5.4, 95% CI: 2.3-12.4, for the 4th quartile) and a high intake of polyunsaturated fatty acids (adjusted OR: 2.3, 95% CI: 1.1-4.6). Moreover, the authors observed a stronger association for early onset PE. (98)

The discrepancies between the findings from our study and those of other published studies may be partially explained by the methods used to estimate dietary intake, the time in pregnancy at which diet is assessed, different definitions of PE and GH, or population differences (i.e. lifestyle, heterogeneity in nutrient intake, socio-demographic factors). Our project is the first to assess maternal diets longitudinally in both early and late pregnancy and to conduct parallel analyses to evaluate dietary factors in the development of GH and PE in two different ecological settings. The present research project makes a novel contribution to the understanding of the role of maternal nutrient intakes in early pregnancy in relation to the risk of PE.

Several clinical studies have been carried out to examine the associations between maternal α-tocopherol concentrations and the risk of PE with inconsistent results. (154, 155, 164, 169, 170, 172) No clear patterns have been observed between the serum or plasma concentrations of γ-tocopherol and the risk of PE in previous studies. (172, 302, 303) Ours is the first study to investigate longitudinal measurements of plasma α-and γ-tocopherols in relation to the risk of PE. We found no association between maternal plasma α-tocopherol levels and the risk of PE, but an increased risk of PE was associated

with high plasma γ-tocopherol levels during pregnancy. It has been suggested that γ-tocopherol could be a more potent antioxidant than α-tocopherol.(304) However, it is not known why γ-tocopherol is associated with an increased risk for PE. Recently, studies on non pregnant populations have reported that elevated γ-tocopherol levels were associated with the increased risk of cancer and cardiovascular disease. (305) (306) It is possible that in addition to its antioxidant effect, it may also have pro-inflammatory effect. (307) (308) Another possible explanation for our findings is that elevated plasma γ-tocopherol levels could be a marker of trans fat intake which in turn has been shown to represent a risk factor for PE. (286) Plasma concentrations of tocopherols are influenced by the plasma lipoproteins that act as transport molecules of the antioxidants. Concentrations of trans fatty acids and lipoproteins were not measured and therefore were not adjusted in our study. It has been suggested that it may be optimal to evaluate plasma concentrations of tocopherol corrected for apolipoprotein B (apo B). In our previous study, the lipid composition did not vary between PE cases and controls. The plasma levels of triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol and LDL apolipoprotein B (apo B) were not statistically different between PE and normotensive controls. (309) Furthermore, correlations between ratio of prostacyclin (PGI2)-a vasodilator / thromboxane A2 –a potent vasoconstrictor and total vitamin E were similar when plasma vitamin E concentration corrected for apoB or not.(309) Therefore, it is very likely that there is a real association between γ-tocopherol and PE risk although plasma lipoprotein was not measured in the present study.

7.2 Application of FFQ in epidemiological studies

Dietary intake measurements only provide estimates of the amounts of energy and nutrients available for metabolism. Several methods have been developed to measure dietary intake, including dietary records, the 24-hour dietary recall, diet history, and FFQs.(262)

The dietary measurement instrument used most often in large-scale epidemiological studies, particularly prospective cohort studies, is the FFQ. Compared to quantitative methods such as food records or recalls, the FFQ is more powerful to capture day-to-day variability in food intakes. This is particularly relevant in a dietary assessment among pregnant women whose diet is likely to be highly variable as food intakes generally change from usual pre-pregnant patterns, and may fluctuate according to their state of comfort and well-being as well as changes in food preferences during the course of pregnancy. Therefore, the food records or recalls may not be enough to capture this dietary variability. On other hand, FFQs are designed to cover a broader period than food records or recalls and thus are more suited to capturing usual intake of foods and nutrients that may be missed by the quantitative methods.(310) It also provides a practical, cost-effective way of collecting information from a large number of respondents.

We used a pre-validated self-administered semi-quantitative 78-item FFQ in the Canadian study population, which was modified for INTAPP to reflect the previous three months' usual food consumption rather than the standard 12 month period. The FFQ was further validated among 107 pregnant women from

a subset of the Canadian INTAPP cohort.(280) The results of validation study suggest that the FFQ is a relatively valid instrument for determining usual diet in pregnant women.(280)

In the Mexican study population, the Canadian FFQ was modified to reflect local foods and dietary habits and information from the second Mexican National Nutrition Survey published data.(281).The FFQ was developed and tested in Spanish and it was validated against three non-consecutive 24 hour food recalls among 85 pregnant women. The results of the validation study suggested that the Mexican FFQ could adequately measure habitual nutritional intakes in Mexican pregnant women and capture enough variability in the population studied.(282)

Nevertheless, it is important to recognize the limits of the FFQs in accurately estimating subjects' habitual dietary intakes. Random errors and uncorrelated measurement errors exist, which can cause attenuations of risk estimates and reduce statistical power.(271) As described by Beaton, there is not, and probably never will be, a method that can estimate dietary intake without error.(275) It does not mean that dietary data with measurement errors should not be collected.

7.3 Methods for analyzing repeated dietary measurements

We used logistic regression for analyses of repeated dietary measures, which is similar to the approaches suggested by Hu et al.(311). Biologically, the diet in early pregnancy may be more important to the development of PE, as opposed to the diet in late pregnancy which may be more important for fetal growth. Thus, it

is not appropriate to simply treat FFQ data as repeated measurements, in which the generalized estimating equation model (e.g. SAS PROC GENMOD) or mixed effects model can be used (e.g.SAS PROC MIXED). Instead, the effects at each exposure time window should be assessed separately, although the effects at the late gestational age window may need to consider early exposures as covariates.

Advantages and disadvantages of survival analysis with time-dependent covariates have been discussed in the literature.(312) One of main advantages of time-dependent covariates is that you can incorporate important events that occur during the study period. The main disadvantages include over-adjustment and decreased usefulness for clinicians. With time-dependent covariates, "effect-cause" may be a problem by including factors that are proximal to outcomes than baseline exposure measurements. One way to deal with this problem is to "lag" the time-dependent measurements substantially before the outcome but still after the baseline. In our study, all participants are pregnant women, with relatively short and approximately constant follow up time (from trial entry to delivery). Furthermore, unlike chronic disease such as cancer for which the incidence tends to rise over time, it may be inappropriate to apply cox regression model in modelling PE as an outcome since the assumption of increasing risk over time does not hold– typical PE cases tend to occur earlier.

Another conventional method for analyzing repeated measures on risk factors is the pooling of repeated observations (PRO) method, which pools observations over all intervals to examine the short term development of disease. There are

several important assumptions underlying PRO method including 1) the underlying risk of outcome in each interval is the same; 2) the relationship between risk factors and outcome is the same for every interval; and 3) only current risk profile is needed to predict outcome.(313) Thus, it is inappropriate to apply the PRO method in our study since such assumptions do not hold.

7.4 Strengths and limitations

Measurement error in explanatory variables and unmeasured confounders can cause considerable problems in epidemiological studies. In the present project, the FFQ in the present study was pre-tested and validated in pregnant women and the results of our validation studies indicated that the FFQ is a relatively valid instrument for determining usual diet in pregnant women. (280) However, it is still possible that some nondifferential misclassification of nutrient intakes from the FFQ may have occurred and therefore may lead a bias towards the null. Nutrient intakes in our study were grouped into quartiles, and results are therefore less likely to be affected by errors in intake estimates. In our study, nutrients included in the models were adjusted for the potential confounding effect of total energy intake.(283) Energy requirements depend on body size, physical activity, and metabolic efficiency of each individual, which may confound absolute total intake in relation to disease risk. Adjustments for energy intake may reduce such confounding effects. (283) In addition, measurements of absolute intake that contain a substantial degree of measurement errors may be eliminated by energy adjustments.

Our effect estimates may be further biased by unmeasured or unknown confounders and/or measurement error in the covariate data we collected. Indeed, many of our measured covariates such as smoking status, alcohol use, income, parity, pre-pregnancy BMI (pre-pregnancy weight), and family history of PE, were from interview-based self report and misclassification was possible. Information on certain variables such as physical activity, infection, and psychosocial profiles was not collected in our study. For instance, it has been suggested that the risk of PE was approximately doubled among women with stress or anxiety during pregnancy.(250) Studies have suggested that the pregnant women with depressive disorders tended to have lower or undesirable nutrient intakes.(314) Therefore, the reported ORs in our study may be overestimated without adjusting for psychosocial stress as a potential confouding factor. The validity of our study may be threatened and care should be taken in generalizing our results.

Another common issue encountered in the analysis of a cohort study is missing data. Missed visits and/or loss to follow up can be extremely problematic if missingness is related to the outcome and exposure of interest. In the INTAPP trial, approximately 4% of participants were lost to follow up in the Canadian arm and 20% were lost to follow up in the Mexican arm. Participants with missing outcomes due to withdrawal or loss to follow-up were excluded from the analysis. However, it is unlikely that loss to follow up was affected by treatment group or the study outcomes and thus it is unlikely to have greatly biased our risk estimations. We further compared the baseline characteristics of

women who were included in the final analysis of the INTAPP trial with that of women who were lost to follow up. There were no significant differences in most baseline characteristics. Canadian women included in the analysis had slightly higher maternal age and were less likely to smoke before pregnancy or during pregnancy compared to women who were lost to follow up. Mexican women included in the analysis had lower education level compared to Mexican women lost to follow up. It is also true that we have a small proportion of missing data with respect to several important covariates such as pre-pregnancy BMI and family history of PE or GH. However, the proportion of missind data with respect to these covariates was less than 5%. Our results indicated that baseline characteristics were comparable between two treatment groups (antioxidant versus placebo).

To assess the association between nutrient intakes during pregnancy and the risk of GH or PE, we analyzed nutrient intake data from a prospective pregnancy cohort of women enrolled in the INTAPP study. A total of 2336 women had complete and plausible FFQ data at the first study visit. Nonresponse is not an issue in the analyses using baseline diet only because only complete baseline dietary data were included in all analyses (n=2336). Approximately 80 percent of baseline population completed the repeated dietary questionnaires at the third trimester (n=1887). Analyses were conducted in this subgroup of population to assess whether nutrient intakes in late pregnancy or changes of nutrient intakes are associated with the risk of PE and GH. We found no associations between nutrient intakes in late pregnancy and changes of nutrient intakes from early to.

late pregnancy and the risks of GH and PE. The main multivariate analysis was on the basis of baseline diet only.

Several methods have been proposed for handling missing data in epidemiological studies such as multiple imputation and weighting methods like inverse probability weighted augmented (IPWA) estimating equations and doubly robust locally efficient IPWA methods. (315) In our study, participants with missing outcomes due to withdrawal or loss to follow-up were excluded from the analysis. It is unlikely that loss to follow up was affected by treatment group or the study outcomes and thus it is unlikely to have greatly biased our risk estimations. However, power in our study may further be compromised with such approach and external validity of our results may be threatened by missing data and care should be taken into consideration in the generalization of our results.

It is well known that the incidence odds ratio approximates the risk ratio (RR) when the disease of interest is rare, but increasingly overestimates the risk ratio as the disease becomes more common. The OR is a very convenient measure of effect with many appealing statistical properties including estimability in a case-control study. The cumulative incidences of GH and PE are approximately 20% and 5% respectively in our study. Thus, the ORs reported in our study tend to overestimate true RRs.

Nonetheless, our study had several notable strengths, including its large sample size, prospective design, and adjudicated PE outcome. The main study outcomes of PE and GH were carefully documented in the INTAPP trial Case

Report Forms (CRFs). Measurements and evaluations of the study outcomes were standardized across clinical centres. The diagnosis of GH and PE was further adjudicated by a team of three independent investigators. Unlike most investigations, our study assessed nutrient intakes in early pregnancy, which provides novel data in relation to PE. INTAPP participants were recruited from different regions with varying socio-demographic characteristics, which increases the generalizability of study results. On the other hand, results may only be generalized to women with those characteristics similar to those of study participants.

7.5 Recommendations and future directions

Encouraged by the positive results of a small pilot randomized trial by Chappell et al, (165) several large clinical trials, including the INTAPP trial, were subsequently conducted and all yielded negative results. The available evidence does not support the use of combined vitamin C and E supplementation during pregnancy for the prevention of PE. Furthermore, the safety of such supplementation, more specifically the effects on infant outcome (e.g. low birth weight), is still uncertain. At this stage, the supplementation of vitamin C and E during pregnancy should be discouraged. In the context of negative results from several trials, it is unlikely that further trials of Vitamins E will be conducted.. An important lesson here is that, additional evidence of biological plausibility of an effect and data on the dose-response is needed before undertaking a large-scale trial. Further studies are necessary required to investigate the potential adverse effects of an intervention, including both short and long-term effects.

It has been suggested that maternal characteristics at enrolment could affect the risk of PE as well as potentially modifying the effect estimate of studied treatment. The characterization of high-risk populations will permit the documentation of the nutritional profiles of these women. This may lead to new intervention strategies to prevent PE. Currently, studies are underway that aim to develop predictive models of PE. For example our research group was recently funded to develop a biochemical screening model based on circulating maternal pro- and antiangiogenic factors in combination with an endothelial injury marker (e.g. free vEGF, PlGF, cFN and endostatin).

More studies including both animal models and human studies are needed to better understand the etiology of PE. As knowledge regarding the underlying mechanism of PE advances, there will be opportunities to explore the impact of nutritional factors on specific causal pathways. Moreover, we found in our study that elevated γ-tocopherol was associated with the risk of PE. Further epidemiologic and intervention studies are needed to better understand the potential role of γ-tocopherol in the risk of PE. In addition, further studies are needed to confirm the association between potassium intake and the risk of PE reported in our study.

Studies exploring the role of maternal vitamin D status in adverse pregnancy outcomes are scarce. In our study, we did not find an association between vitamin D intake and the risk of PE. To date, only one observational study observed that there is an association between vitamin D status and the risk of PE. Further studies are necessary to unravel the association between vitamin D status

during pregnancy and adverse pregnancy outcomes.

We did not find an association between calcium intake or supplementation and the risk of PE or GH. Based on our review, calcium supplementation during pregnancy was found to significantly reduce the risk of PE for women at high risk and among those with low baseline dietary calcium intake. There was no evidence of a protective effect of prenatal calcium supplementation on adverse neonatal outcomes (e.g. preterm birth, neonatal death, IUGR). Further studies are required to substantiate the evidence that calcium supplementation during pregnancy significantly reduces the risk of perinatal mortality or morbidity without showing any long-term adverse effects. Current evidence suggests that calcium supplementation in pregnancy would appear to be justified, particularly in patients with low nutritional intake. However, additional data is required in terms of long-term effects as well as the optimal dose of supplementation.

Given the promising effect of folic acid on the risk of PE shown in previous observational studies, well-designed randomized controlled trials are urgently needed to assess the effect of folic acid supplementation during early pregnancy on PE. It seems that there is a limited time window to implement such a trial, as there is tendency that more and more pregnant women receive folic acid supplementation during early pregnancy. In addition, to prevent neural tube defects, commercial baking and pasta products have been fortified with folic acid since the late 1990s.

In summary, it is important to conduct well designed studies and obtain data on the following aspects: 1) delineation of the dose-response relationship of

important nutrient candidates (e.g. folate, Omega-3 fatty acids) with physiological markers of PE as well as with the risk of PE; 2) examination of the potential nutrient-nutrient, nutrient-environment (e.g. tobacco, alcohol and drug use) and nutrient-genetic interactions as well as elucidation of their interplay on the risk modifications of PE; and 3) identification of the critical time windows during which nutrient intake or supplementation may alter the risk of PE. As new knowledge emerges, we must recall that large randomized clinical trials of nutritional interventions to prevent PE are extremely costly and should be undertaken only when there is strong plausibility of a potential benefit of a novel intervention.

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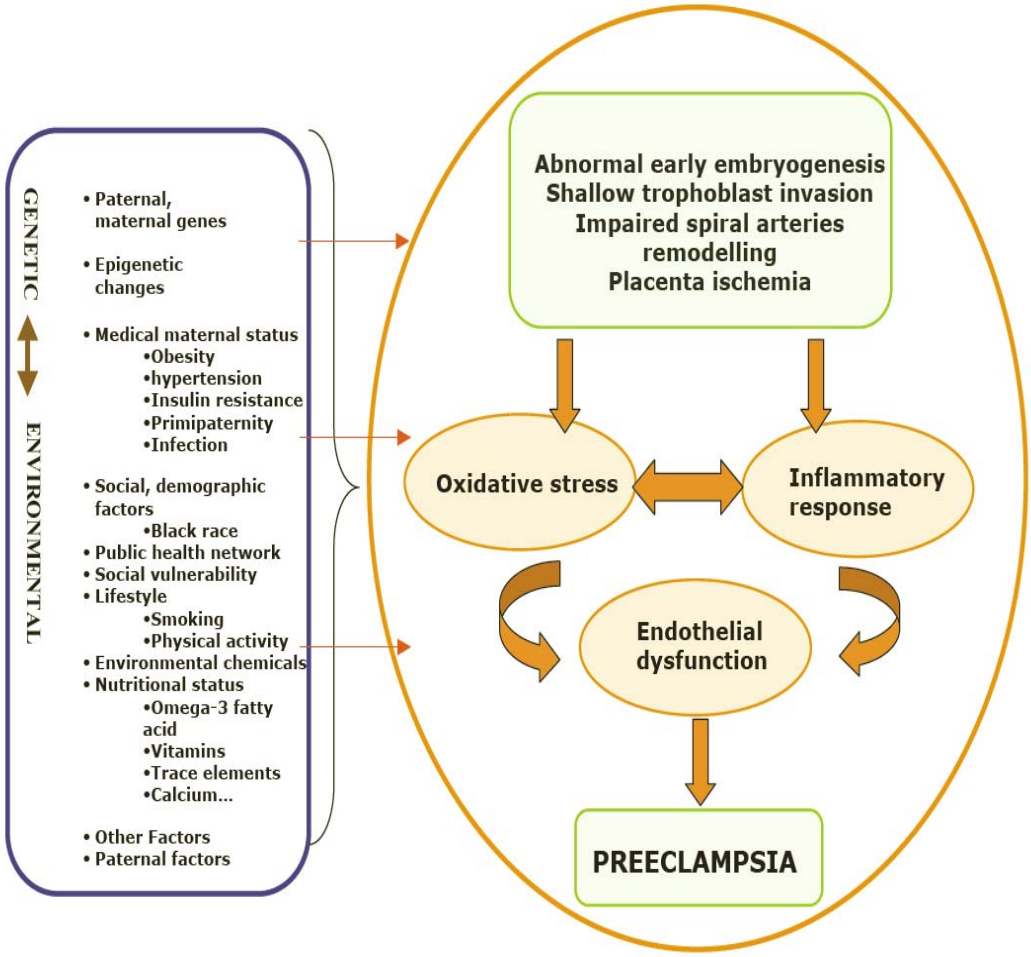
APPENDIX

Figure 1. Hypothetical framework on Pathogenesis of Preeclampsia



Xu H, Shatenstein B, Luo ZC, Wei S, Fraser W. Role of nutrition in the risk of preeclampsia. Nutr Rev 2009; 67(11): 639-57.

Figure 2. Multivariate analysis approach for nutrient intakes during pregnancy and the risk of GH and PE.

Force entry in each step: random assignment (treatment group and risk stratum)

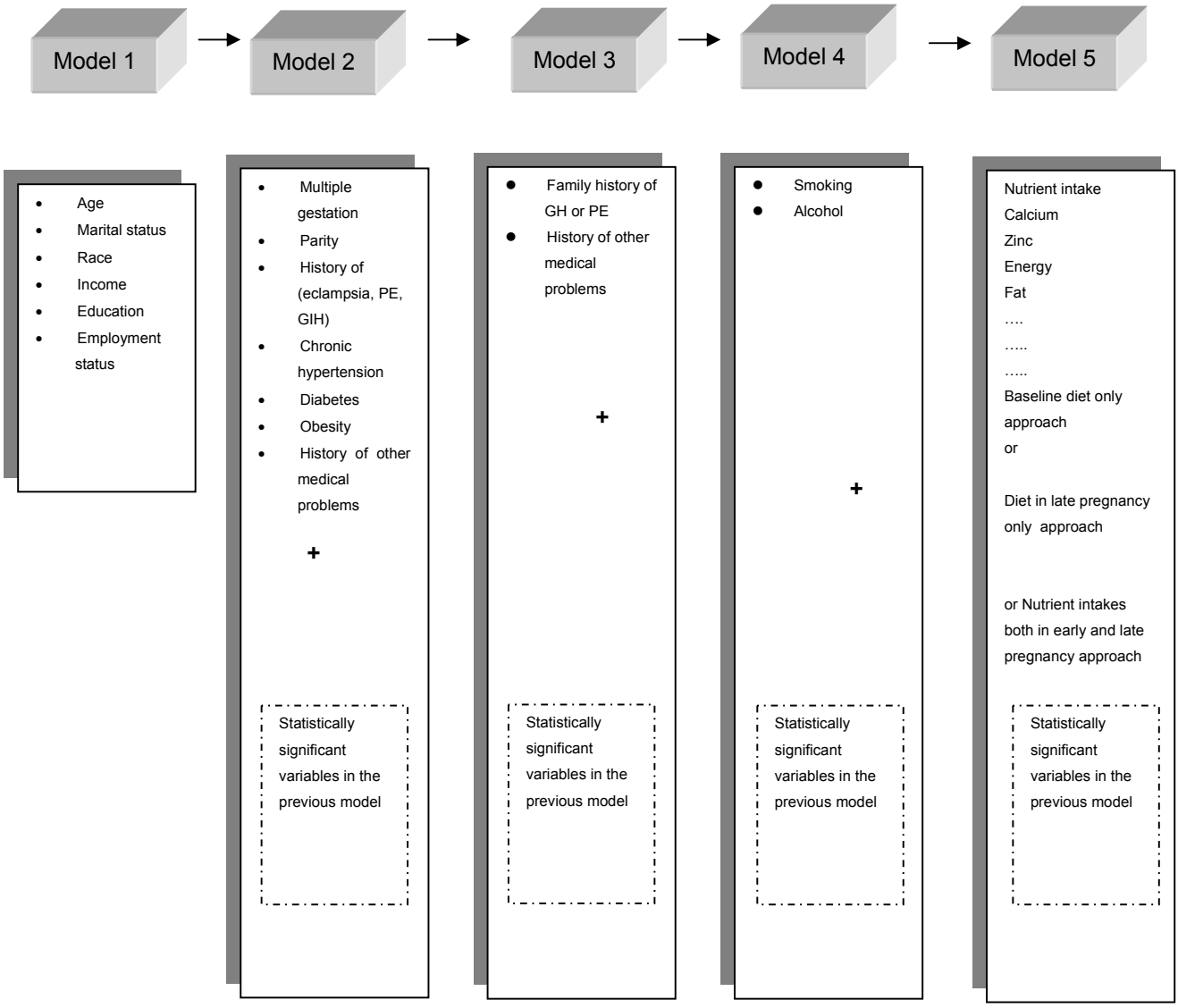


Figure 3. Analytical framework for the case control study of plasma tocopherol concentrations in relation to the risk of PE

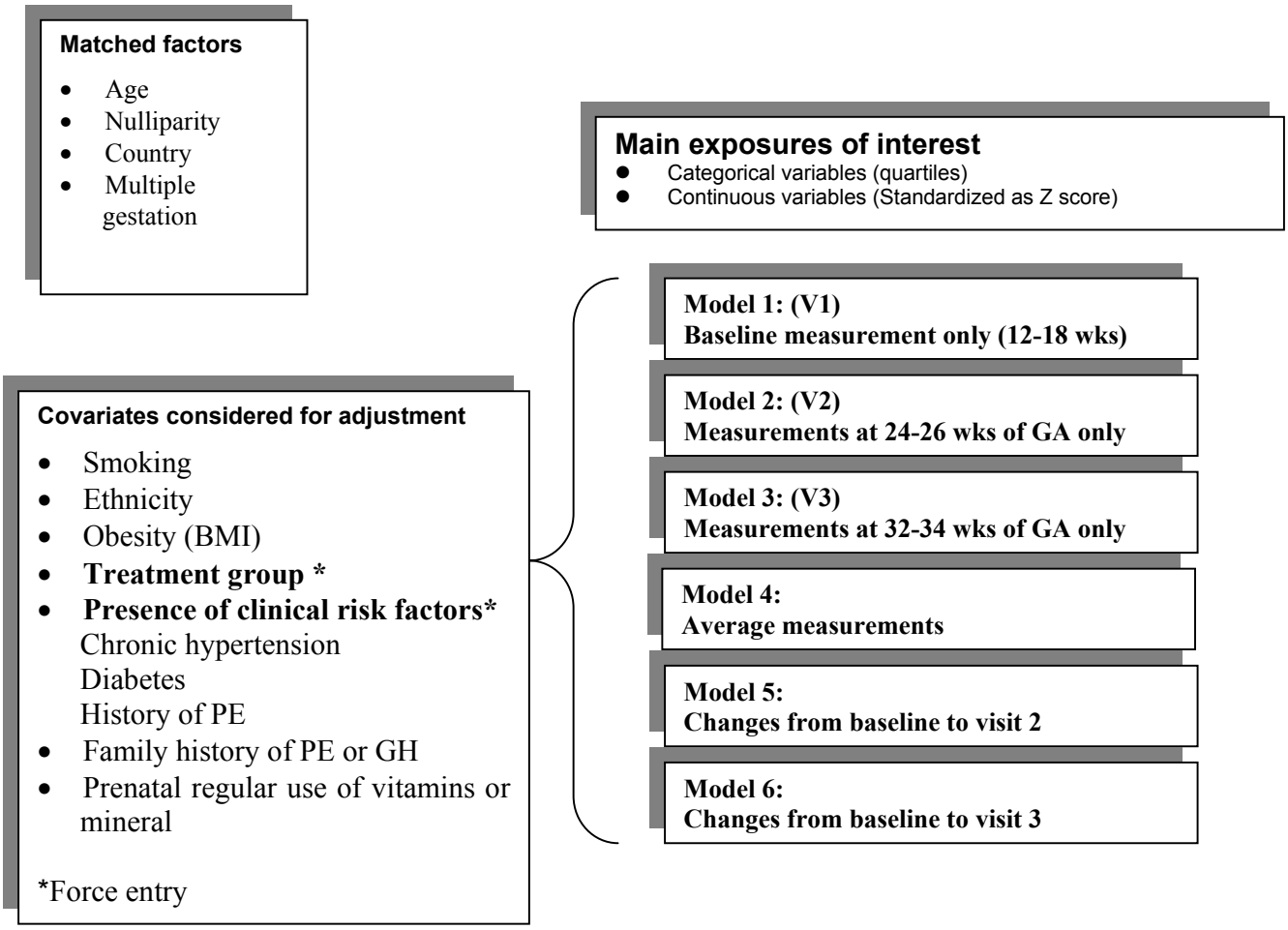


Table A: A summary of RCTs of certain micronutrient supplementations during pregnancy and the risk of Preeclampsia

Micronutrient	Intervention	Participants	Summary of findings
Calcium	Type: Calcium (dose varied from 1.5 to 2.0 grams) versus placebo; Initiation: varied from 18-22 weeks of gestation.	Most women were at low risk for PE and with low calcium intake	A total of 12 trials have been carried out. Most trials conducted in women with low calcium status showed the protective effect of calcium supplementation on the risk of PE. Most trials conducted in women with adequate calcium status failed to show any beneficial effects of calcium supplementation.[142]
Vitamin C only	Type: vitamin C (500mg daily) versus placebo; Initiation: less than 26 weeks of gestation	Women were at high risk for preterm birth (previous abortion or preterm birth)	Only one trial was conducted and found no reduced risk of PE associated with vitamin C supplementation.[294]
Vitamins C and E	Type: 1000mg vitamin C and 400 IU vitamin E daily versus placebo Initiation: Trial entry varied at each trial (18-22,14-20,14-21,12-20 of weeks of gestation)	Women at high and low risk for PE	A total of six trials evaluated the combined effects of vitamins C and E supplementation.[165, 167, 276, 277, 287, 288] Except the first trial by Chappell et al.[165]reported a significant reduction of PE associated with vitamins C and E supplementation, the following trials failed to provide evidence of beneficial effects of vitamins C and E.[167, 276, 277, 287, 288]

Table A. (Continued)

Micronutrient	Intervention	Participants	Summary of findings
Vitamin C, E, fish oil	Type: 500 mg vitamin C per day, 400 IU vitamin E per day, 1g fish oil three times per day and 100 mg aspirin three times a week versus placebo Initiation: Less than 29 weeks of gestation	Women at high risk for PE	Rivas et al. reported that there was a significant reduction of PE associated with supplementation of vitamins C, E and fish oil.[295]
Vitamin A	To date, no trial has been conducted to assess the effects of vitamin A on the risk of PE.		
Vitamin D	Evidence from trial is not available.		
Folic acid (folate)	Direct evidence from trials is not available. A re-analysis of a large RCT indicated that folate supplementation (200μg/day and 5mg/day versus placebo) was associated with a reduced risk of PE.[186]		

Table A. (Continued)

Micronutrient	Intervention	Participants	Summary of findings
Zinc	Type: zinc versus no zinc (dose varied from 20 mg to 44 mg daily) or placebo Initiation: trial entry varied at each trial (<20,<24, <26,<27,15-25 weeks of gestation)	Normal pregnant women with no systemic illness with normal and low zinc status	A total of seven trials were conducted including 5 trials in women with low zinc status and 2 trials of women with normal zinc status.[199] Only one trial conducted in women with low zinc status found that zinc supplementation significantly reduced the risk of PE.
Magnesium	Type: Magnesium (dose of 500 mg or 365 mg daily) versus control; Initiation: <4 months of gestation, 13-24 weeks of gestation)	Nulliparous and multiparous women	Two trials reported no reduction of PE associated with magnesium supplementation. However, methodological quality is questionable.[193]
Iron	Type: Iron (27 mg daily) versus placebo; Initiation: <13 weeks of gestation	Healthy pregnancy women	Eskeland et al. reported no reduction of PE associated with oral iron supplementation. However, the sample size is very small (N=90).[200]

Table B. Nutrients estimated by Food Frequency Questionnaire (FFQ) and average of three non-consecutive Food Records (3D-FR)[1] (FFQ validation study in Canada)

Nutrients	FFQ	Average of 3D-FR	Median differences in means (%)[2]
Energy (kcal)	1963±610 (1860)	2320±607 (2354)	-13.1
Carbohydrate (g)	237±83 (220)	298±97 (301)	-21.0
Total fat (g)	77±28 (72)	88±30 (85)	-14.3
Protein (g)	91±29 (87)	98±28 (97)	-7.1
Saturated fatty acids (g)	26±9.7 (24)	30±12 (29)	-11.6
Polyunsaturated fatty acids (g)	14±6.9 (12)	14±5.8 (14)	0.7
Monounsaturated fatty acids (g)	30±11.9 (28)	33±13.6 (31)	-10.3
Cholesterol (mg)	259±101 (248)	290±123 (273)	-13.1
Fibre (g)	17±6.9 (15)	22±11.7 (20)	-23.1
Calcium (mg)	1180±461 (1135)	1358±567 (1327)	-13.0
Iron (mg)	11.4±4.1 (10.9)	18.1±10.5 (16.7)	-31.8
Zinc (mg)	11.1±3.9 (10.6)	13.3±7.1 (12.1)	-12.8
Potassium (mg)	3231±1084 (3099)	3894±1333 (3800)	-16.6
Sodium (mg)	3006±1102 (2802)	3531±1206 (3414)	-18.6

Table B. Continued (FFQ validation study in Canada)

Nutrients	FFQ	Average of 3D-FR	Median differences in means (%)[2]
Magnesium (mg)	305±107 (283)	405±195 (372)	-20.8
Vitamin A (IU)	8544±4970 (7589)	14540±20001 (10452)	-27.3
Vitamin E (mg)	2.4±2.1 (1.9)	2.7±3.2 (1.8)	-4.4
Vitamin C (mg)	184±103 (161)	204±101 (192)	-10.2
Vitamin D (ug)	5.2±2.9 (5.1)	5.4±3.3 (4.7)	-10.7
Thiamine (mg)	1.6±0.6 (1.4)	2.1±0.9 (2.0)	-28.6
Riboflavin (mg)	1.9±0.7 (1.9)	2.5±0.9 (2.4)	-17.2
Niacin (mg)	18.9±7.1 (17.3)	23.3±7.3 (22.9)	-15.6
Vitamin B6 (mg)	2.1±0.7 (1.9)	2.3±1.3 (2.1)	-4.8
Folate (ug)	343±138 (318)	409±210 (371)	-15.5
Vitamin B12 (ug)	4.5±1.8 (4.3)	5.9±7.0 (4.5)	-11.9

1. Data presented as Mean±Standard Deviation (Median)
2. Determined as ([(FFQ-3DFR)/3DFR] *100)

Shatenstein B, Xu H, Luo Z-C, Fraser W. Relative validity of a food frequency questionnaire for Canadian pregnant women. Canadian Journal of Dietetic Practice and Research (accepted) 2010.

Table C. Association between nutrients estimated by Food Frequency Questionnaire (FFQ) and three non-consecutive Food Records (3D-FRs)- (FFQ validation study in Canada)

Nutrients	Spearman correlation coefficient (r)
Energy (kcal)	0.36**
Carbohydrate (g)	0.31**
Total fat (g)	0.41**
Protein (g)	0.44**
Saturated fatty acids (g)	0.45**
Polyunsaturated fatty acids (g)	0.35**
Monounsaturated fatty acids (g)	0.43**
Cholesterol (mg)	0.38***
Fibre (g)	0.41**
Calcium (mg)	0.46**
Iron (mg)	0.17
Zinc (mg)	0.37**
Potassium (mg)	0.45**
Sodium (mg)	0.19
Magnesium (mg)	0.45**
Vitamin A (IU)	0.30**

Shatenstein B, Xu H, Luo Z-C, Fraser W. Relative validity of a food frequency questionnaire for Canadian pregnant women. Canadian Journal of Dietetic Practice and Research (accepted) 2010.

Table C (Continued)

Nutrients	Spearman correlation coefficient (r)
Vitamin E (mg)	0.17
Vitamin C mg)	0.44**
Vitamin D (ug)	0.36*
Thiamine (mg)	0.19*
Riboflavin (mg)	0.39**
Niacin (mg)	0.41**
Vitamin B6 (mg)	0.34**
Folate (ug)	0.49**
Vitamin B12 (ug)	0.29**
Mean correlation (energy and 24 nutrients)	0.36

* $p<0.05$

** $p<0.01$

Shatenstein B, Xu H, Luo Z-C, Fraser W. Relative validity of a food frequency questionnaire for Canadian pregnant women. Canadian Journal of Dietetic Practice and Research (accepted) 2010.

Table D. Proportions (%) of participants ranked into the same quartile of the distribution according to nutrient estimates obtained from the Food Frequency Questionnaire (FFQ) and three non-consecutive Food Records (3D-FR) (FFQ validation study in Canada)

Nutrients	Identical quartile (%)	Identical and contiguous quartile (%)	Opposite quartile[1] (%)
Energy (kcal)	40	74	5
Carbohydrate (g)	36	71	7
Total fat (g)	37	74	5
Protein (g)	35	76	4
Saturated fatty acids (g)	37	83	3
Polyunsaturated fatty acids (g)	37	79	7
Monounsaturated fatty acids (g)	36	79	2
Cholesterol (mg)	39	78	5
Fibre (g)	25	76	0
Calcium (mg)	33	79	4
Iron (mg)	34	69	11
Zinc (mg)	34	74	4
Potassium (mg)	33	77	3
Sodium (mg)	35	72	9
Magnesium (mg)	37	79	6

Shatenstein B, Xu H, Luo Z-C, Fraser W. Relative validity of a food frequency questionnaire for Canadian pregnant women. Canadian Journal of Dietetic Practice and Research (accepted) 2010.

Table D (Continued)

Nutrients	Identical quartile (%)	Identical and contiguous quartile (%)	Opposite quartile[1] (%)
Vitamin A (IU)	34	71	6
Vitamin E (mg)	29	66	9
Vitamin C mg)	37	80	6
Vitamin D (ug)	38	78	7
Thiamine (mg)	34	72	11
Riboflavin (mg)	38	76	6
Niacin (mg)	33	78	6
Vitamin B6 (mg)	37	71	6
Folate (ug)	38	78	6
Vitamin B12 (ug)	31	66	6
Mean % classification (energy and 24 nutrients)	35	75	6

[1] Frank misclassification: lowest quartile in one method but classified as highest quartile in the other method.

Table E: Nutrients estimated by Food Frequency Questionnaire (FFQ) and three non-consecutive Food Recalls[1]

(FFQ validation study in Mexico)

Nutrients	FFQ (n=105)	1st Food Recall (n=86)	2nd Food Recall (n=86)	3rd Food Recall (n=72)	Average of Food Recalls (n=72)
Energy (kcals)	2098.0±545.0	2089.1± 778.0	1875.8± 709.6	2041.7± 771.1	2507.3±507.3
Carbohydrate (g)	264.6±78.5	289.6±78.5	250.6±120.5	263.8±107.7	267.1±74.0
Protein (g)	71.3±18.0	68.5±28.2	66.1±30.2	68.8±30.2	67.2±19.9
Total lipid (g)	89.1±27.2	77.1±39.1	70.6±30.7	83.2±43.7	77.6±27.1
Total fiber (g)	22.6±8.9	21.5±13.4	17.3±10.6	18.6±11.5	19.0±8.7
Calcium (mg)	1211.6±444.2	1110±762.9	976.6±532.6	1129.0±659.6	1077.0±444.5
Iron (mg)	11.4±3.3	15.3±17.8	12.1±13.6	16.8±18.9	14.9±11.1
Magnesium (mg)	325.3±96.0	298.2±167.2	280.5±177.8	287.2±143.1	281.3±102.2
Potassium(mg)	3245.1±996.8	2962.1±1381.6	2678.9±1806.2	2735.7±1596.5	2706.8±981.7
Zinc (mg)	8.6±2.1	9.5±7.1	7.6±3.4	8.6± 4.0	8.6±3.3
Vitamin A (IU)	6296.1±3560.3	5003.1±5463.1	5046.3±10246.1	6564.1±11640.7	5711.0±5896.3
Vitamin D (mcg)	26.9±15.4	51.1±94.7	42.3±67.5	66.0±217.6	39.4±82.2
Vitamin E (mg)	12.5±5.5	10.1±7.5	9.6±6.9	10.9±9.1	10.0±4.6
Vitamin C (mg)	231.7±124.0	30.8±497.4	164.8±172.8	165.4±148.1	214.1±199.8
Thiamine (mg)	1.3±0.4	1.4±1.1	1.1±0.7	1.5± 1.1	1.3±0.6
Riboflavin (mg)	1.8±0.6	1.8±1.1	1.6±1.0	2.0±1.4	1.8±0.7

1. Data presented as Mean (SD)

Table E. (Continued)[1]

Nutrients	FFQ (n=105)	1st Food Recall (n=86)	2nd Food Recall (n=86)	3rd Food Recall (n=72)	Average of Food Recalls (n=72)
Niacin (mg)	15.0±4.3	17.4±13.1	17.7±14.6	19.4±14.8	17.7±8.1
Pantotenic acid (mg)	5.5±1.8	4.7±2.2	4.7±2.6	4.7±1.6	4.7±1.6
Vitamin b6 (mg)	1.7±0.5	2.0±1.4	1.7±1.1	2.2±1.6	2.0±0.9
Folate(mcg)	348.7±113.8	396.1±306.9	306.4±202.5	405.7±303.0	371.3±171.1
Vitamin B12 (mg)	8.0±5.8	4.2±3.4	4.4±12.3	4.9±11.1	4.5±5.7
Saturated fatty acid (g)	32.4±10.7	29.9±16.2	26.5±12.8	33.0±19.1	30.1±11.2
Polyunsaturated fatty acid (g)	20.2±10.2	15.5±13.2	15.5±11.1	18.1±15.3	16.1±8.0
Monounsaturated fatty acid (g)	23.9±7.9	21.5±12.1	19.1±9.8	22.2±13.9	21.1±8.5

1. Data presented as Mean (SD)

Table F. Pearson's correlation coefficients between FFQ and the 24-hour recalls for energy and selected nutrients (FFQ validation study in Mexico)

Nutrients	Pearson's correlation coefficients			
	1st Food Recall	2nd Food Recall	3rd Food Recall	Average of Food Recalls
Energy (Kcals)	0.24 [1]	0.28 [2]	0.17	0.62 [2]
Carbohydrate (g)	0.23	0.25[1]	0.02	0.48 [2]
Protein (g)	0.16	0.06	0.37[2]	0.77 [2]
Total lipid (g)	0.13	0.30 [2]	0.28 [2]	0.75 [2]
Total fiber (g)	0.31[2]	0.28[2]	0.22	0.74[2]
Calcium (mg)	0.11	0.25[1]	0.15	0.60[2]
Iron (mg)	0.14	0.22[1]	0.71[2]	1.00
Zinc (mg)	0.24[1]	0.002	0.17	0.60[2]
Vitamin A (IU)	0.03	-0.02	0.01	0.72[2]
Vitamin E (mg)	0.01	0.16	0.06	0.632
Vitamin C (mg)	0.18	0.24[1]	0.11	0.25[1]

1. $P<0.05$
2. $P<0.01$

Table F. (Continued)

	Pearson's correlation coefficients			
Nutrients	**1st Food Recall**	**2nd Food Recall**	**3rd Food Recall**	**Average of Food Recalls**
Thiamine (mg)	0.14	0.005	-0.03	0.64[2]
Riboflavin (mg)	0.03	0.04	0.01	0.67[2]
Niacin (mg)	-0.01	-0.03	0.04	0.69[2]
Pantotenic acid (mg)	0.25[1]	0.18	0.20	0.69[2]
Vitamin B6 (mg)	0.16	0.04	0.14	0.71[2]
Folate (mcg)	0.11	0.00	-0.01	0.65[2]
Magnesium (mg)	0.23	0.09	0.16	0.74[2]
Potassium (mg)	0.23	0.08	0.33[2]	0.79[2]
Vitamin D (mcg)	-0.31[1]	0.61[2]	0.09	0.86[2]
Saturated fatty acid (mg)	0.31[2]	0.28[2]	0.21	0.73[2]
Vitamin B12 (mcg)	0.31	0.28[2]	0.21	0.73[2]
Monounsaturated fatty acid (g)	0.12	0.18	0.25[1]	0.76[2]
Polyunsaturated fatty acid (g)	-0.11	0.12	-0.08	0.64[2]

Table G. Proportions (%) of participants ranked into the same tertile of the distribution according to nutrient estimates obtained from the Food Frequency Questionnaire (FFQ) and three non-consecutive Food Recalls (FFQ validation study in Mexico)

	Identical tertiles (%)		
Nutrients	**2st Food Recall**	**2 nd Food Recall**	**3rd Food Recall**
Energy	53	35	45
Carbohydrate	52	36	43
Protein	41	33	33
Total lipid	50	53	43
Total fiber	59	33	74
Calcium	46	42	47
Iron	57	29	48
Magnesium	56	47	56
Potassium	59	39	28
Zinc	42	28	26
Vitamin A	50	39	32
Vitamin D	32	50	31
Vitamin E	38	30	39
Vitamin C	42	35	55
Thiamine	56	41	52
Riboflavin	41	43	40
Pantotenic acid	42	35	45
Vitamin B6	50	33	53
Folate	48	36	43
Vitamin B12	35	38	26
Saturated fatty acid	34	33	26

Table H : Baseline characteristics of women included in the analysis of INTAPP trial and women lost to follow up[1]

	Canada		Mexico	
Characteristics	**Lost[2]**	**Included[3]**	**Lost[2]**	**Included[3]**
Maternal age, y	28.35(6.03)	30.03(5.14)[4]	26.50(5.25)	26.02(5.21)
Maternal education (years)	15.06(3.20)	15.92(3.02)	12.73(3.27)	11.76(2.79)[4]
pre-pregnancy BMI	24.74(5.05)	25.53(6.27)	25.20(4.62)	25.33(4.79)
Gestational age, wk	14.98(1.88)	15.15(2.11)	15.32(2.09)	15.40(2.06)
Nulliparous	62(86.11)	1245(79.71)	173(84.39)	646(80.65)
Employed	54(75.0)	1288(82.62)	135(65.85)	513(64.13)
Smoking before pregnancy	30(41.67)	372(23.85)[4]	81(39.71)	298(37.25)
Current smoker	16(22.22)	151(9.77)[4]	5(2.45)	13(1.63)
Current drinker	4(5.56)	33(2.12)	2(0.98)	4(0.50)
Blood pressure				
Systolic	112.1(12.98)	113.1(12.81)	102.5(10.27)	101.3(10.64)
Diastolic	67.56(9.28)	68.56(9.18)	65.92(8.49)	65.19(8.36)
Dipstick proteinuria				
Normal or trace	68(100)	1487(97.64)	199(97.07)	759(94.76)
1+	0	33(2.17)	6(2.93)	40(4.99)
2+	0	3(0.20)	0	2(0.25)

1.Data presented as Mean (S.D.) or n (%); 2. Women who were lost to follow up or withdrew from the trial; 3. Women who were included in the final analysis of the INTAPP trial; 4. $P<0.05$

Table H. Continued[1]

Characteristics	Canada		Mexico	
	Lost [2]	Participants[3]	Lost[2]	Participants[3]
High risk group (stratum)	20(27.78)	499(31.95)	41(20.0)	185(23.10)
Chronic hypertension	6(8.33)	101(6.47)	10(4.88)	47(5.87)
Diabetes	6(8.33)	132(8.45)	5(2.44)	26(3.25)
Multiple pregnancy	5(6.94)	147(9.41)	6(2.93)	13(1.62)
History of preeclampsia	4(5.56)	185(11.84)	21(10.24)	110(13.73)
Multiple risk factors	1(1.39)	60(3.84)	1(0.49)	11(1.37)
Family history of PE, eclampsia or GH	5(6.94)	177(11.33)	30(14.63)	122(15.23)
Use of supplements	70(97.22)	1479(94.99)	179(87.32)	710(88.64)
Multivitamins	62(86.11)	1300(83.66)	49(23.90)	167(20.85)
Vitamin C	4(5.56)	23(1.48)[4]	0	7(0.88)
Vitamin E	3(4.17)	2(0.13)[4]	0	1 (0.12)
Folate	12(16.67)	470(30.26)[4]	148(72.20)	576(71.91)
Calcium	13(18.06)	250(16.10)	9(4.39)	77(9.61)
Iron	5(6.94)	57(3.67)	110(53.66)	486(60.75)

1.Data presented as Mean (S.D.) or n (%); 2. Women who were lost to follow up or withdrew from the trial; 3. Women who were included in the final analysis of the INTAPP trial; 4. $P<0.05$

Table I: The risk (cumulative incidence, %) of GH or PE according to quartile distributions of nutrient intakes estimated from FFQ administered at 12-18 weeks of gestational age[1]

	Canada		Mexico	
Characteristics	**PE**	**GH**	**PE**	**GH**
Protein				
1Q[2]	3.69	15.04	9.95	29.56
2Q	6.44	20.62	6.97	26.34
3Q	2.59	16.28	10.94	26.29
4Q	4.96	21.93	6.74	24.87
Chi-Square test P	NS	<0.05	NS	NS
Trend P	NS	NS	NS	NS
Lipoprotein				
1Q	3.60	19.79	10.19	26.79
2Q	6.25	19.27	4.76	28.50
3Q	3.40	15.93	9.18	27.98
4Q	4.46	18.90	9.48	23.86
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Carbohydrate				
1Q	4.45	19.37	5.73	22.96
2Q	4.42	17.88	10.00	30.41
3Q	5.22	19.32	9.55	26.50
4Q	3.63	17.36	9.22	27.27
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Fiber				
1Q	5.67	18.04	10.99	25.77
2Q	5.76	20.68	4.76	28.87
3Q	3.36	19.33	9.18	24.24
4Q	2.90	15.83	9.48	28.17
Chi-Square test P	NS	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Total cholesterol				
1Q	4.13	16.80	9.80	26.09
2Q	4.72	15.97	10.88	27.27
3Q	4.72	20.47	7.89	28.80
4Q	4.13	20.67	6.00	25.12
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1. Data only reflect food intake only, nutrient intakes from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table I- Continued [1]

	Canada		Mexico	
Characteristics	**PE**	**GH**	**PE**	**GH**
Saturated fatty acids				
1Q[2]	4.19	17.02	9.62	31.43
2Q	3.86	18.97	5.74	23.92
3Q	5.84	16.45	8.33	25.38
4Q	3.87	21.39	11.24	26.23
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Pantotenic acid				
1Q	5.68	18.35	9.13	28.10
2Q	5.51	16.54	6.32	24.35
3Q	2.93	16.71	7.46	27.94
4Q	3.57	22.19	11.70	26.56
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Monounsaturated fatty acid				
1Q	4.38	18.30	10.84	28.02
2Q	6.23	21.56	9.64	27.14
3Q	3.42	15.53	7.61	28.86
4Q	3.66	18.49	6.32	22.92
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Polyunsaturated fatty acid				
1Q	4.40	22.28	8.42	25.73
2Q	5.66	21.59	6.67	25.95
3Q	3.40	14.36	11.17	29.15
4Q	4.22	15.57	8.17	26.32
Chi-Square test P	NS	<0.05	NS	NS
Trend P	NS	<0.05	NS	NS
Calcium				
1Q	5.74	18.28	5.64	26.90
2Q	3.94	17.59	10.15	29.50
3Q	4.12	16.97	8.70	25.71
4Q	3.91	21.09	10.11	25.00
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1.Data only reflect food intake only, nutrient intakes from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table I- Continued [1]

Characteristics	Canada		Mexico	
	PE	GH	PE	GH
Iron				
1Q[2]	5.17	20.93	9.57	28.27
2Q	4.21	19.16	8.08	27.09
3Q	4.13	17.05	7.46	28.92
4Q	4.19	16.75	9.50	22.89
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Magnesium				
1Q	7.24	19.64	5.61	25.76
2Q	3.65	17.45	8.25	28.14
3Q	3.94	17.80	10.53	24.21
4Q	2.86	19.01	10.14	28.77
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Potassium				
1Q	7.79	18.70	9.66	29.05
2Q	4.19	18.54	6.63	25.00
3Q	2.58	16.02	6.77	24.87
4Q	3.14	20.68	11.46	28.06
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Sodium				
1Q	4.15	19.43	10.77	31.31
2Q	2.62	18.32	5.85	24.08
3Q	4.69	17.66	10.34	26.57
4Q	6.25	18.49	7.46	25.12
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Zinc				
1Q	6.08	18.52	8.08	27.86
2Q	5.43	16.28	9.50	27.36
3Q	2.35	17.71	9.18	25.76
4Q	3.87	21.39	7.77	26.13
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS

1. Data only reflect food intake only, nutrient intakes from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table I- Continued [1]

	Canada		Mexico	
Characteristics	**PE**	**GH**	**PE**	**GH**
Vitamin A				
1Q[2]	6.91	19.18	5.70	21.94
2Q	4.26	18.09	10.34	30.24
3Q	3.35	18.25	10.36	30.30
4Q	3.15	18.37	8.08	24.50
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Vitamin C				
1Q	6.81	18.85	9.23	27.36
2Q	3.94	19.37	7.73	25.51
3Q	4.37	19.28	7.04	25.13
4Q	2.60	16.41	10.55	29.06
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Vitamin E				
1Q	5.77	19.16	10.40	28.99
2Q	5.91	18.46	6.70	23.98
3Q	3.58	17.90	8.65	26.98
4Q	2.40	18.40	8.74	27.05
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Vitamin D				
1Q	4.47	19.18	10.71	25.00
2Q	3.90	18.09	9.19	26.84
3Q	4.69	18.25	6.97	30.69
4Q	4.65	18.37	7.80	24.64
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Vitamin B6				
1Q	5.74	16.71	8.81	28.43
2Q	5.18	20.67	5.21	27.41
3Q	4.20	17.32	11.44	27.94
4Q	2.59	19.17	8.96	23.38
Chi-Square test P	NS	NS	NS	NS
Trend P	<0.05	NS	NS	NS

1. Data only reflect food intake only, nutrient intakes from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table I- Continued [1]

	Canada		Mexico	
Characteristics	**PE**	**GH**	**PE**	**GH**
Vitamin B12				
1Q[2]	5.51	16.80	9.45	26.60
2Q	4.13	18.35	7.25	23.84
3Q	4.44	19.79	8.21	27.41
4Q	3.64	18.96	9.60	30.20
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Folate				
1Q	6.84	21.05	6.91	28.65
2Q	4.83	18.02	8.02	26.18
3Q	2.58	13.95	10.42	27.18
4Q	3.46	21.01	9.09	25.34
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Thiamine				
1Q	4.97	17.49	9.69	29.50
2Q	6.25	21.09	5.73	26.29
3Q	4.42	17.92	9.14	27.59
4Q	2.08	17.40	9.90	23.76
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Riboflavin				
1Q	5.25	17.32	7.88	30.58
2Q	5.48	16.97	7.14	26.37
3Q	2.87	18.23	8.90	24.23
4Q	4.11	21.34	10.66	25.76
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Niacine				
1Q	4.68	17.66	8.42	27.32
2Q	4.95	18.23	7.04	28.22
3Q	4.38	19.02	10.84	28.50
4Q	3.69	19.00	8.21	22.96
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1. Data only reflect food intake only, nutrient intakes from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table J: Unadjusted Odds ratios of dietary nutrients intake in association with preeclampsia (PE) and gestational hypertension (GH) in Canadian and Mexican pregnancy cohorts[1] (FFQ administered at 12-18 weeks of gestational age)

	Canada: OR (95%CI)		Mexico: OR (95%CI)	
Characteristics	**PE**	**GH**	**PE**	**GH**
Protein				
1Q[2]	0.74(0.36-1.49)	0.63(0.44-0.91)[1]	1.53(0.74-3.17)	1.27(0.82-1.97)
2Q	1.32(0.71-2.44)	0.93(0.66-1.31)	1.04(0.47-2.27)	1.08(0.69-1.69)
3Q	0.51(0.23-1.11)	0.69(0.48-0.99)	1.70(0.83-3.50)	1.08(0.68-1.70)
Lipoprotein				
1Q	0.80(0.39-1.65)	1.06(0.74-1.52)	1.89(0.89-4.03)	1.17(0.75-1.83)
2Q	1.43(0.75-2.70)	1.02(0.71-1.47)	1.26(0.56-2.85)	1.27(0.81-1.99)
3Q	0.75(0.36-1.58)	0.81(0.56-1.18)	2.21(1.04-4.69)	1.24(0.79-1.95)
Carbohydrate				
1Q	1.24(0.60-2.55)	1.14(0.79-1.65)	0.60(0.28-1.29)	0.80(0.51-1.25)
2Q	1.23(0.60-2.53)	1.04(0.72-1.50)	1.09(0.56-2.14)	1.17(0.76-1.79)
3Q	1.46(0.73-2.94)	1.14(0.79-1.64)	1.04(0.53-2.03)	0.96(0.62-1.49)
Fiber				
1Q	2.01(0.96-4.21)	1.17(0.80-1.71)	1.18(0.62-2.25)	0.89(0.57-1.37)
2Q	2.04(0.98-4.28)	1.39(0.96-2.01)	0.48(0.21-1.08)	1.04(0.67-1.59)
3Q	1.16(0.51-2.63)	1.27(0.88-1.85)	0.97(0.50-1.89)	0.82(0.53-1.27)
Cholesterol				
1Q	1.00(0.49-2.03)	0.78(0.54-1.11)	1.70(0.81-3.58)	1.05(0.68-1.64)
2Q	1.15(0.58-2.29)	0.73(0.51-1.05)	1.91(0.91-4.00)	1.12(0.72-1.75)
3Q	1.15(0.58-2.29)	0.99(0.70-1.40)	1.34(0.61-2.95)	1.21(0.77-1.88)

1. Data only reflect food intake only, intake from supplements not included. 2. Quartile distributions of nutrient intakes.

Table J-Continued[1]

	Canada: OR (95%CI)		Mexico: OR (95%CI)	
Characteristics	**PE**	**GH**	**PE**	**GH**
Saturated fatty acids				
1Q[2]	1.09(0.53-2.23)	0.75(0.53-1.08)	0.84(0.44-1.62)	1.29(0.83-2.00)
2Q	1.00(0.48-2.07)	0.86(0.61-1.22)	0.48(0.23-1.01)	0.88(0.56-1.40)
3Q	1.54(0.79-3.02)	0.72(0.50-1.04)	0.72(0.36-1.43)	0.96(0.60-1.52)
Pantotenic acid				
1Q	1.63(0.82-3.23)	0.79(0.56-1.12)	0.76(0.40-1.45)	1.08(0.70-1.68)
2Q	1.58(0.79-3.15)	0.69(0.48-1.00)	0.51(0.24-1.06)	0.89(0.56-1.40)
3Q	0.81(0.37-1.82)	0.70(0.49-1.01)	0.61(0.31-1.21)	1.07(0.69-1.67)
Monounsaturated fatty acid				
1Q	1.21(0.59-2.49)	0.99(0.69-1.42)	1.80(0.87-3.75)	1.31(0.83-2.06)
2Q	1.75(0.89-3.44)	1.21(0.85-1.73)	1.58(0.75-3.36)	1.25(0.79-1.98)
3Q	0.90(0.43-2.01)	0.81(0.56-1.18)	1.22(0.56-2.69)	1.36(0.87-2.15)
Polyunsaturated fatty acid				
1Q	1.05(0.52-2.10)	1.56(1.08-2.24)	1.03(0.51-2.08)	0.97(0.63-1.50)
2Q	1.36(0.70-2.63)	1.49(1.03-2.16)	0.80(0.37-1.73)	0.98(0.63-1.54)
3Q	0.80(0.38-1.69)	0.91(0.61-1.36)	1.41(0.73-2.75)	1.15(0.75-1.78)
Calcium				
1Q	1.50(0.77-2.94)	0.84(0.59-1.20)	0.53(0.25-1.15)	1.10(0.70-1.74)
2Q	1.01(0.49-2.09)	0.80(0.56-1.14)	1.01(0.52-1.95)	1.26(0.80-1.96)
3Q	1.06(0.52-2.17)	0.76(0.53-1.10)	0.85(0.43-1.67)	1.04(0.66-1.63)

1. Data only reflect food intake only, intake from supplements not included. 2. Quartile distributions of nutrient intakes

Table J-Continued [1]

Characteristics	Canada: OR (95%CI)		Mexico: OR (95%CI)	
	PE	GH	PE	GH
Iron				
1Q[2]	1.25(0.64-2.44)	1.32(0.91-1.89)	1.01(0.51-1.99)	1.33(0.84-2.09)
2Q	1.01(0.50-2.04)	1.18(0.81-1.71)	0.84(0.42-1.68)	1.25(0.80-1.97)
3Q	0.99(0.49-2.00)	1.02(0.70-1.49)	0.77(0.38-1.56)	1.37(0.88-2.14)
Magnesium				
1Q	2.65(1.30-5.39)[3]	1.04(0.73-1.49)	0.53(0.25-1.12)	0.86(0.56-1.33)
2Q	1.28(0.58-2.86)	0.90(0.62-1.30)	0.80(0.40-1.58)	0.97(0.63-1.49)
3Q	1.39(0.63-3.07)	0.92(0.64-1.33)	1.04(0.55-1.99)	0.79(0.51-1.24)
Potassium				
1Q	2.61(1.31-5.17)[3]	0.88(0.62-1.26)	0.83(0.44-1.57)	1.05(0.68-1.62)
2Q	1.35(0.63-2.89)	0.87(0.61-1.25)	0.55(0.27-1.12)	0.86(0.55-1.34)
3Q	0.82(0.35-1.92)	0.73(0.51-1.06)	0.56(0.27-1.15)	0.85(0.54-1.33)
Sodium				
1Q	0.65(0.34-1.24)	1.06(0.74-1.53)	1.50(0.75-3.00)	1.36(0.88-2.10)
2Q	0.40(0.19-0.86)[3]	0.99(0.69-1.43)	0.77(0.35-1.72)	0.95(0.60-1.50)
3Q	0.74(0.39-1.38)	0.95(0.66-1.37)	1.43(0.72-2.86)	1.08(0.69-1.68)
Zinc				
1Q	1.61(0.83-3.14)	0.84(0.59-1.19)	1.04(0.50-2.17)	1.09(0.70-1.70)
2Q	1.43(0.72-2.81)	0.72(0.50-1.03)	1.25(0.61-2.53)	1.07(0.68-1.66)
3Q	0.60(0.26-1.38)	0.79(0.55-1.13)	1.20(0.59-2.46)	0.98(0.63-1.54)

1. Data only reflect food intake only, intake from supplements not included.
2. Quartile distributions of nutrient intakes
3. $P<0.05$

Table J-Continued [1]

Characteristics	Canada: OR (95%CI)		Mexico: OR (95%CI)	
	PE	GH	PE	GH
Vitamin A				
1Q [2]	2.28(1.14-4.57)[3]	1.05(0.74-1.51)	0.69(0.31-1.52)	0.87(0.54-1.38)
2Q	1.37(0.64-2.93)	0.98(0.68-1.42)	1.31(0.66-2.60)	1.34(0.86-2.07)
3Q	1.07(0.48-2.37)	0.99(0.69-1.43)	1.32(0.66-2.62)	1.34(0.86-2.09)
Vitamin C				
1Q	2.73(1.30-5.75)[3]	1.18(0.82-1.72)	0.86(0.44-1.67)	0.92(0.60-1.42)
2Q	1.53(0.68-3.46)	1.22(0.85-1.77)	0.71(0.36-1.42)	0.84(0.54-1.30)
3Q	1.71(0.77-3.78)	1.22(0.84-1.76)	0.64(0.32-1.30)	0.82(0.53-1.27)
Vitamin E				
1Q	2.49(1.13-5.49)[3]	1.05(0.73-1.51)	1.21(0.63-2.35)	1.10(0.72-1.69)
2Q	2.56(1.17-5.60)[3]	1.00(0.70-1.45)	0.75(0.36-1.58)	0.85(0.54-1.33)
3Q	1.51(0.65-3.53)	0.97(0.67-1.40)	0.99(0.49-2.00)	1.00(0.64-1.55)
Vitamin D				
1Q	0.96(0.49-1.89)	0.79(0.56-1.13)	1.42(0.72-2.80)	1.02(0.65-1.60)
2Q	0.83(0.41-1.67)	0.66(0.46-0.95)	1.20(0.59-2.44)	1.12(0.72-1.76)
3Q	1.01(0.52-1.97)	0.74(0.52-1.06)	0.89(0.42-1.86)	1.36(0.88-2.09)
Vitamin B6				
1Q	2.29(1.07-4.91)[3]	0.85(0.59-1.22)	0.98(0.49-1.97)	1.30(0.83-2.04)
2Q	2.06(0.95-4.45)	1.10(0.77-1.56)	0.56(0.25-1.24)	1.24(0.79-1.95)
3Q	1.65(0.74-3.68)	0.88(0.61-1.28)	1.31(0.69-2.52)	1.27(0.81-1.99)

1. Data only reflect food intake only, intake from supplements not included.
2. Quartile distributions of nutrient intakes
3. $P<0.05$

Table J-Continued [1]

Characteristics	Canada: OR (95%CI)		Mexico: OR (95%CI)	
	PE	GH	PE	GH
Vitamin B12				
1Q[2]	1.55(0.77-3.09)	0.86(0.60-1.25)	0.98(0.50-1.92)	0.84(0.54-1.29)
2Q	1.14(0.55-2.38)	0.96(0.67-1.38)	0.74(0.36-1.52)	0.68(0.44-1.07)
3Q	1.23(0.60-2.53)	1.06(0.74-1.51)	0.84(0.42-1.69)	0.87(0.57-1.35)
Folate				
1Q	2.05(1.04-4.06)[3]	1.00(0.71-1.42)	0.74(0.36-1.54)	1.18(0.77-1.83)
2Q	1.42(0.69-2.92)	0.83(0.58-1.18)	0.87(0.43-1.76)	1.05(0.67-1.63)
3Q	0.74(0.32-1.71)	0.61(0.42-0.89)	1.16(0.61-2.23)	1.10(0.71-1.70)
Thiamine				
1Q	2.47(1.07-5.70)[3]	1.01(0.69-1.46)	0.98(0.50-1.89)	1.34(0.86-2.09)
2Q	3.14(1.39-7.08)[3]	1.27(0.89-1.82)	0.55(0.26-1.19)	1.14(0.73-1.80)
3Q	2.18(0.92-5.11)	1.04(0.72-1.50)	0.92 (0.47-1.79)	1.22(0.78-1.91)
Riboflavin				
1Q	1.29(0.66-2.53)	0.77(0.54-1.11)	0.72(0.36-1.42)	1.27(0.82-1.96)
2Q	1.35(0.70-2.63)	0.75(0.53-1.08)	0.65(0.32-1.31)	1.03(0.66-1.61)
3Q	0.69(0.32-1.51)	0.82(0.58-1.17)	0.82(0.42-1.60)	0.92(0.58-1.46)
Niacine				
1Q	1.28 (0.63-2.61)	0.92(0.63-1.32)	1.03(0.50-2.12)	1.26(0.80-2.00)
2Q	1.36 (0.67-2.75)	0.95(0.66-1.37)	0.85(0.40-1.79)	1.32(0.84-2.07)
3Q	1.20(0.58-2.46)	1.00(0.70-1.44)	1.36(0.69-2.68)	1.34(0.85-2.10)

1. Data only reflect food intake only, intake from supplements not included.
2. Quartile distributions of nutrient intakes
3. $P<0.05$

Table K: The risk (cumulative incidence, %) of GH or PE according to quartile distributions of nutrient intakes estimated from FFQ administered at 32-34 weeks of gestational age[1,3]

	Canada		Mexico	
Characteristics	**PE**	**GH**	**PE**	**GH**
Protein				
1Q[2]	5.32	20.60	7.14	23.84
2Q	4.92	15.74	11.45	29.34
3Q	3.28	12.13	10.32	32.08
4Q	4.26	23.53	6.47	23.26
Chi-Square test P	NS	<0.05	NS	NS
Trend P	NS	NS	NS	NS
Lipoprotein				
1Q	4.97	18.21	8.67	26.44
2Q	7.28	18.48	11.11	31.29
3Q	2.92	19.16	6.79	23.81
4Q	2.63	16.12	8.64	26.67
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Carbohydrate				
1Q	2.93	19.22	9.09	26.79
2Q	3.29	17.05	5.59	22.42
3Q	6.23	18.36	11.73	31.52
4Q	5.33	17.33	8.77	27.33
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Fiber				
1Q	4.89	17.92	7.59	27.50
2Q	5.88	21.90	8.02	23.49
3Q	3.68	16.33	11.24	26.16
4Q	3.29	15.79	8.24	30.81
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Total cholesterol				
1Q	3.96	16.83	7.74	25.00
2Q	5.67	16.67	10.78	24.85
3Q	4.89	17.86	10.24	30.00
4Q	3.27	20.59	6.33	28.22
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1. Data only reflect food intake only, intake from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table K-Continued [1,3]

Characteristics	Canada		Mexico	
	PE	**GH**	**PE**	**GH**
Saturated fatty acids				
1Q[2]	5.90	17.70	8.29	27.47
2Q	3.31	16.17	11.25	27.16
3Q	3.97	18.21	7.55	29.27
4Q	4.56	19.87	8.18	24.07
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Pantotenic acid				
1Q	3.28	15.74	4.71	23.56
2Q	5.26	16.45	8.75	23.46
3Q	5.26	18.75	14.65	34.16
4Q	3.96	21.05	7.56	27.17
Chi-Square test P	NS	NS	<0.05	NS
Trend P	NS	NS	NS	NS
Monounsaturated fatty acid				
1Q	5.96	18.54	10.00	22.81
2Q	6.27	18.75	8.93	31.18
3Q	2.61	18.57	9.26	26.19
4Q	2.96	16.12	6.92	27.95
Chi-Square test P	<0.05	NS	<0.05	NS
Trend P	<0.05	NS	<0.05	NS
Polyunsaturated fatty acid				
1Q	3.63	17.16	7.74	24.85
2Q	5.63	19.21	8.28	27.95
3Q	4.92	16.99	9.43	27.61
4Q	3.59	18.63	9.71	27.68
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Calcium				
1Q	4.28	13.16	6.55	26.32
2Q	6.91	20.72	10.00	29.70
3Q	3.29	20.00	10.37	28.14
4Q	3.29	18.09	8.38	23.95
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1. Data only reflect food intake only, intake from supplements not included; 2.Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table K-Continued [1,3]

Characteristics	Canada		Mexico	
	PE	**GH**	**PE**	**GH**
Iron				
1Q[2]	3.27	18.63	8.28	26.74
2Q	5.57	19.67	9.20	27.88
3Q	4.67	13.62	10.98	29.17
4Q	4.26	20.00	6.75	24.24
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Magnesium				
1Q	5.84	19.28	6.75	25.75
2Q	5.21	17.76	8.92	26.09
3Q	3.59	17.76	10.30	28.31
4Q	2.95	17.16	9.20	27.84
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Potassium				
1Q	5.57	19.67	7.23	26.74
2Q	3.92	16.34	6.79	23.93
3Q	4.95	19.41	12.12	29.76
4Q	3.31	16.56	9.04	27.54
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Sodium				
1Q	3.92	17.97	10.00	28.65
2Q	3.31	16.17	12.42	29.94
3Q	3.96	16.83	5.36	25.58
4Q	6.56	20.98	7.74	24.12
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Zinc				
1Q	4.61	16.45	8.75	28.66
2Q	6.93	20.13	9.58	25.88
3Q	3.62	15.13	10.84	28.57
4Q	2.62	20.26	6.02	25.00
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1. Data only reflect food intake only, intake from supplements not included; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table K-Continued [1,3]

Characteristics	Canada		Mexico	
	PE	GH	PE	GH
Vitamin A				
1Q[2]	5.59	17.43	6.83	23.64
2Q	5.26	17.76	10.90	28.48
3Q	5.54	20.52	8.09	27.01
4Q	1.33	16.23	9.47	28.90
Chi-Square test P	<0.05	NS	NS	NS
Trend P	<0.05	NS	NS	NS
Vitamin C				
1Q	4.93	19.74	8.43	25.88
2Q	3.96	16.50	7.41	28.31
3Q	5.56	19.54	9.64	27.38
4Q	3.30	16.17	9.70	26.51
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Vitamin E				
1Q	4.59	19.02	6.17	27.27
2Q	4.92	16.07	11.66	28.05
3Q	4.95	17.76	7.50	26.06
4Q	3.30	19.14	9.77	26.70
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Vitamin D				
1Q	2.60	12.99	9.41	26.74
2Q	6.62	18.54	11.80	29.70
3Q	4.97	20.20	9.38	23.31
4Q	3.62	20.33	4.76	28.24
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	<0.05	NS	NS
Vitamin B6				
1Q	4.59	20.66	5.56	23.03
2Q	4.58	18.63	15.00	36.59
3Q	5.02	19.00	7.69	25.58
4Q	3.59	13.75	7.14	23.08
Chi-Square test P	NS	NS	<0.05	NS
Trend P	NS	NS	<0.05	NS

1. Data only reflect food intake only, intake from supplements not included.; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table K-Continued [1,3]

	Canada		Mexico	
Characteristics	**PE**	**GH**	**PE**	**GH**
Vitamin B12				
1Q[2]	3.97	15.56	6.43	24.71
2Q	6.56	20.66	10.97	27.67
3Q	2.95	14.10	7.78	23.81
4Q	4.28	21.64	10.24	31.95
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Folate				
1Q	4.28	18.42	7.05	28.40
2Q	6.21	18.63	8.54	27.71
3Q	4.30	19.14	10.43	26.83
4Q	2.96	15.79	9.09	25.28
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Thiamine				
1Q	4.26	16.72	5.39	22.81
2Q	5.26	19.02	14.91	34.76
3Q	4.92	20.98	9.82	27.88
4Q	3.31	15.23	5.36	22.94
Chi-Square test P	NS	NS	<0.05	<0.05
Trend P	NS	NS	NS	NS
Riboflavin				
1Q	4.25	15.03	8.82	27.75
2Q	5.94	17.49	10.83	31.48
3Q	3.96	21.38	8.43	23.35
4Q	3.62	18.09	7.23	25.60
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS
Niacine				
1Q	2.95	18.36	7.27	24.40
2Q	6.56	17.70	10.26	25.79
3Q	4.29	17.82	9.77	31.46
4Q	3.96	18.09	7.93	26.06
Chi-Square test P	NS	NS	NS	NS
Trend P	NS	NS	NS	NS

1. Data only reflect food intake only, intake from supplements not included.; 2. Quartile distributions of nutrient intakes; 3. Data presented as risk (%)

Table L. Unadjusted Odds ratios of changes in nutrient intakes (standardized as Z score) in association with preeclampsia (PE) and gestational hypertension (GH) in Canadian and Mexican cohorts [1]

	Canada: OR (95%CI)		Mexico: OR (95%CI)	
Characteristics	**PE**	**GH**	**PE**	**GH**
Z-scores				
Protein	0.91(0.66-1.25)	0.94(0.81-1.11)	0.82(0.59-1.14)	0.92(0.75-1.13)
Total carbohydrate	0.94(0.70-1.27)	0.96(0.82-1.12)	0.80(0.57-1.12)	0.96(0.78-1.19)
Total lipid	0.89(0.65-1.21)	0.95(0.81-1.12)	0.99(0.72-1.37)	1.02(0.83-1.26)
Total fiber	0.99(0.72-1.35)	0.94(0.80-1.11)	1.05(0.77-1.42)	1.05(0.86-1.28)
Total cholesterol	1.01(0.75-1.36)	1.00(0.85-1.17)	0.78(0.55-1.12)	0.95(0.78-1.17)
Calcium	0.93(0.69-1.26)	0.96(0.82-1.12)	0.88(0.64-1.22)	0.96(0.79-1.17)
Iron	0.97(0.70-1.32)	0.99(0.85-1.17)	0.72(0.55-0.95)	1.00(0.82-1.21)
Magnesium	0.97(0.71-1.32)	0.92(0.78-1.08)	0.94(0.68-1.29)	0.98(0.80-1.20)
Potassium	1.04(0.77-1.42)	0.91(0.78-1.07)	0.91(0.66-1.26)	0.97(0.80-1.18)
Sodium	1.01(0.77-1.33)	1.04(0.88-1.21)	0.78(0.57-1.08)	0.99(0.81-1.21)
Zinc	0.91(0.67-1.26)	0.98(0.84-1.15)	0.72(0.51-1.02)	0.94(0.77-1.15)
Vitamin A	0.71(0.49-1.03)	1.03(0.88-1.21)	0.93(0.68-1.26)	1.11(0.91-1.36)

1. Data only reflect food intake only, intake from supplements not included.

Table L. Continued [1]

	Canada: OR (95%CI)		Mexico: OR (95%CI)	
Characteristics	**PE**	**GH**	**PE**	**GH**
Z-scores				
Vitamin C	1.02(0.74-1.41)	0.92(0.78-1.09)	0.94(0.69-1.29)	1.01(0.84-1.23)
Vitamin E	0.91(0.65-1.29)	0.97(0.82-1.14)	1.13(0.84-1.52)	1.05(0.86-1.29)
Vitamin D	0.99(0.73-1.33)	1.01(0.87-1.17)	0.84(0.63-1.13)	1.08(0.91-1.29)
Vitamin B6	1.04(0.77-1.41)	0.92(0.78-1.08)	0.73(0.55-0.97)	0.99(0.81-1.20)
Vitamin B12	0.98(0.71-1.37)	1.00(0.86-1.17)	1.03(0.76-1.40)	1.05(0.87-1.27)
Folate	1.10(0.82-1.48)	0.98(0.84-1.15)	0.75(0.55-1.02)	0.98(0.80-1.20)
Thiamine	0.99(0.72-1.35)	0.93(0.79-1.10)	0.70(0.53-0.92)	0.99(0.81-1.20)
Riboflavin	0.97(0.72-1.33)	0.95(0.81-1.11)	0.69(0.51-0.92)	0.99(0.81-1.20)
Niacin	1.02(0.74-1.42)	0.96(0.82-1.13)	0.72(0.54-0.95)	0.98(0.80-1.19)
Pantotenic acid	1.07(0.78-1.44)	0.98(0.84-1.14)	0.87(0.63-1.19)	0.99(0.82-1.21)
Saturated fatty acid	0.91(0.68-1.23)	0.93(0.80-1.09)	0.82(0.60-1.13)	0.91(0.75-1.11)
Monounsaturated fatty acid	0.88(0.64-1.19)	0.97(0.83-1.14)	0.89(0.64-1.24)	1.02(0.83-1.26)
Polyunsaturated fatty acid	0.96(0.71-1.31)	1.01(0.86-1.20)	1.15(0.86-1.54)	1.11(0.91-1.34)

1. Data only reflect food intake only, intake from supplements not included.

9.1 FOOD FREQUENCY QUESTIONNAIRE

Canadian FFQ (English, French)

Mexican FFQ (Spanish)

INTAPP
International Trial
of Antioxidants for the Prevention
of Preeclampsia

Évaluation de votre alimentation

Nous aimerions connaître votre alimentation habituelle au cours des 3 derniers mois. L'alimentation habituelle comprend les aliments consommés à la maison, au travail, au restaurant, en visite, les jours de semaine et de fin de semaine durant les repas et les collations. Nous voulons connaître votre alimentation et non pas une diète idéale, ni l'alimentation des autres membres de votre famille.

La plupart des questions auxquelles nous vous demandons de répondre ont plusieurs choix de réponses possibles. Choisissez celle qui vous convient le mieux. Répondez au meilleur de votre connaissance.

Nom de l'investigateur principal	
Numéro d'identification du patient	
Numéro de la visite	
Date de la visite (JJ/MM/AAAA)	

Instructions for completing the food consumption form

1 Answer question 1 for the food in the first row by filling in the circle corresponding to your answer.

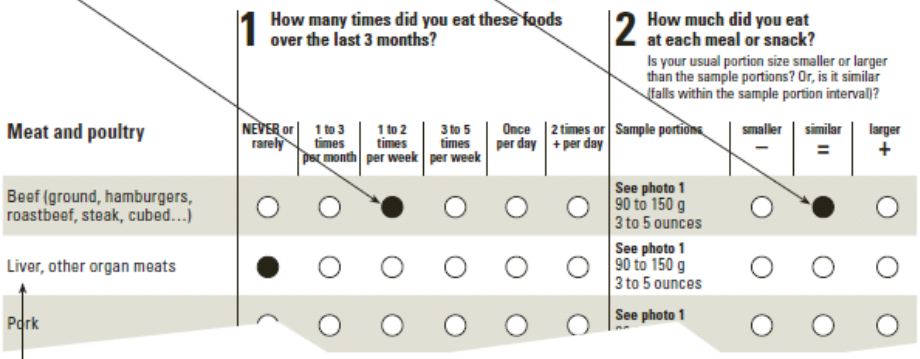
For example, if over the last 3 months you ate beef twice a week, you fill in the circle corresponding to the answer "1 to 2 times per week".

2 Then answer question 2 for the same food by filling in the circle corresponding to your answer.

For example, if at the same meal or snack you usually ate a 4-ounce portion of cooked meat, your serving falls within the sample portion "3 to 5 ounces". You should fill in the circle corresponding to the answer "similar".

Some foods have photos on the opposite page which will help you compare your usual serving size to the sample portions.

If you never ate a particular food or ate it only rarely, do not fill in any portion size for that food. The example below for liver and organ meats illustrates this tip.



3 Go to the food in the second row, and repeat steps 1 and 2 for this food.

Repeat for all foods.

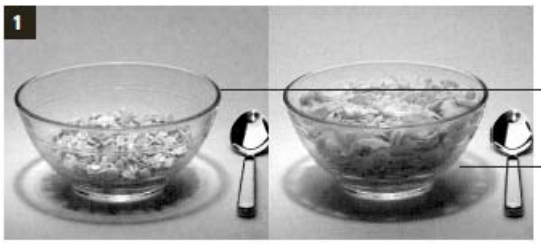
Note that it is important to put an answer to the question 1 at each line.

Don't forget to look at photos on the opposite page when it is written *See photo*.

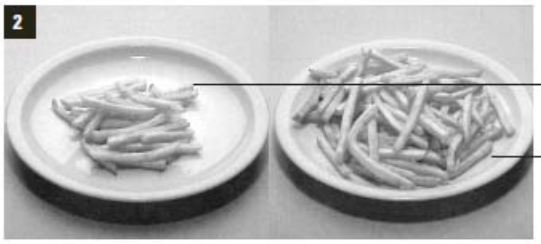
At the end of the questionnaire, you will find a brief checklist to help you verify whether you have completed the questionnaire adequately.

This questionnaire will be handled in strict confidence.

Thank you for your participation

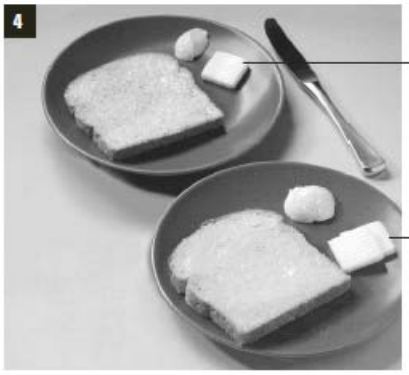


High fiber and other breakfast cereals
125 to 500 ml
½ to 2 cups



French fries or pan-fried potatoes
250 to 500 ml
1 to 2 cups
50 to 100 g

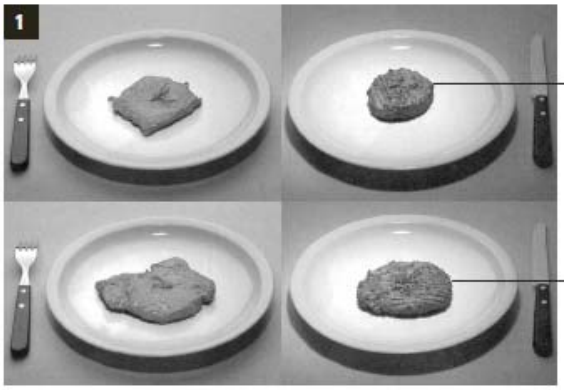
Rice, rice noodles, couscous
125 to 250 ml
½ to 1 cup



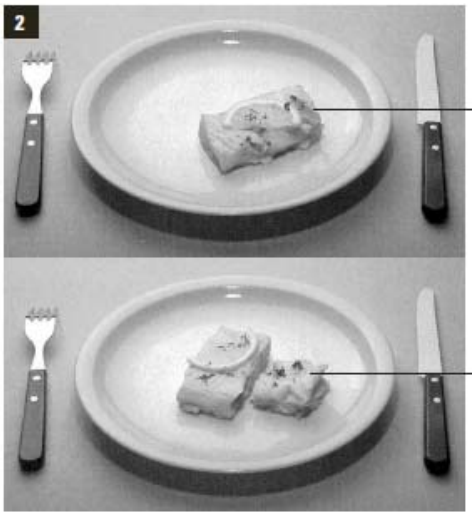
Margarine or butter on bread or on cooked vegetables
5 to 10 ml
1 to 2 full tsp
Note : consider the total quantity spread on all slices of bread eaten at the same meal.

Breads and cereals and related foods	1 How many times did you eat these foods over the last 3 months? NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	2 How much did you eat at each meal or snack? Is your usual portion size smaller or larger than the sample portions? Or, is it similar (falls within the sample portion interval)? Sample portions	smaller −	similar =	larger +
High fiber breakfast cereals (All Bran, 100% Bran, Bran Flakes…)	○	○	○	○	○	○	**See photo 1** 125 to 500 ml ½ to 2 cups	○	○	○
Other breakfast cereals, hot cereals	○	○	○	○	○	○	**See photo 1** 125 to 500 ml ½ to 2 cups	○	○	○
White or brown sugar added to cereals	○	○	○	○	○	○	5 ml 1 full tsp	○	○	○
Commercial sliced white breads	○	○	○	○	○	○	1 to 2 slices	○	○	○
Other white breads (bagels, pita, hamburger/hot dog rolls, tortillas, crusty bread…)	○	○	○	○	○	○	1 small bread, bagel, pita, etc.	○	○	○
Commercial sliced whole wheat breads, with bran, multigrain, rye bread	○	○	○	○	○	○	1 to 2 slices	○	○	○
Other whole wheat breads (bagels, pita, hamburger/hot dog rolls, tortillas, crusty bread…)	○	○	○	○	○	○	1 small bread, bagel, pita, etc.	○	○	○
Peanut butter	○	○	○	○	○	○	15 ml 1 full tbsp	○	○	○
Jam, honey, sweet spreads, maple products	○	○	○	○	○	○	5 to 25 ml 1 to 5 full tsp	○	○	○

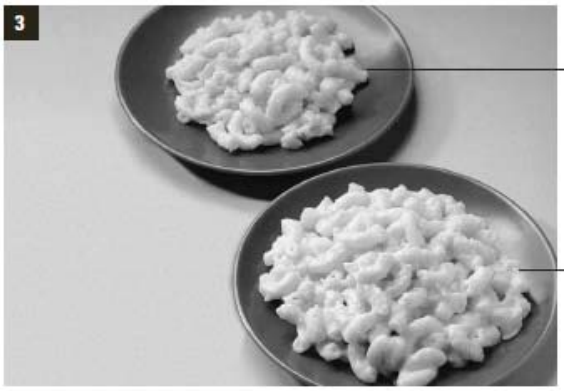
Soups, potatoes and rice	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Tomato or vegetable soups	○	○	○	○	○	○	250 ml 1 cup	○	○	○
Other soups	○	○	○	○	○	○	250 ml 1 cup	○	○	○
French fries or pan-fried potatoes	○	○	○	○	○	○	**See photo 2** 250 to 500 ml 1 to 2 cups	○	○	○
Boiled, mashed or baked potatoes	○	○	○	○	○	○	½ to 1 big potato 1 to 2 scoops	○	○	○
Rice, rice noodles, couscous	○	○	○	○	○	○	**See photo 3** 125 to 250 ml ½ to 1 cup	○	○	○
Margarine on bread or on cooked vegetables	○	○	○	○	○	○	**See photo 4** 5 to 10 ml 1 to 2 full tsp	○	○	○
Butter on bread or on cooked vegetables	○	○	○	○	○	○	**See photo 4** 5 to 10 ml 1 to 2 full tsp	○	○	○



Beef, liver and organ meats, pork, other meats
90 to 150 g cooked
3 to 5 ounces cooked



Fish
90 to 150 g cooked
3 to 5 ounces cooked



Pasta with tomato sauce with or without meat
Pasta with creamy sauce
250 to 500 ml cooked
1 to 2 cups cooked

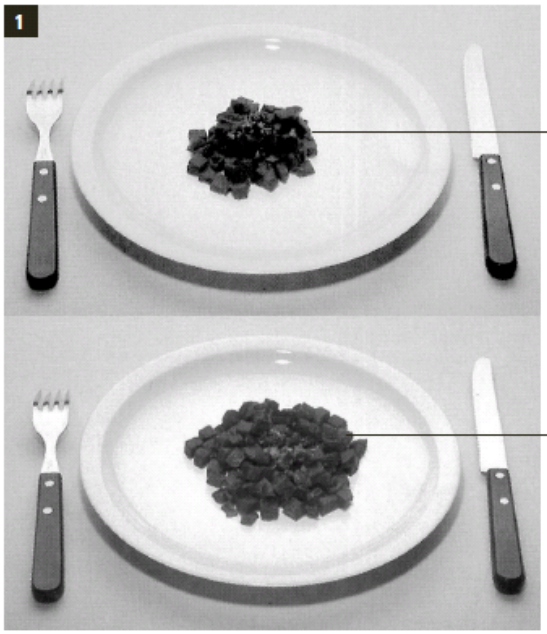
	1 How many times did you eat these foods over the last 3 months?						2 How much did you eat at each meal or snack? Is your usual portion size smaller or larger than the sample portions? Or, is it similar (falls within the sample portion interval)?			
Meat and poultry	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Beef (ground, hamburgers, roast beef, steak, cubed…)	○	○	○	○	○	○	**See photo 1** 90 to 150 g 3 to 5 ounces	○	○	○
Liver, other organ meats	○	○	○	○	○	○	**See photo 1** 90 to 150 g 3 to 5 ounces	○	○	○
Pork	○	○	○	○	○	○	**See photo 1** 90 to 150 g 3 to 5 ounces	○	○	○
Other meats (veal, lamb, game…)	○	○	○	○	○	○	**See photo 1** 90 to 150 g 3 to 5 ounces	○	○	○
Chicken, turkey	○	○	○	○	○	○	1 leg or 1 breast 125 to 250 ml ½ to 1 cup	○	○	○
Sausages, hot dogs	○	○	○	○	○	○	2 sausages or 2 hot dogs	○	○	○
Ham, cold cuts (smoked meat, bacon…)	○	○	○	○	○	○	2 to 3 slices	○	○	○
Sauces (brown, white, BBQ, gravy…)	○	○	○	○	○	○	10 to 50 ml 2 tsp to ¼ cup	○	○	○

Fish and seafood fresh, frozen, canned	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Salmon, trout, sardines, herring, tuna	○	○	○	○	○	○	90 to 150 g 3 to 5 ounces 1 can	○	○	○
Other fish (sole, cod, fish sticks…)	○	○	○	○	○	○	**See photo 2** 90 to 150 g 3 to 5 ounces	○	○	○
Seafood (shrimp, crab, oyster…)	○	○	○	○	○	○	60 to 90 g 2 to 3 ounces	○	○	○

Mixed dishes	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Pasta with tomato sauce with or without meat (spaghetti, lasagna…)	○	○	○	○	○	○	**See photo 3** 250 to 500 ml 1 to 2 cups	○	○	○
Pasta with creamy sauce (cheese macaroni, alfredo sauce, …)	○	○	○	○	○	○	**See photo 3** 250 to 500 ml 1 to 2 cups	○	○	○
Pizza	○	○	○	○	○	○	2 to 3 slices	○	○	○

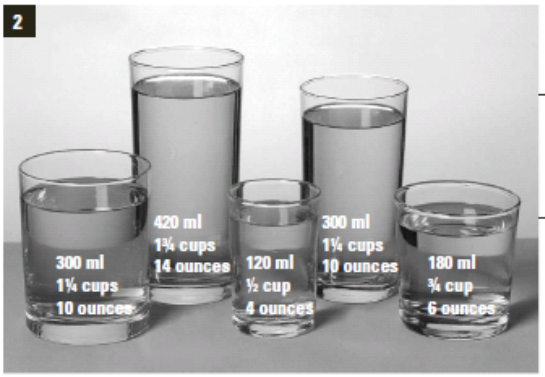
Some sample portion photos

1



Green/yellow beans, green peas, corn
Broccoli, cauliflower, cabbage, brussel sprouts
Carrots
Sweet peppers
All other vegetables
50 to 125 ml
¼ to ½ cup

2

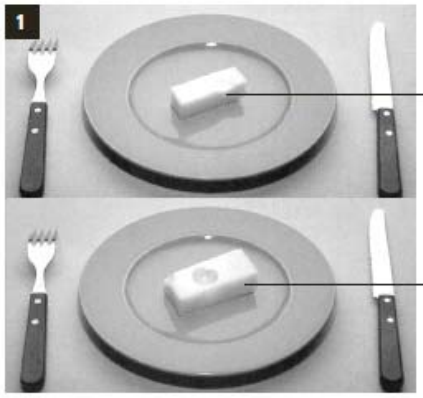


Refer to this photo to estimate your usual portion size of tomato or vegetable juices.

Tomato or vegetable juices
125 to 175 ml
½ to ¾ cup
4 to 6 ounces

	1 How many times did you eat these foods over the last 3 months?						2 How much did you eat at each meal or snack? Is your usual portion size smaller or larger than the sample portions? Or, is it similar (falls within the sample portion interval)?			
Vegetable protein foods and eggs	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Beans, peas, lentils (legumes), hummus, beans with pork	○	○	○	○	○	○	125 to 175 ml ½ to ¾ cup	○	○	○
Tofu and foods with soya or vegetable proteins	○	○	○	○	○	○	125 to 175 ml ½ to ¾ cup	○	○	○
Eggs, omelette, quiche…	○	○	○	○	○	○	2 large eggs 1 slice of quiche	○	○	○
Sunflower seeds	○	○	○	○	○	○	50 ml ¼ cup	○	○	○
Nuts, peanuts, other seeds	○	○	○	○	○	○	50 ml ¼ cup	○	○	○

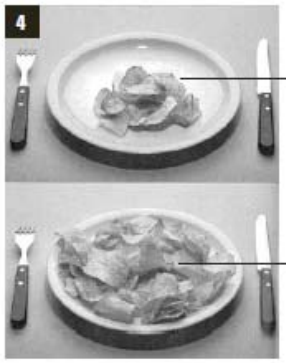
Vegetables–raw, frozen, canned, cooked (don't forget vegetables included in mixed meat, pasta dishes, ethnic foods…)	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Green/yellow beans, green peas, corn	○	○	○	○	○	○	**See photo 1** 50 to 125 ml ¼ to ½ cup	○	○	○
Tomatoes	○	○	○	○	○	○	½ to 1 tomato 50 to 125 ml ¼ to ½ cup	○	○	○
Tomato or vegetable juices	○	○	○	○	○	○	**See photo 2** 125 to 175 ml ½ to ¾ cup	○	○	○
Broccoli, cauliflower, brussel sprouts, cabbage and coleslaw	○	○	○	○	○	○	**See photo 1** 50 to 125 ml ¼ to ½ cup	○	○	○
Carrots	○	○	○	○	○	○	**See photo 1** ½ to 1 carrot 50 to 125 ml ¼ to ½ cup	○	○	○
Lettuce, leafy greens, spinach, green salad	○	○	○	○	○	○	250 ml 1 cup	○	○	○
Green, red, yellow sweet peppers	○	○	○	○	○	○	**See photo 1** 50 to 125 ml ¼ to ½ cup	○	○	○
All other vegetables different from those above	○	○	○	○	○	○	**See photo 1** 50 to 125 ml ¼ to ½ cup	○	○	○
Salad dressings, mayonnaise, dips	○	○	○	○	○	○	15 ml 1 tbsp	○	○	○



Cheeses
1 to 2 cubes of 2.5 cm (1 inch)
25 to 50 g
1 to 2 slices

Berries
All other fruits
125 ml
½ cup

Cakes, pies, doughnuts and pastries
1 slice
1 to 2 small cakes
1 doughnut
1 pastry

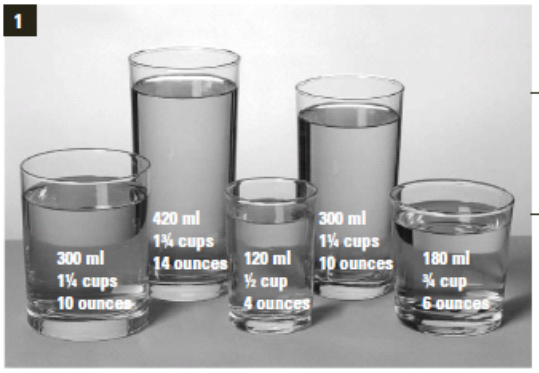


Salty snack foods
125 ml to 500 ml
½ to 2 cups

	1 How many times did you eat these foods over the last 3 months?						2 How much did you eat at each meal or snack? Is your usual portion size smaller or larger than the sample portions? Or, is it similar (falls within the sample portion interval)?			
Milk products	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Cheese	○	○	○	○	○	○	**See photo 1** 25 to 50 g 1 to 2 slices	○	○	○
Yogurt	○	○	○	○	○	○	125 to 175 ml ½ to ¾ cup	○	○	○
Milk-based desserts (puddings, blanc mange…)	○	○	○	○	○	○	125 to 175 ml ½ to ¾ cup	○	○	○
Ice cream, ice milk, frozen yogurt	○	○	○	○	○	○	1 scoop 125 to 175 ml ½ to ¾ cup	○	○	○
Fruits–fresh, canned, frozen, stewed **Do not include juices**	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Apples, pears	○	○	○	○	○	○	1 fruit	○	○	○
Bananas	○	○	○	○	○	○	1 banana	○	○	○
Melons (watermelon, cantaloupe, honeydew…)	○	○	○	○	○	○	125 ml to 250 ml ½ to 1 cup	○	○	○
Oranges, grapefruits, tangerines (citrus fruits)	○	○	○	○	○	○	1 orange ½ grapefruit	○	○	○
Berries (strawberries, raspberries, blueberries…)	○	○	○	○	○	○	**See photo 2** 125 ml ½ cup	○	○	○
Any fruits other than those specified above	○	○	○	○	○	○	**See photo 2** 125 ml ½ cup	○	○	○
Baked goods, sweets and salty snacks	NEVER or rarely	1 to 3 times per month	1 to 2 times per week	3 to 5 times per week	Once per day	2 times or + per day	Sample portions	smaller −	similar =	larger +
Cakes, pies, doughnuts, pastries	○	○	○	○	○	○	**See photo 3**	○	○	○
Muffins	○	○	○	○	○	○	1 muffin	○	○	○
Cookies	○	○	○	○	○	○	2 to 4 cookies	○	○	○
Granola bars, chewy bars, cereal bars	○	○	○	○	○	○	1 bar	○	○	○
Candies, chocolate	○	○	○	○	○	○	2 to 6 candies ½ chocolate bar	○	○	○
Salty snacks (chips, crackers, popcorn, pretzels…)	○	○	○	○	○	○	**See photo 4** 125 ml to 500 ml ½ to 2 cups 5 to 20 crackers	○	○	○

Some sample portion photos

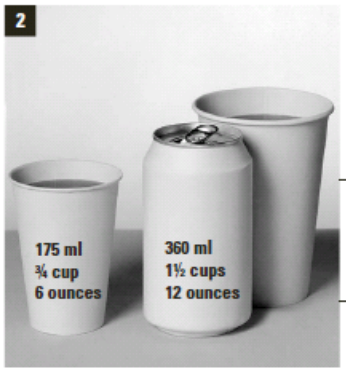
1



Refer to this photo to estimate your usual portion size of milk, soya drink, fruit juice, and fruit drinks

Milk
Fruit juices
Fruit drinks
125 to 250 ml
½ to 1 cup
4 to 8 ounces

2



Regular and diet soft drinks
175 to 360 ml
¾ to 1½ cups
6 to 12 ounces
1 can

Juices and drinks	1 How many times did you eat these foods over the last 3 months? NEVER or rarely	1 to 7 times per month	2 to 6 times per week	Once per day	2 to 3 times per day	4 times or + per day	2 How much did you drink at each meal or snack? Sample portions	smaller −	similar =	larger +
Fruit juices (no sugar added, 100% pure)	○	○	○	○	○	○	**See photo 1** 125 to 250 ml 4 to 8 ounces	○	○	○
Fruit drinks (sugar added: punch, cocktails, with artificial flavors, lemonade…)	○	○	○	○	○	○	**See photo 1** 175 to 325 ml 6 to 10 ounces	○	○	○
Regular soft drinks (cola, 7-Up…)	○	○	○	○	○	○	**See photo 2** 1 can 175 to 360 ml 6 to 12 ounces	○	○	○
Diet soft drinks (cola, 7-Up…)	○	○	○	○	○	○	**See photo 2** 1 can 175 to 360 ml 6 to 12 ounces	○	○	○

Milk and similar beverages (if you put a lot of milk in your coffee, count it with milk for drinking)	NEVER or rarely	1 to 7 times per month	2 to 6 times per week	Once per day	2 to 3 times per day	4 times or + per day	Sample portions	smaller −	similar =	larger +
Whole milk (3.25% m.f.) for drinking	○	○	○	○	○	○	**See photo 1** 125 to 250 ml 4 to 8 ounces	○	○	○
1%, 2% milk for drinking	○	○	○	○	○	○	**See photo 1** 125 to 250 ml 4 to 8 ounces	○	○	○
Skim milk for drinking	○	○	○	○	○	○	**See photo 1** 125 to 250 ml 4 to 8 ounces	○	○	○
Soya drink for drinking	○	○	○	○	○	○	**See photo 1** 125 to 250 ml 4 to 8 ounces	○	○	○

Coffee , alcoholic drinks and related items	NEVER or rarely	1 to 7 times per month	2 to 6 times per week	Once per day	2 to 3 times per day	4 times or + per day	Sample portions	smaller −	similar =	larger +
Coffee, tea	○	○	○	○	○	○	1 cup	○	○	○
Milk or cream in coffee/tea	○	○	○	○	○	○	1 milk/cream	○	○	○
Sugar in coffee/tea	○	○	○	○	○	○	1 sugar	○	○	○
Beer	○	○	○	○	○	○	1 regular size beer	○	○	○
Table wine, aperitifs	○	○	○	○	○	○	125 to 250 ml ½ to 1 cup 1 to 2 glasses	○	○	○
Spirits (hard liquor)	○	○	○	○	○	○	30 to 50 ml 1 to 2 ounces	○	○	○

Food habits and life style habits

1 What type of fat do you usually use in cooking?
Fill in the **two** most frequently used

- ○ Butter
- ○ Margarine
- ○ Low-calorie margarine
- ○ Olive oil
- ○ Canola oil
- ○ Corn oil
- ○ Sunflower oil
- ○ Other vegetable oils
- ○ Vegetable oil shortening
- ○ Lard
- ○ PAM
- ○ None of these choices
- ○ Don't know

2 How often do you use fat or oil in cooking?
Fill in your answer

- ○ Less than once per week
- ○ 1 to 2 times per week
- ○ 3 to 4 times per week
- ○ 5 to 6 times per week
- ○ Once per day
- ○ Twice per day
- ○ 3 times per day
- ○ Don't know

3 Among these particular foods, which ones do you eat several times per month?
Fill in only appropriate answers

- ○ Chicken skin
- ○ Fried chicken
- ○ Lean or extra-lean ground beef
- ○ Breaded fried fish
- ○ Low-calorie or oven-baked chips or crackers
- ○ Low-fat cheese
- ○ Fat-free yogurt (0.1% m.f.)
- ○ Low-calorie margarine
- ○ Low-fat or fat-free mayonnaise or salad dressings
- ○ Calcium-fortified juices
- ○ Decaffeinated coffee
- ○ Meal substitute (bars or beverages)
- ○ High energy or high protein bars or beverages

4 Are you on a special diet?
Fill in your answer

○ No ○ Yes

If yes, why?

_____
_____
_____
_____

5a What is your usual weight (before your pregnancy)?
_____ pounds or _____ kilos

5b What is your current weight?
_____ pounds or _____ kilos

6 How tall are you?
____ feet and _____ inches or ___ . _____ metres

7 How old are you? _____ years

8 Gender

- ○ Male
- ○ Female

Checklist

Please, be sure that…

1. All answers relate to your diet over the **last 3 months**.
2. **All** questions have been answered, including the section on your food habits.
3. Only **one** frequency and **one** portion have been filled in for each food. On the other hand, there **should not** be any portion if the frequency is "never or rarely".
4. You have clearly identified which is the right answer, if you made mistakes that you can't erase.
5. Any notes or comments (see next page) that you have added are clear and understandable.

If you have any comments or suggestions about this questionnaire, please note them on this page.

Thank you for completing this questionnaire. We would like to remind you that all your information will remain strictly confidential.

INTAPP
International Trial
of Antioxidants for the Prevention
of Preeclampsia

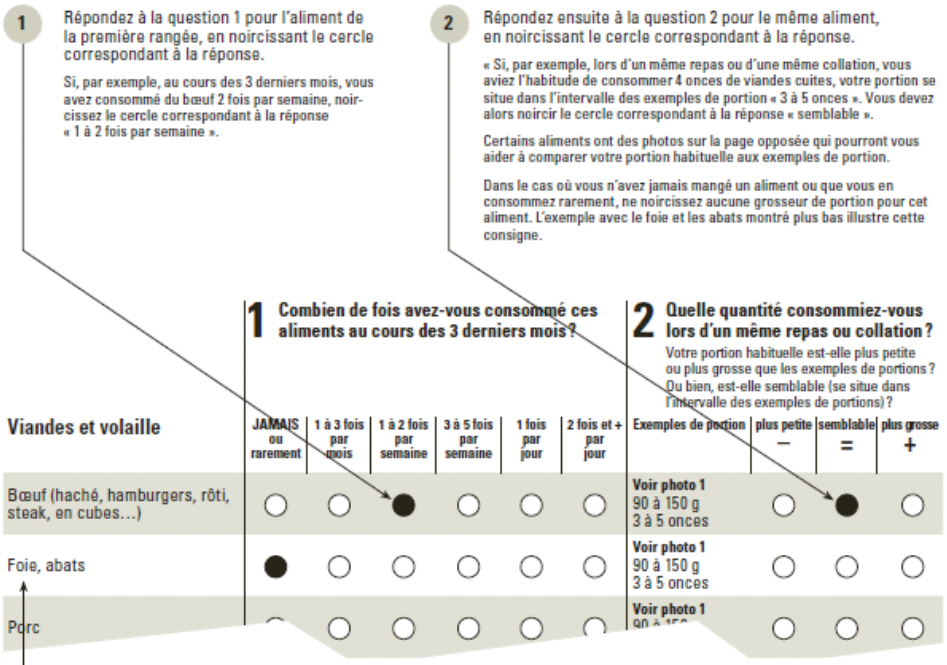
Évaluation de votre alimentation

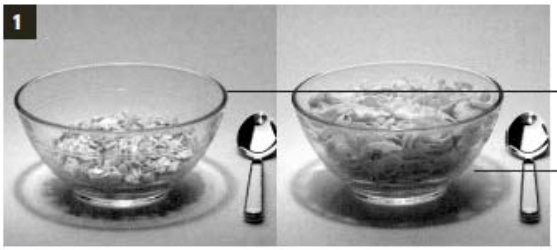
Nous aimerions connaître votre alimentation habituelle au cours des 3 derniers mois. L'alimentation habituelle comprend les aliments consommés à la maison, au travail, au restaurant, en visite, les jours de semaine et de fin de semaine durant les repas et les collations. Nous voulons connaître votre alimentation et non pas une diète idéale, ni l'alimentation des autres membres de votre famille.

La plupart des questions auxquelles nous vous demandons de répondre ont plusieurs choix de réponses possibles. Choisissez celle qui vous convient le mieux. Répondez au meilleur de votre connaissance.

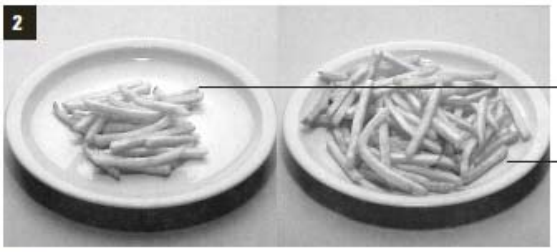
Nom de l'investigateur principal	
Numéro d'identification du patient	
Numéro de la visite	
Date de la visite (JJ/MM/AAAA)	

Instructions pour remplir le tableau de consommation d'aliments





Céréales à déjeuner riches en fibres et autres céréales à déjeuner
125 à 500 ml
½ à 2 tasses



Pommes de terre frites ou rissolées
250 à 500 ml
1 à 2 tasses
50 à 100 g

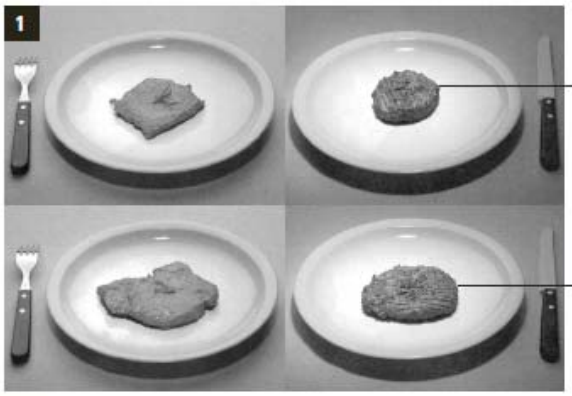
Riz, nouilles de riz, couscous
125 à 250 ml
½ à 1 tasse

Margarine ou beurre sur le pain ou sur les légumes cuits
5 à 10 ml
1 à 2 c.thé rases

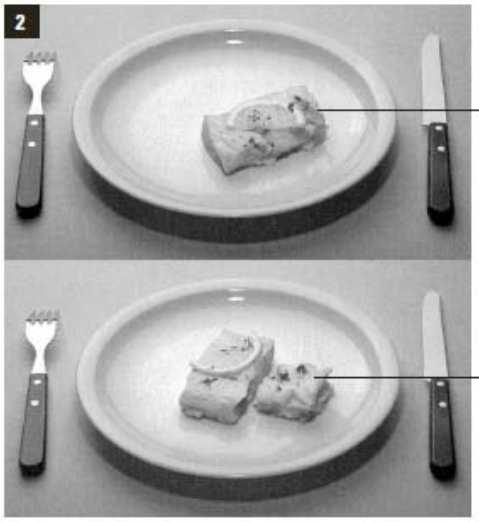
Note : Pensez à la quantité totale comprise sur toutes les tranches de pain mangées à un même repas.

Pains, céréales et accompagnements	1 Combien de fois avez-vous consommé ces aliments au cours des 3 derniers mois?						2 Quelle quantité consommiez-vous lors d'un même repas ou collation? Votre portion habituelle est-elle plus petite ou plus grosse que les exemples de portions? Ou bien, est-elle semblable (se situe dans l'intervalle des exemples de portions)?			
	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Céréales à déjeuner riches en fibres (All Bran, 100% son, Bran Flakes...)	○	○	○	○	○	○	**Voir photo 1** 125 à 500 ml ½ à 2 tasses	○	○	○
Autres céréales à déjeuner, céréales chaudes	○	○	○	○	○	○	**Voir photo 1** 125 à 500 ml ½ à 2 tasses	○	○	○
Sucre blanc ou brun ajouté aux céréales	○	○	○	○	○	○	5 ml 1 c.thé rase	○	○	○
Pains blancs commerciaux tranchés	○	○	○	○	○	○	1 à 2 tranches	○	○	○
Autres pains blancs (pain croûté, bagels, pitas, pains hamburgers/hot dog, tortillas)	○	○	○	○	○	○	1 petit pain, bagel, pita ou tortilla	○	○	○
Pains de blé entier, au son, multigrains, de seigle commerciaux tranchés	○	○	○	○	○	○	1 à 2 tranches	○	○	○
Autres pains de blé entier (pain croûté, pains hamburgers/hot dog, tortillas, bagels, pitas...)	○	○	○	○	○	○	1 petit pain, bagel, pita ou tortilla	○	○	○
Beurre d'arachides	○	○	○	○	○	○	15 ml 1 c.table rase	○	○	○
Confitures, miel, tartinades sucrées, produits de l'érable	○	○	○	○	○	○	5 à 25 ml 1 à 5 c.thé rases	○	○	○

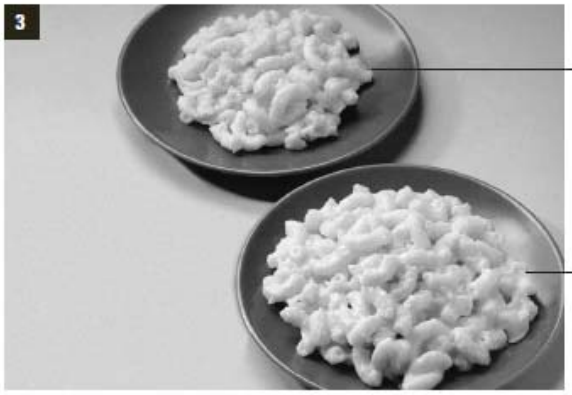
Soupes, pommes de terre et riz	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Soupes aux tomates ou aux légumes	○	○	○	○	○	○	250 ml 1 tasse	○	○	○
Autres soupes	○	○	○	○	○	○	250 ml 1 tasse	○	○	○
Pommes de terre frites ou rissolées	○	○	○	○	○	○	**Voir photo 2** 250 à 500 ml 1 à 2 tasses	○	○	○
Pommes de terre bouillies, pilées, au four	○	○	○	○	○	○	½ à 1 grosse pomme de terre 1 à 2 boules en purée	○	○	○
Riz, nouilles de riz, couscous	○	○	○	○	○	○	**Voir photo 3** 125 à 250 ml ½ à 1 tasse	○	○	○
Margarine sur le pain ou sur les légumes cuits	○	○	○	○	○	○	**Voir photo 4** 5 à 10 ml 1 à 2 c.thé rases	○	○	○
Beurre sur le pain ou sur les légumes cuits	○	○	○	○	○	○	**Voir photo 4** 5 à 10 ml 1 à 2 c.thé rases	○	○	○



Bœuf, foie et abats, porc, autres viandes
90 à 150 g cuit
3 à 5 onces cuit



Poissons
90 à 150 g cuits
3 à 5 onces cuits



Pâtes avec sauce tomate avec ou sans viande
Pâtes avec sauce crémeuse
250 à 500 ml cuites
1 à 2 tasses cuites

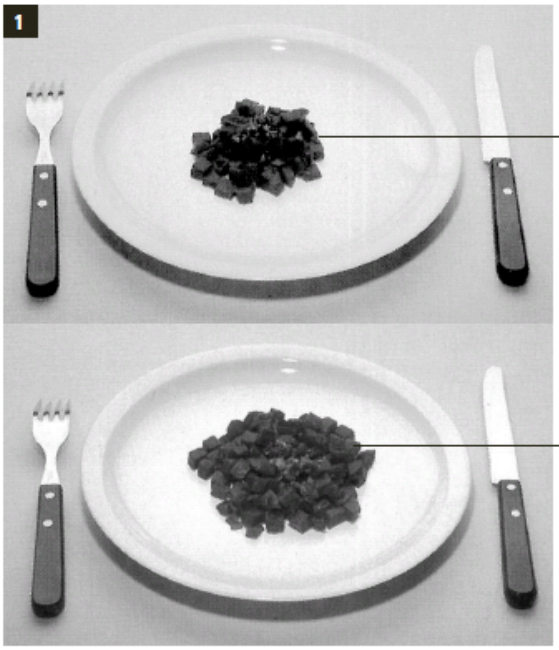
	1 Combien de fois avez-vous consommé ces aliments au cours des 3 derniers mois?						2 Quelle quantité consommiez-vous lors d'un même repas ou collation? Votre portion habituelle est-elle plus petite ou plus grosse que les exemples de portions? Ou bien, est-elle semblable (se situe dans l'intervalle des exemples de portions)?			
Viandes et volaille	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Bœuf (haché, hamburgers, rôti, steak, en cubes…)	○	○	○	○	○	○	**Voir photo 1** 90 à 150 g 3 à 5 onces	○	○	○
Foie, abats	○	○	○	○	○	○	**Voir photo 1** 90 à 150 g 3 à 5 onces	○	○	○
Porc	○	○	○	○	○	○	**Voir photo 1** 90 à 150 g 3 à 5 onces	○	○	○
Autres viandes (veau, agneau, gibier…)	○	○	○	○	○	○	**Voir photo 1** 90 à 150 g 3 à 5 onces	○	○	○
Poulet, dinde	○	○	○	○	○	○	1 cuisse ou 1 poitrine 125 à 250 ml ½ à 1 tasse	○	○	○
Saucisses, hot-dog	○	○	○	○	○	○	2 saucisses ou 2 hot-dog	○	○	○
Jambon, charcuteries (viandes froides ou fumées, bacon)	○	○	○	○	○	○	2 à 3 tranches	○	○	○
Sauces brunes, BBQ, blanches, jus de cuisson…	○	○	○	○	○	○	10 à 50 ml 2 c.thé à ¼ tasse	○	○	○

Poissons et fruits de mer frais, congelés, en boîte	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Saumon, truite, sardine, hareng, thon	○	○	○	○	○	○	90 à 150 g 3 à 5 onces 1 boîte	○	○	○
Autres poissons (sole, morue, en bâtonnets…)	○	○	○	○	○	○	**Voir photo 2** 90 à 150 g 3 à 5 onces	○	○	○
Fruits de mer (crevette, crabe, huîtres…)	○	○	○	○	○	○	60 à 90 g 2 à 3 onces	○	○	○

Mets composés	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Pâtes avec sauce tomate avec ou sans viande (spaghetti, lasagne…)	○	○	○	○	○	○	**Voir photo 3** 250 à 500 ml 1 à 2 tasses	○	○	○
Pâtes avec sauce crémeuse (macaroni au fromage, avec sauce alfredo, …)	○	○	○	○	○	○	**Voir photo 3** 250 à 500 ml 1 à 2 tasses	○	○	○
Pizza	○	○	○	○	○	○	2 à 3 pointes	○	○	○

Photos de quelques exemples de portions

1

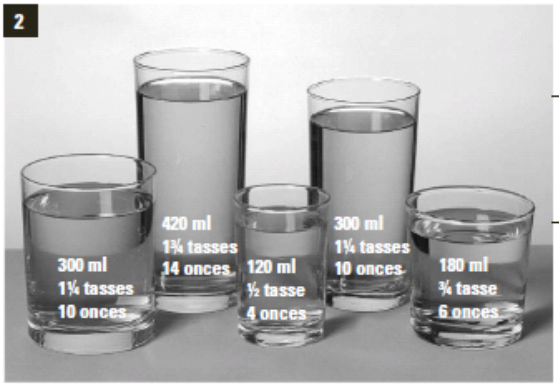


Haricots jaunes/verts, pois, maïs
Brocoli, chou-fleur, chou de bruxelle, chou
Carottes

Poivrons
Tous les autres légumes

50 à 125 ml
¼ à ½ tasse

2



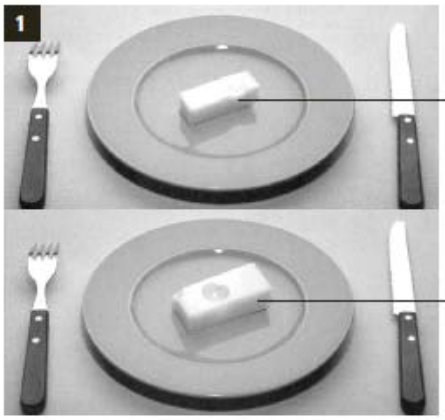
Référez-vous à cette photo pour estimer votre grosseur de portion habituelle de jus de légumes ou de tomates.

Jus de légumes ou de tomates

125 à 175 ml
½ à ¾ tasse
4 à 6 onces

Aliments sources de protéines végétales et œufs	**1** Combien de fois avez-vous consommé ces aliments au cours des 3 derniers mois ? JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	**2** Quelle quantité consommiez-vous lors d'un même repas ou collation ? Votre portion habituelle est-elle plus petite ou plus grosse que les exemples de portions ? Ou bien, est-elle semblable (se situe dans l'intervalle des exemples de portions) ? Exemples de portion	plus petite −	semblable =	plus grosse +
Haricots secs, pois secs, lentilles (légumineuses), hummus, fèves au lard	○	○	○	○	○	○	125 à 175 ml ½ à ¾ tasse	○	○	○
Tofu, aliments à base de soya ou de protéines végétales	○	○	○	○	○	○	125 à 175 ml ½ à ¾ tasse	○	○	○
Œufs, omelette, quiche, ...	○	○	○	○	○	○	2 gros œufs 1 pointe de quiche	○	○	○
Graines de tournesol	○	○	○	○	○	○	50 ml ¼ tasse	○	○	○
Noix, arachides, autres graines	○	○	○	○	○	○	50 ml ¼ tasse	○	○	○

Légumes frais, en conserve, congelés, cuits (n'oubliez pas les légumes qui sont inclus dans les mets composés de viande, de pâtes, mets ethniques...)	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Haricots jaunes/verts, pois verts, maïs	○	○	○	○	○	○	**Voir photo 1** 50 à 125 ml ¼ à ½ tasse	○	○	○
Tomates	○	○	○	○	○	○	½ à 1 tomate 50 à 125 ml ¼ à ½ tasse	○	○	○
Jus de légumes ou de tomates	○	○	○	○	○	○	**Voir photo 2** 125 à 175 ml ½ à ¾ tasse	○	○	○
Brocoli, chou-fleur, chou de bruxelle, chou, salade de chou	○	○	○	○	○	○	**Voir photo 1** 50 à 125 ml ¼ à ½ tasse	○	○	○
Carottes	○	○	○	○	○	○	**Voir photo 1** ½ à 1 carotte 50 à 125 ml ¼ à ½ tasse	○	○	○
Laitues, légumes verts feuillus, épinards, salades vertes	○	○	○	○	○	○	250 ml 1 tasse	○	○	○
Poivrons verts, rouges, jaunes	○	○	○	○	○	○	**Voir photo 1** 50 à 125 ml ¼ à ½ tasse	○	○	○
Tous les autres légumes différents de ceux spécifiés plus haut	○	○	○	○	○	○	**Voir photo 1** 50 à 125 ml ¼ à ½ tasse	○	○	○
Vinaigrettes, sauces à salade, mayonnaises, trempettes maison ou commerciales	○	○	○	○	○	○	15 ml 1 c.table	○	○	○

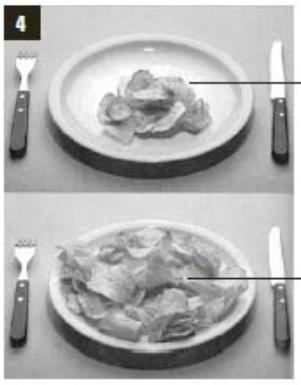


Fromages
1 à 2 cubes de
2,5 cm (1 pouce)
25 à 50 g
1 à 2 tranches

ÉCHANTILLON
Petits fruits

Tous les autres fruits
125 ml
½ tasse

Gâteaux, tartes, beignes, pâtisserie
1 pointe
1 à 2 petits gâteaux
1 beigne
1 pâtisserie

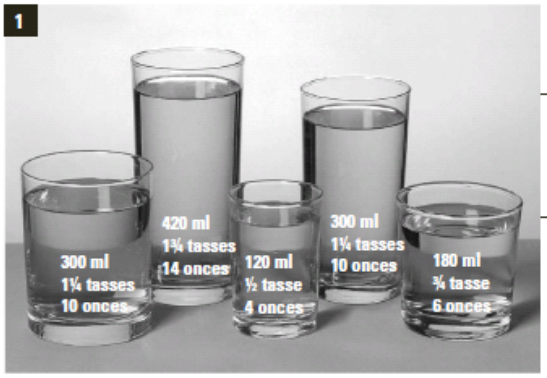


Grignotines
125 ml à 500 ml
½ à 2 tasses

	1 Combien de fois avez-vous consommé ces aliments au cours des 3 derniers mois?						**2** Quelle quantité consommiez-vous lors d'un même repas ou collation? Votre portion habituelle est-elle plus petite ou plus grosse que les exemples de portions? Ou bien, est-elle semblable (se situe dans l'intervalle des exemples de portions)?			
Produits laitiers	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Fromages	○	○	○	○	○	○	**Voir photo 1** 25 à 50 g 1 à 2 tranches	○	○	○
Yogourts	○	○	○	○	○	○	125 à 175 ml ½ à ¾ tasse	○	○	○
Desserts au lait (poudings, blanc-manger…)	○	○	○	○	○	○	125 à 175 ml ½ à ¾ tasse	○	○	○
Crème glacée, lait glacé, yogourt glacé	○	○	○	○	○	○	1 boule 125 à 175 ml ½ à ¾ tasse	○	○	○
Fruits frais, en conserve, congelés, en compote **Ne pas compter les jus**	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Pommes, poires	○	○	○	○	○	○	1 fruit	○	○	○
Bananes	○	○	○	○	○	○	1 banane	○	○	○
Melons (melons d'eau, cantaloup, melon miel…)	○	○	○	○	○	○	125 ml à 250 ml ½ à 1 tasse	○	○	○
Oranges, pamplemousses, clémentines (agrumes)	○	○	○	○	○	○	1 orange ½ pamplemousse	○	○	○
Petits fruits (fraises, framboises, bleuets…)	○	○	○	○	○	○	**Voir photo 2** 125 ml ½ tasse	○	○	○
Tous les autres fruits différents de ceux spécifiés plus haut	○	○	○	○	○	○	**Voir photo 2** 125 ml ½ tasse	○	○	○
Aliments sucrés et collations salées	JAMAIS ou rarement	1 à 3 fois par mois	1 à 2 fois par semaine	3 à 5 fois par semaine	1 fois par jour	2 fois et + par jour	Exemples de portion	plus petite −	semblable =	plus grosse +
Gâteaux, tartes, beignes, pâtisseries	○	○	○	○	○	○	**Voir photo 3**	○	○	○
Muffins	○	○	○	○	○	○	1 muffin	○	○	○
Biscuits	○	○	○	○	○	○	2 à 4 biscuits	○	○	○
Barres tendres, barres granola, barres à déjeuner	○	○	○	○	○	○	1 barre	○	○	○
Bonbons, chocolat	○	○	○	○	○	○	2 à 6 bonbons ½ tablette de chocolat	○	○	○
Grignotines (croustilles, craquelins, bretzel, popcorn…)	○	○	○	○	○	○	**Voir photo 4** 125 ml à 500 ml ½ à 2 tasses 5 à 20 craquelins	○	○	○

Photos de quelques exemples de portions

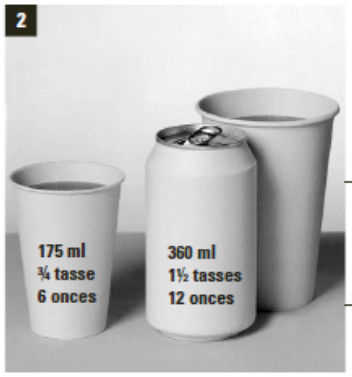
1



Référez-vous à cette photo pour estimer votre grosseur de portion habituelle de lait, boisson de soya, de jus ou de boissons aux fruits

Laits
Jus de fruits
Boissons aux fruits
125 à 250 ml
½ à 1 tasse
4 à 8 onces

2



Boissons gazeuses régulières et boissons gazeuses diètes
175 à 360 ml
¾ à 1½ tasses
6 à 12 onces
1 cannette

	1 Combien de fois avez-vous consommé ces aliments au cours des 3 derniers mois? Attention: les catégories de fréquence sont différentes des pages précédentes						**2** Quelle quantité buviez-vous lors d'un même repas ou collation? Votre portion habituelle est-elle plus petite ou plus grosse que les exemples de portions? Ou bien, est-elle semblable (se situe dans l'intervalle des exemples de portions)?			
Jus et boissons	**JAMAIS ou rarement**	**1 à 7 fois par mois**	**2 à 6 fois par semaine**	**1 fois par jour**	**2 à 3 fois par jour**	**4 fois et + par jour**	**Exemples de portion**	**plus petite −**	**semblable =**	**plus grosse +**
Jus de fruits (sans sucre ajouté, 100 % jus)	○	○	○	○	○	○	**Voir photo 1** 125 à 250 ml 4 à 8 onces	○	○	○
Boissons aux fruits (sucre ajouté: punchs, boissons à saveurs de fruits, limonade…)	○	○	○	○	○	○	**Voir photo 1** 175 à 325 ml 6 à 10 onces	○	○	○
Boissons gazeuses régulières (liqueurs douces, cola, 7-UP…)	○	○	○	○	○	○	**Voir photo 2** 1 cannette 175 à 360 ml 6 à 12 onces	○	○	○
Boissons gazeuses diètes (liqueurs douces , cola, 7-UP…)	○	○	○	○	○	○	**Voir photo 2** 1 cannette 175 à 360 ml 6 à 12 onces	○	○	○
Breuvages laitiers et équivalents (si vous mettez beaucoup de lait dans votre café, comptez-le avec le lait pour boire)	**JAMAIS ou rarement**	**1 à 7 fois par mois**	**2 à 6 fois par semaine**	**1 fois par jour**	**2 à 3 fois par jour**	**4 fois et + par jour**	**Exemples de portion**	**plus petite −**	**semblable =**	**plus grosse +**
Lait 3,25 % m.g. pour boire	○	○	○	○	○	○	**Voir photo 1** 125 à 250 ml 4 à 8 onces	○	○	○
Lait 1 % , 2 % m.g. pour boire	○	○	○	○	○	○	**Voir photo 1** 125 à 250 ml 4 à 8 onces	○	○	○
Lait écrémé pour boire	○	○	○	○	○	○	**Voir photo 1** 125 à 250 ml 4 à 8 onces	○	○	○
Boisson de soya pour boire	○	○	○	○	○	○	**Voir photo 1** 125 à 250 ml 4 à 8 onces	○	○	○
Café et boissons alcoolisées	**JAMAIS ou rarement**	**1 à 7 fois par mois**	**2 à 6 fois par semaine**	**1 fois par jour**	**2 à 3 fois par jour**	**4 fois et + par jour**	**Exemples de portion**	**plus petite −**	**semblable =**	**plus grosse +**
Café, thé	○	○	○	○	○	○	1 tasse	○	○	○
Lait, crème dans le café ou le thé	○	○	○	○	○	○	1 lait/crème	○	○	○
Sucre dans le café ou le thé	○	○	○	○	○	○	1 sucre	○	○	○
Bière	○	○	○	○	○	○	1 bière format régulier	○	○	○
Vin de table, apéritif	○	○	○	○	○	○	125 à 250 ml ½ à 1 tasse 1 à 2 verres	○	○	○
Spiritueux (boissons alcoolisées fortes)	○	○	○	○	○	○	30 à 50 ml 1 à 2 onces	○	○	○

Habitudes alimentaires et habitudes de vie

1 Quelle sorte de gras utilisez-vous habituellement pour la cuisson ?

Noircissez les **deux** les plus fréquemment utilisés

- ○ Beurre
- ○ Margarine
- ○ Margarine réduite en calories
- ○ Huile d'olive
- ○ Huile de canola
- ○ Huile de maïs
- ○ Huile de tournesol
- ○ Autres huiles
- ○ Shortening d'huile végétale
- ○ Lard
- ○ Huile en vaporisateur (ex.PAM)
- ○ Aucun de ces choix
- ○ Ne sais pas

2 À quelle fréquence utilisez-vous du gras ou de l'huile pour la cuisson ?

Noircissez votre réponse

- ○ Moins d'une fois par semaine
- ○ 1 à 2 fois par semaine
- ○ 3 à 4 fois par semaine
- ○ 5 à 6 fois par semaine
- ○ 1 fois par jour
- ○ 2 fois par jour
- ○ 3 fois par jour
- ○ Ne sais pas

3 Parmi les aliments particuliers indiqués ci-dessous, lesquels consommez-vous plusieurs fois par mois ?

Noircissez seulement les réponses qui s'appliquent

- ○ Peau du poulet
- ○ Poulet frit
- ○ Bœuf haché maigre ou extra-maigre
- ○ Poisson pané frit
- ○ Chips ou craquelins réduits en gras ou cuits au four
- ○ Fromages réduits en gras
- ○ Yogourts sans gras (0,1 % m.g.)
- ○ Margarines réduites en gras
- ○ Mayonnaises ou vinaigrettes réduites en gras ou sans gras
- ○ Jus enrichis de calcium
- ○ Café décaféiné
- ○ Substituts de repas (en barres ou en breuvages)
- ○ Barres ou breuvages énergétiques ou élevés en protéines

4 Suivez-vous une diète spéciale ?

Noircissez votre réponse

○ Non ○ Oui

Si oui, pour quelles raisons ?

_____

_____

_____

_____

5a Quel est votre poids habituel (avant votre grossesse)?

_____ livres ou _____ kilos

5b Quel est votre poids actuel ?

_____ livres ou _____ kilos

6 Combien mesurez-vous ?

____pieds et _____pouces ou ___ , _____ mètres

7 Quel est votre âge ? _____ ans

8 Sexe

- ○ Homme
- ○ Femme

Liste de points de repère

SVP, vous assurer que…

1. Les réponses concernent bien **votre** alimentation durant les **3 derniers mois**.
2. **Toutes** les questions ont été répondues incluant celles de la section concernant vos habitudes alimentaires.
3. **Une** fréquence et **une** portion ont été noircies pour chaque aliment. Par contre, il ne devrait **pas** y avoir de portion lorsque la fréquence est « jamais ou rarement ».
4. Les bonnes réponses sont clairement identifiées si vous avez fait des erreurs et que vous ne pouvez pas les effacer.
5. Les notes et commentaires (voir page suivante) que vous avez rajoutés, si nécessaire, sont compréhensibles.

Si vous avez des commentaires ou des suggestions concernant ce questionnaire, veuillez les indiquer dans l'espace suivant.

Nous vous remercions d'avoir bien voulu répondre à ce questionnaire et nous vous rappelons que toute l'information qui y est incluse demeurera confidentielle.

Su evaluación dietética

INTAPP

ENSAYO CLINICO INTERNACIONAL DE ANTIOXIDANTES PARA LA PREVENCION DE LA PREECLAMPSIA

Queremos conocer sus hábitos de alimentación en los pasados 3 meses. Su dieta habitual incluye comidas realizadas en la casa, el trabajo, en restaurantes, durante días entre semana, fines de semana, durante las comidas fuertes y entre comidas.

Nosotros necesitamos conocer lo que usted realmente come, no una dieta ideal, ni lo que otros miembros de su familia consumen.

La mayoría de nuestras preguntas tienen muchas respuestas posibles. Escoja la respuesta que mejor refleje sus hábitos de alimentación. Conteste con tanta precisión como le sea posble.

Nombre del investigador a cargo:	
Número de identificación del paciente:	
Número de visita:	
Fecha (DD/MM/AAAA):	
Hora de inicio:	Hora de finalización:

Instrucciones para el llenado de su evaluación dietética

Para responder la pregunta de cada alimento, marque el círculo en la línea que corresponde a dicho alimento; por ejemplo: si en los pasados tres meses ha consumido frijoles dos veces a la semana, sombree o tache el círculo correspondiente a la respuesta de "1 a 2 veces por semana".

Para responder la pregunta sobre el tamaño de porción del mismo alimento, sombree el círculo de la línea correspondiente a dicho alimento.
Por ejemplo: si la ración de frijoles que comunmente consume es una porción de 1 plato de frijoles o 100 g. marque o tache el círculo que pertenece a la respuesta "similar".

Algunas de las porciones cuentan con fotografías de alimentos para la comparación de tamaños de porción y usted puede ayudarse con ellas.

Si rara vez o nunca consume el alimento, no marque ninguna opción de tamaño de porción.

	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la porción señalada ?			
Leguminosas y huevos	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor -	Similar =	Mayor +
33 Frijoles.	○	○	●	○	○	○	100 g, 1 plato	○	●	○
34 Huevos en sus diferentes presentaciones, Omelet.	○	○	○	○	○	○	150 g, 2 huevos grandes	○	○	○

Vaya al alimento en la segunda línea y repita los pasos 1 y 2 para este producto alimenticio.

Repita el mismo procedimiento para todos los alimentos.

Note que es importante poner una respuesta en cada una de las lineas. Y solo es válida una respuesta por renglón para cada frecuencia y para cada tamaño respectivamente.

No olvide ver las fotos de los alimentos.

Este cuestionario es estrictamente confidencial.

Agradecemos su participación.

Bolillo o Telera

38 gr a 142 gr
½ a 1-½ bolillos

Pan dulce

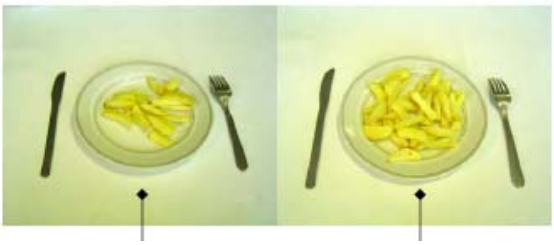
35 gr a 128 gr
1 a 2 piezas medianas

Cereal

32 gr a 120 gr
¼ a 1 taza mediana

Espagueti u otras Pastas secas

50 gr a 100 gr
½ a 1 plato mediano

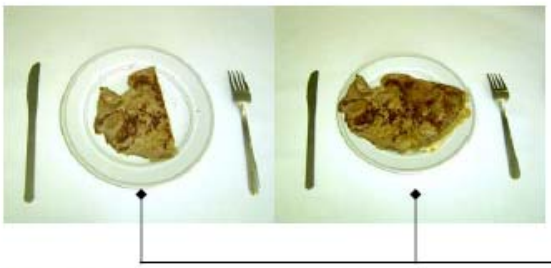


Papas fritas a la francesa

38 gr a 211 gr
½ a 1 ½ tazas

Pan cereales y alimentos relacionados	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la porción señalada ?			
	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Porción muestra	Menor —	Similar =	Mayor +
1 Rebanadas de pan blanco	○	○	○	○	○	○	26 a 75 gr. 1-3 rebanadas	○	○	○
2 Otro tipo de panes blancos (bolillo, telera)	○	○	○	○	○	○	Ver foto 1 38 a 142 gr. 1/2 a 1 1/2 bolillos	○	○	○
3 Pan dulce	○	○	○	○	○	○	Ver foto 2 35 a 128 gr. 1-2 pzas. medianas	○	○	○
4 Tortilla de harina de trigo (como tortillinas)	○	○	○	○	○	○	30 a 90 gr. 1-3 pzas.	○	○	○
5 Tortilla de maiz	○	○	○	○	○	○	36 a 132 gr. 1-5 pzas. tortilleria	○	○	○
6 Mantequilla	○	○	○	○	○	○	5 a 15 gr. 1-2 cdas. cafeteras	○	○	○
7 Margarina	○	○	○	○	○	○	5 a 15 gr. 1-2 cdas. cafeteras	○	○	○
8 Mermelada, miel, cajeta, leche condensada (como la lechera)	○	○	○	○	○	○	8 a 23 gr. 1-2 cdas. soperas	○	○	○
9 Cereales (como Corn Flakes, Chococrispis, Zucaritas)	○	○	○	○	○	○	Ver foto 3 32 a 120 gr. 1/4 - 1 taza	○	○	○

Sopas, papas y arroz	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
10 Sopa de pasta con caldo	○	○	○	○	○	○	86 a 319 gr. 1/2 - 2 platos hondos	○	○	○
11 Espagueti u otras pastas secas (en jitomate ó en crema con ó sin carne)	○	○	○	○	○	○	Ver foto 4 50 a 100 gr. 1/2 - 1 plato mediano, 1/2 - 1 cda. servir	○	○	○
12 Arroz	○	○	○	○	○	○	54 a 190 gr 1/2 - 2 platos medianos, 1 -2 cdas. servir	○	○	○
13 Papas fritas a la francesa	○	○	○	○	○	○	Ver foto 5 38 a 211 gr. 1/2 - 1 1/2 tzas., 2 pzas. medianas	○	○	○
14 Papas cocidas, en puré o al horno.	○	○	○	○	○	○	20 a 114 gr. 1/2 - 1 1/2 tzas., 2 pzas. medianas	○	○	○



Carne de res

62 gr a 182 gr
½ a 1-½ bisteces

Pollo

45 gr a 185 gr
½ a 1½ piezas con hueso

Atun

20 gr a 156 gr
¼ a ½ taza
1 taza – 1 lata

	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la porción señalada ?			
Carnes y aves	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
15 Carne de res (asada, bistec, en trozo, molida en hamburguesa)	○	○	○	○	○	○	Ver foto 1 60 a 182 gr ½ - 1½ bisteces (piezas.)	○	○	○
16 Hígado u otras vísceras	○	○	○	○	○	○	50 a 82 gr. de res 1-3 hígados de pollo	○	○	○
17 Carne de cerdo (bistec, lomo, trozo)	○	○	○	○	○	○	39 a 185 gr. 1-3 trozos chicos, ½ - 1½ bisteces	○	○	○
18 Chicharrón	○	○	○	○	○	○	20 a 100 gr. 1 - 5 trozos medianos	○	○	○
19 Longaniza y / o chorizo	○	○	○	○	○	○	12 a 79 gr. ¼ - 1 trozo frito	○	○	○
20 Barbacoa de borrego	○	○	○	○	○	○	118 a 320 gr. 1 trozo mediano - 2 trozos gdes.	○	○	○
21 Pollo (cualquier pieza)	○	○	○	○	○	○	Ver foto 2 45 a 185 gr. ½ - 1½ piezas. con hueso	○	○	○
22 Salchichas	○	○	○	○	○	○	24 a 105 gr. 1-3 piezas. medianas	○	○	○
23 Jamón y otras carnes frías	○	○	○	○	○	○	20 a 61 gr. 1-3 rebanadas.	○	○	○
24 Salsas (roja, verde)	○	○	○	○	○	○	64 a 110 gr. ½ - 1 cda. servir	○	○	○

Pescados y mariscos	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
25 Atún en aceite o en agua	○	○	○	○	○	○	Ver foto 3 20 a 156 gr. ¼ - ½ taza, ½ - 1 lata	○	○	○
26 Otros pescados frescos (mojarra, trucha)	○	○	○	○	○	○	80 a 120 gr. 1 pieza. chica - 1 pieza. mediana	○	○	○
27 Sardina en jitomate o en aceite	○	○	○	○	○	○	20 a 145 gr. ½ - 3½ piezas.	○	○	○

Platillos combinados	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
28 Enchiladas o chilaquiles verdes	○	○	○	○	○	○	150 a 250 gr. 2 - 3 piezas.	○	○	○
29 Mole poblano	○	○	○	○	○	○	24 a 50 gr. ¼ - ½ taza	○	○	○
30 Tacos (suadero, maciza, canasta)	○	○	○	○	○	○	76 a 380 gr. 2 - 5 piezas.	○	○	○

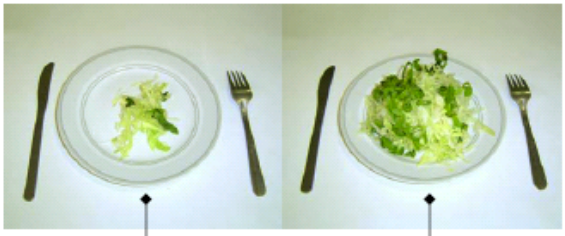
Frijoles

50 gr a 100 gr
½ a 1plato mediano

	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la porción señalada ?			
Platillos combinados	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
31 Tamal	○	○	○	○	○	○	120 a 240 gr. 1-2 pzas.	○	○	○
32 Fritangas (sope, quesadilla, pambazo)	○	○	○	○	○	○	50 a 150 gr. ½ - 1½ pzas. preparado	○	○	○

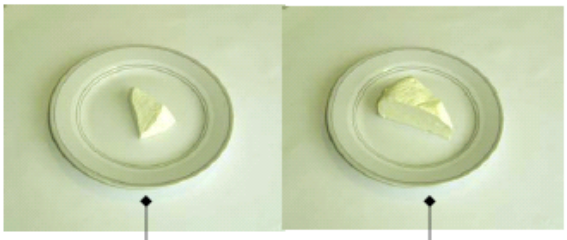
Leguminosas y huevos	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
33 Frijoles (cocidos o refritos)	○	○	○	○	○	○	Ver foto 1 50 a 336 gr. ½ - 3 cdas. servir gdes.	○	○	○
34 Lentejas	○	○	○	○	○	○	20 a 140 gr. ¼ - 1 taza mediana	○	○	○
35 Huevos en sus diferentes presentaciones, Omelet.	○	○	○	○	○	○	50 a 117 gr. 1 - 2 pzas. medianas	○	○	○

Vegetales crudos o cocidos. Aceites y aderezos.	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
36 Elote	○	○	○	○	○	○	40 a 153 gr ¼ - 1 taza mediana	○	○	○
37 Chile serrano, jalapeño	○	○	○	○	○	○	5 a 29 gr ½ - 2 piezas medianas	○	○	○
38 Jitomate crudo	○	○	○	○	○	○	12 a 102 gr. ¼ - 1 pza. mediana	○	○	○
39 Jitomate en salsa ó guisado	○	○	○	○	○	○	11 a 96 gr. ¼ - 1 chuchara de servir	○	○	○
40 Calabacita italiana	○	○	○	○	○	○	13 a 52 gr. ¼ - ½ taza	○	○	○
41 Zanahorias	○	○	○	○	○	○	9 a 86 gr. 1 - 9 cdas. soperas	○	○	○
42 Chayote sin espinas	○	○	○	○	○	○	25 a 50 gr. ¼ - ½ pieza mediana cocida	○	○	○
43 Nopales	○	○	○	○	○	○	27 a 100 gr. ¼ - 1 pza. mediana	○	○	○
44 Aguacate	○	○	○	○	○	○	12 a 65 gr. ¼ - 1 pza. sin hueso	○	○	○
45 Lechuga	○	○	○	○	○	○	11 a 50 gr. ½ - 1 ½ hojas	○	○	○



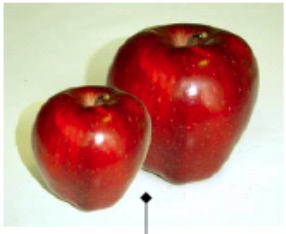
Vegetales de hojas verdes

10 gr a 50gr
1 a 5 cucharadas soperas



Quesos frescos

18 gr a 60 gr
1 a 4 rebanadas pequeñas



Manzana

79 gr a 213 gr
1 pieza chica a 1 pieza grande
¼ a 1 taza mediana

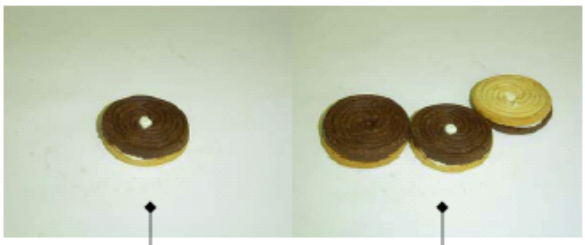
	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la			
Vegetales crudos o cocidos. Aceites y aderezos.	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
46 Vegetales de hojas verdes (acelgas, espinacas, quintoniles)	○	○	○	○	○	○	Ver foto 1 10 a 50 gr. 1 - 5 cdas. soperas	○	○	○
47 Aderezos para ensaladas	○	○	○	○	○	○	25 a 100 gr. ¼ - 1 taza chica	○	○	○
48 Mayonesa	○	○	○	○	○	○	4 a 30 gr. ½ - 3 cdas. soperas	○	○	○
49 Aceite canola (como Capullo)	○	○	○	○	○	○	10 a 30 gr. 1 - 3 cdas. soperas	○	○	○
50 Aceite de cártamo (como 1-2-3)	○	○	○	○	○	○	10 a 28 gr. 1 - 3 cdas. soperas	○	○	○
51 Aceite de Maíz (como Mazola)	○	○	○	○	○	○	10 a 29 gr. 1 - 3 cdas. soperas	○	○	○
52 Aceite de oliva	○	○	○	○	○	○	20 a 60 gr. 2 - 6 cdas. soperas	○	○	○

Productos lácteos	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
53 Quesos frescos: panela y/o cotija	○	○	○	○	○	○	Ver foto 2 18 a 60 gr. 1 - 4 rebanadas pequeñas	○	○	○
54 Quesos fuertes: oaxaca, manchego, chihuahua	○	○	○	○	○	○	32 a 70 gr. 1 - 2 ½ trozos medianos	○	○	○
55 Crema y queso doble crema	○	○	○	○	○	○	20 a 57 gr. 2 - 6 cdas. soperas	○	○	○

Frutas- frescas, enlatadas, congeladas o en almíbar no incluye jugos de frutas	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
56 Manzana	○	○	○	○	○	○	Ver foto 3 79 a 213 gr. 1 pza. chica - 1 pza. grande	○	○	○
57 Plátano	○	○	○	○	○	○	43 a 163 gr. ½ - 2 pzas. medianas	○	○	○
58 Papaya	○	○	○	○	○	○	85 a 250 gr. 1 - 3 rebanadas medianas	○	○	○
59 Naranja	○	○	○	○	○	○	72 a 178 gr. ½ - 1½ pzas. medianas	○	○	○
60 Mandarina	○	○	○	○	○	○	60 a 142 gr. ½ -1½ pzas. medianas	○	○	○
61 Guayaba	○	○	○	○	○	○	50 a 90 gr. 1 pza. chica - 1 pza. grande	○	○	○

Pasteles

70 gr a 172gr
1 rebanada chica
1 rebanada grande



Galletas Dulces

12 gr a 40 gr
1 a 3 piezas medianas

	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la porción señalada ?			
Frutas- frescas, enlatadas, congeladas o en almíbar no incluye jugos de frutas	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
62 Mango	○	○	○	○	○	○	70 a 200 gr. 1 - 2 pzas. chicas	○	○	○
63 Tuna	○	○	○	○	○	○	56 a 120 gr. ½ - 1 pza. sin cáscara	○	○	○

Conservas, dulces y botanas saladas	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
64 Pasteles	○	○	○	○	○	○	Ver foto 1 70 a 172 gr. 1 rebanada chica - 1 rebanada grande	○	○	○
65 Galletas dulces	○	○	○	○	○	○	Ver foto 2 12 a 40 gr. 1 - 3 pzas. medianas	○	○	○
66 Chocolate en polvo (como Chocomilk)	○	○	○	○	○	○	6 a 20 gr. 1 - 4 cdas. cafeteras	○	○	○
67 Dulces y chocolates	○	○	○	○	○	○	18 a 54 gr. 1 - 3 bolsas pequeñas 1 - 3 barritas	○	○	○
68 Botanas saladas (papas fritas, palomitas, frituras)	○	○	○	○	○	○	30 a 88 gr. 1 bolsa pequeña - 1 bolsa mediana	○	○	○
69 Nueces de la india y/o cacahuates y/o pistaches	○	○	○	○	○	○	15 a 58 gr. 1 bolsa pequeña - 1 bolsa mediana, 1 ½ - 6 cdas. soperas	○	○	○

Leche y bebidas similares	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
70 Leche entera pasteurizada (Lala, alpura, etc.)	○	○	○	○	○	○	171 a 360 ml. ½ - 1 ½ vasos grandes	○	○	○
71 Leche semidescremada (Lala, alpura, etc.)	○	○	○	○	○	○	148 a 293 ml. 1 - 2 vasos chicos	○	○	○
72 Leche Liconsa o Conasupo.	○	○	○	○	○	○	108 a 300 ml. 1 vaso chico - 1 vaso grande	○	○	○
73 Yogurt	○	○	○	○	○	○	170 a 250 ml. 1 - 2 botes indiv.	○	○	○
74 Atole con leche	○	○	○	○	○	○	140 a 370 ml. 1 - 2 ½ tazas chicas	○	○	○

Jugos de fruta y bebidas

	1 ¿Cuántas veces ha consumido estos alimentos en los últimos 3 meses?						2 ¿Qué tanto comió usted en cada comida o entre comidas? ¿El tamaño habitual de su porción ingerida es similar, menor o mayor a la porción señalada ?			
Jugos y bebidas	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 6 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	—	=	+
75 Jugo de naranja	○	○	○	○	○	○	150 a 300 ml. ½ - 1 vaso gde.	○	○	○
76 Jugos de frutas industrializados (Boing, Del Valle, Botellín, etc.)	○	○	○	○	○	○	163 a 242 ml. ½ - 1 vaso mediano	○	○	○
77 Refresco con gas (refrescos de cola, 7-up, Jarritos, etc.)	○	○	○	○	○	○	177 a 340 ml. 1 vaso chico - 1 vaso grande	○	○	○
78 Bebidas de frutas industrializadas (Frutsi, Kool-Aid, Frisco ya preparado, etc.)	○	○	○	○	○	○	120 a 250 ml. 1 vaso chico - 1 vaso mediano	○	○	○
79 Agua de fruta casera	○	○	○	○	○	○	201 a 720 ml. 1 - 3 vasos medianos, 1 - 3 tazas grandes	○	○	○

Bebidas alcohólicas, café y otras bebidas	Nunca o rara vez	1 a 3 veces por mes	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción	Menor —	Similar =	Mayor +
80 Cerveza, vino de mesa	○	○	○	○	○	○	100 a 300 ml. 1/3 - 1 lata, 1 - 2 copas	○	○	○
81 Café	○	○	○	○	○	○	4 a 14 gr. 1 - 3 cdas. cafeteras en polvo, 1 - 2 tazas (líquido)	○	○	○

Datos complementarios

1. ¿Cuántas cucharaditas de azúcar le agrega usted a sus alimentos?

_____ cds.

2. ¿Consume usted suplementos de calcio?

☐ SI ☐ NO

Indique ¿Cuál? _____

Frec. Consumo

- ☐ Menos de una vez al mes
- ☐ 1 a 3 veces al mes
- ☐ 1 vez a la semana
- ☐ 2 a 4 veces a la semana
- ☐ 5 a 6 veces a la semana
- ☐ Una vez al día

3. ¿Consume usted ácido fólico?

☐ SI ☐ NO

Indique ¿Cuál? _____

Frec. Consumo

- ☐ Menos de una vez al mes
- ☐ 1 a 3 veces al mes
- ☐ 1 vez a la semana
- ☐ 2 a 4 veces a la semana
- ☐ 5 a 6 veces a la semana
- ☐ Una vez al día

4. ¿Consume usted un suplemento vitáminico?

☐ SI ☐ NO

Indique ¿Cuál? _____

Frec. Consumo

- ☐ Menos de una vez al mes
- ☐ 1 a 3 veces al mes
- ☐ 1 vez a la semana
- ☐ 2 a 4 veces a la semana
- ☐ 5 a 6 veces a la semana
- ☐ Una vez al día

Hábitos de alimentación y hábitos de estilo de vida.

1. ¿Qué tan frecuentemente usa grasa o aceite para cocinar?

Marque la respuesta más cercana a su realidad

- ☐ Al menos una vez por semana
- ☐ 1 a 2 veces por semana
- ☐ 3 a 4 veces por semana
- ☐ 5 a 6 veces por semana
- ☐ 1 vez por día
- ☐ 2 veces por día
- ☐ 3 veces por día
- ☐ No sabe

2. ¿Qué tipo de aceite utiliza más comunmente para cocinar?

Marque la respuesta más cercana a su realidad

- ☐ Mantequilla
- ☐ Margarina
- ☐ Margarina baja en calorías
- ☐ Aceite de olivo
- ☐ Aceite de canola
- ☐ Aceite de maíz
- ☐ Aceite de girasol
- ☐ Aceite de cártamo
- ☐ Manteca de cerdo
- ☐ PAM
- ☐ Ninguno de los anteriores
- ☐ No sabe

3. ¿Cuál de los siguientes alimentos come regularmente (muchas veces al mes)?

Marque todas las respuestas apropiadas.

- ☐ Pollo con piel
- ☐ Pollo frito (rostizado o tipo Kentucky)
- ☐ Carne de res magra (sin grasa)
- ☐ Pescado frito empanizado
- ☐ Galletas o panes bajos en calorías
- ☐ Queso bajo en grasa (panela, cottage)
- ☐ Yogurt libre de grasa (Light)
- ☐ Margarina baja en calorías
- ☐ Mayonesa o aderezo baja en grasa
- ☐ Bebidas light (Clight, Levite, etc.)
- ☐ Café descafeinado
- ☐ Suplementos alimenticios (barra o bebida tipo slim fast)
- ☐ Bebidas o barras altas en proteína o en energía (Bran Fruit o Energy)

Hábitos de alimentación y hábitos de estilo de vida.

4. ¿Le agrega usted sal a sus alimentos antes de comerselos?

☐ SI ☐ NO

5. Lleva una dieta especial

Marque su respuesta

☐ NO

☐ SI

6. Sí su respuesta es sí ¿Por qué?

_____

_____

_____

_____

_____

_____

7. ¿Qué tipo de ejercicio practica usted?

- ☐ Ninguno
- ☐ Correr
- ☐ Aeróbicos
- ☐ Caminar
- ☐ Bailar
- ☐ Tennis
- ☐ Voleibol
- ☐ Basquetbol
- ☐ Futbol
- ☐ Spinning
- ☐ Nadar

8. ¿Cuántas veces a la semana?

- ☐ 1 a 2 veces por semana
- ☐ 3 a 4 veces por semana
- ☐ 5 a 6 veces por semana
- ☐ 7 veces por semana

9. ¿Cuántos minutos

- ☐ 10 a 19 minutos
- ☐ 20 a 29 minutos
- ☐ 30 a 39 minutos
- ☐ 40 a 49 minutos
- ☐ 50 a 59 minutos
- ☐ 1 hr. ó mas

10. Sí tiene alguna sugerencia o comentario sobre este cuestionario, por favor anótelo en estas líneas:

_____

_____

_____

_____

_____

_____

ci

Otros alimentos

Alimentos	1 a 2 veces por semana	3 a 5 veces por semana	Una vez por día	2 veces o más al día	Tamaño de porción (en gramos o medidas caseras)
1	◯	◯	◯	◯	
2	◯	◯	◯	◯	
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9.2 ROLE OF NUTRITION IN THE RISK OF PREECLAMPSIA

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Role of nutrition in the risk of preeclampsia

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Preeclampsia (PE) accounts for about one-quarter of the cases of maternal mortality and ranks second among the causes of pregnancy-associated maternal deaths in Canada and worldwide. The identification of an effective strategy to prevent PE is a priority and a challenge for research in obstetrics. Progress has been hampered by inadequate understanding of the underlying etiology of the disease. The role of maternal diet in the etiology of PE has recently received increased attention. The objective of this paper is to provide an overview of the literature concerning 1) the current understanding of the pathogenesis of PE, 2) the biological plausibility and potential mechanisms underlying the associations between maternal dietary exposures, nutrition, and the risk of PE, and 3) the epidemiological findings of maternal nutrient intake in relation to the risk of PE.

INTRODUCTION

Preeclampsia (PE), defined as pregnancy-induced hypertension and proteinuria, is a syndrome that is unique to human pregnancy, affecting between 2% and 8% of pregnancies.[1–3] It accounts for about 10–15% of direct maternal deaths in low- and middle-income countries as well as in high-income countries.[4,5] Gestational hypertension, especially PE, is a primary cause of low birth weight (<2,500 g) in infants and thereby perinatal death through both preterm delivery and intrauterine growth restriction.[6–10] Since delivery is the only known cure, PE is a leading cause of indicated premature delivery[11] and accounts for about 15% of infants with growth restriction.[12] As many as 60% of extremely-low-birth-weight (≤800 g) infants suffer learning disabilities and low IQ,[13] increasing the hidden costs of the disease. The study of the etiology, prevention, and outcome of PE and other hypertensive disorders of pregnancy remains a research priority for healthcare. Effective prevention of PE would have major health benefits and result in considerable savings in health budgets.

CAUSAL MECHANISMS OF PREECLAMPSIA

PE is a multisystem disorder that is specific to human pregnancy and can only be resolved by delivery. Generally, the etiology of PE can be conceptualized in broad categories: PE of placental origin and PE of maternal origin.[14] Placental PE arises from a hypoxic placenta and progresses in two stages, described as preclinical (poor placentation) and clinical.[14] Maternal PE arises from the interaction between normal placenta and maternal constitutional factors such as microvascular disease, long-term hypertension, obesity, inflammation, or diabetes. Thus, pregnancy may represent a metabolic and vascular "stress test" that unmasks latent cardiovascular risk.[15] However, involvement of both placental and maternal constitutional factors is very common in the development of PE, and these broad categories are likely not mutually exclusive. Most patients are somewhere on a continuum between these two pathways.

Several etiologic theories of PE have been proposed and extensively investigated.[14,16–21] During normal pregnancy, cytotrophoblasts invade the maternal decidua and spiral arteries and completely remodel the maternal spiral

arteries into large capacitance vessels with low resistance. In preeclampatic pregnancies, shallow endovascular cytotrophoblast invasion of the spiral arteries results in a hypoxic and dysfunctional placenta along with the release of factors such as cytokines, growth factors, and certain chemicals into the maternal circulation.[14–26] These maternal circulating factors mediate endothelial dysfunction, leading to the clinical signs of PE. Increased levels of factor VIII-related antigen, total and cellular fibronectin, thrombomodulin, and endothelin and disturbances of the prostacyclin-to-thromboxane A2 ratio all support the hypothesis that a more global endothelial dysfunction is centrally involved in the pathogenesis of preeclampsia.[27–36] Several lines of evidence support the hypothesis that the ischemic placenta induces an alteration in the balance of circulating levels of angiogenic/antiangiogenic factors such as vascular endothelial growth factor (VEGF), free placental growth factor (PlGF), soluble fms-like tyrosine kinase (sFlt1), and soluble endoglin (sEng), which contributes to endothelial cell dysfunction in the maternal vasculature.[19,37–40] Recent studies suggest that women with clinically established PE have significantly lower levels of PlGF and VEGF compared with gestational age-matched normotensive controls.[16,41–46] Circulating sFlt1, a receptor binding VEGF and PlGF, is significantly increased before the onset of preeclampsia.[46–48] However, it remains unclear whether impaired placental perfusion initiates symptoms such as hypertension, endothelial dysfunction, and increased sFlt1 expression, or whether inadequate placental development occurs initially and is followed by a pathological rise in sFlt1 expression and secretion.[49]

The role of oxidative stress in the pathogenesis of PE is also increasingly recognized.[21,50,51] Oxidative stress is an imbalance between pro-oxidant and antioxidant forces, resulting in an accumulation of free radicals or of reactive oxygen or reactive nitrogen species. Deleterious effects of free radicals include lipid peroxidation, oxidative damage to bimolecules, and cellular dysfunction. It has been hypothesized that hypoxia stimulates the activity of xanthine or NAD(P)H oxidase in placenta, which leads to superoxide generation. Oxidative stress likely contributes to maternal endothelial cell activation and enhanced apoptosis of trophoblasts, and is believed to underlie the intense vasoconstriction and procoagulant state of PE.[14,16–21] Markers of oxidative stress, such as isoprostanes and malondialdehyde, are increased in the plasma,[52,53] small arteries,[54] and decidua basalis[55] of women with PE.

Experimental and epidemiological data support the role of maternal-fetal immune maladaptation in the etiology of PE.[56–59] There are reports of altered immune status in PE.[60–62] A significantly lower proportion of T-helper cells was demonstrated in women who later developed PE.[60] Deposition of immunoglobulin (IgM), complement (C3), and fibrin has been observed in the walls of spiral arteries in women who develop PE.[61,63] Studies have shown that mothers lacking most or all activated killer cell immunoglobulin-like receptors (KIRs, AA genotype) when the fetus had human leukocyte antigens (HLA-C) were at a substantial risk of PE.[63] These findings are supported by epidemiological studies investigating the relationship between parity, paternity, and the risk of PE.[64,65] It has been demonstrated that multiparity is associated with a reduced risk of PE, which suggests an immune tolerance phenomenon. Interestingly, some studies suggest that the protective role of primiparity is lost with the change of partner, suggesting that primpaternity, rather than primiparity, is related to the risk of PE.[64,65]

PE is associated with an increase in systematic inflammatory responses. The causes of these responses remain unknown. One attractive concept is that placental ischemia and reperfusion with oxidative stress may induce the higher proliferation of cytotrophoblasts and increase the deportation of syncytiotrophoblasts.[14,20] Thus, the altered balance between proliferation and apoptosis of trophoblasts may cause aponecrotic or even necrotic release of trophoblasts, accentuating maternal inflammatory burdens. It has been reported that there are increased amounts of trophoblast debris, comprised of syncytiotrophoblast membrane microparticles, cytokeratin fragments, and soluble fetal proteins, in maternal circulation in women with PE.[14,16–21] Enhanced activation of cytokine mediators of apoptosis (especially interferon, tumor necrosis factor) has been found in PE.[14,16–21] It is well known that severe PE and eclampsia have a familial tendency. Nilsson et al.[66] reported a heritability of 31% for PE and 20% for gestational hypertension. Chesley et al.[67] reported a 26% incidence of PE in daughters of women with PE, compared with only an 8% incidence in the daughters-in-law. It seems that a number of maternal susceptibility genes or perhaps fetal genes contribute to the pathogenesis of PE by interacting with the maternal cardiovascular or hemostatic system or by regulating endothelial activation and inflammatory responses.[68–71]

In summary, there are numerous theories of the pathogenesis of PE (Figure 1). These different underlying mechanisms are not mutually exclusive, but rather likely interactive. A vast array of initiating agents and multiple pathogenic mechanisms have been implicated in the development of PE, including increased systematic vascular resistance, enhanced platelet aggregation, activation of coagulation systems, and endothelial dysfunction.

INVOLVEMENT OF NUTRITIONAL FACTORS IN THE PATHOGENESIS OF PREECLAMPSIA

The role of maternal diet in the etiology of PE has recently received increased attention. Information largely

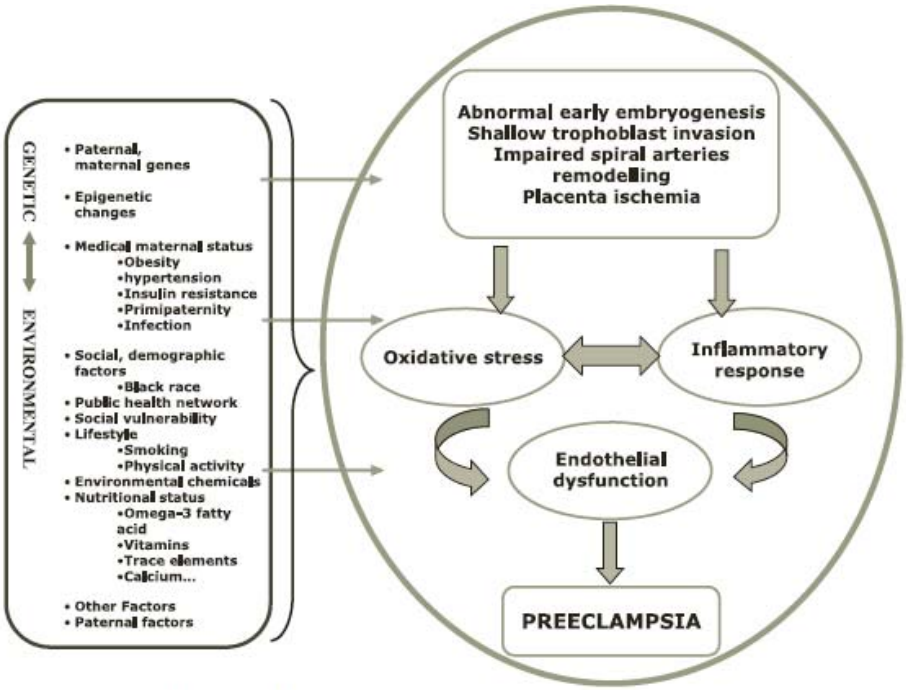

Figure 1 **Hypothetical framework of the pathogenesis of preeclampsia.**

derived from studies external to pregnancy indicates that certain nutrients may be involved in several important steps in the current proposed concepts of the pathogenesis of PE.[71–74] Several nutrients, particularly omega-3 (n-3) fatty acids, antioxidants, folic acid, and L-arginine, have important roles in modulating endothelial function.[71,72] Higher intake or supplementation of these nutrients is associated with decreased expression of endothelium adhesion molecules (VCAM-1) but increased levels of endothelium-dependent vasodilation and nitric oxide production.[75–80] The influence of these nutrients on endothelial function is multi-faceted and complex and includes inhibition of monocyte adhesion and platelet activation, improvement of vasodilation, and blockage of lipid oxidation.[71,72,74]

Nutrients can affect oxidative stress by increasing or decreasing free radicals or antioxidants, by providing substrates for the formation of free radicals, or by modulating functions of antioxidant enzymes. For instance, lipids are extensively involved in the generation of free radicals.[81] Antioxidants (vitamin C, E, alpha- or beta-carotene, copper, selenium, zinc, etc.) can indirectly or directly scavenge free radicals or function as essential substrates or cofactors for the adequate functioning of antioxidant enzymes. Therefore, adequate dietary antioxidant intake is crucial for maintaining pro-oxidant and antioxidant balance, since some nutrients are not synthesized in humans.

Compelling evidence suggests that nutrients may modify certain inflammatory responses.[82–85] For example, certain nutrients can affect the production of monocyte tumor necrosis factor-α (e.g., antioxidants and fatty acids), modulate proinflammatory cytokine production and actions (e.g., iron, fatty acids), or activate genes involved in the inflammatory responses (e.g., polyunsaturated fatty acids).[82–85] These mediators are essentially implicated in the pathogenesis of PE via processes such as trophoblast apoptosis, inflammatory response, and endothelial activation.

It has also been suggested that nutrients such as trace elements, fatty acids, and folic acid can contribute to insulin resistance, a risk factor for PE.[86–89] Experimental

as well as epidemiological studies have indicated that n-3 fatty acids can improve glucose tolerance and prevent insulin resistance.[90,91]

ENERGY AND DIET COMPOSITION

It is proposed that high-energy diets can affect endothelial function and inflammatory responses by activating oxidative stress-responsive transcription factors, inflammatory cytokine production, and the expression of adhesion molecules.[92,93] Abnormal lipid metabolism is present in women with mild or severe PE: these anomalies are characterized by increased levels of triglycerides, low-density lipoprotein cholesterol (LDL-C), LDL-III (small dense lipoprotein), and apolipoprotein A-I.[94,95]

A large case-control study was conducted in Jerusalem, involving 180 women with PE and 360 healthy control subjects who were matched for country of origin, parity, month of delivery, age, year of immigration, and years of schooling. A diet history was obtained at the time of delivery, and results indicated that preeclamptic women had significantly lower intakes of protein, fat, and energy. However, further investigations suggested that these differences might be secondary to the disease rather than causal.[96] Atkinson et al.[97] published a case-control study in Zimbabwe using a crude (simple/qualitative/nonquantitative) food frequency questionnaire and found no significant differences between 180 women with PE and 194 normotensive controls. Only a few prospective population-based studies have examined the relationship between energy intake and the risk of PE, and they have yielded inconsistent findings.[98,99] A US study evaluated diet using a 24-hour dietary recall at 13–21 weeks gestation in 4,157 women who had been enrolled in a randomized controlled trial of calcium supplementation in the prevention of PE.[99] There was no evidence of an increased risk of PE in women with a higher intake of energy. Moreover, there was no difference between cases and controls in the intake of any of the 28 nutrients that were studied.[99] A Norwegian team administered a semiquantitative food frequency questionnaire to 3,771 women at 17–19 weeks of gestation.[98] The risk of PE was increased among women with a high energy intake (adjusted OR 5.4; 95% CI 2.3–12.4, for the 4th quartile) and a high intake of polyunsaturated fatty acids (adjusted OR 2.3; 95% CI 1.1–4.6). Differences persisted even after adjusting for age, smoking, and body mass index. Moreover, the authors observed a stronger association for early-onset PE. The discrepancy between these studies may be partially explained by the methods used to estimate dietary intake, the time in pregnancy at which diet is assessed, different definitions of PE and gestational hypertension, or population differences (i.e., lifestyle, heterogeneity in nutrient intake, sociodemographic factors).

It is worth pointing out that in both the Norwegian[98] and the American studies,[99] women who later developed PE had a higher prepregnancy body weight, suggesting the potential role of energy balance before pregnancy in the development of PE.

FIBER

Evidence derived from randomized controlled trials indicated that dietary fiber may have beneficial effects on plasma lipid and lipoprotein profiles, postprandial glucose metabolism, insulin sensitivity, and blood pressure.[100,101] The clinical data on the role of fiber in pregnancy, however, are quite limited.

In 1991, Skajaa et al.[102] found no differences in mean daily fiber intake during the third trimester between PE cases and controls. Frederick et al.[103] conducted a case-control study of 172 preeclamptic women and 339 normotensive controls to explore the relation between PE risk and maternal intake of dietary fiber, potassium, magnesium, and calcium. They reported that fiber intake was inversely associated with the risk of PE. They found that women with fiber intake in the highest quartile (>24.3 g/day) had a reduced risk of 51% for the occurrence of PE (OR 0.46; 95% CI 0.23–0.92) compared with women in the lowest quartile (<13.1 g/day). In this study, the food frequency questionnaires were administered at the end of pregnancy; therefore, the possibility of recall bias cannot be excluded.[103] More recently, Qiu et al.[104] carried out a prospective cohort study of 1,538 pregnant women in Washington State in which a 121-item food frequency questionnaire was administered at a mean gestational age of 13.1 weeks. The adjusted relative risk of PE for women in the highest (≥21.2 g/day) versus the lowest (<11.9 g/day) quartile was 0.28 (95% CI 0.11–0.75). The authors observed similar magnitudes of associations for the highest versus the lowest quartiles of water-soluble fiber (RR 0.30; 95% CI 0.11–0.86) and insoluble fiber (RR 0.35; 95% CI 0.14–0.87).[104] Furthermore, mean triglyceride concentrations were significantly lower and high-density lipoprotein cholesterol concentrations were insignificantly higher for women in the highest quartile compared to those in the lowest quartile.[104] More well-designed cohort studies and clinical trials that assess the relationship between fiber intake prior to or during pregnancy and the risk of PE are needed to further explore the role of fiber as well as of obesity, insulin resistance, and dyslipidemia in the development of PE.

PROTEIN INTAKE

It has been suggested that some amino acids such as arginine, citrulline, glycine, taurine, and histidine, as well as

small peptides that directly scavenge oxygen free radicals, are essential for normal endothelial vasomotion.[71,72] However, epidemiological studies have not yielded compelling evidence to support an association between protein deficiency and the increased risk of PE.[96–99] Furthermore, trials of protein supplementation have failed to demonstrate a reduction in the risk of PE.[105,106] The effect of high protein supplementation (protein/energy supplementation in which the protein content of the supplement provided >25% of its total energy content) on pregnancy outcome was assessed in a Cochrane systematic review. No significant benefits of protein supplementation were observed.[107] Another systematic review to assess the effects of the balanced protein-energy supplementation on pregnancy outcome (protein content <25% of total energy content) showed no effects on pregnancy outcome, including the risk of PE.[108] It should be noted that the trials included in these systematic reviews had methodological flaws. Alternate treatment allocation rather than a solid randomization method was used, and a large proportion of women were lost to follow-up for assessing the main outcome.

On the other hand, a high-protein diet may increase the risk of PE by contributing to oxidative stress via homocysteine production and increased whole-body nitric oxide production from nitric oxide synthase induction.[109] However, a published meta-analysis showed that, in three trials involving 384 women, energy/protein restriction had no effect on pregnancy-induced hypertension or PE, although women who were overweight or exhibited high weight gain significantly reduced weekly maternal weight gain and mean birth weight.[110]

LIPID INTAKE

Several studies have documented dyslipidemia in women with PE. Reduced HDL[111,112] and increased triacylglycerols,[113] LDL cholesterol,[114,115] and small dense LDL[116] were demonstrated in women with PE. Increases in serum triglycerides and free fatty acids among women who later developed PE were evident before 20 weeks of gestation.[117]

Increased levels of polyunsaturated and total free fatty acids and of other lipids and reduced levels of (n-3) fatty acids have been observed in women with PE.[118,119] One study prospectively assessed dietary fatty acid intake and fatty acid composition in maternal, fetal, and umbilical blood.[120] Maternal blood was sampled in a large cohort of women at less than 16 and at 22–32 weeks of gestation, as well as within 24 h of delivery. A subset of women underwent dietary assessment in each trimester. The results showed that there were no differences between groups (gestational hypertension with or without proteinuria versus normotensive women) in maternal fatty acid and nutrient intake at 16 and 32 weeks of gestation. After delivery, levels of essential fatty acids 18:2 (n-6) linoleic acid and 18:3 (n-3) α-linoleic acid were significantly lower, whereas the sum of (n-6) long-chain polyenes, which are polyunsaturated fatty acids with 20 or more carbon atoms and three or more double bonds, were significantly higher in hypertensive women compared to controls.[120] In another prospective study by Clausen et al.,[98] an increased intake of polyunsaturated fatty acids was demonstrated in women who later developed PE.

Omega-3 (n-3) fatty acids have been suggested to have a preventive effect on early delivery and hypertensive disorders of pregnancy.[121,122] Omega-3 (n-3) fatty acids are known to reduce fasting and postprandial triglycerides and to decrease platelet and leukocyte reactivity. It has been suggested that high-dose n-3 fatty acid intake could reduce maternal thromboxane A_2 synthesis and enhance maternal refractoriness to angiotensin II, which may reduce the risk of PE.[123] Low erythrocyte levels of omega-3 fatty acids and high levels of omega-6 fatty acids, particularly arachidonic acid, appear to be associated with an increased risk of PE.[124] Wang et al.[125] observed a significantly decreased level of total n-3 and n-6 polyunsaturated fatty acids in women with PE. However, recent clinical trials failed to detect any significant effect of fish oil supplementation on PE risk in women at high risk of gestational hypertension.[126–129] Interestingly, a recent study reported that dietary intake of polyunsaturated fatty acids (n-3 and n-6) was positively related to glutathione peroxidase (GSH-Px) activity in healthy pregnant women, suggesting a possible prooxidant effect.[130] Moreover, a recent prospective study indicated that the odds ratio for hypertensive disorders presented a U-shaped curve across different intake levels of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs).[131] The authors concluded that excessive consumption of n-3 LCPUFAs in early pregnancy or other nutrients (e.g., vitamins A, D, E) found in liquid cod-liver oil may increase the risk of developing hypertensive disorders in pregnancy.[131] Horvath et al.[132] carried out a meta-analysis of randomized controlled trials to evaluate the effects of long-chain polyunsaturated fatty acids on pregnancy outcomes. There were no differences in the rates of pregnancy-induced hypertension and PE.

CALCIUM

Calcium is the micronutrient that has been most extensively studied in relation to PE. Numerous studies have demonstrated reduced levels of serum or urinary calcium in PE.[133–136] Several epidemiological studies indicate an association between low dietary intake of calcium and increased risk of PE.[103,137]

Encouraged by the results of observational studies, a number of controlled trials have been conducted to confirm the beneficial effects of calcium supplementation, but with conflicting results.[138–140] A recent large trial investigated whether calcium supplementation of pregnant women with low calcium intake reduced PE and preterm delivery.[141] Calcium supplementation was associated with a small reduction in the incidence of PE and/or eclampsia (4.1% versus 4.5%; RR 0.91, 95% CI 0.69–1.19), early-onset PE and/or eclampsia (RR 0.77; 95% CI 0.54–1.11), and gestational hypertension (RR 0.96, 95% CI 0.86–1.06); however, null effects were not excluded. A life-table analysis indicated that effects on PE and/or eclampsia were evident by 35 weeks of gestation (1.2% in calcium group versus 2.8% in placebo group, $P = 0.04$). Furthermore, calcium supplementation was associated with a reduced risk of eclampsia (RR 0.68, 95% CI 0.48–0.97) and severe gestational hypertension (RR 0.71, 95% CI 0.61–0.82).[141] Overall, there was a statistically significant reduction in the severe preeclamptic complications index, including any of the following: severe PE, early-onset PE, eclampsia, placental abruption, HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome, or severe gestational hypertension (RR 0.76, 95% CI 0.66–0.89, life-table analysis, log rank test $P = 0.04$).[141] Hofmeyr et al.[142] recently conducted a meta-analysis of 12 randomized controlled trials, including 15,528 women, in which 66% women had a low dietary calcium intake and 96% women were at low risk for gestational hypertension or PE. The dose of calcium administered varied from 1.5 to 2.0 g per day. The random effects model indicated that calcium supplementation significantly reduced the risk of high blood pressure (11 trials, 14,946 women: relative risk 0.70, 95% CI 0.57–0.86), PE (12 trials, 15,206 women: RR 0.48, 95% CI 0.33–0.69), and maternal death or serious morbidity was reduced (4 trials, 9,732 women: RR 0.80, 95% CI 0.65–0.97). The effect was greatest for women at high risk for hypertensive disorders of pregnancy (5 trials, 587 women: RR 0.22, 95% CI 0.12–0.42) and for those with low baseline calcium intake (7 trials, 10,154 women: RR 0.36, 95% CI 0.18–0.70).[142] However, the HELLP syndrome was increased in the calcium supplementation group compared to the placebo group (2 trials, 12,901 women: RR 2.67; 95% CI 1.05–6.82). There were no differences in neonatal outcomes such as preterm birth or stillbirth or death before discharge from hospital.[142]

SODIUM

A Cochrane review indicated that manipulating sodium intake does not affect the frequency of PE.[143] In addition, a study in Japan indicated that a low-salt diet is not only ineffective for the prevention of PE but also accelerates volume depletion in PE.[144] The reduction in sodium intake may cause a significant reduction in the intake of energy, protein, carbohydrates, fat, calcium, and other essential nutrients.[145] Therefore, based on recent evidence, salt restriction is not recommended in pregnancy.

VITAMINS C AND E

Vitamins C and E are two essential nutrients that can scavenge free radicals and constitute a strong line of defense in retarding reactive oxygen species (ROS)-induced cellular damage. Vitamin C (ascorbic acid) is an essential water-soluble vitamin and serves as a nonenzymatic antioxidant by delivering a hydrogen atom with a single electron to a reactive oxygen molecule. Adequate dietary intake is required to prevent oxidative stress. Vitamin E is the major peroxyl radical scavenger in biological lipid molecules, such as those in membranes or low-density lipoproteins (LDLs). Its antioxidant action has been ascribed to its ability to chemically act as a lipid-based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation and oxidized LDL formation.[146] Vitamins C and E also play a key role in the modulation of enzymes involved in the vascular endothelial damage known to contribute to the pathophysiological mechanisms of the clinical expression of PE.[147] In vitro and in vivo studies demonstrate a synergistic effect between two vitamins.[148]

Numerous studies reported reduced levels of vitamin C in women with PE.[149–151] A case-control study (99 women with PE compared with 99 controls) found that women with both elevated oxidized LDL and low vitamin C concentration had a 9.8-fold risk of PE (95% CI 3.0–32.2).[151] Sagol et al.[152] reported that plasma levels of ascorbic acid and serum antioxidant activities were significantly decreased in mild and severe PE. Serum alpha-tocopherol levels were significantly decreased only in severe PE.[152] Furthermore, a case-control study using a semiquantitative food frequency questionnaire found that women who consumed <85 mg of vitamin C daily, as compared with others, experienced a twofold risk of PE. Women with plasma ascorbic acid <34.6 µmol/L had a 3.8-fold increased risk of PE compared with those in the highest quartile. Analyses were conducted by adjusting for maternal age, parity, prepregnancy body mass index, and energy intake.[153]

A reduced level of vitamin E in association with PE has been reported in certain,[154–156] but not in all, studies.[149,150,157–160] Reduced levels of vitamin E have been most consistently demonstrated in severe cases of PE.[152,154,161,162] The variation across studies may be explained by the fact that concentrations of lipid-soluble vitamin E have not been adjusted for lipid concentrations, although the elevation of total cholesterol and triglycer-

ides is one of the characteristics of PE.[163] Moreover, the measurement of plasma vitamin E is a far from satisfactory estimate of the focal site of vitamin E activity, namely the cell membrane. To date, there is no study to measure red cell vitamin E concentrations or those in any other tissue in women with PE. It is striking that the diversion from normal values correlated with severity of disease in the reports describing either lowered or elevated plasma vitamin E concentration in women with PE.[152,154,156,164]

The first clinical trial to investigate the effects of vitamin C and E on the risk of PE was conducted by a UK research group.[165] Patients were included in the study if they were at increased risk of PE, as defined by abnormal uterine artery Doppler waveform or by past history of the disease. The investigators reported a 54% reduction in PE with the supplementation of vitamin C (1,000 mg/day) and vitamin E (400 IU alpha-tocopherol/day) for women who were at risk (RR 0.39; 95% CI 0.17–0.90). The ratio of PAI-1 (plasminogen activator inhibitor-1, a marker of endothelial cell activation) to PAI-2 (a marker of placental function) was significantly decreased in the vitamin-treated group. High-risk women who developed PE in the placebo group had lower plasma vitamin C concentrations ($P < 0.002$) compared with normal pregnant controls, and these returned to normal levels on supplementation.[166] Plasma concentrations of the isoprostane, 8-epi-prostaglandin F2alpha, a marker of lipid peroxidation, were raised in the high-risk placebo group but fell to concentrations comparable to those in low-risk subjects after vitamin C and E supplementation.[166] Another small trial of women at high risk on the basis of their clinical history found no evidence of benefits with the same antioxidants.[167]

Several large trials to evaluate the effects of vitamins E and C in the prevention of PE have been conducted, are under way, or have been recently reported. These trials are from several countries, including Canada, Mexico, the United Kingdom, the United States, and developing countries. The study (VIP trial) enrolled 2,410 women identified to be at increased risk of PE.[168] Women were randomly assigned to either the treatment group (1,000 mg vitamin C and 400 IU RRR-alpha-tocopherol) or the matched placebo group. Analysis was conducted by intention to treat. The incidence of PE was similar in the treatment and control groups (15% versus 16%; RR 0.97; 95% CI 0.80–1.17). However, the incidence of low birth weight was higher in women with antioxidant treatment than in controls (28% versus 24%; RR 1.15; 95% CI 1.02–1.30). With respect to plasma analysis, plasma concentrations of vitamins C and E did not differ between treatment groups. In the placebo group, plasma concentrations of vitamin C were lower throughout the gestational period studied in women who developed PE than in those who did not. Furthermore, the highest quartile of baseline vitamin C intake was associated with a reduced risk of a small-for-gestational-age infant (OR 0.42; 95% CI 0.26–0.67), low birth weight (OR 0.39; 95% CI 0.24–0.63), and PE (OR 0.59; 95% CI 0.38–0.93) after adjusting for risk group, degree of education, housing status, and smoking status. An Austrian multicenter trial was conducted among 1,877 nulliparous women recruited between 14 and 22 weeks of gestation.[169] Women were assigned to either daily supplementation with 1,000 mg of vitamin C and 400 IU of vitamin E (935 in the treatment group) or placebo (microcrystalline cellulose) until delivery (942 in the placebo group). Primary outcomes were the risks of maternal PE, death or serious outcome in the infant, and delivery of an infant with birth weight below the 10th percentile for gestational age. The results indicated that there were no significant differences between the vitamin and placebo groups in the risk of PE (6.0% versus 5%; RR 1.20; 95% CI 0.82–1.75), death or serious infant outcome (9.5% versus 12.1%; RR 0.79; 95% CI 0.61–1.02), or delivery of a small-for-gestational-age infant (8.7% versus 9.9%; RR 0.87; 95% CI 0.66–1.16). Women in the vitamin group were more likely than those in the placebo group to be admitted antenatally with hypertension and to be treated with antihypertensive drugs. This finding may be due only to chance. However, research has suggested that antioxidants may promote DNA oxidation by interacting with metal ions.[170] Spinnato II et al.[171] reported the results of a trial in women recruited between 12 and 19 weeks of gestation and diagnosed to have chronic hypertension or a prior history of PE. There was no significant reduction in the risk of PE in the supplementation group compared with the placebo group (adjusted RR 0.87; 95% CI 0.61–1.25). There were no differences in mean gestational age or rates of perinatal mortality, abruptio placentae, preterm delivery, or small-for-gestational-age or low-birth-weight infants.[171] The World Health Organization recently completed a multicenter trial that was conducted among pregnant women with low socioeconomic status and low nutritional status from developing countries (e.g., India, Peru, South Africa, and Vietnam).[172] The trial followed the research protocol that was used in the VIP trial,[168] with only minor adaptation to local resources. The results showed that supplementation with vitamins C and E did not reduce the risk of PE (RR 1.0; 95% CI 0.9–1.3), eclampsia (RR 1.5; 95% CI 0.3–8.9), or gestational hypertension (RR 1.2; 95% CI 0.9–1.7).[172]

In addition to the trials of combined vitamins C and E supplementation, Steyn et al.[173] reported no effects of vitamin C supplementation alone on the risk of PE (RR 1.00; 95% CI 0.21–4.84). Rivas-Echeverria[174] et al. conducted a trial in which supplements of aspirin, vitamin C, vitamin E, and fish oil were used. They found that such supplements significantly reduced the risk of PE (RR

0.07; 95% CI 0.01–0.54). However, it is hard to infer whether such an effect was due to vitamins C and E, fish oil, or aspirin, or the effects of their interaction. Our group has recently updated the meta-analysis by Polyzos et al.,[175] which involved eight trials, one of which assessed the effect of vitamin C alone,[173] six of which evaluated the effect of combined vitamin C and E supplementation,[165,167–169,171,172] and one of which examined the benefits of vitamin C and E with fish oil.[174] Overall, we were unable to detect any benefits of vitamin C alone, or vitamin C combined with vitamin E, or vitamin C and E combined with other supplements in reducing the risk of PE (random effects model, RR 0.93; 95% CI 0.76–1.13).

It is clear from the literature that vitamin E exerts both potentially beneficial and potentially detrimental effects. The ineffectiveness of vitamins C and E in the prevention of PE emphasizes the need for understanding the underlying mechanisms and metabolism of both vitamins C and E in the human body. It is noteworthy that vitamin E also has nonantioxidant pleiotropic effects in addition to its antioxidant capacity.[176] Exogenous vitamin E may prevent an immunologic switch (Th1 to Th2) that is considered crucial for early-to-late transition in normal pregnancy, and it could be a potential interferon-gamma (IFN-γ) mimic, facilitating proinflammatory responses at the maternal-fetal interface. Therefore, vitamin E treatment might have undesirable side effects, which may partially explain the conflicting results of previously published trials.[165,167–169] There is some evidence that high doses of alpha-tocopherol (primary form of vitamin E in supplementation) could deplete plasma and tissue gamma-tocopherol (major forms in plant seeds and in the American diets).[177–180] Therefore, the efficacy of vitamin E supplementation (alpha-tocopherol) may be offset by deleterious changes of other nutrients.

VITAMIN A

The role of vitamin A and beta-carotene (provitamin A) in pregnancy-induced hypertension and PE is also a subject of controversy. Many clinical studies have found significantly lower levels of vitamin A and beta-carotene in preeclamptic women than in healthy women.[181–185] However, the decreased levels of retinol and beta-carotene might be secondary to disease as a part of an acute-phase reaction rather than a causal relationship. Further studies are needed to determine the temporal relationship between carotenoids and the risk of adverse pregnancy outcomes. Unlike beta-carotene, vitamin A in high doses could be toxic, and there is concern about its teratogenicity.[186–190] Given that it is unlikely that a safety threshold of vitamin A consumption in early pregnancy would be established over the next few years, it is ethically difficult to conduct human trials to assess the effects of vitamin A supplementation in early pregnancy on the risk of PE.

FOLATE (FOLIC ACID)

Folate is the generic term for this water-soluble B-complex vitamin. It functions as a coenzyme in single-carbon transfers in the metabolism of amino acids and nucleic acids and is therefore required by all cells for growth. Folic acid (pteroylmonoglutamic acid, or PGA), which is the common form used in vitamin supplements and fortified food products, is the most oxidized and stable form of folate. Most naturally occurring folates, called food folate, are pteroylmonoglutamates, which contain one to six additional glutamate molecules joined in a peptide linkage to the γ-carboxyl of glutamate.

The importance of an adequate folate supply during pregnancy and lactation is increasingly recognized. It has been suggested that folic acid from food intake and routine supplementation may be sufficient during the periconceptional period, but larger doses may be required in early gestation, particularly for women with a higher risk of adverse pregnancy outcomes (e.g., PE). Folate may reduce the risk of developing PE by improving endothelial function at both the placental and systemic levels,[191] or by lowering homocysteine, a risk factor for PE.[192]

Epidemiologic studies have found that supplementation with multivitamins containing folic acid is associated with a reduced risk of PE.[193,194] Bodnar et al.[193] examined the association between regular use of multivitamins containing folic acid at less than 16 weeks of gestation and the risk of PE in 1,835 women in Pittsburgh, Pennsylvania, between 1997 and 2001. They found that regular use of multivitamins containing folic acid was associated with a 45% reduction in PE risk compared with nonusers (OR 0.55; 95% CI 0.32–0.95) Hernandez-Diaz et al.[194] also observed a significant reduction of risk for gestational hypertension after supplementation with multivitamins containing folic acid (adjusted OR 0.55; 95% CI 0.39–0.79). Wen et al.[195] carried out a prospective cohort study of 2,951 women in Ottawa and Kingston, Canada. They found that supplementation with multivitamins containing folic acid early in the second trimester was associated with increased serum folate, lowered plasma homocysteine, and reduced risk of PE (adjusted OR 0.37; 95% CI 0.18–0.75). Catov et al.[196] examined the associations between supplementation with multivitamins containing folate or with folate only during a 12-week periconceptional period using the Danish National Birth Cohort data. They found that regular periconceptional use of a multivitamin containing folate was associated with a 20% reduction in the risk of PE among normal-weight women. However, such a reduction was not observed for

folate-only supplements.[196] Furthermore, Ray and Mamdani[197] found a small reduction in the rates of PE in Canada after folic acid food fortification in 1998 (prevalence ratio 0.96, 95% CI 0.94–0.98). Evidence from trials assessing the effects of folate supplements on the risk of PE is very limited. Taylor et al.[198] conducted a randomized trial to assess the effects of supplementation with elemental iron (65 mg/day) and folic acid (350 µg/day) on adverse pregnancy outcomes in 48 healthy pregnant women. They found no effect of iron-folic acid supplementation on the risk of PE. Charles et al.[199] reanalyzed data from a large randomized controlled trial performed between 1966 and 1967 and found that the risk of PE was lower in groups supplemented with folic acid (200 µg/day and 5 mg/day) than in the placebo group.

VITAMIN D

The immunomodulatory properties of the hormonal vitamin D system could potentially have beneficial effects for successful maintenance of pregnancy.[200] Impaired vitamin D metabolism is demonstrated in preeclamptic pregnancy.[200,201] Therefore, adequate vitamin D status/intake could potentially contribute to the prevention of PE.[202]

Studies exploring the role of maternal vitamin D status in adverse pregnancy outcomes are scarce. Bodnar et al.[203] conducted a nested case-control study of pregnant women followed from less than 16 weeks of gestation to delivery (1997–2001) to assess the association of maternal serum 25(OH) D levels with the risk of PE. Their results indicated that serum 25(OH) D concentrations in early pregnancy were lower in women who subsequently developed PE compared with controls. There was a monotonic dose-response relation between serum 25(OH) D concentration prior to 22 weeks of gestation and the risk of PE. A 50 nmol/L decline in 25(OH) D concentration doubled the risk of developing PE (adjusted OR 2.4; 95% CI 1.1–5.4). Newborns of preeclamptic women were twice as likely as control newborns to have a 25(OH) D concentration of less than 37.5 nmol/L (adjusted OR 2.2; 95% CI 1.2–4.1).[203] A recently published paper by Haugen et al.[204] examined the association between vitamin D intake during pregnancy and the risk of PE in 23,423 nulliparous pregnant women taking part in the Norwegian Mother and Child Cohort Study. They found an odds ratio of PE of 0.76 (95% CI 0.60–0.95) for women with a total vitamin D intake of 15–20 µg/d versus those with an intake of less than 5 µg/d. Moreover, they reported a 27% reduction in the risk of PE (OR 0.73; 95% CI 0.58–0.92) for women taking 10–15 µg/d vitamin D supplementation as compared with no supplementation. However, no association was found between vitamin intake from the diet alone and the risk of PE.[204] There is potential correlation between vitamin D supplementation and intake of other nutrients (e.g., calcium, omega-3 fatty acid).[204] Further studies with data on other nutrient intake and vitamin D status will be necessary to disentangle the effect of each on PE risk.

MAGNESIUM

Magnesium is an essential mineral needed by humans in relatively large amounts. It is crucial for regulating temperature and protein synthesis and maintaining electrical potential in nerves and muscle membranes. In a prospective observational study in which a food frequency questionnaire was used to assess diet at 30 weeks of gestation in pregnant Danish women, no difference in magnesium intake was found in women who developed PE compared with controls.[102] Observational studies show that supplementation with magnesium is associated with a reduced risk of PE.[205] However, a Cochrane systematic review found no evidence of a benefit of magnesium supplementation on the risk of PE.[206] The methodological quality of trials included in the review was poor.

OTHER MICRONUTRIENTS

Certain trace elements are essential cofactors for adequate activation of antioxidant enzymes. These trace elements (e.g., copper, iron, and selenium) are directly implicated in oxidative/antioxidative balance – a key pathogenic process in PE – and are highly dependent on dietary habits and supplements.[207–209] Serum concentrations of magnesium, copper, and zinc have been reported to be significantly lower in women with PE compared with controls.[136,210]

Epidemiological studies have suggested that deficiencies of zinc, iron, and selenium are associated with an increased risk of PE. However, randomized trials have failed to demonstrate a beneficial effect of supplementation with trace elements in the prevention or management of PE.[211–213] Little information is available with respect to the specific roles of these trace elements in early pregnancy for PE susceptibility.

OBESITY, WEIGHT GAIN, AND RISK OF PREECALMPSIA

Obesity is an independent risk factor for PE.[17] The link between obesity and PE is complex. The metabolic changes in obese women, such as increased lipid availability, higher cholesterol and triglyceride levels, or insulin resistance, may lead to a derangement of the very-low-density lipoprotein (VLDL)/toxicity-preventing activity balance and enhance cytokine-mediated oxidative stress,

subsequently leading to endothelial cell dysfunction.[214–216] Moreover, elevated cardiac output with compensatory vasodilation in women with obesity may also lead to endothelial cell dysfunction. Most observational studies consistently demonstrate that maternal obesity or a higher prepregnancy body mass index (BMI) is associated with an increased risk of PE or gestational hypertension.[217–220]

Bodnar et al.[217] reported that prepregnancy adiposity is a strong independent risk factor for PE. The authors further explored the dose-dependent relationship between prepregnancy BMI and the risk of PE. The results indicated that PE risk rises through most of the BMI distribution. Compared with women with a BMI of 21, the risk of PE doubled for women at a BMI of 26, and nearly tripled risk for those at a BMI of 30.[217] A systematic review identifying three cohort studies in 1.4 million women (from the United States, Sweden, the Netherlands, Latin America, the Caribbean, Taiwan, and the United Kingdom) demonstrated that the risk of PE typically doubled with each 5–7 kg/m^2 increase in prepregnancy BMI.[218]

The prevalence of obesity is rapidly increasing worldwide, and the epidemic is especially pronounced in women of childbearing age.[221,222] It has been reported that the prevalence of prepregnancy obesity increased by 69% over a 10-year period, from 13% in 1993–1994 to 22% in 2002–2003.[222] As obesity confers a significant risk for PE, the epidemic of obesity will undoubtedly increase the incidence of PE. Wallis et al.[223] analyzed public-use data from the National Hospital Discharge Survey and reported that rates of PE and gestational hypertension increased by 25% and 184%, respectively, from 1987 to 2004. Thus, public health programs to promote the reduction of overweight or obesity as well as further research to evaluate their effectiveness are needed to suppress the epidemic of obesity and, thereby, the significant increase of PE.

Over the past 25 years, several authors have demonstrated a significant association between excessive weight gain and hypertensive disorders of pregnancy.[219,220,224–226] Brennand et al.[227] showed that obese women with excessive weight gain had a higher prevalence of PE (14.9%) than obese women with low (3.7%) or acceptable (6.3%) weight gain. Saftlas et al.[228] did not find an association between excessive weight gain and the risk of PE, although the risk of transient hypertension was increased more than twofold among women in the highest quartile of the weight gain index (OR 2.55; 95% CI 1.66–3.92). A prospective population-based cohort study by Cedergren[229] of 245,526 singleton term pregnancies showed that obese women with low gestational weight gain had a decreased risk of PE (OR 0.52; 95% CI 0.42–0.62). There was a twofold increased risk of PE among average-weight and overweight women with excessive weight gain. Kiel et al.[230] carried out a population-based cohort study of 120,251 pregnant obese women to examine the associations between gestational weight change and adverse outcomes. The authors reported that a gestational weight gain in overweight or obese pregnant women of less than the currently recommended 15 pounds was associated with a significantly lower risk of PE. The authors concluded that limited or no weight gain in obese pregnant women is associated with favorable pregnancy outcomes. Langford et al.[231] conducted a population-based cohort study to examine the association between gestational weight gain and adverse outcomes among overweight women (BMI 26.0–29.0 kg/m^2). Compared with women who gained 15–25 lbs, women who gained <15 lbs were 0.8 (95% CI 0.6–1.0) times as likely to have PE, but women who gained >25 lbs were 1.7 (95% CI 1.5–1.9) times as likely to have PE.[231] The Institute of Medicine of the National Academies has recently revised its guidelines for healthy ranges of weight gain in pregnancy for overweight or obese women: 15–25 lb of weight gain for overweight women (BMI 25–29.9 kg/m^2), and 11–20 lb of weight gain for obese women (BMI ≥30 kg/m^2).[232] Continued research and health policy should promote the implementation of the new guidelines and determine their impact on healthcare.

DIETARY MEASUREMENT

The measurements of subjects' dietary intake and the related methodological considerations remain at the center of discussion in nutritional studies. The dietary measurement instrument used most often in large-scale epidemiological studies, particularly prospective cohort studies, is the food frequency questionnaire (FFQ). The major obstacle in using the questionnaire assessment is the lack of accuracy of the subjects' self-reported estimations of their habitual dietary intakes. Random errors and uncorrelated measurement errors can cause attenuations of risk estimates and reduce the statistical power.[233] These problems have prompted researchers to incorporate validation and calibration substudies that include more intensive, presumably more accurate reference methods, typically multiple-day food records or multiple 24-hour dietary recalls.[234] Nevertheless, It may be overly optimistic to assume that these reference measurements can provide truly unbiased dietary intake levels. Therefore, it is essential to consider all of these issues in the design and analysis of nutritional studies as well as in the interpretation of nutritional data.

COMMENT

A causal network of socioeconomic, genetic, maternal health, and nutritional factors likely plays a crucial role in

the etiology of PE. The underlying mechanisms are complex and remain unclear. It has been suggested that maternal nutritional imbalance may lead to altered gene methylation and expression, altered homocysteine metabolism, inflammatory responses, and oxidative stress. However, nutritional intervention studies have not yet provided unequivocal evidence in favor of an association between maternal nutrient intake, in particular that of micronutrients (e.g., folate, vitamins), during pregnancy and the risk of PE (Table 1). Several knowledge gaps remain, including: 1) the delineation of the dose-response relationship between important nutrient candidates (e.g.,

Table 1 **A summary of randomized controlled trials of micronutrient supplementation during pregnancy and the risk of preeclampsia.**

Micronutrient	Intervention	Participants	Summary of findings
Calcium	Type: calcium (daily dose varied from 1.5 to 2.0 g) versus placebo Initiation: varied from 18 to 22 weeks of gestation	Most women were at low risk for PE and had low calcium intake.	A total of 12 trials have been carried out. Most trials conducted in women with low calcium status showed a protective effect of calcium supplementation on the risk of PE. Most trials conducted in women with adequate calcium status failed to show any beneficial effects of calcium supplementation.[142]
Vitamin C only	Type: vitamin C (500 mg daily) versus placebo Initiation: <26 weeks of gestation	Women at high risk for preterm birth (previous abortion or preterm birth)	Only 1 trial was conducted and found no reduced risk of PE associated with vitamin C supplementation.[173]
Vitamins C and E	Type: 1,000 mg vitamin C and 400 IU vitamin E daily versus placebo Initiation: trial entry varied with each trial (18–22, 14–20, 14–21, and 12–20 of weeks of gestation)	Women at high and low risk for PE	A total of 6 trials evaluated the combined effects of vitamins C and E supplementation.[165,167–169,171,172] Although the first trial by Chappell et al.[165] reported a significant reduction of PE associated with vitamins C and E supplementation, the following trials failed to provide evidence of beneficial effects of vitamins C and E.[167–169,171,172]
Vitamins C and E and fish oil	Type: 500 mg vitamin C per day, 400 IU vitamin E per day, 1 g fish oil 3 times per day and 100 mg aspirin 3 times per week versus placebo Initiation: <29 weeks of gestation	Women at high risk for PE	Rivas et al.[174] reported a significant reduction of PE associated with supplementation of vitamins C and E and fish oil.
Vitamin A	To date, no trial has been conducted to assess the effects of vitamin A on the risk of PE.		
Vitamin D	Evidence from trial is not available.		
Folic acid (folate)	Direct evidence from trials is not available. A reanalysis of a large RCT indicated that folate supplementation (200 µg/day and 5 mg/day versus placebo) was associated with a reduced risk of PE.[199]		
Zinc	Type: zinc versus no zinc (dose varied from 20 to 44 mg daily) or placebo Initiation: trial entry varied with each trial (<20, <24, <26, <27, and 15–25 weeks of gestation)	Normal pregnant women with no systemic illness with normal and low zinc status	A total of 7 trials were conducted, including 5 trials in women with low zinc status and 2 trials in women with normal zinc status.[212] Only 1 trial conducted in women with low zinc status found that zinc supplementation significantly reduced the risk of PE.
Magnesium	Type: magnesium (dose of 500 mg or 365 mg daily) versus control Initiation: <4 months of gestation, 13–24 weeks of gestation	Nulliparous and multiparous women	Two trials reported no reduction of PE associated with magnesium supplementation. Methodological quality, however, is questionable.[206]
Iron	Type: iron (27 mg daily) versus placebo Initiation: <13 weeks of gestation	Healthy pregnant women	Eskeland et al.[213] reported no reduction of PE associated with oral iron supplementation. The sample size, however, was very small ($n = 90$).

Abbreviations: PE, preeclampsia; RCT, randomized controlled trial.

folate, omega-3 fatty acids) and the risk of PE, 2) the examination of the potential nutrient-nutrient, nutrient-environmental (e.g., tobacco, alcohol, and drug use), and nutrient-genetic interactions as well as elucidation of their interplay on the risk modifications of PE, and 3) the identification of the critical time windows during which nutrient intake or supplementation may alter the risk of PE.

On the basis of our review, calcium supplementation during pregnancy was found to significantly reduce the risk of PE for women at high risk and for those with low baseline dietary calcium intake. However, there was no evidence of a protective effect of prenatal calcium supplementation on adverse neonatal outcomes (e.g., preterm birth, neonatal death, intrauterine growth restriction). Further studies are required to substantiate the evidence that calcium supplementation during pregnancy significantly reduces the risk of perinatal mortality or morbidity without showing any long-term adverse effects. Current evidence suggests that calcium supplementation in pregnancy would appear to be justified, particularly in patients with low nutritional intake. However, additional data is required in terms of long-term effects. Furthermore, the optimal dose of supplementation should be further studied.

With the success of folic acid in the prevention of neural tube defects, the health benefits of folic acid are increasingly recognized by health professionals and women of reproductive age. Given the promising effect of folic acid on the risk of PE shown in previous observational studies, a randomized controlled trial with solid methodology is urgently needed to assess the effect of folic acid supplementation during early pregnancy on PE. It seems that there is a limited time window to implement such a trial, as there is tendency for more and more pregnant women to receive folic acid supplementation during early pregnancy because of proven effects in the prevention of neural tube defect.

The available evidence does not support the use of combined vitamin C and E supplementation during pregnancy for the prevention of PE. Furthermore, the safety of such supplementation, more specifically the effect on infant outcome (e.g., low birth weight), is still uncertain. At this stage, the supplementation of vitamin C and E during pregnancy should be discouraged unless solid supporting data from randomized controlled trials become available.

Studies exploring the role of maternal vitamin D status in adverse pregnancy outcomes are scarce. To date, only one observational study observed that there is an association between vitamin D status and the risk of PE. Further studies are necessary to unravel the association between vitamin D status during pregnancy and adverse pregnancy outcomes. Furthermore, more studies to investigate the adverse effects of vitamin D intake during pregnancy, including both short-term and long-term effects, are required in the near future. Additional observational data and evidence of biological plausibility of an effect should be obtained before undertaking a large-scale trial of vitamin D supplementation for the prevention of PE.

It has been suggested that maternal characteristics at enrollment could affect the risk of PE as well as potentially modify the estimated effect of the treatment studied. Currently, studies are underway that aim to develop predictive models of PE. The characterization of high-risk populations will permit the documentation of the nutritional profiles of these women. This may lead to new intervention strategies to prevent PE. Furthermore, as knowledge regarding the underlying mechanism of PE advances, there will be opportunities to explore the impact of nutritional factors on specific causal pathways. As new knowledge emerges, we must recall that large randomized clinical trials of nutritional interventions to prevent PE are extremely costly and should be undertaken only when there is a strong plausibility of a potential benefit of a novel intervention.

CONCLUSION

For many years, diet has been suggested to play a role in the risk of PE. Much of the clinical and basic research into nutritional causes of hypertensive disorders of pregnancy has paralleled research conducted on hypertension focused on nutrients such as calcium, sodium, magnesium, and fatty acids. Studies to investigate the relationships between nutrients such as folate, vitamins A and D, beta-carotene, and trace elements and the risk of hypertensive disorders during pregnancy are very limited. Additional clinical research, including well-designed clinical trials and prospective cohort studies in particular, are warranted.

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9.3 An international trial of antioxidants in the prevention of preeclampsia

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OBSTETRICS

An international trial of antioxidants in the prevention of preeclampsia (INTAPP)

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OBJECTIVE: We sought to investigate whether prenatal vitamin C and E supplementation reduces the incidence of gestational hypertension (GH) and its adverse conditions among high- and low-risk women.

STUDY DESIGN: In a multicenter randomized controlled trial, women were stratified by the risk status and assigned to daily treatment (1 g vitamin C and 400 IU vitamin E) or placebo. The primary outcome was GH and its adverse conditions.

RESULTS: Of the 2647 women randomized, 2363 were included in the analysis. There was no difference in the risk of GH and its adverse conditions between groups (relative risk, 0.99; 95% confidence interval, 0.78–1.26). However, vitamins C and E increased the risk of fetal loss or perinatal death (nonprespecified) as well as preterm prelabor rupture of membranes.

CONCLUSION: Vitamin C and E supplementation did not reduce the rate of preeclampsia or GH, but increased the risk of fetal loss or perinatal death and preterm prelabor rupture of membranes.

Key words: preeclampsia, randomized controlled trial, vitamins C and E

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Preeclampsia (PE), defined as gestational hypertension (GH) and proteinuria, is a syndrome unique to, and that complicates, 2-8% of human pregnancies.[1-3] It accounts for about 10-15% of direct maternal deaths in low-, middle-, and high-income countries and is associated with low birthweight (<2500 g) infants and thereby perinatal deaths through both preterm birth and intrauterine growth restriction (IUGR).[4-10]

Several lines of evidence support the hypothesis that oxidative stress, an imbalance between prooxidant and antiox-

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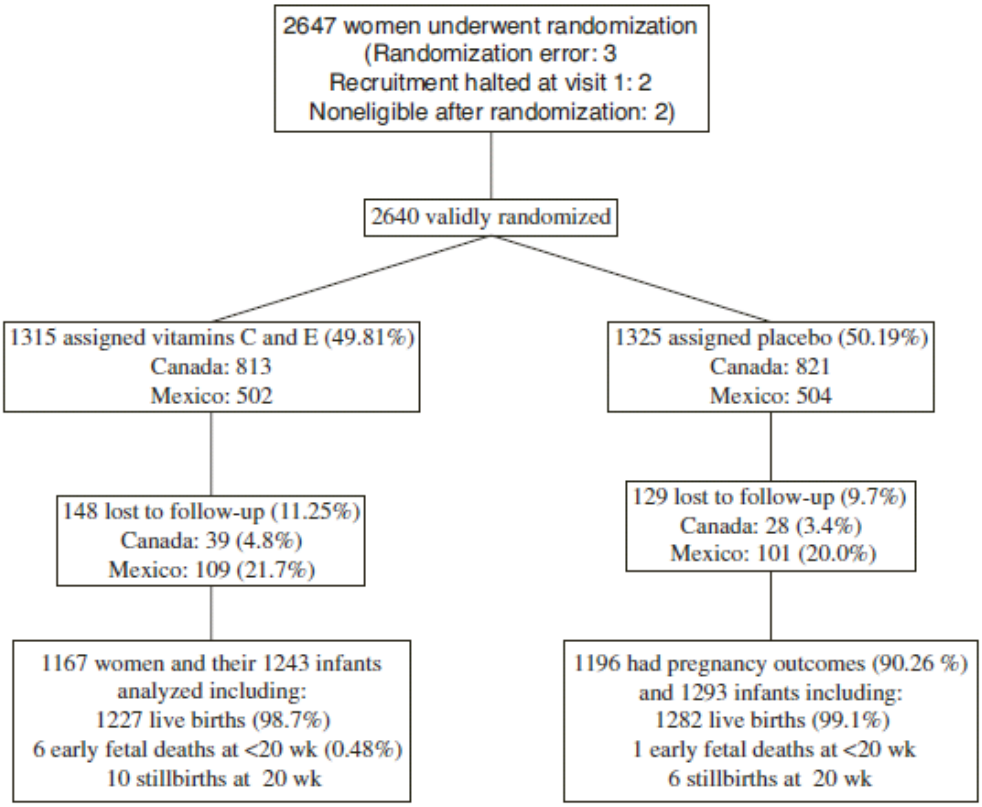
FIGURE
Trial profile



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idant forces, plays an essential role in the development of hypertensive disorders of pregnancy.[11-15] Markers of oxidative stress, such as isoprostanes and malondialdehyde, are increased in plasma,[16,17] small arteries,[18] and decidua basalis[19] of women with PE. In response to these findings, several clinical studies have been conducted that attempt to improve the antioxidant capability of pregnant women and thereby reduce the risk of PE.[20-25] A pilot randomized trial by Chappell et al[20] reported a 54% reduction in PE in the group that was supplemented with vitamins C and E (relative risk [RR], 0.39; 95% confidence interval [CI], 0.17–0.90) compared with the placebo group. Most women included in the trial were at an increased risk for PE as defined by abnormal uterine artery Doppler waveform finding or by history of the disease.[20]

In response to the trial by Chappell et al,[20] we designed the International Trial of Antioxidants in the Prevention of PE (INTAPP) to assess whether or not vitamin C and E supplementation during pregnancy reduces the risk of developing GH and its adverse conditions in: (1) nulliparous women without additional identified major risk factors; and (2) nulliparous and multiparous women having those risk factors.

MATERIALS AND METHODS

From January 2004 through March 2006, we conducted a double-blinded, multicenter trial in Canada (17 centers) and Mexico (10 centers). Women were eligible for the trial if they were between 12 and 18 completed weeks of pregnancy on the basis of last menstrual period and confirmed by early ultrasound examination. The exclusion criteria were: (1)

women who regularly consumed supplements >200 mg/day for vitamin C and/or 50 IU/day for vitamin E; (2) women who took warfarin; (3) women who had known fetal abnormalities (eg, hydatidiform mole), or known fetal chromosomal or major malformations in the current pregnancy; (4) women who had a history of medical complications including endocrine disease (eg, thyroid disease), renal disease with altered renal function, epilepsy, any collagen vascular disease (eg, systemic lupus erythematosus and scleroderma), active and chronic liver disease (eg, hepatitis), heart disease, serious pulmonary disease, cancer, or hematologic disorder (eg, anemia or thrombophilia); (5) women with repeated spontaneous abortion (women with a previous bleeding in the first trimester were included if the site documented a viable fetus at the time of recruitment); and (6) women who used an illicit drug during the current pregnancy.

Randomization was performed through an electronic data management platform, which enabled randomization and data entry over the Internet through a secured and restricted-access World Wide Web site and stored the data in a centralized database. Randomization was stratified by center and by risk status according to prespecified clinical risk criteria. Women were at high risk if they were nulliparous or multiparous with prepregnancy chronic hypertension (or diastolic blood pressure >90 mm Hg at <20 gestational weeks or use of antihypertensive medication), prepregnancy diabetes (insulin-dependent or hypoglycemic agents), multiple pregnancy, or a history of PE in the previous pregnancy. Women were stratified into the low-risk stratum if they were nulliparous without any identified clinical risk factors. They were randomly allocated at a ratio of 1:1 to antioxidant supplementation (vitamins C and E) group or to placebo group through an electronic data management platform. None of the trial staff or any other person involved in the trial knew the treatment allocation for any women until after completion of the trial analysis. The Hôpital Ste-Justine Ethics Research Committee in Montreal, Quebec, Canada (No. 1863, Jan. 12, 2003) and the Instituto Mexicano del Seguro Sosial (IMSS) Ethics Board in Mexico City, Mexico, provided ethics approval, and we acquired ethics approval from each participating center. All participants gave written consent.

Women were provided either with the vitamins C and E or placebo (Carlson Laboratories Inc, Arlington Heights, IL). Women assigned to the vitamin group were advised to take 2 soft gel capsules, each containing 500 mg of vitamin C (as

TABLE 1
Women's baseline demographic and obstetric characteristics by treatment group

Characteristic	Vitamins C and E n = 1167	Placebo n = 1196
Maternal age, y	28.66 (5.57)	28.68 (5.44)
Maternal education, y	14.48 (3.47)	14.53 (3.62)
Maternal prepregnancy BMI[a]	25.45 (5.69)	25.47 (2.09)
Maternal visit-1 BMI[a]	26.69 (5.81)	26.75 (6.21)
Ethnic origin		
Asian	26 (2.23)	12 (1.01)
South Asian	6 (0.51)	4 (0.34)
Caucasian	364 (31.22)	406 (34.00)
French Canadian	285 (24.44)	279 (23.37)
African	10 (0.86)	16 (1.34)
Hispanic	403 (34.56)	416 (34.84)
First Nation	7 (0.60)	5 (0.42)
Other	65 (5.57)	56 (4.69)
Gestational age, wk	15.19 (2.10)	15.28 (2.09)
Gravidity	1.65 (1.02)	1.67 (1.10)
Nulliparous	934 (80.03)	957 (80.02)
Employed	890 (76.33)	911 (76.36)
Smoking before pregnancy	340 (29.16)	330 (27.64)
Current smoker	76 (6.56)	88 (7.43)
Current drinker	14 (1.20)	23 (1.93)
Blood pressure		
Systolic	108.92 (13.13)	109.27 (13.57)
Diastolic	67.50 (9.03)	67.33 (9.07)
Dipstick proteinuria		
Normal or trace	1109 (96.43)	1137 (96.85)
1+	40 (3.48)	33 (2.81)
2+	1 (0.09)	4 (0.34)
High-risk group (stratum)	338 (28.96)	346 (28.93)
Chronic hypertension	78 (6.68)	70 (5.85)
Diabetes	82 (7.03)	76 (6.35)
Multiple pregnancy[b]	67 (5.74)	93 (7.78)[b]
History of PE	147 (12.60)	148 (12.37)
Multiple risk factors	33 (2.83)	38 (3.18)

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TABLE 1
Women's baseline demographic and obstetric characteristics by treatment group *(continued)*

Characteristic	Vitamins C and E n = 1167	Placebo n = 1196
Use of supplements		
Multivitamins	711 (61.29)	756 (63.26)
Vitamin C	20 (1.72)	10 (0.84)
Vitamin E	2 (0.17)	1 (0.08)
Folate	527 (45.35)	519 (43.54)
Calcium	164 (14.11)	163 (13.67)
Iron	272 (23.41)	271 (22.75)
Family history of PE, eclampsia, or GH	156 (13.37)	143 (11.96)
Family history of PE	95 (8.14)	84 (7.02)
Family history of eclampsia	16 (1.37)	10 (0.84)
Family history of GH	89 (7.63)	82 (6.86)
Obstetric history		
History of abortion	316 (27.10)	315 (26.36)
History of stillbirth	17 (1.46)	11 (0.92)
History of preterm birth	97 (8.32)	84 (7.03)
History of low birthweight	60 (5.15)	48 (4.02)

BMI, body mass index; *GH*, gestational hypertension; *PE*, preeclampsia.
Data are presented as mean (SD) or n (%).
[a] BMI = weight (kg)/height^2 (m^2); [b] $P < .05$.
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ascorbic acid) and 200 IU of vitamin E (100 IU d-α-tocopherol, 100 IU d-α-tocopheryl acetate). The total daily dose of vitamin C was 1000 mg, and that of vitamin E was 400 IU. Women in the placebo group were advised to take capsules that were identical in appearance to the active treatment capsules. Women were asked to swallow the capsules whole without crushing or chewing them and were advised not to take other antioxidant supplements.

Women and their infants received care according to standard practice in each center, with surveillance for hypertension using standardized measurements of blood pressure. Systolic and diastolic blood pressure were measured by the clinical staff at each visit using a sphygmomanometer and were assessed in a sitting position, with the cuffed arm resting on a desk at the level of the heart. Korotkoff phase V was used to measure diastolic blood pressure and Korotkoff phase IV was utilized when a phase V was absent.

The composite primary outcome was defined as GH and its adverse conditions. Our choice of the primary outcome for the trial relied on definitions proposed in the Canadian Consensus Statement of 1997.[1] The goal was to assess the impact of antioxidants on clinically significant hypertensive disorders of pregnancy, whether or not proteinuria was present. GH was defined as ≥2 readings of diastolic blood pressure ≥90 mm Hg taken 4 hours apart but within 72 hours occurring at >20 weeks of gestation.[1,26] Severe GH was defined as ≥2 readings of diastolic blood pressure systolic ≥110 mm Hg or systolic blood pressure ≥160 mm Hg at least 4 hours apart.[1,26] Proteinuria was defined as the urinary excretion of ≥0.3 g/24 hours, or ≥2+ on diagnostic strips. PE was defined as GH or severe GH with proteinuria.[1,26] For women with preexisting hypertension, PE is classified as new or worsening proteinuria as defined above. For women with preexisting proteinuria (eg, diabetes with renal involvement), the diagnosis of PE was made on clinical or biochemical grounds by identifying at least 1 additional adverse condition (eg, abnormal liver enzymes, low platelets, and eclampsia).[1,26] All cases of GH and PE were further adjudicated by 2 independent investigators working in the trial coordinating center with failure to achieve consensus resolved by a third independent investigator.

Adverse conditions were defined as ≥1 of the following selected medical conditions: (1) diastolic pressure ≥110 mm Hg or systolic pressure ≥160 mm Hg; (2) proteinuria ≥300 mg/24-hour urine collection or ≥2+ on diagnostic strips; (3) convulsion (eclampsia); (4) thrombocytopenia (platelet count $<100{,}000 \times 10^9$/L); (5) elevated liver enzyme levels (aspartate aminotransferase or alanine aminotransferase >70 U/L); (6) hematocrit <24% or blood transfusion; (7) IUGR (ie, birthweight <3rd centile for gestational age using Canadian population-based birthweight for gestational age as reference[27]); and (8) perinatal death (fetal death >20 weeks or neonatal death within 7 days). Other maternal outcomes included death, severe GH, severe PE, prelabor rupture of membranes (PROM), preterm PROM (PPROM), and hospitalization prior to giving birth. Severe PE was defined as PE and the presence of at least 1 of the following adverse conditions: (1) diastolic pressure ≥110 mm Hg or systolic pressure ≥160 mm Hg; (2) proteinuria ≥5 g/24-hour urine collection or dipstick ≥3+; (3) convulsion; (4) thrombocytopenia; (5) elevated liver enzyme levels; (6) hematocrit <24% or blood transfusion; (7) IUGR; (8) perinatal death; or (9) preterm delivery (<34 weeks of gestational age).[1,26,28] PROM was defined as spontaneous at ≥37 weeks of gestation and before onset of labor. PPROM was defined as spontaneous at <37 weeks of gestation and before onset of labor.

A composite outcome "fetal loss or perinatal death" was defined as any fetal loss at <20 weeks, stillbirth, or neonatal death. Other fetal or neonatal outcomes

included: (1) preterm birth <37 weeks of gestational age (gestational age corrected by early ultrasound scan); (2) preterm birth <34 weeks of gestational age; (3) small for gestational age (defined as <5th or 10th centile); (4) perinatal mortality; (5) spontaneous abortion; and (6) neonatal morbidity indicators such as Apgar score <4 at 5 minutes, retinopathy of prematurity, periventricular leukomalacia, thrombocytopenia, neutropenia, sepsis, necrotizing enterocolitis, hypotonia, intraventricular hemorrhage, convulsion, sepsis, respiratory distress requiring oxygen therapy and/or assisted ventilation for >24 hours, and the need for intensive care for >4 days.

Based on published data from the trial of calcium to prevent PE in low-risk women[29] and 1-year delivery records from 2 collaborating tertiary obstetric centers (the Royal Alexandra Hospital, Edmonton, Alberta, Canada [1996] and St-Francois d'Assise Hospital, Quebec, Quebec, Canada [1999]), we estimated 4% and 15% incidences of the primary outcome in the low- and high-risk strata, respectively. We planned to recruit 5000 patients per group in stratum I (low risk) for a total of 10,000 patients and 1250 women per group in stratum II (high risk) for a total of 2500 patients to detect 30% reduction of PE, with a power of 90% and alpha error of 5%. After reviewing the evidence from the trials conducted by a United Kingdom research group (vitamin C and vitamin E in pregnant women at risk for PE-VIP trial)[22] and the Australian Collaborative Trial of Supplements Study group[23] as well as our internal data on serious adverse events, and in accordance with the recommendations of the data safety and monitoring committee, the trial steering committee decided to terminate the trial. A total of 2640 consenting eligible women were recruited. The last woman was recruited on March 30, 2006, and the last infant was born on Sept. 1, 2006.

The analysis was carried out on an intention-to-treat basis. We used Student *t* tests to compare continuous variables and the χ^2 test or Fisher's exact test for categorical variables, as appropriate. The effects of the intervention were expressed as RR (95% CI). Participants with missing outcomes due to withdrawal or loss to follow-up were excluded from the analysis of outcomes. We assessed twin and triplet infants as if cluster randomized (the cluster being the mother). Neonatal outcomes were analyzed by adjusting for the multiplicity of the pregnancy as the main neonatal outcomes were strongly affected by multiple births except for the outcome of preterm birth. Stratified analysis and multivariable logistic regression were performed to assess whether the effect estimates differed according to country, ethnic group and socioeconomic status, smoking, maternal age (<20 and >35 years), commercial and dietary vitamin C and E consumption (estimated by the Food Frequency Questionnaire) at the time of randomization and at 26 weeks of gestation, or patient compliance that was: (1) calculated as the proportion of tablets not returned in the bottles over the total number of tablets given to each woman; and (2) defined as compliant to treatment if >80% of tablets were used.

TABLE 2
Primary outcomes

Characteristic	Vitamins C and E n = 1167	Placebo n = 1196	RR (95% CI)	*P*
GH and its adverse conditions[a]	118 (10.11)	122 (10.20)	0.99 (0.78–1.26)	.94
GH	253 (21.68)	249 (20.82)	1.04 (0.89–1.22)	.61
Preeclampsia	69 (5.95)	68 (5.71)	1.04 (0.75–1.44)	.81
Eclampsia	1 (0.10)	0	–	.50
Diastolic pressure ≥110 mm Hg	32 (2.74)	27 (2.26)	1.21 (0.73–2.01)	.45
Systolic pressure ≥160 mm Hg	53 (4.54)	68 (5.69)	0.80 (0.56–1.13)	.21
Hematocrit <24%	3 (0.26)	5 (0.42)	0.61 (0.15–2.57)	.50
Blood transfusion	3 (0.26)	6 (0.50)	0.51 (0.13–2.04)	.33
Thrombocytopenia	7 (0.60)	7 (0.59)	1.02 (0.36–2.91)	.96
Elevated liver enzyme levels (AST or ALT >70 U/L)	9 (0.77)	7 (0.59)	1.32 (0.49–3.53)	.58
IUGR (<3rd percentile)[b]	18 (1.54)	15 (1.25)	1.23 (0.62–2.43)	.55
Perinatal death[c]	5 (0.43)	1 (0.08)	5.12 (0.60–43.79)	.10

ALT, alanine aminotransferase; *AST*, aspartate aminotransferase; *CI*, confidence interval; *GH*, gestational hypertension; *IUGR*, intrauterine growth restriction; *RR*, relative risk.
Data are presented as mean (SD) or n (%).

[a] GH and ≥1 of the following: (1) diastolic pressure ≥110 mm Hg or systolic pressure ≥160 mm Hg; (2) proteinuria >300 mg/24-hour urine collection or dipstick ≥2+; (3) convulsion (eclampsia); (4) thrombocytopenia (platelet count <100,000 × 10^9/L); (5) elevated liver enzyme levels (AST or ALT >70 U/L); (6) hematocrit <24% and blood transfusion; (7) IUGR <3rd centile; and (8) perinatal death (fetal death >20 wk or neonatal death within 7 days); [b] Only singleton pregnancy was considered in primary composite outcome; [c] Counted as number of pregnancies (mothers).

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The study was registered as an International Standard Randomized Controlled Trial (RCT), No. ISRCTN 85024310.

RESULTS

The Figure displays the trial profile. Of the 2647 eligible women who were randomized, a total of 2640 women were validly randomized (randomization error in 3 women, recruitment halted at randomization visit in 2 women, 2 women noneligible after randomization). Of these, 1315 (49.81%) women were assigned to vitamins C and E group and 1325 women (50.19%) were allocated to the placebo group. Patients who were lost to follow-up were excluded from the analyses and a total of 2363 women and their 2536 infants (vitamin group: 1167 women and 1243 infants; placebo group: 1196 women and 1293 infants) were included in the final analyses.

At study entry, maternal baseline characteristics were similar in 2 groups, ex-

TABLE 3
Primary outcome, gestational hypertension, and preeclampsia stratified by risk at enrollment

Characteristic, n (%)	Vitamins C and E	Placebo	RR (95% CI)
GH and its adverse conditions			
High-risk stratum	68 (20.12)	70 (20.23)	0.99 (0.74–1.34)
Chronic hypertension	28 (35.90)	27 (38.75)	0.93 (0.61–1.41)
Diabetes	11 (13.41)	15 (19.74)	0.68 (0.33–1.39)
Multiple pregnancy	9 (13.43)	13 (13.98)	0.96 (0.44–2.12)
History of PE	33 (22.45)	29 (19.59)	1.15 (0.74–1.79)
Multiple risk factors	11 (33.33)	13 (34.21)	0.97 (0.51–1.87)
Low-risk stratum	50 (6.03)	52 (6.12)	0.99 (0.68–1.44)
GH			
High-risk stratum	114 (33.73)	119 (34.39)	0.98 (0.80–1.21)
Chronic hypertension	44 (56.41)	39 (55.71)	1.01 (0.76–1.35)
Diabetes	20 (24.39)	23 (30.26)	0.81 (0.48–1.34)
Multiple pregnancy	13 (19.40)	15 (16.13)	1.20 (0.61–2.36)
History of PE	57 (38.78)	57 (38.51)	1.01 (0.76–1.34)
Multiple risk factors	18 (54.55)	14 (36.84)	1.48 (0.88–2.49)
Low-risk stratum	139 (16.77)	130 (15.29)	1.10 (0.88–1.37)
PE			
High-risk stratum	41 (12.17)	38 (11.05)	1.10 (0.73–1.67)
Chronic hypertension	16 (20.51)	11 (15.71)	1.31 (0.65–2.62)
Diabetes	6 (7.32)	11 (14.47)	0.51 (0.20–1.30)
Multiple pregnancy	4 (6.06)	6 (6.52)	0.93 (0.27–3.16)
History of PE	24 (16.33)	16 (10.88)	1.50 (0.83–2.71)
Multiple risk factors	7 (21.21)	5 (13.16)	1.61 (0.56–4.60)
Low-risk stratum	28 (3.40)	30 (3.55)	0.96 (0.58–1.59)

CI, confidence interval; *GH*, gestational hypertension; *PE*, preeclampsia; *RR*, relative risk.
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cept that there was a slightly higher proportion of multiple pregnancies in the placebo group (Table 1). There was no significant difference in patient compliance between the vitamin and the placebo groups (85.5% vs 86.5%; $P = .3640$).

There was no statistically significant difference in the risk of the primary outcome, GH and its adverse conditions, in the treatment group and the placebo group (10.11% and 10.20%, respectively; RR, 0.99; 95% CI, 0.78–1.26). The incidence of PE was similar between the 2 groups (5.95% vs 5.71%; RR, 1.04; 95% CI, 0.75–1.44) and there was no statistically significant difference in the risk of GH between the 2 groups (21.68% vs 20.82%; RR, 1.04; 95% CI, 0.89–1.22). Furthermore, there were no significant differences for any individual outcomes included in the primary composite outcome (Table 2).

Table 3 provides the results of the primary outcome, GH, and PE stratified by specific risk factor at enrollment. Vitamins C and E did not reduce the risk of GH and its adverse conditions, GH or PE, irrespective of risk at enrollment.

Compared with the placebo group, women in the vitamin supplementation group had statistically significantly higher rates of PROM (10.17% in the vitamin group vs 6.15% in placebo group; RR, 1.65; 95% CI, 1.23–2.22) and PPROM (5.97% in the vitamin group vs 3.03 in placebo group; RR, 1.97; 95% CI, 1.31–2.98). There were no differences in the rates of severe GH and severe PE. There was no reported maternal death in the study. There were no differences in other maternal adverse outcomes in the vitamin group compared with the placebo group (Table 4).

The rate of total death of the composite outcome "fetal loss or perinatal death" was significantly higher in the vitamin supplemented group (1.69% vs 0.78; RR, 2.20; 95% CI, 1.02–4.73) (Table 5). The rates of spontaneous abortion, stillbirth, and neonatal death before discharge were higher in the supplementation group, however these differences were not statistically significant. There were no statistically significant differences in the rates of preterm birth, IUGR (<3rd centile), or small for gestational age (<5th or 10th centile) in 2 groups. There were no differences between the vitamin supplementation and placebo groups in neonatal morbidity indicators including respiratory distress requiring supplemental oxygen therapy, assisted ventilation for >24 hours, the need for intensive care for >4 days, Apgar score <4 at 5 minutes, convulsion, sepsis, intraventricular hemorrhage, necrotizing enterocolitis, hypotonia, hypertonia, retinopathy of prematurity, leukomalacia, or neutropenia.

Stratification analysis by risk status for PE (low vs high) and country (Canada and Mexico) indicated no evidence of heterogeneity between countries. We further assessed the effects of vitamin supplementation on the risk of PE adjusted by preselected covariates (ie, smoking, maternal age [<20 or >35 years], vitamin C and E intake at the time of randomization, and the proportion of patients compliant with treatment). The effect estimates remained similar.

COMMENT

Taking into account risk profile, the rate of PE in our study was similar to that reported in previous trials.[22,23] We did not find that supplementation with vitamins C and E reduced the risk of GH and its adverse conditions among patients at high and low risk for PE. The results were

TABLE 4
Secondary maternal outcomes

Characteristic, n (%)	Vitamins C and E n = 1167	Placebo n = 1196	RR (95% CI)
Severe GH[a]	70 (6.0)	78 (6.52)	0.92 (0.67–1.26)
Severe PE[b]	33 (2.83)	39 (3.26)	0.87 (0.55–1.37)
PROM[c]	109 (10.17)	67 (6.15)	1.65 (1.23–2.22)*
PPROM[d]	64 (5.97)	33 (3.03)	1.97 (1.31–2.98)*
Infection	20 (1.73)	29 (2.46)	0.71 (0.40–1.24)
Delivery method			
Spontaneous delivery	526 (45.11)	551 (46.15)	
Instrumental delivery	120 (10.29)	126 (10.55)	–
Cesarean	520 (44.60)	517 (43.30)	
Antepartum hemorrhage	4 (0.34)	2 (0.17)	2.05 (0.38–11.17)
ICU admission	16 (1.37)	18 (1.51)	0.91 (0.47–1.78)
Predelivery hospitalization	333 (28.63)	341 (28.66)	0.99 (0.88–1.14)

CI, confidence interval; *GH*, gestational hypertension; *ICU*, intensive care unit; *PE*, preeclampsia; *PROM*, prelabor rupture of membranes; *PPROM*, preterm prelabor rupture of membranes; *RR*, relative risk.

[a] Defined as ≥2 readings of diastolic blood pressure ≥110 mm Hg or systolic blood pressure ≥160 mm Hg at least 4 hours apart; [b] Defined as PE and ≥1 of following adverse conditions: (1) diastolic pressure ≥110 mm Hg or systolic pressure ≥160 mm Hg; (2) proteinuria ≥5 g/24-hour urine collection or dipstick ≥3 +; (3) convulsion (eclampsia); (4) thrombocytopenia (platelet count $<100{,}000 \times 10^9$/L); (5) elevated liver enzyme levels (aspartate aminotransferase or alanine aminotransferase >70 U/L); (6) hematocrit <24% or blood transfusion; (7) intrauterine growth restriction (ie, birthweight <3rd centile for gestational age); (8) perinatal death (fetal death >20 wk or neonatal death within 7 days); or (9) preterm delivery <34 wk of gestation; [c] Defined as ≥37 wk of gestation and before onset of labor; [d] Defined as <37 wk of gestation and before onset of labor; * $P < .05$.

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consistent with those of other recently reported RCTs,[20-25] with the exception of the first small trial by Chappell et al.[20] Poston et al[22] reported no reduction of PE risk associated with vitamin supplementation (RR, 0.97; 95% CI, 0.80–1.17) in 2410 women identified at increased risk of PE. Rumbold et al[23] found no differences between the vitamin and placebo groups in the risk of PE (RR, 1.20; 95% CI, 0.82–1.75), and other adverse birth outcomes (eg, perinatal death, small for gestational age) in 1877 nulliparous women recruited between 14 and 22 weeks of gestation. The World Health Organization recently completed a multicenter trial and indicated that supplementation of vitamins C and E did not reduce the risk of PE, eclampsia, or GH among pregnant women with low socioeconomic status and low nutritional status in developing countries.[25]

In addition to the trials of combined vitamin C and E supplementation, Rivas-Echeverria et al[30] conducted a trial to assess the effects of aspirin, vitamins C and E, and fish oil supplements. They found that such supplements significantly reduced the risk of PE (RR, 0.07; 95% CI, 0.01–0.54). However, it is hard to infer whether such an effect was due to vitamins C and E, fish oil, or aspirin, or the effects of their interaction. Steyn et al[31] reported no effects of vitamin C supplementation on the risk of PE (RR, 1.00; 95% CI, 0.21–4.84). The study was stopped early because of an increase in spontaneous preterm labor in the treatment group.

Women in the group receiving vitamin supplementation had higher rates of PROM and PPROM than did the placebo group. The treatment differences remained significant after covariate adjustment for maternal age, smoking, body mass index, patient compliance, and presence of medical risk factors (eg, chronic hypertension, multiple pregnancy, history of PE, history of diabetes). Of the previously reported large RCTs of vitamin C and E supplementation in the prevention of PE risk, only 2 trials reported and examined the effects of vitamin C and E supplementation and the risk of PPROM.[23,24] Rumbold et al[23] reported a slight increase of PPROM risk in the supplemented group (3.2% vs 2.4%; RR, 1.31; 95% CI, 0.77–2.25). However the difference was not statistically significant. Spinnato et al[24] observed a significant increased risk of PPROM in the antioxidant group (10.6% vs 5.5%; adjusted RR, 1.89; 95% CI, 1.11–3.23). Casanueva et al[32] conducted a randomized trial in which 109 women were randomly assigned at 20 weeks of gestation either to 100 mg vitamin C or placebo. Despite the fact that there was no measured difference in plasma vitamin C concentration between 2 groups, there was a significantly lower rate of PROM in the supplemented group compared with the placebo group.[32]

We did note an increased risk of the composite outcome "fetal loss or perinatal death" (1.69% vs 0.78%; RR, 2.20; 95% CI, 1.02–4.73). While this was not a prespecified outcome in the study protocol, it was used as an outcome for the monitoring of morbidity by the data safety monitoring board, and contributed to the decision to stop the trial early. We noted that there were more perinatal deaths in the antioxidant group than the placebo group, but the effect did not reach statistical significance. We did not find evidence of differences between groups in the rates of low birthweight, preterm birth, small for gestational age, or low Apgar score. Poston et al[22] found vitamin supplements to be positively associated with the risk of low birthweight compared with controls. This effect was particularly strong among women with prepregnancy diabetes who were in the vitamin supplement group (RR, 1.15; 95% CI, 1.02–1.30).

The daily doses of 1000 mg vitamin C and 400 IU vitamin E (RRR α-tocopherol) are certainly below the maximum recommended intake in pregnant women. We do not know why supplementation of vitamins C and E at these doses did not reduce the risk of PE, or GH, but increased the rates of PROM and PPROM in our study. The dose of vitamin E that is required to suppress isoprostane levels (a marker of oxidative stress) has been documented in men,[33] but not in pregnant women. Exogenous

TABLE 5
Secondary neonatal outcomes

Characteristic, n (%)	Vitamins C and E n = 1243	Placebo n = 1293	RR (95% CI)
Fetal loss or death of infants[a,b]	21 (1.69)	10 (0.78)	2.20 (1.02–4.73)
Spontaneous abortion	6 (0.48)	1 (0.08)	6.25 (0.72–54.52)
Stillbirth	10 (0.80)	6 (0.47)	1.73 (0.63–4.78)
Neonatal death before discharge	5 (0.40)	3 (0.23)	1.73 (0.41–7.25)
IUGR (<3rd percentile)	60 (4.87)	60 (4.67)	1.05 (0.72–1.51)
Preterm birth[c]			
<37 wk	193 (16.57)	184 (15.48)	1.07 (0.89–1.29)
<34 wk	67 (5.75)	65 (5.47)	1.05 (0.76–1.47)
Small for gestational age			
<5th percentile	91 (7.38)	102 (7.93)	0.93 (0.69–1.25)
<10th percentile	173 (14.03)	194 (15.09)	0.92 (0.73–1.15)
Convulsion	5 (1.07)	2 (0.42)	2.55 (0.49–13.19)
Respiratory distress requiring oxygen	267 (21.87)	281 (22.02)	0.99 (0.81–1.21)
Assisted ventilation ≥24 h	55 (4.51)	54 (4.25)	1.07 (0.67–1.69)
NICU care >4 d	46 (4.08)	48 (4.09)	1.00 (0.62–1.61)
Congenital anomalies	37 (3.03)	30 (2.35)	1.30 (0.78–2.19)
Sepsis	13 (1.07)	6 (0.47)	2.28 (0.77–6.80)
Intraventricular hemorrhage	11 (0.90)	8 (0.63)	1.44 (0.53–3.94)
Necrotizing enterocolitis[b]	1 (0.08)	9 (0.71)	0.12 (0.01–0.91)
Hypertonia	6	0	–
Hypotonia	7 (0.57)	6 (0.47)	1.22 (0.41–3.63)
Retinopathy of prematurity	4 (0.33)	3 (0.24)	1.39 (0.16–12.46)
Leukomalacia	1 (0.08)	0	–
Neutropenia	6 (0.50)	3 (0.24)	2.09 (0.52–8.38)
Apgar score <4 at 5 min	9 (0.73)	6 (0.47)	1.57 (0.56–4.44)

CI, confidence interval; *IUGR*, intrauterine growth restriction; *NICU*, neonatal intensive care unit; *RR*, relative risk.

[a] Defined as spontaneous abortion, stillbirth, or neonatal death; [b] $P < .05$; [c] Counted as number of pregnancies.

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vitamin E may prevent an immunologic switch (T helper 1 to T helper 2) that is considered as crucial for early to late transition in normal pregnancy and it could be a potential interferon-γ mimic, facilitating proinflammatory responses at the maternal-fetal interface.[34] It is possible that vitamin E exerts both potentially beneficial and detrimental effects. Therefore, vitamin E treatment might have undesirable side effects and may partially explain the unexpected results of the increased risks of PROM and PPROM. There is some evidence that high doses of α-tocopherol (primary form of vitamin E in supplementation) could deplete plasma and tissue γ-tocopherol (major form in plant seeds and in the North American diet).[35-38] Therefore, the efficacy of vitamin E supplementation (α-tocopherol) may be offset by deleterious changes in the levels of other nutrients. In fact, our preliminary data analyses using the INTAPP population found that women in the supplementation group had a significantly higher level of α-tocopherol at 32 weeks of gestation (58%; $P < .001$) and lower ratio of γ-/α-tocopherol (–58%; $P = .001$) compared with the baseline visit (visit 1: week 12-16 of gestation). While levels of α-tocopherol were significantly increased compared to baseline (34%; $P < .001$) among women in the placebo group, the ratios of γ-/α-tocopherol were not affected.[39] The ineffectiveness of vitamin C and E in the prevention of PE and the potentially harmful effects emphasize the need for a better understanding of the underlying mechanisms and metabolism of both vitamins C and E in the human body.

Witztum[40] hypothesized that only individuals under oxidative stress are likely to benefit from antioxidant supplementation. Meagher et al proposed that only people deficient in vitamin E may benefit from vitamin E supplementation.[41] To date, a series of trials have been conducted, including the present study, involving both low- and high-risk patients (eg, presence of chronic hypertension, history of PE, multiple gestation, diabetes, low socioeconomic status, and low nutritional status). It is clear that irrespective of study population (ie, risk profile and nutritional status), supplementation with vitamins C and E during pregnancy is unlikely to prevent PE, GH, preterm birth, or low birthweight.

The definitions for GH and PE retained for this trial are those of the Canadian Consensus Statement on Hypertensive Disorders of Pregnancy.[1] This definition focuses on the diastolic blood pressure value for diagnosis. There is no universal consensus regarding the definition of GH or PE, nor are there studies comparing the relative validity of the different definitions. However, it is unlikely that the choice of an alternative definition would have modified the results of the trial.

The trial was prematurely stopped with a total of 2640 eligible pregnant women included in the final analysis. This resulted in a significant decrease in power relative to the initially planned sample size. Nevertheless, in light of our results and those of other investigators, it is unlikely that further recruitment would have identified a difference in treatment group. Furthermore, given the increased risk of certain adverse outcomes (fetal loss/perinatal deaths and PPROM), we considered it unethical to continue the study. We also acknowledge that an approximate 20% loss to

follow-up occurred in Mexican centers. This is because a high proportion of Mexican women beginning prenatal care in the IMSS centers change health care provider in the course of prenatal care and deliver in non-IMSS hospitals where data could not be accessed. However, the proportion of loss to follow-up was balanced between treatment and placebo groups and stratification analysis by country did not result in any difference for effect estimates.

Despite the fact that the underlying mechanisms remain largely unclear, there is increasing concern that supplementation of vitamins C and E at the doses studied [ie, 1000 mg vitamin C and 400 IU vitamin E (RRR α-tocopherol)] may increase the risk of other adverse pregnancy outcomes such as low birthweight[22] and PPROM. Therefore, based on our present knowledge, vitamin C and E supplementation at the above doses cannot be recommended for pregnant women to prevent adverse pregnancy outcomes including PE. ■

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