Relation of body-mass index to blood folate and total homocysteine concentrations in Japanese adults

Mio Nakazato[a], Takahiro Maeda[a], Noboru Takamura[a], Mitsuhiro Wada[a], Hironori Yamashita[a], Kelley E.Johnston[b], Tsunenobu Tamura[b]

[a] Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[b] University of Alabama at Birmingham, Birmingham, AL, USA

Corresponding author: T. Tamura, Room 455, 1675 University Blvd., Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, 35294 USA
Tel.: (205) 934-7478; Fax: (205) 934-7049; E-mail: tamurat@uab.edu

Abstract

Purpose Plasma folate concentrations are suggested to be negatively associated with body-mass index (BMI, kg/m^2), although these findings are controversial. Our objective was to evaluate the association of BMI to blood folate and total homocysteine (tHcy) concentrations.

Methods We measured plasma and erythrocyte folate and plasma tHcy concentrations in 434 healthy adults (343 women and 91 men; mean age of 63.8 ± 10.7 [SD, range 23 – 88] years old), who participated in a 2007 population-based survey in western Japan.

Results The overall mean plasma and erythrocyte folate and tHcy were 21.6 (± 11.0, SD) nmol/L, 844 (± 291) nmol/L and 11.6 (± 3.9) μmol/L, respectively. The mean BMI was 22.8 (± 3.0; 15.6 – 33.3) kg/m^2 and only 72 subjects (17%) had BMI > 26.0 kg/m^2. Mean plasma folate decreased as BMI increased (*P*-trend < 0.01), whereas mean erythrocyte folate and plasma tHcy were similar regardless of BMI (*P*-trends = 0.49 and 0.28, respectively).

Conclusion Our data indicate that the interpretation of plasma folate concentrations to assess folate nutritional status is complicated by BMI, although the impact of BMI on plasma folate was relatively small. It is important to take this association into account for the selection of subjects for future large-scale studies. The mechanism of this inverse association between BMI and plasma folate concentrations should be investigated.

Keywords Folate – Blood – Homocysteine – Body-mass index – Human

Introduction

Plasma/serum folate concentrations are the most commonly used means to assess folate status in humans, although they are affected by various factors [1]. One of these factors is body-mass index (BMI) that has been suggested to be negatively correlated with plasma/serum folate, although the findings are controversial [2-14]. For erythrocyte folate, another commonly used index of folate status, however, no such significant correlation are reported [4, 5]. The finding of an association between BMI and plasma total homocysteine (tHcy) concentrations, one of the functional indicators of folate status, have been conflicting [4, 7-10, 12, 13]. The mechanism(s) of the association of BMI to plasma folate is unclear; however, a few putative explanations have been proposed including changes in plasma volume, folate distribution in different tissues, folate transport (absorption or excretion) and endocrine functions with varying BMI [4]. Laurence et al. [6] reported that there were racial/ethnic differences in serum folate concentrations in pregnant women. Therefore, we evaluated the associations of BMI to plasma and erythrocyte folate and plasma tHcy in healthy Japanese adults.

Methods

Subjects and samples

This study was approved by the ethical committee for human use of Nagasaki University. A total of 434 apparently healthy subjects (343 women and 91 men) were selected based on the availability of folate values from those who participated in a 2007 population-based survey in western Japan and were non-smokers who resided on Goto Islands and had no history of serious disease. The mean age was 63.8 (± 10.7, SD; range 23 – 88) years old, and body weight and height were measured by an automatic body composition analyzer (BF-220, Tanita, Tokyo, Japan) at the time of blood drawing. Fasting blood samples were collected using evacuated tubes with EDTA (Terumo, Tokyo, Japan). Portions of whole blood were mixed with 9 volumes of ascorbic acid solution (56.8 mmol/l, final pH of 4.6) for the whole-blood folate assay, and plasma samples were mixed with an equal volume of 56.8 mmol/l ascorbic acid after the separation and kept frozen at –30ºC. Ascorbic acid was added as a cautionary measure to protect folate degradation. Within 6 months of sample collection, both ascorbic acid-containing whole blood lysate and plasma were sent on dry ice to the University of Alabama at Birmingham (USA), where these were kept at –80ºC until folate assay.

Laboratory analysis

For the *L. rhamnosus* microbiological assay, 5-formyltetrahydrofolate (5-HCO-H_4folate) was used as a standard [15, 16]. Whole blood lysate (pH 4.6) were incubated for 1 hour at 37ºC to hydrolyze polyglutamyl folates in erythrocyte by endogenous

plasma folyl-poly-γ-glutamate carboxypeptidase II (folate conjugase) [15]. The inter-assay coefficient of variation (CV) for folate assay was ~6% using two pooled human plasma samples, and our assay values of Standard Reference Material 1955 (National Institute of Standards and Technology, Gaithersburg, MD, USA) were within the specified values by the Institute for all three levels. Erythrocyte folate concentrations were calculated using the following formula: [whole blood concentration – plasma concentration x (1 – hematocrit (%)/100)] ÷ hematocrit (%)/100. Plasma tHcy concentrations were measured by the HPLC-fluorescent detection method that was developed in our laboratory, and the inter-assay CV for the tHcy analysis ranged from 5.2 – 7.8% [17]. Hemoglobin and hematocrit were measured using an automatic hematology analyzer (XE-2100, Syntex, Kobe, Japan).

Statistical analysis

The data are expressed as mean ± SD, where appropriate. For linear regression analysis, dependable variables included plasma and erythrocyte folate, and plasma tHcy concentrations, all of which were logarithmically transformed due to skewed distribution. Independent variables for the analysis were age, BMI, and alcohol drinking, which were not logarithmically transformed. In addition, Pearson correlation analysis was used to evaluate association between two factors that were not logarithmically transformed. Differences in folate, tHcy, hemoglobin and hematocrit values in BMI quartiles were evaluated by Kruskal-Wallis test in combination with Kolmogorov-Smirnov test. Gender differences were evaluated by Mann-Whiney U-test and Chi square test. A *P* value <

0.05 was considered statistically significant. All statistical analyses were done with software (SPSS Inc., Tokyo, Japan).

Results

The characteristics of subjects are shown in Table 1. The mean BMI of our subjects was 22.8 kg/m^2 with only 5 subjects (1.2%) having BMI >30.0 kg/m^2. Only 17% and 4.6% of all subjects had plasma and erythrocyte folate below the cutoffs for inadequate folate status in the countries without the folic acid fortification program [18]. Only~17% of the subjects exceeded the normal cutoff of plasma tHcy [18]. Of 343 women, 55 (16%) and 25 (7%) women had hemoglobin and hematocrit values below normal ranges, respectively, and of 91 men, 40 (44%) and 12 (13%) had below normal ranges, respectively. Plasma tHcy concentrations were significantly negatively correlated with plasma and erythrocyte folate (r = -0.25 and -0.19, P < 0.0001 and 0.001, respectively).

We evaluated the association between BMI quartiles and blood indices. There was a significant negative association (P< 0.01, Figure 1-A) for all subjects combined, indicating that the higher the BMI, lower the plasma folate concentrations. However, no such associations were found between BMI and erythrocyte folate or tHcy (P = 0.49 and 0.28, respectively: Figures 1-B and C). We also evaluated these associations in women and men independently and found similar associations.

Since there was a wide age variation in our subjects, we analyzed plasma and erythrocyte folate and plasma tHcy concentrations and BMI independently by dividing the subjects into three age groups (< 50, n = 50; 51-70, n = 177; and >71 years old, n = *207*) using the Krustal-Wallis method. We found that plasma folate and tHcy concentrations showed positive correlations with advancing age (P < 0.002 and 0.0001, respectively), whereas there were no significant associations between age groups and

erythrocyte folate concentrations and BMI (data not shown). In addition, there were significant positive correlations between BMI and hemoglobin and hematocrit values (r = 0.11, $P < 0.05$ and r = 0.13, $P < 0.02$, respectively).

Discussion

We found the inverse correlation between BMI and plasma folate concentrations in Japanese adults with a mean BMI of only 22.8 kg/m^2. In contrast, no such associations were found between BMI and erythrocyte folate or plasma tHcy. Our findings on the relationship between BMI and plasma folate are consistent with those reported by others [4-10, 12-14, 19]. In contrast, there have been reports indicating that no such association exists [2, 3, 11]. Both our data and those in the literature are summarized in Table 2, where we limited the number of citations to only those where the subjects were subdivided into more than two BMI groups [4-6, 8, 13]. Based on these data, it may be reasonable to conclude that there is a negative correlation between BMI and plasma folate.

The mechanism(s) of the significant inverse association between BMI and plasma folate is unknown. However, a few putative explanations are available including changes in plasma volume, folate distribution in different tissues, folate transport (absorption or excretion) and endocrine functions, since these can be affected by body size or body composition [4]. Considering our finding of the significant positive correlations between BMI and hemoglobin or hematocrit, hemodilution is unlikely the reason; however, this may be too simplistic because we did not measure actual plasma volumes. An argument can be made that dietary folate intakes differ due to the types of food consumed by individuals with different BMIs, and this issue remains to be clarified in the future studies [4, 5]. Furthermore, future studies are warranted to elucidate the mechanisms especially from the viewpoint of the observed worldwide trend of increasing BMI in the population.

To our knowledge, there have been only two studies where no significant association between BMI and erythrocyte folate was found [4, 5], and our data are consistent with these data. Winkels et al. [20] reported that the increase in erythrocyte folate concentrations in response to folic acid administration was larger in women than men, and suggested that this is explained by the gender difference in the body size and in folate distribution over a larger volume in men than women. The ages of subjects reported by Winkles et al. [20] ranged from 50 to 70 years old that was similar to ours; however, we did not observe gender difference in our subjects. Further studies are warranted to investigate the association between BMI and folate absorption/distribution.

There have been studies on the relationship between BMI or waist circumference and plasma tHcy concentration. The data are, however, not consistent; two indicated a significantly positive correlation [7, 9], whereas one showed an inverse correlation [12] and four showed no association [5, 8, 10, 13]. van Driel et al. [21] reported in women of reproductive age that BMI is the positive determinant of plasma S-adenosylmethionine and S-adenosylhomocysteine that are closely related to homocysteine metabolism via the transmethylation pathway. Although they did not find any association between BMI and tHcy, they suggested that BMI is related to methylation reactions. Further studies are warranted on the association between BMI and homocysteine metabolism.

There have been reports indicating that the development of neural-tube defects (NTDs) is non-responsive to periconceptional folic acid supplementation in obese women [22, 23], suggesting that obese pregnant women may have different folate metabolism or transport, particularly at the site of placental folate transfer. Obese women may have constantly low plasma folate concentration, which is likely to be proportional to the rate of placental folate transport to the fetus against the concentration

gradient [24]. We believe that it is urgent to study the mechanism of such an association of low plasma folate to folic acid non-responsible NTDs in obese women. This may lead to further reduction in the rate of pregnancies complicated with NTDs.

The weaknesses of our study include that the number of subjects was small, and they were limited to elderly Japanese population. In addition, we do not have dietary folate intake data, and as noted above, this issue is important to be clarified in the future research, although conflicting data were found in two reports [4, 5]. Furthermore, it is important to adjust various lifestyle factors in such investigations, since these factors are likely to differ among individuals with different BMI.

The amount of change in plasma folate concentrations based on BMI was small. However, if one does not take BMI-related differences in plasma folate concentrations into the account, there may be unexpected consequences. For example, in a selection of subjects for a trial to evaluate the effect of prenatal zinc supplementation on fetal growth, we chose those with plasma zinc concentration below the median of the group. We did not know at the time that heavier subjects have lower plasma zinc than thinner subjects [25]. This resulted in unexpectedly selecting a group of women with a higher mean BMI.

In summary, we found that plasma folate concentrations inversely correlated with BMI. Our data indicate that the interpretation of plasma folate concentrations to assess folate nutritional status is complicated by BMI, although the impact of BMI on plasma folate concentrations was relatively small. It is important to take this association into account in the selection of subjects for future large-scale studies. The mechanism of this inverse association should be investigated.

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Table 1. Characteristics of subjects

Characteristics [normal range]	Women (*n* = 343)	Men (*n* = 91)	Both combined (*n* = 434)
Age (years)**	62.8 ± 11.0 (23 – 88)	67.6 ± 10.7 (30 – 84)	63.8 ± 10.7 (23 – 88)
Body-mass index (kg/m^2)*	22.6 ± 3.0 (15.6 – 33.3)	23.5 ± 2.7 (17.1 – 30.1)	22.8 ± 3.0 (15.6 – 33.3)
Alcohol drinking (%)*	8.7	46.2	16.6
Plasma folate (nmol/l)* [> 12.0 nmol/l]	22.2 ± 10.7 (6 – 60)	19.3 ± 11.7 (5 – 58)	21.6 ± 11.0 (5 – 60)
Erythrocyte folate (nmol/l) [> 450 nmol/l]	848 ± 288 (247 – 2073)	829 ± 300 (373 – 1749)	844 ± 291 (247 – 2073)
Plasma total homocysteine (μmol/l)** [< 15 μmol/l]	11.2 ± 3.8 (4.2 – 31.6)	13.0 ± 4.2 (4.9 – 24.9)	11.6 ± 3.9 (4.2 – 31.6)
Hemoglobin (g/l)** [female, > 120 g/l; male, > 140 g/l]	129 ± 12 (69 -157)	142 ± 11 (115 -167)	131 ± 13 (69 -167)
Hematocrit (%)** [female, > 35%; male > 40%]	40.2 ± 3.4 (26.9 – 48.2)	43.8 ± 3.3 (35.8 – 51.0)	41.0 ± 3.7 (26.9 – 51.0)

Values are presented as mean ± SD (range) or % of our population. Normal values were based on those by Tamura et al. [18] and by Japan Hematological Society. Differences between women and men were significant (*$P < 0.01$; and **$P < 0.0001$) by Mann-Whitney U-test and Chi-square test.

Table 2. Studies to evaluated correlation between body-mas index (BMI) and plasma and erythrocyte (RBC) folate and homocysteine (tHcy) concentrations

Investigators, year, country	Subjects (n)	Correlation with BMI		
		Plasma folate	RBC folate	tHcy
Kant [4], 2003, USA	adults (> 13,000)	significantly negative	NS	–*
Mojtabai [5], 2004, USA	non-pregnant adult females (> 6,000)	significantly negative	NS	–
Lawrence et al. [6], 2006, USA	pregnant adults (> 6,000)	significantly negative	–	–
Papandreou et al. [8], 2007, Greece	school children (524)	NS	–	NS
Mahabir et al. [13], 2008, USA	adults (51)	significantly negative	–	NS
Our study, Japan	adults (434)	significantly negative	NS	NS

We limited the number of citations to only those where the subjects were subdivided into more than two groups based on BMI.

NS represents Not significant association

*Not determined

Figure legends

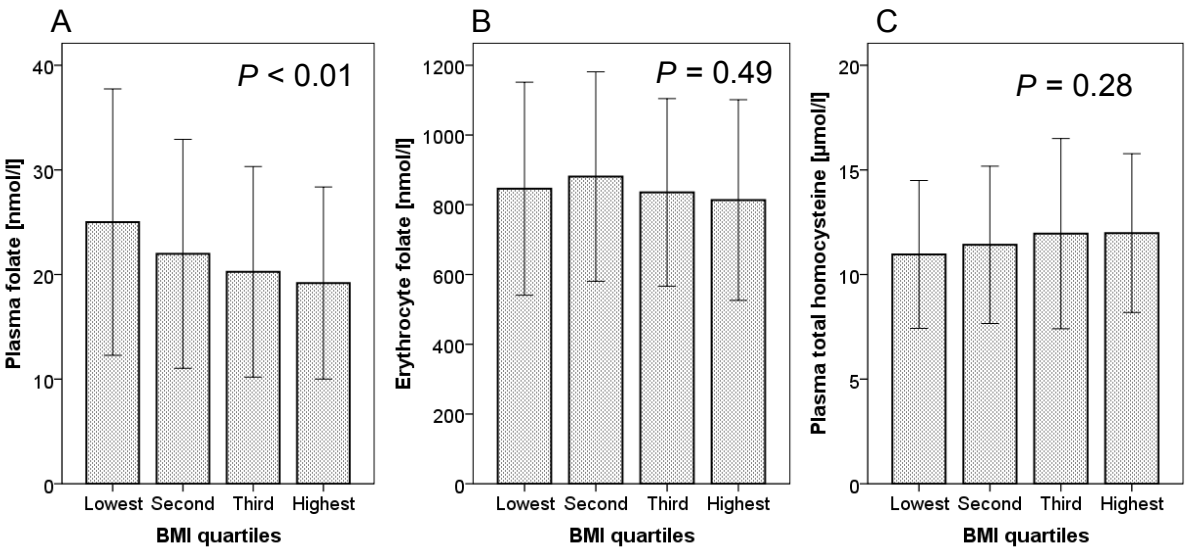

Figure 1. Plasma (A) and erythrocyte (B) folate and plasma total homocysteine (tHcy) (C) concentrations based on the quartiles of body-mass index (BMI) in all subjects combined (*n* = 434). Bars represent the mean and vertical lines represent SD for each quartile. The ranges of BMI for quartiles were; lowest, ≤ 20.6 kg/m^2; second, 20.7 – 22.4 kg/m^2; third, 22.5 – 24.6 kg/m^2; highest, ≥ 24.7 kg/m^2. The differences in four values for each BMI quartile are significant, if the *P* value is less than 0.05.