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ARE OBESE WOMEN A RISK GROUP FOR VITAMIN B12 AND FOLIC ACID DEFICIENCIES ?

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Abstract

Background and Aims: *To evaluate the relationship between vitamin B_{12} and folic acid levels of overweight and obese women along with insulin resistance.* ***Materials and Methods:*** *A total of 384 women with normal weight (n=72) overweight (n=125) and obese (n=187) were included in the study. Body weight and height were measured and BMI was calculated. Obesity classification was made according to WHO criteria. Biochemical parameters (serum fasting blood glucose, fasting blood insulin, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol) were collected, and HOMA-IR was calculated.* ***Results:*** *Although not statistically significant, vitamin B_{12} levels were lower in obese women than overweight and normal weight women. Serum folic acid levels of the women with insulin resistance were significantly lower, whereas there was no significant difference between B_{12} levels of obese and overweight women with and without insulin resistance. There was a weak negative correlation between BMI and vitamin B_{12} levels. While a weak negative correlation was detected between folic acid, HOMA-IR and fasting insulin levels, no such correlation was observed between B_{12}, HOMA-IR and fasting insulin levels.* ***Conclusion:*** *Our data suggest that overweight and obesity are risk factors for vitamin B_{12} deficiency in women that should be routinely tested in these individuals.*

key words: *obesity, vitamin B_{12} deficiency, folic acid deficiency*

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Background and Aims

Obesity is a globally significant public health problem. Epidemiologic researches showed that increased body weight and abdominal body fat accumulation cause metabolic and cardiovascular diseases such as type 2 diabetes, insulin resistance (IR), dyslipidemia, hypertension, and coronary heart disease [1]. World Health Organization (WHO)

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reported in 2015 that worldwide 39.0% of adults are overweight and 13.0% are obese [2]. According to the data obtained from Turkey Nutrition, Health and Food Consumption research conducted in 1974, obesity prevalence was 7.6% for men, and 25% for women, while the prevalence reported in 2010 raised to 20.5% and 41.0%, respectively in a similar countrywide research [3,4].

Although the total energy intake of overweight and obese individuals is sufficient, micronutrient element consumption may be low because of inadequate intake and/or nutrient element absorption metabolism [5-7]. In recent studies, especially vitamin D, vitamin B_{12} and folic acid deficiency have been detected in obese individuals [8-10].

Vitamin B_{12} is an essential vitamin found in enriched nutrients and in nutrients of animal origin has an important role in terms of DNA synthesis, optimal hematopoiesis and neurological functions [11]. On the other hand, folic acid found in green-leafy vegetables, legume, some fruits and enriched grains is responsible for nucleic acid synthesis and interconversion of some amino acids by functioning in single carbon metabolism [12-13].

In folic acid deficiency, homocystein level increases, whereas hyperhomocysteinaemia may emerge in vitamin B_{12} insufficiency since B_{12} plays a role as a cofactor in methionine to homocystein synthesis [14]. Hyperhomocysteinaemia is reported to be associated with IR and cardiovascular disease development [15].

For these reasons, concerns similar to those of folic acid deficiency come into question for B_{12} insufficiency. Moreover, B_{12} levels should be evaluated in order to prevent potential complications in obese individuals [16].

Despite the fact that there are studies in the literature about this subject, vitamin B_{12} and folic acid levels of overweight and obese individuals and their relationship with IR have not been clearly put forward yet. This research is planned with the purpose of determining the relationship between vitamin B_{12} and folic acid levels of overweight and obese women in the reproductive age group and anthropometric measurements and IR.

Materials and methods

Study population

This cross-sectional study was conducted between March 2012 and May 2013. A total of 384 women, aged 20-49 years, who attended the Outpatient Clinics of İzmir Bozyaka Training and Research Hospital of Internal Medicine, Endocrinology and Diet were included in the study.

Exclusion criteria

Women having any of the following conditions were excluded from the study: women younger than 20 and older than 49 years, vegans, individuals using corticosteroid, metformin, vitamin-mineral support and proton-pump inhibitor and individuals with diabetes mellitus.

Written consent was obtained from all participants at the beginning of study which was approved by the Ethics Committee of the Faculty of Medicine, İzmir Katip Celebi University, İzmir, Turkey (Approval number 03/07/2014-135).

Anthropometric Measurements

All anthropometric measurements were performed without shoes and with clothes as light as possible. Body weight was measured with a regularly calibrated TANİTA TBF300 (Tanita Corporation®, Tokyo, Japan), body height was measured using a tape measure with women standing barefoot, keeping their shoulders in a relaxed position, arms hanging freely and head in Frankfort horizontal plane. Body Mass Index (BMI) was calculated using the weight/height formula in kg/m^2 units. Weight status was evaluated according to the classification of WHO as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥ 30 kg/m^2) [17].

Waist (WC) and hip circumferences (HC) were measured while the individuals were standing, arms were open on both sides and feet were together. WC was measured with a non-elastic measuring tape as the circumference of the midpoint between the lowest rib and iliac crest. WHO WC≥88 cm for women, was classified as abdominal obesity [17]. HC was determined from the highest point of the side of the hip. Waist/hip ratio (WHR) was calculated from the values of WC and HC. WHO WHR ≥0.85 for women, was considered to diagnose abdominal obesity [17].

Assessment of biochemical parameters

Following an 8-hour overnight fast, blood samples were collected between 08.30 and 10.30 am. Blood tests including serum fasting blood glucose (FBG), fasting blood insulin (FBI), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), vitamin B_{12} and folic acid levels were analysed in the Izmir Bozyaka Training and Research Hospital Laboratory. Serum FBG, TG, HDL-C, and LDL-C were determined with kits using Architect c16000 autoanalyzer (Abbott Diagnostics®, USA). Vitamin B_{12} and folic acid levels were assayed using the solid-phase chemiluminescent immunometric assay and competitive immunoassay (IMMULITE® 2000, Siemens Medical Solutions Diagnostics, Flanders), respectively. FBI was measured using commercial radioimmunoassay kits (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). We used 3 descriptive cutoff points for the status of vitamin B_{12}, as suggested by Tucker et al. [18]. Accordingly, the current clinical cutoff was 200 pg/mL (148 pmol/L), the intermediate point was 250 pg/mL (185 pmol/L), and a point at which individuals may be at risk of deficiency was 350 pg/ mL (258 pmol/L), although further testing is then needed.

In this study, IR was determined with the "Homeostasis model assessment IR index (HOMA-IR)" method using the [(FBG x FBI)/405] formula. HOMA-IR ≥2.7 is accepted as an IR indicator [19].

Statistical analysis

Data were evaluated using SPSS 21.0 software. Descriptive statistics (average, mean, standard deviation) were used in the evaluation of the quantitative data, while frequency distribution was used for qualitative data. For assessing the differences among anthropometric measurements, biochemical data, B_{12} and folic acid levels of individuals classified according to WHO obesity criteria, ANOVA was used for normally distributed data, whereas Kruskal-Wallis or Mann Whitney U tests were used for the non-normally distributed data. When an overall significance was observed, pairwise post-hoc tests were performed using Tukey's test for normally distributed data, whereas the Mann-Whitney U test was used for the non-normally distributed data to test the significance of differences between the groups.

The relation of the individuals' B_{12} and folic acid concentrations with BMI, HOMA-IR and FBI levels was analysed using the Pearson correlation coefficient. The results were assessed in 95% confidence interval on a p significance level and considered statistically significant at $p<0.05$.

Results

The mean age of women in normal weight (BMI= 18.5-24.9 kg/m^2), overweight (BMI= 25.0- 29.9 kg/m^2) and obese (BMI= ≥30 kg/m^2) were 30.2±7.14, 31.6±7.58 and 34.7±7.77 years, respectively. The biochemical parameters and anthropometric measurements which are classified according to BMI values are presented in Table 1. According to Table 1, 18.8% (n=72) of individuals were normal weight whereas 32.5% and 48.7% of them were overweight (n=125) and obese (n=187). WC, HC and WHR of obese and overweight women were higher than in women with normal weight (p<0.001).

While FBG, FBI, HOMA-IR and TG levels were significantly lower in normal-weight women than their overweight and obese fellows, HDL-C levels were observed to be significantly higher in normal-weight women. Although statistically non-significant, the level of LDL-C was lower in women with normal weight (p>0.05). Similarly, vitamin B_{12} levels were lower in obese women than overweight and normal weight women (p>0.05).

Table 1. Biochemical parameters and anthropometric measurements of individuals according to BMI status.

	Normal Weight (n=72)	Overweight (n=125)	Obese (n=187)	p
	Mean±SD	Mean ±SD	Mean ±SD	
Age (year)	30.2±7.14^{c}	31.6±7.58^{c}	34.7±7.77^{a,b}	<0.001*
Anthropometric Measurements				
BMI (kg/m^2)	22.7±1.81^{b,c}	27.3±1.45 ^{a,b}	35.5±4.79^{a,b}	<0.001**
WC (cm)	80.2±7.30^{b,c}	91.2±6.21^{a,c}	104.5±9.86 ^{a,b}	<0.001**
HC (cm)	102.7±5.72^{b,c}	107.9±5.78^{a,c}	120.6±10.3^{a,b}	<0.001**
WHR	0.8±0.06	0.8±0.05	0.9±0.07	0.005
Biochemical Status				
FBG (mg/dL)	77.6±26.94^{b,c}	83.0±23.04^{a,c}	89.3±18.16^{a,b}	<0.001**
FBI (µU/mL)	6.7±3.09^{b,c}	8.2±3.91^{a,c}	11.9±7.44^{a,b}	<0.001**
HOMA-IR	1.3±0.81^{b,c}	1.7±0.98^{a,c}	2.6±1.92^{a,b}	<0.001*
TG (mg/dL)	85.3±40.35^{b,c}	103.0±50.2^{a,c}	125.5±75.2^{a,b}	<0.001**
LDL-C (mg/dL)	109.4±31.39	116.6±34.4	119.0±40.47	0.186
HDL-C (mg/dL)	60.8±13.20^{b,c}	55.9±13.34^{a,c}	49.8±11.84^{a,b}	<0.001*
Vitamin B_{12} (pg/mL)	234.5±102.70	219.3±36.89	207.8±88.98	0.128
Folic acid (pg/mL)	8.9±4.41	9.3±4.05	9.0±3.65	0.786

*SD-standard deviation, *ANOVA (p<0.05) Tukey HSD Test, **Kruskal-Wallis (p<0.05) Mann Whitney U test*
^{a} for Normal weight, ^{b} for Overweight, ^{c} for Obese

Vitamin B_{12} deficiency (<100 pg/mL) was present in 4.3% of the obese and 1.4% of the normal-weight. In addition, vitamin B_{12} insufficiency (100-200 pg/mL) was detected in 41.7% of women in normal weight and 48.7% of the obese. However, the difference between the three groups is not statistically significant as shown in Table 2 (p>0.05).

Table 2. Vitamin B_{12} levels of participants according to the BMI group

	Normal Weight (n=72)		Overweight (n=125)		Obese (n=187)		p
	n	%	n	%	n	%	
Vitamin B_{12} (pg/mL)							
Deficiency (<100 pg/mL)	1	1.4	3	2.4	8	4.3	
Inadequate (100-200 pg/mL)	30	41.7	61	48.8	91	48.7	0.512
Adequate (≥ 200 pg/mL)	41	56.9	61	48.8	88	47.0	

Pearson chi-square (p<0.05)

In Table 3, vitamin B_{12} and folic acid levels in the overweight and obese group were compared according to the status of HOMA- IR. Serum folic acid levels of the women with IR were reported to be significantly low whereas there was no significant difference between B_{12} levels of obese and overweight women with and without IR ($p<0.001$) as shown in Table 3.

Table 3. Vitamin B_{12} and folic acid concentration according to HOMA-IR status in women with obesity and overweight.

	HOMA-IR		p
	<2.7 (n=228)	≥2.7 (n=84)	
	Mean±SD	Mean±SD	
Vitamin B_{12} (pg/mL)	205.3±78.95	221.1±107.11	0.324
Folic acid (pg/mL)	9.5±4.03	7.6±2.70	<0.001*

**Mann-Whitney U (p<0.05)*

There was a weak negative correlation between BMI and B_{12} levels (r= -0.113, p=0.028); however, no correlation was encountered between folic acid and BMI (r=-0.029, p=0.570) as shown in Table 4. While a weak negative correlation was detected among folic acid, HOMA-IR (r=-0.163, p=0.002) and FBI (r=-0.219, p<0.001), no such correlation was observed among B_{12}, HOMA-IR (r=-0.012, p=0.827) and FBI levels (r=-0.007, p=0.894).

Table 4. Correlation between vitamin B_{12} and folic acid concentration with BMI, HOMA-IR and FBI level.

	BMI (kg/m^2)		HOMA-IR		FBI (µU/ml)	
	r	p	r	p	r	p
Vitamin B_{12} (pg/mL)	-0.113	0.028*	0.012	0.827	0.007	0.894
Folic acid (pg/mL)	-0.029	0.570	-0.163	0.002	-0.219	<0.001*

**Pearson chi-square (p<0.05)*

Discussion

In this study in which we included 384 women of reproductive age, no statistically significant difference was detected between B_{12} and folic acid levels of normal-weight women and their obese and overweight fellows. However, a negative correlation was observed between B_{12} and BMI, folic acid and HOMA-IR and folic acid and FBI. In spite of this, no correlation was reported between folic acid level and BMI whereas folic acid levels of the individuals with IR was detected to be significantly lower than in those without IR.

Studies conducted on this subject indicate that vitamin and mineral levels (particularly water-soluble B_{12}, folic acid and vitamin C) of overweight and obese individuals are significantly lower than normal-weight ones [20-22]. It is speculated that wrong feeding habits, common among the obese, cause vitamin and mineral levels to be low [16].

It is known that there is an increase in cytochrome P450 activity in obesity which uses folic acid as a substrate [23]. Also, increased serum MeFox (5-methylenetetrahydrofolate oxidation product) level in the obese supports the increased degradation of folic acid [24]. Studies conducted on the obese demonstrate that although their folic acid levels are low, folate and MeFox levels in their red blood cells are higher than those of non-obese individuals [25,26]. While BMI has a converse relation with unmetabolised folic acid and other serum folate forms (5-methyltetrahydrofolate, non-methyl folate, 5-formyltetrahydrofolate, 5,10-methenyl-tetrahydrofolate), it is positively associated with serum MeFox and red blood cells folate levels [26].

Bradbury et al. [27] reported an 1% decrease in serum folate concentration as a result of each 1 unit BMI increase. Similarly, Ortega et al. observed that folic acid levels of obese women were higher than that of their non-obese fellows although they consume same amounts of folic acid [28]. Different from these studies, Aasheim et al. stated in their research on the morbidly obese that B_{12} and folic acid levels were not different from those of the normal-weight, which is similar to our study [20].

IR is a "metabolic attack", diseases associated with IR triggering a proinflammatory state. Not every obese individual has IR [29,30]. In addition to this, the relationship between folic acid and IR is not clear [31]. In this study, it was observed that folic acid levels of individuals with IR were significantly lower than those of patients without IR, whereas some studies do not detect any difference between the folic acid levels of individuals with and without IR [10,32].

Vitamin B_{12}, whose main source is foods of animal origin, is a required nutrient also known as cobalamin. Decreased consumption, abnormal nutrient absorption and inborn errors of B_{12} metabolism cause vitamin B_{12} insufficiency which is linked with hematologic, neurological and psychiatric manifestations [33]. Moreover, hyperhomocystinemia, which is an independent risk element of atherosclerotic disease, is a result of vitamin B_{12} deficiency [34]. In the study of Sánchez et al. [9], a B_{12} deficiency prevalence of 10.6% was detected in the obese, whereas whereas de Luis [8] observed a frequency of 9.5%.

We preferred to use a classification for vitamin B_{12} levels put forward by Tucket et al. [18] for it was majorly used in clinical practices. This classification indicates that the majority of the overweight and obese patients were below the vitamin B_{12} level cutoff value. Observing the 3 vitamin B_{12} level cutoff values, we encountered a significant variation in the number of women between the groups. This study detected 48.8% B_{12} deficiency in overweight and 53.0% in obese women, which are higher than the studies conducted in other countries. In Baltaci et al.'s [10] study in Turkey, similar to this one, B_{12} deficiency was detected in 37.7% of the overweight individuals and in 40.1% of the obese ones.

Although overweight and obese women were detected to have lower levels of B_{12} than their normal-weight fellows in this study, the difference between the groups was not statistically significant. Likewise, obese and overweight individuals among Thai adults demonstrated no statistically significant difference regarding their B_{12} concentrations compared to normal control subjects [35]. On the other hand, Reitman et al. [36] worked on vitamin B_{12}, plasma antioxidants and homocysteine levels in obese individuals; however, he could not detect a difference in B_{12} levels between the obese and the lean.

Like the one between folic acid and IR, there is no clear link between vitamin B_{12} levels and IR. Kaya et al. [32] carried out a research on the connection between IR and plasma vitamin B_{12} levels of women with polycystic ovary syndrome. What they found was that obese women with IR had lower levels of vitamin B_{12} than those without IR.

Similar to the research in which B_{12} levels of middle-aged women with and without metabolic syndrome were analysed [37], this study reported no difference in B_{12} levels of women with and without IR and detected no correlation between B_{12} levels and IR.

Similar to the study of Vaya et al. [38], this one detected that overweight and obese women had statistically higher WC, FBG, FBI, HOMA-IR and TG levels than their normal-weight fellows. However, no statistically significant difference was observed between the groups in terms of LDL-C levels. Vaya et al. [38] indicated that folic acid and FBG levels and WC measurement were connected with homocystein level which is related to IR. In this research, homocystein level was not focused on; nevertheless, a similar relation between folic acid and IR was reported.

Since the majority of the applicants of the diet polyclinic are women and they requested attendance, this study is conducted only on women. This is a limitation of the study. The results can't be generalized and the recommendations only apply to women. The other limitations of our study are: the number of normal-weight indviduals is lower than the obese ones, mean age among the groups is statistically different and serum homocysteine level was not analysed.

Conclusion

The results of our study show that being overweight or obese is a risk factor for vitamin B_{12} deficiency in females and suggest that overweight and obese individuals with IR should be evaluated in terms of vitamin B_{12} deficiency. In addition, we showed there is no correlation between folic acid level and IR thus contesting that folic acid supplementation can increase insulin sensitivity.

Conflicts of interest. The authors declare no conflicts of interest.

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