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ORIGINAL ARTICLE



Influence of testosterone substitution on glycemic control and endothelial markers in men with newly diagnosed functional hypogonadism and type 2 diabetes mellitus: a randomized controlled trial

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ABSTRACT

Effects of testosterone (T) on the cardiovascular system of men remain controversial. The impact of T-replacement therapy (TRT) in men with functional hypogonadism and type 2 diabetes mellitus (T2DM) has to be elucidated. This study included 80 men (mean age 51.5 ± 6.3 years) with newly diagnosed T2DM (according to ADA criteria) and functional hypogonadism (according to EAU criteria). Randomization: Group1 ($n=40$): TRT using 1%-transdermal T-gel (50 mg/day), Group2 ($n=40$) no TRT (controls). Dietary treatment applied to both. Parameters at baseline/after 9 months: anthropometric parameters, lipids and indicators of carbohydrate metabolism (fasting glucose, insulin, HbA1c, HOMA-IR), markers of adipose tissue and EnD (leptin, resistin, p- and e-selectin, ICAM-1, VCAM-1 and CRP). ANCOVA for repeated measurements revealed TRT to cause a significant decrease in waist circumference (WC), HOMA-IR and HbA1c vs controls ($p < .001$, $p = .002$, $p = .004$, respectively). Leptin declined in subjects receiving TRT vs controls ($p = .04$). Concentrations of resistin, ICAM-1, p-selectin and CRP decreased significantly vs controls (all $p < .001$); no effects for e-selectin and VCAM-1. Advanced age attenuated effects, higher delta testosterone levels augmented effects. Decrement of WC was related to decreasing markers of adipose tissue secretion/EnD. TRT in men with functional hypogonadism and T2DM improved carbohydrate metabolism and markers of endothelial dysfunction.

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Introduction

In men, serum testosterone (T) levels decrease about 1% per year after the third decade of life [1]. Factors detrimenting general health contribute markedly to this decline, also irrespectively of age, paramount comorbidities in this context being obesity and type 2 diabetes mellitus (T2DM) [1–3]. Simultaneously, lower T levels can promote fat accumulation, suggesting a bidirectional relationship between obesity and low T, creating a self-reinforcing mechanism [4,5].

Weight gain is mostly and progressively associated with a decline in T levels without a concomitant change in LH [6]. The phenomenon is referred to as functional hypogonadism, as this particular type of testosterone deficiency exists without pathological correlate in the testes or hypothalamic/pituitary region (these conditions are known as classical hypogonadism) [7]. Functional hypogonadism accounts for more than 50% of men with low T in the general population

[8–10] and even more in patients with sexual dysfunction [11–13]. In general, complaints regarding sexuality and general wellbeing are pivotal in diagnosing functional hypogonadism and identifying an indication for testosterone replacement therapy (TRT) [7,14–18].

Simultaneously, there are strong indications that low testosterone levels can promote insulin resistance and the development of T2DM [19–21] and that, vice versa, TRT is able to mitigate insulin resistance [22,23] and, possibly, attenuate the detrimental effects of T2DM promoting vascular morbidity; there are observations that ultimately also promote mortality in men with T2DM and concomitant hypogonadism might be reduced by TRT [24,25]. There is still debate whether such findings can be applied to men with functional hypogonadism as such [26]. Recent trials in such men have demonstrated at least a decrease in insulin resistance (IR) in hypogonadal men with and without T2DM, but also markers of cardiovascular health, such

as intima-media thickness or lipid parameters [27–29]. In this context, hypogonadal men presenting with T2DM being already under effective antidiabetic therapy are not likely to exhibit further declines in HbA1c when started on TRT [30].

Hypogonadism has been demonstrated to have a negative impact on numerous factors of cardiovascular risk in general, such as IR/T2DM, dyslipidemia and visceral obesity [31]. The presence of each of these factors separately contributes to endothelial dysfunction (EnD) and their combination synergistically aggravates pathological processes within endothelial cells [31]. EnD is seen as the universal starting mechanism of any vascular pathology and marker of its progression [32]. The earliest signs of EnD are impairments of secretory function of the endothelium, which are present before the deterioration of vasomotor functions and clinical manifestations of cardiovascular diseases [33–35].

A pathogenetic mechanism linking components of the disturbed carbohydrate metabolism found in IR and T2DM with hypogonadism is the synthesis of pro-inflammatory substances by the visceral adipose tissue [5,19]. These substances trigger the pathological expression cascade of numerous adhesion molecules that can activate and damage the endothelium [36]. Further on, secreted by adipocytes, the hormone resistin not only inhibits insulin-mediated glucose uptake by target tissues and acts as an insulin antagonist, it also participates in stimulation of inflammatory processes. In addition, it can activate endothelial cells, which further on leads to proliferation of smooth muscle cells of vessel walls, promoting the formation of foam cells [37]. Resistin can enhance the expression of the adhesion molecules VCAM-1 and ICAM-1 on the surface of endotheliocytes, a process contributing to the aggravation of EnD and ultimately leading to vascular damage [38].

This is a process related to the shedding of selectins by endothelial cells: P-selectin, the largest selectin, is stored in α -granules of platelets and in Weibel–Palade bodies of endothelial cells, and is translocated to the cell surface of activated endothelial cells and platelets. E-selectin is not expressed under baseline conditions, but is rapidly induced by acute inflammatory processes. Generally, selectins are involved in constitutive lymphocyte homing in chronic and acute inflammation processes, including atherosclerosis [39,40].

Leptin, secreted by visceral fat tissue, can be considered as a pro-atherotic agent, as well [41]. It regulates the production of nitric oxide (NO) synthase in endotheliocytes and the accumulation of reactive oxygen species, which stimulates the migration of

endothelial cells [42]. Leptin promotes the deposition of cholesterol in macrophages and stimulates atherogenesis [19]. Moreover, the accumulation of metabolically active visceral fat on the one hand contributes to the development of IR and EnD, and on the other hand suppresses T production by Leydig's cells via inhibitory Leptin signaling and disrupts the feedback to the pituitary gland and hypothalamus via the kisspeptin signaling pathway, causing the progression of functional hypogonadism, characterized by low serum levels of testosterone and inadequately low levels of gonadotropins without organic correlate [5,19,43].

Summarizingly, the vascular endothelium is an important target for the (indirect) action of testosterone, and that EnD is one of the key factors of cardiovascular risk in men. Therefore, research of TRT in men with factors compromising endothelial integrity, such as T2DM, is paramount. Men with untreated T2DM are likely to present a pivotal population in this regard.

Aim

Evaluation of the effects of TRT on carbohydrate metabolism and the biochemical markers of EnD in men with newly diagnosed T2DM and functional hypogonadism.

Patients and methods

The trial was registered at EGISU-NIOKTR (114090170004).

The study included 80 men newly diagnosed with T2DM according to the criteria of the American Diabetes Association and referring to HbA1c levels as leading parameter (HbA1c levels had to be measured two times within 4 weeks above 6.5%) [44]. HbA1c levels above 9.0% led to exclusion of subjects for this trial: these men were treated on an individual basis and not started on a dietary change alone [45].

Men were simultaneously also diagnosed for potential functional hypogonadism according to the diagnostic criteria of the EAU guideline on male hypogonadism as of 2015 (serum levels of total testosterone two times below 12.1 nmol/L or serum levels of free testosterone two times below 243 pmol/L in combination of at least two symptoms or complaints of sexual or psychological nature) [46]. Exclusion criteria were primary or secondary hypogonadism of any origin (testicular damage, diagnosis of pituitary/hypothalamic malfunction), as well as hyperprolactinemia and hypothyroidism. Also any history of a malignant disease as well as elevated levels of PSA ($>4 \mu\text{g/dL}$) and the wish for paternity led to exclusion.

Patients were randomized into 2 groups using a random number list. The first group included 40 men (mean age 53.3 ± 5.4 years) who received T-Gel at a dose of 50 mg per day (testosterone replacement therapy, TRT), which was applied to the skin in the morning on a daily basis (serum sampling was performed 3–4 hours after application of gel). The second group served as a control cohort and was represented by 40 men as well (mean age 54.1 ± 5.6 years), who were not assigned to TRT.

Antidiabetic therapy in all subjects was restricted to dietary changes, respective counseling was homogeneously applied. A complete re-assessment of baseline parameters was performed at the end of the trial after 9 months.

The number of patients completing the trial was $n=76$. Within the TRT group as well as the control group, 2 subjects each were lost to follow-up. The analysis was thus performed as per protocol analysis in 2×38 men.

All patients gave written informed consent according to protocol approved by the Ethics Committee of the Rostov State Medical University.

Patients underwent anthropometric measurements such as height, body weight, body mass index (BMI), waist circumference (WC), blood pressure (BP) and heart rate. The laboratory tests included parameters of carbohydrate metabolism (fasting blood glucose, glycosylated hemoglobin (HbA1c), immunoreactive insulin (IRI), and calculation of HOMA1-IR). Hormone analysis included the serum levels of total T, sex hormone-binding globulin (SHBG) with the calculation of the level of free T according to Vermeulen [47], the assessment of luteinizing hormone (LH), follicle stimulating hormone (FSH). In addition, serum levels of leptin, resistin, p- and e-selectin, intracellular adhesion molecule type 1 (ICAM-1), and vascular adhesion molecule type 1 (VCAM-1) as well as c-reactive protein (CRP) were determined.

Blood was collected from the ulnar vein before 10 am after 12 hours of fasting. Biochemical tests were performed from fresh blood serum; for enzyme immunoassays, the blood was centrifuged at 3000 rpm for 20 min and serum was frozen at -20°C for analysis of all parameters in one batch.

The Bayer ADVIA 1650 analyzer was used to assess biochemical markers. The determination of HbA1c was performed on the Siemens Healthcare Diagnostics DCA 2000+ analyzer.

To determine insulin resistance, the HOMA1-Index using the homeostasis model was used as described (HOMA1: fasting insulin ($\mu\text{U/ml}$) \times fasting glucose (mmol/l)/22.5) [48].

Enzyme-linked immunosorbent assays (ELISA) included the assessment of sex hormones, adipohormones as well as markers of endothelial function. The serum levels of LH, FSH, were determined using the Alcor-Bio kits (Russia). Determination of total T and SHBG was performed using the DRG Elisa test system (Germany). To assess serum levels of leptin laboratory kits from Mediagnost (Germany), for resistin from BioVendor (Czech Republic), for IRI from Monobind Inc. (USA) were used. The serum concentration of endothelial markers was assessed by laboratory kits for ELISA: ICAM-1, VCAM-1, p-selectin, e-selectin assays by Bender Medisystems GmbH (Austria).

Intra- and interassay coefficients of variation were less than 5% and less than 10%, respectively for all analytes.

Statistics

All variables were checked for normality of distribution according to Kolmogorov–Smirnov. Since most data were not normally distributed, they are presented as median and interquartile range (IQR). Statistical analysis was carried out using the non-parametrical Mann–Whitney *U*-test for paired groups for comparison of baseline to follow-up levels within each group. The Mann–Whitney *U*-test for two independent samples was used to compare follow-up levels between the treatment group and the control group.

Variables were log-transformed to achieve normalization to perform ANCOVA for repeated measurements to compare treatment effects between both groups over time using stepwise models involving covariates (age, delta serum testosterone concentrations, delta waist circumference). Further on, a backward stepwise multiple regression model was established to elucidate putative parameters of influence on the observed decrement of IR (calculated as HOMA1) and on HbA1c on the effects within the active treatment arm.

The critical level of significance in testing statistical hypotheses was assumed to be 0.05. Statistical analysis was carried out using the STATISTICA software package (StatSoft 10), Berikon, Switzerland and SPSS 24.0, Chicago, Illinois, USA.

Results

Regarding safety of subjects, during the entire period of observation no serious adverse events were registered, PSA levels remained below 4 ng/dL for all subjects, hematocrit remained below 52% for all subjects.

Serum sex hormone concentrations and safety parameters are presented in Table 1. Overall, there was a marked increase in serum testosterone concentrations within the treatment arm vs baseline as well as compared to the control group at the time point of follow-up. The patients receiving TRT also exhibited a significant decline in serum gonadotropin levels. Men applying T-gel showed a marked increase in hemoglobin and hematocrit remaining within the acceptable normal range, but they showed no change in PSA.

Baseline and follow-up anthropometric parameters are displayed in Table 2. There was a slight but significant reduction of weight as well as BMI within the active treatment arm vs baseline as well as compared to the control group at the time point of follow-up. A more pronounced effect was seen in terms of reduction of WC in patients receiving TRT.

Parameters reflecting carbohydrate metabolism are exhibited in Table 3. Fasting blood glucose as well as insulin levels decreased markedly in men receiving TRT, this is reflected by a significant loss in IR (calculated as HOMA1) and also HbA1c in men applying T-Gel vs baseline as well as compared to the control group at the time point of follow-up.

Markers of endothelial function and inflammation are shown in Table 4. There was a marked reduction of leptin, resistin; ICAM-1, p-selectin and CRP vs baseline as well as compared to the control group at the time point of follow-up. There were no differences in serum levels of VCAM-1 and e-selectin.

Parameters of lipid metabolism exhibited a significant decrease for total cholesterol, triglycerides and LDL-cholesterol in patients receiving TRT vs baseline as well as compared to the control group at the time point of follow-up. Levels HDL-Cholesterol increased in men receiving T-gel (Table 5).

The stepwise ANCOVA models for repeated measurements displayed in Table 6 demonstrate a time-dependent significant effect of TRT vs control for HOMA1, HbA1c, resistin, leptin, ICAM-1, p-selectin and CRP as detected by the tests displayed in Tables 3 and 4. Advanced age attenuated the effects, delta serum testosterone concentrations augmented the effects. Loss of WC contributed independently to a lower expression of EnD-markers and CRP. This effect was not visible regarding IR or HbA1c.

Multiple regression models in stepwise backwards mode regarding influence of treatment by T-gel vs control and putative parameters of influence on delta HOMA1 and delta HbA1c are displayed in Figure 1(a,b). Overall, treatment is effective. Age attenuates the treatment effects, while a higher delta of testosterone levels as well as higher delta levels of resistin, leptin as well as CRP (all lower) contribute to the effect of T-gel.

Discussion

In general, our study corroborates data on changes of weight loss, BMI, WC and especially markers of IR such

Table 1. Sex hormone concentrations and safety parameters.

Parameter (Unit) given as median (IQR)	Group 1 (TRT) baseline, <i>n</i> = 38	Group 1 (TRT) follow-up, <i>n</i> = 38	Group 1 (TRT) baseline vs follow- up, <i>p</i>	Group 2 (control) baseline, <i>n</i> = 38	Group 2 (control) follow-up, <i>n</i> = 38	Group 2 (control)baseline vs follow-up <i>p</i>	Group 1 (TRT) vs Group 2 (control) at follow-up, <i>p</i>
Total T (nmol/l)	9.6 (2.7)	15.0 (6.4)	<.001	9.9 (2.6)	9.8 (2.9)	ns	<.001
Free T (pmol/l)	208 (142)	323 (167)	.001	223 (140.0)	230 (134)	ns	.008
SHBG (nmol/l)	20.8 (20.7)	22.8 (20.2)	ns	18.5 (17.6)	21.2 (16.0)	ns	ns
LH (IU/l)	3.5 (2.3)	2.4 (2.1)	.03	4.0 (2.4)	4.5 (2.8)	ns	<.001
FSH (IU/l)	4.9 (2.6)	2.7 (2.2)	<.001	4.6 (2.4)	4.4 (2.3)	ns	.001
Hemoglobin	14.6 (1.3)	15.7 (1.5)	<.001	14.7 (1.2)	15.0 (1.4)	ns	.03
Hematocrit	44.2 (2.7)	46.4 (3.2)	.003	44.4 (2.8)	44.8 (2.9)	ns	.04
PSA (ng/ml)	0.9 (1.0)	0.9 (1.3)	ns	0.8 (0.9)	0.9 (1.0)	ns	ns

IQR: interquartile range, follow-up at 9-months, *p* values according to Wilcoxon-*U*-Tests (for matched pairs within groups, for independent samples between groups), per protocol analysis. Baseline data between groups were statistically not different ($p \geq .05$) for all hormone parameters. Not significant: "ns": $p > .1$, *p* values between .05 and .1 are reported, but are not significant, as well.

Table 2. Anthropometric parameters.

Parameter, Unit given as median (IQR)	Group 1 (TRT) baseline, <i>n</i> = 38	Group 1 (TRT) follow-up, <i>n</i> = 38	Group 1 (TRT) baseline vs follow-up, <i>p</i>	Group 2 (control) baseline, <i>n</i> = 38	Group 2 (control) follow-up, <i>n</i> = 38	Group 2 (control) baseline vs follow-up <i>p</i>	Group 1 (TRT) vs Group 2 (control) at follow-up, <i>p</i>
Age (years)	53 (7)	—	—	54 (7)	—	—	—
Weight (kg)	105.0 (12)	100.0 (9)	.03	105.5 (13)	106.0 (12)	ns	.01
BMI ($\text{kg} \times \text{m}^{-2}$)	34.0 (2.6)	32.3 (2.8)	.006	33.6 (2.9)	33.9 (2.8)	ns	.01
WC (cm)	114.3 (9.5)	108.1 (8.2)	.003	114.7 (9.8)	115.5 (10.1)	ns	<.001

IQR: interquartile range, follow-up at 9-months, *p* values according to Wilcoxon-*U*-Tests (for matched pairs within groups, for independent samples between groups), per protocol analysis. Baseline data between groups were statistically not different ($p \geq .05$) for all anthropometric parameters. Not significant: "ns": $p > .1$, *p* values between .05 and .1 are reported, but are not significant, as well.

Table 3. Parameters of carbohydrate metabolism.

Parameter (Unit) given as median (IQR)	Group 1 (TRT) baseline, <i>n</i> = 38	Group 1 (TRT) follow-up, <i>n</i> = 38	Group 1 (TRT) baseline vs follow-up, <i>p</i>	Group 2 (control) baseline, <i>n</i> = 38	Group 2 (control) follow-up, <i>n</i> = 38	Group 2 (control) baseline vs follow-up <i>p</i>	Group 1 (TRT) vs Group 2 (control) at follow-up, <i>p</i>
Fasting blood glu- cose (mmol/l)	8.1 (3.7)	6.3 (2. 2)	.01	8.7 (5.0)	8.8 (5.0)	ns	.004
IRI (μU/ml)	26.7 (12.8)	17.5 (7.6)	<.001	24.2 (13.7)	26.2 (13.9)	ns	<.001
HOMA1	9.8 (5.3)	6.3 (4.8)	.003	10.2 (5.7)	11.6 (5.9)	ns	<.001
HbA1c (%)	7.8 (2.4)	6.7 (1.9)	.03	7.9 (2.4)	8.4 (3.1)	ns	.004

IQR: interquartile range, follow-up at 9-months, *p* values according to Wilcoxon-*U*-Tests (for matched pairs within groups, for independent samples between groups), per protocol analysis. Baseline data between groups were statistically not different ($p \geq .05$) for all parameters regarding carbohydrate metabolism. Not significant: "ns": $p > .1$, *p* values between .05 and .1 are reported, but are not significant, as well.

It should be noted that within the treatment group 17 subjects had an HbA1c level <6.5% after nine months.

Table 4. Markers of endothelial function/inflammation.

Parameter (Unit) given as median (IQR)	Group 1 (TRT) baseline, <i>n</i> = 38	Group 1 (TRT) follow-up, <i>n</i> = 38	Group 1 (TRT) baseline vs follow-up, <i>p</i>	Group 2 (control) baseline, <i>n</i> = 38	Group 2 (control) follow-up, <i>n</i> = 38	Group 2 (control) baseline vs follow-up <i>p</i>	Group 1 (TRT) vs Group 2 (control) at follow-up, <i>p</i>
Leptin (ng/ml)	12.4 (8.6)	8.7 (5.7)	.03	11.7 (8.8)	11.9 (8.1)	ns	.04
Resistin (ng/ml)	11.5 (7.3)	4.0 (5.2)	<.001	10.9 (7.6)	10.4 (7.2)	ns	<.001
ICAM-1 (ng/ml)	323 (183)	198 (80)	<.001	297 (169)	321 (193)	ns	<.001
VCAM-1 (ng/ml)	825 (321)	819 (306)	ns	843 (344)	847 (325)	ns	ns
P-selectin (ng/ml)	591 (201)	77 (53)	<.001	414 (187)	408 (179)	ns	<.001
E-selectin (ng/ml)	45 (36)	42 (35)	ns	46 (33)	47 (30)	ns	ns
CRP (mg/l)	6.8 (3.3)	1.3 (0.8)	<.001	7.1 (3.8)	9.2 (4.5)	.03	<.001

IQR: interquartile range, follow-up at 9-months, *p* values according to Wilcoxon-*U*-Tests (for matched pairs within groups, for independent samples between groups), per protocol analysis. Baseline data between groups were statistically not different ($p \geq .05$) for all endothelial markers. Not significant: "ns": $p > .1$, *p* values between .05 and .1 are reported, but are not significant, as well.

Table 5. Serum lipid parameters.

Parameter (Unit) given as median (IQR)	Group 1 (TRT) baseline, <i>n</i> = 38	Group 1 (TRT) follow-up, <i>n</i> = 38	Group 1 (TRT) baseline vs follow-up, <i>p</i>	Group 2 (control) baseline, <i>n</i> = 38	Group 2 (control) follow-up, <i>n</i> = 38	Group 2 (control) baseline vs follow-up <i>p</i>	Group 1 (TRT) vs Group 2 (control) at follow-up, <i>p</i>
Total cholesterol (mmol/l)	6.1 (1.2)	5.1 (1.2)	<.001	5.9 (1.5)	5.5 (1.8)	ns	ns
Triglycerides (mmol/l)	2.4 (1.7)	1.7 (1.1)	.03	2.3 (1.6)	2.2 (1.5)	ns	.09 (ns)
HDL-cholesterol (mmol/l)	1.41 (0.12)	1.52 (0.13)	<.001	1.42 (0.12)	1.43 (0.13)	ns	.003
LDL-cholesterol (mmol/l)	3.41 (1.28)	2.86 (1.04)	.04	3.35 (1.30)	3.34 (1.32)	ns	.07 (ns)

IQR: interquartile range, follow-up at 9-months, *p* values according to Wilcoxon-*U*-Tests (for matched pairs within groups, for independent samples between groups), per protocol analysis. Baseline data between groups were statistically not different ($p \geq .05$) for all lipid parameters. Not significant: "ns": $p > .1$, *p* values between .05 and .1 are reported, but are not significant, as well.

as HOMA1 and HbA1c in hypogonadal men with newly diagnosed T2DM receiving TRT vs controls (previous meta-analysis [22]). Our data also correspond to the results of a meta-analysis that shows a decrease in fasting glycemia, fasting serum insulin, an index of IR, and HbA1c level on TRT in men with T2DM and functional hypogonadism [49].

One of the most fundamental studies in diabetology, UKPDS, has shown that a 1% decrease in HbA1c is associated with a 14% reduction in the risk of myocardial infarction, peripheral vascular peritoneal by 43%, and stroke by 12% [50], which underscores the importance of our results in terms of reduction in cardiovascular risk in men with T2DM and T deficiency.

In addition to these data, we demonstrate that adipohormones such as leptin and resistin, known to play a pivotal role in the development and exacerbation of IR/T2DM, can be influenced by TRT in hypogonadal

men and seem to be of paramount importance in facilitating the effects of androgen repletion in these patients on their diabetic status. Respective theories and findings indicated this in the past [5,19,20,43]. Leptin causes exacerbation of IR and decrease in not only the function, but also the mass of pancreatic β -cells [51]. Therefore, it can be assumed that a decrease in leptin concentrations may lead to an improvement in the carbohydrate metabolism and may be one of the additional mechanisms implementing the effects of TRT.

Resistin, an adipocyte-specific hormone, is suggested to be an important link between obesity and diabetes. Recent studies have suggested an association between resistin and atherogenic processes. The adhesion of circulating monocytes to endothelial cells is a critical step in the early stages of atherosclerosis. Furthermore, resistin increases the expression of ICAM-1

Table 6. Stepwise ANCOVA models for repeated measurements on parameters related to the main target of the trial.

Predictor	HOMA1	HbA1c	Resistin	Leptin	ICAM-1	p-Selectin	CRP
MODEL 1 (no covariates)							
Interaction of Treatment \times time	0.002	0.004	0.001	0.02	0.003	0.002	0.005
MODEL 2 (between-subject contrasts)							
Treatment yes/no	0.003	0.008	0.006	0.03	0.009	0.007	0.008
Age	0.02 ↓	0.03 ↓	0.04 ↓	0.04 ↓	0.01 ↓	0.008 ↓	0.03 ↓
MODEL 3 (between-subject contrasts)							
Treatment yes/no	0.003	0.009	0.007	0.03	0.009	0.008	0.008
Age	0.03 ↓	0.04 ↓	0.04 ↓	0.03 ↓	0.02 ↓	0.009 ↓	0.03 ↓
Delta total serum testosterone levels	0.01 ↑	0.02 ↑	0.03 ↑	0.04 ↑	0.02 ↑	0.006 ↑	0.005 ↑
MODEL 4 (between-subject contrasts)							
Treatment yes/no	0.004	0.009	0.007	0.04	0.01	0.008	0.009
Age	0.04 ↓	0.06, ns	0.07, ns	0.08, ns	0.05, ns	0.04 ↓	0.06, ns
Delta total serum testosterone levels	0.02 ↑	0.03 ↑	0.03 ↑	0.04 ↑	0.03 ↑	0.008 ↑	0.01 ↑
Delta waist circumference	0.18, ns	0.32, ns	0.02 ↑	0.008 ↑	0.04 ↑	0.01 ↑	0.002 ↑

Results of analysis of covariance (ANCOVA) for repeated measurements. p values and the direction of influence are given, ns: not significant. Treatment vs control was calculated as dichotomous variable (yes/no). Delta of total serum testosterone levels was calculated as follow-up value minus baseline value. Delta of waist circumference was calculated as baseline value minus follow-up value to maintain the putative direction of influence (higher value representing possible higher impact).

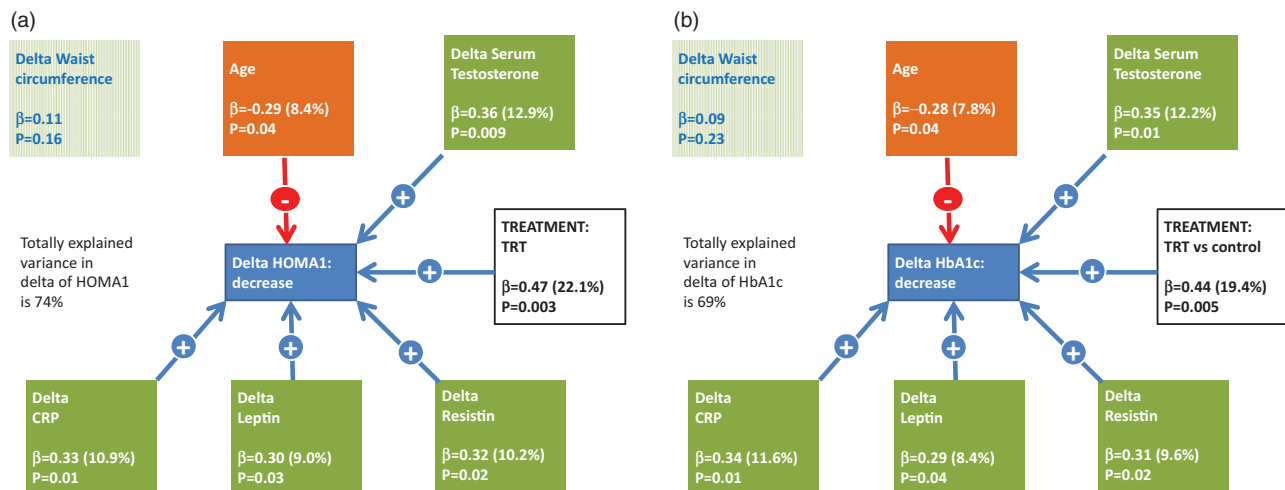


Figure 1 (a,b): Graphic display of putative effects of variables on delta HOMA1 (a) and HbA1c (b) as a marker of insulin resistance (as calculated by backward stepwise multiple regression models). The variables were identified by non-parametrical analyses (as shown in Tables 2 and 4) and log-transformed for normalization. Waist circumference was excluded by the model (shaded box). Standardized coefficients of variation (β) are given along with p values for their significance. The square of β explains the influence of the variable on the whole model (given as percent values behind the β value). Treatment itself was included as dichotomous variable (yes/no) and had the strongest effect. Delta of total serum testosterone levels was calculated as follow-up value minus baseline value. Deltas of waist circumference, leptin, resistin and CRP were calculated as baseline value minus follow-up value to maintain the putative direction of influence (higher value representing possible higher impact).

and VCAM-1 [52]. Resistin causes not only microvascular, but also severe macrovascular complications of diabetes and is secreted by the endothelium, it did markedly change in a favorable fashion during TRT in this trial. It is known that, in addition to the negative effect on carbohydrate metabolism, resistin activates the endothelium, triggering the proliferation of smooth muscle cells of vessel walls and promoting the formation of foam cells, thus facilitating the initiation and progression of atherosclerosis [37]. Therefore, the decrease in the level of resistin detected in this trial

within the background of TRT is most likely not only related to a reduction in the volume of visceral adipose tissue, but may also reflect an attenuation of its negative impact on the endothelium.

Moreover, in addition to studies demonstrating the stimulation of adhesion molecules by resistin, we obtained data on a significant decrease in the levels of ICAM-1, secreted directly by endothelial cells after their activation. ICAM-1 is a type of intercellular adhesion molecule continuously present in low concentrations in the membranes of leukocytes and endothelial

cells. Upon inflammatory stimulus, the concentrations greatly increase. When activated, leukocytes bind to endothelial cells via the ICAM-1 signal and then trans-migrate into tissues [53]. It has been established that the concentration of serum ICAM-1 correlates with the severity of the clinical manifestations of atherosclerosis and can serve as a sign of its activity [54]. This allows us to suggest that the endothelial damage decreases in case of treatment of androgen deficiency in men with T2DM. In contrast, the VCAM-1 gene contains six or seven immunoglobulin domains, and is expressed on both large and small blood vessels only after the endothelial cells are markedly stimulated by strong inflammatory processes [55]. As our subjects did not exhibit such events, it can be assumed that the lack of impact of TRT on VCAM-1 concentrations is due to low, not high grade vessel inflammatory status.

P-selectin also decreased significantly during TRT in a similar way. It is known that p-selectin is synthesized by endotheliocytes even at rest, but is released from special granules upon damage of the endothelium, thereby causing adhesion and rolling of leukocytes on its surface [39,40,54]. Hence, our data indicate that TRT can significantly reduce the damage to endotheliocytes accompanied by the release of p-selectin. In addition, it is not surprising that the concentration of e-selectin remained practically unchanged, since its synthesis is triggered immediately after damage of endotheliocytes, which, was shown earlier, significantly decreased.

It is also important to mention the significant reduction of serum levels of CRP during TRT in hypogonadal men with T2DM. It is known that CRP enhances the apoptosis of endothelial cells and inhibits angiogenesis by blocking the differentiation of endothelial progenitor cells (EPCs) that have escaped from the bone marrow [56]. Thus we conclude that during the compensation of androgen deficiency in patients with T2DM using TRT, damage of the endothelium and endothelial dysfunction are reduced. One may further speculate that apoptosis of endotheliocytes is also likely to be decreased, being accompanied by increased differentiation of new cells from EPCs. This hypothesis requires confirmation in the course of conducting new studies.

Overall, the stepwise model for repeated measurements as well as the multiple regression model on changes during treatment demonstrate that a reduction of CRP and substances indicating endothelial damage (ICAM-1, p-selectin) was not only induced by TRT vs control but that the effect was augmented by both higher testosterone levels achieved by treatment and by the amount of decrease of waist circumference

achieved during medication. This might be explained by the direct influence of adipokines (leptin, resistin) on these substances [5,19,43].

The observed changes in insulin resistance as well as HbA1c were not directly influenced by a reduction of waist circumference. The finding corroborates the results of a meta-analysis, which attributes these changes on carbohydrate metabolism under TRT to the gain of fat-free mass (i.e. muscle mass) and not to the loss of fat mass [22]. Nevertheless, markers of adipose tissue secretion contributed markedly to the effect on HOMA1/HbA1c and these substances are directly correlated to waist circumference. Hence, the effect of waist circumference could have been lost due to the direct inclusion of adipose tissue products. The mitigating effect of advanced age might be explained by a reduced overall metabolism, but has to be further elucidated.

The study has some limitations. First of all, the design was not double-blind and placebo-controlled but had open controls not receiving mock-medication. In addition, it would have been interesting to study putative changes in body composition during treatment, possibly by DXA. Also, estradiol levels were not reliably available. It was also not known how T2DM and/or hypogonadism had existed in the patients.

A strength is the cohort of subjects consisting of newly diagnosed patients for T2DM and focusing on newly detected functional, not classical hypogonadism. The potential to observe changes in the studied parameters is higher than in patients already receiving effective antidiabetic treatment.

Conclusion

TRT in men with functional hypogonadism with T2DM leads to a decrease in BMI and WC.

TRT in men with T2DM and functional hypogonadism leads to a significant improvement in carbohydrate metabolism, namely improving insulin resistance, lowering fasting blood glucose levels and, ultimately, HbA1c.

In men with T2DM and androgen deficiency, TRT is accompanied by a decrease in the levels of adipohormones, such as leptin and resistin, which, together with a reduction of EnD markers and indicators of inflammation (ICAM-1, p-selectin and CRP), seems to facilitate a reduction in cardiovascular risk.

Disclosure statement

No potential conflict of interest was reported by the authors.

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