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CLINICAL STUDY

Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes

D Kapoor[1,3], E Goodwin[1], K S Channer[2] and T H Jones[1,3]

[1]*Centre for Diabetes and Endocrinology, Barnsley NHS Foundation Trust Hospital, Gawber Road, Barnsley S75 2EP, UK and* [2]*Department of Cardiology, Royal Hallamshire Hospital, Sheffield, UK and* [3]*Academic Unit of Endocrinology, Division of Genomic Medicine, University of Sheffield, UK*

(Correspondence should be addressed to T H Jones; Email: hugh.jones@bdgh-tr.trent.nhs.uk)

Abstract

Objective: Low levels of testosterone in men have been shown to be associated with type 2 diabetes, visceral adiposity, dyslipidaemia and metabolic syndrome. We investigated the effect of testosterone treatment on insulin resistance and glycaemic control in hypogonadal men with type 2 diabetes.

Design: This was a double-blind placebo-controlled crossover study in 24 hypogonadal men (10 treated with insulin) over the age of 30 years with type 2 diabetes.

Methods: Patients were treated with i.m. testosterone 200 mg every 2 weeks or placebo for 3 months in random order, followed by a washout period of 1 month before the alternate treatment phase. The primary outcomes were changes in fasting insulin sensitivity (as measured by homeostatic model index (HOMA) in those not on insulin), fasting blood glucose and glycated haemoglobin. The secondary outcomes were changes in body composition, fasting lipids and blood pressure. Statistical analysis was performed on the delta values, with the treatment effect of placebo compared against the treatment effect of testosterone.

Results: Testosterone therapy reduced the HOMA index (-1.73 ± 0.67, $P=0.02$, $n=14$), indicating an improved fasting insulin sensitivity. Glycated haemoglobin was also reduced ($-0.37 \pm 0.17\%$, $P=0.03$), as was the fasting blood glucose (-1.58 ± 0.68 mmol/l, $P=0.03$). Testosterone treatment resulted in a reduction in visceral adiposity as assessed by waist circumference (-1.63 ± 0.71 cm, $P=0.03$) and waist/hip ratio (-0.03 ± 0.01, $P=0.01$). Total cholesterol decreased with testosterone therapy (-0.4 ± 0.17 mmol/l, $P=0.03$) but no effect on blood pressure was observed.

Conclusions: Testosterone replacement therapy reduces insulin resistance and improves glycaemic control in hypogonadal men with type 2 diabetes. Improvements in glycaemic control, insulin resistance, cholesterol and visceral adiposity together represent an overall reduction in cardiovascular risk.

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Introduction

Hyperinsulinaemia and insulin resistance are antecedents to clinically established type 2 diabetes. Insulin resistance is also an essential component of the metabolic syndrome, which is defined as the presence of three or more of the following factors: central obesity, hypertriglyceridaemia, low high-density lipoprotein (HDL), hypertension and a raised fasting blood glucose (1). This syndrome is associated with an increased risk of the premature development of coronary artery disease.

Studies in healthy men have shown an inverse relationship between total testosterone levels and insulin concentrations (2, 3). Low testosterone levels in men have been found to predict insulin resistance and the future development of type 2 diabetes (4–6).

Furthermore, in diabetic men observational studies have reported a higher prevalence of hypogonadism when compared with non-diabetics (7–10). Dhindsa and colleagues found the prevalence of hypogonadism in type 2 diabetic men to be as high as 33% using free testosterone measurement by the equilibrium dialysis method (10).

Visceral obesity is an important cause of insulin resistance and is strongly linked to the development of impaired glucose tolerance. Studies have shown that free testosterone levels are low in obese men and inversely correlated with the degree of obesity (11, 12). There is an increase in deposition of abdominal adipose tissue in hypogonadal subjects, which in turn leads to a further decrease in testosterone concentrations, through conversion to oestradiol by aromatase, hence facilitating

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fat deposition and a greater degree of hypogonadism (12). In addition, leptin levels increase with obesity and this hormone causes a further reduction in androgen levels in men (12, 13).

Further evidence for the association between hypogonadism and insulin levels in men has been reported in studies on patients undergoing treatment for prostate carcinoma where androgen ablation is the main treatment. Two studies have shown an increase in insulin levels in patients treated with gonadotrophin-releasing hormone (GnRH) agonists (14, 15). Another study reported an increase in insulin and glucose levels after surgical castration for prostate carcinoma (16). These observations suggest that an inverse relationship exists between serum androgens and insulin sensitivity.

Low testosterone levels have also been found to be associated with dyslipidaemia and hypertension (17). Furthermore, Laaksonen and colleagues have shown that hypotestosteronaemia is associated not only with components of the metabolic syndrome but also with the metabolic syndrome itself, independent of body mass index (BMI) (18).

Interventional studies have been limited. A study in male castrated rats demonstrated impaired insulin sensitivity that was corrected with physiological testosterone replacement treatment but not with supraphysiological therapy (19). Similarly, a study in healthy men with low total testosterone levels (< 11.8 nmol/l), reported an improvement in insulin sensitivity and a decrease in insulin levels using testosterone or dihydrotestosterone treatment (20). Testosterone treatment has also been shown to reduce insulin resistance in obese men (21, 22) and to decrease total cholesterol in hypogonadal men with coronary artery disease, even in those taking statins (23). A study of type 2 diabetic men using oral testosterone showed an improvement in glycaemic control (24) although Corrales *et al.* have reported a neutral effect using i.m. testosterone treatment (25).

To our knowledge, this is the first study to assess the effect of testosterone treatment on insulin resistance and glycaemic control in hypogonadal men with type 2 diabetes.

Subjects and methods

This was a double-blind placebo-controlled crossover study performed at the Centre for Diabetes and Endocrinology, Barnsley NHS Foundation Trust Hospital, Barnsley, UK. The primary outcomes were changes in the homeostasis model assessment (HOMA) index of insulin resistance, fasting blood glucose and glycated haemoglobin. The secondary outcomes were changes in fasting lipids, blood pressure and anthropometric measurements including waist circumference, waist/hip ratio, BMI and % body fat.

The trial was 7 months in duration in which patients had two treatment phases of 3 months each with a washout period of 1 month in between. Each patient was randomised to receive either placebo or testosterone therapy first and after the washout period patients crossed over to the alternate therapy.

Subjects were men aged over 30 years with type 2 diabetes and with hypogonadism. All patients gave written informed consent and the local research ethics committee approved the protocol. Inclusion criteria were type 2 diabetic men with HbA1c up to 9.5% showing no significant symptoms of hyperglycaemia. Hypogonadism was defined as total testosterone level <12 nmol/l (on two separate occasions) and symptoms of hypogonadism (positive ADAM score) (26). Patients were excluded if they had any inflammatory disease or infection with elevation of C-reactive protein >10 mg/l, were already on hormone therapy or had any contraindication to testosterone therapy such as elevation of prostate-specific antigen (PSA) beyond the age-adjusted normal range. HOMA was not measured in those patients treated with insulin.

Randomisation and drug treatment

Patients were randomised to 'testosterone first' or 'placebo first' using a computer-generated random number. Treatment was with Sustanon 200 mg (testosterone propionate 30 mg, testosterone phenylpropionate 60 mg, testosterone isocaproate 60 mg, and testosterone decanoate 100 mg/ml, Organon Laboratories, Cambridge, UK), a depot preparation of testosterone given by deep i.m. injection. Intramuscular injections were given once every 2 weeks, with patients receiving a total of six injections in each phase. The final assessment in each treatment phase was 12–14 days after the previous injection. This regimen is commonly used as standard physiological testosterone replacement therapy in men with androgen deficiency and represents 3 months of testosterone treatment. Placebo was given as 0.9% normal saline. Drugs were drawn in identical syringes by a research nurse in a separate clinical room away from the patient and the doctor assessing the patient.

Assessment

Patients were screened initially with a questionnaire detailing their medical history and their concomitant medications were noted. Assessments were always made between 0800 and 1000 h after an overnight fast. All concomitant oral hypoglycaemic, anti-hypertensive and lipid-lowering medications were permitted and continued throughout the study without dose adjustment. However, those patients who were on insulin were permitted to make insulin dose adjustments to avoid hypoglycaemia. Subjects' height and weight were recorded, BMI was calculated using the equation (BMI = weight (kg)/height (metres)^2). Waist/hip ratio

was measured: waist was defined as the point midway between the iliac crest and the costal margin (lower rib) and hip as the widest circumference over the buttocks and below the iliac crest. Body composition including fat free mass and percentage body fat were also recorded in the fasting state by the bioelectrical impedance technique using a TANITA BF-300 body fat analyser (TANITA Corporation, Tokyo, Japan). Measurement of body composition with the TANITA body fat analyser has recently been validated against underwater weighing, which is the perceived gold standard method of body fat analysis (27). Systolic and diastolic blood pressures were recorded at baseline at 3 and 6 months either manually or using a 24 h blood pressure monitor.

Insulin sensitivity was calculated using the HOMA index (28). The perceived ideal investigation of insulin sensitivity is hyperinsulinaemic euglycaemic clamp. This has the disadvantage of being invasive, intensive and technically difficult but is still used in specialist research centres. The HOMA index is a simpler test and is calculated from the equation $((I_f \times G_f/22.5)$, where I_f is fasting insulin and G_f is fasting glucose. As insulin secretion is pulsatile, we used the mean of three samples taken every 5 min after an overnight fast to measure the fasting insulin. The homeostatic model assessment has been validated against the glucose clamp technique and found to provide a useful and repeatable index of insulin resistance (29).

Serum samples were obtained by centrifugation (10 min at 3500 r.p.m.) and immediately frozen at -20 °C pending further analysis. Total testosterone and sex hormone-binding globulin (SHBG) were measured by ELISA technique (DRG Diagnostics, Marburg, Germany). Luteinising hormone (LH), follicle-stimulating hormone (FSH) and PSA were measured by chemiluminescent microparticle immunoassay (Abbott Laboratories, Illinois, USA). Serum lipids and glucose were measured by Olympus analysers (Olympus Diagnostics, Hamburg, Germany) and insulin was measured by ELISA technique (Mercodia Diagnostics, Uppsala, Sweden). Bioavailable testosterone was determined by a modification of the method described by Tremblay & Dube (30).

Statistical analysis

Data were analysed using the GraphPad Instat package (version 3.05, GraphPad Software, San Diego, USA). All data were tested against a normal distribution using the Kolmogorov–Smirnov test. The data are presented as the mean $\pm$ S.E.M. unless indicated otherwise. The data were initially examined to exclude treatment/period interaction by ensuring that the baseline data of the treatment group (placebo and testosterone) were not statistically different, using the *t*-test between the baseline values in each case. The primary and secondary outcomes were compared by analysis of the delta between group analysis of the difference with placebo vs testosterone using the *t*-test. Results were considered statistically significant at $P<0.05$.

Results

Twenty-seven patients were randomised; three patients had to be withdrawn because of protocol violation – two of them were put on oral steroids, which can affect insulin sensitivity, and one patient did not adhere to the study protocol. The baseline data are presented in Tables 1 and 2. The sample comprised a group of hypogonadal men with type 2 diabetes. Fourteen were on oral hypoglycaemic agents and 10 were on insulin treatment. Eight had primary hypogonadism, one of whom was diagnosed with Klinefelter's syndrome, and two had secondary hypogonadism who were further investigated and found to have normal pituitary hormones and MRI pituitary. Fourteen had mixed primary and secondary hypogonadism. The mean baseline total testosterone was 8.83 ± 0.55 nmol/l and the mean trough testosterone level on treatment was 12.79 ± 0.79 nmol/l (Table 3). The treatment was well tolerated by the subjects. There were no adverse effects on haematological, PSA and biochemical parameters. The baseline values of the placebo and testosterone group were not statistically different.

Table 1 Baseline characteristics of whole population.

Parameter	Mean $\pm$ S.E.M.	Sample range
Age (years)	64 ± 1.34	52–76
Total testosterone (nmol/l)	8.63 ± 0.51	2.34–11.62
SHBG (nmol/l)	27.37 ± 2.59	11.67–63.45
Bioavailable testosterone (nmol/l)	2.73 ± 0.18	0.6–4
FSH (U/l)	12.95 ± 2.6	2.9–58.1
LH (U/l)	7.63 ± 1.1	2.2–24.7
PSA (μg/l)	1.35 ± 0.23	0.09–3.91
BMI	33 ± 0.86	26.4–45
Waist circumference (cm)	115.1 ± 2.4	97.5–141
Waist/hip ratio	1.02 ± 0.01	0.9–1.14
HbA1c (%)	7.28 ± 0.19	5.8–9.4

Table 2 Pharmacological profile of patients at baseline.

Medication*	Total population (*n*)	Patients receiving placebo first (*n*)	Patients receiving testosterone first (*n*)
None (diet only)	3	1	2
Metformin	4	2	2
Metformin + gliclazide	2	1	1
Metformin + rosiglitazone	3	2	1
Metformin + gliclazide + rosiglitazone	2	1	1
Insulin + metformin	7	3	4
Insulin	3	2	1
ACE inhibitors/angiotensin receptor antagonist	18	10	8
Statins	17	7	10
Fibrates	1	0	1

*Patients had been stabilised on these medications for at least 6 months prior to the study.

Fasting insulin sensitivity and glycaemic control

In the 14 patients taking oral agents, insulin sensitivity, as measured by the HOMA index, improved on testosterone treatment as compared with placebo. The mean treatment effect and 95% confidence intervals of testosterone on the HOMA index was a reduction (-1.73 ± 0.67, $P=0.02$) (-0.28 to -3.18) as compared with placebo (Fig. 1). This effect was explained by a reduction in both fasting glucose (-1.58 ± 0.68 mmol/l, $P=0.03$) (-0.17 to -2.99) and fasting insulin (-1.9 ± 1.1 mIU/l, $P=0.1$) (0.49 to -4.3), though only the effect on fasting glucose was statistically significant (Table 3). Glycated haemoglobin (HbA1c) was reduced as a consequence of improved insulin sensitivity. The mean treatment effect of testosterone on

Table 3 Comparison of the effects of placebo and testosterone on insulin sensitivity, glycaemic control, lipid profile, body composition and blood pressure in type 2 diabetic men.

	Placebo		Testosterone		Analysis of the difference: testosterone vs placebo (delta)		
Parameter	Baseline	Post-treatment	Baseline	Post-treatment	Mean effect	*P*	95% confidence intervals
Total testosterone (nmol/l)	8.14 ± 0.59	8.38 ± 0.58	8.83 ± 0.55	12.79 ± 0.79	3.7 ± 0.93	**0.001**	1.8 to 5.6
Bioavailable testosterone (nmol/l)	2.59 ± 0.18	2.65 ± 0.17	2.75 ± 0.17	3.8 ± 0.23	1.09 ± 0.28	**0.001**	0.51 to 1.67
Fasting insulin (mIU/l)*	12.37 ± 1.87	12.36 ± 2.13	13.68 ± 1.95	11.76 ± 1.76	−1.9 ± 1.1	0.1	0.49 to −4.3
Fasting glucose (mmol/l)	7.6 ± 0.43	8.73 ± 0.61	7.83 ± 0.49	7.38 ± 0.37	−1.58 ± 0.68	**0.03**	−0.17 to −2.99
Total cholesterol (mmol/l)	4.95 ± 0.15	5.07 ± 0.17	5.11 ± 0.17	4.83 ± 0.2	−0.4 ± 0.17	**0.03**	−0.04 to −0.75
HDL cholesterol (mmol/l)	1.04 ± 0.04	1.02 ± 0.04	1.02 ± 0.04	0.97 ± 0.04	−0.03 ± 0.04	0.3	−0.11 to 0.04
LDL cholesterol (mmol/l)†	2.64 ± 0.16	2.81 ± 0.17	2.79 ± 0.15	2.74 ± 0.18	−0.23 ± 0.15	0.2	−0.55 to 0.1
Triglyceride (mmol/l)	2.7 ± 0.2	2.76 ± 0.26	2.9 ± 0.25	2.56 ± 0.26	−0.4 ± 0.3	0.2	−1.03 to 0.23
% Body fat	33.73 ± 1.04	33.14 ± 1.09	33.79 ± 1.13	32.77 ± 1.1	−0.85 ± 0.55	0.1	−1.99 to 0.29
BMI	32.85 ± 0.88	32.97 ± 0.95	33.28 ± 0.92	33.62 ± 0.91	0.23 ± 0.21	0.3	−0.2 to 0.66
Fat free mass	66.99 ± 2.17	67.66 ± 2.24	67.08 ± 2.17	68.31 ± 2.14	0.56 ± 0.76	0.4	−1.01 to 2.13
Systolic blood pressure (mm Hg)‡	131 ± 3.1	127.5 ± 2.9		127.6 ± 2.8	0.43 ± 2.7	0.8	−5.18 to 6.05
Diastolic blood pressure (mm Hg)§	74 ± 1.4	72.7 ± 1.7		72.6 ± 1.5	0.26 ± 1.5	0.8	−2.7 to 3.2

*Patients on diet/oral hypoglycaemics only.
LDL, low-density lipoprotein.
†$n=18$, LDL could not be measured in the other patients in view of high triglyceride levels.
‡, §Thirteen patients had a 24 h blood pressure monitor and there was no significant difference between systolic and diastolic blood pressure on treatment with placebo or testosterone.
Statistically significant *P* values are expressed in bold.

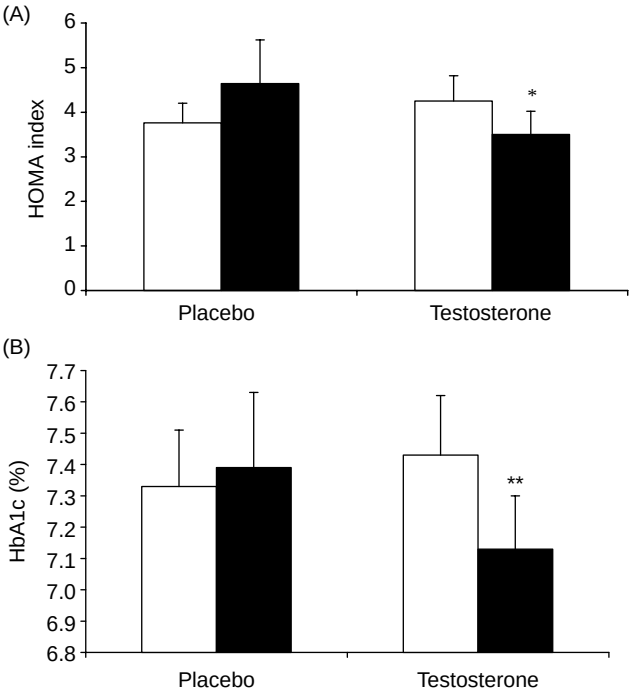

Figure 1 Effect of testosterone replacement compared to placebo on (A) HOMA index and (B) HbA1c. White, baseline; black, after 3 months of treatment (mean ± S.E.M.) *$P=0.02$, **$P=0.03$.

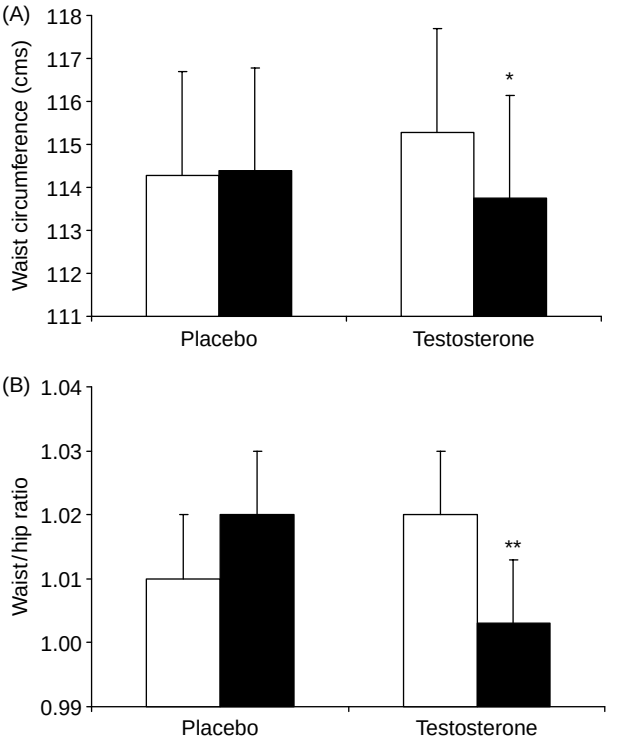

Figure 2 Effect of testosterone replacement compared to placebo on (A) waist circumference and (B) waist/hip ratio (mean ± S.E.M.) *$P=0.03$, **$P=0.01$ vs placebo.

HbA1c was a reduction ($-0.37 \pm 0.17\%$, $P=0.03$) (-0.03 to -0.71) as compared with placebo (Fig. 1). Of the 10 patients on insulin, 5 patients reduced their daily insulin dosages, whilst on testosterone treatment, by a mean of 7 ± 1.9 units.

Body composition

There was a significant reduction in waist circumference (-1.63 ± 0.71, $P=0.03$) (-3.1 to -0.15) and waist/hip ratio (-0.03 ± 0.01, $P=0.01$) (-0.05 to -0.01) following testosterone treatment (Fig. 2). The percentage of body fat also decreased but the change did not reach statistical significance. No significant changes were observed in BMI or fat free mass.

Fasting lipids (Table 3)

There was a small but significant decrease in total cholesterol with testosterone therapy. However, no significant changes were seen with HDL cholesterol, low-density lipoprotein (LDL) cholesterol or triglycerides.

Blood pressure (Table 3)

No significant changes were seen in either systolic or diastolic blood pressure following testosterone treatment. Thirteen patients had 24 h blood pressure monitoring and no significant changes in systolic or diastolic blood pressure were observed.

ADAM questionnaire

There was a reduction in mean ADAM score from a baseline of 6.5 ± 0.4 to a score of 4 ± 0.3 with testosterone therapy ($P<0.05$).

Discussion

We have shown that testosterone replacement therapy improves insulin resistance and glycaemic control in hypogonadal men with type 2 diabetes. The sample size was small, but previous studies (non-diabetic populations) have shown a statistically significant effect on insulin sensitivity with small numbers. Simon *et al.* reported an improvement in insulin sensitivity in 12 men treated with testosterone or dihydrotestosterone (20). Similarly, Marin *et al.* showed a beneficial effect of androgen therapy on insulin resistance with sample sizes in the low twenties (21, 22).

It is well known that a reduction in insulin resistance in type 2 diabetes results in an

improvement in glycaemic control. We have reported a significant reduction in fasting blood glucose and HbA1c with testosterone treatment. Boyanov and colleagues have reported a greater reduction in HbA1c after 3 months using oral testosterone treatment in poorly controlled diabetics (24) as compared with our group who were reasonably controlled. However, their study was a non-blinded, non-placebo-controlled study and the changes observed in HbA1c were much larger than would be expected within 3 months using conventional anti-diabetic medications. It is possible that we would have observed a greater reduction if the study had been continued for a longer period of time. Moreover, five of our patients on insulin did decrease their insulin dosages whilst on testosterone treatment, thus affecting the overall reduction in the glycated haemoglobin result.

The mechanism by which testosterone reduces insulin resistance are uncertain. There is considerable evidence linking abdominal obesity to insulin resistance and the excess visceral fat results in the liver being exposed to higher amounts of free fatty acids leading to hepatic and eventually systemic insulin resistance (12). We have shown that testosterone therapy results in a significant reduction in waist circumference and waist/hip ratio. Waist circumference is a practical indicator of visceral fat (31). This is comparable with a study by Marin *et al.* who showed that testosterone treatment for 8 months in abdominally obese men resulted in a decrease in visceral fat mass (measured by computerised tomography) without a change in body mass or lean body mass (21). Similarly, Rebuffe-Scrive *et al.* demonstrated that moderate doses of testosterone led to a reduction in waist/hip ratio and abdominal lipoprotein lipase activity (32). Even though the percentage of body fat did decrease in our study, this did not reach statistical significance. The plausible explanation for this is that our patients received testosterone treatment for 3 months only and others have shown significant decreases in body fat with testosterone therapy over a longer period of time (33, 34). We believe that testosterone improves insulin resistance chiefly through reduction in visceral adiposity, although other mechanisms may be involved. Firstly, androgen receptor levels are greater in visceral adipocytes than in subcutaneous tissues (35). Testosterone inhibits lipoprotein lipase activity, which reduces triglyceride uptake into adipocytes (36). Secondly, pro-inflammatory cytokines are produced by adipocytes, such as TNF α and IL6, and these have been shown to be associated with insulin resistance (37). An anti-inflammatory effect of testosterone replacement therapy in men with low androgen levels has been demonstrated, showing that testosterone reduces TNF α, Il-1b levels and increases IL-10, the anti-inflammatory cytokine (23). However, changes in cytokine activation have not been studied in hypogonadal men with type 2 diabetes.

Holmang & Bjorntorp have also shown that testosterone is an important regulator of muscular insulin sensitivity in male rats (19), and a similar effect on muscle insulin sensitivity could be another potential mechanism. Our study also shows that testosterone replacement therapy decreases total cholesterol in hypogonadal men, with no significant changes in HDL cholesterol. This is in agreement with two other studies which reported a similar effect of androgen supplementation on total and HDL cholesterol (38, 39). Testosterone treatment has also been shown to reduce total cholesterol in hypogonadal men with coronary artery disease, even in patients already on statins (23).

Serum testosterone levels have been reported to be lower in men with hypertension (17). Testosterone replacement has been shown to acutely reduce peripheral vascular resistance and improve cardiac index in men with chronic heart failure (40). Our results did not show a significant change in mean systolic or diastolic blood pressure with testosterone treatment in hypogonadal diabetic men. However, blood pressure was well controlled in our population at baseline and 18 patients were on ACE inhibitors/angiotensin receptor antagonist and thus an effect of testosterone treatment on blood pressure may have been masked. Others have also not shown a significant effect on blood pressure with testosterone therapy (24).

Conclusions

Our data show that testosterone treatment reduces insulin resistance and improves glycaemic control in hypogonadal men with type 2 diabetes. The UK Prospective Diabetes Study (UKPDS) reported that a reduction in HbA1c in type 2 diabetic patients was associated with reduced microvascular complications as well as myocardial infarction (41). Furthermore, men with coronary artery disease do have significantly lower levels of bioavailable and free testosterone (42), and testosterone therapy can improve ischaemia in men with chronic stable angina (43). Thus androgen replacement therapy in hypogonadal type 2 diabetic men could potentially improve glycaemic control and reduce microvascular and cardiovascular events in these patients. However, the benefits of male hormone replacement therapy in diabetic men would have to be considered against long-term risks that need to be evaluated in future, larger long-duration studies.

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