**Homocysteine and folate plasma concentrations in mother and baby at delivery after pre-eclamptic or normotensive pregnancy; influence of parity.**

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**Abstract**

Pre-eclampsia affects between 2-7% of pregnant women. There are conflicting data on plasma homocysteine and folate in pre-eclampsia, and little about fetal concentrations.

**Objectives:** To compare the concentrations of homocysteine and folate in maternal and paired fetal (umbilical venous) plasma samples from normotensive or pre-eclamptic pregnancies at delivery and to identify any effect of parity on these concentrations.

**Study design:** Hospital based cross-sectional study of 24 normotensive and 16 pre-eclamptic pregnant women from whom maternal and fetal plasma samples were collected at delivery.

**Main outcome measures:** Maternal and fetal plasma homocysteine and folate concentrations between normotensive and pre-eclamptic pregnancies with varying parity.

**Results:** There were no significant differences in either maternal or fetal plasma homocysteine or folate concentrations between normotensive and pre-eclamptic pregnancies. In both the normotensive and pre-eclamptic women, plasma folate concentration was higher in paired fetal compared to maternal plasma ($P < 0.001$ and $P = 0.047$ respectively). Both maternal and fetal plasma folate concentrations were lower in parous women ($P = 0.001$; $P = 0.017$ respectively), the lowest concentrations being in pre-eclamptic parous women ($P = 0.004$), but homocysteine concentrations were similar.

**Conclusions:** The low plasma folate in parous women is an interesting finding and, when intake is also low, may contribute to adverse pregnancy outcomes, particularly in relation to pre-eclampsia.

## Introduction

Pre-eclampsia is estimated to occur in 2-7% of all pregnancies and is a leading cause of maternal and perinatal mortality and morbidity in the Western world [1]; together with other hypertensive disorders of pregnancy it is responsible for approximately 60,000 deaths each year [2]. Pre-eclampsia is now commonly regarded as being a state of oxidative stress [3]. It is thought that primarily inadequate placental perfusion results in excessive production of reactive oxygen species giving rise to endothelial cell dysfunction and thus clinical manifestations of pre-eclampsia [4].

Homocysteine is a metabolic product of methyl-group donation by the amino acid methionine; remethylation is catalysed by folate. Small increases in plasma homocysteine concentrations are associated with increased risk of vascular disease [5], Alzheimer's disease [6] and neural tube defects [7] in the general population. Elevated homocysteine concentrations contribute to oxidative stress and endothelial dysfunction [8] and are thus also potentially implicated in the pathogenesis of pre-eclampsia. Some studies report raised homocysteine concentrations in pre-eclampsia (e.g. [9, 10]) while others have shown no significant differences [11, 12]. Maternal and fetal plasma homocysteine concentrations have been reported to be directly correlated in healthy nulliparae [13] and do not appear to change significantly during the course of pregnancy [14]. If plasma homocysteine concentrations are indeed raised in pre-eclampsia, and there is similar parallelism, then the fetus will be exposed to potentially damaging levels of homocysteine even before birth, which might have long-term consequences. Important factors influencing homocysteine concentrations are folate and vitamin $B_{12}$ status and the methylenetetrahydrofolate reductase (MTHFR) polymorphisms [15]; once again the data on these in pre-eclampsia is conflicting [16, 17].

There is considerably less, but similarly conflicting, evidence linking folate concentrations and pre-eclampsia [18, 19]. The fetus must receive an adequate supply of folate for growth and development; inadequate concentrations will also, *inter alia*, impede the remethylation of homocysteine. Both metabolites have active transport systems in the placenta [20] and impaired placentation is believed to

be central to the pathogenesis of pre-eclampsia. Folate has recently been shown to possibly play a direct role in extravillous trophoblast invasion [21], thus highlighting the need for adequate folate concentrations pre-pregnancy and during the early stages of pregnancy. Studies linking homocysteine and folate in the mother and fetus are very limited [17]. We therefore felt it to be important to measure fetal, as well as maternal, concentrations of homocysteine and folate at delivery.

Folate intake among women of reproductive age in the UK is reported to be low [22]. There is an increased demand for folate during pregnancy, which can result in suboptimal folate status [23], although there is some evidence to suggest that folate turnover during pregnancy does not appear to change if folate intake is adequate [24]. Increasing parity has been associated with decreasing plasma folate concentrations [25, 26], presumably because lactation is a further drain on folate reserves [27]. If dietary intake is suboptimal, these stores may not be replenished before a subsequent pregnancy, especially in cases of short inter-pregnancy intervals [25, 28]. We are unaware of any studies relating these low folate levels to increased plasma homocysteine concentrations in either parous women as a group, or parous women who develop pre-eclampsia. However, there is indirect evidence for an interaction between parity and raised plasma homocysteine concentrations which has been related to increased risk of pre-eclampsia [29, 30]. We hypothesised that plasma folate concentrations would be lower, and homocysteine higher, in pre-eclamptic women and their babies than in normotensive pregnant controls. We also opportunistically examined the effect of parity on maternal plasma folate concentrations in normal and pre-eclamptic pregnancy.

## Methods

*Subjects:* These investigations formed part of a detailed study of selenium and glutathione peroxidases in pregnancy, requiring recruitment of 25 pregnant women to each arm (see: [31]). The current study had a power of 80% to detect a difference of 1SD in maternal plasma folate concentration between the 24 normotensive and 16 pre-eclamptic women at the 5% level from whom paired maternal and fetal samples were available. Approval for these investigations was given by the Nottingham Hospital Ethics Committee and written informed consent was obtained from each participant. The study population consisted of two groups of White European women: normotensive and pre-eclamptics (Table 1). Paired fetal samples were also obtained via umbilical venous plasma. Cases were defined on admission with a clinical diagnosis of pre-eclampsia, using the International Society for the Study of Hypertension in Pregnancy definition of blood pressure ≥ 140/90 mm Hg (Korokoff V) on 2 occasions after 20 weeks gestation and proteinuria > 300 mg/L, 500 mg/day or 2+ on dipstick [32]. Controls were healthy with no pregnancy or medical complications.

*Sample collection:* Non-fasting venous blood samples were taken from mothers before delivery; where possible, umbilical venous samples were also taken immediately after placental delivery, in EDTA. Samples were transported on ice to the laboratory and centrifuged at 1,400 x g for 10 minutes at 4$^{o}$C and plasma was immediately stored at -80$^{o}$C until analysis.

*Homocysteine measurements:* Measurements of plasma homocysteine concentrations were obtained via fluorescence polarization immunoassay on the Abbott AxSym analyser. This assay has an analytical range of 1-50 μmol/L and reproducibility (CV) of 5.5% at 7.4 μmol/L, 6.2% at 13.5 μmol/L, and 5.4% at 25.9 μmol/L.

*Folate assay:* A microbiological technique with a chloramphenicol-resistant strain of *Lactobacillus rhamnosus (L. rhamnosus)* was used to assess the plasma folate concentrations as described previously [33]; 15 mg/ml manganese sulphate was added to the buffer to avoid artificially low plasma folate values [33, 34]. The inter- and intra-assay CVs were 5% and 4% respectively.

*Statistical analysis:* All tests were performed using SPSS for Windows version 14.0. Summary data are presented as medians [interquartile range (IQR)] or means ± SD depending on the distribution. The Kolmogorov-Smirnov test was used to assess the distribution of the data. Between group comparisons were made using Kruskal-Wallis tests or 2-tailed Student's *t* tests/Mann-Whitney *U*-tests depending on the distribution. Wilcoxon Signed Ranks tests were used to carry out paired comparison. Multiple regression analysis for maternal folate and parity correcting for inter-pregnancy interval and number of previous pregnancies were conducted. Correlations between the parameters were tested using Spearman's Ranks correlation test. The null hypothesis was rejected where $P < 0.05$.

## Results

*Subjects:* Both groups conceived spontaneously, carried singleton pregnancies and all neonates survived. Normotensive pregnant women delivered without developing hypertension or proteinuria, having infants weighing > 2500 g, delivered at 37 weeks' gestation or later. The systolic and diastolic blood pressure levels were, by definition, significantly raised in pre-eclampsia compared to normal pregnancy ($P < 0.0001$ for both; Table 1). Overall, the pre-eclamptic women all had moderate-to-severe disease (see[31]).

*Biochemical and molecular measurements:* Maternal and fetal plasma homocysteine and folate concentrations are given in Table 1. Plasma homocysteine concentrations were very similar in normotensive and pre-eclamptic women; fetal concentrations were also very similar in the two groups. Maternal and fetal homocysteine concentrations were significantly positively correlated ($r = 0.68$, $R^2 = 0.68$, $P < 0.0001$). Fetal homocysteine concentrations were higher than maternal in normotensive ($P = 0.002$), but not pre-eclamptic ($P > 0.1$) pregnancy.

Maternal folate appeared higher in normal than pre-eclamptic pregnancy, but there this was not statistically significant due to the large range of data. Fetal plasma folate concentration did not differ across groups (Table 1) and were positively correlated with maternal concentrations ($r = 0.44$, $R^2 = 0.20$, $P = 0.005$). Fetal folate concentrations were higher than maternal in normotensive ($P < 0.001$) and pre-eclamptic ($P = 0.047$) pregnancies. Correlation analysis showed no evidence for an association between plasma homocysteine and folate concentrations in either maternal or umbilical venous plasma ($r = 0.07$, $R^2 = 0.03$, $P > 0.6$; $r = 0.09$, $R^2 = 0.023$, $P > 0.4$ respectively).

*Folate concentrations and parity:* Overall, parous women and their babies had significantly lower plasma folate concentrations (Maternal: 6.0 [4.9, 6.8]; Fetal 18.2 [10.8, 22.2]) than nulliparous women (13.5 [7.5, 19.3]; $P = 0.001$) and babies (23.8 [17.2, 33.4]; $P = 0.017$; Figure 1a and b). Moreover, in both normotensive and pre-eclamptic women, parous women had significantly lower folate concentrations (normotensive: 6.2 [4.8, 10.4] cf pre-eclamptic: 5.4 [5.0, 6.2]; $P = 0.006$) compared to

nulliparous women (normotensive: 14.3 [10.2, 20.8] cf pre-eclamptic: 11.4 [6.2, 17.6]; $P = 0.015$; Figure 1b). Parous pre-eclamptic women had the lowest plasma folate concentrations (ANOVA; $P = 0.004$). This difference was maintained when considered with respect to fetal plasma folate but only in the normotensive group (parous: 13.8 [10.4, 21.8] cf nulliparous 17.3 [11.5, 25.5]; $P = 0.015$). A Kruskal-Wallis test showed that maternal folate concentrations were significantly different ($P = 0.006$) between nulliparous women and women who had had one or two previous pregnancies. Subsequent Mann-Whitney *U* tests indicated that the maternal folate concentrations were significantly higher in nulliparous women compared to parous women with 1 ($P = 0.005$) and 2 previous ($P = 0.037$) pregnancies (Table 2). Multiple regression analysis correcting for confounding factors thought to influence maternal plasma folate concentrations (inter-pregnancy intervals, number of previous pregnancies) indicated parity as an independent factor. No similar effect was seen with respect to homocysteine.

## Discussion

Although small, this cross-sectional study reports both maternal and fetal homocysteine and folate concentrations in normotensive and pre-eclamptic pregnancies; there appears to be little comparable maternal:fetal data from European populations [17]. Our maternal data are consistent with the results of other European studies in which no association was found with respect to homocysteine or folate concentrations and normotensive and pre-eclamptic pregnancies [11, 12]. Homocysteine has been potentially linked to processes such as inflammation [35] and hypercoagulation [8], which are present in severe pre-eclampsia. However, this does not mean that hyperhomocysteinaemia is a requirement for the development of pre-eclampsia and the elevated homocysteine concentrations seen in some pre-eclamptic patients may be an associated, or pre-disposing, rather than a causal factor.

Folate plays an essential role in the growth of the placenta from early pregnancy; folate deficiency has been linked to placental abruption and thus restricted fetal growth [36]. We observed no significant differences between maternal or fetal samples from normotensive pregnancy or pre-eclampsia. Most folate concentrations were within the reference range (7 – 46 nmol/L [37]) for non-pregnant UK adults, using this methodology, but 9 normotensive pregnant (7 parous) and 12 pre-eclamptic (6 parous) women had concentrations below this level and the pre-eclamptic patients had the lowest median values, which is consistent with previous studies examining folate concentrations [10, 17, 38]. Having folate concentrations at the lowest end of the range may contribute to the pathological consequences of oxidative stress as folate has been reported to have antioxidant properties [39]. The placenta takes up folate from the maternal circulation against a concentration gradient through a high-affinity binding site, the folate receptor-$\alpha$ [40], and releases it into the fetal circulation through the reduced folate carrier. Further functional studies are required to ascertain the changes folate transport in adequate and deficient folate concentrations.

Women in the UK have moderately low folate intakes for the developed world [41]; in our laboratory, a group of normotensive, non-pregnant women of comparable age had a median folate concentration of 13.9 nmol/L, relatively low in the reference range [37]. Periconceptual dietary supplementation with folic acid is widely advocated for the prevention of neural tube defects. However, only four women in normotensive and two women in the pre-eclamptic group (Table 1) took folic acid supplementation in this study. Unless there is dietary supplementation with folic acid, maternal folate concentrations in both plasma and red blood cells fall from about the fifth month of pregnancy [42].

Although sample sizes were small, we observed lower plasma folate concentrations in parous mothers and their babies in our study (Fig 1a), a trend exacerbated when pre-eclampsia supervened (Fig 1b). We do not know whether the parous women began pregnancy with lower stores or whether the uptake or renal reabsorption of folate differs in parous women. Larger studies are required to investigate this further especially by the findings from our group to indicate that folate may be essential early in pregnancy possibly directly promoting extravillous trophoblast invasion [21]. As only two women had 3 previous pregnancies, statistical tests could not be performed. These women did however have maternal plasma concentrations of 6.2 and 6.6 nmol/L respectively, which are all approximately half of the nulliparous folate concentrations (Table 2). This suggests that regardless of how many previous pregnancies a woman has had, her folate concentrations appear to be significantly lower than nulliparous women. Qvist *et al* also showed that by 2 -3 months after delivery, a third of all mothers can have subnormal concentrations of folate in serum and red blood cells, both those who are and those who are not breast-feeding [42]. Furthermore, it has been reported that by 6 months postpartum, 20% of mothers were still folate deficient [43]. The low plasma folate concentration in parous women independent of inter-pregnancy interval suggest possible beneficial effects of pre-pregnancy folic acid supplementation particularly in parous women who have previously suffered from pre-eclampsia.

Despite public health campaigns recommending the use of pre-conceptual folic acid supplementation,

the dietary intake of folic acid is still inadequate in about 13 million people within the UK [44]. Further investigations using larger cohorts are underway to further examine these fascinating initial data.

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**Contributors**

HDM completed this study as part of a PhD funded by BBSRC and wrote the majority of this article. JM completed the main laboratory experiments supervised by HDM and LOK as part of her BMedSci research project. FBP and MES were the principal investigators; MMR provided the clinical input for this study.

**Conflict of Interest**

None of the authors had a personal or financial conflict of interest.

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**Table 1.** Maternal and fetal venous plasma homocysteine and folate and participant details

| Parameter | Normotensive pregnant<br>n = 24 | Pre-eclampsia<br>n = 16 |
|---|---|---|
| Maternal age (years)[†] | 29 ± 6.6 | 31 ± 5.8 |
| Booking body mass index (kg/m$^2$) [†] | 25.9 ± 5.6 | 25.7 ± 3.8 |
| Primipara n (%) | 15 (62.5) | 11 (68.8) |
| Max. systolic blood pressure (mm Hg)[†] | 116 ± 4.3 | 157 ± 7.4[#] |
| Max. diastolic blood pressure (mm Hg)[†] | 76 ± 2.5 | 98 ± 4.9[#] |
| Proteinuria (g/L) [‡] | - | 1.0 [0.5, 1.8] |
| Gestation age at delivery, wks | 39.8 ± 1.0 | 38.1 ± 2.0[#] |
| Folate supplementation n (%) | 4 (16.7) | 2 (12.5) |
| Maternal homocysteine (µmol/L)[‡] | 8.7 [ 6.8, 11.0] | 8.2 [7.0, 10.6] |
| Fetal homocysteine (µmol/L) [‡] | 10.5 [9.0, 12.4]* | 9.5 [7.4, 11.9] |
| Maternal folate (nmol/L) [‡] | 10.7 [6.2, 19.4] | 6.8 [5.3, 14.1] |
| Fetal folate (nmol/L) [‡] | 22.2 [17.8, 32.7]* | 16.4 [11.3, 23.4]* |

[#] $P < 0.05$ between normotensive and pre-eclamptic pregnancies; *$P < 0.05$ between maternal and fetal samples. [†]Values represented as means ± SD; [‡]values represented as median [IQR]. Further demographic and pregnancy outcome details have been previously published[31].

**Table 2.** Maternal plasma folate concentrations in women with different parities.

| Parity | Maternal Folate, nmol/L (median [IQR]) |
|---|---|
| 0 (n = 24) | 13.5 [6.9, 19.4] |
| 1 (n = 11) | 5.6 [ 4.5, 8.0]** |
| 2 (n = 5) | 6.0 [6.0, 6.8]* |

*$P$ = 0.037 between nulliparous and two previous pregnancies; **$P$ = 0.005 between nulliparous and one previous pregnancy.

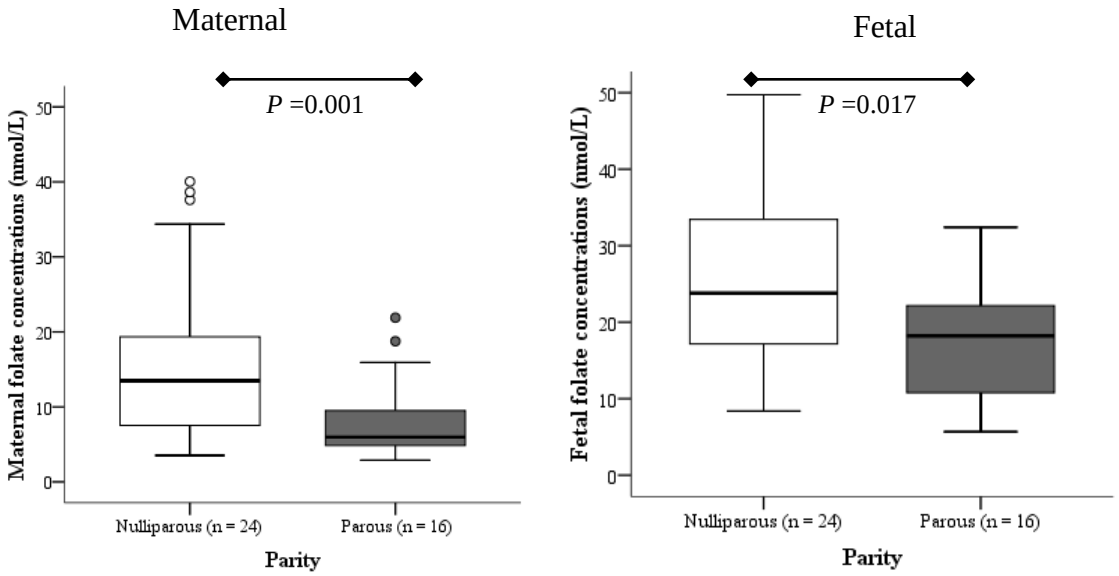
## Figure Legends

**Figure 1.** a) Maternal and fetal plasma folate concentrations by parity (nulliparous: white or parous: grey) in all pregnancies.

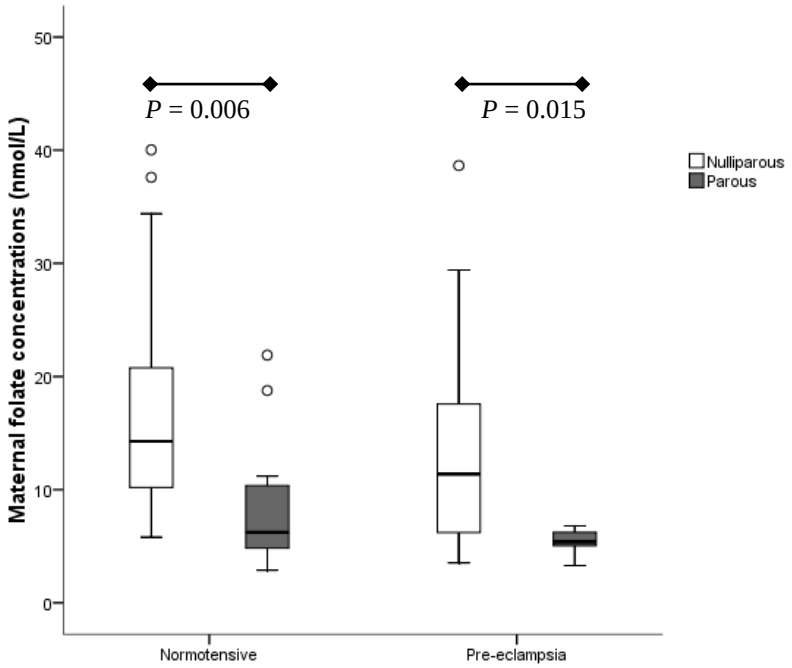
b) Maternal plasma folate concentrations by both parity (nulliparous: white or parous: grey) and the presence or absence of pre-eclampsia. There was a significant effect across parity:group for both maternal ($P = 0.006$) and fetal ($P = 0.015$) samples. Maternal and plasma folate concentrations were lowest in pre-eclamptic, parous women.

**Figure 1**

**a)**


Maternal
Fetal
P =0.001
P =0.017
Maternal folate concentrations (nmol/L)
Fetal folate concentrations (nmol/L)
0
10
20
30
40
50
Nulliparous (n = 24)
Parous (n = 16)
Parity

**b)**


P = 0.006
P = 0.015
Nulliparous
Parous
Maternal folate concentrations (nmol/L)
0
10
20
30
40
50
Normotensive
Pre-eclampsia