Review

Obesity is associated with folate metabolism

A Systematic Review

Süleyman Köse, Saniye Sözlü, Hatice Bölükbaşi, Nüket Ünsal, and Makbule Gezmen-Karadağ

Gazi University, Faculty of Health Sciences, Department of Nutrition and Dietetics, 06560 Beşevler, Ankara, Turkey

Received: November 15, 2017; Accepted: June 20, 2018

Abstract: *Objective*: The aim of the present study is to perform a systemic review of the previous studies executed on the association between obesity and folate.
Method: In the present research, the selected keywords were scanned on the PubMed, Web of Science, Cochrane and Lilac databases between May and June, 2017 through Gazi University's network. In total, 4236 clinical, randomized controlled, cross-sectional and prospective studies were determined and 17 of these that specifically fit the aims of the present research were reviewed. This study involved an electronic literature search of databases on folic acid and obesity published in the English language between 2000 and 2016.
Results: Of the 17 studies, 5 were based on folic acid supplementation and 12 were related with participants' folate status. As a general consequence of both intake and serum/status measurements of folic acid supplementation: It was found that obesity-associated metabolic changes might affect individual folate use and obese individuals had lower serum folate levels, although there was no change in folate intake.
Conclusion: Overweight and obese individuals have lower serum folate concentrations when compared with individuals with normal weight. It is explained by increased use of folic acid, urinary excretion, dilution of blood volume, different levels in different tissues and changes in the endocrine functions of folate. Individuals with higher Body Mass Indexes have less supplement use, unhealthier diets and donot consume sufficient vegetables and fruits, all of which can affect decrease in folate levels. Furthermore, adiposity may affect folate absorption by intestinal epithelium.

Keywords: Adipose tissue, body mass index, folate, homocysteine, obesity

Introduction

Folate and folate derivatives are involved in important biochemical reactions, such as metabolism of purine, pyrimidine, homocysteine and methionine amino acids. Therefore, folate is among the essential vitamins in tissues with rapid DNA formation and catabolism [1]. It is known that obese individuals have lower folate and vitamin B_{12} levels [2–4]. Serum folate level is affected primarily by diet and some studies indicate an association between a lower *serum* folate level and higher body mass index (BMI) [5–8]. Bradbury et al. [9] reported that a 1% decrease in serum folate concentration associated with every increase of 1 unit in BMI [9]. Although there are many study about the association between serum folate level and BMI; these studies are insufficient to explain the relationship. Some hypotheses suggest that modifications in plasma volume may affect the distribution, transportation and endocrine functions of folate [10, 11]. Some hypothesis suggest that obese individuals requirements are greater than individuals with normal body mass index [12]. There is an additional recommendation for all women of childbearing age to consume 400 mg folic acid/day. These recommendations are valid for all women regardless of BMI [13].

Also it has been shown that in women of reproductive age BMI is a positive indicator for S-adenosylmethionine and S-adenosylhomocysteine; the transmethylation pathways of these have close associations with the metabolism of homocysteine [14]. Another study detected higher plasma concentrations of homocysteine in obese individuals when compared with the control group [15]. Another explanation for the association between low folate status and obesity is the dietary habits. Obese individuals eating less fruit, vegetables and cereals result in low folic acid intake [12]. Lower folate levels, which are associated with higher BMI levels, have significant health effects, such as neuraltubedefects [16] and other birth defects [17, 18]. Therefore, research on methylenetetrahydrofolate reductase (MTHFR) enzyme

gene polymorphism is important to determine the cause and direction of the association between low folate levels and higher BMI levels [12]. MTFHR plays a central role in folate metabolism as a catalyst during the conversion of 5,10-methylenetetrahydrofolateto 5-methyltetrahydrofolate, which is the primary circulating form of folate and acts as a co-substrate in the methylation of homocysteine (Hcy) [19]. Researchers have observed a relation between (MTHFR) C677T TT genotype and reduced folate levels and obesity [19–21]. It is notable that low folate levels affect DNA methylation negatively in humans [22, 23]. Epigenetic modifications which is including methylation, are connected with adulthood obesity [24]. We analyzed the studies with the intent of identifying differences in approaches, rather than to define a golden standard. A primary aim of the present study is to perform a systemic review on data obtained from studies investigating the association between obesity and folate. A secondary aim was to describe the inevitable inaccuracy that arises from the use of different definitions of, for example, red-blood cells folate levels, folate concentrations in blood serum, folate concentrations in plasma and folate intake.

Method

A retrospective scan was performed without any limitation on dates and appropriate articles were reviewed. The literature search of databases involved articles on folic acid and obesity published in the English language between 2000 and 2016. The keywords "folic acid, folate, tetrahydrofolate, tetrahydrofolic acid, folic acid supplementation, pteroylglutamicacid, homocysteine, obesity, obese, adipose tissue, adiposity, weight gain and BMI" were scanned on the PubMed, Web of Science, Cochrane and Lilac databases between May and June, 2017 through the access network of Gazi University. The scan was performed by inserting "and" and "or" between the keywords to assess association of folate with obesity. Articles which did not allow an automatic scan were searched manually.

The scans were conducted by two different researchers (4236 articles). Thus, 102 articles were identified as duplicates and 4134 articles were evaluated. Later, article titles were reviewed during evaluation of the articles and articles with inappropriate titles were excluded. As a result of this process, remaining 102 articles were reviewed within the scope of the present study. The titles and abstracts of the identified reports were used to exclude studies that clearly did not meet the inclusion criteria. For studies deemed potentially eligible for inclusion, we obtained the full paper. We screened the full articles of selected studies to confirm eligibility and resolved any disagreements by discussion. Our intent was not to exclude studies based on strict scientific criteria, or to perform a traditional quality assessment, but to make the studies as comparable as possible. Finally, the summaries and methods of the articles were evaluated and included as appropriate (17 articles) (Figure 1). The present review involved cohort, case-control, intervention, clinical, randomized controlled, cross sectional and prospective studies carried out with animals.

This review excluded reviews, meta-analyses, in-vitro studies, animals, theses, verbal and poster assertions presented in conferences and articles written in other languages than English. This paper complies with the PRISMA recommendations [25].

Results

The electronic literature searches identified 4236 citations. Once duplicates were deleted, 4134 articles remained. Seventeen articles met the inclusion criteria and 17 unique studies representing 15966 adults (morbidly obese, obese or non-obese) were included in the systematic review.

The designs of the studies evaluated within the scope of the research are cohort (2), cross-sectional (6), randomized controlled study (3), intervention (1) and case-control (1). Four studies designs are not specified. In these studies, observational study (Table I) and supplementation studies (Table II) are given separately.

When observational studies are evaluated, the follow-up period varies between 1 year [26, 27] and 6 weeks [28]. Supplementation studies showed that a single dose of 1.1–12.5 mg folate [29] or 400 mg folate [13] was given 1–5 mg/dayfolate (Asami) for 8 weeks and 5 mg folate [30] for 12 weeks.

The studies were about dietary intake or additional supplementation of folate in the case of obesity. The results of cross-sectional or randomized controlled studies, which formed the majority, were found to be similar.

Folate intake and serum folate levels were evaluated through food consumption records; these were associated with body weight and BMI in the studies. In addition, some studies analysed different biochemical indicators as well as blood lipids, homocysteine and insulin levels. As a result of the review, folate intake were adjusted for BMI and it was found that folate intake was reversely associated with BMI, that folate levels decreased when BMI increased and that folate requirements increased in case of obesity.

Discussion

Obesity causes metabolic disorders that contribute to the pathogenesis of diabetes and cardiovascular diseases;

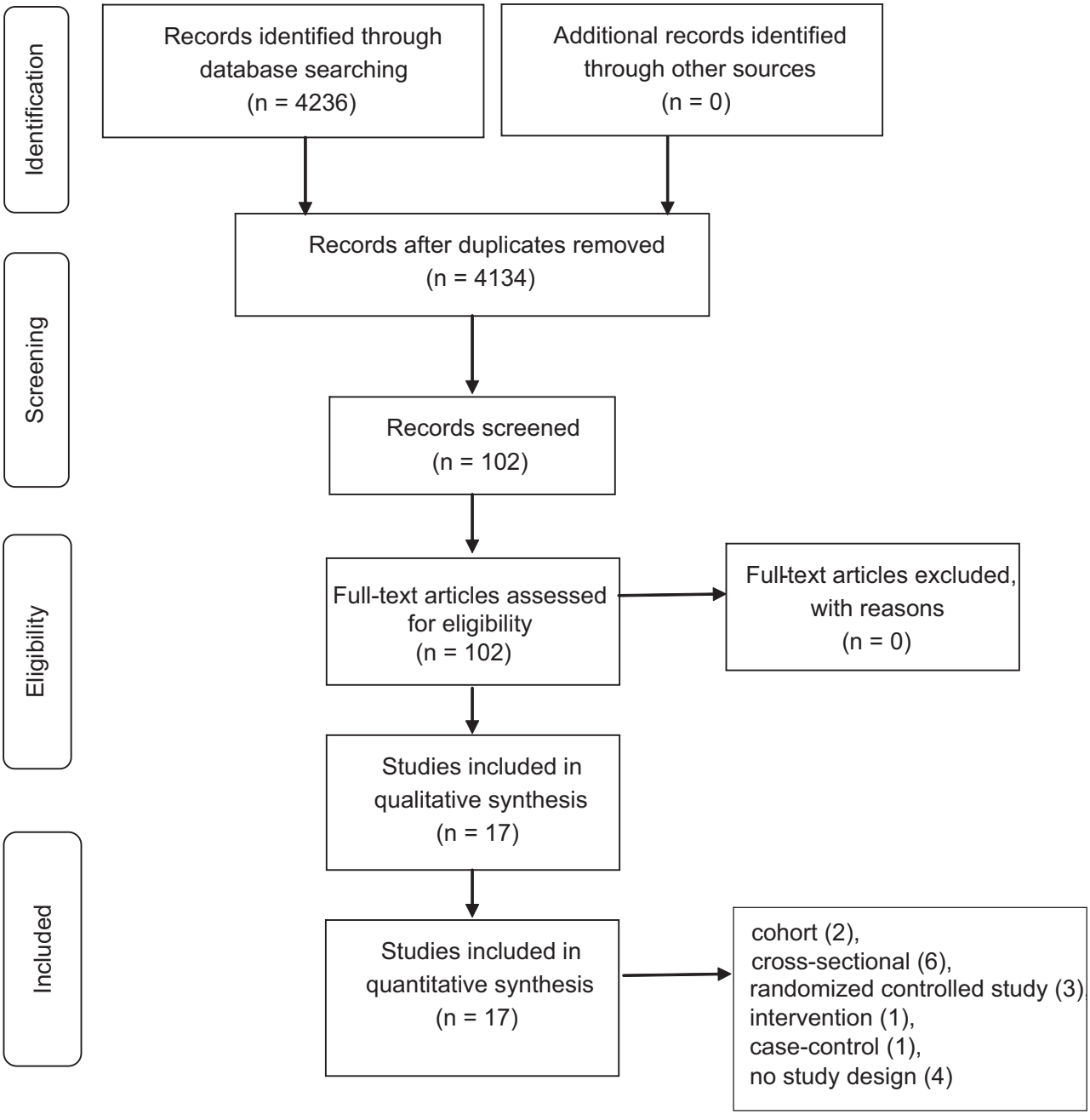

Figure 1. The literature search of PubMed, Web of Science, Cochrane and Lilac involved articles on folic acid and obesity published in the English language between 2000 and 2016.

furthermore, it is associated with a significant increase in risk of mortality and morbidity [27]. Obesity-induced metabolic modifications may affect individual's folate use and increase their folate requirements. The studies showed that obese individuals have lower serum folate levels, although they did not have any difference in folate intake [6–8]. Although their folate intake was at suggested levels, the suggested dose might be insufficient due to the obesity-induced modification in folate metabolism. The risk of insufficiency may develop even when folate is supported by diet and supplement [31].

Serum folic acid level and obesity

There is a reverse relation between obesity and folate insufficiency [27]. Recent studies on folic acid fortification show

Table I. Overview of Observational Studies Included in the Review

Human studies	Year	Sample	Study design	Aim	Method	Duration	Country	Outcome	Inclusion	Exclusion	Results	Mechanism
Martínez, J. G., Ruiz, F. A., & Candil, S. D. [26]	2006	Morbidly obese n = 182; group 1 (responding to the treatment) n = 21; group 2 (not responding to the treatment); n = 161	Cohort (a longitudinal prospective study)	Evaluation of the outcomes of diet and exercise treatment in morbidly obese individuals for one year.	National Institutes of Health Recommendations and a diet program adopted from the Mediterranean diet were applied to the individuals. Orlistat and sibutramine were prescribed for those who could not achieve a weight loss of 5% in 3 months. Basic indicators of a weight loss of 10% were investigated.Serum folate levels were analysed through Electrochemiluminometric methods.	1 year	Spain	Serum folate; BMI; Albumin; Thyroid hormone tests; OGTT; transferrin, prealbumin,RBP, Zn, B12, Ca, P, Mg, 25-dihydroxyvitamin D3.	Those between 18 and 55 years of age with a BMI $\geq$ 40 kg/m^2 with high motivation	Individuals with medical or psychiatric contraindications for bariatric surgery	An increase of 1 ng/ml in serum folate increased the achievement of the treatment by 28% (95% CI 4.58)	Higher serum folate level indicates a healthy nutrition habit as well as an increase in vegetable-fruit consumption.
Mojtabai, R. [7]	2004	NHANES III n = 5018; NHANES1999-2000 n = 1351; female individuals 4 groups depending on BMI values; G1: < 20.0, G2:20.0–26.9, G3:27.0–29.9, G4: $\geq$ 30.0	Data from cross-sectional research was used (NHANES III, NHANES1999-2000)	Although the mechanism is unclear, higher BMI is a risk factor for neural tube defects. Evaluation of the association between folate level and BMI.	Individuals with different BMI values were assessed by diving them into 4 groups. Food consumption records for 24 hours were obtained through the reminding technique. Those with folate supplementation were investigated and reported. Folate (Bio-Rad Laboratories Quantaphase Folate/vitamin B12 radioassay Kit) was measured.	–	USA	BMI; RBC folate; Serum folate; Homocysteine	Non-pregnant female individuals between 17 and 49 years of age whose serum folate and BMI levels could be accessed through NHANES studies	Pregnant	The increase of BMI in women at reproductive age was found to be associated with lower serum folate levels ($p < 0.001$).	Women with higher BMI have lower possibilities of folate supplementation and sufficient folate intake by diet.
Ortega, R. M., López-Sobaler, A. M., Andrés, P. et al. [28]	2006	Overweight/obese individuals Diet V: more vegetable consumption n = 28; Diet C: More consumption of enriched cereal for breakfast n = 29	Cohort (a longitudinal prospective study)	Determination of folate levels in overweight/obese women who implemented two different hypocaloric diets.	Food consumption records for 3 days from individuals who were assessed within two groups according to vegetable and grain consumption. Physical activity records were obtained. The individuals in the grain group received 250–300 μg/100 g folate. Drugs, supplements, alcohol and smoking, which affect folate status, were investigated. Folate intakes were recorded as diet folate equivalent. Serum folate levels were analysed through RIA.	6 weeks	Spain	BW; Body length; BMI; Serum folate; Homocysteine	Females between 20 and 35 years of age; BMI 24–35 kg/m^2 Women who have not quit smoking for last 2 months and have a regular menstrual cycle	Diabetes, hyperthyroidism, metabolic disease, hypertriglyceridemia, lactose and gluten intolerance. Those who were on a diet and lost more than 4.5 kg 2 months before the study. Those who had weight change more than 3 kg between the first interview and onset of the study. Those who drink more than 2 glasses of alcohol per day. Pregnant and breastfeeding.	The weight loss in the group who had enriched breakfast grains was higher than those who had vegetable consumption; however, a significant increase was also detected in serum folate levels ($p < 0.05$).	Although folate content of the vegetables and the fruits is high, the folate losses during the cooking process may have caused the result. Weight gain may increase requirements of folate.
Sheu, W. H. H., Hung-Sheng, W. U., Chen-Wen, W. A.N.G., Chu-Jen, W.A.N., & Wen-Jane, L.E.E. [27]	2001	Morbidly obese individuals; Male n = 3; Female n = 9		Evaluation of homocysteine levels at 6th month after gastroplasty that caused insufficient food intake and severe weight loss.	The individuals were assessed at months 0, 6 and 12 after gastroplasty. Serum folate levels were analysed through RIA.	Month 12	China	Fasting blood glucose; Lipoprotein; Homocysteine; Serum Folate and $B_{12;}$ HOMA IR	BMI $\geq$ 35 kg/m^2 individuals	Individuals with major psychiatric disease, liver and kidney failure, below 20 years of age	There was not any significant change in serum folate levels after gastroplasty	The number of individuals might be insufficient and the period might be limited. Early and long-term diets were suggested to prevent an increase in postoperative homocysteine levels.
Casanueva, E., Drijanski, A., Femfindez-Gaxiola, A.C., Meza, C., & Pfeffer, F. [38]	2000	117 females	Cross-sectional study	Evaluation of the association between anaemia and obesity caused by folate insufficiency.	Different biochemical parameters were analysed in a 5-ml blood sample collected from non-pregnant and non-breastfeeding participants.	–	Mexico	Folate; Iron; Vitamin B12	Oestrogen levels were normal	Pregnant and breastfeeding women with any metabolic diseases (DM, hypothyroidism) which cause infertility. Women who use Fe, B12 and any drug or alcohol that may affect folate metabolism. Those who use vitamin supplements. Women using oral contraceptive agents.	The prevalence of folate insufficiency was 20.5% and folate insufficiency in case of obesity was found to be 50-fold higher (95% CI = 13,39–186,95).	A reverse association exists between obesity and folate insufficiency.

(Continued on next page)

Table I. (Continued)

Human studies	Year	Sample	Study design	Aim	Method	Duration	Country	Outcome	Inclusion	Exclusion	Results	Mechanism
Vaya, A. Rivera, L. Hernandez-Mijares, A. Fuente, M., Sola, E., Romagnoli, M., Alisc, R., & Laiz, B. [41]	2012	66 morbidly obese individuals (47 females, 19 males) 66 controls (43 females, 23 males)	A randomized controlled study	To analyse homocysteine levels of morbidly obese individuals and individuals with normal BMI and evaluate homocysteine levels according to metabolic syndrome.	Blood samples were collected for metabolic syndrome indicators and anthropometric measurements were taken.	–	Spain	Glucose HOMA, Leptin, Triglycerides HDL Fibrinogen, C reactive protein (CRP) B12 vitamin; Folic acid waist circumference		Those with malign, haematological, infectious or inflammatory diseases. Individuals with a medical history of ischemic heart disease or stroke, thromboembolism or secondary obesity. Individuals who use folate or vitamin B12 supplement.	Higher homocysteine levels were generally found associated with abdominal obesity ($p = 0,031$) and insulin resistance ($p = 0.042$) and homocysteine was found reversely associated with folate levels.	Since folate is used as a cofactor in metabolism of homocysteine, the decrease in folate levels increases homocysteine levels.
Mahabir, S., Ettinger, S., Johnson, L., Baer, D.J., Clevidence, B. A., Hartman, T.J., & Taylor, P.R. [39]	2008	51 postmenopausal women	Cross-sectional study	Evaluation of the association between serum folate concentrations and adiposity in postmenopausal women.	Orange juice including 0, 15 or 30 grams of ethanol were given to the participants randomly with three different controlled diets for 8 weeks. Each diet period was separated with a two- to five-week discharge period. Body composition was measured with DXA. Each diet period was assessed through biochemical parameters at the end.	30 weeks	USA	Folate; B_{12} Homocysteine; Methylmalonic acid	Females $\geq$ 49 years of age. Postmenopausal. Those who do not use hormone replacement therapy or any other drug that would affect working. Those who are disposed to follow the diet and other foods and beverages, without any alcohol use or smoking personally and in the family.		Although they had the same diets, overweight women had lower serum folate levels by 12% ($p = 0.08$) and obese women had lower serum folate levels by 22% ($p = 0.02$) when compared with normal weight women. The increase in BMI and body fat percentage, as well as abdominal and peripheral lipidosis, are associated with a decrease in serum folate levels.	Lower serum folate concentrations observed in obese individuals may be explained by increased use and urinary excretion; furthermore, oestrogen concentrations derived from increased adipose tissue may be another cause of folate deficiency.
Settin, A.A., Algasham, A., Dowaidar, M., & Ismail, H. [19]	2009	130 mildly overweight and obese individuals; 111 normal individuals	Cross-sectional case control study	To evaluate the association of obesity and	methylenetetrahydrofolate reductase (MTHFR) and angiotensin converting enzyme (ACE) gene-associated polymorphism.	DNA of	participants was analysed through real-time PCR for MTHFR	polymorphisms.	–	Saudi Arabia	677C/T 1298 A/C ACE; I/D genes	
		There were not any statistically significant differences detected in genotypes and alleles of overweight/obese individuals when compared with the control group; however, BMI was observed as higher in polymorphism status, but was not statistically significant.	Genetic polymorphisms are not usually associated with obesity only, but also with obesity-associated diseases. Furthermore, differences may appear according to ethnicity and genetic formation.									

(Continued on next page)

Table I. (Continued)

Human studies	Year	Sample	Study design	Aim	Method	Duration	Country	Outcome	Inclusion	Exclusion	Results	Mechanism
Thawnashom, K., Tungtrongchitr, R., Petmitr, S., Pongpaew, P., Phonrat, B., Tungtrongchitr, A., & Schelp, F.P. [21]	2005	149 obese and 113 normal individuals	Cross-sectional case control study	MTHFR (C677T) To evaluate the association between gene polymorphism and serum concentrations of homocysteine, serum folate and vitamin B_{12}	After one night of fasting, 10 ml of venous blood samples were collected and vitamin B12, folate and homocysteine levels were analysed. The DNA was analysed from buffy coat fraction, which is rich in peripheral blood leukocytes of the whole blood, and was centrifuged and treated by EDTA.	–	Thailand		Healthy individuals who have mild diseases such as hypertension, mild cardiovascular disease and insulin-independent diabetes.	Physical and biochemical laboratory tests were assessed for inclusion criteria.	The association between folate and gene polymorphism was found significant in the overweight/obese and control groups ($p < 0.05$).	High homocysteine may cause mutation in the genes searched.
Bird et al. [4]	2015	Healthy adults (n = 3767, NHANES)	-	To determine the association between blood folate status and BMI, obesity and metabolic factors.	Serum and RBC folate levels, Bio-Rad radiological measurements, food consumption records for last 2 days.	–	USA	Serum folate, RBC, serum triglyceride, glucose level, waist circumference.		Children, adolescents, pregnant women and thin individuals.	Serum folate levels were found lower in obese individuals when compared with normal and overweight ($p < 0.001$) individuals; a positive association between BMI and RBC folate levels and a negative association with BMI and folate levels.	Dilution of blood volume adiposity affects folate absorption from folate epithelium. Lower serum folate levels may stimulate folate uptake of the RBC.
Mehmetoğlu et al. [3]	2012	Morbidly obese (n = 96) and normal weight (n = 65) individuals (21 to 60 years of age)	–	To evaluate cardiovascular risk factors including plasma w-3 FA and w-6 FA and tHcy in morbidly obese individuals.	Serum B12 and folate levels were analysed using E170 analyser. Serum tHcy HPLC.	–	Turkey	Plasma fatty acid composition, serum tHcy, vitamin B12 and folate.		Those with malign diseases, chronic liver disease, chronic kidney disease, infectious diseases, hypertension, cardiovascular disease history and those using fish oil supplements.	Serum vitamin B12 ($p < 0.01$) and folate ($p < 0.05$) levels were lower in obese individuals; furthermore, there was a positive correlation between w-3 FAs of serum folate level ($p < 0.01$).	Eating habits, genetic and autoimmune factors.
Nakazato et al. [37]	2011	Healthy adults (n = 434)		Evaluation of the association between BMI and folate and total homocysteine concentrations in the blood.	Healthy females (n = 343) and males (n = 91). Serum tHcy HPLC.	–	Japan	Plasma folate, tHcy, erythrocyte folate level		Smoking, those with severe diseases	A negative correlation was detected between BMI and plasma folate levels ($p < 0.001$); no difference was observed in BMI levels by mean erythrocyte folate and plasma tHcy.	Dilution in the blood volume, different folate levels in different tissues.

BMI: Body mass index; OGTT: Oral glucose tolerance test; RBP: Retinol-binding protein; Zn: Zinc; Ca: Calcium; P: Phosphor; Mg: Magnesium; CI: Confidence interval; G1-4: Grup 1-4; RBC folate; Red-blood cell folate; BW: Body weight DM: Diabetes Mellitus; MTHFR: Methylenetetrahydrofolate reductase; ACE: Angiotensin converting enzyme; tHcy: Total homocysteine.

Table II. Overview of Supplementation Studies Included in the Review

Human studies	Year	Sample	Study design	Aim	Method	Supplement	Duration	Country	Outcome	Inclusion	Exclusion	Results	Mechanism
Stern, S.J., Matok, I., Kapur, B., & Koren, G. [29]	2011	Obese (n = 12) Non-obese (n = 12)	Case-control	To determine the effects of single dose folate administration in normal weight and obese individuals.	Additional folate was administrated with a diet including a standard folate levels (0.06 mg). Serum folate. The folate was analysed via assay system. AUC was calculated according to trapeze rule.	In equal quantities per kg for all individuals (0.0153 to 0.0917 mg/kg) or (1.1 to 12.5 mg folate)	Single dose	Canada	Folate concentrations. Lean body mass. AUC values.	Women of reproductive age between 18 and 45 years of age. Individuals who did not have any multivitamin or folate supplements for at least 6 months.	Those with chronic diseases and those using any drug that affects metabolism and pharmacokinetics of folate.	C_{max} AUC and LBW were found higher in obese individuals (p = 0.008).	Free distribution of folate in adipose tissue may be effective in different distribution between tissues. Determination of periconceptional folate supplement dosages according to lean body mass would be more accurate in obese individuals.
Tinker, S.C., Hamner, H.C., Berry, R.J., Bailey, L.B., & Pfeiffer, C. M. [2]	2012	n = 4272	Data from cross-sectional research. NHANES 2003–2004, 2005–2006, 2007–2008	Evaluation of folate state and use of folate supplements in individuals with different BMI levels.	Supplement use within last 1 month (type, dose, frequency) was assessed. Serum and RBC folate. Bio-Rad Quantaphase II radioassay (BR) and microbiological tests were used for evaluation. Food consumption records for 24 hours were obtained through reminding technique. Folate intakes were recorded as diet folate equivalent.	Group 1: those who did not use folate supplements. Group 2: ≤ 400mcg/d folate. Group 3: > 400mcg/d folate.	–	USA	Serum folate. RBC folate. Total plasma homocysteine concentration.	Female individuals between 15 and 44 years of age	Women who did not participate in physical examination and could not provide food consumption records, Pregnant women.	Women who did not use any supplements, including folate. had a reverse association between BMI and serum folate levels. There was not any significant difference detected in those using supplements. Homocysteine levels were not significant between the groups.	An increase in BMI may change cellular folate uptake and distribution in tissues. Change in tissue distribution may result along with a decrease in folate.
Asemi, Z., Karamali, M., & Esmaillzadeh, A. [42]	2014	81 obese women	A randomized double-blind placebo controlled study	The aim of this study was to determine the effects of folate supplementation on metabolic profiles in obese women with polycystic ovarian syndrome.	The participants were divided into three groups which were selected randomly, including two study groups and one placebo group. Blood samples were collected from the participants at the beginning and end of the study.	Group 1: 1 mg/day folate Group 2: 5 mg/day folate Group 3: Placebo	8 weeks	Iran	Food consumption. Cholesterol. Vitamin B6. Folate. Vitamin B12.	Women diagnosed with PCOS according to Rotterdam criteria.	Women < 18 years of age. Women > 40 years of age. Individuals with a BMI of > 25 kg/m^2. Those who have neoplastic, hepatic, renal or cardiovascular disorders and malabsorptive disorders. Those who use hormones, antidiabetic or anti-obesity drugs.	Folate supplement of 5 mg per day was positively effective on plasma Hcy (p = 0.009), serum insulin, total-, LDL(p = 0.007)– and HDL-cholesterol (p < 0.001) levels as well as HOMA-IR level in the women with PCOS. Body weights and BMI differences of the participants were found to be lower in the study groups than the placebo group; however, this was not statistically significant.	
				Phosphatidylinositol 3-kinase is inhibited in folate deficiency. Serum insulin and insulin resistance thereby increase.									

(Continued on next page)

Table II. (Continued)

Human studies	Year	Sample	Study design	Aim	Method	Supplement	Duration	Country	Outcome	Inclusion	Exclusion	Results	Mechanism
Sheu et al. [30]	2005	Obese women (n = 74)	A randomized double-blind placebo controlled study	To determine effect of folate supplementation on serum tHcy concentrations.	Group 1: a weight-loss diet for 12 weeks + folate (n = 36) Group 2: a weight-loss diet for 12 weeks + placebo (n = 38)	Folate 5 mg	12 weeks	Taiwan	Serum folate, fasting serum homocysteine levels		Smoking	The weight loss was 7.7% in the study group and 8.9% in the control group. There was not any correlation between decrease of serum homocysteine levels and serum folate levels in obese individuals with higher homocysteine levels (p = 0.646).	
Silva et al. [13]	2013	Healthy women (n = 32, 18 to 35 years of age)	Intervention study	To assess the association between short-term oral folate administration and BMI.	Healthy obese (≥30.0 kg/ m^2 BMI, n = 16) and normal weight (18.5-24.9 kg/ m^2 BMI, n = 16) individuals.	Oral folate 400 µg	Single dose	Greece	Serum folate, red blood cell folate level	Caucasian race, those who were not pregnant during last year, did not use any supplements for last 1 month, without any chronic disease or drug use, without smoking and alcohol use, without weight loss of more than 10% during last 6 months.		Initial fasting folate levels were lower in obese individuals (p < 0.005); red blood cell folate levels were higher (p = 0.05). In general, serum folate response is lower in obese individuals (p = 0.001).	Dilution of blood volume and differences in cellular folate use are affected by larger adiposity.

AUC: Area under the time–concentration curve; LBW: Lean body weight; BMI: Body mass index; RBC folate: Red-blood cell folate; PCOS: Polycystic ovary syndrome; Hcy: Homocysteine.

that obese individuals also show lower fasting serum folate concentrations, but, paradoxically, their red blood cell (RBC) folate concentrations and MeFox (5-methyltetrahydrofolate oxidation product) are significantly higher, when compared with non-obese individuals [4, 32]. Furthermore, obesity is also found to be associated with increased activity of cytochrome P450 (CYP) 2E1, a monooxygenase enzyme that can use folic acid as a substrate [33].

Many obesity risk factors, such as being a member of black race [4], having a low birth weight/preterm birth [34], a winter (or cold weather) birth [35] or physical inactivity [36], are related to decreased sweat-gland function. This is also supported by the finding that an equivalent dose of folic acid (by body weight) caused a greater increase in serum folate in obese people than non-obese individuals [29]. Given that obesity is associated with folate-degrading enzyme CYP2E1 [33], the association of increased serum MeFox and RBC folate levels and low fasting serum folate levels in obesity may reflect a severe folic acid overload.

Nakazato et al. [37] detected a negative correlation between BMI and mean plasma folate levels in their study executed on 434 healthy individuals ($p < 0.01$). Another research revealed that folate insufficiency was 50-fold higher in obese individuals and noted an increase in folate requirements following an increase in body weight [38]. Mahabir et al. demonstrated that overweight and obese individuals have lower serum folate concentrations when compared with individuals with normal weight. Such effects are explained by increased use of folic acid, urinary excretion [39], dilution of the blood volume, different levels in different tissues and changes [37] in the endocrine functions for folate. Furthermore, the use of folate increases by methylation requirements through catechol-o-methyltransferase (COMT). The increase in oestrogen concentrations due to increased fat tissue was reported as another cause for increased folate deficiency [40]. Martinez et al. evaluated the treatment outcomes of obese individuals who had diet and exercise therapies for 1 year in their prospective study and detected that the individuals with the highest serum folate levels (7.2 ng/ml) experienced a weight loss of ≥10% from their initial body weights; this weight loss was 8.5-times more in the group with a folate level <4.7 ng/ml [24]. Another study found no significant changes in individuals' serum folate levels (n = 12) at the sixth month after gastroplasty with insufficient food intake and severe weight loss. However it was stated that the results might have been affected by the number of the individuals and the short study period [27].

In a study comparing serum B_{12} and folate levels of morbidly obese (n = 96) and normal weight (n = 65) individuals, serum B_{12} ($p < 0.01$) and folate (0.05) levels were found to be lower in the former group. Furthermore, it was also found

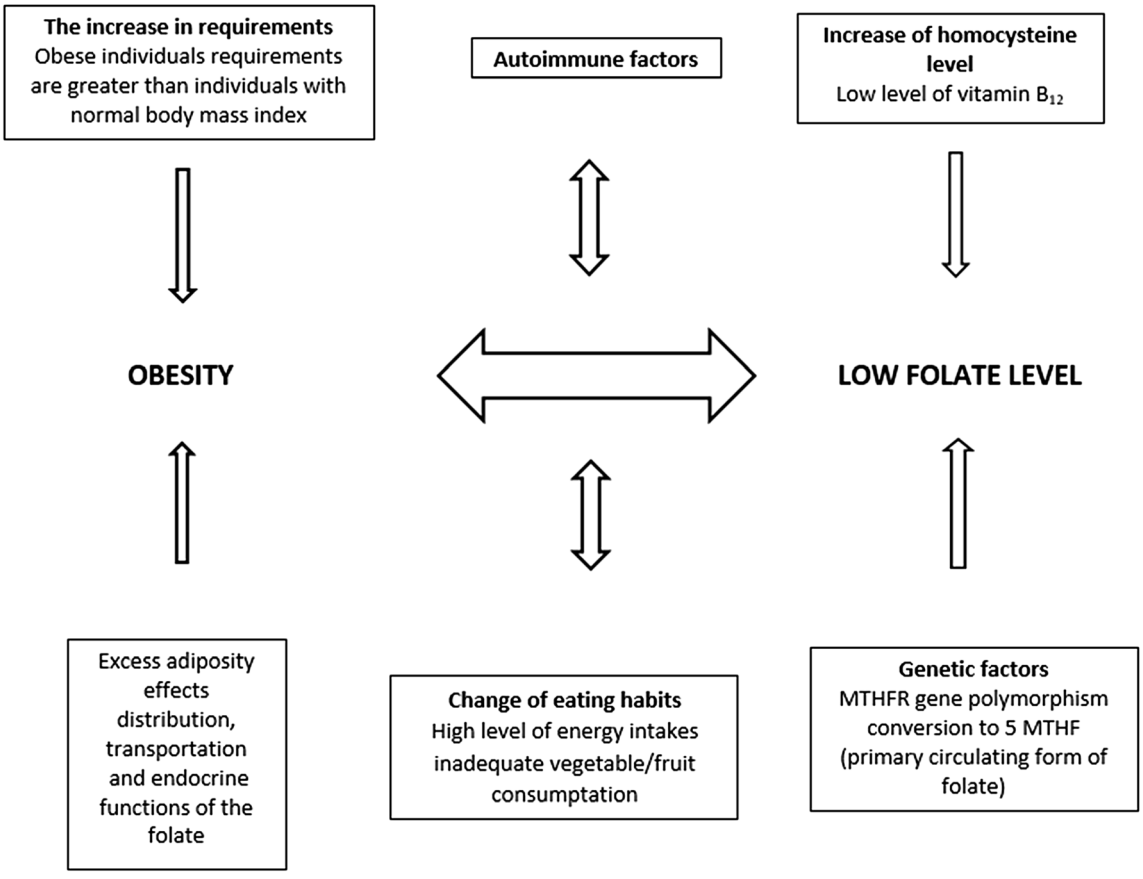

Figure 2. Mechanism between obesity and low folate status.

that obese individuals' total homocysteine levels were higher than those of normal weight individuals ($p > 0.05$) [3]. Higher homocysteine levels were generally found to be associated with abdominal obesity and insulin resistance; it is known that homocysteine is reversely associated with folate levels. Decreases in individuals' folate levels led to increased homocysteine levels [41]. Asemi et al. found that folate supplementation had positive effects on plasma homocysteine, serum insulin, total cholesterol, LDL-K and HDL-K levels and HOMA-IR. Phosphatidylinositol 3-kinase is inhibited in folate deficiency. Folate insufficiency thereby increases serum insulin and insulin resistance [42]. In another study, there was not any significant difference in plasma tHcy levels, though severely obese individuals had lower vitamin B_{12} and folate levels. Although the underlying mechanism of the finding was not clearly explained, it is possible that obese individuals prefer fatty foods instead of vegetables and fruit [8].

Serum folate levels have a positive correlation with a diet including fruits, vegetables, grains and poultry meat and a negative correlation with excessive intake of red meat, fatty milk products and refined grains [43]. Ortega et al. [28] assessed folate levels in mild overweight/obese women, applied two different hypocaloric diets and evaluated these in two groups according to vegetable ($n = 28$) and grain ($n = 29$) consumption. The first group was asked to eat breakfast cereals at least 3 times a day and the second group was asked to eat vegetables at least 3 times a day; all individuals were informed to avoid foods with high energy content. Increases in folate intake were expected by increasing the aforesaid food consumption groups. At the end of the study, the decrease in body weight was higher in the group who had enriched breakfast cereal in their diet. Furthermore, a significant increase was detected in the homocysteine and serum folate levels in the group consuming breakfast cereal. The grain group was asked to eat grains, including 250 to 300 mg/100 g folate but there was not any quantity mentioned in the vegetable group. Furthermore, homocysteine levels are affected by group B vitamins and the increase of vitamin B intake was assumed to have affected the decrease in homocysteine levels. In addition, although the vegetables and fruits were rich sources of folate, the serum folic acid level increased more in the cereal consuming group [28]. It is thought that the increase in folic acid levels in the cereal consuming group may be due to possible folic acid losses during cooking [44].

Folic acid supplementation and obesity

Along with the assessment of studies, supplementation studies also explored the relationship between body weight and folate levels. In a study of the association between BMI levels and folate levels in women of reproductive age, lower serum folate levels were found in individuals with higher BMI. Each 10 kg/m^2 increase in BMI decreased serum folate levels by 15.6%. It was predicted that obese individuals with a BMI > 30 kg/m^2 should be supported by 350 mg additional folate per day to match the folate levels of the individuals with a BMI < 20 kg/m^2 [7]. Tinker et al. [2] assessed folate levels and folate supplement use in individuals with different BMI values by investigating type, dose and frequency. A reverse association was found between BMI and serum folate levels of the women who did not use supplements, whereas no difference was detected in serum folate levels among the women with different BMI levels who used supplements [2]. These outcomes do not support the association between folate intake, folate indicators and BMI. In line with increases in BMI, increases in fat mass may affect cellular uptake and the tissue distribution of folate; this may be compensated by supplement use [44].

Silva et al. [13] assessed serum and red blood cell folate levels in 32 women according to body weight after oral folate supplementation at 400 μg/day. The initial fasting folate levels were determined lower ($p < 0.05$), red blood cell folate was found higher ($p = 0.05$) and overall serum folate responses were found lower ($p = 0.01$) in obese individuals in comparison with individuals with normal weight. This study was the first experimental study to evaluate the pharmacokinetic effect of currently recommended folate doses to obese and normal weight women at pre-reproductive age. It has not been mentioned which measurement is better. However, Folate content in RBC is known to reflect long-term average consumption and tissue stores because RBC only accumulates folate during erythropoiesis [45]. It is not clear if the lower serum folate concentrations observed in obese women were caused by diluted blood volume or by differences of serum folate uptake affected by high adiposity. The higher red blood cell folate levels observed in obese women when compared to normal weight women [2] are like those detected in the NHANES study and the results support the potential differences in cellular uptake. Folate concentrations in red blood cellsare an indicator for long term folate intake; this is considered an indicator for folate tissue concentrations [46]. Furthermore, high basal folate concentrations in the red blood cells indicated that obesity did not disrupt absorption of folate in the diet and may cause redistribution of the vitamin into the tissues [13].

Sheu et al. [30] included obese women in their study and divided participants into two groups. The researchers applied a weight loss diet to both groups and folate supplementation was applied to one group (5 mg folate for 12 weeks); they assessed the effects on serum tHcy concentrations and serum folate levels. The weight loss was 7.7% in the study group and 8.9% in the control group There was not any association between the decrease of serum homocysteine levels in obese individuals with higher homocysteine levels and serum folate levels ($p = 0.646$). Therefore, it was considered that the folate requirements of obese individuals might change depending on obesity-induced metabolic changes; this should be assessed during calculation of the requirements. Stern et al. [29] assessed the pharmacokinetic effects of single dose folate supplementation on obese individuals ($n = 12$) in their case-control study; each individual received amounts of folate supplementation for each 1 kg of body weight. Furthermore, a standard diet, including 0.06 μg folate, was implemented to individuals. The $C_{max,}$ AUC values and lean body weight (LBW) were found higher in obese individuals when compared with the control group. This was considered to be caused by free distribution of the folate in adipose tissue, which might be effective in changing distribution between tissues. It was reported that administration of the periconceptional folate supplementation dose according to the total body weight resulted in an administration higher than the requirement; therefore, calculation should be carried out according to lean body weight rather than total body weight in obese individuals [29].

Lower serum folate and vitamin B12 levels may be affected by eating habits in obese individuals when compared with normal weight individuals; genetic and autoimmune factors may also be influential (Figure 2). MTHFR irreversibly catalyses the transformation of 5,10methylenetetrahydrofolate into 5-methyltetrahydrofolate and plays a central role in folate metabolism [47]. Thawnashom et al. [21] reported that folate and gene polymorphism were significantly associated in overweight and obese individuals when compared with control groups; higher homocysteine levels might cause mutation in MTHFR genes. Settin et al. [19], in their study of MTHFR polymorphism in overweight and obese individuals, detected that individuals with higher BMI had higher MTFHR 677 T allelepolymorphism rates. ($p > 0.05$) However, such genetic polymorphism may not be associated with obesity only, but also with obesity-related diseases; further studies on this topic are needed [19].

This study is important in terms of establishing the relationship between folic acid and obesity that mainly based on the result of the negative energy balance. There are limited studies with sufficient data to support whether serum folate levels are lower in obese individuals in terms of noted

an increase in folate requirements following an increase in body weight. Moreover, these studies used different folate levels (serum, blood), which cause the evaluation of activity of folate to be difficult and these studies involve quite heterogeneous data. Therefore, larger well-designed, randomised controlled studies are needed to examine the effects of folic acid levels on obesity. The elucidation of folate and homocysteine and their mechanisms in obese individuals are also worthy for further researches.

Conclusion and Suggestions

The underlying paradox in the mechanism for folate metabolism in overweight and obese individuals has not been clearly understood. As a result of the assessment studies, it is possible that individuals with higher body mass indexes have unhealthy eating habits and prefer high energy foods rather than vegetables and fruits, which are significant sources of folate. It was found that individuals with higher body mass indexes presented symptoms of insufficiency as a result of obesity-induced metabolic changes, even when they had folate supplementation. This is important for calculating the required doses. Folic acid supplementation does not only increase folic acid levels. It is also thought that folic acid supplementation may also help to improve metabolic markers such as insulin resistance and lipid profile disorders related to obesity.

Consequently, adoption of healthy diet habits, supplementation for individuals who cannot meet the requirement with foods, detection of supplementation dose based on lean body weight and under supervision of a physician are recommended. Further studies on this topic are also needed.

References

1. Clarok, W., Brater, I., & Johnson, A. (1988) Antianemic drugs. Goth's Medical Pharmacology (pp. 3–612). St. Louis: CV Mosby Company.
2. Tinker, S.C., Hamner, H.C., Berry, R.J., Bailey, L.B., & Pfeiffer, C.M. (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 A Clin Mol Teratol. 94(10), 749–755.
3. Mehmetoglu, I., Yerlikaya, F.H., Kurban, S., & Polat, H. (2012) Plasma ω-3 fatty acid levels negatively and ω-6 fatty acid levels positively associated with other cardiovascular risk factors including homocysteine in severe obese subjects. Asia Pac J Clin Nutr. 21(4), 519–525.
4. Bird, J.K., Ronnenberg, A.G., Choi, S.-W., Du, F., Mason, J.B., & Liu, Z. (2015) Obesity is associated with increased red blood cell folate despite lower dietary intakes and serum concentrations. J Nutr. 145(1), 79–86.
5. Kimmons, J.E., Blanck, H.M., Tohill, B.C., Zhang, J., & Khan, L. K. (2006) Associations between body mass index and the prevalence of low micronutrient levels among US adults. Meds Gen Med. 8(4), 59.
6. Tungtrongchitr, R., Pongpaew, P., Tongboonchoo, C., Vudhivai, N., Changbumrung, S., Tungtrongchitr, A., & Schelp, F.P. (2003) Serum homocysteine, B12 and folic acid concentration in Thai overweight and obese subjects. Int J Vitam Nutr Res. 73(1), 8–14.
7. Mojtabai, R. (2004) Body mass index and serum folate in childbearing age women. Eur J Epidemiol. 19(11), 1029–1036.
8. Hirsch, S., Poniachick, J., Avendaño, M., Csendes, A., Burdiles, P., Smok, G., & ia de la Maza, M.P. (2005) Serum folate and homocysteine levels in obese females with non-alcoholic fatty liver. Nutrition. 21(2), 137–141.
9. Bradbury, K.E., Williams, S.M., Mann, J.I., Brown, R.C., Parnell, W., & Skeaff, C.M. (2014) Estimation of serum and erythrocyte folate concentrations in the New Zealand adult population within a background of voluntary folic acid fortification. J Nutr. 144, 68–74.
10. Fattal-Valevski, A., Bassan, H., Korman, S.H., Lerman-Sagie, T., Gutman, A., & Harel, S. (2000) Methylenetetrahydrofolate reductase deficiency: importance of early diagnosis. J Child Neurol. 15(8), 539–543.
11. Kant, A. (2003) Interaction of body mass index and attempt to lose weight in a national sample of US adults: association with reported food and nutrient intake, and biomarkers. Eur J Clin Nutr. 57(2), 249.
12. Lewis, S.J., Lawlor, D.A., Nordestgaard, B.G., Tybjærg-Hansen, A., Ebrahim, S., Zacho, J., & Smith, G.D. (2008) The methylenetetrahydrofolate reductase C677T genotype and the risk of obesity in three large population-based cohorts. Eur J Endocrinol. 159(1), 35–40.
13. Da Silva, V., Hausman, D., Kauwell, G., Sokolow, A., Tackett, R., Rathbun, S., & Bailey, L. (2013) Obesity affects short-term folate pharmacokinetics in women of childbearing age. Int J Obes. 37(12), 1608–1610.
14. Van Driel, L.M., Eijkemans, M.J., de Jonge, R., de Vries, J.H., van Meurs, J.B., Steegers, E.A., & Steegers-Theunissen, R.P. (2009) Body mass index is an important determinant of methylation biomarkers in women of reproductive ages. J Nutr. 139(12), 2315–2321.
15. Narin, F. (2005) leptin and apolipoprotein B levels in childhood obesity. Ann Saudi Med. 25(3), 209–214.
16. Strohle, A., & Bohn, T. (2015) Folate and Prevention of Neural Tube Defects: New Insights from a Bayesian Model. Int J Vitam Nutr Res. 85, 109–111.
17. Ginsburg, H.J., Mendez, R.V., & Haskar-Zolnierek, K.B. (2017) Cautionary Note About Magnitude of Decline in Neural Tube Birth Defects Following Folic Acid Fortification in the United States. Int J Vitam Nutr Res. 1(3).
18. Lawrence, J.M., Watkins, M.L., Chiu, V., Erickson, J.D., & Petitti, D.B. (2006) Do racial and ethnic differences in serum folate values exist after food fortification with folic acid? Am JObstet Gyneco. 194, 520–526.
19. Settin, A.A., Algasham, A., Dowaidar, M., & Ismail, H. (2009) Methylene tetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms related to overweight/obesity among Saudi subjects from Qassim Region. Dis Markers. 27(2), 97–102.
20. Van der Put, N.M., Gabreëls, F., Stevens, E.M., Smeitink, J.A., Trijbels, F.J., Eskes, T.K., & Blom, H.J. (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 62(5), 1044–1051.

21. Thawnashom, K., Tungtrongchitr, R., Petmitr, S., & Pongpaew, P. (2005) Methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) in relation to homocysteine concentration in overweight and obese Thais. Southeast Asian J Trop Med Public Health. 36(2), 459.
22. Rampersaud, G.C., Kauwell, G.P., Hutson, A.D., Cerda, J.J., & Bailey, L.B. (2000) Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. Am J Clin Nutr. 72(4), 998–1003.
23. Jacob, R.A., Pianalto, F.S., Henning, S.M., Zhang, J.Z., & Swendseid, M.E. (1995) In vivo methylation capacity is not impaired in healthy men during short-term dietary folate and methyl group restriction. J Nutr. 125(6), 1495.
24. Gesta, S., Blüher, M., Yamamoto, Y., Norris, A.W., Berndt, J., Kralisch, S., & Kahn, C.R. (2006) Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc Natl Acad Sci. 103(17), 6676–6681.
25. Moher, D., Liberati, A., Tetzlaff, J., & Altman, D.G. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 6.
26. Martínez, J.G., Ruiz, F.A., & Candil, S.D. (2006) Baseline serum folate level may be a predictive factor of weight loss in a morbid-obesity-management programme. Br J Nutr. 96(05), 956–964.
27. Sheu, W.H.-H., Hurng-Sheng, W., Chen-Wen, W., Chu-Jen, W., & Wen-Jane, L. (2001) Elevated plasma homocysteine concentrations six months after gastroplasty in morbidly obese subjects. Int Med. 40(7), 584–588.
28. Ortega, R., López-Sobaler, A., Andrés, P., Rodriguez-Rodriguez, E., Aparicio, A., Bermejo, L., & López-Plaza, B. (2006) Changes in folate status in overweight/obese women following two different weight control programmes based on an increased consumption of vegetables or fortified breakfast cereals. Br J Nutr. 96(04), 712–718.
29. Stern, S.J., Matok, I., Kapur, B., & Koren, G. (2011) A comparison of folic acid pharmacokinetics in obese and nonobese women of childbearing age. Ther Drug Monit. 33(3), 336–340.
30. Sheu, W.H.-H., Chin, H.-M.L., Lee, W.-J., Wan, C.-J., Su, H.-Y., & Lang, H.-F. (2005) Prospective evaluation of folic acid supplementation on plasma homocysteine concentrations during weight reduction: a randomized, double-blinded, placebo-controlled study in obese women. Life Sci. 76(18), 2137–2145.
31. Stern, S.J., Matok, I., Kapur, B., & Koren, G. (2012) Dosage requirements for periconceptional folic acid supplementation: accounting for BMI and lean body weight. J Obstet Gynaecol Can. 34(4), 374–378.
32. Pfeiffer, C.M., Sternberg, M.R., Fazili, Z., Lacher, D.A., Zhang, M., Johnson, C.L., Hamner, H.C., Bailey, R.L., et al. (2015) Folate status and concentrations of serum folate forms in the US population: National Health and Nutrition Examination Survey 2011–2. BrJ Nutr. 113(2), 1965–1977.
33. Hanley, M.J., Abernethy, D.R., & Greenblatt, D.J. (2010) Effect of obesity on the pharmacokinetics of drugs in humans. Clinical Pharmacokinet. 49(2), 71–87.
34. Casey, P.H., Bradley, R.H., Whiteside-Mansell, L., Barrett, K., Gossett, J.M., & Simpson, P.M. (2012) Evolution of obesity in a low birth weight cohort. Journal Perinatol. 32 (2), 91–96.
35. Wattie, N., Ardern, C.I., & Baker, J. (2008) Season of birth and prevalence of overweight and obesity in Canada. Early Hum Dev. 84(8), 539–547.
36. Liou, T.H., Pi-Sunyer, F.X., & Laferrere, B. (2005) Physical disability and obesity. Nutr Revi. 63(10), 321–331.
37. Nakazato, M., Maeda, T., Takamura, N., Wada, M., Yamasaki, H., Johmston, K.E., & Tamura, T. (2011) Relation of body mass index to blood folate and total homocysteine concentrations in Japanese adults. EurJNutr. 50(7), 581–585.
38. Casanueva, E., Drijanski, A., Fernández-Gaxiola, A.C., Meza, C., & Pfeffer, F. (2000) Folate deficiency is associated with obesity and anemia in mexican urban women. Nutr Res. 20 (10), 1389–1394.
39. Mahabir, S., Ettinger, S., Johnson, L., Baer, D.J., Clevidence, B. A., Hartman, T.J., & Taylor, P.R. (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(5), 644–650.
40. Goodman, J.E., Lavigne, J.A., Wu, K., Helzlsouer, K.J., Strickland, P.T., Selhub, J., & Yager, J.D. (2001) COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. Carcinogenesis. 22(10), 1661–1665.
41. Vayá, A., Rivera, L., Hernández-Mijares, A., de la Fuente, M., Solá, E., Romagnoli, M., & Laiz, B. (2012) Homocysteine levels in morbidly obese patients. Its association with waist circumference and insulin resistance. Clin Hemorheol Micro. 52(1), 49–56.
42. Asemi, Z., Karamali, M., & Esmaillzadeh, A. (2014) Metabolic response to folate supplementation in overweight women with polycystic ovary syndrome: A randomized double-blind placebo-controlled clinical trial. Mol Nutr Food Res. 58(7), 1465–1473.
43. Melse-Boonstra, A., Verhoef, P., Konings, E.J., van Dusseldorp, M., Matser, A., Hollman, P.C., & West, C.E. (2002) Influence of processing on total, monoglutamate and polyglutamate folate contents of leeks, cauliflower, and green beans. J Agr Food Chem. 50(12), 3473–3478.
44. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. (US): National Academies Press.
45. Shane, B. (2011) Folate status assessment history: implications for measurement of biomarkers in NHANES. Am JClin Nutr. 94(1), 337–342.
46. Wald, D.S., Law, M., & Morris, J.K. (2002) Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. Bmj. 325(7374), 1202.
47. Friso, S., Choi, S.W., Girelli, D., Mason, J.B., Dolnikowaki, G.G., Bagley, P.J., Olivieri, O., et al. (2002) A common mutation in the 5, 10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Nati Acad Sci USA. 99(8), 5606–5611.

Conflict of Interest
The authors declare that there are no conflicts of interest.

Süleyman Köse
Gazi University, Faculty of Health Sciences
Department of Nutrition and Dietetics
Emniyet Mahallesi, Muammer Yaşar Bostancı Caddesi
No: 16, 06500 Beşevler, Ankara
Turkey

suleyman.kose@windowslive.com

