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ORIGINAL ARTICLE

# Evaluation of adipocytokine levels and vascular functions in young aged to middle aged men with idiopathic hypogonadotrophic hypogonadism

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## Abstract

**OBJECTIVE:** Hypogonadism has major effects on the urogenital system, in addition to other systems, the cardiovascular system in particular. There have been few studies conducted on markers of atherosclerosis, such as flow mediated dilatation (% FMD), carotid intima-media thickness (CIMT) and adipocytokine levels in idiopatic hypogonadotropic hypogonadal (IHH) males mostly in adult patients. The aim of this study was to evaluate the relationship between androgens and adipocytokines and parameters of vascular functions in hypogonadal men.

**MATERIALS AND METHODS:** The study population consisted of 11 treatment naive IHH patients (group 1) and 15 age-matched healthy control males (group 2). A fasting blood sample was obtained for leptin, adiponectin and resistin. The endothelial functions were evaluated by studying % FMD and CIMT by high resolution B-mode ultrasound.

**RESULTS:** No significant differences in age, body mass index, systolic and diastolic blood pressure were recorded between the two groups. The leptin level was significantly higher in group 1, whereas adiponectin and resistin levels were same between two groups. There was a negative correlation between total testosterone and carotid intima-media thickness (r=–0.656, $p$=0.008), and a negative correlation between total testosterone and leptin level (r=–0.794, $p$<0.001). No correlation was found between leptin and CIMT ($p$=0.184).

**CONCLUSION:** Testosterone deficiency in hypogonadal men is associated with vascular parameters of atherosclerosis. The findings may establish indications for testosterone replacement therapy in hypogonadal men.

## INTRODUCTION

Hypogonadism has major effects on the urogenital system, in addition to other systems, the cardiovascular system in particular. Flow mediated dilatation (% FMD) of the brachial artery and carotid intima-media thickness (CIMT) are parameters used to assess endothelial functions. Impaired FMD and increased CIMT are an early marker of atherosclerosis and have been shown to be correlated with coronary endothelial dysfunction (Neunteufl *et al.* 2000; Kablak-Ziembicka *et al.* 2010). The relationship between adipocytokines (adiponectin, leptin and resistin) and obesity, insulin resistance, atherosclerosis, and cardiovascular disease have been shown previously (Chiodini *et al.* 2010; Yenigün *et al.* 2005; Steppan & Lazar 2002). However, data demonstrating the connection between adipokines and the development of insulin resistance or atherosclerosis in patients with hypogonadism are completely lacking. Very few studies have been conducted on markers of atherosclerosis, such as % FMD, CIMT and adipocytokine levels in idiopatic hypogonadotropic hypogonadal (IHH) males. The data concerning the association of testosterone (T) and vascular function are conflicting. The aim of this study was to evaluate the relationship between androgens and adipocytokines and parameters of vascular functions in hypogonadal men.

## MATERIALS AND METHODS

The study population consisted of 11 treatment naive IHH male patients (group 1) and 15 age matched healthy male controls (group 2). We excluded patients with diabetes mellitus, coronary heart disease, impaired renal and liver functions, hyperthyroidism, hypothyroidism and smoking. All hypogonadal males were evaluated with pituitary and cranial magnetic resonance imaging and were found to be normal. Additionally, all hypogonadal males were evaluated with regard to pituitary functions, and found to be normal except hypogonadotropic hypogonadism. Body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were evaluated. BMI was computed using body mass in kilograms divided by height in meters squared (BMI = $kg/m^2$). A fasting blood sample was obtained for leptin, adiponectin and resistin. Venous blood samples were collected in vacutainer tubes and centrifuged at 1 300 *g* for 10 minutes. Sera were separated and stored at –20 °C until analysis. Human resistin, leptin and adiponectin levels were determined quantitatively by enzyme linked immunosorbent assay (ELISA) with an ELISA microplate strip washer (ELX50; BioTek Instruments, USA) and ELISA microplate reader (Chromate; Awareness Technology, USA). Human resistin and adiponectin concentrations were determined with an eBioscience ELISA kit (Bender MedSystems GmbH; Austria). Intra and inter-assay precisions were 5.1% and 8.1% for resistin; 4.2% and 3.1% for adiponectin. Leptin concentrations were measured with a DRG ELISA kit (DRG Instruments GmbH, Germany). The range of the assay was 1.0–100 ng/mL. Mean intra and inter-assay precisions were 6.43% and 10%; respectively. The endothelial function was evaluated by studying CIMT and FMD, measurements were made by high resolution B mode doppler ultrasonograpy, by the same technician. Three measurements at three sites of the common carotid artery were averaged to assess mean CIMT. A standard protocol was used to assess FMD, according to guidelines (Corretti *et al.* 2002). The brachial artery of the right arm was visualized longitudinally. The brachial artery diameter was determined in end-diastole. After three baseline measurements were obtained, a cuff was inflated to suprasystolic pressure (20 to 50 mm Hg above systolic arterial pressure) to produce ischemia in the forearm. The cuff is deflated after three minutes, thus causing a reactive hyperemia and shear stress stimulus that induces the endothelium to release nitric oxide. After the deflation of the cuff, diameter measurements were performed after one minute. % FMD was calculated using the formula: % FMD = (maximum diameter – baseline diameter) / baseline diameter × 100.

### *Ethical Committee approval*

The present study was approved by the local ethics committee of Ankara Numune Education and Research Hospital.

### *Statistical analyses*

SPSS (Statistical Package for the Social Sciences) for Windows 18.0 was used for statistical analyses of the data in this study. The Shapiro-Wilk test was used to test the normality of the continuous data. Normally distributed continuous data were presented as mean ± standard deviation; non-normally distributed continuous data were presented as median [minimum–maximum]; and categorical variables were presented as number of cases (percentage). Group comparisons were performed using Student's t test for normally distributed data and the Mann Whitney U test for non-normally distributed data. For those associations that were statistically significant we tested several multiple regression models. Categorical variables were compared between groups using Pearson's Chi-Square test. Pearson correlation coefficients were calculated for normally distributed variables. Significance level was accepted as 0.05, significant difference was called when $p<0.05$, and non-significant difference was called when $p>0.05$.

## RESULTS

Clinical features of the subjects are reported in Table 1. No significant differences in age, BMI, systolic and diastolic blood pressure were recorded between the two groups. All participants were normotensive. Serum HDL, LDL, triglyceride, uric acid levels and MPV were similar between the two groups. Leptin

level was significantly higher in group 1, whereas adiponectin and resistin level were the same between two groups (Table 1). IMT of the carotid artery was significantly higher in hypogonadal males than in controls (0.80±0.21 mm, 0.55±0.11 mm, $p$=0.018). FMD % was significantly impaired in group1 than in group 2 (3.9±5.3%, 21.3±11.7%, respectively, $p$=0.009) (Table 2). There was a negative correlation between total testosterone and carotid intima-media thickness (r=–0.656, $p$=0.008), and a negative correlation between total testosterone and leptin level (r=–0.794, $p$<0.001). No correlation was found between total testosterone and % FMD, also between leptin and CIMT ($p$=0.184, $p$=0.24, respectively).

**Tab. 1.** Clinical features and adipocytokine levels of hypogonadal (group 1) and eugonadal males (group 2). (Results are given as mean ± standard deviation).

| | Group 1 (n=11) | Group 2 (n=15) | *p*-value |
|---|---|---|---|
| Age (years) | 34.9±8.57 | 31.57±11.84 | 0.212 |
| BMI (kg/m²) | 22.6±2.3 | 21.8±2.9 | 0.263 |
| Total testosterone (nmol/ l) | 0.34±0.18 | 5.4±1.2 | **<0.01** |
| Adiponectin (ng/ml) | 4410±3780 | 5252±2402 | 0.815 |
| Leptin (ng/ml) | 14.92±9.33 | 4.36±1.71 | **0.015** |
| Resistin (pg/ml) | 4633±2062.77 | 4058.29±1962.51 | 0.561 |

**Tab. 2.** The results of endothelial function studies in both groups. (Results are given as mean ± standard deviation).

| | Group 1 (n=11) | Group 2 (n=15) | *p*-value |
|---|---|---|---|
| CIMT (mm) | 0.80±0.21 | 0.55±0.11 | **0.018** |
| Brachial artery diameter baseline (mm) | 4.1±0.7 | 3.5±0.9 | **0.015** |
| Brachial artery diameter posthyperemia (mm) | 4.3±1.1 | 4.1±0.8 | 0.191 |
| % FMD | 3.9±11.3 | 21.3±11.7 | **0.009** |

## DISCUSSION

There is growing data to suggest that low testosterone levels are associated with reduced insulin sensitivity and type 2 diabetes in males (Kapoor *et al.* 2005). Further, in the Rancho Bernardo study, men who had total T levels below normal limits at baseline, had a 38% chance of CV mortality, in comparison with those with a higher T level (Laughlin *et al.* 2008). Similar results were also reported in the Norfolk Prospective Population Study (Khaw *et al.* 2007). It is also well known that ımpaired FMD and increased CIMT are early markers of atherosclerosis and coronary endothelial dysfunction (Neunteufl *et al.* 2000; Kablak-Ziembicka *et al.* 2010). Zitzmann *et al.* demonstrated a significant association between androgen deficiency and increased FMD in hypogonadal males. Also, FMD improved significantly after testosterone replacement therapy in this study (Zitzmann *et al.* 2002). In another study, Bernini *et al.* reported impaired endothelium-independent and -dependent vasodilation in hypogonadal males (Bernini *et al.* 2006). In our study, % FMD was significantly lower in hypogonadal males than in healthy subjects, similar to previous study. Hypogonadal males had also a significantly higher CIMT than that in controls, in our study. In the Turku Male Aging Study, a beneficial effect of endogenous testosterone on CIMT was shown in middle-aged and older men, similar to the findings of our study (Mäkinen *et al.* 2005). Adiponectin, an adipocyte-derived cytokine, reduces levels of blood free fatty acids and has been associated with improved lipid profiles, better glycemic control, and reduced inflammation in diabetic patients (Mantzoros *et al.* 2005). Adiponectin has also been inversely associated with the development of diabetes in the non-diabetic population (Li *et al.* 2009), also it improves insulin sensitivity and has a vascular-protective effect (Yamauchi *et al.* 2002, Ouchi *et al.* 1999). Exogenous testosterone treatment reduces adiponectin levels in both eugonadal and hypogonadal mice (Nishizawa *et al.* 2002). Testosterone levels are negatively correlated with body fat mass and testosterone replacement therapy can significantly reduce body fat content in hypogonadal males (Bhasin *et al.* 2001). It was shown previously that serum concentrations of adiponectin in hypogonadal men, including those with Klinefelter syndrome, are higher than in eugonadal men and a significant reduction is observed after testosterone replacement therapy (Lanfranco *et al.* 2004). On the other hand, in a recently published study, they found no correlation between testosterone and adiponectin similar to our findings, also after testosterone replacement, adiponectin levels did not differ (Tsujimura *et al.* 2009). The underlying control mechanisms of synthesis and secretion of adiponectin have not been fully elucidated. It seems that several factors play a role in its metabolism. Further studies with a larger number of patients is necessary to clarify this association. Another important adipocytokin is leptin, which controls food intake and energy expenditure, storage of fat and insulin signalling (Moreno-Aliaga *et al.* 2010). Leptin improves insulin sensitivity through activation of AMP protein kinase (Minokoshi *et al.* 2002). It was shown that testosterone replacement therapy suppresses leptin synthesis both *in vivo* and *in vitro* (Jockenhovel *et al.* 1997; Kiess *et al.* 1999; Simon *et al.* 2001). Testosterone substitution therapy normalises elevated leptin serum levels in hypogonadal men (Jock-

enhovel *et al.* 1997). The mechanisms by which testosterone reduces leptin levels are uncertain. But this is considered that testosterone lowers leptin levels by reducing adipose tissue mass (Kapoor *et al.* 2007). Our data confirm the strong inverse association between testosterone and leptin.

Resistin is associated with obesity and insulin resistance in rodents, but human data is conflicting (Lee & Kim 2012). While some studies show an association with obesity and insulin resistance, others do not show any association (Lee & Kim 2012; Mirrakhimov 2012). We observed only one study that evaluates resistin levels in hypogonadism which was done in hypogonadal males with type 2 diabetes. In that study, there were no significant correlations between baseline resistin levels and testosterone concentrations. Moreover, no significant effect of testosterone therapy on resistin levels was observed (Kapoor *et al.* 2007). Our study, which is the first study to examine resistin levels in young aged to middle aged men with IHH without diabetes, revealed that resistin level was not different between hypogonad males and healthy subjects.

The strengths of our study include; a population that consisted of young and treatment naive patients with idiopatic hypogonadotropic hypogonadism, with none of the patients having traditional cardiovascular risk factors that can influence vascular reactivity and adipocytocin levels. The relatively limited number of subjects was the limitation of our study.

In conclusion, testosterone deficiency in hypogonadal men is associated with vascular parameters of atherosclerosis. The findings may establish indications for testosterone replacement therapy in hypogonadal men.

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### *Conflicts of Interest*

*The authors declare no conflicts of interest.*

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