CLINICAL STUDY

Do reproductive hormones modify insulin sensitivity and metabolism in older men? A randomized, placebo-controlled clinical trial of recombinant human chorionic gonadotropin

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Abstract

Objective: In order to assess the hormonal determinants of insulin sensitivity and related components of the metabolic syndrome, we evaluated the effect of subcutaneous recombinant human chorionic gonadotropin (r-hCG; Ovidrel) on insulin sensitivity, vascular reactivity, leptin, insulin-like growth factor-I (IGF-I) and lipids in ambulant, community dwelling men 60 years of age with serum testosterone \( \leq 15 \) nmol/l on two occasions.

Design: Forty eligible men were randomized to receive 250 \( \mu \)g (5000 IU) r-hCG subcutaneously twice each week \((n = 20)\) or placebo \((n = 20)\) injections for 3 months, and all subjects (mean age 67 (range 60–85) years) completed the study.

Methods and results: Groups were well matched for height, weight, anthropometry and insulin sensitivity. Insulin sensitivity was assessed by homeostasis model (HOMA) and euglycemic hyperinsulinemic clamp at baseline and at the end of the treatment period in the first 30 men who did not have diabetes mellitus. Insulin sensitivity (HOMA and euglycemic clamp) or \( \beta \) cell function (HOMA) were not significantly changed by r-hCG despite a significant increase in lean body mass \((\sim 2 \text{ kg}, P < 0.001)\) and reduced fat mass \((\sim 1 \text{ kg}, P < 0.05)\). Subcutaneous fat (skinfold measurements), abdominal girth and serum leptin all decreased and IGF-I tended to increase, but these changes were not significant. Recombinant hCG significantly reduced total and low density lipoprotein cholesterol, and triglycerides, but did not significantly alter high density lipoprotein cholesterol. Endothelial function (vascular reactivity) was not significantly worsened. We conclude that three-months of treatment with r-hCG demonstrates expected hormonal effects, improved lipids and did not worsen vascular endothelial function. Insulin sensitivity was not altered despite suggestive changes in body composition.

Conclusions: These findings suggest short-term metabolic and cardiovascular safety and argue against an important role for androgens in the hormonal control of insulin sensitivity in older men.

European Journal of Endocrinology 148 55–66

Introduction

There is considerable interest in examining androgen supplementation in ageing men since increasing age is associated with increased frailty and falling circulating testosterone \((1,2)\), but whether androgen supplementation improves physical function is not yet supported by objective evidence \((3)\). Studies investigating the metabolic and cardiovascular effects of androgen replacement therapy in older men are lacking but are also of interest \((4)\) in order to determine whether improvements in function can be achieved without adverse effects on cardiovascular disease \((5)\) and to elucidate the possible hormonal control of insulin sensitivity. Insulin resistance in healthy individuals \((6)\) can independently predict the development of many age-related diseases including non-insulin dependent diabetes mellitus (NIDDM), hypertension, coronary artery disease, stroke and cancer. Interventions that improve insulin sensitivity \((7)\) particularly in those at high risk can prevent NIDDM and may have major public health implications.

Epidemiological studies suggest that androgen supplementation may improve insulin sensitivity. In older men, low serum testosterone can predict future development of NIDDM \((8–10)\) and central obesity \((11)\). Since normal ageing is associated with progressive impairment of carbohydrate tolerance \((12)\) which is
partly due to increasing insulin resistance (13), older individuals are an appropriate target group in whom timely intervention (perhaps with androgen supplementation) may delay progression to NIDDM and associated morbidity. Conversely, interventions that worsen insulin resistance may be particularly harmful due to increased background risk and reduced compensatory reserve.

However, few randomized placebo-controlled studies (5, 14–18) using recommended measures of insulin sensitivity (19) have been performed in men, and none in older men. The limited data that exist favor the possibility that moderate dose testosterone supplementation results in stable pharmacokinetics improves insulin sensitivity particularly in those with lower baseline total serum testosterone (17, 18). In addition, determination of insulin sensitivity by euglycemic hyperinsulinemic clamp (17, 18) may be critical since such findings have not been replicated using minimal model (5, 14, 15). Furthermore, the effect of androgens on other metabolically important hormones such as leptin and insulin-like growth factor-I (IGF-I), and how these may control insulin sensitivity is not well understood. Since both leptin (20) and IGF-I (21) are important in glucose homeostasis and can be altered by androgen supplementation in older men (22, 23), these may be important mechanisms by which androgens alter insulin sensitivity.

In this study, we examined the effect of androgenic supplementation on insulin sensitivity and other components of the metabolic syndrome (vascular reactivity and lipids) in healthy older men with lower circulating testosterone concentrations. Leptin and IGF-I were assessed since these could be important mechanisms by which androgens might alter insulin sensitivity. Androgenic supplementation was achieved by administering recombinant human chorionic gonadotropin (r-hCG), which has theoretical advantages over existing steroidal pharmacokinetics and can still target a group that is more likely to be androgen responsive (18, 23, 32).

Subjects and methods

Subjects and treatment

Healthy, ambulatory and community-dwelling men older than 60 years of age were recruited if they had a plasma testosterone ≤ 15 nmol/l on two separate occasions. Such a definition is consistent with previous studies (22, 27–31) that have recognized that lower baseline total testosterone, even within the young eugonadal range, can still target a group that is more likely to be androgen responsive (18, 23, 32).

Men were excluded due to (a) prostate cancer or disease requiring further treatment, (b) unstable, uncontrolled or severe chronic medical disease, (c) medical conditions that might interfere with muscle testing or (d) medication use known to interact with sex steroid action.

Study procedures

Volunteers were recruited through public advertisement or referred from primary care physicians. Respondents were provided with an explanation of the study and a written information sheet, and signed a consent form prior to screening drafted in accordance with the Declaration of Helsinki. Standardized medical history, physical examination and blood samples were obtained at entry (and then monthly whilst on study). Eligible subjects who satisfied all entry criteria and provided written informed consent were randomly assigned a study number that corresponded with individually numbered drug supplies. Randomization was performed in blocks of four according to a predetermined randomization list held by Serono Australia. Subjects
were noted and coded at each visit. The randomization code was not broken until all data were collected and the resulting database cleaned and locked. Fasting blood samples were taken between 0830 and 1030 h and participants were asked not to vary their diet or exercise patterns.

Participants were studied twice at baseline prior to commencing treatment, monthly during the 3-month treatment period and then one month after cessation of treatment. A single observer performed all study procedures. Treatment consisted of self administration of 250 µg (5000 IU) recombinant hCG (Ovidrel; Serono Australia, French’s Forrest, Sydney, Australia) subcutaneously on Tuesday and Friday mornings, or matching placebo. A dose of 250 µg (5000IU) r-hCG administered twice each week was chosen based on the combined literature which suggests an increase in testosterone of 50% (33–35) to 150% (36–38). Such a dose would increase circulating testosterone concentrations, even in normal men. Injection sites were rotated around the abdomen. A monthly diary was kept noting the date and time, and whether any adverse event (stinging, bruising or spillage) occurred with each injection. Boxes and diaries were returned at each monthly visit and medication compliance was based on diary entries and the number of unused vials returned. Adverse events or intercurrent illness and their likely relationship with drug administration were noted and coded at each visit.

Assays
Hormones and biochemical variables were measured as previously reported (24). Briefly, total testosterone (Immulyte, Los Angeles, CA, USA; coefficient of variation (CV) 7.8–12.7%) and estradiol (DELFIA, Perkin Elmer Life Sciences, Rowville, Australia; CV 1.2–5.8%) were measured from unextracted serum samples. Percentage unbound testosterone was determined by an in-house centrifugal ultrafiltration assay (CV 9.6–11.7%) using Centrifree columns (Millipore Australia, North Ryde, Australia) and tritiated testosterone. Free testosterone was calculated from this percentage being unbound to simultaneously directly measured total testosterone (39). In addition, insulin was measured by the Assymy autoanalyzer (Abbott Laboratory, IL, USA; CV 4–6%), leptin was measured by double antibody radioimmunoassay using 125I-labeled human leptin as tracer and rabbit antihuman leptin antibodies (Linco Research Inc, St Louis, MO, USA; CV 7–9%) assay (40) and IGF-I was measured by radioimmunooassay after acid–ethanol extraction (CV 9–11%) (41). Hormones were measured within the same assay where feasible. Blood glucose was determined by the hexokinase method using the Hitachi 917 autoanalyzer (Roche Diagnostics, Castle Hill, Australia; CV 1–2%) from blood collected in sodium fluoride (Vacutainer; Becton Dickinson, Rutherford, NJ, USA) during the previous 2 h. Biochemical variables (total and high density lipoprotein (HDL) cholesterol, triglycerides) were measured by routine autoanalyzer methods. Low density lipoprotein (LDL) cholesterol was calculated according to the Friedwald equation.

Anthropometric measurements
Anthropometric and bioimpedance measurements were taken immediately after micturition, with participants dressed in a gown as previously reported (24). In brief, height was measured to the nearest 0.5 cm using a standard fixed stadiometer and weight to the nearest 0.1 kg on calibrated scales. Skinfold thicknesses were measured at the biceps, triceps, subscapular and suprailiac locations using Harpenden Skinfold Calipers (British Indicators Ltd) at standard sites on the right side of the body (42). Bioelectrical impedance (BIA) was measured with a SEAC Model BIM 3.0 Bio-Impedance meter (Inderlec, Brisbane, Australia) and lean mass was estimated from bio-impedance readings according to Lukaski’s formula for men and fat mass, obtained by subtraction from body weight (43). Fat mass (and lean mass by subtraction) was also calculated from Siri’s equations (44) using body density (45). To examine regional effects, upper limb fat and muscle area were calculated from arm circumference (C) and triceps skinfold thickness (T) (46). BIA has been successfully used in studies of androgen therapy (29, 31, 47), and testosterone supplementation over a wide dosage range does not cause fluid retention (48).

Physical activity
Physical activity was determined by accelerometry (TriTrac R3D Ergometer, ZMD Reining Inc., Madison, WI, USA) (49, 50) using the manufacturer’s proprietary software (TriTrac R3D software V6.05).

Vascular reactivity
Arterial endothelial function was measured non-invasively at baseline, at the end of the treatment period and one month after treatment (51). Brachial artery diameter was recorded on B-mode ultrasound images, using a 12-5 MHz linear array transducer with a standard ATL HDI 5000CV system (Bothell, WA, USA) at rest, after flow-mediated dilatation and in response to a 400 µg spray of sublingual glyceryl trinitrate. Flow-mediated dilatation measures the vascular dilatation due to vascular hyperemia following release of high-pressure occlusion of the brachial artery by an inflated sphygmanomanometer cuff. Comparing arterial diameter measurements during reactive hyperemia with baseline allows calculation of the values

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for flow-mediated dilatation which is predominantly due to nitric oxide release by the endothelium (52). Increased blood flow shear leads to endothelial release of nitric oxide, which is cleared by both direct, endothelial-independent effects of direct nitric oxide delivery by glyceryl trinitrate. Recordings were later measured by a single observer who was blinded to treatment and stage of study. 

**Insulin sensitivity**

Insulin sensitivity was determined by euglycemic hyperinsulinemic clamp and by homeostasis (25) and modified homeostasis (53) models since clamp- and homeostasis-determined insulin sensitivity measures peripheral and hepatic insulin action respectively (54).

Insulin sensitivity was determined before and after the 3-month treatment period by euglycemic hyperinsulinemic clamp. For 3 days prior to each study, these participants consumed a weight maintaining diet containing at least 200 g carbohydrate per day (which corresponds to the usual Australian diet) and did not perform atypical exercise. All studies were performed at 0800 h following a 10 to 14 h overnight fast. A polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into the contralateral antecubital vein for blood sampling, and the forearm was kept heated (55). Blood glucose was measured every 5–10 min by the glucose oxidase method at the patient’s bedside (YSI 2300 stat plus glucose analyser, YSI Incorporated, Yellow Springs, OH, USA). Fifty units recombinant human insulin (Actrapid; Novo Nordisk, North Rocks, Australia) were added to 500 ml of 4% succinylated gelatin solution (Gelofusine; B Braun, Castle Hill, Australia) and was maintained in a diet containing at least 200 g carbohydrate per day (which corresponds to the usual Australian diet) and did not perform atypical exercise. All studies were performed at 0800 h following a 10 to 14 h overnight fast. A polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into the contralateral antecubital vein for blood sampling, and the forearm was kept heated (55). Blood glucose was measured every 5–10 min by the glucose oxidase method at the patient’s bedside (YSI 2300 stat plus glucose analyser, YSI Incorporated, Yellow Springs, OH, USA). Fifty units recombinant human insulin (Actrapid; Novo Nordisk, North Rocks, Australia) were added to 500 ml of 4% succinylated gelatin solution (Gelofusine; B Braun, Castle Hill, Australia) and was maintained in a diet containing at least 200 g carbohydrate per day (which corresponds to the usual Australian diet) and did not perform atypical exercise. All studies were performed at 0800 h following a 10 to 14 h overnight fast. A polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into the contralateral antecubital vein for blood sampling, and the forearm was kept heated (55). Blood glucose was measured every 5–10 min by the glucose oxidase method at the patient’s bedside (YSI 2300 stat plus glucose analyser, YSI Incorporated, Yellow Springs, OH, USA).

Characteristics and disposition of participants

The details of the subject population have been published (24). Briefly, 40 eligible men aged 67.5 ± 0.8

**Statistical analysis**

Analysis was performed blinded to the treatment group allocation. Response variables were calculated as the difference from baseline. The effects of r-hCG on continuous response variables were estimated by the main effects of treatment (r-hCG vs placebo), time and treatment × time interaction terms in a repeated measures analysis of variance (ANOVA) model. The r-hCG treatment effects were identified from treatment main effects or interactions and reversibility of treatment effects by an appropriate linear contrast. Data were analyzed in all 40 men for all variables initially and then restricted to the first 30 consecutive men without diabetes mellitus to ensure consistent results. Missing data were not imputed. In order to determine the effect of important baseline variables, repeated measures analysis of covariance (ANCOVA) including either baseline total or free testosterone, or estradiol was also performed where appropriate. Insulin sensitivity was adjusted using baseline measures of central adiposity, specifically waist circumference, body mass index and waist–hip ratio, as well as fat mass and physical activity (60) as covariates. Data were analyzed and graphed by using StatXact version 4 (Cytel Software Corporation, Cambridge, MA, USA), SPSS version 10 (SPSS Inc., Chicago, IL, USA) and SigmaPlot 2000 (SPSS Inc.). P < 0.05 (two-tailed) was considered significant.

**Results**

**Characteristics and disposition of participants**

The details of the subject population have been published (24). Briefly, 40 eligible men aged 67.5 ± 0.8
(S.E.M.) years were recruited, with 20 being randomized to each group. Among the 30 men who underwent clamp studies, 13 received r-hCG. The treatment groups did not differ significantly with respect to age, height, weight, baseline testosterone levels, fat mass, lean mass or insulin sensitivity amongst all 40 men as well as within the subgroup of 30 men who underwent euglycemic hyperinsulinemic clamp studies (see Table 1).

**Compliance** Drug dispensed was calculated so as to finish on the morning of the next appointment when subjects were asked to return unused drug and empty drug vials. Compliance was assessed by counting the number of unused drug vials in comparison with their diary. Non-compliance to treatment occurred in <1% for all participants and was due to spillage of drug during the injection procedure.

**Data and adequacy of blinding** All 40 men completed the study. There were no missing appointments and minimal missing data (<1%) arising from equipment failure.

Subjects were unable to correctly predict their treatment assignments at the end of the treatment period (only 40% guessed correctly, $P = 0.26$) suggesting adequate blinding.

**Hormonal effects**

Recombinant hCG treatment significantly suppressed luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (not shown) and markedly increased serum total and free testosterone and estradiol (Fig. 1) as previously reported (24). All hormonal changes had returned to baseline by one month after cessation of treatment. There was no significant treatment effect on leptin or IGF-I (Fig. 1). When leptin was adjusted for total body fat in ANCOVA (since leptin is secreted by all, although predominantly by subcutaneous, adipose tissue (61)), there was no change in these findings (data not shown). There was no change in any finding when the analysis was restricted to those men who underwent clamp studies.

![Graph showing changes in serum hormone concentrations](https://www.eje.org)

**Table 1** Characteristics of the subject population in the r-hCG and placebo treatment groups. Results are means ± S.E.M.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-hCG (n = 13)</th>
<th>Placebo (n = 17)</th>
<th>P</th>
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<td>Age (years)</td>
<td>68.6±1.2</td>
<td>65.6±1.2</td>
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<td>Height (cm)</td>
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<td>173±1</td>
<td>NS</td>
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<td>Weight (kg)</td>
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<td>99±3</td>
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<td>Hip circumference (cm)</td>
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</tr>
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<td>Fat mass (kg)</td>
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<td>Total testosterone (nmol/l)</td>
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<td>NS</td>
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<td>Free testosterone (pmol/l)</td>
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<td>NS</td>
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<td>LH (lIU/l)</td>
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<td>FSH (lIU/l)</td>
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<td>SHBG (nmol/l)</td>
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<td>Estradiol (pmol/l)</td>
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<td>Leptin (ng/ml)</td>
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<tr>
<td>IGF-I (nmol/l)</td>
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<td>11.7±0.8</td>
<td>NS</td>
</tr>
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BMI, body mass index; SHBG, sex hormone-binding globulin; NS, not significant.

**Figure 1** Plot of changes in serum hormone concentrations before, during and after twice-weekly subcutaneous injection of 250 μg (5000 IU) recombinant hCG for three months. Note significant increase in total testosterone (T), free testosterone and estradiol with no consistent changes in IGF-I or leptin. Data plotted as the mean and standard error of the mean of differences from each individual’s own baseline. In some instances the error bar is smaller than the data point symbol. Dashed lines indicate no change from baseline. Significant differences between the treatment (○) and placebo (○) groups are indicated by the asterisk which appears next to the hormone name. For further details see text. (Inset) For total and free testosterone and for estradiol, the same data are shown as the actual (not change in) hormone concentrations on the y axis. The dotted/dashed lines indicate the reference range for a young population.
Body compositional effects

Anthropometric measures During treatment, r-hCG significantly increased weight (~1 kg) and lean mass (~2 kg) and significantly reduced fat mass (~1 kg) as previously reported (24). Midthigh skinfold thickness fell (treatment × time interaction, P = 0.02), with similar nonsignificant trends in all other skinfold thicknesses. No effects on body circumference, or waist/hip ratio were detected (Fig. 2). Recombinant hCG did not alter calculated upper limb fat or muscle area (not shown). When the analysis was restricted to those men who underwent euglycemic hyperinsulinemic clamp studies, the significant effect on midthigh skinfold thickness disappeared, but there was no change in any other finding. There were no significant differences in findings using baseline testosterone as a covariate.

Metabolic and cardiovascular parameters

Insulin sensitivity Despite randomization, fewer subjects (n = 13) received r-hCG than placebo (n = 17). Glucose disposal rate (or glucose utilisation) remained unchanged when measured by euglycemic clamp (Table 2). Adjusting glucose disposal rate by lean body mass (glucose R d), total body mass or by steady state plateau serum insulin levels did not alter results. Steady state insulin levels and the metabolic clearance

Figure 2 Plot of changes in body circumferences and skinfolds before, during and after twice weekly subcutaneous injection of 250 µg (5000 IU) recombinant hCG for three months. Note significant decrease in midthigh skinfold thickness, but no consistent effect in any other anthropometric variable. Data plotted as the mean of differences from each individual’s own baseline. In some instances the error bar is smaller than the data point symbol. Dashed lines indicate no change from baseline. Significant differences between the treatment (●) and placebo (○) groups are indicated by the asterisk which appears next to the variable name. For further details see text.
rate of insulin were not different between hCG and placebo (Table 2).

Neither insulin sensitivity nor β cell function were significantly different between the two treatment groups when determined by either the homeostasis or modified homeostasis models (Fig. 3, modified homeostasis data similar, hence not shown). Fasting insulin and glucose did not change in these men (Fig. 3).

Results were not changed when adjusted by baseline testosterone or estradiol, by any measure of central adiposity, namely waist circumference or waist–hip ratio or body mass index, by fat mass or by physical activity.

**Vascular reactivity** Three months of r-hCG treatment produced no changes in brachial artery size or in endothelial or smooth-muscle dependent vascular function measured by flow-mediated or glyceryl trinitrate-induced dilatation (Table 2). These findings did not change when the analysis was restricted to those men who were examined by euglycemic hyperinsulinemic clamp (data not shown).

**Blood pressure and pulse** No significant differences in systolic or diastolic blood pressure, pulse or pulse pressure were detected between the two treatments at any stage during the study period (not shown).

**Lipids** Total and LDL cholesterol and triglycerides were decreased by r-hCG treatment. HDL cholesterol was unchanged (Fig. 3). These findings did not change when the analysis was restricted to those men who were examined by euglycemic hyperinsulinemic clamp.

**Discussion**

Our data demonstrate that r-hCG therapy in older men sufficient to increase serum testosterone within the physiological range does not alter glucose disposal rate, glucose Rd or euglycemic hyperinsulinemic clamp-determined insulin sensitivity. The metabolic clearance rate of insulin and steady state serum insulin were also unchanged. Similarly, homeostasis model-derived measures of insulin sensitivity and β cell function did not change with r-hCG therapy. Since homeostasis- and clamp-determined insulin sensitivity measure hepatic and peripheral insulin action respectively (54), we conclude that r-hCG does not alter insulin action at various sites. Furthermore, variation in baseline measures of central adiposity, total fat mass or physical activity did not alter results.

This neutral effect on overall insulin sensitivity is surprising since androgens have long been suspected of worsening insulin resistance in men (62–66). These studies highlighted the induction of insulin resistance by oral 17α alkylated androgens (oxymetholone and methandienone), but measured insulin sensitivity by simpler glucose tolerance methods. Subsequent reports confirmed this finding by minimal model (67). However, insulin resistance appears to have been induced by the known hepatotoxicity of these particular oral androgens since nonhepatotoxic orally administered androgens do not worsen insulin sensitivity (15, 18). Again, early uncontrolled reports (68, 69) inferring insulin sensitivity from glucose tolerance testing reported that oral testosterone undecanoate markedly worsened insulin sensitivity. But subsequent randomized placebo-controlled studies (15, 18) reported that oral non-17α alkylated androgens improved (18) or did not worsen (15) insulin sensitivity measured by clamp (18) or minimal model (15). Similarly, other randomized placebo-controlled studies using well-validated measures of insulin sensitivity have also reported no worsening of insulin sensitivity after oral dehydroepiandrosterone administration in young (70) or older (71) healthy men.

The studies using well-validated methods (57, 72) to determine insulin sensitivity (5, 14, 15, 17, 18, 67,
73–75) have examined the effect of a wide range of aromatizable (testosterone, oxandrolone) and minimally aromatizable (dihydrotestosterone, nandrolone) androgens in a wide range of men. Although the design and power of these studies vary, minimally aromatizable androgens (14, 15, 17, 73) and intramuscular androgens (5, 14, 15, 67, 73–75), do not worsen (but also do not improve) insulin sensitivity over the broad physiological range. Aromatization to estrogens may be an important step in improving insulin sensitivity, although estrogens per se when administered orally at high doses worsen insulin sensitivity (76). We therefore cannot exclude the possibility that the larger increase in estradiol induced by r-hCG may have negated an androgen-induced improvement in insulin sensitivity. Stable androgen pharmacokinetic profile may also be an important step in improving insulin sensitivity since greater visceral fat reduction results (15). Oral and transdermal (but not intramuscular) androgens result in such a profile, and improve insulin sensitivity when administered to middle-aged abdominally obese men with borderline low baseline serum testosterone (17, 18). The steroidal response induced by r-hCG is comparable with transdermal testosterone therapy (77) because of parenteral administration (avoiding first pass hepatic effects), associated increase in circulating estradiol and stable pharmacokinetics. Since we also measured insulin sensitivity by euglycemic hyperinsulinemic clamp, the differences cannot be explained by varying methods.

Figure 3: Plot of changes in fasting lipids and glucose homeostasis variables before, during and after twice weekly subcutaneous injection of 250 μg (5000 IU) recombinant hCG for three months. Note significant decrease in total and LDL cholesterol and triglycerides but no significant decrease in HDL cholesterol. Note that fasting glucose is not significantly different between the r-hCG and placebo groups, and there are no consistent changes in fasting insulin nor in homeostasis-determined insulin sensitivity or β cell function. Data plotted as the mean and standard error of the mean of differences from each individual’s own baseline. In some instances the error bar is smaller than the data point symbol. Dashed lines indicate no change from baseline. Significant differences between the treatment (●) and placebo (○) groups are indicated by the asterisk which appears next to the variable name. For further details see text.
of determination of insulin sensitivity (5). Although we also examined men who were more insulin resistant (due to older age), we cannot exclude the possibility that men who are more insulin resistant specifically from abdominal obesity may be particularly susceptible to the insulin sensitizing effects of aromatizable androgens. This may be because chronic administration of androgens favors reduction of visceral fat due to differential effects on lipoprotein lipase (17, 18, 78) or triglyceride assimilation (79, 80) in visceral compared with subcutaneous fat. If this effect is specific to abdominally obese men, we can therefore conclude that androgens are not important hormonal regulators of insulin sensitivity in older age. This concurs with the insulin resistance typically found at puberty (81) which has also recently been shown not to be attributable to gonadal hormones (73, 74).

Although insulin sensitivity did not improve, a significant improvement in body composition was observed with r-hCG. Lean mass was increased, and total fat mass was reduced – a finding which is consistent with the literature (23, 31, 68). Importantly, no anthropometric measure of central obesity, such as waist circumference, was improved by r-hCG suggesting that visceral fat may not have been reduced. This may explain the lack of effect on insulin sensitivity.

r-hCG did not significantly alter leptin or IGF-I, which are important mediators of glucose homeostasis action (20, 21, 82). Although androgen administration lowers serum leptin in young and old, eugonadal and hypogonadal men (22, 83, 84), this has not been reported universally (85). Furthermore, we cannot exclude the possibility that the marked increases in serum estradiol may have attenuated the effects of testosterone on IGF-I and leptin. Critically, in this latter study, no effect of gonadotropins on leptin was detected in young gonadotropin-deficient men after three months of therapy. Similarly, in healthy middle-aged men, urinary hCG does not acutely alter circulating leptin (86).

Hence, the lack of effect of r-hCG on leptin may be specific to gonadotropins (87). Similarly, testosterone increases IGF-I in young and old, eugonadal and hypogonadal men (23, 88, 89), but nandrolone does not (89). Whether the lack of observed effect with r-hCG is also due to a specific hCG action is unclear.

r-hCG improved the overall lipid profile since total and LDL cholesterol were reduced without any significant change in HDL cholesterol. This is in contrast with androgen administration which usually, although not always, also results in a reduction in HDL cholesterol (90). Furthermore, vascular reactivity and blood pressure were not worsened. Since arterial flow-mediated dilatation (91) correlates significantly with coronary endothelial function (92) and coronary atherosclerosis (93), short term cardiovascular safety is suggested. This confirms previous studies using non-aromatizable androgen supplementation (31, 94) which also reported no worsening of endothelial function.

Since insulin sensitivity, arterial flow-mediated dilatation and lipids are important and independent early indicators of presymptomatic vascular damage that can precede overt atherosclerosis, cardiovascular risk does not appear to be worsened with r-hCG treatment. Although r-hCG did markedly increase circulating estradiol, which may have an impact on cardiovascular risk or thromboembolic event rates (76), this may not be true for parenteral rather than oral administration due to differences in hepatic exposure. Furthermore, our data suggest that there does not appear to be a net adverse effect of r-hCG administration on cardiovascular risk.

We conclude that three months of treatment with subcutaneous r-hCG improved body composition and lipid profile. Insulin sensitivity, endothelial function and blood pressure were not worsened. Although long term studies are needed (95), our data do not support the hypothesis that r-hCG administration in older men sufficient to increase serum testosterone within the physiological range will necessarily increase the risk of atheromatous heart disease since important and independent risk factors such as insulin sensitivity, arterial flow-mediated dilatation, and lipids were not worsened. It is possible that the increase in circulating estradiol may have counteracted any beneficial effect arising from the increase in serum testosterone. Furthermore, although older (and hence more insulin resistant) subjects were targeted, it is possible that men with insulin resistance arising specifically from central obesity may have responded more favorably. Although r-hCG increased testosterone and estradiol, there was no resultant net effect on leptin or IGF-I suggesting that gonadal hormones are not important hormonal regulators of insulin sensitivity in older men, concurring with similar results in pubertal boys.

Acknowledgements

We thank Serono Australia, French’s Forrest, Sydney, Australia for financial support and for providing the r-hCG (Ovidrel) and matching placebo and Prof. Dr E Nieschlag (Institute of Reproductive Medicine, University of Münster, Germany) for stimulating our interest in this approach. We are grateful to the participants whose interest, patience and good humor made this study possible, particularly those under the care of Dr Charles Ovadia and his colleagues at the Leichhardt General Practice, Sydney, Australia. We are indebted to Janet Bryson, Dorota Pawlak and Dennis Yue for the loan of the YSI glucose analyzer, and to Seng Kee Gan and Fay Bacha for their assistance in establishing the euglycemic hyperinsulimemic clamp technique in our laboratory. We are also indebted to the staff of the Department of Andrology (Concord Hospital) and Cardiovascular Research Laboratory (Royal Prince Alfred Hospital) for their valued help. This
study was supported by the National Health and Medi-
cal Research Council (postgraduate medical research
scholarship to P Y L) and was presented in part during
the Clinical Trials Symposium of the US Endocrine
Society meeting, Denver, Colorado, June 20–23 2001
and at the Endocrine Society of Australia Scientific

References

1 Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB,
Clebben AD et al. Age trends in the level of serum testosterone
and other hormones in middle-aged men: longitudinal results
from the Massachusetts Male Aging Study. Journal of Clinical
Endocrinology and Metabolism 2002 87 589–598.

2 Harman SM, Metter EJ, Tobin JD, Pearson J & Blackman MR.
Longitudinal effects of aging on serum total and free testosterone

3 Snyder PJ. Effects of age on testicular function and consequences
of testosterone treatment. Journal of Clinical Endocrinology and
Metabolism 2001 86 2369–2372.

4 National Institute on Ageing Advisory Panel, Report of National
Institute on Ageing Advisory Panel on testosterone replacement
86 4611–4614.

5 Singh AB, Hsia S, Alaupovic P, Sinha-Hikim I, Woodhouse L,
and other hormones in middle-aged men: longitudinal results
from the Massachusetts Male Aging Study. Journal of Clinical
Endocrinology and Metabolism 2002 86 136–143.

6 Facchini FS, Hua N, Abbati F & Reaven GM. Insulin resistance as
a predictor of age-related diseases. Journal of Clinical Endocrinology
and Metabolism 2001 86 1574–1578.

7 Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin
JM, Walker EA et al. Reduction in the incidence of type 2 diabetes
with lifestyle intervention or metformin. New England Journal

8 Stellato RK, Feldman HA, Hamdy O, Horton ES & McKinlay JB.
Testosterone, sex hormone-binding globulin, and the develop-
ment of type 2 diabetes in middle-aged men: prospective results
from the Massachusetts Male Aging Study. Diabetes Care 2001
24 490–494.

9 Haffner SM, Shaten J, Stern MP, Davey Smith G & Kuller L. Low
levels of sex hormone-binding globulin and testosterone predict
the development of non-insulin-dependent diabetes mellitus in

10 Tibblin G, Adlerberth A, Lindstedt G & Björntorp P. The pituitary–
gonadal axis and health in elderly men: a study of men born in

11 Khaw KT & Barrett-Connor E. Lower endogenous androgens
2 754–755.

12 Davidson MB. The effect of aging on carbohydrate metabolism:
a review of the English literature and a practical approach to the
diagnosis of diabetes mellitus in the elderly. Metabolism: Clinical
and Experimental 1979 28 688–705.

13 Fink RL, Koltermann OG, Griffin J & Olefsky JM. Mechanisms of
insulin resistance in aging. Journal of Clinical Investigation 1983
71 1523–1535.

14 Hobson RJ, Jones RE & Pymate SR. Nandrolone, a 19-nortestos-
terone, enhances insulin-independent glucose uptake in normal
81 1582–1585.

15 Lovejoy JC, Bray GA, Greenson CS, Klemperer M, Morris J,
Partington C et al. Oral anabolic steroid treatment, but not
parenteral androgen treatment, decreases abdominal fat in
obese, older men. International Journal of Obesity and Related

16 Marin P. Testosterone and regional fat distribution. Obesity
Research 1993 1 (Suppl 4) 609S–612S.

17 Marin P, Holmang S, Gustafsson C, Jonsson L, Kivist H, Elander A
et al. Androgen treatment of abdominally obese men. Obesity

The effects of testosterone treatment on body composition and
metabolism in middle-aged obese men. International Journal

19 Anonymous. Consensus Development Conference on Insulin

20 Kamohara S, Burcellin R, Halaas JL, Friedman JM & Charon MJ.
Acute stimulation of glucose metabolism in mice by leptin treat-

21 Lewitt MS. Role of the insulin-like growth factors in the endocrine
control of glucose homeostasis. Diabetes Research and Clinical

22 Shi R, Morley JE, Kaiser FE, Perry HM, Patrick P & Ross C.
Testosterone replacement in older hypogonadal men: a
12-month randomized controlled trial. Journal of Clinical
Endocrinology and Metabolism 1997 82 1661–1667.

et al. Effect of testosterone treatment on body composition and
muscle strength in men over 65 years of age. Journal of Clinical
Endocrinology and Metabolism 1999 84 2647–2653.

24 Liu PY, Wishart SM & Handselman DJ. A double-blind, placebo-
controlled, randomized clinical trial of recombinant human chor-
ionic gonadotropin on muscle strength and physical function and
activity in older men with partial age-related androgen deficiency.

25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF
& Turner RC. Homeostasis model assessment: insulin resistance
and beta-cell function from fasting plasma glucose and insulin

26 Anonymous. Guidelines for Good Clinical Practice (GCP) for trials
on Pharmaceutical Products. Geneva: World Health Organisation,
1993.

27 Tenover JS. Effects of testosterone supplementation in the aging
male. Journal of Clinical Endocrinology and Metabolism 1992
75 1092–1098.

28 Morley JE, Perry HM, Kaiser FE, Kraenagle D, Jensen J, Houston K
et al. Effects of testosterone replacement therapy in old hypo-
gonadal males: a preliminary study. Journal of the American
Geriatric Society 1993 41 149–152.

29 Clague JE, Wu FCW & Horan DA. Differences in measuring the
effect of testosterone replacement therapy on muscle function in

30 Kenny AM, Prestwood KM, Gruman CA, Marcello KM & Raisz LG.
Effects of transdermal testosterone on bone and muscle in older
men with low bioavailable testosterone levels. Journals of Geron-
tology Series A – Biological Sciences and Medical Sciences 2001
56 M266–M272.

31 Ly LP, Jimenez J, Zhuang TN, Celermajer DS, Conway AJ &
Handelsman DJ. The effects of varying doses of T on insulin
sensitivity, plasma lipids, apolipoproteins, and C-reactive protein
in healthy young men. Journal of Clinical Endocrinology and
Metabolism 2002 87 116–113.

32 Hendrix SL, Dallal GE,倅, and others. Calcium, vitamin D, and
physical activity for prevention of osteoporosis in postmenopausal
women. A randomized controlled trial. Journal of the American

33 Nieschlag E, Lammers U, Freischelm CW, Langer K & Wickings EJ.
Androgen replacement therapy improves muscle strength, function,
and quality of life in older men with partial androgen deficiency.
Metabolic effects of r-hCG in ageing men


81 Goran MI & Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes 2001 50 2444–2450.

Received 24 July 2002
Accepted 30 September 2002