# Levels of Plasma Homocysteine in Obese Women Subjects Homocysteine and Obesity

## *Obez Kadın Hastalarda Plazma Homosistein Seviyeleri Homosistein ve Obezite*

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## ABSTRACT

**OBJECTIVE:** An increased homocysteine level is an independent risk factor for vascular diseases. The present study was designed to evaluate plasma homocysteine levels in obese women compared with non-obese healthy women.

**MATERIAL and METHODS:** We selected 55 obese women (mean age 47.2±9.2 years) having a body mass index ≥ 30 kg/m$^2$ and 50 non-obese healthy women matched for age (mean age 46.3±9.5 years) who attended our outpatients clinic. We measured levels of homocysteine in obese and non-obese groups.

**RESULTS:** No significant difference was observed between obese and non-obese control groups regarding the homocysteine levels (10.3±3.5 $\mu$mol/l vs. 10.1±3.8 $\mu$mol/l , p>0.05). In this selected study population as a whole, the correlation between homocysteine levels and body mass index did not attain statistical significance (r=0.12, p>0.05).

**CONCLUSION:** We found that homocysteine levels were comparable between middle-aged obese and non-obese women. Our data may suggest that increased cardiovascular risk in obese women is probably not related to the homocysteine level.

**KEY WORDS:** Obesity, Homocysteine, Body mass index, Cardiovascular risk

## ÖZ

**AMAÇ:** Artmış homosistein seviyesi vasküler hastalıklar için bağımsız bir risk faktörüdür. Bu çalışma, obez ve obez olmayan kadınlarda plazma homosistein düzeylerinin değerlendirilmesine yönelik tasarlanmıştır.

**GEREÇ ve YÖNTEMLER:** Çalışmaya polikliniğimize başvuran, vücut kitle indeksi ≥ 30 kg/m$^2$ olan 55 obez kadın (ortalama yaş 47,2±9,2 yıl) ve aynı yaş grubundan (ortalama yaş 46,3±9,5 yıl) 50 obez olmayan sağlıklı kadın seçtik. Obez ve obez olmayan gruplarda homosistein düzeylerini ölçtük.

**BULGULAR:** Obez ve obez olmayan kontrol grubu arasında homosistein düzeyleri (10.3±3.5 $\mu$mol/l vs. 10,1±3,8 $\mu$mol/l , p>0,05) açısından anlamlı farklılık tespit edilmedi. Çalışmaya seçilmiş populasyonda, homosistein seviyeleri ile vücut kitle indeksi arasında istatistiksel olarak anlamlı korelasyon tespit edilmedi (r=0,12, p>0,05).

**SONUÇ:** Orta yaş obez ve obez olmayan kadınlarda homosistein düzeylerinin benzer olduğunu saptadık. Verilerimiz obezitede artmış kardiyovasküler risk ile homosistein düzeyleri arasında ilişki olmadığına işaret edebilir.

**ANAHTAR SÖZCÜKLER:** Obezite, Homosistein, Vücut kitle indeksi, Kardiyovasküler risk

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## INTRODUCTION

Obesity is a chronic metabolic disorder associated with cardiovascular disease (i.e., insulin resistance and type 2 diabetes mellitus, dyslipidemia, hypertension), and increased morbidity and mortality (1-3).

Homocysteine (Hcy) is a metabolic product of methyl group donation by the amino acid methionine. Hcy is controlled both by mutations in its regulating enzymes and by the B vitamins, folic acid, B12 and B6 (4,5). Hcy has several potentially deleterious vascular actions such as oxidative stress, endothelial dysfunction, and stimulation of thrombosis (6). There are various studies on the association between homocysteine and vascular diseases. Hyperhomocysteinemia has been linked to an increased risk of cardiac events, sudden death, stroke, essential hypertension, coronary, carotid, cerebral, and peripheral arterial disease, and venous and pulmonary thromboembolism (7-12).

Conflicting data have been published on the association of Hcy and obesity. Therefore, the present study was designed to evaluate plasma Hcy levels in obese women compared with non-obese healthy women.

## PATIENTS and METHODS

This study was performed at the outpatients clinic of the Department of Internal Medicine of Akdeniz University Hospital. Our study was conducted on obese women who wanted to lose weight. We selected 55 obese women (mean age 47.2±9.2 years) having a body mass index (BMI) ≥ 30 kg/m$^2$ and 50 non-obese healthy women matched for age (mean age 46.3±9.5 years) and occupation who attended our outpatients clinic. All patients gave their informed consent to participate in the study.

Exclusion criteria for entry into the study were drug use (including vitamin supplements), alcohol and/or coffee abuse, smoking habit, dyslipidemia, sustained hypertension, diabetes mellitus, renal failure, heart failure, cerebrovascular disease, ischaemic heart disease, peripheral vascular disease, hypothyroidism, high serum uric acid, psoriasis, confirmed macrocytic anemia and heavy physical activity. Folic acid and vitamin B12 levels are within the normal range in all subjects.

Eligible subjects underwent a comprehensive assessment including documentation of medical history, physical examination, and measurement of laboratory variables. Body weight and height were measured with the subjects in light clothes and without shoes. BMI was calculated as the weight (kg)/height squared (m)$^2$.

Blood samples were collected from an antecubital vein without the use of a tourniquet, between 08.30 and 09.00 h. The enzymatic colorimetric assay method (Roche Diagnostic GmbH, Mannheim, Germany) was used to measure triglyceride, cholesterol and HDL-C levels. The LDL-C level was calculated according to Friedewald formula (13). Fasting plasma glucose level was measured by the enzymatic colorimetric assay method (GLU, Roche Diagnostic GmbH, Mannheim, Germany).

The plasma specimens were drawn after a fasting period of 12 h and were kept in tubes containing EDTA. Plasma got separated from the red blood cells within 1 h of collection as synthesis and excretion of Hcy continued in the cells after sampling. Prior to analysis, all specimens were stored in a frozen state (–20°C). We applied two levels of internal quality standards before the assay. Level 1 had a value in the normal range and level 2 had a value above the threshold.

The disulphide bands in the calibrant/sample were reduced using the reducing agent. Protein was precipitated from solution and the thiol groups in the supernatant were than derivatised with a fluorescent thiol-specific dye. The fluorescent derivative mixture was then separated using the Drew DS 30 Hcy analyser which automatically calculates the Hcy concentration using suitable derivates which are separated and detected by their fluorescence (λex=385 nm, λem=515 nm). Quantitative evaluation of the Hcy concentration was achieved by comparison with two-point calibration.

### Statistical Analysis

Statistical analysis was performed using SSPS 10.0 statistical software. For α=0,05 (between each group) and power=80%, a sample size per group >36 subjects was needed to detect an actual difference. Two-group comparisons (obese vs. non-obese) were performed with independent t-tests. Pearson's correlation was used to evaluate the association between Hcy levels and BMI. The values were given as mean ± standard deviation. P<0.05 was accepted as statistically significant.

## RESULTS

Clinical and laboratory parameters of the study population are reported in Table I. Metabolic parameters were not different between obese and non-obese control groups, as a results of the selection process (p>0.05). BMI was significantly higher in the obese group than in the non-obese control group (34.1 ± 3.3 kg/m$^2$ vs. 23.4± 5.1 kg/m$^2$, p<0.001).

No significant difference was observed between the obese group and control group regarding the Hcy levels (10.3±3.5 μmol/l vs. 10.1±3.8 μmol/l , p>0.05). In this selected study population as a whole, the correlation between Hcy levels and BMI did not attain statistical significance (r=0.12, p>0.05).

## DISCUSSION

Although obesity is one of the most important risk factors for cardiovascular disease and generally the Hcy levels increase in obesity, the Hcy levels in our study were similar between the obese and non-obese groups.

Many studies have shown that elevated Hcy and obesity are both associated with increased cardiovascular disease risk. However it is unclear if these two risk factors are interrelated. Marchesini et al. reported that Hcy levels were moderately increased in obese individuals when compared with the normal

**Table I:** Clinical and laboratory parameters of the study groups.

| Parameters | Obese group (n=55) | Non-obese group (n=50) | p values |
|---|---|---|---|
| Age (years) | 47.2±9.2 | 46.3±9.5 | NS |
| Body mass index (kg/m$^2$) | 34.1±3.3 | 23.4± 5.1 | <0.001 |
| Fasting glucose (mg/dl) | 86.5± 5.3 | 85.48±10.34 | NS |
| HDL-cholesterol (mg/dl) | 51.6± 15.2 | 52.8± 16.7 | NS |
| LDL-cholesterol (mg/dl) | 120.0± 29.5 | 118.6± 30.2 | NS |
| Triglyceride (mg/dl) | 113.4± 37.8 | 111.9± 39.1 | NS |
| Homocysteine ($\mu$mol/l) | 10,3±3.5 | 10.1±3.8 | NS |

**HDL:** High-density lipoprotein, **LDL:** Low-density lipoprotein, **NS:** Not significant.

population and higher in males than in females ( p< 0.0002), but not different in relation to the severity of obesity (14). Similarly in Tungtrongchitr et al's study, statistically significantly higher levels of serum Hcy concentrations were found in the overweight subjects, and serum Hcy concentrations in overweight and obese males were significantly higher than females. In addition, this study demonstrated that this result may caused by insufficiency dietary folic acid intake (15). Konukoglu et al. reported that plasma Hcy concentrations were higher in obese diabetics than in non-obese diabetics (16). Akoglu et al found a positive correlation between plasma Hcy levels and BMI in liver transplant recipients (17). Jacques et al. reported that persons with the largest weight-for-height (BMI≥ 30.7) had slightly greater plasma Hcy concentrations than did those with a BMI< 30.7 (18). Koehler et al also reported a weak positive relation between BMI and Hcy concentrations (19), but Lussier-Cacan et al. observed no association. In this study, while serum folic acid levels were higher in females than males, serum Hcy levels were higher in males than females. This result may indicate that hyperhomocysteinemia was due to lower levels of folic acid (20). The Hordaland Homocysteine Study investigators reported a U-shaped association between BMI and Hcy concentrations that disappeared after adjustment for other determinants of Hcy concentrations (21). Uysal et al. reported that Hcy levels were comparable between obese and non-obese subjects (22). Brasilerio et al demonstrated that obesity was not a determinant factor of Hcy levels (23). In Fonseca et al's study, there was no relationship between Hcy and BMI (24). The different results of the studies may be due to factors such as different sample size, subject characteristics, equipment and/or technique for measuring Hcy levels and genetic or nutritional factors (involved in Hcy metabolism).

Certain factors are related to total Hcy levels. Sex is one of the stronger determinants of Hcy. Previous studies reported that Hcy levels were higher in obese men than in obese women. Whenever reported, the sex difference has been ascribed to various factors; including different rates of Hcy formation, the presence of larger muscle mass and greater creatinine phosphate synthesis in men and a lowering effect of estrogen in women (20). Our study was conducted on obese women who wanted to lose weight. Therefore, we have not performed an analysis of Hcy levels as regards gender.

The present study has some limitations. First, we excluded patients with clinically overt cardiovascular disease (such as coronary artery disease, cerebrovascular disease and renal failure) to clarify the specific levels of BMI-related abnormalities. However, obesity is associated with numerous comorbidities, including hypertension, dyslipidemia, cardiovascular disease, non-insulin-dependent diabetes mellitus, gallbladder disease, respiratory dysfunction, gout, and osteoarthritis (1,25,26). Our results therefore cannot be extrapolated to all obese subjects. Second, since we did not evaluate genotypes of enzymes involved in Hcy metabolism and vitamin B6 status, it is unclear whether the present findings are related to genetic or nutritional factors. The third limitation of this study is that our analysis was based on a simple baseline determination that may not reflect subject status over long periods.

In conclusion, we found that Hcy levels were comparable between middle-aged obese and non-obese women (without other cardiovascular disease). Our data may suggest that increased cardiovascular risk in obesity may not be related to the Hcy level.

## REFERENCES

1. Poirier P, Eckel RH: Obesity and cardiovascular disease. Curr Atheroscler Rep 2002; 4: 448-453
2. Eckel RH, Krauss RM: American Heart Association call to action: Obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. Circulation 1998; 97: 2099-2100

3. Kumanyika S, Jeffery RW, Morabia A, Ritenbaugh C, Antipatis VJ: Obesity prevention: The case for action. Int J Obes Relat Metab Disord 2002; 26: 425-436
4. Bostom AG, Selhub J: Homocysteine and arteriosclerosis. Subclinical and clinical disease associations. Circulation 1999; 99: 2361-2363
5. Scott JM: Homocysteine and cardiovascular risk. Am J Clin Nutr 2000; 72: 333-334
6. Haynes WG: Hyperhomocysteinemia, vascular function and atherosclerosis: Effects of vitamins. Cardiovascular Drugs Ther 2002; 16: 391-399
7. Plazar N, Jurdana M: Hyperhomocysteinemia: Relation to Cardiovascular Disease and Venous Thromboembolism. www.intechopen.com/download/pdf/37048
8. Kalra DK: Homocysteine and Cardiovascular Disease. Curr Atheroscler Rep 2004; 6: 101-106
9. Pierdomenico SD, Bucci A, Lapenna D, Lattanzio FM, Talone L, Cuccurullo F, Mezzetti A: Circulating homocysteine levels in sustained and white coat hypertension. J Hum Hypertens 2003; 17: 165-170
10. Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S: Homocysteine and cardiovascular disease: A critical review of the epidemiologic evidence. Ann Intern Med 1999; 131: 363-375
11. Graham IM, Daly LE, Refsum HM, Robinson K, Brattström LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Lúis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Andria G: Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 1997; 277: 1775-1781
12. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE: Plasma homocysteine levels and mortality in patients with coronary arter disease. N Engl J Med 1997: 337: 230-236
13. Friedewald WT, Levi RI, Fredricksen DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502
14. Marchesini S, Manini R, Bianchi G, Sassi S, Natale S, Chierici S, Visani F, Baraldi L, Forlani G, Melchionda N: Homocysteine and psychological traits: A study in obesity. Nutrition 2002; 18: 403-407
15. Tungtrongchitr R, Pongpaew P, Tongboonchoo C, Vudhivai N, Changbumrung S, Tungtrongchitr A, Phonrat B, Viroonudomphol D, Pooudong S, Schelp FP: Serum homocysteine, B12 and folic acid concentration in Thai overweight and obese subjects. Int J Vitam Nutr Res 2003; 73: 8-14
16. Konukoglu D, Serin O, Turhan MS: Plasma total homocysteine concentrations in obese and non-obese female patients with type 2 diabetes mellitus; its relations with plasma oxidative stress and nitric oxide levels. Clin Hemorheol Microcirc 2005; 33: 41-46
17. Akoglu B, Wondra K, Casparu WF, Faust D: Determinants of fasting total serum homocysteine levels in liver transplant recipients. Exp Clin Transplant 2006; 4: 462-466
18. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J: Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001; 73: 613-621
19. Koehler KM, Romero LJ, Stauber PM, Pareo-Tubbeh SL, Liang HC, Baumgartner RN, Garry PJ, Allen RH, Stabler SP: Vitamin supplementation and other variables affecting serum homocysteine and methylmalonic acid concentrations in elderly men and women. J Am Coll Nutr 1996;15: 364-376
20. Lussier-Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest J Jr: Plasma total homocysteine in healthy subjects: Sex-specific relation with biological traits. Am J Clin Nutr 1996; 64: 587-593
21. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M, Kvåle G: Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 1995; 274: 1526-1533
22. Uysal O, Arikan E, Cakir B: Plasma total homocysteine level and its association with carotid intima-media thickness in obesity. J Endocrinol Invest 2005; 28: 928-934
23. Brasilerio RS, Escrivão MA, Taddei JA, D'Almeida V, Ancona-Lopez F, Carvalhaes JT: Plasma total homocysteine in Brazilian overweight and non-overweight adolescents: A case-control study. Nutr Hosp 2005; 20: 313-319
24. Fonseca VA, Fink LM, Kern PA: Insulin sensitivity and plasma homocysteine concentrations in non-diabetic obese and normal weight subjects. Atherosclerosis 2003; 167: 105-109
25. Pi-Sunyer FX: A review of long-term studies evaluating the efficacy of weight loss in ameliorating disorders associated with obesity. Clin Ther 1996; 18: 1006-1036
26. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH: The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. BMC Public Health 2009; 9: 88