The Relationship between Dietary Intake and Pre-eclampsia

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Abstract: Background: Pre-eclampsia (PE) is a major complication of pregnancy associated with hypertension and proteinuria. This work aimed to determine the relationship between dietary micronutrients, macronutrients, and PE in a large cohort of women known as risk of PE. **Subjects and Methods:** 104 women who developed PE and 206 women at high risk but did not develop the disorder were studied in the second trimester of pregnancy. 7-day food diaries were analyzed for macronutrient and micronutrient content using a comprehensive UK compositional food database and associations between dietary intake and pregnancy outcome were explored. Associations between plasma micronutrient, lipid status, diet, clinical risk factors and development of PE (and other abnormal outcomes) were also investigated. The mean daily intake for each nutrient was determined and compared with the appropriate Dietary Reference Values. Conditional logistic regression was used to determine the association of each nutrient with PE. **Results:** There was no association between nutrients intake and the development of PE (or any other outcome) when assessed by 7-day diaries. There was some evidence of deficiency in the intake of some nutrients among high-risk, ex., vitamin D and selenium and retinol. Mean dietary intakes of vitamin E and C exceeded the Estimated Average Requirements (EAR). Associations between plasma indices of nutrient status and dietary intake were poor. The only risk factor influenced by intake of any nutrient was obesity; there was an association between obesity and the dietary intake of protein (p<0.01). **Conclusions:** This study does not support the hypothesis that dietary nutrient intake influences the development of PE in high risk women.

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Key words: Pre-eclampsia, pregnancy, micronutrients, macronutrients.

1.Introduction:

Preeclampsia is a complex multisystem disorder seen exclusively in the human species. Worldwide, it is a leading cause of maternal and fetal morbidity and mortality. Reduced perfusion as a result of abnormal placentation is thought to lead to ischemia reperfusion injury to the placenta. Placental oxidative stress, which results from the ischemia reperfusion injury, is increasingly reported to be involved in the etiopathogenesis of gestational hypertension/preeclampsia and associated with impaired glucose tolerance that occurs after 20 weeks of gestation **(Walsh *et al.*, 2000 and Sibai, 2003)**. The exact incidence of PE is unknown, but it has been reported to affect between 0.4% to 2.8% of all pregnancies in developed countries, and more in developing countries **(Villar *et al.*, 2000)**. As a multisystem disorders that affecting many organs including the kidney (renal failure), liver (cell death and hemorrhage) and brain (eclamptic convulsion) **(Sattar, 2004)**. Pre-eclampsia (PE) is the leading indication for elective premature delivery, it is also responsible for the death of approximately 600 babies each year **(Confidential Enquiries into Maternal Deaths in the United Kingdom, 2004)**, and infants have an increased risk of respiratory distress syndrome **(Akkoyun *et al.*, 2006)**. Preeclampsia is also coupled to fetal growth retardation with long-term physical and neurological problems. Thus, an increased incidence of diabetes, high blood pressure and cardiovascular mortality may arise in later life as a consequence of the low birth weight **(Barker *et al.*, 1989)**. Due to its devastating consequences, strategies have been proposed and investigated to prevent PE.

The definition of PE varies widely. PE is defined as a pregnancy-specific syndrome observed after the 20th week of pregnancy with systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg and 300 mg of protein in a 24-hour urine sample. A diagnosis of severe PE is made with one or more of the following criteria; high blood pressure, proteinuria, and medical complications *e.g.* Pulmonary edema, acute renal insufficiency, cerebral edema, haemolysis, elevated liver enzymes and low platelet count, symptoms of PE include swelling of the face and hands, visual disturbances, headache, and renal lesion **(Sibai, 2003)**. PE remains a major cause of maternal and neonatal morbidity and mortality, which represents (28.2%) of the main causes of death **(Davison *et al.*, 2004, Rodrigo *et al.*, 2005 and Romero-Gutiérrez *et al.*, 2007).** Despite PE being a major concern in both developed and developing countries, there is no satisfactory treatment to prevent the development of the disease, except steps to avoid or reduce complications such as hypertension and eclamptic seizures **(Afifi and Churchill, 2003)**. In women with PE, blood pressure usually returns to baseline within days to weeks after delivery.

Therefore, this work aims to determine the relationship between dietary intake and the incidence of PE and investigate the relationships between nutrient intakes and pregnancy outcomes, birth weight and preterm birth. The aims also extended to determine the association between the intake of certain micronutrients and their plasma concentrations.

2. Subjects and Methods

Subjects:

From a total of 310 subjects, 104 women developed PE and 206 women at high risk but did not develop the disorder were studied in the second trimester of pregnancy. The study was conducted over a period of three months between January and March 2006. All of the participants in this study were over 18 years old and had a gestational age greater than 14 weeks. The women recruited were divided into two groups; those identified as being at "high-risk" of developing PE, using the same eligibility criteria as the recent VIP trial **(Poston *et al*., 2006),** and those in the control or "low-risk" group, with no identifiable risk factors for PE. The women approached for this study were provided with information sheets about the study. They were made aware of the aims and objectives of the study and also of what would be required of them. Informed written consent was obtained from those women who agreed to take part in the study, after which they were asked to fill in the questionnaire. All women were interviewed, to prevent literacy and vision problems from hampering participation.

Ethical approval:

Pregnant women were recruited with Local Ethical Committee approval from St Thomas' Hospital. (St Thomas' Hospital Research Ethics Committee, REC reference number 05/Q0702/100).

Methods:

Criteria for inclusion of subjects

The study was designed on a one case to two control basis (1 case to 2 controls). Cases were all those women who developed PE (n=104) from the women who completed and returned the 7-day diaries. Each participant was matched to two controls for parity (primiparous or multiparous), gestational age at recruitment (within 2 weeks), for maternal age (within 5 years), and for measures of socio-economic status (working/maternity leave, home maker, student, unemployed, and length of education). As the VIP trial did not show any significant effect of the antioxidant supplements (vitamins C and E) on the incidence of PE (**Poston *et al*., 2006),** it was therefore possible to interrogate the whole database for the evaluation of dietary intake in the women who developed PE.

The study questionnaire:

The first part of the questionnaire concentrates on personal information. Participants in the study were asked their age, parity, gestational age, country of birth and ethnicity. This information enabled me to match subjects from the high-risk group to their control group counterparts, in order to make suitable comparisons between the two groups and adjust for any confounding variables. The final questions were pertained to health advice.

Dietary assessment (7-day diaries):

Food diaries (records) are typically obtained for 3 to 7-days. All women were asked if they would take the 7-day record booklet and fill it in within the following week after the randomization interview. The women were asked to mail the 7-day diary back to the trial office in a pre-paid envelope. The participants were asked to record, in as much detail as possible, all food and beverages consumed over 7 consecutive days. Subjects were also asked to answer a number of general questions regarding their dietary intake, such as the type of fat and milk most requently used during the recording period . The questions served as a cross check and were intended to confirm the accuracy of the account.

Power considerations:

This study was designed to assess associations of dietary deficiency or excess with the development of PE. It was difficult to power the study since there are at this time very few reports in the literature which have investigated associations between parameters of dietary intake and pregnancy outcomes. We carried out a power calculation on the basis of the Glasgow MONICA study (**Wrieden *et al*., 2000)**. However, it is not possible to say how much the power would be increased without more detailed background information of the effects on the diet of the variables used in matching cases with controls, e.g., gestational age.

Nutrient database:

Nutritional assessment by diet analysis is a two-stepped process consisting of evaluation of food consumption, and conversion of food into nutrient intake by using a food composition database, which lists the mean nutritional values for a given food portion. The food intake data obtained from 7-day diaries were entered manually into a Microsoft Excel spreadsheet for each subject. The descriptions of the portion sizes were converted into weights using the standard portion weights in **Crawley (2002)**, and the EPIC-Oxford food dairy photo portion weights in grams. Each food item described in 7-day diary were coded using **McCancaend and Widdowson (2002)**. To estimate the nutrient content of mixed dishes, women were asked to write down the typical recipes of the dishes that were eaten. Recipes contained the weight in grams of raw ingredients. When the portion size had not been entered in the 7-day diary it was entered as an average weight, i.e., medium. As the subjects were asked to answer a number of general questions regarding their

dietary intake, missing food types such as milk, bread, and fat were obtained from these general questions. Missing nutrient data were added where possible, using a combination of food labels and published food composition tables. Food compositional information about new foods was added to the original databank and given an identifying code (**Odutuga *et al*., 1992).**

The evaluation of nutrient intakes:

Recommended daily amount (RDAs) were replaced with the Dietary Reference Values (DRVs) as published in the 1991 report **(Dietary Reference Values for Food Energy and Nutrients for the United Kingdom Department of Health, 1991).**

Quality control:

For quality control, food diaries were screened for errors prior to analysis. Data were cross-checked for input errors, duplicate entries and values outside the expected range for weight of portion size, energy (kcal) and a few selected nutrients. These nutrient values were then used to analyze the subjects' nutrient intake. On average each 7-day diary took 2 hours to code, including checking and editing of the entered data. In addition, a further 1 hour of work was required for creating new food codes and checking of nutritional data, and running the analysis programs followed by further checks on the data.

Biochemical analysis:

Blood samples were collected for the measurement of biomarkers at 14^{+0} to 21^{+6} weeks of gestation. A total of 41 subjects (14 cases and 27 controls) who provided both blood sample and 7-day diaries were available for this analysis. Plasma concentrations of vitamin E/total cholesterol (α-tocopherol), vitamin C, γ-tocopherol, triglycerides, uric acid, MDA, total cholesterol, high-density lipoprotein (HDL-cholesterol), and low-Density Lipoprotein (LDL-cholesterol) were compared between cases and controls.

Data entry:

The study was designed on the basis that 85 women would be recruited to each group. As described above, it was anticipated that 170 participants would give the study a 90% power at the 5% significance level to detect differences in diet and lifestyle changes between the high-risk and low-risk groups. Microsoft Access was used for data entry.

Statistical analysis

All statistical analyses were performed using Stata, version 9.1 (Stata Corp, College Station Texas) statistical software package. A paired Student's t-test was used to detect the significances of differences between groups and between dietary methods. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion:

Demographic characteristics and risk categories of subjects

From a total of 310 subjects, 104 women developed PE and 206 women (two per case) were identified as a high-risk control as identified by **(Poston *et al*., 2006)**, were involved in evaluation of dietary assessment. Table (1) showed the demographic characteristics of the women involved in dietary assessment. In women developed PE the mean age was 32 ± 5 years, 30% classified as obese and a total of 31 and 33 % taken either folic acid or multivitamin supplementation respectively. While in high-risk women (control) 28% classified as overweight and the same percentage as obese, the majority of women were multiparous, 7% recorded smokers and a total of 29 and 30% taken either folic acid or multivitamin supplementation respectively. The choice of the specific gestational age period in which the women were studied was opportunistic women at this gestational age are much less likely to be affected by nausea and vomiting, which is known to occur in the first trimester and to decrease at the beginning of the second trimester and this may indicate that the data obtained reflect habitual intake. Distributions of the women according to multiple risk factors are presented in Table 2. In this study in PE women 12.5% with a BMI >30 kg/m² in their first pregnancy, 16% had a multiple pregnancy (twins), 7% diabetes, 1% had chronic renal disease and 21% had multiple risk factors, these distributions incomparable with the high risk women represented 13%, 16%, 6%, 1% and 17%, for the same mentioned risk factors, respectively.

Table (3) demonstrated all neonatal outcomes by diagnosis allocation of all women involved in the dietary assessment study (n=310), a total of 120 babies were born to 104 women with PE and 237 babies were born to 206 high-risk women (controls). As expected PE were associated with several differences in neonatal outcomes. Birth weight was significantly lower among PE cases (2601 g *vs*. 2983 g). The incidence of preterm birth (< 37 weeks gestation) was also significantly higher in cases than controls (46% *vs*. 23%; *p*=0. 01). Also there was a significant difference in the incidence of low birth weight (< 2500 g) babies born to cases than controls (42% *vs*. 20%; *p*=0. 01). The number of small for gestational age babies ($<5^{th}$ centile) was also significantly higher among cases than controls (48% *vs*. 23%, *p*=0. 001).

Dietary macronutrients and micronutrients intakes of subjects:

The habitual nutrient intakes, from food and dietary supplements during the second trimester in a population of PE pregnant women when compared with high-risk controls are illustrated in Table 4..

Table 1. Demographic characteristics of women (PE cases and control groups).

Demographic category	Cases (n=104)	Controls (n=206)	Difference (95%CI)
Age (years)	32±5	32± 5	-0.33 (-1.56 to 0.90)
Height (cm)	165±6	164±7	0.76 (-0.75 to 2.27)
Weight (kg)	76±18	73±18	2.70 (-1.45 to 6.86)
Body-mass index (kg/m²)	28±6	27±6	0.71 (-0.74 to 2.16)
<18.5	2 (2%)	4 (2%)	1.14 (0.36 to 3.65)
18.5-24.9	36 (34.5%)	87 (42%)	Reference
25-29.9	36 (34.5%)	57 (28%)	1.32 (0.91 to 1.92)
≥ 30	30 (29%)	58 (28%)	1.16 (0.78 to 1.74)
Parity			
Primiparous (0) *	46 (44%)	76 (37%)	Reference
Multiparous (1) **	40 (38.5%)	94 (46%)	0.79 (0.56 to 1.12)
> 1***	18 (17.5%)	36 (17%)	0.88 (. 57 to 1.37)
Employed			
Working/Maternity	78 (75%)	151 (73%)	Reference
A home maker	14 (13%)	38 (18%)	0.79 (0.49 to 1.28)
Student	2 (2%)	3 (1%)	1.17 (0.40 to 3.49)
Unemployed	10 (10%)	14 (7%)	1.22 (0.74 to 2.03)
Employed			
Working/Maternity	78 (75%)	151 (73%)	Reference
A home maker	14 (13.5%)	38 (18.5%)	0.79 (0.49 to 1.28)
Student	2 (2%)	3 (1.5%)	1.17 (0.40 to 3.49)
Unemployed	10 (9.5%)	14 (7%)	1.22 (0.74 to 2.03)
Education			
GCSE	49 (47%)	99 (48%)	Reference
None	8 (7%)	13 (6.5%)	1.11 (0.59 to 2.09)
A level	19 (18%)	27 (13%)	1.25 (0.82 to 1.89)
College/University	28 (27%)	67 (32.5%)	0.89 (0.60 to 1.31)
Current smoker ≥ 1/day	5 (5%)	14 (7%)	
Gestational age (weeks) at recruitment	18±2	18±3	0.07 (-0.51 to 0.64)
Use of supplements			
Folate	32 (31%)	60 (29%)	1.05 (0.75 to 1.48)
Multivitamins	34 (33%)	61 (30%)	1.10 (. 79 to 1.53)

Data are given as mean or percentage (%) of the total. *Primiparous (0): Women who had never given birth to a live Infant. **Multiparous (1): Woman with one or more previous pregnancies > 24 week and *** >1: Women pregnant with more than one fetus.

Table 2. Distribution of risk factors among women (PE cases and control groups).

Risk group	Cases (n=104)	Controls (n=206)	All (n=310)	Risk Ratio (CI 95%)
Chronic hypertension	48 (46%)	92 (45%)	140 (46%)	1.03 (0.80 - 1.34)
BMI>30 kg/m² in first pregnancy	13 (12.5%)	27 (13%)	40 (13%)	0.95 (0.51 -1.77)
Previous PE	35 (34%)	70 (34%)	105 (34%)	0.99 (0.71 - 1.38)
Multiple pregnancy twins	17 (16%)	32 (16%)	49 (16%)	1.06 (0.62 -1.81)
Diabetes	7 (7%)	13 (6 %)	20 (7%)	1.07 (0.44 - 2.59)
Abnormal uterine artery	3 (3%)	5 (2%)	8 (3%)	1.19 (0.29 - 4.88)
Chronic renal disease	1 (1%)	2 (1%)	3 (1%)	0.99 (0.09-10.80)
Multiple risk factors	21(20%)	36 (17%)	57 (18%)	1.12 (0.77to 1.65)

Data are given as mean or percentage (%) of the total.

Table 3. Neonatal outcomes by maternal (PE cases and control groups).

Neonatal outcomes	PE Number of babies=120	Control Number of babies=237	Risk Ratio (CI 95%)
*Pre-term birth (<37 wks)	55 (46%)	54 (23%)	2.16 (1.55 to 3.01)
Pre-term birth (<34 wks)	23 (19%)	22 (9%)	1.74 (1.16 to 2.60)
Birth weight (g)	2601±892	2983±773	N/A
Low birth weight <2500g	52 (42%)	49 (20%)	2.32 (1.70 to 3.15)
Low birth weight <1500g	13 (11%)	16 (7%)	1.35 (0.82 to 2.21)
**IUGR (below 5th centile)	55 (43%)	55 (23%)	2.02 (1.48 to 2.75)
IUGR (below 10th centile)	59 (48%)	80 (33%)	1.61 (1.17 to 2.20)

Data are given as mean or (%) of total. Outcome data from 3 babies of PE cases, and 3 babies of high-risk control were not available. * Data were available for 189 babies. **Intrauterine growth restriction (IUGR) is the pathologic counterpart of small-for-gestational-age, small-for-gestational-age was defined as <5th centile for gestation as defined within the population and by customized birth weight centile charts, adjusting for gestational age, maternal height, weight at booking, parity and ethnicity.

An indication of adequacy was assessed by evaluating intakes in relation to the Estimated Average Requirements (EAR) values **(Department of Health, 1991),** the 7-day diaries was used in this analysis. For some nutrients, the scientific evidence on requirements is insufficient to define an EAR In this study the reference nutrient intake (RNI) for vitamin D was used to estimate adequacy of vitamin D intake equated with the USA-derived AI **(Prentice, 2002).** Of the 310 women after energy adjustment and determination of those women who were calculated to have underreported their dietary intake, there were remained 84 completed diaries from PE women (cases group), and 169 from the high-risk control group. When a rigorous matching criterion was employed for age, parity, ethnicity, housing, occupation, education, and risk factors, 26 cases and 38 controls remained well matched, and have been compared with data sets for nutritional intake from all the cases and controls (Table 4) and Figures (1-4). Taking food and supplements into account, the mean intakes of energy, macro- and micronutrients exceeded the EAR in all cases and controls, except for selenium and retinol, which did not meet the EAR. Also, the mean intake of vitamin D did not meet the RNI. There were no significant differences between mean energy and nutrient intakes between the 84 PE women and 169 controls. Although there was a trend towards a higher mean intake of all macronutrients and some of the micronutrients, and a lower mean intake of vitamin C and carotene amongst the cases compared with controls, at the same time these did not reach statistical significance, when the groups of matched cases and controls were compared. As in the whole cohort there were no significant differences between cases and controls in the matched women. As shown in Figure 1, for macronutrients, 50% of control subjects did not achieve the population average for MUFA. Figure 2 more than 50% of all cases and controls did not achieve the EAR for selenium, retinol, nor the RNI for vitamin D, all the women (approximately 100 %) had total vitamin D intake below the RNI. The data were analysed for the matched cases (n=26), and controls (n=38) (Figures 3 and 4). More than 50% of matched cases and controls achieved dietary intakes greater than the EAR or population average for all macronutrients except for TFA where more than 50% (for both matched cases and controls) did not achieve the population average. Also, for micronutrients, more than 50% of all matched cases and controls did not achieve the EAR for selenium and neither retinol nor the RNI for vitamin D, about 100 % of women had total vitamin D intake below the RNI. The results of the present study also showed that 50% of all cases and controls, and matching groups as well, were below the population average of *trans* fatty acids (TFA) intake. This may be a benefit to health, as *trans* fats have been implicated in coronary heart disease by raising levels of LDL cholesterol and lowering levels of HDL cholesterol. Furthermore, **Williams *et al.* (1998)** have suggested that diets high in elaidic acid (one of the most abundant dietary *trans* fatty acids), might be associated with an increased risk of PE. Furthermore, **Mahomed *et al.* (2007)** examined the association between maternal erythrocyte *trans* fatty acids and risk of PE in a population from Zimbabwe and observed a strong positive association between *trans* fatty acids (particularly diunsaturated trans fatty acids), and the risk of PE.

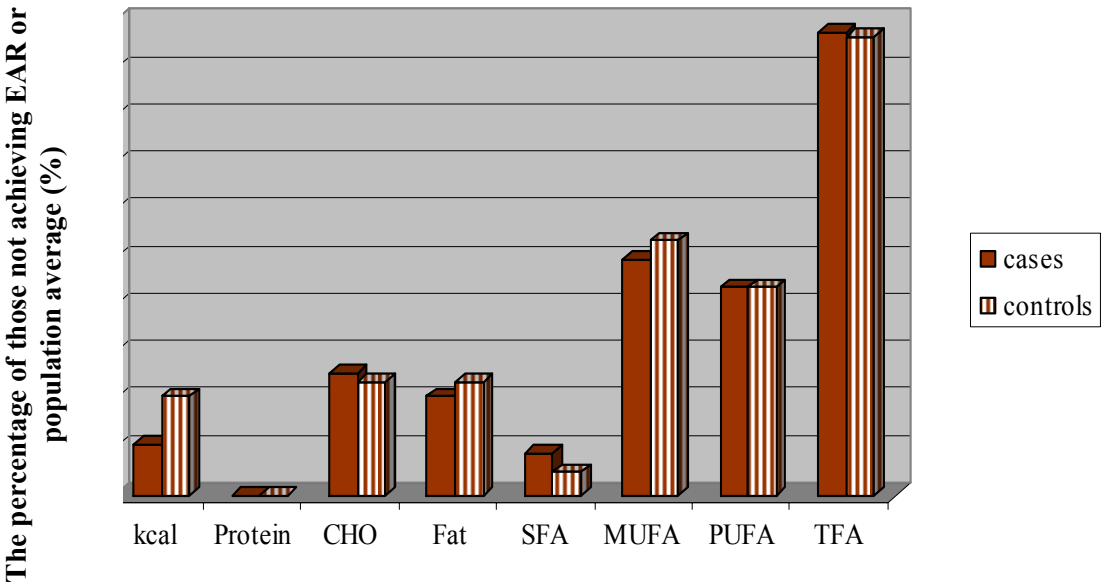
On average, high-risk women in this study received adequate amounts of the examined vitamins and minerals from food with the exception of vitamin D, selenium and retinol. The results imply a problem with selenium deficiency, because the intake of high-risk women was approximately equal to the LRNI of the DRVs (40 µg). The LRNI is defined as the amount of nutrient that will only satisfy the needs of a few individuals (about 2.5%). Thus, if the intake is at or below the LRNI the majority of the group will not have their needs satisfied so must be classified as "at risk" of selenium deficiency **(Department of Health, 1991)**. The relationship between selenium intake/status and PE may have implications for pregnancy outcome, including PE **(Rayman *et al.*, 1996 and Rayman *et al.*, 2003)**. Importantly, as selenium is a cofactor for the glutathione peroxidase defence pathway, a deficiency could lead to an inadequate capability to counteract oxidative stress, both in physiological and pathophysiological circumstances. Thus, the present study adds further evidence to the observation that low selenium status occurs in the UK population **(Rayman, 2000)** and to the suggestion that selenium intake in the UK diet should be re-addressed.

Retinol intakes were below the EAR for all cases and controls, and also for all cases and controls in the matched women. A problem with retinol deficiency was implied because the mean intake of high-risk women was approximately equivalent to the LRNI i.e., 350 µg. Vitamin A deficiency during pregnancy can have devastating effects. It can impact on the mother's bone reserve, and these mothers may also be at higher-risk for PE **(Lee *et al.*, 2008)**, although the present study provides no evidence for an association with PE. Vitamin A has an antioxidant and protective properties, it can protect against cell mutation. Furthermore, **Radhika *et al.* (2002)** reported the incidence of night blindness was 2.9% in women with subclinical vitamin A deficiency. The results showed that 100% of cases and controls did not achieve RNI for vitamin D. In the United Kingdom between 5-20% of most age groups have serum 25(OH)2D concentrations <10 ng/ml (<25

nmol/l) **(Prentice, 2008)**, and the present study adds to the evidence for inadequate vitamin D status in this population. In pregnancy this could influence both maternal and fetal bone health **(Bodnar *et al.*, 2007)**. The high level of nutritional adequacy for most micronutrients noted in this study is unusual, but it is probably due, at least in part, to the high usage of prenatal supplements which included in the analysis, education, aware of the importance of adopting healthy behavior (smoking less, etc.) and may have consumed more milk, fruit or colour vegetables during pregnancy. Indeed, the typical dietary pattern of women included a high intake of vegetable and fruits.

Table 4: Comparison of the mean daily energy, macronutrient and micronutrient intakes between all cases and controls, and between matched cases and controls, as assessed using the 7-day diaries.

Nutrients	PE cases and Controls				All matched PE cases and controls			
	Cases (n=84)		Controls (n=169)		Cases (n=26)		Controls (n=38)	
	Mean	*CI (95%)*	Mean	*CI (95%)*	Mean	*CI (95%)*	Mean	*CI (95%)*
Energy intake (kcal)	2447	2338-2561	2258	2193-2325	2556	2345-2785	2294	2160-2437
Protein (g)	87	82- 91	82	79-84	85	77-93	82	77-88
Total Fat (g)	100	94-105	91	87-94	103	92-115	92	84-102
Carbohydrate (g)	312	295- 330	290	280- 300	334	301-369	295	279-312
Saturated fatty acids (g)	37	35-40	34	33-36	39	33-45	35	32-39
Trans-fatty acids (g)	2	2-3	2	2-2.4	3	2-3	2	2-3
Monounsaturated fatty acids (g)	32	30-34	29	28-31	33	29-38	30	27-33
Cholesterol (mg)	263	240-289	241	229-255	263	218-316	235	212-261
Polyunsaturated fatty acids (g)	17	16-18	16	15-16	18	16-19	16	15-18
Vitamin C (mg)	130	116-146	134	123-147	135	103-171	148	125-177
Vitamin E (mg)	12	11-14	12	11-13	14	11-16	13	11-17
Vitamin D (µg)	0.48	0.38-0.59	0.44	0.39-0.51	0.51	0.33-0.81	0.46	0.35-0.61
Vitamin B_6 (mg)	3	3-3	3	3-3	3	2.3-3.4	3	3-4
Vitamin B_{12}(µg)	5	5-6	5	5-6	6	5-7	6	5-6.4
Iron (mg)	20	17-24	18	16-20	23	17-31	19	15-23
Selenium (µg)	45	42-48	43	41-47	44	39-50	43	39-47
Folate (µg)	492	431-563	455	419-495	548	428-689	507	423-605
Calcium (mg)	1154	1080-1233	1089	1041-1139	1182	1036-1334	1099	969-1197
Sodium (mg)	3197	3002-3404	3060	2956-3167	3270	2949-3622	3178	2920-3385
Potassium (mg)	3185	3052-3324	3117	3015-3222	3252	2972-3500	3221	2998-3385
Zinc (mg)	12	11-13	12	11-12	12	10-14	12	10-14
Retinol (µg)	369	330-413	332	306-360	392	317-484	328	275-383
Carotene (µg)	2519	2148-2953	2538	2272-2836	2525	1861-3251	2379	1669-2951



†CHO= Carbohydrate

Figure 1. The percentage of all cases (n=84) and controls (n=169) not achieving dietary EAR or population average.

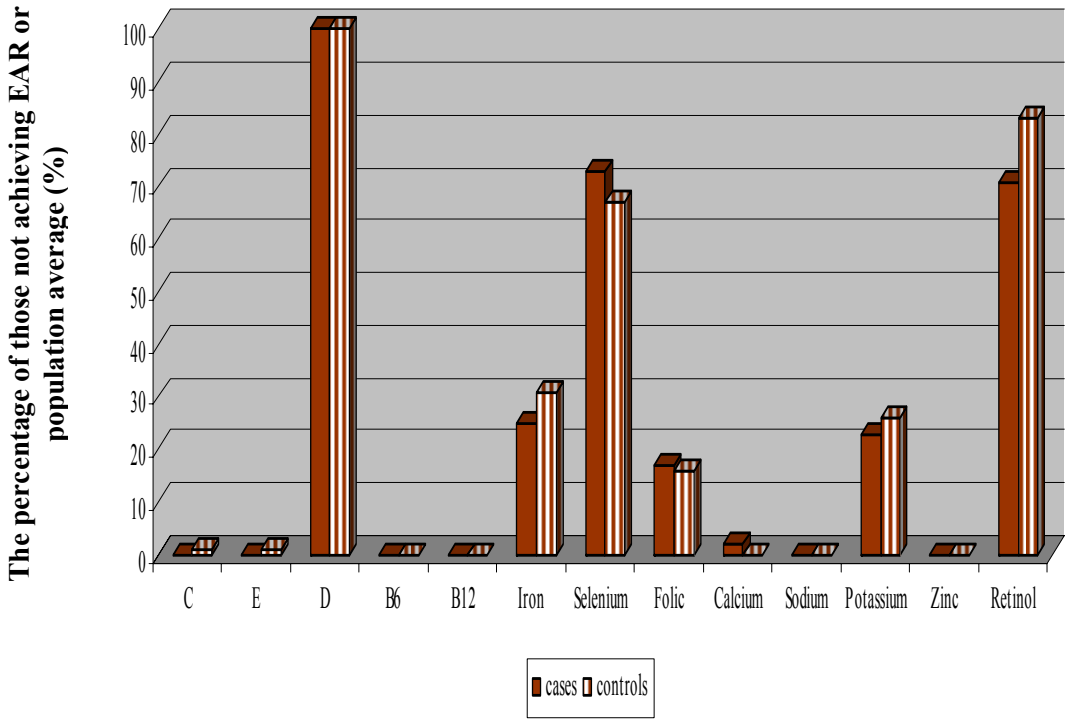
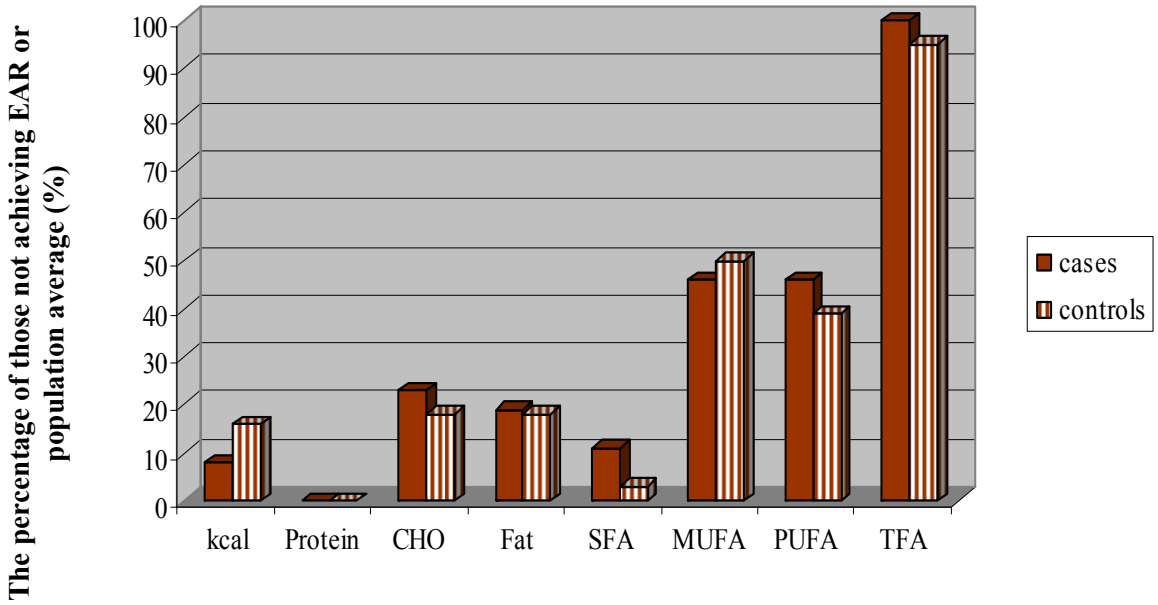


Figure 2. The percentage of all cases (n=84) and controls (n=169) not achieving dietary EAR, or the RNI for vitamin D.



†CHO= Carbohydrate

Figure3. The percentage of matched cases (n=26), and controls (n=38) not achieving dietary EAR or population average.

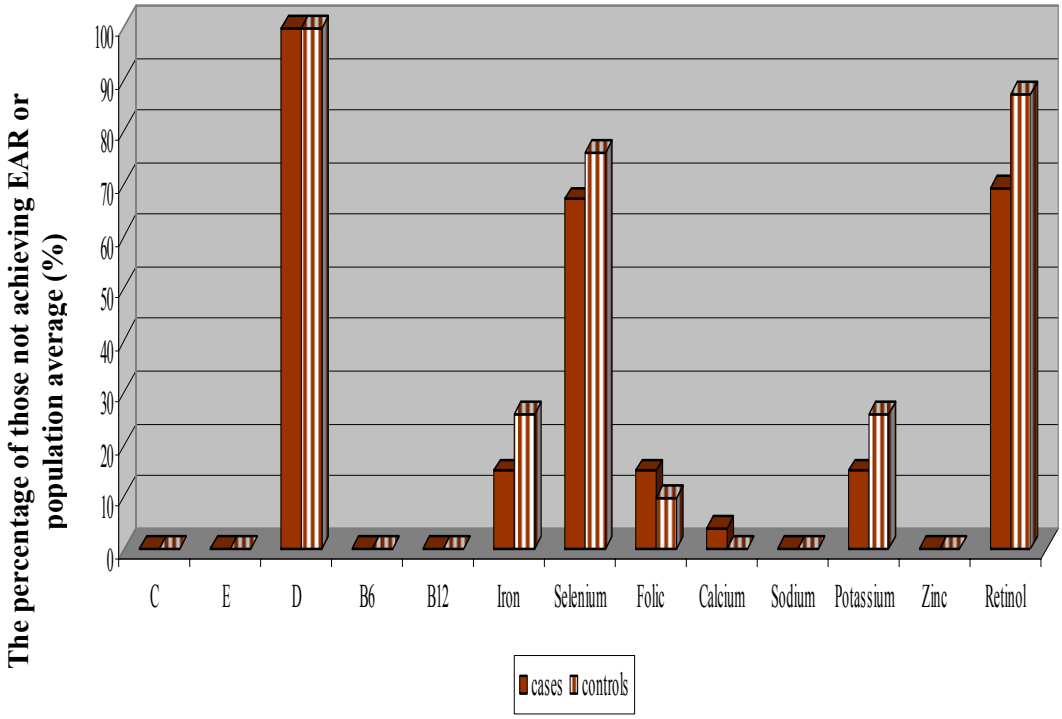


Figure 4. The percentage of matched cases (n=26), and controls (n=38) not achieving dietary EAR, or the RNI for vitamin D.

Association between nutrient intake and PE

The potential association between intake of selective macro and micronutrients and the development of PE are presented Table 5. Only the mean intake of retinol (Odds Ratio 1.96, CI 1.06 to 3.64; *p*= 0.032) was significantly associated with the risk of developing PE, but none of the other nutrient intakes were significantly associated with the risk of developing PE when cases and high-risk controls were considered (n= 253). There was also no association between the PE and the use of pregnancy-specific multivitamin supplements (Odds Ratio 1.03, CI 0.59 to 1.82, adjusting for ethnicity and principal risk factors). For the tightly matched cases versus controls (n=64), the same analysis for selective nutrients also showed no statistically significant predictor of PE with any dietary factor.

Bodnar *et al.* (2006) have suggested a protective effect of multivitamin use on the development of PE in lean women only. Results showed no significant differences when all cases and controls were considered or when only the tightly matched cases and controls were analyzed, therefore, there were no protective effect of multivitamin use in either group. Generally, there was no association between dietary intake and PE. These findings are not directly comparable with the results of all other studies in pregnant groups. This could reflect differences in general nutritional status of the population studied. Also, differences in sample size, stages of pregnancy, socioeconomic status, and the dietary assessment methods used may contribute to such divergent findings. Furthermore, not all previous studies have taken the use of prenatal multivitamins and other nutritional supplements into account, as we did in this study. However, in contrast to the present study, a recent report has noted a lower rate of PE amongst a population of USA women taking multivitamins **(Bodnar *et al.*, 2006)**. Additionally, the differences may have reflected a greater intrinsic variability in the diets of women from different countries compared with those in the UK.

In agreement with the present study, some reports show no association between PE and diet. These include **Morris *et al.* (2001)** who found no difference in dietary intake amongst women who did and did not develop PE amongst a cohort of 4313 nulliparous American low risk women taking part in a study to assess the role of calcium supplements in the prevention of PE. The women were supplemented with 2 g calcium or placebo from early in pregnancy and the calcium supplements had no significant effect on the subsequent incidence of PE. Moreover, **Schiff *et al.* (1996)** did not report any evidence that low vitamin E consumption was related to the development of PE and we showed similar results. Furthermore, the results of a prospective cohort study of 299 Australian pregnant women who completed a FFQ showed that the mean dietary vitamin E consumption was similar for both the pre-eclamptic and control women **(Rumbold *et al.*, 2005)**.

Other reports have however shown an association between increased the risk of PE and poor diet. These included **Zhang *et al.* (2002)** who, using a FFQ, reported low intakes of vitamin C in a case-control study of 93 women with PE and 234 controls in Washington, USA. In another report, **Clausen *et al.* (2001)** suggested that high intakes of energy, sucrose, and polyunsaturated fatty acids independently increase the risk for PE. Several important limitations must be considered when interpreting the results of the different studies. With a few rare exceptions, dietary assessments have not usually been validated for pregnancy or the stage of pregnancy. We have confidence in our study because we used the 7-day diaries.

Table 5: Associations between the nutrient intake/ day and PE

Nutrients	Odds ratio for PE and controls (n=253)	*P*	Odds ratio for all matched PE and controls (n=64)	*p*
Macronutrients				
Energy intake (kcal)	2.00 (0.95 -4.65)	0.065	2.5 (0.27-22.60)	0.418
Total Fat (g)	1.15 (0.60 -2.15)	0.667	0.87 (0.25-3.01)	0.822
Carbohydrate (g)	0.84 (0.46 -1.55)	0.578	0.77 (0.25-2.40)	0.653
Saturated fatty acids (g)	0.46 (0.17 -1.26)	0.131	0.27 (0.02-2.70)	0.267
Trans-fatty acids (g)	0.69 (0.14 -3.50)	0.656	1.13 (0.44-2.87)	0.800
Monounsaturated fatty acids (g)	1.20 (0.72 -2.03)	0.483	0.87 (0.34-2.22)	0.769
Polyunsaturated fatty acids (g)	1.03 (0.60 -1.70)	0.926	2.5 (0.27-22.60)	0.418
Micronutrients				
Iron (mg)	1.37 (0.76-2.47)	0.301	1.56 (0.45-5.38)	0.479
Selenium (µg)	0.80 (0.45 -1.50)	0.481	1.07 (0.28-4.01)	0.927
Folate (µg)	0.89 (0.48- 2.01)	0.956	0.71 (0.17-3.10)	0.653
Potassium (mg)	1.23 (0.66- 2.30)	0.301	1.86 (0.45-7.68)	0.392
Retinol (µg)	1.96 (1.06- 3.64)	0.032	0.70 (0.63-20)	0.125
Carotene (µg)	0.96 (0.75 -1.20)	0.895	1.56 (0.45-5.38)	0.479

Association between dietary intake and risk factors of PE

The association between dietary intake and obesity as critical risk factors for PE have been identified amongst the study cohort. As shown in Table 6 the analysis showed that the dietary intake of protein was associated with obesity as measured by BMI. This is further shown in a plot of protein intake against BMI (Figure 5). Dietary constituents have also been implicated in pregnancy outcomes other than PE, especially birth weight. An analysis of the relationship between birth weight and dietary intakes of retinol, carotene, vitamin E, C, B_6, B_{12}, folic acid, and protein (264 babies) using multiple regression analysis showed no association between the intake of these nutrients and birth weight. In this study 31% of the women reported that they were taking prescribed multivitamin/mineral supplementation at enrolment, and 30% reported folic acid supplementation. Multiple regression analysis showed there were no associations between the use of pregnancy-specific multivitamins ($F=1.0$, $p=0.4$) with birth weight, very low birth weight (<1500 g), small for gestational age (below 5th percentile), or small for gestational age (below 10th percentile), but there was a marginally positive association with the use of multivitamins and the incidence of low birth weight <2500 g (OR 0.39, 0.2-0.9). Additionally, results presented as odds ratios shows that there was no significant relationship between the use of folic acid (183 babies), and birth weight. ($F=0.43$, $p=0.73$), low birth weight <2500 g (186 babies), or very low birth weight <1500 g (169 babies), or small for gestational age deliveries (below 5th percentile), but there was a marginal positive relationship between the use of folic acid and small for gestational age deliveries (below 10th percentile) (OR 0.5, 0.3-0.98).

Whilst there was no indication that the intake of any nutrient was related to the incidence of PE, we also investigated whether there were any interactions between risk group and diet. Unexpectedly, there was no association between energy intake and BMI, despite the exclusion of women who under-reported energy intake. Some inaccuracy of the reporting of energy intake amongst those included may contribute and also the wide confidence intervals in the data for macronutrient intake with the numbers studied. However, it found that BMI of cases and high-risk controls (n=253) was associated with protein intake. Few previous studies have investigated associations between pregnancy BMI and nutrient intakes in the second trimester. The relationship we observed agrees indirectly with a study by **Lauro *et al.* (2000)** who showed that pre-eclamptic women consumed a greater daily intake ($p<0.05$) of energy, proteins and lipids than women with normal pregnancy. **Irles Rocamora *et al.* (2003)** described the level of nutritional adequacy of the regular diet of Spanish pregnant women (n=49) during the first trimester, food intake was assessed using a 24-hour reminder sheet, and a weekly consumption survey, The normal diet of these pregnant women from Seville was rich in animal protein (88 ±21 g/day), and fat (97 ±27 g/day).

Table 6: Associations between daily nutrient intake and BMI (n=253)

Nutrients	Change (95% CI)	*p*
Macronutrients		
Energy intake (kcal)	-1.88 - 3.25	0.581
Total Fat (g)	-2.43 - 2.60	0.944
Carbohydrate (g)	-4.81 - 1.39	0.260
Protein (g)	0.67 - 3.94	0.008
Cholesterol (mg)	-0.61 - 1.65	0.348
Saturated fatty acids (g)	-1.57 - 1.93	0.828
Trans-fatty acids (g)	-0.52 - 1.10	0.455
Monounsaturated fatty acids (g)	-1.56 - 2.48	0.635
Polyunsaturated fatty acids (g)	-0.54 - 1.75	0.280
Micronutrients		
Vitamin E (mg)	-1.03 - 0.85	0.837
Vitamin C (mg)	-1.50 - 0.11	0.085
Iron (mg)	-1.36 - 0.19	0.132
Selenium (µg)	-0.31 - 2.27	0.126
Folate (µg)	-1.71 - 0.93	0.541
Zinc (mg)	-1.00 - 1.15	0.880
Sodium (mg)	-1.39 - 4.38	0.290
Potassium (mg)	-0.72 - 3.94	0.163
Calcium (mg)	-2.06 - 0.91	0.423
Retinol (µg)	-1.68 - 0.33	0.173
Vitamin B_6 (mg)	-0.81 - 0.71	0.894
Vitamin B_{12} (µg)	-1.29 - 1.16	0.909
Carotene (µg)	-1.03 - 0.34	0.298

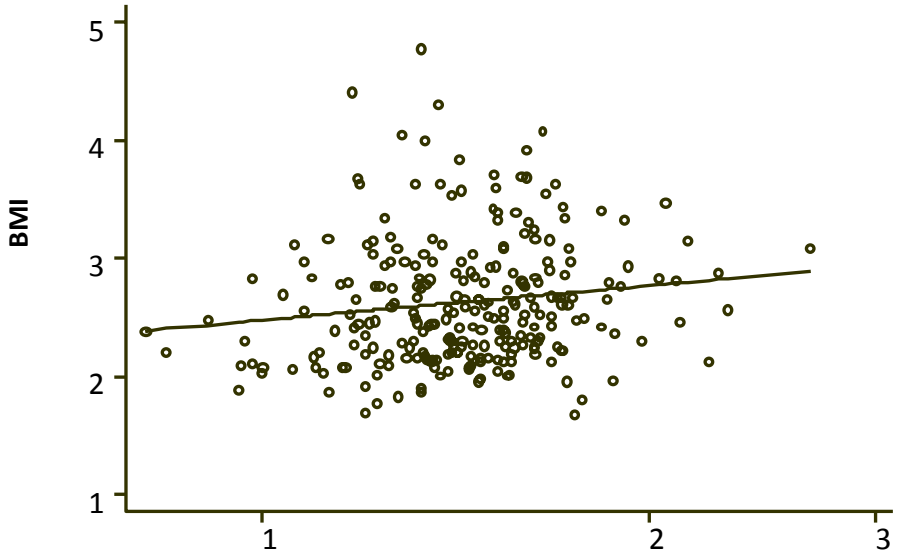


Figure 5. Association between protein intake and BMI in cases and high-risk controls

These agree with the results of **Johnson *et al.* (1994)** who also found no associations between nutrient intake and birth weight in a prospective observational study of urban African American women. There are however numerous studies describing a relationship between micronutrient deficiency and low birth weight in developing countries, particularly in association with folate, vitamin B_{12}, zinc and magnesium status **(Ramakrishnan *et al*., 1999, Ladipo, 2000, Siega-Riz *et al*., 2004 and Evans *et al.,* 2004)**. According to the World Health Organization (WHO), 95% of total low birth weight babies in the world are born in developing countries **(King, 1981)**.

In the present study there were no associations between the use of folic acid supplements and birth weight, low birth weight <2500 g, or very low birth weight <1500 g, but there was a marginal positive relationship between gestational age (below 10^{th} percentile), and the use of folic acid. **Charles *et al.* (2005)** found no association between folic acid supplementation and mean birth weight, placental weight or gestational age, and no effect of multivitamin on the risk of preterm births (34-37 weeks) or SGA (5th-10th percentiles; **Catov *et al*., 2007**). By contrast, **Scholl *et al.* (1997)** found that the risk of low birth weight was reduced approximately two-fold with multivitamin supplement use during the first and second trimester of pregnancy. **Poston *et al.* (2006)** reported that use of pregnancy-specific multivitamins was associated with lower rates of low birth weight, and better birth weight percentiles, but after correction for risk group at baseline, degree of education, and housing and smoking status this remained significant only for birth weight percentiles. In addition **Haider and Buhutta (2006)** when compared with supplementation of two or fewer micronutrients or no supplementation or a placebo, multiple-micronutrient supplementation resulted in a statistically significant decrease in the number of low birth weight babies, and SGA babies. The remits of the present study do not therefore agree with this result, indeed we found marginal associations with multivitamin use, of folic acid and low birth weight.

Biochemical analysis of women (PE cases and control groups)

Plasma concentration of nutrients between cases and controls were illustrated in Table 7. Plasma concentrations of vitamin C, vitamin E/total cholesterol (α-tocopherol), γ-tocopherol, retinol , total cholesterol, high density lipoprotein cholesterol (HDL-c), and low density lipoprotein cholesterol (LDL-c), uric acid and MDA, were compared between cases and controls, the results revealed that, there were no significant difference in the plasma concentrations of any of the variables measured of high-risk women who eventually developed PE late in their pregnancy (cases) and those who did not (controls).

In this study we found no significant differences in plasma concentration of nutrients between cases and controls. In women with established PE there are inconsistent reports on the plasma concentrations of vitamin C; some suggest it is reduced compared to controls **(Zhang *et al.,* 2002 and Gupta *et al.,* 2005)**, or have shown no

difference **(Mutlu-Turkoglu *et al.*, 1998)**. In the present study this relationship was not apparent although numerically the values were lower in cases than controls and the mean values similar. Similarly, the results of our study showed no significant differences between cases and controls in plasma α-tocopherol. In women with established PE there are varying reports of the plasma concentrations of vitamin E some suggesting it is reduced compared to controls **(Madazli *et al.*, 1999, Sagol *et al.*, 1999, Kharb, 2000 and Zhang *et al.*, 2002)**, and some showing no difference **(Bowen *et al.*, 2001 and Llurba *et al.*, 2004)**. Different dietary vitamin E intakes may explain the paucity of consistent results reported in the literature. It has been demonstrated that vitamin E levels rise progressively throughout normal pregnancy, probably due to the parallel increase in circulating lipids **(Roberts, 2000 and Chen *et al.*, 2003)**, which can influence fat-soluble antioxidant concentrations and may confound the interpretation of vitamin E as indicators of antioxidant status. Given the small numbers of subjects involved, these results do not permit us to conclude that the risk of PE is, or is not, associated with oxidative stress as estimated using plasma concentrations of Vitamins C, E or A.

Uric acid is the final product of purine metabolism in humans. The final two reactions of its production i.e., conversion of hypoxanthine to xanthine and the latter to uric acid, are catalysed by the enzyme xanthine oxidoreductase, this enzyme may attain two inter-convertible forms, namely xanthine dehydrogenase or xanthine oxidase. The latter uses molecular oxygen as an electron acceptor and generates superoxide anion and other reactive oxygen products **(Poston and Raijmakers, 2004)**. The role of uric acid in conditions associated with oxidative stress is not entirely clear. Evidence based on epidemiological studies, suggests that increased serum levels of uric acid are a risk factor for cardiovascular disease where oxidative stress plays an important pathophysiological role , in women with PE it has been shown that the uric acid level are elevated **(Many *et al.*, 1996).** Furthermore, a study by **D'Anna *et al.* (2000)** who found that compared with normal, the median serum uric acid levels in women with PE or transient hypertension were significantly elevated. Differences in median serum uric acid concentrations between women with PE and with transient hypertension were statistically significant didderence. In our study mean uric acid concentrations were 182 μM among PE cases, and 178 μM, among high-risk controls with no significant difference. This is in general agreement with **Thangaratinam *et al.* (2006)** who showed that serum uric acid was a poor predictor of maternal and fetal complications in women with PE.

There is substantial evidence to suggest that lipid changes in PE are associated with increased oxidative stress and endothelial activation **(Sattar, 2004 and Ray *et al.*, 2006)**. Moreover, **Ware-Jauregui *et al.* (1999)** studied the relationship between maternal plasma lipid concentrations and risk of PE, the results suggested that high triglyceride and low HDL-c concentrations were important risk factors for PE among Peruvian women. In the present study we found no association. Numerous other studies have confirmed that plasma lipid peroxide levels are also significantly elevated in PE including serum MDA, or the TBARS **(Uotila *et al.*, 1993 and Takacs *et al.*, 2001)**. The findings in our study do not show any evidence of deficiency in the maternal protective antioxidant systems or increased production of lipid peroxidation products, and MDA in women who developed PE as compared with high-risk pregnancy without PE. However, our findings differ from those obtained in other studies, which indicated higher serum concentrations of MDA in women with PE compared to controls **(Ilhan *et al.*, 2002, Aydin *et al.*, 2004 and Atamer *et al.*, 2005)**. This difference may relate to our control group, which were high-risk pregnant women; also our groups were recruited at 14^{+0} to 21^{+6} weeks of gestation and blood samples were collected before the development of PE. However, our data are consistent with several previously published studies using methods comparable to ours, which also failed to find evidence that circulating markers of oxidative stress are significantly elevated in plasma of pregnant women with PE. **(Morris *et al.*, 1998, Diedrich *et al.*, 2001 and Bowen *et al.*, 2001)**. Also, **Llurba *et al.* (2004)** showed no evidence of increased MDA in PE women.

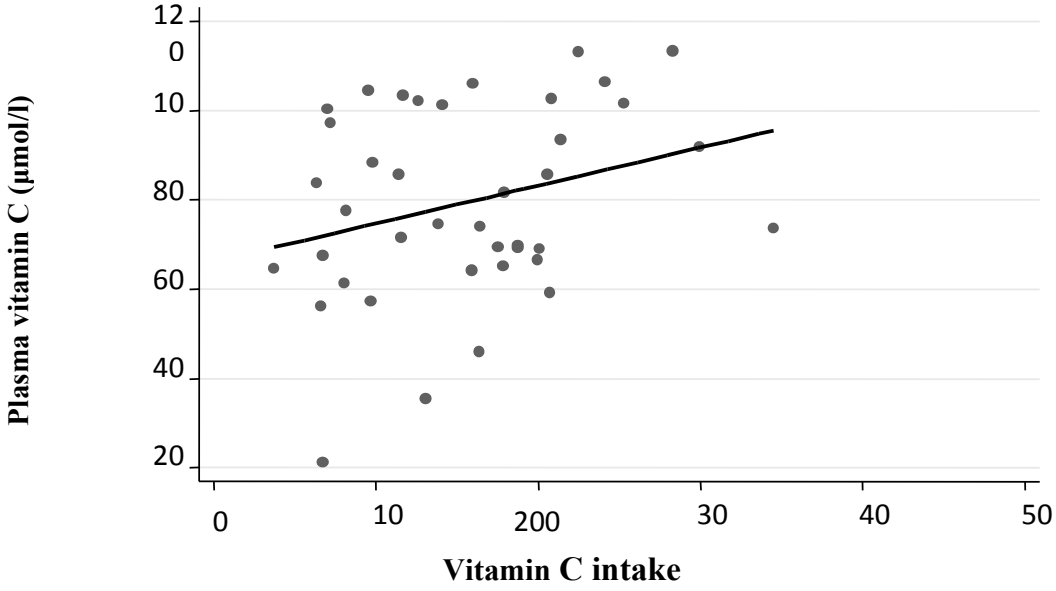
6. Relationship between selected nutrients assessed by 7-day diaries and their plasma concentration.

The results showed no significant relationship between plasma concentration of any nutrient and intake of the same nutrient for all cases and controls, and no significant relationship between plasma concentration of any nutrient and intake of the same nutrient for cases and controls considered separately (Figures 6 to 10). The obtained results in agreement with **Lee *et al.* (2004)** who reported no significant association between the dietary intake of vitamins C and E, and the level of these vitamins in the maternal serum from normal pregnant women. This may be due to the fact that even normal pregnancy is associated with oxidative stress, leading to a biological depletion of antioxidants in addition to other contributory genetic and environmental factors, for example oxidative damage caused by sunlight, environmental toxins, exercise, stress and other factors. Also, the lack of association between the plasma concentration of nutrients and intakes of the same nutrients may very well relate to the errors inherent in the estimation of dietary intakes. It is well known that the correlation is usually poor for many nutrients.

Table 7: Plasma concentrations of nutrients in case and control women recruited at 14^{+0} to 21^{+6} weeks of gestation.

Serum	Cases (n=14)		Controls (n=27)		Difference between means (95% CI)	*P* value of difference
	Mean ± SD	**Range**	**Mean ± SD**	**Range**		
Vitamin C (µmol/l)	74±24	21-113	81±20	36-113	-20.71-8.75	0.426
Vitamin E/total cholesterol (µmol/mmol)	6±1	5-7	6±1	3-9	-0.57- 0.733	0.811
Gamma-tocopherol (µmol/l)	0.27±0.08	0.15-0.43	0.23±0.09	0.11-0.44	-0.01-0.09	0.139
Retinol (µmol/l)	1.81±0.49	1.20-2.70	1.70±0.43	-0.86 -2.61	-0.17-0.43	0.392
Total cholesterol (mmol/l)	5.04±0.94	3.74-6.91	5.20±0.89	3.37-6.96	-0.73-0.46	0.658
HDL-cholesterol (mmol/l)	1.50±0.08	1.31-1.64	1.45±0.12	1.23-1.72	-0.00-0.09	0.083
LDL-cholesterol (mmol/l)	2.90±0.72	1.94-3.59	3.00±0.73	1.85-4.78	-0.60-0.32	0.553
Triglyceride (mmol/l)	1.37±0.67	0.50-3.10	1.43±0,65	0.47 -3.20	-0.41 -0.41	0.993
Uric acid (µmol/l)	182±81	70-376	178±44	116-257	-40.66-49.33	0.850
MDA (µmol/l)	1.88±0.24	1.40-2.30	1.82±0.21	1.50-2.40	-0.09-0.21	0.430

(a) r =0.2674, *p*=0.091.



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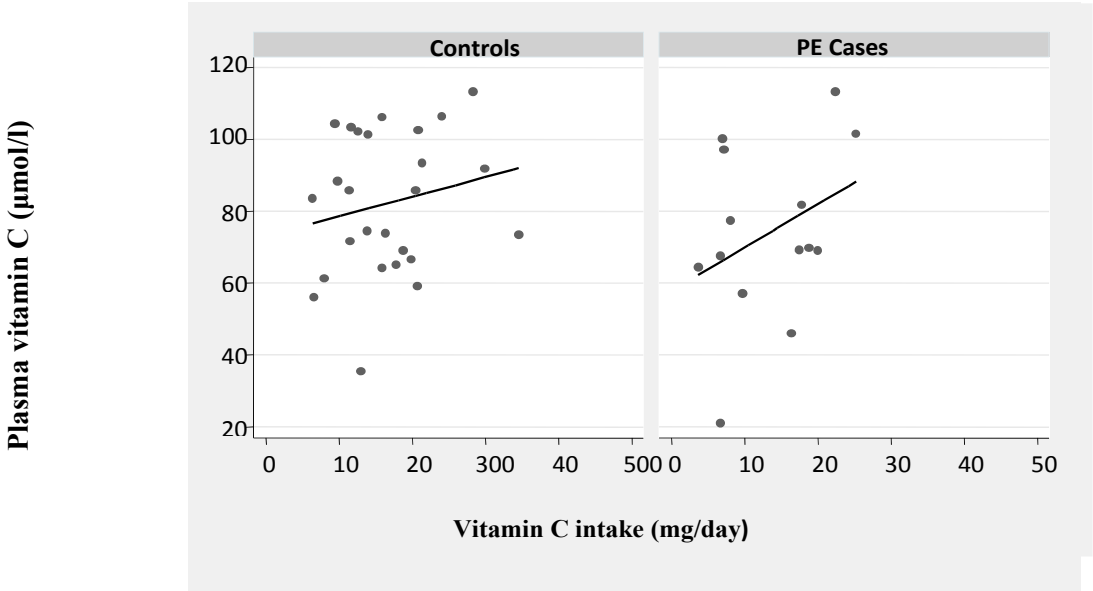
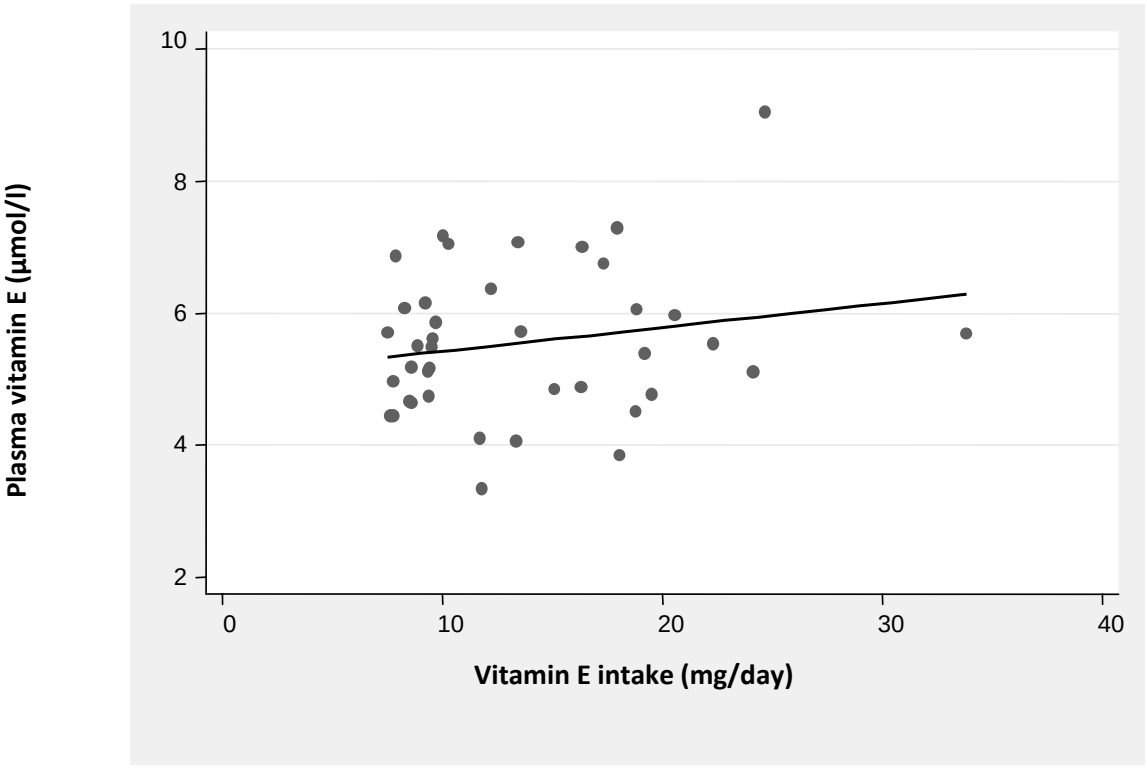


Figure 6: (a) Correlation between the daily intakes of vitamin C assessed by 7-day diaries, and plasma vitamin C for all cases and controls. (b) Correlations between the daily intakes of vit C assessed by 7-day diaries, and plasma vitamin C for PE cases and controls separately.

(a) r=0.1827, *p*=0.253.



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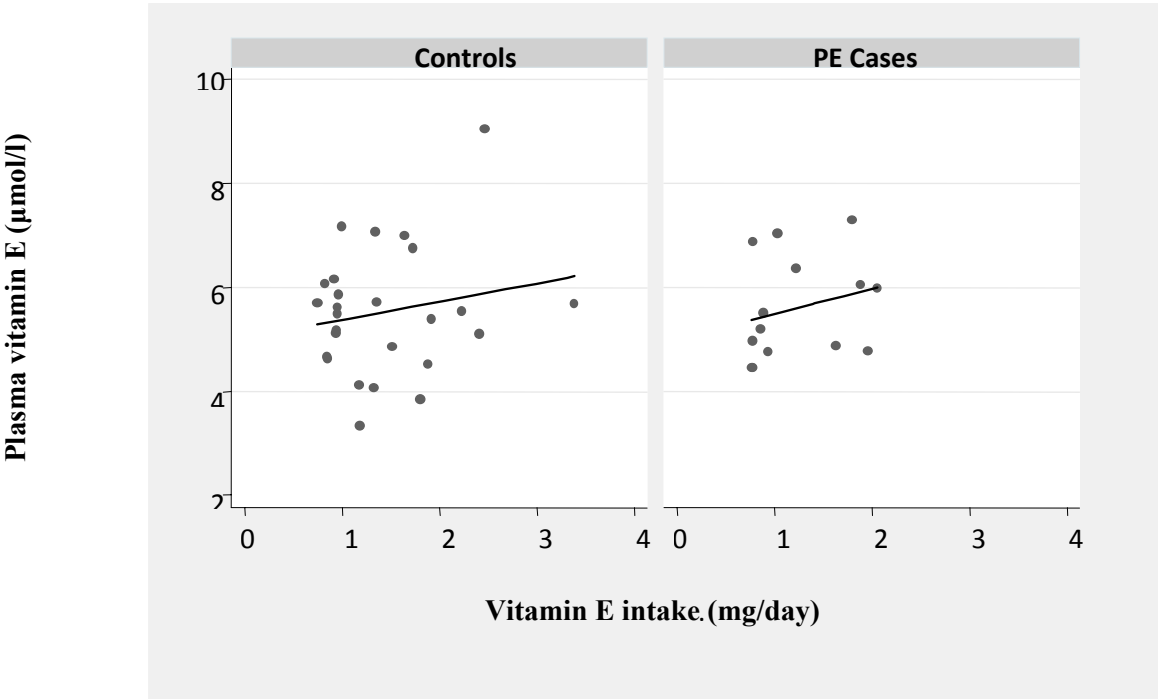
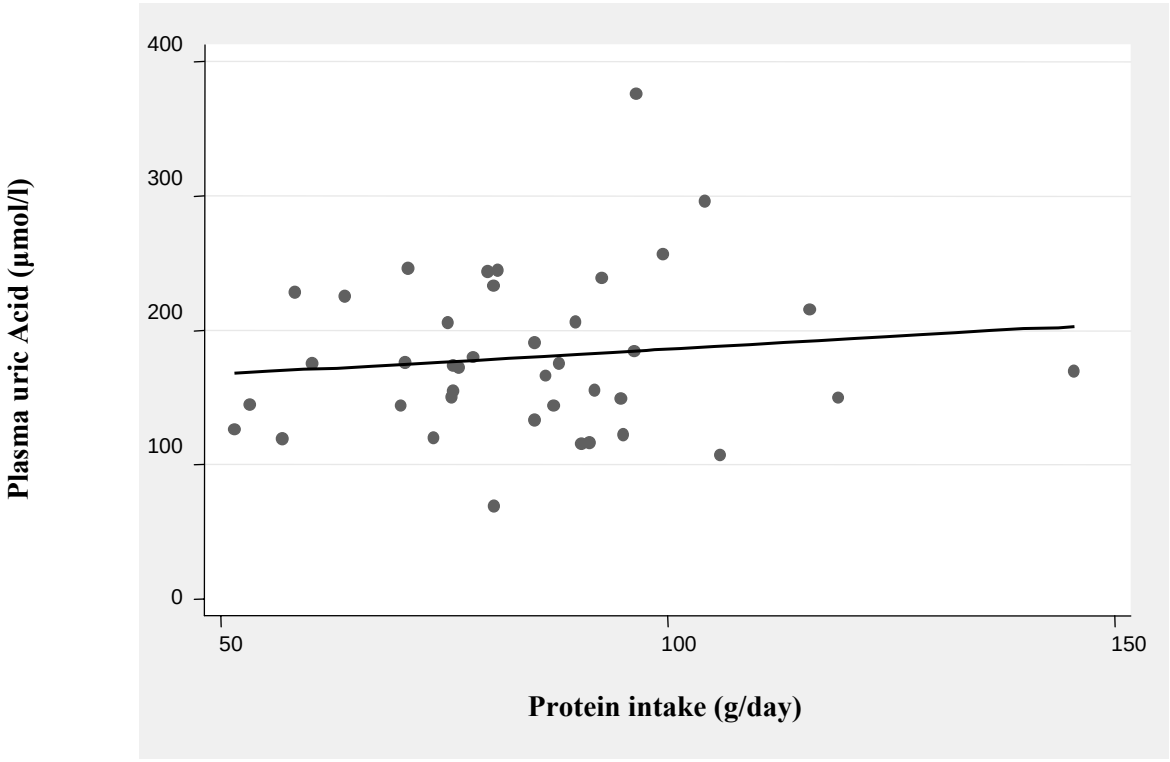


Figure 7: (a) Correlation between the daily intakes of vitamin E assessed by 7-day diaries, and plasma vitamin E for all cases and controls. (b) Correlations between the daily intakes of vitamin E assessed by 7-day diaries, and plasma vitamin E for PE cases and controls separately.

(a) r=0.1468, p=0.360.



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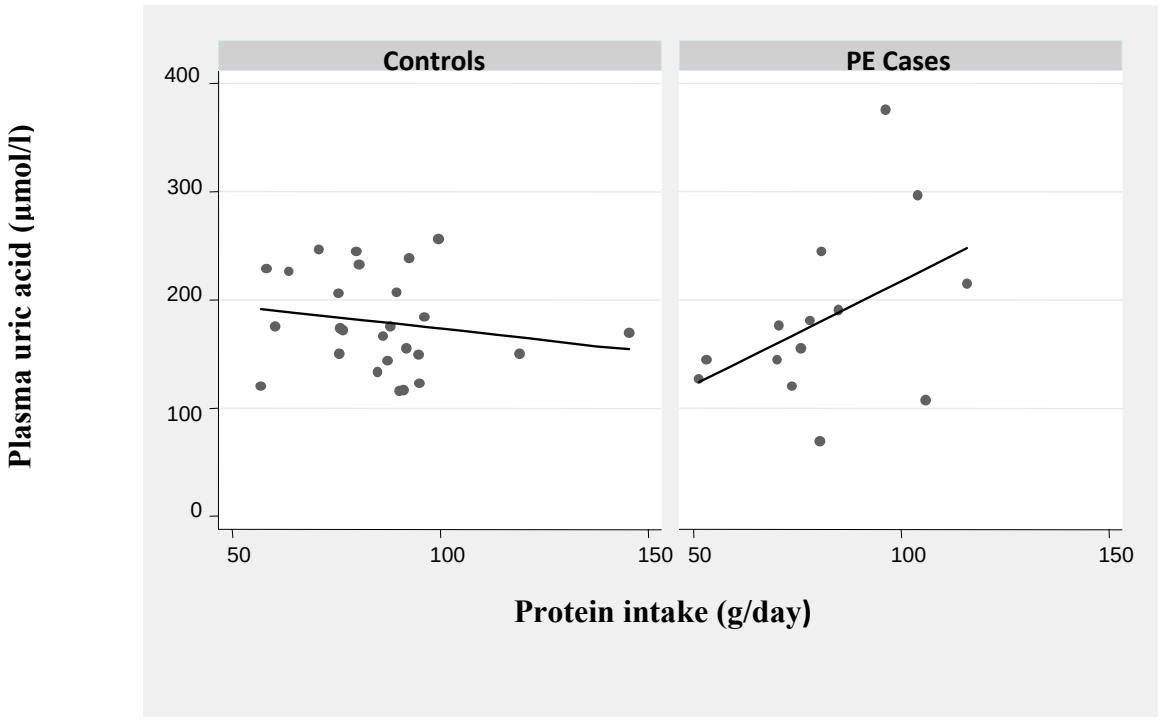
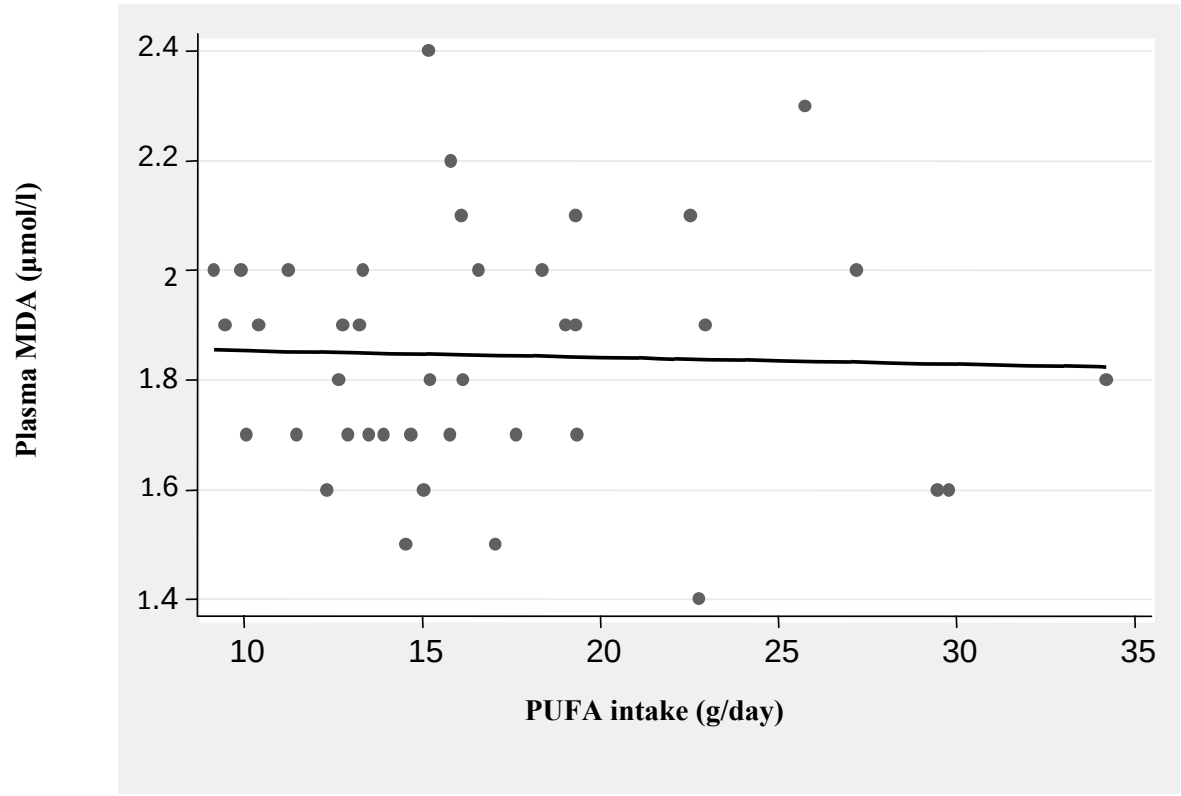


Figure 8: (a) Correlation between the daily intakes of protein assessed by 7-day diaries, and plasma uric acid for all cases and controls. (b) Correlations between the daily intake of protein assessed by 7-day diaries, and plasma uric acid for all PE cases and controls separately.

(a) r=-0.0347, p=0.830



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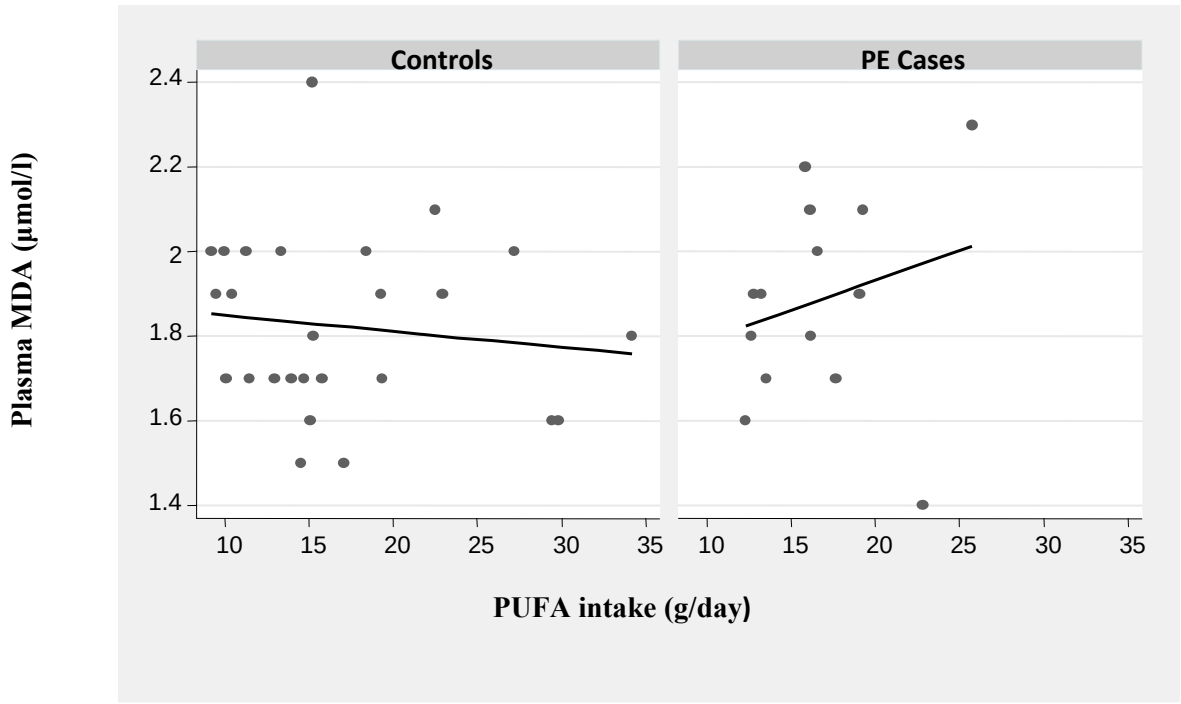
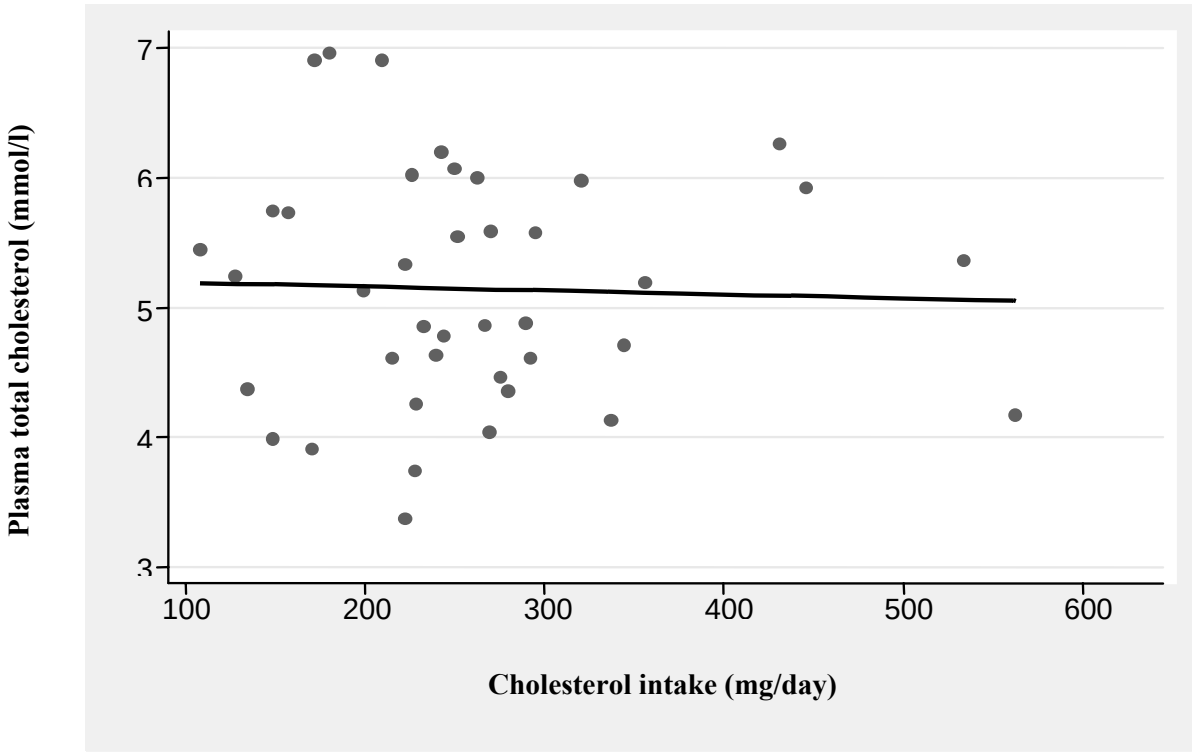


Figure 9: (a) Correlation between the daily intakes of PUFA assessed by 7-day diaries, and plasma MDA for all cases and controls. (b) Correlations between the daily intakes of PUFA assessed by 7-day diaries, and plasma MDA for PE cases and controls separately.

(a) r=-0.0322, p=0.842.



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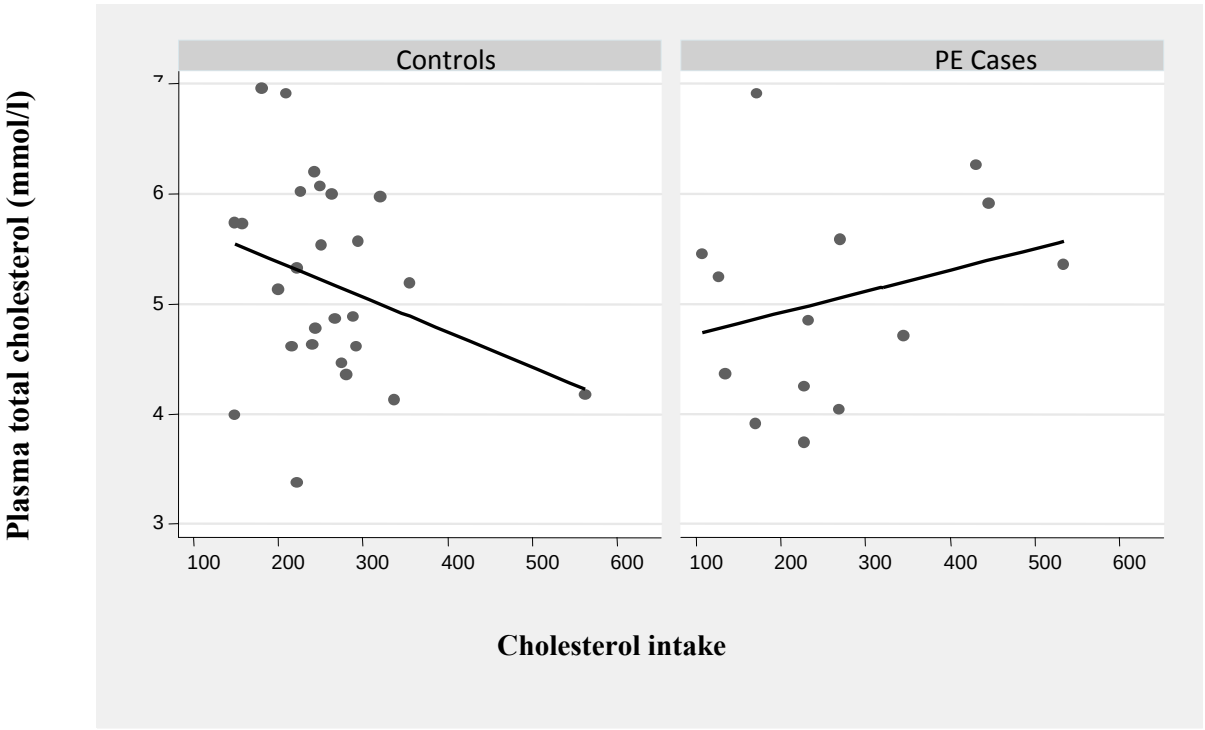

Figure 10: (a) Correlation between the daily intake of cholesterol assessed by 7-day diaries, and plasma total cholesterol for all cases and controls. (b) Correlations between the daily intakes of cholesterol assessed by 7-day diaries, and plasma total cholesterol for PE cases and controls separately.

In conclusion, this study has refuted our hypothesis that dietary intake is a major determinant of PE in high-risk women. This does not exclude a potential role in less advantaged individuals in the developing world. The results of the present study indicate that the mean dietary intakes of all macronutrients and micronutrients of PE cases and high-risk controls women in our study were unrelated to the development of PE. However, vitamin D, retinol and selenium intake may need further investigation in this high-risk population. The results indicate, however, that the dietary intake of protein was associated with obesity as measured by BMI, but that this was not independently related to PE risk.

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