Serum and red-blood-cell folate demonstrate differential associations with BMI in pregnant women

Minxue Shen[1,2,3], Shazia Hira Chaudhry[2,3,4], Amanda J MacFarlane[5], Laura Gaudet[2,3], Graeme N Smith[6], Marc Rodger[3,4,7], Ruth Rennicks White[2,3], Mark C Walker[2,3] and Shi Wu Wen[1,2,3,4],*

[1]Department of Epidemiology and Health Statistics, School of Public Health, Central South University, Changsha, Hunan, People's Republic of China: [2]OMNI Research Group, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada: [3]Ottawa Hospital Research Institute, Clinical Epidemiology Program, 501 Smyth Road, Ottawa, Ontario, Canada, K1H 8L6: [4]School of Epidemiology, Public Health, and Preventive Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada: [5]Nutrition Research Division, Health Canada, Ottawa, Ontario, Canada: [6]Department of Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada: [7]Department of Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Abstract

Objective: To examine the association between BMI and folate concentrations in serum and red blood cells (RBC) in pregnant women.
Design: A cross-sectional comparison of folate concentrations in serum and RBC sampled simultaneously from the same individual.
Setting: The Ottawa Hospital and Kingston General Hospital, Ontario, Canada.
Subjects: Pregnant women recruited between 12 and 20 weeks of gestation.
Results: A total of 869 pregnant women recruited from April 2008 to April 2009 were included in the final analysis. Serum folate was inversely associated and RBC folate positively associated with BMI, after adjusting for folic acid supplementation, age, gestational age at blood sample collection, race, maternal education, annual income, smoking and *MTHFR* 677C→T genotype. In stratified analyses, this differential association was significant in women with the *MTHFR CC* variant. In women with the *CT* and *TT* variants, the differential associations were in the same direction but not significant. Folic acid supplementation during pregnancy did not alter the differential association of BMI with serum and RBC folate concentration. This indicates that the current RBC folate cut-off approach for assessing risk of neural tube defects in obese women may be limited.
Conclusions: BMI is inversely associated with serum folate and positively associated with RBC folate in pregnant women, especially for those with the *MTHFR CC* variant.

Folate status in women of childbearing age is important due to its role in the prevention of neural tube defects (NTD). Previous studies have observed a differential association between obesity and serum and red-blood-cell (RBC) folate concentrations in men, non-pregnant women of childbearing age and postmenopausal women[1–5]. Specifically, serum folate was found to be negatively associated, while RBC folate was positively associated, with BMI among non-pregnant adults[1–5]. The mechanisms for this differential association of obesity with serum and RBC folate concentrations are not clearly understood. Some investigators have suggested that the altered pharmacokinetics and distribution of folate in obese *v.* normal-weight persons may explain, at least in part, these differences[6,7]. It was observed that the peak serum folate concentrations of obese and normal-weight women were statistically different after a single oral dose of folic acid (0·4 mg), but there was no difference in overall area under the curve, indicating that obesity does not impair the absorption of folic acid but may lead to redistribution of folate from circulation into tissue. Folate requirements are higher in pregnancy in order to maintain placental and fetal growth, and accelerated folate catabolism and haemodilution during pregnancy can also result in decreased circulating folate concentrations[8–10]. Given the potential physiological and pharmacokinetic differences in pregnant *v.* non-pregnant populations,

Corresponding author: Email swwen@ohri.ca

the association of obesity with serum and RBC folate concentrations may be altered. Owing to the observed differential associations of folate with BMI in previous studies, the standard RBC folate cut-off approach for assessing NTD risk in obese women may be limited. Lower serum folate in obese women may reflect a redistribution of folate in response to the higher mass of maternal tissue, potentially limiting the amount of folate available to the developing embryo which accesses folate through serum folate rather than RBC folate, thus increasing the risk of NTD. The identification of subgroups of women who may be susceptible to low folate status or respond differently to folic acid supplementation will ensure that public health interventions can be tailored to meet the needs of the entire population. We therefore examined the association of BMI with serum and RBC folate concentrations in pregnancy using data collected in the Ottawa and Kingston (OaK) Birth Cohort study[11].

Method and materials

Data source

We used a subset of data from the OaK Birth Cohort, a prospective cohort study that recruited pregnant women at The Ottawa Hospital and Kingston General Hospital, Ontario, Canada, from September 2002 to April 2009. The subset included participants recruited from April 2008 to April 2009, from whom both serum and RBC folate were measured (Fig. 1). Participants were recruited between 12 and 20 gestational weeks and later delivered in the Ottawa–Carleton and Kingston regions. Participants were referred to the research team by clinic staff and asked to participate at their first antenatal visit. They were recruited into the study after information about the purpose of the study was provided and their written consent was obtained.

At the time of recruitment, participants' self-reported demographic and lifestyle information including age, race, education, household income, folic acid supplementation and smoking. Nurses measured current height and weight at recruitment. Participants provided the brand name(s) of vitamin supplement(s) that were being consumed at the time of recruitment. Total daily supplemental folic acid intake was calculated based on the frequency of use and folic acid dose from all vitamin supplements. For women who were using more than one vitamin supplement, the total daily supplemental folic acid dose was calculated as the combined intake from all supplement sources.

Fasting blood samples were collected at the time of routine blood work, in order to avoid additional venepuncture. The blood samples were drawn from the antecubital vein or from the hand of the participating women at gestational age between 12 and 20 weeks, and measured in batches every month (i.e. the samples were stored for up to 1 month). Blood samples for serum folate testing were collected in serum separator tubes (Becton

Dickinson, Franklin Lakes, NJ, USA) and allowed to clot. Samples were centrifuged for 10 min at 3000g to separate serum. Serum was then removed and stored at −20°C until analysis. Folate concentrations were measured using the Beckman Coulter Access 2 and Unicel DxI 800 immunoassay analysers using the manufacturer's reagents (Beckman Coulter, Brea, CA, USA).

Blood samples for measuring RBC folate concentration were collected in 3 ml purple-top EDTA tubes. Haematocrit was determined in fresh samples using an OSM3 hemoximeter (Radiometer, Copenhagen, Denmark). Samples were frozen at −20°C until analysis, at which point they were thawed and cells were lysed using Access RBC Folate Lysing Agent (Beckman Coulter). RBC folate concentrations were quantified using the Beckman Coulter UniCel DxI 800 Access Immunoassay System measured by competitive protein-binding assay. RBC folate concentrations were adjusted for haematocrit as described by the manufacturer.

Blood samples for genotyping the 677C→T polymorphism in the methylenetetrahyrofolate reductase gene (*MTHFR*) were collected in K_2EDTA Vacutainer tubes. Samples were centrifuged at 1100g and DNA was extracted using the BioRobot M48 and MagAttract DNA Blood Midi Kit (Qiagen, Hilden, Germany). PCR was used to amplify DNA regions of interest and genotyping was performed using the ABI 3130xl Genetic Analyzer and the ABI Prism SnaPshot Multiplex Kit (Applied Biosystems Inc., Foster City, CA, USA), which allows genotyping of several SNP in the same reaction[12]. The laboratory procedures for genotyping have been described previously[11,13]. For quality control, all assays were tested in hospital laboratories that are accredited by Ontario Laboratory Accreditation (OLA) and the Institute for Quality Management in Healthcare (IQMH), and are ISO certified for quality, competence and beyond.

Statistical analysis

Biomarker concentrations were non-normally distributed; therefore the geometric mean was used in our analysis. In regression analyses, logarithmic transformations were applied to variables with skewed distributions.

BMI was calculated and categorized into normal and underweight (<25·0 kg/m^2), overweight (25·0–29·9 kg/m^2) and obese (≥30·0 kg/m^2). Underweight cases (n 41) were combined with normal-weight cases due to limited sample size. Use of folic acid supplements was categorized as: (i) no supplement; (ii) supplement with folic acid intake ≤1 mg/d; and (iii) supplement with folic acid intake >1 mg/d.

Characteristics of the participants were described and compared across BMI categories using χ^2 tests. ANCOVA was used to examine folate concentrations associated with participant characteristics, including BMI, daily folic acid supplement intake, *MTHFR* 677C→T genotype (*CC, CT* and *TT*), maternal age, gestational age at blood sample collection, race, maternal education, household annual income and smoking.

Multiple linear regression analysis was used to examine associations of folate concentrations with BMI, in all samples and in subgroups stratified by *MTHFR* 677C→T genotype or folic acid supplementation. Interaction between BMI and genotype was tested in the overall model. Supplementary analysis using the pre-gravid weight (self-reported) was also performed. The level of significance was set at 0·05. Statistical analyses were performed using the statistical software package SAS version 9.2.
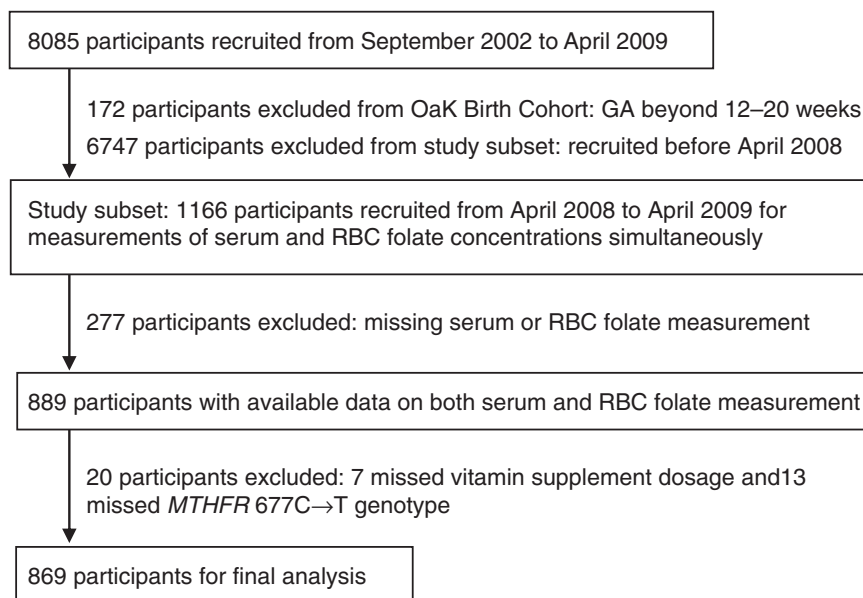
Results

A total of 889 participants who had complete records for both serum and RBC folate concentrations were identified in the cohort. Seven participants reported the use of vitamin supplements but did not include information on dose, and thirteen women missed information on *MTHFR* 677C→T genotype, leaving 869 participants for the final analysis (Fig. 1).

The overall geometric mean concentration of serum folate was 45·7 (SD 18·7) nmol/l and the geometric mean concentration of RBC folate was 1549 (SD 378) nmol/l. Serum folate <7·0 nmol/l was non-existent, and only one participant's serum folate was below 10 nmol/l. Demographic characteristics are shown in Table 1. Thirty-five participants (4%) were not taking any vitamin supplements. The proportion of overweight and obesity were 26% and 23%, respectively. Twelve per cent of participants were homozygous for the *MTHFR* 677C→T variant (*TT*). Gestational age at recruitment, education, annual income, folic acid supplementation and *MTHFR* 677C→T

genotype were statistically different among the BMI categories. Women with BMI ≥ 30·0 kg/m^2 had a higher gestational age at recruitment, lower education, lower income, lower supplement use and were more likely to have the *MTHFR* CC variant.

Table 2 shows the determinants of serum and RBC folate concentrations. BMI showed an inverse association with serum folate but a positive association with RBC folate whether adjusted for or not (Table 2). Serum folate was lower with each additional variant allele such that *CC* > *CT* > *TT*; however, the trend was not statistically significant. Conversely, unadjusted and adjusted RBC folate was higher in participants homozygous for the T allele such that *TT* > *CC* and *TT* > *CT*. Folic acid supplementation was associated with higher serum and RBC folate concentrations. Gestational age at recruitment was a determinant of serum folate, and maternal age, gestational age at recruitment, education and smoking were determinants of RBC folate.

Table 3 shows the association between BMI and serum or RBC folate, for all participants and in subgroups stratified by *MTHFR* 677C→T genotype or folic acid supplementation, adjusted for demographic variables. Overall, serum folate was inversely associated with BMI and RBC folate was positively associated with BMI. Interaction between BMI and genotype was not significant ($P > 0.05$) in the overall model (data not shown). When stratified by *MTHFR* genotype, serum folate was inversely associated with BMI in women with *CC* and *CT* variants, while RBC folate was positively associated with BMI in women with the *CC* variant. In women with the *TT* variant, both associations were insignificant, but the direction remained consistent with that observed for the *CC* and *CT* variants.

```
┌─────────────────────────────────────────────────────────────────┐
│ 8085 participants recruited from September 2002 to April 2009     │
└─────────────────────────────────────────────────────────────────┘
        │
        │  172 participants excluded from OaK Birth Cohort: GA beyond 12–20 weeks
        │  6747 participants excluded from study subset: recruited before April 2008
        ▼
┌─────────────────────────────────────────────────────────────────┐
│ Study subset: 1166 participants recruited from April 2008 to      │
│ April 2009 for measurements of serum and RBC folate               │
│ concentrations simultaneously                                     │
└─────────────────────────────────────────────────────────────────┘
        │
        │  277 participants excluded: missing serum or RBC folate measurement
        ▼
┌─────────────────────────────────────────────────────────────────┐
│ 889 participants with available data on both serum and RBC        │
│ folate measurement                                                │
└─────────────────────────────────────────────────────────────────┘
        │
        │  20 participants excluded: 7 missed vitamin supplement dosage and13
        │  missed MTHFR 677C→T genotype
        ▼
┌─────────────────────────────────────────────┐
│ 869 participants for final analysis          │
└─────────────────────────────────────────────┘
```

Fig. 1 Flowchart of participants, illustrating the procedure of study group identification (OaK, Ottawa and Kingston; GA, gestational age; RBC, red blood cells; *MTHFR*, methylenetetrahyrofolate reductase gene)

Table 1 Demographic characteristics of the study participants by BMI category‡: pregnant women (*n* 869) recruited between 12 and 20 weeks of gestation, Ottawa and Kingston (OaK) Birth Cohort study, April 2008–April 2009

	Overall		BMI (kg/m²)						P§
			< 25·0		25·0–29·9		≥ 30·0		
BMI (kg/m²)	869	100·0	446	51·3	226	26·0	197	22·7	
Age (years)									
≤29	420	48·3	223	50·0	107	47·3	90	45·7	0·181
30–34	293	33·7	145	32·5	70	31·0	78	39·6	
≥35	156	18·0	78	17·5	49	21·7	29	14·7	
GA at recruitment (week)									
12	584	67·2	308	69·1	158	69·9	118	59·9	0·048
13–15	239	27·5	113	25·3	55	24·3	71	36·0	
16–20	46	5·3	25	5·6	13	5·8	8	4·1	
Maternal education									
High school and below	170	19·6	91	20·4	34	15·0	45	22·8	0·010
College/university not completed	77	8·8	31	6·9	19	8·4	27	13·7	
College/university completed	622	71·6	324	72·7	173	76·6	125	63·5	
Race									
Caucasian	796	91·6	400	89·7	211	93·4	185	93·9	0·111
Other races	73	8·4	46	10·3	15	6·6	12	6·1	
Household annual income ($CAN)									
Declined	46	5·3	31	6·9	6	2·6	9	4·6	0·005
≤49 999	244	28·1	128	28·7	54	23·9	62	31·5	
50 000–79 999	274	31·5	123	27·6	77	34·1	74	37·5	
≥80 000	305	35·1	164	36·8	89	39·4	52	26·4	
Smoking									
No	734	84·5	367	82·3	200	88·5	167	84·8	0·110
Yes	135	15·5	79	17·7	26	11·5	30	15·2	
Folic acid supplement‖									
Non-users	35	4·0	10	2·2	9	4·0	16	8·1	0·009
≤1 mg/d	710	81·7	377	84·6	183	81·0	150	76·1	
>1 mg/d	124	14·3	59	13·2	34	15·0	31	15·8	
MTHFR 677C→T genotype									
CC	371	42·7	181	40·6	90	39·8	100	50·8	0·036
CT	394	45·3	212	47·5	112	49·6	70	35·5	
TT	104	12·0	53	11·9	24	10·6	27	13·7	

GA, gestational age; *MTHFR*, methylenetetrahyrofolate reductase gene.
‡Data are presented as *n* and %. BMI = [weight (kg)]/[height (m)]². BMI was measured in pregnancy (between 12 and 20 weeks of gestation); this may lead to misclassification of BMI category.
§*P* value of χ² test.
‖Folic acid from supplements, not including folic acid from fortified foods or naturally occurring food folate.

Supplementary analysis using self-reported pre-gravid weight yielded similar results (data not shown).

Discussion

Our study confirmed a differential association of BMI with serum and RBC folate concentrations in pregnant women, such that BMI and serum folate concentration had an inverse relationship, and BMI and RBC folate concentration had a positive relationship. The differential associations were not significant in women with *CT* or *TT* genotype, although the direction of these associations was consistent in women with *MTHFR CC* genotype. Our data indicate that the differential association between serum and RBC folate and BMI observed in non-pregnant obese women likely persists into pregnancy. Serum folate was also found to be inversely associated with BMI at mid and late pregnancy in Korean women, although no RBC folate was measured simultaneously in that study[14]. In both Canadian and American populations, non-pregnant obese

women, including those of childbearing age, have been shown to have lower plasma folate and higher RBC folate in comparison to normal-weight women[2,5,7]. These associations remained even after adjustment for dietary and supplemental folate intake, an important consideration given the observed pattern of lower folate intake among obese women[2,5].

The associations were significant only for women with *CC* genotype and not significant for those with *TT* owing to limited sample size in the *TT* group, as well as increased measurement error attributed to the use of a competitive protein-binding assay to measure RBC folate, which is an important limitation of the present study. We observed a *CC* > *CT* > *TT* pattern in serum folate concentration, but a reversed *TT* > *CC* and *TT* > *CT* pattern for RBC folate concentration. In a meta-analysis, Tsang *et al.* reported a *CC* > *CT* > *TT* pattern in serum and RBC folate concentrations measured by microbiological assay, which was reversed for RBC folate concentrations measured by protein-binding assay[15]. The differences between the assays are due to differences in affinity of the assays for

Table 2 Geometric mean concentrations of serum and RBC folate by characteristics of participants: pregnant women (*n* 869) recruited between 12 and 20 weeks of gestation, Ottawa and Kingston (OaK) Birth Cohort study, April 2008–April 2009

	Serum folate (nmol/l)				RBC folate (nmol/l)			
	Unadjusted geometric mean	95 % CI	Adjusted geometric mean‡	95 % CI	Unadjusted geometric mean	95 % CI	Adjusted geometric mean‡	95 % CI
Overall	45·7	44·1, 47·4	–	–	1549	1521, 1577	–	–
BMI (kg/m²)								
<25·0	47·5	45·2, 50·0	47·4	45·2, 49·6	1500	1460, 1541	1503	1467, 1540
25·0–29·9	45·4	42·6, 48·3	45·0	42·2, 48·0	1549	1494, 1606	1532	1481, 1585
≥30·0	40·3	37·5, 43·3	41·0*	38·3, 44·0	1591	1527, 1657	1604*	1546, 1663
MTHFR 677C→T genotype								
CC	46·4	44·0, 49·0	46·6	44·3, 49·0	1552	1504, 1602	1553	1513, 1594
CT	44·8	42·6, 47·0	44·3	42·2, 46·5	1471	1432, 1512	1473*	1436, 1510
TT	43·0	39·2, 47·3	44·1	40·1, 48·5	1712*	1629, 1799	1704*,†	1622, 1790
Folic acid supplement								
Non-users	24·1	21·8, 26·6	25·8	21·9, 30·5	1275	1155, 1409	1306	1197, 1426
≤1 mg/d	44·2*	42·9, 45·7	44·1*	42·6, 45·8	1512*	1485, 1540	1514*	1517, 1613
>1 mg/d	61·4*,†	53·7, 70·3	61·0*,†	55·9, 66·5	1743*,†	1666, 1824	1722*,†	1645, 1803
Age (years)								
≤29	42·3	40·2, 44·4	43·4	41·3, 45·6	1430	1392, 1469	1470	1433, 1509
30–34	47·5*	44·8, 50·3	46·5	43·9, 49·3	1603*	1560, 1647	1564*	1517, 1613
≥35	49·7*	45·8, 53·9	48·0	44·4, 51·9	1700*	1633, 1771	1650*	1583, 1720
GA at recruitment (week)								
12	45·9	44·1, 47·8	45·7	43·9, 47·5	1512	1478, 1546	1512	1481, 1544
13–15	44·9	41·8, 48·3	45·7	42·9, 48·6	1554	1498, 1611	1555	1504, 1607
16–20	39·2*	35·0, 43·8	38·0*	33·0, 43·8	1707*	1581, 1843	1693*	1571, 1825
Maternal education								
High school and below	40·2	36·9, 43·7	42·1	38·6, 45·9	1327	1269, 1388	1408	1345, 1473
College/university not completed	42·6	38·1, 47·7	43·2	38·6, 48·4	1502*	1403, 1607	1496	1410, 1589
College/university completed	47·1*	45·2, 49·0	46·4	44·5, 48·4	1599*	1563, 1635	1574*	1540, 1608
Race								
Caucasian	44·8	43·3, 46·5	44·9	43·4, 46·5	1531	1503, 1558	1532	1504, 1560
Other races	49·9	43·8, 56·9	49·0	43·6, 54·9	1559	1456, 1668	1545	1454, 1642
Household annual income ($CAN)								
≤49 999	40·7	37·9, 43·8	43·2	40·1, 46·6	1375	1320, 1432	1490	1432, 1550
50 000–79 999	46·4*	43·7, 49·2	46·2	43·5, 49·0	1576*	1527, 1626	1544	1496, 1593
≥80 000	47·6*	44·9, 50·5	45·5	42·9, 48·4	1615*	1571, 1659	1540	1492, 1590
Declined	48·9	41·9, 57·0	49·3	42·7, 56·9	1645*	1537, 1760	1656	1535, 1786
Smoking								
No	45·5	43·9, 47·1	45·1	43·5, 46·7	1569	1541, 1597	1553	1524, 1583
Yes	44·1	39·9, 48·7	46·2	42·3, 50·6	1352*	1280, 1427	1426*	1360, 1495

RBC, red blood cell; *MTHFR*, methylenetetrahyrofolate reductase gene; GA, gestational age.
*Significantly different from the first category of the listed variables (*P* < 0·05).
†Significantly different from the second category of the listed variables (*P* < 0·05).
‡Adjusted for all other variables listed.

various folate forms. Plasma folates are exclusively monoglutamates and the majority are in the form of 5-methyltetrahydrofolate, whereas the majority of RBC folates are variable-length, long-chain polyglutamate 5-methyltetrahydrofolate. RBC folates must be hydrolysed into the monoglutamate form. The differences in affinities among these folate forms, as well as inconsistent proportion of folate forms among those with *MTHFR* 677C→T homozygous genotype (*TT*), impair the interpretation of RBC folate concentration measured by protein-binding assay[16].

There are several other limitations to our study. First, the study design is cross-sectional, and serum and RBC folate were measured in early pregnancy; therefore, it is not possible to determine whether these associations hold through late pregnancy when haemodilution could have a significant effect. Second, the type and dose of supplements were self-reported and may be subject to measurement error due to inaccurate patient report and irregular pattern of use. We did not conduct dietary surveys on participants. Previous studies reported that obese women tended to under-report their food intake[17,18], and folate from naturally occurring food sources exhibits variable and incomplete bioavailability, which can be affected by physiological conditions, pharmaceuticals and genetic polymorphisms[19–21]. Third, BMI was measured in early pregnancy (95 % were measured before 15 weeks) and this may lead to misclassification of obesity, although the weight gain in early second trimester is generally small[22]. Finally, samples were measured in batches and the storage

Table 3 Association of BMI with serum and RBC folate, stratified by *MTHFR* 677C→T genotype and folic acid supplementation‡; pregnant women (*n* 869) recruited between 12 and 20 weeks of gestation, Ottawa and Kingston (OaK) Birth Cohort study, April 2008–April 2009

	Serum folate‡			RBC folate‡		
	β	95 % CI	*P*	β	95 % CI	*P*
All participants§	−0·32	−0·50, −0·16	<0·001	0·14	0·05, 0·22	0·001
MTHFR 677C→T genotype‖						
CC	−0·42	−0·66, −0·17	<0·001	0·19	0·06, 0·32	0·003
CT	−0·25	−0·49, −0·02	0·037	0·10	−0·02, 0·22	0·107
TT	−0·16	−0·64, 0·32	0·521	0·10	−0·16, 0·36	0·449
Folic acid supplementation‖						
Non-users	−0·62	−1·09, −0·14	0·011	0·44	−0·12, 1·00	0·124
≤1 mg/d	−0·32	−0·48, −0·16	<0·001	0·12	0·02, 0·22	0·015
>1 mg/d	−0·21	−0·78, 0·37	0·478	0·22	0·03, 0·41	0·021

RBC, red blood cell; *MTHFR*, methylenetetrahyrofolate reductase gene; β, the partial regression coefficient of log-transformed BMI.
‡BMI, serum folate and RBC folate concentrations were log-transformed.
§Regression coefficient of BMI in all participants, adjusted for *MTHFR* 677C→T genotype, folic acid supplementation, age, gestational age at recruitment, race, maternal education, household annual income and smoking.
‖Regression coefficient of BMI in subgroups stratified by *MTHFR* 677C→T genotype or folic acid supplementation, adjusted for age, gestational age at recruitment, race, maternal education, household annual income and smoking.

time varied, but was no longer than 1 month. Serum folate stored in a frost-free freezer at −20°C for even a short period may be relatively unstable and sensitive to minor temperature fluctuations[23]. This may contribute to the variation in folate concentrations.

There are several strengths of the present study. First, to our knowledge, it is the first study to examine the association of obesity with serum and RBC folate concentrations in pregnant women. Because of the difference in folate metabolism between the non-pregnant and pregnant populations, it is important to examine if the observed differential association between obesity and folate concentrations measured from serum *v.* RBC would persist in a pregnant population[1–5]. Second, our study was based on data collected in a prospective cohort, which mitigates recall bias. Third, serum folate and RBC folate concentrations in our study were derived from the same fasted participants, thereby mitigating bias/confounding when examining the association between serum and RBC folate with BMI.

It is not clearly understood why obesity was inversely associated with serum folate concentration but positively associated with RBC folate concentration. Obese women may demonstrate lower serum folate concentrations due to volumetric dilution of the blood. Alternatively, higher RBC folate may be due to the redistribution of folate from circulation into tissues. Cell culture and animal studies have shown that the expression of tissue folate receptors and transporters is responsive to folate concentrations: folate deficient conditions induce the expression of reduced folate carrier in rat intestine and cultured human Caco2 cells[24,25] whereas supplementation reduces its expression[26]. It could be hypothesized that the delay in absorption of folate observed in obese women may alter the expression of cellular folate transporters/receptors such that it promotes cellular uptake.

Folate status in women of childbearing age is important due to the role of folate in the prevention of NTD.

The placenta takes up folate from the maternal plasma by the reduced folate carrier, the proton-coupled folate transporter and the folate receptor α[27,28]. Placental uptake of circulating folate ensures an adequate folate supply to the developing fetus. Obese women are at increased risk for NTD-affected pregnancy[29–32]. A proportion of the NTD risk associated with maternal obesity may be due to lower circulating folate in obese women, which can be corrected to some degree with higher folate intake[29]. On the other hand, it must be noted that a proportion of the NTD risk associated with obesity appears to be independent of folate status and may be due to other metabolic disturbances[33]. Obese women of childbearing age may therefore require supplemental folic acid to increase or maintain a plasma folate status that is associated with maximal protection from NTD. However, folate status associated with NTD protection is currently expressed in terms of RBC folate[34]. Because of the observed differential association of RBC folate with BMI, the standard RBC folate cut-off approach for assessing NTD risk in obese women may be limited. The relationship between RBC folate and serum folate, and how they relate to NTD risk reduction, may need to be determined for women of all BMI categories to determine appropriate 'cut-offs' for NTD protection.

A second consideration is whether obese women respond to folic acid supplementation similar to normal-weight women. Folic acid supplement use did not necessarily mitigate the observed differential association of BMI with serum and RBC folate in these pregnant women. A pharmacokinetic study found that obese women demonstrated a lower peak serum folate concentration after a 400 μg bolus folic acid dose and a lower area under the curve in the absorptive phase (0–3 h post-bolus), but no difference in the overall area under the curve[7]. The data indicate that obesity may delay the absorption of folic acid but that overall absorption is not impaired. It should be noted that the women in our study had a folate status

associated with maximal protection from NTD risk ($\geq$906 nmol/l)[35]. In our study, the overall mean RBC folate was 1549 (sd 378) nmol/l and 1275 (95 % CI 1155, 1409) nmol/l in supplement non-users. This is comparable to findings from the Canadian Health Measures Survey (2007–2009) where mean RBC folate (also measured by immunoassay) was 1142 (95 % CI 1153, 1239) nmol/l in women of childbearing age who did not consume a folic acid supplement[36]. These findings are reflective of the mandatory folic acid fortification of white wheat flour in Canada. The relationship of obesity with folate status may be more of a concern in countries where fortification is not widespread and supplement use is lower.

The differential association of obesity with serum folate concentration *v.* RBC folate concentration observed in the present study deserves attention in research and perinatal health practice alike. The identification of subgroups of women who may be susceptible to low folate status or respond differently to folic acid supplementation will ensure that public health interventions can be tailored to meet the needs of the entire population. Obesity is a known risk factor for a number of adverse maternal and infant outcomes including pre-eclampsia[37,38], gestational diabetes[39,40] and NTD[41]. Folic acid deficiency is a known risk factor for NTD[42,43] and may be associated with other pregnancy complications such as pre-eclampsia[44,45] and other congenital anomalies[46,47]. As a result, in the study of the association between folate status and adverse maternal and infant outcomes, it is critical to use the appropriate biological specimens for the measurement of folate. Since BMI is positively associated with RBC folate but inversely associated with serum folate, the observed associations between folate status and pregnancy complications may be overestimated if serum folate is used while underestimated if RBC folate is used. Folate status varies substantially during pregnancy[8], which can further complicate the accurate assessment of folate status.

In summary, we confirmed the differential associations of folate with BMI in pregnant women, indicating that the RBC folate cut-off approach for assessing NTD risk may be limited. Because absorption and metabolism of folate are modified by adiposity, RBC folate may not reflect freely available folate at cellular level in pregnant women.

Acknowledgements

References

1. Kimmons JE, Blanck HM, Tohill BC *et al.* (2006) Associations between body mass index and the prevalence of low micronutrient levels among US adults. *Med Gen Med* **8**, 59.
2. Bird JK, Ronnenberg AG, Choi SW *et al.* (2015) Obesity is associated with increased red blood cell folate despite lower dietary intakes and serum concentrations. *J Nutr* **145**, 79–86.
3. Mojtabai R (2004) Body mass index and serum folate in childbearing age women. *Eur J Epidemiol* **19**, 1029–1036.
4. Mahabir S, Ettinger S, Johnson L *et al.* (2008) Measures of adiposity and body fat distribution in relation to serum folate levels in postmenopausal women in a feeding study. *Eur J Clin Nutr* **62**, 644–650.
5. Tinker SC, Hamner HC, Berry RJ *et al.* (2012) Does obesity modify the association of supplemental folic acid with folate status among nonpregnant women of childbearing age in the United States? *Birth Defects Res Part A Clin Mol Teratol* **94**, 749–755.
6. Stern SJ, Matok I, Kapur B *et al.* (2011) A comparison of folic acid pharmacokinetics in obese and nonobese women of childbearing age. *Ther Drug Monit* **33**, 336–340.
7. da Silva VR, Hausman DB, Kauwell GP *et al.* (2013) Obesity affects short-term folate pharmacokinetics in women of childbearing age. *Int J Obes (Lond)* **37**, 1608–1610.
8. Higgins JR, Quinlivan EP, McPartlin J *et al.* (2000) The relationship between increased folate catabolism and the increased requirement for folate in pregnancy. *BJOG* **107**, 1149–1154.
9. Walker MC, Smith GN, Perkins SL *et al.* (1999) Changes in homocysteine levels during normal pregnancy. *Am J Obstet Gynecol* **180**, 660–664.
10. Açkurt F, Wetherilt H, Löker M *et al.* (1995) Biochemical assessment of nutritional status in pre- and post-natal Turkish women and outcome of pregnancy. *Eur J Clin Nutr* **49**, 613–622.
11. Walker MC, Finkelstein SA, Rennicks White R *et al.* (2011) The Ottawa and Kingston (OaK) Birth Cohort: development and achievements. *J Obstet Gynaecol Can* **33**, 1124–1133.
12. Di Cristofaro J, Silvy M, Chiaroni J *et al.* (2010) Single PCR multiplex SNaPshot reaction for detection of eleven blood group nucleotide polymorphisms: optimization, validation, and one year of routine clinical use. *J Mol Diagn* **12**, 453–460.
13. Ferraro ZM, Barrowman N, Prud'homme D *et al.* (2012) Excessive gestational weight gain predicts large for gestational age neonates independent of maternal body mass index. *J Matern Fetal Neonatal Med* **25**, 538–542.
14. Kim H, Hwang JY, Kim KN *et al.* (2012) Relationship between body-mass index and serum folate concentrations in pregnant women. *Eur J Clin Nutr* **66**, 136–138.

15. Tsang BL, Devine OJ, Cordero AM et al. (2015) Assessing the association between the methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism and blood folate concentrations: a systematic review and meta-analysis of trials and observational studies. Am J Clin Nutr 101, 1286–1294.

16. Bagley PJ & Selhub J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci U S A 95, 13217–13220.

17. Scagliusi FB, Polacow VO, Artioli GG et al. (2003) Selective underreporting of energy intake in women: magnitude, determinants, and effect of training. J Am Diet Assoc 103, 1306–1313.

18. Scagliusi FB, Ferriolli E, Pfrimer K et al. (2009) Characteristics of women who frequently under report their energy intake: a doubly labelled water study. Eur J Clin Nutr 63, 1192–1199.

19. Prinz-Langenohl R, Brönstrup A, Thorand B et al. (1999) Availability of food folate in humans. J Nutr 129, 913–916.

20. Babu S & Srikantia SG (1976) Availability of folates from some foods. Am J Clin Nutr 29, 376–379.

21. Obeid R, Koletzko B & Pietrzik K (2014) Critical evaluation of lowering the recommended dietary intake of folate. Clin Nutr 33, 252–259.

22. Institute of Medicine (2009) Weight Gain During Pregnancy: Reexamining the Guidelines. Washington, DC: National Academies Press, National Academy of Sciences.

23. Bailey LB (2009) Folate in Health and Disease, 2nd ed. Boca Raton, FL: CRC Press.

24. Said HM, Chatterjee N, Haq RU et al. (2000) Adaptive regulation of intestinal folate uptake: effect of dietary folate deficiency. Am J Physiol Cell Physiol 279, C1889–C1895.

25. Subramanian VS, Chatterjee N & Said HM (2003) Folate uptake in the human intestine: promoter activity and effect of folate deficiency. J Cell Physiol 196, 403–408.

26. Ashokkumar B, Mohammed ZM, Vaziri ND et al. (2007) Effect of folate oversupplementation on folate uptake by human intestinal and renal epithelial cells. Am J Clin Nutr 86, 159–166.

27. Yasuda S, Hasui S, Yamamoto C et al. (2008) Placental folate transport during pregnancy. Biosci Biotechnol Biochem 72, 2277–2284.

28. Solanky N, Requena Jimenez A, D'Souza SW et al. (2010) Expression of folate transporters in human placenta and implications for homocysteine metabolism. Placenta 31, 134–143.

29. McMahon DM, Liu J, Zhang H et al. (2013) Maternal obesity, folate intake, and neural tube defects in offspring. Birth Defects Res Part A Clin Mol Teratol 97, 115–122.

30. Gao LJ, Wang ZP, Lu QB et al. (2013) Maternal overweight and obesity and the risk of neural tube defects: a case–control study in China. Birth Defects Res Part A Clin Mol Teratol 97, 161–165.

31. Shaw GM, Velie EM & Schaffer D (1996) Risk of neural tube defect-affected pregnancies among obese women. JAMA 275, 1093–1096.

32. Waller DK, Shaw GM, Rasmussen SA et al. (2007) Prepregnancy obesity as a risk factor for structural birth defects. Arch Pediatr Adolesc Med 161, 745–750.

33. Parker SE, Yazdy MM, Tinker SC et al. (2013) The impact of folic acid intake on the association among diabetes mellitus, obesity, and spina bifida. Am J Obstet Gynecol 209, 239.e1–e8.

34. World Health Organization (2015) Optimal Serum and Red Blood Cell Folate Concentrations in Women of Reproductive Age for Prevention of Neural Tube Defects. Geneva: WHO.

35. Daly LE, Kirke PN, Molloy A et al. (1995) Folate levels and neural tube defects. Implications for prevention. JAMA 274, 1698–1702.

36. Shi Y, De Groh M & MacFarlane AJ (2014) Socio-demographic and lifestyle factors associated with folate status among non-supplement-consuming Canadian women of childbearing age. Can J Public Health 105, e166–e171.

37. Lisonkova S & Joseph KS (2013) Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol 209, 544.e1–544.e12.

38. Roberts JM, Bodnar LM, Patrick TE et al. (2011) The role of obesity in preeclampsia. Pregnancy Hypertens 1, 6–16.

39. Baci Y, Üstüner I, Keskin HL et al. (2013) Effect of maternal obesity and weight gain on gestational diabetes mellitus. Gynecol Endocrinol 29, 133–136.

40. Chu SY, Callaghan WM, Kim SY et al. (2007) Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care 30, 2070–2076.

41. Rasmussen SA, Chu SY, Kim SY et al. (2008) Maternal obesity and risk of neural tube defects: a metaanalysis. Am J Obstet Gynecol 198, 611–619.

42. Czeizel A & Dudas I (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 327, 1832–1835.

43. MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 338, 132–137.

44. Wen SW, Champagne J, Rennicks White R et al. (2013) Effect of folic acid supplementation in pregnancy on preeclampsia: the folic acid clinical trial study. J Pregnancy 2013, 294312.

45. Wen SW, Chen XK, Rodger M et al. (2008) Folic acid supplementation in early second trimester and the risk of preeclampsia. Am J Obstet Gynecol 198, 45.e1–e7.

46. Pei L, Zhu H, Zhu J et al. (2006) Genetic variation of infant reduced folate carrier (A80G) and risk of orofacial defects and congenital heart defects in China. Ann Epidemiol 16, 352–356.

47. Feng Y, Wang S, Chen R et al. (2015) Maternal folic acid supplementation and the risk of congenital heart defects in offspring: a meta-analysis of epidemiological observational studies. Sci Rep 5, 8506.