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VITAMIN DEFICIENCY AND INSULIN RESISTANCE IN NONDIABETIC OBESE PATIENTS

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Abstract

Objective. Obese people may have nutritional deficiencies, although they are exposed to excessive food intake. We aim to assess relationship of vitamin D, B12, and folic acid levels and dietary vitamin intake and insulin resistance in obese people.

Design. This case-control study was performed at the obesity outpatient clinics between March 2014 and April 2015.

Subjects and Methods. We included 304 non-diabetic obese subjects in patient group and 150 normal weight individuals in control group. Patients were questioned in detail about their food intake.

Results. Mean age of obese patients was 37.3±10.1 years, the mean duration of obesity was 7.9±5.4 years, and the percentage of female patients was 65.8%. Mean vitamin D, B12, and folic acid levels were significantly lower in patients than in controls. Vitamin D deficiency (<20 ng/mL) in 69.7%, vitamin B12 deficiency (<200 pg/mL) in 13.5%, and folic acid deficiency (<4 ng/mL) was found in 14.2% of the patients. BMI negatively correlated with vitamin D, B12, and folic acid levels. B12 levels negatively correlated with duration of obesity. Insulin resistance was found in 55.9% of patients and HOMA-IR levels negatively correlated with vitamin D and B12 levels. While dietary vitamin D and folic acid intakes were inadequate in all of patients, only 28.3% of patients had inadequate vitamin B12 intake. There was no relation between vitamin levels and dietary vitamin intakes.

Conclusions. The study reveals that vitamin D, B12, and folic acid levels were low and poor vitamin D and B12 status were associated with insulin resistance in nondiabetic obese patients.

Key words: Obesity, Vitamin D deficiency, Vitamin B deficiency, Folic Acid deficiency, Diet, Insulin Resistance.

INTRODUCTION

Obesity is a significant risk factor for chronic life-threatening conditions. The most important determinants for the development of obesity include a sedentary life style, low socioeconomic status, and excessive consumption of high-calory and low fiber foods (1-5). Recent studies have shown that obesity and metabolic syndrome rates have increased in the past decade (2, 5, 6).

Deficiency of several vitamins, iron, and iodine is a common health problem in developing countries (7-10). As obesity has become more prevalent, the rate of vitamin and micronutrient deficiency has increased even in developed countries. In a recent meta-analysis, the prevalence of vitamin D deficiency was reported to be 35% higher in obese people compared to nonobese individuals (11). Low levels of vitamin D have also been found to be associated with diabetes and metabolic syndrome (12, 13). Furthermore, improvement of insulin resistance and glycemic parameters has been shown following vitamin D replacement as well as folate and vitamin B12 therapy (14, 15). Any causal relationship between vitamin deficiency and obesity has not been clarified. Studies of obesity and vitamin deficiency in adults have been mostly conducted in patients before and after bariatric surgery (16-18).

There are only limited studies investigating association between intake of naturally vitamin containing foods and vitamin deficiency in obesity (19-21). The objective of the present study was to compare the serum vitamin D, vitamin B12, and folic acid levels of nondiabetic obese patients and nonobese healthy controls, and to investigate the relationship between vitamin deficiencies, insulin resistance, and glucose metabolism. We also evaluated dietary vitamin intakes in the obese patients in order to determine any role of nutrition on vitamin levels in obesity.

METHODS

Study population

We conducted the study at the obesity

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outpatient clinics of Baskent University in Istanbul, between March 2014 and April 2015. The study included 304 nondiabetic obese patients (body mass index [BMI] ≥30 kg/m^2) aged 18 to 55 years old as the patient group; and 150 age and sex-matched normal weight nondiabetic individuals (BMI, 18.5 to 24.9 kg/m^2) as the control group. The patient group was divided into the following subgroups; obese and morbidly obese (BMI ≥40 kg/m^2).

Exclusion criteria in the study were having chronic disease (diabetes, Cushing syndrome, malabsorption disorders, inflammatory bowel disease, liver and/or kidney failure), bariatric surgery, alcohol addiction, pregnancy, vegetarian diet or protein restricted diet and the use of vitamin supplements or regular medications such as metformin, orlistat, proton pump inhibitors, anticonvulsants, oral contraceptives, corticosteroids, or chemotherapeutic agents. All study subjects were in the euthyroid state and had normal calcium concentrations.

We collected information on previous medical and family history, tobacco use, and diet and exercise habits for all subjects. Regular exercise was defined on the basis of aerobic and/or strength exercises at least 30 to 60 minutes a day, 3 to 5 times a week during the last 4 weeks. During the initial visit, the investigators performed a physical examination, anthropometric measurements, and collected a blood sample for biochemical analyses.

Measurements of anthropometric parameters

We used a body composition analyzer to measure weight in light clothing and barefoot (Tanita BC- 418 MA; Tokyo, Japan), a stadiometer to measure height to the nearest 0.5 cm, and a computer to calculate the Body mass index (BMI) [weight in kilograms divided by height in square meters (kg/m^2)]. To define obesity we used the WHO criteria (22). Body fat percentage was calculated by bioelectric impedance analysis method using the body composition analyzer.

To measure systolic (sBP) and diastolic (dBP) blood pressure we used both arms in sitting position after a 15-min of rest, and higher measurement was taken for all subjects. Measurements for sBP≥140 mmHg, dBP≥90 mmHg, or the use of antihypertensive agents were defined as hypertensive state. Serum LDL-cholesterol levels of ≥130 mg/dL and triglyceride levels of ≥150 mg/dL, and/or on statin or fibrate therapy were defined as the Hyperlipidemic state.

Assay of vitamin levels and laboratory parameters

According to the WHO criteria, male subjects with hemoglobin levels <13.5 g/dL and female subjects with hemoglobin levels <12 g/dL were defined as having hemoglobin deficiency or anemia (23).

Vitamin D [25 (OH) D] levels were evaluated according to seasonal differences. Serum 25(OH) D levels, <20 ng/mL (50 nmol/L) were classified as deficiency, 20–30 ng/mL as insufficiency and ≥30 ng/mL (80 nmol/L) as sufficiency (24, 25). Patients with serum vitamin B12 level <200 pg/mL (148 pmol/L) were defined as having vitamin B12 deficiency (26, 27). Based on the literature, vitamin B12 status was categorized as marginal if vitamin B12 between 200-400 pg/mL and as adequate if ≥400 pg/mL (26). Patients with folic acid level <4 ng/mL (10 nmol/L) were defined as having folic acid deficiency (27). To calculate insulin resistance we used the HOMA-IR (Homeostasis Model of Assessment-Insulin Resistance) score [fasting glucose (mg/dL) x fasting insulin (μU/mL) / 405], and patients with a HOMA-IR score ≥2.7 were classified to have insulin resistance (IR) (28).

An at least 8-hour overnight fasting was necessary to collect blood samples by venipuncture. The same biochemistry laboratory was responsible to analyze all of the collected blood samples. Serum 25(OH)D levels were measured with a chemiluminescent immunoassay method (Architect i1000system, Abbott, USA) with normal laboratory ranges of 15.7–60.3 ng/mL (summer) and 8.8–46.3 ng/mL (winter); and the intra-assay and inter-assay coefficients of variation were 2.6-4.0%. Serum B12 levels were measured with a chemiluminescent immunoassay method (Architect i2000system, Abbott, USA); normal ranges were 187-883 pg/mL and intra- and inter-assay CV were 3.4% and 8%, respectively. Serum folic acid levels were measured with a chemiluminescent immunoassay method (Architect i2000system, Abbott, USA); normal ranges were 3.1-20.5 ng/mL and intra- and inter-assay CV were 1.5% and 4.0%, respectively.

Serum insulin levels were analysed by chemiluminescent immunoassay (Architect i2000 system, Abbott, USA); normal range 2.6–24.9 mU/mL and intra-assay CV 2.3-4.2%. Levels of Hs-CRP were measured with an immunoturbidimetric assay (C8000 system Abbott, USA); normal level, <0.5 mg/dL, intra- and inter-assay CV 0.65-4.00% and 0.26-2.38 %, respectively. Serum glucose levels were measured by enzymatic colorimetric assay (C8000 system Abbott, USA), intra- and inter-assay CV were 0.65-1.98% and

0.84-0.93 %, respectively. HbA1c was detected with a turbidimetric assay method (C4000, Architect cSystem Abbott, USA), intra- and inter-assay CV were 0.77-0.88% and 1.45-1.88 %, respectively.

Blood cell count was determined using an automated blood cell counter (Cell-Dyn 3700, Abbott, USA). Serum PTH levels measured by electrochemiluminescent immunoassay method (Architect i2000 system) normal range 15–68 pg/ml; intra-assay CV3.0-6.5%. TSH was measured with a chemiluminescent immunoassay method (CMIA) (Architect i2000 system, Abbott, USA), intra-assay CV 4.3%. Serum calcium (Ca) levels were measured with an enzymatic colorimetric assay (C8000 system); intra- and inter-assay CV were 0.5-0.6% and 0.3-0.5%, respectively.

Assessment of food intake in patient group

Patients recorded the type and quantity of food and liquid intake in a 3-day food log (two weekdays and one weekend within a single week). They also completed a food consumption questionnaire developed for the purpose of this study, which included specific questions regarding consumption of each food group based on the intake frequency and specified portion size.

Adequate food intake amounts were defined according to the recommendations of the US Food and Nutrition Board (29). Adequate food intake was defined as consumption of 20 to 30 g/day of fiber, five or more servings of fruit and vegetables per day, 1-2 portions from the meat group per day (60-90 g/day), 2-3 servings of dairy products per day (400-600 g/day) and 2-4 portions of leguminosae per week. The Dietary Reference Intake, or daily requirement, for vitamin D is 5 µg/day, for vitamin B12=2.4 µg/day, for folic acid 400 µg/day, for calcium 1000-1200 mg/day, for iron 10-15 mg/day, and dietary fiber intake 25-30 g/day for adults (29).

Nutrient Data Base (BEBIS) Program (EBISpro for Windows; Stuttgart, Germany; Turkish version BeBis, Version 5, 2006) was used to analyze the patient's nutrient intake (30). The results were defined as adequate or inadequate nutrient and vitamin intake.

Ethical approval

The Baskent University Institutional Ethics Committee and Institutional Review Board approved the study (Project No: KA13/177). All subjects gave written informed consent.

Statistical analysis

Statistical analysis was conducted using the SPSS 18.0 version. Student T test, Fisher's exact test and Spearman correlation test were used for comparisons and correlations between two variables. Data are expressed as mean ± SD. P-value ≤0.05 was considered as statistically significant.

RESULTS

Mean age of the nondiabetic obese patients was 37.3±10.1 years (range 17-55), the mean duration of obesity was 7.9±5.4 years (range 1-30), and the percentage of female patients was 65.8% (n=200). Mean BMI was 35.8±6.2 kg/m^2 in patients and 22.7±2.0 kg/m^2 in controls (p<0.001). There was no significant difference in age, gender, smoking status, exercise habit, and family history of coronary heart disease between obese patients and controls. Insulin resistance (HOMA-IR≥2.7) was found in 55.9% (n=170) of nondiabetic obese patients and none of the controls. The anthropometric parameters and demographic features in the study group are shown in Table 1. The comparison of laboratory findings between patients and controls is

Table 1. Demographics and comorbidities of nondiabetic obese patients and normal weight control subjects

	Obese (n=304)	Control (n=150)	*p*
Age (year, mean ± SD, range)	37.3±10.5	37.1±9.5	0.886
Gender (F/M, n)	200/104	112/38	0.055
BMI (kg/m^2)	35.8±6.2	22.7±2.1	**<0.001**
Body-fat (%)	40.3±9.3	26.2±7.3	**<0.001**
Systolic blood pressure (mmHg)	130.1±17.6	115.8±12.7	**<0.001**
Diastolic blood pressure (mmHg)	82.2±9.7	74.5±8.2	**<0.001**
Hypertension (n, %)	74 (24.3)	11 (7.4)	**<0.001**
Hyperlipidemia (n, %)	88 (28.9)	21(14.1)	**<0.001**
Family history of diabetes (n, %)	176 (57.9)	71 (47.7)	**0.025**
Family history of coronary heart disease (n, %)	87(28.6)	33(22.1)	0.087
Regular exercise (n, %)	48 (15.8)	21 (14)	0.363
Current smoker (n, %)	85(28)	38 (25.3)	0.317

Table 2. Comparison of biochemical laboratory findings in nondiabetic obese patients and controls

	Males			Females			Total		
	Obese (Mean ±SD) (n=104)	**Control (Mean ±SD) (n=38)**	***p***	**Obese (Mean ±SD) (n=200)**	**Control (Mean ±SD) (n=112)**	***p***	**Obese (Mean ±SD) (n=304)**	**Control (Mean ±SD) (n=150)**	***p***
Fasting plasma glucose (mg/dL)	97.2±8.9	92.5±6.7	**0.004**	94.6±8.5	89.9±7.3	**<0.001**	95.5±8.7	90.6±7.2	**<0.001**
Fasting plasma insulin (µU/mL)	16.0±7.1	9.2±3.1	**<0.001**	13.4±6.8	7.2±3.4	**<0.001**	14.2±6.7	7.7±3.4	**<0.001**
HOMA-IR* index	3.8±1.9	2.1±0.1	**<0.001**	3.1±1.7	1.6±0.8	**<0.001**	3.4±1.8	1.7±0.8	**<0.001**
HbA1c (%)	5.5±0.4	5.4±0.3	0.080	5.4±0.8	5.2±0.4	**<0.001**	5.4±0.4	5.2±0.4	**<0.001**
Total-chol (mg/dL)	205.5±36.9	212.2±45.3	0.499	204.1±43.9	211.2±44.9	0.394	204.6±41.3	211.6±44.7	0.277
LDL-chol (mg/dL)	135.9±33.3	135.9±36.7	0.998	130.0±35.2	125.6±40.7	0.338	132.1±34.6	128.5±39.8	0.365
HDL-chol (mg/dL)	39.1±6.9	46.8±8.4	**<0.001**	48.1±11.8	58.4±10.3	**<0.001**	45.0±11.3	55.3±11.1	**<0.001**
Triglyceride (mg/dL)	163.2±81.5	116.3±51.6	**<0.001**	119.7±66.2	85.5±40.9	**<0.001**	134.6±74.6	93.8±46.0	**<0.001**
Alanine aminotransferase (U/L)	42.4±23.9	26.6±14.9	**<0.001**	20.9±10.3	15.1±6.6	**<0.001**	28.3±19.3	18.1±10.7	**<0.001**
Creatinine (mg/dL)	0.85±0.9	0.89±0.1	**0.030**	0.71±0.1	0.70±0.1	0.727	0.75±0.1	0.75±0.1	0.881
Uric acid (mg/dL)	6.9±1.2	5.6±1.3	**<0.001**	5.3±4.0	4.1±0.7	**0.020**	5.9±3.3	4.6±1.2	**<0.001**
TSH (mIU/L)	1.9±0.9	1.6±1.0	0.128	2.2±1.9	1.9±1.5	0.270	2.1±1.7	1.9±1.4	0.171
Hs-CRP (mg/L)	4.9±4.6	1.6±1.9	**<0.001**	7.8±8.1	1.5±1.5	**<0.001**	6.7±7.1	1.5±1.6	**<0.001**
PTH (pg/mL)	45.6±19.1	38.9±8.0	0.392	53.3±24.6	41.6±13.2	0.053	51.5±23.5	41.0±12.0	**0.032**
Calcium (mg/dL)	9.6±0.4	9.6±0.4	0.882	9.3±0.3	9.5±0.4	0.051	9.4±0.4	9.5±0.4	0.149
Ferritin (ng/mL)	124.5±80.6	145.8±110.8	0.311	34.3±33.3	27.3±24.4	0.063	62.4±67.1	53.5±74.4	0.274
Hematocrit (%)	45.4±2.9	44.6±3.9	0.258	39.4±2.8	38.9±2.5	0.206	41.4±4.0	40.4±3.8	**0.013**
Hemoglobin (g/dL)	15.3±0.9	14.9±1.3	0.136	13.0±1.0	13.1±0.9	0.705	13.8±1.5	13.6±1.3	0.073
	Case-control cross-tabulation						**Obese (n, %)**	**Control (n, %)**	***p***
Hemoglobin/Female				**≤12 g/dL**			28(17.4)	11(11.1)	χ^2:1.896
				>12 g/dL			133(82.6)	88(88.9)	p=0.114
Hemoglobin /Male				**≤13.5 g/dL**			5(6.9)	4(14.8)	χ^2:1.472
				>13.5 g/dL			67(93.1)	23(85.2)	p=0.201

*Homeostasis model assessment of insulin resistance.

Table 3. Comparison of vitamin levels and seasonal differences of vitamin D in nondiabetic obese patients and controls

		Obese (Mean ±SD)	**Control (Mean ±SD)**	***p***
Vitamin B12 (pg/mL)		318.4±94.2	460.2±139.2	**<0.001**
Serum folic acid (ng/mL)		6.7±2.6	7.6±3.0	**0.030**
Vitamin D (ng/mL) (Serum 25(OH)D)		17.6±6.5	22.5±9.4	**<0.001**
Case-control cross-tabulation		**Obese (n, %)**	**Control (n, %)**	***p***
Vitamin D (Serum 25(OH)D) in summer	**<20 ng/ml**	52(59)	16(32)	χ^2:9.362
	≥20 ng/ml	36(41)	34(68)	**p=0.002**
Vitamin D (Serum 25(OH)D) in winter	**<20 ng/ml**	132(75)	41(53.9)	χ^2:10.931
	≥20 ng/ml	44(25)	35(46.1)	p=0.455

shown in Table 2.

Anemia was found in 7.8% of male and 16.8% of female patients. There was no significant difference in hemoglobin levels between patients and controls (Table 2). The mean vitamin B12 levels and folic acid levels were significantly lower in patients having anemia than in other patients (p=0.011, p<0.001, respectively).

Mean serum vitamin D, vitamin B12, and folic acid levels were significantly lower in patients than in controls (p<0.001, p<0.001, p=0.030, respectively) (Table 3). Mean vitamin D level was significantly lower in patients than in controls in summer, but not significantly lower in winter (Table 3). These results were consistent in both genders.

While the mean vitamin B12 levels were significantly lower in females than in male obese patients (307.2±94.3, 339.9±90.5, respectively,

Table 4. Analysis of vitamin status in nondiabetic morbid obese, obese patients, and controls

		Obese			
		Morbid Obese n=63 (%)	Obese n=241 (%)	Total n=304 (%)	Control n=150 (%)
Vitamin D (Serum 25(OH)D)	Deficiency (<20 ng/mL)	73.7	68.6	69.7	45.2
	Insufficiency (20-30 ng/mL)	22.8	26.1	25.4	35.7
	Sufficiency (≥30 ng/mL)	3.5	5.3	4.9	19
Vitamin B12	Deficiency (<200 pg/mL)	14.3	13.3	13.5	0
	Marginal (200-400 pg/mL)	60.3	60.2	60.2	44
	Adequate (≥400 pg/mL)	25.4	26.6	26.3	56
Serum folic acid	Deficiency (<4 ng/mL)	25.5	9.8	14.3	5.5
	Adequate (>4 ng/mL)	74.5	90.2	85.7	94.5

p=0.004), there was no significant difference between genders in controls. There was no gender related difference in folic acid levels in both groups.

Mean serum vitamin D level was 17.6±6.5 ng/mL, vitamin D deficiency (<20 ng/mL) was found in 69.7% and sufficiency (≥30 ng/mL) in only 4.9% of the obese patients. Mean serum vitamin B12 level was 318.4±94.2 pg/mL, vitamin B12 deficiency (<200 pg/mL) was found in 13.5% and adequate vitamin B12 level (≥400 pg/mL) in only 26.3% of the obese patients. Mean serum folic acid level was 6.7±2.6 ng/mL and folic acid deficiency (<4 ng/mL) was found in 14.3% of obese patients. Analysis of vitamin status in morbid obese, obese patients, and controls is shown in Table 4.

Morbid obesity subgroup was present in 13.9% of the obese patients (n=63; 41 female, 22 male). Vitamin D deficiency was found in 73.7%, Vitamin B12 deficiency in 14.3%, and folic acid deficiency in 25.5% of morbid obese patients. There was no significant difference in vitamin D and B12 levels between morbid obese and other obese patients. Anemia was significantly more common in morbid obese patients (27.4%) than in other obese patients (10%) (p=0.001). Folic acid level was not significantly different but folic acid deficiency was more common in morbid obese patients (25.5%) than in other obese patients (9.8%) (Table 4).

Serum vitamin D levels were negatively correlated with BMI, body-fat percentage, insulin, HOMA-IR, parathyroid hormone levels (r=-0.256, p<0.001; r=-0.186, p=0.001, r=-0.153, p=0.004; r=-0.141, p=0.007; r=-0.228, p=0.040, respectively). There was a positive correlation between vitamin D and HDL-chol levels (r=0.159, p=0.002) (Table 5).

Serum vitamin B12 levels were negatively correlated with BMI, duration of obesity, body fat percentage, insulin, HOMA-IR, and triglyceride levels (r=-0.432, p<0.001; r=-0.131, p=0.023; r=-0.304, p<0.001; r=-0.282, p<0.001; r=-0.266, p<0.001; and r=-0.194, p<0.001, respectively). There was a positive correlation between vitamin B12 and HDL-chol levels (r=0.297, p<0.001) (Table 5).

There was a negative correlation between serum folic acid level and BMI (r=-0.190, p=0.002). Folic acid levels were not significantly correlated with HOMA-IR and other metabolic parameters (Table 5).

Hs-CRP levels were negatively correlated with vitamin D, vitamin B12, and folic acid levels (r=-0.138, p=0.033; r=-0.226, p<0.001; r=-0.154, p=0.030, respectively) (Table 5). Hs-CRP levels were positively correlated with BMI, insulin, and HOMA-IR (r=0.627, p<0.001; r=0.273, p<0.001; r=0.274, p<0.001, respectively). There was a positive correlation between serum ferritin levels and insulin, and HOMA-IR (r=0.163, p=0.004; r=0.160, p=0.005, respectively).

Daily mean energy intakes were 2778.1±406.4 kcal in males and 2379.8±235.2 kcal in female obese patients (p=0.002). Mean daily vitamin and nutrient intakes in obese patients and comparison with recommendations is shown in Table 6. Daily dietary vitamin D, folic acid, and some micronutrients such as calcium and iron, as well as fiber intakes were found inadequate in 100% of obese patients. On the other hand, inadequacy of vitamin B12 intake was found in only 28.3% of obese patients (Table 6).

Intake of daily dietary fruit and vegetables was inadequate in 100% of obese patients. Intake was inadequate for meat group (meat, chicken and fish) in 40.8%, for dairy products in 28.3%, and for leguminosae in 21.4% of the obese patients. Fish or sea food intake was reported as "never or less than once a month" by 53% of obese patients.

There were not significant differences in daily vitamin and micronutrient intakes between morbid obese subgroup and obese patients. Daily dietary folic acid, calcium, and fiber intakes were higher in males

Table 5. Correlations between vitamin D, vitamin B12, folic acid and metabolic parameters in nondiabetic obese patients

Pearson Correlation		Vitamin D (ng/mL) (Serum 25(OH)D)	Vitamin B12 (pg/mL)	Serum folic acid (ng/mL)
BMI (kg/m^2)	***r***	**-0.256****	**-0.432****	**-0.190****
	p	**0.000**	**0.000**	**0.002**
Body fat (%)	***r***	**-0.186****	**-0.304****	-0.108
	p	**0.001**	**0.000**	0.182
Duration of obesity (year)	***r***	-0.024	**-0.131***	-0.129
	p	0.692	**0.023**	0.082
Fasting plasma glucose (mg/dL)	***r***	-0.034	**-0.156****	0.009
	p	0503	**0.001**	0.890
HbA1c (%)	***r***	-0.096	**-0.181****	0.038
	p	0.102	**0.001**	0.605
Insulin (µU/mL)	***r***	**-0.153****	**-0.282****	-0.84
	p	**0.004**	**0.000**	0.195
HOMA-IR index	***r***	**-0.141****	**-0.266****	-0.076
	p	**0.007**	**0.000**	0.244
HDL-chol (mg/dL)	***r***	**0.159****	**0.239****	0.124
	p	**0.002**	**0.000**	0.055
Triglyceride (mg/dL)	***r***	-0.056	**-0.194****	0.087
	p	0.289	**0.000**	0.178
Hs-CRP (mg/L)	***r***	**-0.138***	**-0.226****	**-0.154***
	p	**0.033**	**0.000**	**0.030**

**Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 6. Comparisons of daily mean energy, micronutrient, and vitamin intakes in obese male and female patients

	Reference (per day) (Adult) (Sedentary/Moderately Active)	Males (Mean ±SD)	Obese Females (Mean ±SD)	Total (Mean ±SD)	*p*
Energy (kcal)	1800-2000 (Female) 2000-2600 (Male)	2778.1±406.4	2379.8±235.2	2428.4±290.8	**0.002**
Fiber (g)	25-30	12.3±2.6	11.9±1.9	12.0±2.2	0.106
Vitamin D (µg)	5	2.88±0.6	2.80±0.6	2.83±0.6	0.286
Vitamin B12 (µg)	2.4	3.75±1.3	3.78±1.3	3.8±1.3	0.808
Folic acid (µg)	400	141.3±21.8	137.5±23.9	138.8±23.2	0.187
Calcium (mg)	1000-1200	391.8± 91.7	382.7± 96.6	385.9± 94.9	0.432
Iron (mg)	10-15	5.6±0.9	5.4±0.8	5.4±0.9	0.792

* Reference: Food and Nutrition Board, The Dietary Reference Intakes (29).

than in female obese patients, but these differences were not statistically significant (Table 6). There was no significant correlation between serum vitamin levels and mean daily dietary vitamin intakes.

DISCUSSION

Although obese people tend to have an excessive food intake, they may have nutritional deficiencies. Multiple factors might confound the association between obesity and vitamin deficiency, such as lifestyle and dietary habits. Few data regarding vitamin deficiency and dietary vitamin intake in obesity are available (31, 32).

Vitamin D deficiency seems to be the most common vitamin deficiency in obese people (33). Low serum concentrations of vitamin D in obesity may partly be a result of its sequestration in adipose tissue. Consistent with the literature, we found a high prevalence of vitamin D (69.7%), vitamin B12 (13.5%) and folic acid (14.3%) deficiencies in non-diabetic obese patients. We found that dietary vitamin D intake was much lower than the recommended levels in obese patients. There are few vitamin D sources in diet, including fish (especially, salmon, tuna, sardine, and mackerel), vitamin D fortified dairy products and foods. Notably, only half of the obese patients in the present study ate seafood. Although Turkey has a long coastline and inland waters, seafood potential does not seem to be fully utilized. This may be associated with cultural dietary habits. Mean vitamin D level was found to be significantly lower in obese patients than

in controls during summer. Obese people may tend to spend less time in outdoor activities or have limited mobility, and they may tend to dress skin covering clothes in summer and therefore may benefit less from sunlight. Vitamin D deficiency was highly prevalent in obese patients but also in normal weight controls in winter, which suggests that vitamin D deficiency is an important public health problem in our country (34). Cultural habits like clothing style may also explain vitamin D deficiency in Turkey, a sunny country.

Recent studies have drawn attention to the association of Vitamin D deficiency with metabolic syndrome and insulin resistance (35, 36). Reduced vitamin D levels were found to be associated with an increased risk of developing diabetes. This may be caused by an impaired β-cell function (37).The mechanism underlying the association of diabetes mellitus with vitamin D deficiency has not been fully explained although studies have shown that vitamin D receptors are located also in the pancreas and these receptors are linked to the insulin-like growth factor (IGF-1) (36, 38) .

We observed a negative but weak correlation between serum 25(OH)D and BMI, insulin, HOMA-IR. Consistent with our findings, Saneii *et al.* have reported a negative correlation between serum 25(OH) D levels and BMI in adult population in a systematic review and meta-analysis (39). Although a beneficial effect of vitamin D replacement on weight control has been shown in some randomized clinical trials, variable results have been obtained in the improvement of insulin resistance and metabolic syndrome (39).

Dietary folic acid and some micronutrients such as calcium and iron, as well as daily fiber intakes were found to be much lower than the recommended levels in obese patients. On the other hand, mean B12 intake was higher than the recommended levels and most of obese patients (71.7%) had adequate daily dietary vitamin B12 intake. Dhonukshe-Rutten *et al.* reviewed intakes and status of folate and vitamin B12 of general adult population from 15 European countries (40). They observed common low folate intake in Scandinavian countries, but low vitamin B12 intake was rare in European population. Consistent with our results, the mean intake level of vitamin B12 was higher than the recommendations, despite the serum vitamin B12 status were inadequate in the Netherlands and Germany. Although none of our patients had a clinical malabsorption condition, adequate vitamin B12 serum levels were found in only 26.3% (≥400 pg/mL) of the obese patients. Obesity itself may be associated with reduced intestinal absorption of dietary vitamin B12 which might be caused by changes of intestinal microbial flora, reduced biosynthesis of vitamin B12, and/or changes in absorption mechanisms. Recent studies suggest that the gastrointestinal microbiota may contribute to the development of obesity (41, 42). Causal relations may be explained by performing *in vitro* absorption studies and investigation of intestinal flora; however, such investigations are complicated and difficult.

We also found a negative correlation between serum Vitamin B12 levels and BMI, insulin, fasting plasma glucose, HOMA-IR, HbA1c, triglyceride levels. Baltaci *et al.* conducted a study with obese patients and found that vitamin B12 level was significantly lower in the obese group than in the control group in Duzce, Turkey. They also found a negative correlation of vitamin B12 levels with BMI, but not with insulin resistance. Vitamin B12 deficiency was found in 40.1% of their obese patients (43). The higher prevalence of vitamin B12 deficiency in their cohort than in our patients may be explained by different settings. Their patient population was selected from a primary-care setting in a rural area; whereas ours is a private university hospital, a tertiary care center in Istanbul. A better socioeconomic status may be associated with adequate intake of natural food sources of vitamin B12 (animal products such as meat, eggs and milk) in our patients.

In our study, morbidly obese patients were at a greater risk for folic acid deficiency. Schweiger *et al.* have also reported a high prevalence of folic acid (24.3%) and low prevalance of vitamin B12 (3.6%) deficiency among 114 bariatric surgery candidates (44). Consistent with our findings, they found a higher risk for developing folic acid deficiency in morbid obese people.

Setola *et al.* have reported that folate and vitamin B12 therapy resulted in reduction of homocysteine level and improvement of endothelial dysfunction and insulin resistance in patients with metabolic syndrome (14). We found a negative correlation between vitamin D, B12, folic acid and Hs-CRP levels and a positive correlation between BMI and Hs-CRP in our study population, consistent with previous reports of low-grade inflammation in obesity (45, 46). Although literature in this field may not indicate a cause and effect relationship, systemic inflammation and low serum vitamin B12, folic acid and vitamin D concentrations are together risk factors for atherosclerotic disease, a common comorbidity of obesity.

In this study, we did not find an increased frequency of anemia in obese patients compared to controls. On the other hand, there was a positive correlation between serum ferritin levels and insulin and HOMA-IR. Ferritin is an index of body iron stores and is also considered as an acute phase reactant to pro-inflammatory stimuli. These findings are consistent with the recently reported studies showing a significant relationship between insulin resistance and ferritin levels (47, 48).

There are several limitations of our study. The limited size of the control group was one of the limitations of our study. Our patient cohort was selected from obese patients seeking weight loss at a university obesity outpatient clinic which might have an impact on their nutrition status. A community based study would have more accurately shown the nutrition status of obese individuals. Anemia was defined based on WHO criteria, but not classified. Serum homocysteine and methylmalonic acid levels were not measured in determining Vitamin B12 deficiency, and folic acid deficiency was determined without measuring erythrocyte folate concentrations.

In conclusion, this study draws attention to the increased prevalence of vitamin D, vitamin B12, and folic acid deficiency in nondiabetic obese patients. Our findings may suggest improper alimentation regarding vitamin D, folic acid, calcium, iron, fiber dietary intake and possible malabsorption of vitamin B12 in obesity. Food fortification, which is adding deficient vitamins and minerals to commonly used food products, is an important strategy to fight against vitamin deficiency. However, this strategy is not widely used in our country. We recommend weight-loss plans together with evaluation of vitamin deficiencies in obese patients in order to provide a better guidance for healthy and balanced diets and vitamin supplementation.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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