Endothelial dysfunction, insulin resistance and inflammation in congenital hypogonadism, and the effect of testosterone replacement

Alper Sonmez1), Cem Haymana1), Aydogan Aydogdu1), Serkan Tapan2), Yalcin Basaran1), Coskun Meric1), Kamil Baskoy3), Mustafa Dinc1), Mahmut Yazici1), Abdullah Taslipinar1), Cem Barcin4), Mahmut Ilker Yilmaz5), Erol Bolu6) and Omer Azal1)

1) Department of Endocrinology and Metabolism, Gulhane School of Medicine, 06018 Etilik Ankara, TURKEY
2) Department of Biochemistry, Gulhane School of Medicine, 06018 Etilik Ankara, TURKEY
3) Department of Endocrinology and Metabolism, Haydarpasa Training Hospital, 34668 Istanbul, TURKEY
4) Department of Cardiology, Gulhane School of Medicine, 06018 Etilik Ankara, TURKEY
5) Department of Nephrology, Gulhane School of Medicine, 06018 Etilik Ankara, TURKEY
6) Department of Endocrinology and Metabolism, Memorial Atasehir Hospital, 34750 Atasehir Istanbul, TURKEY

Abstract. Patients with hypogonadism have poor cardiovascular and metabolic outcomes, and the effect of testosterone replacement therapy (TRT) is not clear. We investigated the presence of inflammation, insulin resistance and endothelial dysfunction in an unconfounded population of congenital hypogonadotrophic hypogonadism (CHH) and the effect of TRT on these subjects. A total of 60 patients with CHH (mean age 21.82±2.22 years) and 70 healthy control subjects (mean age 21.32±1.13 years) were enrolled. The demographic parameters, Asymmetric dimethylarginine (ADMA), TNF-like weak inducer of apoptosis (TWEAK), high sensitive C reactive protein (hs-CRP) and homeostatic model assessment of insulin resistance (HOMA-IR) levels were measured before and after TRT. The patients had higher Waist Circumferences (WC) (p=0.009), Diastolic Blood Pressures (p=0.02), Triglycerides (p=0.03), ADMA, insulin and HOMA-IR levels (p<0.001 for all) and lower TWEAK levels (p<0.001), compared to the healthy controls. After 5.56 ± 2.04 months of TRT, the patients had significantly elevated systolic blood pressures (p=0.01), body mass indexes and WC (p<0.001 and p=0.001 respectively) and decreased total and HDL cholesterol levels (p=0.032 and p<0.001 respectively). ADMA levels significantly increased (p=0.003), while the alterations in TWEAK, hsCRP and HOMA-IR were not significant. The results of the present study show that endothelial dysfunction, inflammation and insulin resistance are prevalent even in the very young subjects with CHH, who have no metabolic or cardiac problems at present. This increased cardiometabolic risk however, do not improve but even get worse after six months of TRT. Long term follow-up studies are warranted to investigate the unfavorable cardiometabolic effects of TRT.

Key words: Endothelial dysfunction, Insulin resistance, Inflammation, Hypogonadism

However, whether the testosterone replacement treatment (TRT) improves the metabolic and cardiovascular risks of patients with hypogonadism is not clear. Several reports mention favorable metabolic effects of TRT [6-9] while others do not confirm these data [10-13]. The metaanalyses show no significant metabolic benefit but a tendency towards increased cardiovascular events due to TRT [14-16]. Meanwhile, a prospective cohort of elderly hypogonadal men was recently terminated due to the increased cardiovascular mortality in the testosterone replacement arm [13]. There may be several reasons for the inconsistencies of the previous reports. Most of these studies were per-
formed in small groups of middle aged or the elderly patients who already have concomitant metabolic or cardiac disorders and take several potentially confounding medications. Also, different androgen formulations in different dosages were given in varying periods in all these studies.

Recently, in our database of young, treatment naïve CHH patients, we noticed that the metabolic parameters do not improve but worsen in about six months of TRT with triweekly injection of testosterone esters [5, 17]. Whether these unfavorable metabolic effects of TRT in this specific population of hypogonadism increase the cardiovascular risk is not known. Therefore, we designed the following study to search for the answers to the following questions: 1- Is there any difference between the treatment naïve young patients with CHH and healthy control subjects in terms of inflammation, insulin resistance and endothelial functions? 2-What is the effect of TRT on the cardiovascular risk of treatment naïve young patients with CHH? For this purpose, we measured the surrogate markers for endothelial dysfunction, inflammation and insulin resistance as predictors of the cardiovascular mortality and morbidity [18, 19]. We also compared the effects of two different TRT modalities on the above parameters; triweekly injection of testosterone esters vs. daily transdermal testosterone gel.

**Materials and Methods**

The study group was selected from the male subjects with CHH (n=60, mean age 21.82±2.22 years) who were not previously given testosterone or human chorionic gonadotropin (hCG) therapy. The patients did not have any chronic metabolic disorders or organ dysfunction. Seventy, age and body mass index (BMI) matched male volunteers (mean age 21.32±1.13 years) were enrolled as control subjects. All subjects gave informed consents and the Local Ethical Committee of Gulhane School of Medicine approved the study.

Detailed medical histories of the patients were taken before the study. The height, weight waist circumference (WC), diastolic blood pressure (DBP) and systolic blood pressure (SBP) of the patients and control subjects were measured with their underwear. BMI was computed as the ratio of weight to the square of height (kg/m²). WC was measured, after the patients exhaled, on the line between the iliac crest and the lower costal margin parallel to the ground. Pubertal developments of the patients were assessed according to the Tanner stages. The diagnosis of CHH was based on the history of failure to undergo spontaneous puberty before 18 years of age and was confirmed by low serum total testosterone and normal or low gonadotropin levels. Pituitary hormones were evaluated in all patients to exclude panhypopituitarism, and pituitary or hypothalamic mass lesions were excluded by magnetic resonance imaging.

**Testosterone replacement therapy**

The patients enrolled in this study were treated with two different testosterone replacement regimens. The first regimen is the oil-based injectable blend of four esterized testosterone compounds (™Sustanon 250 mg; 30mg Testosterone Propionate, 60mg Testosterone Phenylpropionate, 60 mg Testosterone Isocaproate 100 mg Testosterone Decanoate) injected once every three weeks. The other regimen is the transdermal testosterone gel (Testogel 50 mg gel) applied every night. The allocation was not performed randomly. The injectable testosterone regimen is the first choice in our clinical practice as it is easy to ensure compliance and considerably cheaper than the transdermal regimen. However, when the injectable regimens were inaccessible or when the patients preferred using transdermal regimens, we implemented transdermal regimens and continued as such, till the end of the follow-up period.

The blood samples for the evaluation of the baseline metabolic parameters were taken before the first testosterone dosage. The patients were then reevaluated in the 3rd and/or 6th months of treatment. The follow-up visits for the testosterone injection regimen group were arranged on the days before the next testosterone injection. Therefore, the time points for taking the blood samples were similar for all the patients.

**Sample collection and Laboratory measurements**

For biochemical analyses, all blood samples were collected from the antecubital veins, between 0800 and 0900 h. after overnight fasting. The samples were centrifuged for 15 min at 4000 g, aliquoted and immediately frozen at -80°C for analyses. Fasting plasma glucose, Total Cholesterol, Triglyceride, and High Density Lipoprotein Cholesterol (HDL-C) levels were measured by the enzymatic colorimetric method with Olympus AU2700 auto analyzer using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). Low-density lipoprotein cholesterol (LDL-C) was
calculated by Friedewald’s formula [20]. The serum basal insulin level, total testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured by the chemiluminescence method with Unice I/8I 800 Access Immunoassay System (Miami, FL, ABD). Complete blood count was carried out through autoanalyzer. Insulin sensitivity was calculated by using the Homeostatic model assessment-insulin resistance (HOMA IR) by the formula, HOMA-IR=(insulin x glucose)/405. Plasma asymmetric dimethylarginine (ADMA) levels were determined by ELISA (Immundiagnostik, Bensheim, Germany). The minimum detectable concentration for ADMA was 0.05µmol/L. Tumor Necrosis Factor Weak Inducer of Apoptosis (sTWEAK) was measured in serum by ELISA kit (Bender MedSystems, Lot Nr.BMS2006INST, Vienna, Austria). The calculated overall intra-assay and inter-assay coefficient of variation for sTWEAK were 7.9% and 9.2%, respectively (minimum detectable concentration= 9.7 pg/mL). ELISA measurements were carried out using Bio-Tek Synergy HT plate reader (Biotek Instruments Inc., Winooski, VT, USA). High-sensitivity C-reactive protein (hs-CRP) level was determined in serum by immunoturbidometric fixed rate method by Olympus AU-2700 autoanalyzer (Hamburg, Germany). Intra-assay coefficient of variation (CV) and inter-assay CV were 5.8% to 3.1% respectively. The minimum detectable concentration for hs-CRP was 0.07 mg/L.

Statistical analysis

All data were recorded on a computer database and analyzed using SPSS 15.0 package program (SPSS, Inc., Chicago, IL, USA). Results are expressed as mean ± S.D. The variables were assessed for normality using Kolmogorov-Smirnov test and Levene’s test was used to evaluate the equality of variance. Intragroup changes at two time points were analyzed by paired samples t-test or Wilcoxon signed-rank test as appropriate. Inter-group differences were analyzed by Student’s t-test and Mann–Whitney U test as appropriate. Differences were considered significant at p<0.05.

Results

The demographical and biochemical characteristic of the patients and the control subjects are given in Table 1. When compared to the healthy controls, the

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=70)</th>
<th>Patients (n=60)</th>
<th>Patients after treatment (n=60)</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21.32±1.13</td>
<td>21.82±2.22</td>
<td>24.22±3.13</td>
<td>0.102</td>
<td>-</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.73±2.38</td>
<td>22.38±3.1</td>
<td>24.22±3.13</td>
<td>0.268</td>
<td>&lt;0.001</td>
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<tr>
<td>WC (cm)</td>
<td>79.60±6.94</td>
<td>84.72±10.0</td>
<td>87.75±9.9</td>
<td>0.009</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115±10.7</td>
<td>118±13.5</td>
<td>120±19.9</td>
<td>0.207</td>
<td>0.01</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68.84±5.87</td>
<td>72.33±8.0</td>
<td>71.46±9.1</td>
<td>0.02</td>
<td>0.411</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>83.10±11.76</td>
<td>86.20±6.09</td>
<td>85.93±7.59</td>
<td>0.154</td>
<td>0.814</td>
</tr>
<tr>
<td>T.Chol (mg/dl)</td>
<td>165.3±28.6</td>
<td>159.2±24.4</td>
<td>152.6±26.5</td>
<td>0.210</td>
<td>0.032</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>79.0 (59.0-97.75)</td>
<td>97.0 (66.5-135.0)</td>
<td>89.0 (69.5-142.5)</td>
<td>0.01</td>
<td>0.70</td>
</tr>
<tr>
<td>HDL-Chol (mg/dl)</td>
<td>45.85±9.8</td>
<td>47.63±9.9</td>
<td>41.63±9.1</td>
<td>0.381</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-Chol (mg/dl)</td>
<td>100.0±23.76</td>
<td>89.52±19.35</td>
<td>88.35±23.94</td>
<td>0.007</td>
<td>0.596</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>3.66±2.38</td>
<td>0.76±0.62</td>
<td>0.87±1.50</td>
<td>&lt;0.001</td>
<td>0.486</td>
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<tr>
<td>LH (mIU/ml)</td>
<td>4.91±1.77</td>
<td>0.43±0.54</td>
<td>0.64±1.0</td>
<td>&lt;0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>T.Testosterone (ng/ml)</td>
<td>5.35±1.29</td>
<td>0.26±0.16</td>
<td>2.0±1.57</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulina (µU/ml)</td>
<td>4.85 (3.39-5.78)</td>
<td>9.04 (6.28-13.23)</td>
<td>8.33 (6.73-13.25)</td>
<td>&lt;0.001</td>
<td>0.964</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.92 (0.69-1.27)</td>
<td>1.92 (1.40-2.79)</td>
<td>1.80 (1.39-2.90)</td>
<td>&lt;0.001</td>
<td>0.955</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.13±1.1</td>
<td>1.14±1.0</td>
<td>1.12±1.2</td>
<td>0.158</td>
<td>0.450</td>
</tr>
<tr>
<td>TWEAK (pg/ml)</td>
<td>1282.9±205.5</td>
<td>1043.2±386.5</td>
<td>980.5±314.2</td>
<td>&lt;0.001</td>
<td>0.219</td>
</tr>
<tr>
<td>ADMA (µmol/L)</td>
<td>0.33±0.05</td>
<td>0.65±0.17</td>
<td>0.72±0.2</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
</tbody>
</table>

P1: Students-t test. The comparison of the parameters between the healthy control subjects and the patients.
P2: Paired samples t test. The comparison of the parameters of the patients before and after the TRT

aResults are given as mean (25–75%). BMI, Body mass index; WC, Waist circumference; SBP, Sistolic blood pressure; DBP, Diastolic blood pressure; FBG, Fasting blood glucose; T.Chol, Total cholesterol; TG, Triglyceride; HDL-Chol, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; FSH, Follicle stimulating hormone LH, Luteinizing hormone; T.Testosterone, Total Testosterone; HOMA-IR, Homeostatic model assessment-insulin resistance; hsCRP, High-sensitivity C-reactive protein; TWEAK, Tumor Necrosis Factor Weak Inducer of Apoptosis; ADMA, Asymmetric dimethylarginine
the injectable or transdermal treatment regimen were also compared. According to the results, no significant differences were present between the alterations of the two treatment arms (Table 2).

There were significant correlations between the total testosterone levels and the ADMA (r = -0.703 p< 0.001), TWEAK (r = 0.370 p<0.001), HOMA-IR (r = -0.451 p<0.001), WC (r = -0.410 p< 0.001), TG (r = -0.276 p=0.002) or LDL Cholesterol (r = 0.218 p<=0.018) levels.

Multiple regression analysis was applied to test the independent link between total testosterone levels and the demographical and metabolic parameters after six months of follow up with TRT, the alterations in ADMA levels were significant however the alterations in TWEAK and HOMA-IR were not significant.

WC (p=0.009), DBP (p=0.02), Triglycerides (p=0.03), ADMA, insulin and HOMA-IR levels (p<0.001 for all) were significantly higher, and the TWEAK levels were significantly lower (p<0.001) in patients with CHH. After the six months of follow up with TRT, the patients had significantly elevated ADMA, SBP, BMI and WC (p=0.003, p=0.01, p=0.001 and p=0.001 respectively) and decreased total and HDL cholesterol levels (p=0.032 and p<0.001 respectively) while the alterations in TWEAK, hsCRP and HOMA-IR were not significant (Fig. 1, Table 1). The percent alterations of the demographical and metabolic parameters after the TRT are shown in Table 1 and Fig. 1.

Fig. 1  The comparisons of the plasma ADMA (a), TWEAK (b) and HOMA-IR (c) levels between patients before and after the TRT and the healthy control subjects.

There were significant alterations of plasma ADMA, TWEAK and HOMA-IR levels between hypogonadal patients and healthy controls. After the six months of follow up with TRT, the alterations in ADMA levels were significant however the alterations in TWEAK and HOMA-IR were not significant.

WC (p=0.009), DBP (p=0.02), Triglycerides (p=0.03), ADMA, insulin and HOMA-IR levels (p<0.001 for all) were significantly higher, and the TWEAK levels were significantly lower (p<0.001) in patients with CHH. After the six months of follow up with TRT, the patients had significantly elevated ADMA, SBP, BMI and WC (p=0.003, p=0.01, p=0.001 and p=0.001 respectively) and decreased total and HDL cholesterol levels (p=0.032 and p<0.001 respectively) while the alterations in TWEAK, hsCRP and HOMA-IR were not significant (Fig. 1, Table 1). The percent alterations of the demographical and metabolic parameters after the TRT are shown in Table 1 and Fig. 1.

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Multiple regression analysis was applied to test the independent link between total testosterone levels and
Cardiometabolic risk in hypogonadism

There seems to be a mutual link between hypogonadism and cardiovascular or metabolic disorders. Patients with type 2 diabetes, metabolic syndrome or cardiovascular disease have low testosterone levels [21-23], while patients with hypogonadism have significantly increased risk of cardiovascular or metabolic disorders [24-26]. Several physiological factors including aging [27], obesity [25] or commonly used medications [28, 29] may also cause low testosterone levels and potentially confound the relationship between hypogonadism and chronic metabolic disorders. Therefore, our results are important, as we have potential functional correlates of this outcome variable (ADMA, TWEAK, WC, HOMA-IR, TG, LDL cholesterol). Multivariate models were of adequate statistical power because included at least 10 observations for each covariate in the same models. The multivariate analysis has shown that the total testosterone levels were the significant independent determinants of the plasma ADMA (β = -0.575 p<0.001) and HOMA-IR (β =-384 p=0.004) levels.

**Discussion**

According to the results, the study population of young and previously untreated patients with CHH has higher triglycerides, waist circumferences, diastolic blood pressures and lower LDL cholesterol levels when compared to the healthy control subjects. Moreover, these patients have significantly higher levels of insulin, HOMA-IR, ADMA, and significantly lower levels of TWEAK, indicative of the presence of insulin resistance, inflammation and endothelial dysfunction. According to the multivariate analysis, low testosterone is the significant determinant of the endothelial dysfunction and the insulin resistance. However, none of the above parameters have improved after six months of TRT. Even the previously elevated ADMA levels increased much more significantly after the treatment period. Also, the BMI, WC and systolic blood pressures increased and the total and HDL cholesterol levels decreased in the follow-up period. Below, the implications of these findings will be discussed in detail.

**Table 2** The effect of injectable and transdermal testosterone regimens on the demographic and metabolic parameters

<table>
<thead>
<tr>
<th></th>
<th>Injectable testosterone treatment (n=40)</th>
<th>Transdermal testosterone treatment (n=20)</th>
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<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5±2.17</td>
<td>24.46±3.08</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>85.7±10.08</td>
<td>89.6±9.03</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115.1±10.7</td>
<td>119.1±8.8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72.2±7.9</td>
<td>72.4±9.0</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>85.6±5.9</td>
<td>86.9±7.1</td>
</tr>
<tr>
<td>T.Chol (mg/dl)</td>
<td>156.9±25.3</td>
<td>150.1±28.3</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>96.0 (61.5-130.5)</td>
<td>84.0 (68.0-136.0)</td>
</tr>
<tr>
<td>HDL-Chol (mg/dl)</td>
<td>48.3±11.0</td>
<td>41.1±9.1</td>
</tr>
<tr>
<td>LDL-Chol (mg/dl)</td>
<td>88.2±18.9</td>
<td>88.9±23.9</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>0.7±0.58</td>
<td>0.68±0.58</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>0.33±0.35</td>
<td>0.51±0.64</td>
</tr>
<tr>
<td>T.Testosterone (ng/ml)</td>
<td>0.28±0.16</td>
<td>2.22±1.66</td>
</tr>
<tr>
<td>Insulin* (µU/ml)</td>
<td>9.1 (6.6-13.4)</td>
<td>8.3 (7.0-12.3)</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>1.9 (1.4-2.8)</td>
<td>2.7 (1.6-3.1)</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.3±1.11</td>
<td>1.5±2.48</td>
</tr>
<tr>
<td>TWEAK (pg/ml)</td>
<td>1065.0±389.5</td>
<td>991.9±249.2</td>
</tr>
<tr>
<td>ADMA (µmol/L)</td>
<td>0.62±0.14</td>
<td>0.67±0.16</td>
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</table>

p1: Paired samples t test. The comparison of the parameters of the patients before and after injectable testosterone treatment.

p2: Paired samples t test. The comparison of the parameters of the patients before and after transdermal testosterone treatment.

p3: Mann-Whitney U test. The comparison of the effect of each treatment regimen on the demographic and metabolic parameters.

*Results are given as mean (25–75%)

BMI, Body mass index; WC, Waist circumference; SBP, Sistolic blood pressure; DBP, Diastolic blood pressure; FBG, Fasting blood glucose; T.Chol, Total cholesterol; TG, Triglyceride; HDL-Chol, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; T.Testosterone, Total Testosterone; HOMA-IR, Homeostatic model assessment-insulin resistance; hsCRP, High-sensitivity C-reactive protein; TWEAK, Tumor Necrosis Factor Weak Inducer of Apoptosis; ADMA, Asymmetric dimethylarginine
evaluated the cardiovascular risk status in young and treatment naive hypogonadal patients, who have no other chronic diseases. We have previously reported that these patients, even at the very young ages, have metabolic problems such as visceral obesity, elevated blood pressures and dyslipidemia [5]. The results of the present study not only replicate the previous data, but also show elevated ADMA, HOMA-IR and low TWEAK levels in this specific patient group.

Being an endogenous inhibitor of nitric oxide (NO) synthase, ADMA elevation is indicative of endothelial dysfunction, the initial step in atherosclerosis and cardiovascular mortality [18]. High ADMA levels are well established in metabolic disorders such as T2DM, hypertension, chronic kidney disease and dyslipidemia [30]. Now, our data present high ADMA levels in hypogonadism as well. Furthermore, these subjects have significantly higher HOMA-IR levels indicative of the presence of insulin resistance in the patient group. Moreover, low total testosterone appears to be the significant independent determinant of the ADMA and HOMA-IR levels. Endothelial dysfunction, insulin resistance and inflammation are the major factors in the pathogenesis of atherosclerosis [31-33]. The elevation of these surrogate markers implicates that these young hypogonadal subjects have endothelial dysfunction and insulin resistance. However, there was no difference between the hs-CRP levels of the healthy controls and the patients. CRP is an established marker of inflammation and increased risk of metabolic and cardiovascular disorders [34, 35]. Unaltered hs-CRP levels may imply that inflammation may not be a significant problem in these subjects. However, these patients have significantly lower TWEAK levels. TWEAK is a multifunctional cytokine that controls many cellular activities including proliferation, migration, differentiation, apoptosis, angiogenesis and inflammation [35, 36]. Unlike CRP, TWEAK is produced from wide variety of different tissues and cells other than the liver. TWEAK plays an important role in chronic inflammatory disease and participates in different stages of atherosclerotic plaque development from early stages to progression and subsequent plaque rupture [36, 37]. This is the first study showing low plasma levels of TWEAK in patients with hypogonadism.

Since, the role of low testosterone levels in the pathogenesis of cardiovascular and metabolic outcomes are well established [21-24], doctors from different branches, pharmaceutical companies and even tabloid media have “great expectations” from the androgen replacement therapy [38]. However, there is controversial data and growing skepticism about the effect of TRT on the improvement of the cardiometabolic risk. Recently a prospective study in elderly hypogonadal men was terminated due to the increased cardiovascular mortality in the testosterone replacement arm [13]. Also, a recent retrospective cohort in hypogonadal elderly men showed that TRT is associated with an increased risk of death, MI, or ischemic stroke [39]. So far, no randomized, placebo controlled prospective study has been performed to investigate the long-term metabolic and cardiovascular outcomes of TRT in patients with hypogonadism. Therefore, measuring the alterations of the surrogate markers of inflammation, endothelial dysfunction or insulin resistance is the alternate way to evaluate the cardiovascular and metabolic effects of TRT in patients with hypogonadism. Up to now, the studies searching for the effect of testosterone replacement on endothelial functions yielded inconclusive results. A study performed by measuring flow-mediated dilatation, has not shown any benefit [40], while two other studies, reported improvements in ADMA levels [41, 42]. Certainly, the improvement in ADMA levels after TRT should be a promising finding. However, significant methodological problems are present in these reports. One of these studies reported improved ADMA levels which were measured only a few days after a single injection [41] while the other one measured ADMA levels of 10 middle aged and elderly hypogonadal patients having different etiologies and several comorbidities [42]. Likewise, the previous reports about the effects of TRT on insulin resistance and inflammation are controversial. Some studies mention improvements [6-9] while others mention either trivial [12, 14] or unfavorable effects [11, 13, 39]. The reasons for these conflicting results may be related to the methodological variations such as the inhomogenities of the study populations, using different testosterone regimens in different follow-up periods and the presence of various confounders such as concomitant drugs or metabolic disorders. Along with the editorial bias towards over-publication of the positive results [43], it is likely that the effects of TRT might have been overstated at least in some of these reports [44]. Our data support the previous reports about the unfavorable cardiometabolic effects of TRT [11, 13, 39] and implicate that the worsening of endothelial functions may contribute to the
adverse cardiovascular outcomes of TRT. There may be several potential mechanisms how TRT worsen the cardiometabolic parameters. First, supraphysiological androgen dosages, namely periodic depot injections, may cause the adverse effect [45, 46]. However, our results show that both daily transdermal and triweekly depot injections of TRT have similar cardiometabolic effects in this patient group. Second, effects of endogenous and exogenous testosterone may not be similar. Exogenous testosterone may induce the coagulation and aggravate thrombotic disorders such as polycythemia, hyperviscosity of blood and platelet aggregation [16, 47]. Exogenous testosterone may also increase circulating estrogens which may cause increased cardiovascular events [48].

There may be several limitations of this study. As mentioned in the methods section, the patients were not randomly allocated to the treatment arms. Also, this specific population of treatment naïve young patients with CHH may not represent the general population of hypogonadism. It is likely that, TRT may ignite pubertal growth in these young patients, which is not seen in the elderly hypogonadal patients of other etiologies. This may partly explain the rapid increase in BMI and waist circumference of these patients. As we do not have long term follow up data, insights about the long-term metabolic and cardiac effects of TRT in these patients may not be accurate. Another limitation may be the small sample size. However, being untreated in the adult age is a very uncommon situation. Therefore we think that the number of the patients is adequate due to the unique conditions of the study group. However, despite these drawbacks, this study has significant advantages, regarding the homogeneous study population and lack of confounding factors, such as chronic metabolic disorders and concomitant medications.

In conclusion, the present study shows significantly increased plasma ADMA and HOMA-IR and decreased plasma TWEAK levels in treatment naïve very young patients with congenital hypogonadism. Six months of treatment with two different testosterone replacement regiments do not seem to improve but rather worsen the increased ADMA levels along with the worsening of other metabolic parameters in these patients. Whether these metabolic alterations will result in adverse cardiovascular outcomes in the long term is not clear. Also, the results of the present study do not resolve the controversies about the effect of TRT on the cardiometabolic parameters such as insulin resistance and inflammation. Therefore, prospective studies are needed to find out the long-term effects of testosterone replacement on cardiovascular morbidity and mortality in this specific population.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Conflict of Interest

The authors declare that they have no conflict of interest relevant to this manuscript.

References


