Measures of adiposity and body fat distribution in relation to serum folate levels in postmenopausal women in a feeding study

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Abstract

Objective—To assess the associations between serum folate concentration and measures of adiposity in postmenopausal women.

Design—This study was conducted as a cross-sectional analysis within the control segment of a randomized, crossover trial in which postmenopausal women (n=51) consumed 0g (control), 15g (one drink), and 30g (two drinks) alcohol (ethanol)/d for 8 weeks as part of a controlled diet. Subjects in one treatment arm were crossed-over to another arm after a two to five week washout period. BMI was measured, and dual energy x-ray absorptiometry (DEXA) scan administered to the women during the control (0 g alcohol) treatment, and a blood sample from this group was collected at baseline and week 8 of each diet period and analyzed for folate, B12, homocysteine, and methylmalonic acid.

Setting—This study was conducted at the Beltsville Human Nutrition Research Center, Maryland, USA.

Results—In multivariate analysis, women who were overweight had a 12% lower, and obese women had a 22% lower serum folate concentrations compared to normal weight women (P-trend=0.02). Vitamin B12 also decreased with increasing BMI (*P*-trend=0.08). Increased BMI, percent body fat, and absolute amounts of central and peripheral fat were all significantly associated with decreased serum folate, but were unrelated to serum B12, homocysteine, or methylmalonic acid.

Conclusions—Our data show that adiposity is associated with lower serum folate levels in postmenopausal women. With obesity at epidemic proportions, these data, if confirmed by prospective or randomized controlled studies, have *important* public health implications.

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Descriptors—Adiposity and folate

Keywords

INTRODUCTION

Natural menopause, a normal aspect of the aging process, influences the risk of breast cancer (Edwards *et al.* 2002), the most common new cancer in women in the United States (US). At the onset of menopause a woman's body weight reaches its natural maximum and any subsequent increase in relative body weight is in the form of adipose tissue, particularly abdominal or central fatness that occurs with advancing age (Astrup 1999). There is considerable evidence that the risk of postmenopausal breast cancer is increased by obesity (Calle *et al.* 2003; Lahmann *et al.* 2004), and influenced by body fat distribution (Folsom *et al.* 1990). In prospective studies, elevated central adiposity assessed by waist-hip ratio was associated with increased risk of postmenopausal breast cancer (Ballard-Barbash *et al.* 1990; Folsom *et al.* 1990) as well as postmenopausal breast cancer mortality (Borugian *et al.* 2003).

Epidemiologic studies of folate and vitamin B12 and breast cancer indicate a negative association between folate intake and risk of breast cancer (Graham *et al.* 1991; Freudenheim *et al.* 1996; Ronco *et al.* 1999; Zhang *et al.* 1999; Rohan *et al.* 2000), as well as a negative association between *vitamin B12 levels* and risk of breast cancer (Wu *et al.* 1999) and DNA damage (Fenech *et al.* 1998; Fenech 2001). Since obesity (Calle *et al.* 2003) and folate (Zhang *et al.* 1999; Rohan *et al.* 2000) are considered risk factors for postmenopausal breast cancer, it is possible that obesity may modify physiologic folate levels. However, research on the relationship between obesity and folate levels is only now evolving and includes only a handful of studies to date. Mojtabi found that increased BMI in US women of childbearing age was associated with lower serum folate levels both before and after folic acid fortification (Mojtabai 2004). Tungtrongchitr et al. reported that obese Thai subjects have lower serum folate than normal weight subjects (Tungtrongchitr *et al.* 2003). A study of obese adolescents in Austria (Gallistl *et al.* 2000) reported that serum folate correlated inversely with BMI. However, no significant differences in serum folate in overweight and normal weight Brazilian adolescents (Hirsch *et al.* 2005), or obese and normal weight adults in Israel (Reitman *et al.* 2002) were seen. None of these studies had strict control for the influence of diet. We hypothesized that increased adiposity measured by BMI and DEXA would result in decreased serum folate concentrations.

In the present clinical nutrition feeding study, we investigated the relationship of serum folate, vitamin B12, homocysteine, and methylmalonic acid to obesity as assessed by BMI and other measures of adiposity among healthy postmenopausal women who did not smoke or take hormone replacement therapy (HRT).

SUBJECTS AND METHODS

Study design

This study was conducted as a cross-sectional analysis within the control segment of a randomized, crossover trial in which postmenopausal women (n=51) consumed 0 (control), 15 (one drink), and 30 (two drinks) g of alcohol (ethanol)/d for 8 weeks while consuming a controlled diet. Details of the study design and procedures have been published previously (Dorgan *et al.* 2001; Baer *et al.* 2002). Briefly, subjects were assigned to three separate 8-week diet periods during which they consumed a controlled diet and were provided a beverage (orange juice) each day that contained 0, 15, or 30 g alcohol (95% ethanol) in random order. Each subject completed the three diet periods; each diet period was separated by two to five week washout periods. DEXA (Lunar Corp., Model DPX-L, Madison, WI) whole body scans for adiposity assessments were administered to the women during the fourth week of their 0 g alcohol (control) treatment, and a fasting blood sample was collected at baseline and at week eight of each diet period for folate, vitamin B12, homocysteine, and methylmalonic acid analyses. BMI was measured on the same day.

Subjects

Postmenopausal women were recruited by advertisement from the communities surrounding the Beltsville Human Nutrition Research Center, Beltsville, MD. The eligibility criteria were: *(1)* women ≥ 49 y of age, *(2)* postmenopausal (last menses > 12 mo before the study started or follicle stimulating hormone > 40,000 IU/L, natural menopause or hysterectomy with at least one ovary intact), *(3)* not receiving hormone replacement therapy (HRT), *(4)* not taking prescription medications that might interfere with the study, *(5)* willing and able to consume the diet prepared or approved by the Center and no other foods or beverages, *(6)* with no personal or parental history of alcohol abuse, and *(7)* non-smoker (i.e., have not used tobacco products during the past 12 months). The subjects were evaluated by a physician and determined to be in good health with no signs or symptoms of any disease.

This study was approved by the National Cancer Institute's Institutional Review Board and the Committee on Human Research of the Johns Hopkins University Bloomberg School of Hygiene and Public Health. All subjects were fully informed of the study requirements and were required to read and sign a consent form detailing the objectives, risks, and benefits of the study. The subjects were compensated for their participation.

Diets and feeding

All meals were prepared at the Beltsville Human Nutrition Research Center from typical US foods and served in a seven-day menu cycle. Each day's diet provided 15% energy as protein, 50% energy as carbohydrate, and 35% energy as fat, with a polyunsaturated/monounsaturated/saturated fat ratio of 0.6:1:1. Daily fiber intake was 10 g/1,000 kcal, and daily cholesterol intake was 150 mg/1,000 kcal. Diets provided 100% of the US recommended dietary allowances for vitamins and minerals (NRC 1989). The study participants were weighed each week-day by study investigators at the Beltsville facility, and energy intake was adjusted to maintain constant body weight.

DEXA measurements

Body composition was determined by pencil beam dual energy x-ray absorptiometry (Lunar Corp., Model DPX-L, Madison, WI). Subjects were placed in a supine position with arms and legs close to their body for a whole body scan following the manufacturer's recommended protocol. Whole body and regional lean mass (mass of bone and nonfat soft tissue) and fat mass were determined using the manufacturer's algorithm (software version 1.33).

Biological sample collection and analysis

During the last week of the control treatment, blood samples for folate, vitamin B12, homocysteine, and methylmalonic acid analyses were collected from fasting (>12 hours) subjects before breakfast (6:30 AM to 9:00 AM) on each of three non-consecutive days in each dietary period. An equal volume of serum from each day's blood draw was pooled for analysis. Serum was separated and aliquots were frozen at −70°C. For quality control, 11 aliquots from a pooled serum sample were randomly inserted among study samples, but not within any individual subject's set of three samples. Coefficients of variation on masked quality control samples were as follows: folate = 2.9%, vitamin B12 = 3.7%, homocysteine = 2.9%, and methylmalonic acid = 11.0%.

The assays for folate, B12 and methylmalonic acid have previously been described (Laufer *et al.* 2004). Serum folate and serum B12 assays were done by radioimmunoassay using the method of Gunter et al. (Gunter *et al.* 1996). The assay measured total serum folate concentrations. Serum homocysteine was measured by HPLC with pre-column derivatization and fluorescence detection according to Pfeiffer et al. (Pfeiffer *et al.* 1999b). Serum methylmalonic acid was measured by the gas chromatography-mass spectrometry method validated by Pfeiffer and Gunter (Pfeiffer *et al.* 1999a). All of these assays were performed by the NHANES Laboratory at the Centers for Disease Control and Prevention (Atlanta, GA, USA).

Statistical analysis

Serum folate, B12, homocysteine, and methylmalonic acid concentrations were log transformed using the natural log. Pearson and Spearman correlations between the different DEXA measurements and BMI (kg/m^2 calculated from measured weight and height) were determined. Mean serum folate, B12, homocysteine, and methylmalonic acid concentrations for BMI categories were estimated using linear regression models that included a series of indicator variables for BMI categories (normal weight, ≤25 kg/m^2; overweight, >25 to ≤30 kg/m^2; or obese, >30 kg/m^2). BMI categories were also modeled as ordinal variables with values 0, 1, and 2. Additional models estimated percent changes in serum folate, B12, homocysteine, and methylmalonic acid concentrations per 1000 g of central or peripheral fat, percent total body fat, or single unit change in BMI (all modeled as continuous variables). All models were adjusted for age (continuous), race (indicator for African American), family history of breast cancer (indicator for yes if positive history in mother or full sister), parity (continuous), and menarche (indicator for <12 years). In a second series of models (Model 2), BMI (continuous) was also added to Model 1. The addition of alcohol group assignment order, dietary period, hysterectomy, duration of menses, years since last menses, nulliparity, and age at first birth (for those with children) did not improve the precision of our estimates and were not included in the final models. There was no evidence of effect modification as assessed by likelihood ratio tests of models fit after the addition of cross-product terms to models that included only main effects. *P*-values for BMI and DEXA measurements were determined using likelihood ratio tests that compared models with the BMI or DEXA term of interest to models without that term. Multiple R^2 and F-tests were calculated from the linear regression models. All tests of statistical significance were two-sided. Statistical analyses were performed using S-PLUS (S-PLUS version 6.2 for Windows, Seattle, WA, Insightful Corporation; 2002).

Results

Fifty-one women successfully completed the entire study and are included in this analysis. Characteristics of the subjects at baseline are provided in Table 1. All participants were postmenopausal and ranged in age from 49.2 years to 78.8 years (median = 58.2). Most

(74%) women were white, 22% were black, and 4% were Asian. The median body weight of participants was 73.2 kg (range, 42.1 to 117.4); BMI ranged from 17.7 kg/m^2 to 42.5 kg/m^2 (median, 26.9), and total body fat ranged from 7,942 g to 55,756 g (median, 26,808), while trunk, leg, and arm fat were of progressively lesser magnitude. Descriptive statistics for serum folate, vitamin B12, homocysteine, and methylmalonic acid concentrations are also presented in Table 1.

Table 2 shows the geometric means for serum folate, vitamin B12, homocysteine, and methylmalonic acid concentrations by categories of BMI (ie, normal weight, overweight, and obese). Serum folate concentrations decreased with increasing levels of overweight and obesity. When normal weight was used as the comparison group, serum folate concentrations among the overweight women were 11.2% lower, and obese women had 22.0% lower serum folate (*P*-trend=0.016). There were non-significant decreases in serum concentrations of vitamin B12 among overweight and obese women compared to normal weight women (*P*-trend=0.079). Homocysteine and methylmalonic acid concentrations among overweight and obese women compared to normal weight women were similar.

Table 3 shows how much serum concentrations of folate, vitamin B12, and homocysteine changed for a one-unit increase of BMI, one-percent increase in percent body fat, and a one-kg increase in central or peripheral fat or lean body mass. For model 1, we found statistically significant decreases in the concentrations of serum folate for increasing levels of BMI, percent body fat, central fat, and peripheral fat. For example, after adjusting for age, parity, race, family history of breast cancer, and age of menarche, serum folate decreased 1.7% (*P*<0.05) for each one-unit increase in BMI. The association held true for overall body fat (measured as percent body fat by DEXA), central fat, and peripheral fat. Serum folate decreased 1.4% (*P*<0.05) per one-percent increase in overall body fat; decreased 1.9% (*P*<0.005) per one-kilogram increase in central fat; and decreased 1.8% (*P*<0.05) per one-kilogram increase in peripheral fat. There were no significant changes in serum concentrations of vitamin B12, homocysteine, or methylmalonic acid (data not shown) with increasing levels of adiposity. In model 2, BMI was included as a covariate in the models; there was no evidence that DEXA adiposity measures provided additional predictive information about serum folate concentrations beyond that of BMI and the other (non-obesity related) covariates.

When multiple R^2 was used to assess the strength of the linear associations of the overall models to serum folate (Table 3), the percent fat models explained a slightly higher proportion (up to 29%) of the variance related to serum folate concentrations than BMI (24%). Overall, the multiple R^{2}s shown in Table 3 for all the Model 2s demonstrate that BMI, percent body fat, central fat and peripheral fat models are all associated with serum folate concentrations.

Discussion

The objective of this controlled feeding study was to investigate the relationship between well-defined measures of adiposity and serum folate, vitamin B12, homocyteine, and methylmalomic acid in postmenopausal women. Additional precision was gained by adjusting for breast cancer risk factors (family history) and estrogen exposure (parity and age of menarche) in the models. Serum folate concentrations were inversely related to adiposity as measured by BMI, percent total fat, and both central and peripheral fat mass. Adiposity was not related to serum vitamin B12 levels, nor to indicators of folate/B12 metabolism, methylmalonic acid, or homocysteine.

This type of study is important because it offers potential mechanistic insight into the obesity-cancer association. Folate and vitamin B12 are critical for the maintenance of DNA stability, which is, in turn, required for normal cell function. Depletion of folate and/or vitamin B12 result in alterations of one-carbon metabolism, which may lead to increased cancer susceptibility by mechanisms such as uracil misincorporation, DNA strand breaks, DNA repair abnormalities, and aberrations in DNA methylation (Choi *et al.* 2000; Bailey *et al.* 2001).

It is possible that serum folate concentration is not the appropriate index of folate status. Herbert (Herbert 1962) demonstrated that serum folate levels fell into the deficient range (<3 ng/ml) within 22 days of ingesting a folate deficient diet, while red cell folate levels fell only at 3–4 months, as folate replete red cells senesced. From these data, he concluded that serum folate levels reflected recent intake, while red cell folate concentration was a measure of folate storage. However, serum and red cell folate concentrations are closely correlated (r = 0.5, P<0.0001) (Ettinger 1987). Thus, serum folate concentrations have been taken as an index that reflects steady-state folate equilibration and is routinely measured in clinical laboratories (Galloway et al. 2003).

Because all the women in our study consumed the same diet, the lower serum folate concentrations found in women with higher BMI and fat mass may reflect a perturbation of whole body steady state folate concentrations with increased fat mass. Although our study was conducted within the control (0 g) segment of the alcohol trial, like all crossover studies, there may be residual treatment effects and for these reasons the design included a two to five week washout period. Because of these washout periods between each treatment group, and because biologic samples were collected and tested in the final (eight week) of each dietary period, carryover effects, if any, should have been minimized. There was also a similar washout period prior to enrolling in the study. Further, as described in the statistical analysis section, we specifically tested for carryover effects. The addition of alcohol group assignment order did not affect the precision of our estimates. We have also previously reported that the moderate doses of alcohol in this study did not significantly affect serum folate concentrations (Laufer et al. 2004).

Three possibilities could explain lower steady state serum folate concentrations with increased fat mass: increased cellular uptake, increased intracellular retention, and/or increased renal excretion (Villanueva et al. 1998). Folate enters the cell by at least two independent transport systems. The first system is folate receptor (FR) mediated (Rothberg et al. 1990). FRs have limited distribution in normal tissues, but are highly expressed in some tumors. The second system, used by most normal tissues, is the reduced folate carrier (RFC), a 12-pass transmembrane protein (Ferguson et al. 1999) with high affinity for reduced folates (eg, 5-MeTHF). Cell-specific folate homeostasis mediated by the RFC is regulated by multiple promoters and non-coding exons (Whetstine et al. 2002) as well as dietary folate (Liu et al. 2005), such that each cell type has the flexibility to regulate its intracellular folate concentration according to immediate need.

Serum folate taken up by the cell is demethylated by vitamin B12 dependent methionine synthase and conjugated with variable numbers of glutamate residues by folylpoly-γ-glutamate synthase (conjugase) (Lowe et al. 1993). The methyl group is transferred to homocysteine with the formation of methionine, an essential amino acid. Methionine is adenosylated to S-adenosyl methionine (SAM) and donates its methyl group to a wide range of macromolecules including DNA (Townsend et al. 2004) and estrogens (Goodman et al. 2001). Demethylated tetrahydrofolate (THF) accepts and transfers activated one-carbon units to multiple macromolecules, including uracil in generation of thymine, required for DNA synthesis and repair (Prasannan et al. 2003). Substituted THF is reduced to 5-MeTHF

by the enzyme, methyltetrahydrofolate reductase (MTHFR) and re-enters the serum pool. Polymorphisms of MTHFR and other genes involved in folate metabolism can perturb intracellular and serum folate. We are in the process of genotyping single nucleotide polymorphisms (SNPs) for genes in the folate metabolic pathway for all subjects in the current study.

We have recently reported significant elevation of circulating estrogen metabolites with increased fat mass in overweight and obese women in this study population (Mahabir et al. 2006), and this suggests a third system for the observed folate reductions. Although the women did not take HRT, it is established that postmenopausal women aromatize adrenal androsteinedione to estrogen metabolites in adipose tissue (Nevton et al. 1986; Szymczak et al. 1998). Thus, in our overweight and obese subjects, peripheral conversion in the more abundant adipose tissue is a likely source for the elevated estrogen metabolites. For many years, it has been known that estrogen supplements adversely affect folate status (Krumdieck et al. 1975; Shojania et al. 1975). Oral contraceptive intake reduced serum folate availability sufficiently to produce cervical megaloblastosis (Butterworth et al. 1992) and to potentiate platelet hyperactivity (Durand et al. 1997). Estrogen inhibits glutamate conjugation activity thereby increasing the "free" folate pool that can leave the cell and is readily filtered by the kidney (Krumdieck et al. 1975). Estrogen also increases folate utilization by its requirement for methylation via catechol-O-methyltransferase (COMT) prior to urinary excretion (Goodman et al. 2001). Thus, lower serum folate concentrations observed in obese subjects cold be explained by increased utilization and urinary excretion secondary to increased adipose tissue-derived estrogen concentrations.

It must be noted that serum homocysteine concentration was not associated with obesity in this well nourished study population. Since homocysteine methylation to methionine requires adequate 5-methylTHF, a reduction in serum folate concentration could be expected to compromise this pathway. However, serum folate concentrations, even in the obese subjects were within the normal range, and homocysteine can be remethylated through the independent betaine homocysteine methyltransferase that derives its methyl group from choline (Finkelstein et al. 1988).

Although we used a cross-sectional design with only modest numbers of subjects, the strengths of this study include a homogenous study population (e.g., women who were smoking or taking HRT were excluded) and measurement stability, which resulted from use of a carefully controlled diet and use of fasting blood samples for analysis. The small sample size of our study may have limited our power to detect weaker associations. The few studies to date (described in the introduction section) of adiposity and serum folate levels used only BMI, and made adjustments for dietary folate intake assessed by questionnaires, which have known limitations. The DEXA scans employed in our study are considered a reference method for body composition analysis (Panotopoulos et al. 2001).

In summary, while our data suggest that increased fat mass is associated with reduction in serum folate concentrations, an accepted measure of folate availability, because of the cross sectional design of our study, causality cannot be inferred from our findings. It is not possible to determine from these data whether these lower serum folate levels are physiologically significant, as the mean of the obese group (>14 ng/ml) was still well within the normal range (5–16 ng/ml). With obesity at epidemic proportions and increasing with each new survey, further investigations into the interactions between folate metabolism and obesity appear warranted.

Acknowledgments

References

Astrup A. Physical activity and weight gain and fat distribution chnages with menopause: current evidence and research issues. Med Sci Sports Exerc. 1999; 31:S564–S567. [PubMed: 10593529]

Baer D, Judd J, Clevidence B, Muesing R, Cambell W, Brown E, Taylor P. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr. 2002; 75:593–599. [PubMed: 11864868]

Bailey, L.; Moyers, S.; Gregory, J, III. Folate. In: Bowman, BA.; Russell, RM., editors. Present Knowledge in Nutrition. Eight Edition. Washington, DC: ILSI Press; 2001.

Ballard-Barbash R, Schatzkin A, Carter C, Kannel W, Kreger B, D'Agostino R, Splansky G, Anderson K, Helsel W. Body fat distribution and breast cancer in the Framingham study. J Natl Cancer Inst. 1990; 82:286–290. [PubMed: 2299677]

Borugian M, Sheps S, Kim-Sing C, Olivotto I, Patten C, Dunn B, Coldman A, Potter J, Gallagher R, Hislop T. Waist-to-hip ratio and breast cancer mortality. Am J Epidemiol. 2003; 158:963–968. [PubMed: 14607804]

Butterworth C, Hatch K, Macaluso M, Cole P, Sauberlich H, Soong S, Borst M, Baker V. Folate deficiency and cervical dysplasia. JAMA. 1992; 267:528–533. [PubMed: 1729576]

Calle E, Rodriguez C, Walker-Thurmond K, Thun M. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl Med. 2003; 348:1625–1638.

Choi S, Mason J. Folate and carcinogenesis: an integrated scheme. J Nutr. 2000; 130:129–132. [PubMed: 10720158]

Dorgan J, Baer D, Albert P, Judd J, Brown E, Corle D, Cambell W, Hartman T, Tejpar A, Clevidence B, Giffen C, Chandler D, Stanczyk F, Taylor P. Serum hormones and the alcohol-breast cancer association in postmenopausal women. J Natl Cancer Inst. 2001; 93:710–715. [PubMed: 11333294]

Durand P, Prost M, Blache D. Folic acid deficiency enhances oral contraceptive-induced platelet hyperactivity. Arterioscler Thromb Vasc Biol. 1997; 17:1939–1946. [PubMed: 9351357]

Edwards B, Howe H, Ries L, Thun M, Rosenberg H, Yancik R, Wingo P, Jemal A, Feigal E. Annual report to the nation on the status of cancer, 1973–1999, featuring implications of aging on US cancer burden. Cancer. 2002; 94:2766–2792. [PubMed: 12173348]

Ettinger, S. Doctoral Dissertation. New York: Columbia University; 1987. Alteration of Folate and Zinc Status with Age.

Fenech M. The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutat Res. 2001; 475:57–67. [PubMed: 11295154]

Fenech M, Aitken C, Rinaldi J. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. Carcinogenesis. 1998; 19:1163–1171. [PubMed: 9683174]

Ferguson P, Flintoff W. Topological and Functional Analysis of the Human Reduced Folate Carrier by Hemagglutinin Epitope Insertion. J Biol Chem. 1999; 274:16269–16278. [PubMed: 10347183]

Finkelstein J, Martin J, Harris B. Methionine metabolism in mammals. The methionine-sparing effect of cystine. J Biol Chem. 1988; 263:11750–11754. [PubMed: 3403552]

Folsom A, Kaye S, Prineas R, Potter J, Gapstur S, Wallace R. Increased incidence of carcinoma of the breast associated with abdominal adiposity in postmenopausal women. Am J Epidemiol. 1990; 131:794–803. [PubMed: 2138863]

Freudenheim J, Marshall J, Vena J, Laughlin R, Brasure J, Swanson M, Nemoto T, Graham S. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. J Natl Cancer Inst. 1996; 88:340–348. [PubMed: 8609642]

Gallistl S, Sudi K, Mangge H, Erwa W, Borkenstein M. Insulin and independent correlate of plasma homocysteine levels in obese children and adolescents. Diabetes Care. 2000; 23:1348–1352. [PubMed: 10977031]

Galloway M, Rushworth L. Red cell or serum folate? Results from the National Pathology Alliance benchmarking review. J Clin Pathol. 2003; 56:924–926. [PubMed: 14645351]

Goodman J, Lavigne J, Wu K, Helzlsouer K, Strickland P, Selhub J, Yager J. COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. Carcinogenesis. 2001; 22:1661–1665. [PubMed: 11577006]

Graham S, Hellmann R, Marshall J, Freudenheim J, Vena J, Swanson M, Zielenzy M, Nemoto T, Stubbe N, Raimondo T. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am J Epidemiol. 1991; 134:552–566. [PubMed: 1951261]

Gunter, E.; Lewis, B.; Koncikowski, S. Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988– 1994. Included in CD-ROM 6-0178, NHANES III Reference Manuals and Reports. Hyattsville, MD: Centers for Disease Control and Prevention; 1996. ; p. VII-D-1-VII-D-14.

Herbert V. Experimental nutritional folate deficiency in man. Trans Assoc Am Phys. 1962; 75:307–320. [PubMed: 13953904]

Hirsch S, Poniachick J, Avendano M, Csendes A, Burdiles P, Smok G, Diaz J, de la Maza M. Serum folate and homocysteine levels in obese females with non-alcoholic fatty liver. Nutrition. 2005; 21:137–141. [PubMed: 15723740]

Krumdieck C, Boots L, Cornwell P, Butterworth C. Estrogen stimulation of conjugase activity in the uterus of ovariectomized rats. Am J Clin Nutr. 1975; 28:530–534. [PubMed: 1168985]

Lahmann P, Hoffmann K, Allen N, Van Gils C, Khaw K-T, Tehard B, Berrino F, Tjonneland A, Bigaard J, Olsen A, Overvad K, Clavel-Chapelon F, Nagel G, Boeing H, Trichopoulos D, Economou G, Bellos G, Palli D, Tumino R, Panico S, Sacerdote C, Krogh V, Peeters P, Bueno De Mesquita H, Lund E, Ardanaz E, Amiano P, Pera G, Quiros J, Martinez C, Tormo M, Wirfalt E, Berglund G, Hallmans G, Key T, Reeves G, Bingham S, Norat T, Biessy C, Kaaks R, Riboli E. Body size and breast cancer risk: Findings from the European prospective investigation into cancer and nutrition (EPIC). Int J Cancer. 2004; 111:762–771. [PubMed: 15252848]

Laufer E, Hartman T, Baer D, Gunter E, Dorgan J, Campbell W, Clevidence B, Brown E, Albanes D, Judd J, Taylor P. Effects of moderate alcohol consumption on folate and vitamin B12 status in postmenopausal women. Eur J Clin Nutr. 2004; 58:1518–1524. [PubMed: 15138463]

Liu M, Ge Y, Cabelof D, Aboukameel A, Heydari A, Mohammad R, Matherly L. Structure and regulation of the murine reduced folate carrier gene identification of four noncoding exons and promoters and Regulation by dietary folates. J Biol Chem. 2005; 280:5588–5597. [PubMed: 15579899]

Lowe K, Osborne C, Lin B-F, Kim J-S, Hsu J-C, Shane B. Regulation of folate and one-carbon metabolism in mammalian cells. II. Effect of folylpoly-gamma-glutamate synthetase substrate specificity and level on folate metabolism and folylpoly-gamma-glutamate specificity of metabolic cycles of one-carbon metabolism. J Biol Chem. 1993; 268:21665–21673. [PubMed: 8408019]

Mahabir S, Baer D, Johnson L, Hartman T, Dorgan J, Campbell W, Clevidence B, Taylor P. Usefulness of body mass index as a sufficient adiposity measurement for sex hormone concentration associations in postmenopausal women. Cancer Epidemiol Biomarkers Prev. 2006; 15:2502–2507. [PubMed: 17164376]

Mojtabai R. Body mass index and serum folate in childbearing age women. Eur J Epidemiol. 2004; 19:1029–1036. [PubMed: 15648596]

Nevton C, Samuel D, James V. Aromatase activity and concentrations of cortisol, progesterone and testosterone in breast and abdominal tissue. J Steroid Biochem. 1986; 24:1033–1039. [PubMed: 3724141]

NRC. Recommended dietary allowances. 10th ed.. Washington, DC: National Academy Press; 1989. National Research Council.

Panotopoulos G, Jean C, Bernard G-G, Arnaud B. Dual x-ray absorptiometry, bioelectrical impedance, and near infrared interactance in obese women. Med Sci Sports Exerc. 2001; 33:665–670. [PubMed: 11283446]

Pfeiffer C, Gunter E. Automated assay for methylmalonic acid (MMA) in plasma. Clin Chem. 1999a; 45:A166–A167.

Pfeiffer C, Huff D, Gunter E. Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. Clin Chem. 1999b; 45:290–292. [PubMed: 9931056]

Prasannan P, Pike S, Peng K, Shane B, Appling D. Human mitochondrial C1-tetrahydrofolate synthase gene structure, tissue distribution of the mRNA, and immunolocalization in Chinese hamster ovary cells. J Biol Chem. 2003; 278:43178–43187. [PubMed: 12937168]

Reitman A, Friedrich I, Ben-Amotz A, Levy Y. Low plasma antioxidants and normal plasma B-vitamins and homocysteine in patients with severe obesity. Isr Med Assoc J. 2002; 4:590–593. [PubMed: 12183861]

Rohan T, Jain M, Howe G, Miller A. Dietary folate consumption and breast cancer risk. J Natl Cancer Inst. 2000; 92:266–269. [PubMed: 10655445]

Ronco A, De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Leborgne F. Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. Nutr Cancer. 1999; 35:111–119. [PubMed: 10693163]

Rothberg K, Ying Y, Kolhouse J, Kamen B, Anderson R. The glycophospholipid-linked folate receptor internalizes folate without entering the clathrin-coated pit endocytic pathway. J Cell Biol. 1990; 110:637–649. [PubMed: 1968465]

Shojania A, Hornady G, Scaletta D. The effect of oral contraceptives on folate metabolism. III. Plasma clearance and urinary folate excretion. J Lab Clin Med. 1975; 85:185–190. [PubMed: 1113011]

Szymczak J, Milewicz A, Thijssen J, Blankenstein M, Daroszewski J. Concentration of sex steroids in adipose tissue after menopause. Steroids. 1998; 63:319–321. [PubMed: 9618794]

Townsend J, Davis S, Mackey A, Jesse F, Gregory I. Folate deprivation reduces homocysteine remethylation in a human intestinal epithelial cell culture model: role of serine in one-carbon donation. Am J Physiol: Gastrointest Liver Physiol. 2004; 286:588–595.

Tungtrongchitr R, Pongpaew P, Tongboonchoo C, Vudhivai N, Changbumrung S, Tungtrongchitr A, Phonrat B, Viroonudomphol D, Pooudong S, Schelp F. Serum homocysteine, B12 and folic acid concentration in Thai overweight and obese subjects. Int J Vitam Nutr Res. 2003; 73:8–14. [PubMed: 12690905]

Villanueva J, Ling E-H, Chandler C, Halsted C. Membrane and tissue distribution of folate binding protein in pig. Am J Physiol. 1998; 275:1503–1510.

Whetstine J, Witt T, Matherly L. The human reduced folate carrier gene is regulated by the AP2 and Sp1 transcription factor families and a functional 61-base pair polymorphism. J Biol Chem. 2002; 277:43873–43880. [PubMed: 12228234]

Wu K, Helzsouer K, Comstock G, Hoffman S, Nadeau M, Selhub J. A prospective study on folate, B12, and pyridoxal 5-phospate (B6) and breast cancer. Ca Epidemiol Biomarkers Prev. 1999; 8:209–217.

Zhang S, Hunter D, Hankinson S, Giovannucci E, Rosner B, Colditz G, Speizer F, Willett W. A prospective study of folate intake and the risk of breast cancer. JAMA. 1999; 281:1632–1637. [PubMed: 10235158]

Table 1

Characteristics of the subjects (N=51) at baseline

Characteristics	Mean	Median	(range)
Age (y)	59.7	58.2	(49.2–78.8)
Height (cm)	163.9	163.1	(152.1–179.7)
Weight (kg)	74.8	73.2	(42.1–117.4)
BMI (kg/m^2)	27.8	26.9	(17.7–42.5)
Total body fat (g)	29,744	26,808	(7,942–55,756)
Central fat (g)	13,830	13,234	(3,056–26,396)
Peripheral fat (g)	14,299	12,476	(4,163–29,507)
% Body Fat	41.3%	42.5%	(17.8%–55.7%)
Age at menarche (yrs)	12.7	13	(10–16)
Duration of menses (yrs)	33.3	35	(12–45)
Years since last menses	13.1	12	(0–38)
Parity (# of children)	3	2	(0–8)
[†]Age at first birth (yrs)	23.2	22	(16–36)
Folate, ng/mL	17.51	16.35	(7.30–30.50)
B12, pg/mL	451.0	397.5	(134.0–1404.0)
Homocysteine, umol/L	9.00	9.48	(5.79–19.75)
Methylmalonic acid, umol/L	0.22	0.18	(0.07–1.05)

Characteristics	No	(%)
Race		
White	38	(74.5%)
Black	11	(21.6%)
Asian	2	(3.9%)
Menopause type		
Natural	39	(76.5%)
Hysterectomy	12	(23.5%)
Family history of breast cancer		
Yes	11	(21.5%)

[†]Based on n=43 subjects

Table 2

Geometric mean serum folate, vitamin B12, homocysteine, methylmalonic acid concentrations by categories of BMI

BMI categories N	Normal weight (BMI ≤25) 20	Overweight (BMI >25 to ≤30) 17	Obese (BMI >30) 14	P-trend[†]	R²
	Mean (95% C.I.)	Mean (95% C.I.)	Mean (95% C.I.)		
Folate, ng/mL	18.67 (14.66–23.77)	16.58 (13.44–20.45)	14.57 (11.97–17.73)	0.016	0.25
Vitamin B12, pg/mL	455.08 (355.75–616.80)	375.29 (269.32–522.96)	345.07 (252.85–470.92)	0.079	0.32
Homocysteine, μmol/L	8.77 (7.08–10.86)	9.37 (7.97–11.00)	9.69 (8.31–11.30)	0.197	0.35
Methylmalonic acid, μmol/L	0.18 (0.13–0.25)	0.19 (0.13–0.26)	0.19 (0.14–0.26)	0.828	0.35

Model adjusted for age, race, family history of BC, parity and menarche < 12 yrs

[†]P-trend from linear regression models where trend is measured as a continuous value after assigning the normal weight category 0,overweight category 1, and obese category 2.

Table 3

Associations of adiposity with serum folate, vitamin B12 and homocysteine concentrations

	Folate, ng/mL			Vitamin B12, pg/mL			Homocysteine, µg/mL		
	Δ	(95% C.I.)	R^2	Δ	(95% C.I.)	R^2	Δ	(95% C.I.)	R^2
BMI[†]									
Model 1	−1.72[a]	(−3.07, −0.34)	0.24	−2.12	(−4.24, 0.04)	0.32	0.78	(0.16, −0.30)	0.35
Model 2									
% Fat[‡]									
Model 1	−1.35[b]	(−2.22, −0.46)	0.29	−0.94	(−2.39, 0.54)	0.29	0.26	(−0.46, 0.99)	0.33
Model 2	−1.51	(−3.33, 0.35)	0.29	1.29	(−1.70, 4.38)	0.34	−0.81	(−2.26, 0.67)	0.37
Central fat[‡]									
Model 1	−1.88[a]	(−3.27, −0.47)	0.26	−1.52	(−3.77, 0.78)	0.29	0.49	(−0.64, 1.63)	0.33
Model 2	−1.66	(−5.33, 2.15)	0.26	3.69	(−2.27, 10.02)	0.35	−1.75	(−4.57, 1.14)	0.37
Peripheral fat[‡]									
Model 1	−1.79[a]	(−3.07, −0.49)	0.26	−1.80	(−3.85, 0.29)	0.31	0.62	(−0.42, 1.67)	0.34
Model 2	−1.96	(−5.46, 1.67)	0.26	0.71	(−4.96, 6.72)	0.33	−0.55	(−3.36, 2.33)	0.35
Lean mass[‡]									
Model 1	−1.05	(−3.03, 0.98)		−2.24	(−5.20, 0.81)		1.38	(−0.08, 2.87)	
Model 2	0.82	(−1.69, 3.40)		−0.61	(−4.49, 3.42)		1.17	(−0.74, 3.11)	

Δ- Percent change for folate, B12, and homocysteine for each one-unit change in each of the five adiposity measures (i.e., percent change in folate, B-12, and homocysteine per one unit change in BMI, per 1% increase in total fat, per 1 kg increase in central and peripheral fat).

BMI: per unit increase; % fat: per 1% increase; trunk fat: per 1 kg increase; peripheral fat: per 1 kg increase

[†]Model 1: Adjusted for age, race, family history of breast cancer, parity and menarche < 12 years

[‡]Model 2: Model 2 + BMI

[a]$P < 0.05$

[b]$P < 0.005$