Effect of 2 Years of Testosterone Replacement on Insulin Secretion, Insulin Action, Glucose Effectiveness, Hepatic Insulin Clearance, and Postprandial Glucose Turnover in Elderly Men

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OBJECTIVE — We sought to determine whether, and if so the mechanism by which, testosterone replacement improves carbohydrate tolerance.

RESEARCH DESIGN AND METHODS — Fifty-five elderly men with relative testosterone deficiency ingested a labeled mixed meal and underwent a frequently sampled labeled intravenous glucose tolerance test before and after either placebo or treatment with testosterone patch (5 mg/day) for 2 years.

RESULTS — Despite restoring bioavailable testosterone to values observed in young men, the change (24 months minus baseline values) in fasting and postprandial glucose, insulin, and C-peptide concentrations and meal appearance, glucose disposal, and endogenous glucose production were virtually identical to those observed after 2 years of placebo. The change over time in insulin and C-peptide concentrations post–intravenous glucose injection also did not differ. Furthermore, the change over time in insulin action and glucose effectiveness (measured with the unlabeled and labeled "oral" and "intravenous" minimal models), as well as insulin secretion and hepatic insulin clearance (measured with the C-peptide model), did not differ in the testosterone and placebo groups.

CONCLUSIONS — We conclude that 2 years of treatment with testosterone in elderly men does not improve carbohydrate tolerance or alter insulin secretion, insulin action, glucose effectiveness, hepatic insulin clearance, or the pattern of postprandial glucose metabolism. Thus, testosterone deficiency is unlikely the cause of the age-associated deterioration in glucose tolerance commonly observed in elderly men.

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Abbreviations: DHEA, dehydroepiandrosterone; SHBG, sex hormone–binding globulin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Testosterone concentrations fall and glucose tolerance deteriorates in men as they age (1–5). Currently, it is not known whether the former contributes to the latter. Data from both animal and human studies suggest this may be the case. In rats, castration decreases insulin-stimulated glucose uptake and reduces insulin gene expression in pancreatic β-cells (6,7). In contrast, testosterone replacement restores insulin action, increases islet insulin content, and enhances insulin secretion (6,7). Low testosterone concentrations are associated with insulin resistance and predict the development of diabetes in elderly men (8–12). Furthermore, androgen treatment improves insulin action in abdominally obese middle-aged men (13,14), whereas testosterone has been reported to have no effect on insulin action in normal young men (15) or in men with hypogonadism (16). Additionally, supraphysiologic doses of androgens cause insulin resistance in both humans (17,18) and dogs (19).

We recently reported that 24 months of dehydroepiandrosterone (DHEA) replacement in physiological doses had no beneficial effects on quality of life, body composition, or physical performance in either elderly men or women (20). We also observed that DHEA replacement did not alter net insulin action measured with the unlabeled meal minimal model (21). To our knowledge, the effects of testosterone replacement on insulin secretion, insulin action, and postprandial glucose turnover have not been previously evaluated in elderly men. The present studies, conducted as part of a large randomized trial that also evaluated the effects of testosterone replacement on body composition and muscle strength (20), addressed this question by measuring insulin action, insulin secretion, and hepatic insulin clearance before and after 2 years of treatment with tes-

Table 1—*Subject characteristics at baseline and following treatment*

	Placebo group (n = 29)		Testosterone group (n = 26)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Age (years)	67 (64–73)	—	67 (63–72)	—
BMI (kg/m^2)	27 (26–30)	28 (25–29)	29 (26–30)	29 (26–30)
Body fat (%)	28 (24–32)	28 (22–32)	27 (24–30)	26 (24–29)
Fat-free mass (kg)	61 (57–64)	61 (57–63)	60 (58–64)	61 (58–64)*
Peak Vo_2 (ml/kg fat-free mass)	40 (36–42)	38 (34–46)	40 (37–44)	41 (33–46)
Sulfated DHEA (μg/ml)	0.7 (0.5–1.0)	0.6 (0.4–0.8)	0.7 (0.4–0.9)	0.5 (0.3–0.7)
Total testosterone (ng/dl)	398 (296–465)	395 (342–528)	371 (282–465)	483 (421–651)*
Bioavailable testosterone (ng/dl)	53 (46–62)	53 (40–64)	56 (44–65)	84 (67–118)*
Estradiol (pg/ml)	24 (20–28)	22 (19–28)	20 (17–25)	25 (17–31)
Bioavailable estradiol (pg/ml)	9 (8–12)	9 (6–10)	9 (7–11)	10 (9–17)
SHBG (nmol/l)	42 (36–50)	40 (36–51)	38 (31–41)	36 (32–44)

Data are median (interquartile range) of hormones and biochemical markers before and after placebo and treatment with testosterone. *$P < 0.01$ vs. pretreatment.

tosterone or placebo. Postprandial glucose turnover was also measured to determine whether testosterone replacement lowered the rate of meal appearance, enhanced postprandial suppression of endogenous glucose production, and stimulated postprandial glucose uptake. We report that while 2 years of testosterone replacement resulted in a small but statistically significant increase in muscle mass, it had no effect on insulin action, glucose effectiveness, insulin secretion, hepatic insulin clearance, or postprandial glucose turnover.

RESEARCH DESIGN AND METHODS

— The study design and methods have been previously described in detail (20). In brief, men whose bioavailable testosterone (non–sex hormone–binding globulin [SHBG]-bound) concentration was <103 ng/dl and DHEA concentration <1.57 μg/ml (15th percentile for young men) were eligible for study and were considered as having relative testosterone deficiency. Volunteer characteristics are given in Table 1. The study was a randomized, placebo-controlled, double-blind, 2-year trial. Subjects were assigned to wear either a testosterone (5 mg/day) (D-TRANS; Alza, Mountain View, CA) or placebo patch each day. Baseline data and results of DHEA replacement have been published previously (3,4,11).

Subjects were admitted and given a standard 10 kcal/kg meal. No additional food was eaten until the next morning. On one occasion, a mixed meal (10 kcal/kg) consisting of scrambled eggs, Canadian bacon, and [1-^{13}C]glucose Jell-O was consumed within 15 min (3,4). Infusion of [6-^{3}H]glucose was started with the meal, and infusion of both [6-^{3}H]glucose and [6,6-$^2H_2$]glucose were altered, minimizing the change in plasma glucose enrichment (22). On another occasion, 0.33 g/kg glucose containing [6,6$^2H_2$]glucose was injected at time 0 min, and 0.02 units/kg insulin was injected at time 20 min (4). Arterialized venous blood was collected at frequent intervals, as previously described (3,4).

Plasma glucose concentrations were measured using a glucose oxidase method (YSI, Yellow Springs, OH). Plasma insulin concentrations were measured by chemiluminescence (Access Assay; Beckman, Chaska, MN). Plasma C-peptide concentrations were measured by radioimmunoassay (Linco Research, St. Louis, MO). Levels of sulfated DHEA and total bioavailable testosterone and estrogen were measured by a competitive chemiluminescene immunoassay, and SHBG was measured by solid-phase chemiluminescene assay (Immulite; Diagnostic Products, Los Angeles, CA). In subjects with low testosterone levels, values were obtained with the use of a high-sensitivity chemiluminescence assay. Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI).

Calculations

The oral and intravenous glucose minimal models (23,24) were used to interpret the plasma glucose and insulin concentrations measured following meal ingestion or glucose injection (supplemental Fig. 3 found in an online appendix at http://dx.doi.org/10.2337/dc07-0359).

The systemic rates of meal appearance (R_{aMEAL}), endogenous glucose production, and glucose disappearance (R_d) were calculated using Radziuk's two-compartment model (25). Values from −30 to 0 min were averaged and considered as basal. Area above basal was calculated using the trapezoidal rule. Parameters of all models were estimated using SAAM II software (26). Measurement errors have been assumed to be independent and Gaussian, with 0 mean and variance for glucose and tracer glucose (as previously described [27]) and for C-peptide concentrations (as previously described [28]).

Statistical analysis

Data are presented as means ± SE. Area above basal was calculated using the trapezoidal rule. Changes from baseline (i.e., 24-month values minus baseline values) were compared in the testosterone and placebo groups using Student's t test. Demographic, hormonal, and biochemical data are presented as median and interquartile range. Multiple regression analysis was done in which the dependent variable was the change from baseline (with the use of a rank transformation), and the independent variables were study group, sex, age at the time of randomization, length of follow-up, and baseline values. A P value <0.05 was considered to be statistically significant.

RESULTS

Plasma testosterone and estrogen concentrations and body composition

The effects of testosterone replacement on plasma hormone concentrations and

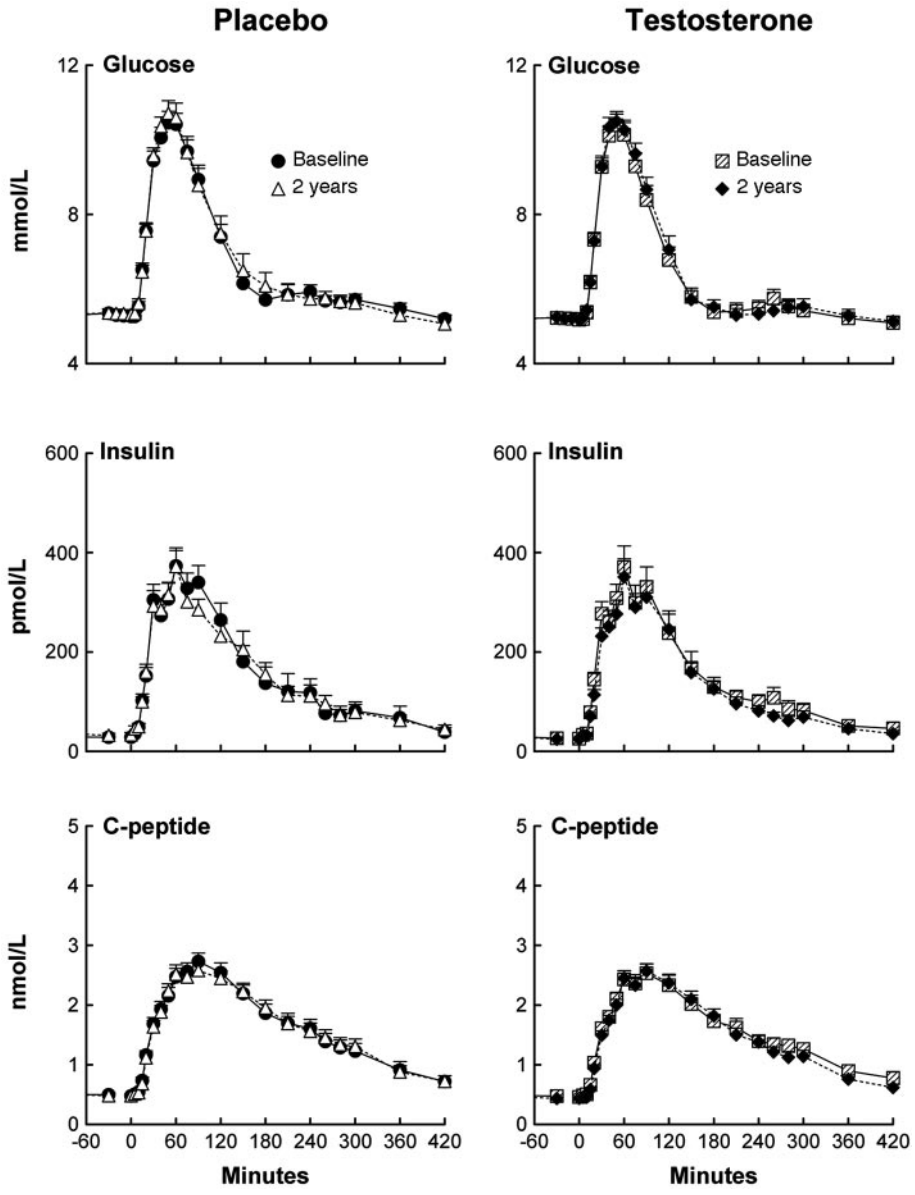

Figure 1—*Plasma glucose, insulin, and C-peptide concentrations observed in elderly men after meal ingestion before (baseline) and after 2 years of either placebo* (left) *or treatment with testosterone* (right)*.*

body composition have been described in detail elsewhere (20). In brief, plasma testosterone concentration was significantly higher during 2 years of treatment with testosterone versus placebo ($P < 0.005$) (Table 1). In contrast, plasma testosterone concentrations did not change following 2 years of treatment with placebo. Estrogen concentrations were not different following treatment with either testosterone or placebo. Two years of treatment with testosterone did not alter visceral or percentage body fat; however, it resulted in a slight but statistically significant increase ($P < 0.01$) in fat-free mass. Even so, treatment with testosterone did not alter peak VO_2, leg isometric knee extension, double leg press, or chest press.

Plasma glucose, insulin, and C-peptide concentrations observed before and following meal ingestion

The change (i.e., 24 months minus baseline) of fasting glucose, insulin, and C-peptide concentrations present on the day of the mixed-meal study did not differ following 2 years of treatment with testosterone or placebo (Fig. 1). The change in the postprandial increments (i.e., area above basal) of glucose, insulin, and C-peptide concentrations also did not differ following treatment with testosterone or placebo.

Meal rate of appearance, endogenous glucose production, and glucose disappearance observed following meal ingestion

The change in fasting rates of endogenous glucose production and glucose disappearance did not differ following 2 years of treatment with testosterone from that observed following 2 years of placebo (Fig. 2). The change in the postprandial increment in meal appearance and glucose disappearance or postprandial decrement in endogenous glucose production also did not differ following 2 years of treatment with testosterone or placebo.

Plasma glucose, insulin, and C-peptide concentrations observed before and following intravenous injection of glucose

The change in fasting glucose, insulin, and C-peptide concentrations present on the day of the intravenous glucose tolerance test did not differ in the testosterone and placebo groups (supplemental Fig. 3). Similarly, the change in glucose, insulin, and C-peptide concentrations observed following intravenous injection of glucose did not differ following 2 years of treatment with testosterone or placebo.

Insulin action, insulin secretion, glucose effectiveness, and hepatic insulin clearance

The change from baseline in net insulin action (S_I) measured with either the unlabeled oral or unlabeled "oral" or "intravenous" glucose minimal models did not differ following 2 years of treatment with either testosterone or placebo. The change in the ability of insulin to stimulate glucose uptake (S_I*) measured with either the labeled oral or labeled intravenous minimal models also did not differ in the testosterone and placebo groups (Table 2).

The change from baseline in meal indexes ($Phi_{dynamic}$, Phi_{static}, and Phi_{total}) intravenous glucose indexes (Phi_1, Phi_2, and Phi_{total}) of insulin secretion did not differ following 2 years of treatment with testosterone or placebo (Table 2). This resulted in no difference in the change from baseline in either the meal ($DI_{dynamic}$, DI_{static}, and DI_{total}) or intravenous glucose (DI_1, DI_2, and DI_{total}) disposition indexes. The change in net glucose effectiveness (GE), the ability of glucose to stimulate its

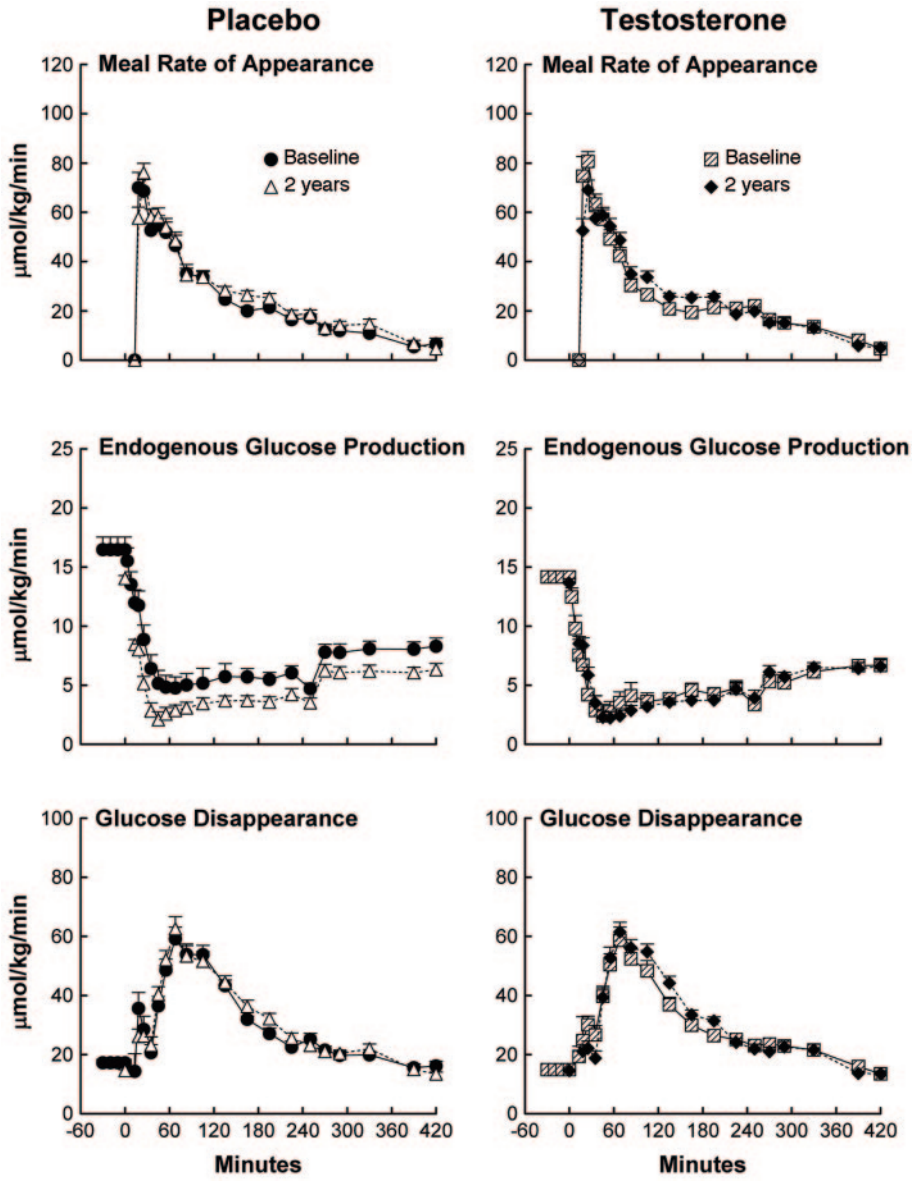

Figure 2—*Meal appearance, endogenous glucose production, and glucose disappearance observed in elderly men after meal ingestion before (baseline) and after 2 years of either placebo* (left) *or treatment with testosterone* (right).

own uptake (GE*), and hepatic insulin clearance measured following intravenous glucose injection also did not differ in the testosterone and placebo groups.

CONCLUSIONS — The present studies indicate that 2 years of testosterone replacement has no detectible effect on insulin secretion, insulin action, glucose effectiveness, hepatic insulin clearance, or postprandial glucose turnover in elderly men. These data strongly imply that relative or absolute testosterone deficiency does not contribute to insulin resistance or the impairment in insulin secretion that is commonly observed in elderly men. It also exemplifies that testosterone replacement does not delay or reverse age-associated deterioration in glucose tolerance.

We have previously reported that postprandial glucose disposal is lower in elderly men than in young men (3). The present data indicate that this defect is not reversed by testosterone replacement, since rates of postprandial glucose disposal were virtually identical before and after 2 years of treatment with testosterone and did not differ from those following placebo. The increment in glucose concentrations following a mixed meal is also influenced by the rate at which the ingested glucose enters the systemic circulation and by the rate at which endogenous glucose production is suppressed. Neither differed from those observed in young men or were influenced by testosterone replacement. Thus, testosterone replacement did not improve glucose tolerance in elderly men and did not restore postprandial rates of glucose disappearance to those observed in healthy younger subjects.

Elderly men commonly have low testosterone concentrations and are insulin resistant (5,8–11). Testosterone receptors are present on muscle (29), and some (6,13,14), but not all (15,16,19), studies have suggested that treatment with androgens can improve insulin action in rats (6) or middle-aged humans (13,14). We have previously reported that testosterone replacement in elderly men does not improve net insulin action measured with the unlabeled oral minimal model (20). The present data extend these observations by showing that testosterone replacement also had no effect on net insulin action (S_i) measured with the unlabeled intravenous glucose tolerance test or any effect on insulin measured with either the labeled oral or intravenous-labeled minimal models. The latter observation is particularly noteworthy since the labeled minimal model specifically measures the ability of insulin to stimulate glucose disposal (S_i*). Furthermore, the oral and intravenous glucose tolerance tests were performed on two separate occasions, and insulin action measured with the unlabeled and labeled minimal models provided independent assessments of insulin action. Therefore, whereas it is possible that an effect of testosterone replacement on insulin action was missed, it must have been small and likely of limited biologic significance. Glucose uptake is regulated by glucose and insulin concentrations (30,31). The former is commonly referred to as glucose effectiveness. Net glucose effectiveness and the ability of glucose to stimulate its own uptake can be measured with the unlabeled and labeled minimal models, respectively.

Experiments in animals suggest that testosterone can regulate islet cell function (7). We have previously reported that insulin secretion is impaired in elderly men (3). The present data indicate that treatment with testosterone does not ameliorate this impairment. Insulin secretion whether evaluated qualitatively by comparing the area above basal of insulin and

Table 2—*Meal and intravenous glucose tolerance test (IVGTT) indexes of insulin action and secretion*

Meal	S_i (10^{-4} dl · kg^{-1} · min^{-1} per μU/ml)	$Phi_{dynamic}$ (10^{-9})	Phi_{static} (10^{-9} · min^{-1})	Phi_{total} (10^{-9} · min^{-1})	DI (10^{-14} dl/kg per min^2 per pmol/l)	GE (dl/kg per min)	S_i* (10^{-4} dl · kg^{-1} · min^{-1} per μU/ml)	GE* (dl/kg per min)
Placebo								
Pretreatment	11.7 ± 1.8	419.2 ± 28.6	34.1 ± 1.9	37.7 ± 2.1	685.0 ± 87.1	0.04 ± 0.00	9.6 ± 1.3	0.02 ± 0.00
Posttreatment	11.9 ± 1.4	411.9 ± 44.5	33.0 ± 2.2	36.6 ± 2.5	673.8 ± 67.1	0.04 ± 0.00	9.0 ± 1.3	0.02 ± 0.00
Δ	0.2 ± 1.5	−7.3 ± 34.4	−1.1 ± 1.7	−1.1 ± 1.5	−11.2 ± 78.5	0.00 ± 0.00	−0.6 ± 1.0	0.00 ± 0.00
Testosterone								
Pretreatment	12.6 ± 1.6	410.5 ± 40.5	33.8 ± 1.9	37.2 ± 2.0	731.0 ± 76.0	0.00 ± 0.00	7.8 ± 1.1	0.02 ± 0.00
Posttreatment	14.1 ± 1.7	386.4 ± 41.3	32.8 ± 2.7	35.0 ± 3.0	759.0 ± 84.0	0.00 ± 0.00	9.4 ± 1.2	0.02 ± 0.00
Δ	1.5 ± 1.6	−24.1 ± 41.5	−1.0 ± 1.8	−2.2 ± 2.2	28.0 ± 83.2	0.00 ± 0.00	1.6 ± 0.5	0.00 ± 0.00
IVGTT	S_i (10^{-4} dl · kg^{-1} · min^{-1} per μU/ml)	Phi_1 (10^{-9})	Phi_2 (10^{-9} · min^{-1})	Phi_{total} (10^{-9} · min^{-1})	DI (10^{-14} dl/kg per min^2 per pmol/l)	GE (dl/kg per min)	S_i* (10^{-4} dl · kg^{-1} · min^{-1} per μU/ml)	GE* (dl/kg per min)
Placebo								
Pretreatment	5.8 ± 0.8	130.3 ± 15.1	10.3 ± 0.8	16.6 ± 1.3	153.3 ± 19.8	0.03 ± 0.00	6.7 ± 1.1	0.02 ± 0.00
Posttreatment	5.5 ± 0.8	135.5 ± 11.7	10.3 ± 0.7	17.2 ± 1.2	150.1 ± 20.2	0.03 ± 0.00	7.6 ± 1.2	0.02 ± 0.00
Δ	−0.3 ± 0.4	5.2 ± 9.5	0.0 ± 0.6	0.6 ± 0.8	−3.2 ± 10.6	0.00 ± 0.00	0.9 ± 0.5	0.00 ± 0.00
Testosterone								
Pretreatment	5.4 ± 0.7	119.9 ± 11.0	10.5 ± 0.8	16.8 ± 1.3	148.3 ± 22.0	0.03 ± 0.00	6.4 ± 0.7	0.02 ± 0.00
Posttreatment	5.7 ± 0.6	119.3 ± 11.0	10.3 ± 0.8	16.4 ± 1.2	144.1 ± 16.0	0.03 ± 0.00	7.9 ± 0.9	0.02 ± 0.00
Δ	0.3 ± 0.5	−0.5 ± 7.0	−0.2 ± 0.6	0.4 ± 0.8	−4.2 ± 10.6	0.00 ± 0.00	1.5 ± 0.5	0.00 ± 0.00

Data are means ± SE. Meal: S_i* pretreatment vs. posttreatment testosterone group, $P = 0.06$. DI (disposition index), the appropriateness of insulin secretion for the prevailing level of insulin resistance; $DI_{dynamic}$, $Phi_{dynamic} \times S_i$; DI_{static}, $Phi_{static} \times S_i$; DI_{total}, $Phi_{total} \times S_i$; GE is the ability of glucose per SE to promote glucose disposal and inhibit glucose production; GE*, effect of glucose to stimulate glucose disposal; Phi_1, first-phase insulin secretion that presumably represents release of previously docked insulin granules following intravenous glucose; Phi_2, slow/second phase of insulin secretion that represents the response to a given increment in glucose following intravenous glucose; $Phi_{dynamic}$, β-cell response to a given increment in glucose concentration following a meal; Phi_{static}, β-cell response to an increment in glucose above basal following a meal; Phi_{total}, overall β-cell response to glucose following a meal or intravenous glucose; S_i, net insulin action is the overall effect of insulin to stimulate glucose disposal and inhibit glucose production; S_i*, effect of insulin to stimulate glucose disposal.

C-peptide concentrations after meal ingestion or glucose injection or quantitatively by using C-peptide models did not differ following treatment with testosterone or placebo. Since there were virtually no changes over time in insulin or C-peptide concentrations, these data also indicate that hepatic insulin clearance did not change. The oral and intravenous glucose tolerance tests evaluate different aspects of insulin secretion (32). The former assesses glucose- and nutrient-induced stimulation of insulin secretion, whereas the latter evaluates the response to glucose alone. Similarly, the intravenous Phi_1 is believed to reflect the acute release of insulin from previously docked insulin granules that occurs during the few minutes following glucose injection. In contrast, $Phi_{dynamic}$ measures the increment in insulin secretion that occurs in response to the progressive increase in glucose concentrations observed during 30–60 min following meal ingestion. Therefore, it presumably is modulated by additional intra-islet events, which include translocation, priming, and docking of insulin granules. In contrast, the intravenous Phi_2 and the meal Phi_{static} are believed to be influenced by the still earlier events in the insulin secretory cascade including insulin synthesis and processing. Taken together, the present data indicate that testosterone replacement had no detectible effect on any aspect of β-cell function, whether considered as the absolute values of the various insulin secretion indexes or in light of the prevailing level of insulin action by calculation of disposition indexes.

The present study has certain limitations. We recruited men whose bioavailable testosterone concentration (not bound to SHBG) was less than the 15th percentile of healthy young men (33). However, none of the men had undetectable levels. Therefore, it is possible and even likely that testosterone treatment would improve carbohydrate tolerance in men who are overtly hypogonadal. The BMI of the subjects averaged ~28 kg/m^2. Therefore, few of the subjects were overtly obese. Of interest, the effects of testosterone on insulin action (S_i and S_i*) and insulin secretion (DIs) did not differ when the subjects whose BMI or bioavailable testosterone in the upper tertile was compared with those in the lower tertile. Due to concern regarding the potential adverse effects of long-term treatment of elderly men with androgens, we sought to raise bioavailable testosterone concentra-

tions to the lower range of normal for healthy young men. While we accomplished this goal, it is possible that larger amounts of testosterone would have altered glucose tolerance. However, use of high-dose androgens has multiple safety concerns and may decrease rather than increase insulin action (18,19). It is possible that 2 years of treatment with androgens was insufficient time to alter carbohydrate metabolism. We doubt this is the case since when such effects have been observed in animals or younger humans, they were detectible within days to weeks (7,13,14).

In summary, the present studies indicate that treatment of elderly men with relative testosterone deficiency for 2 years with doses sufficient to raise their bioavailable testosterone concentrations to the low normal range for healthy young men had no detectible effect on fasting or postprandial glucose concentrations. Testosterone replacement also did not alter meal appearance, postprandial suppression of endogenous glucose production, or stimulation of glucose uptake. Furthermore, testosterone replacement did not alter insulin secretion, insulin action, glucose effectiveness, or hepatic insulin clearance. Thus, testosterone deficiency is unlikely to be the cause of the age-associated deterioration in glucose tolerance commonly observed in elderly men. These data also argue against the premise that testosterone replacement will delay or prevent the progression of the age-associated deterioration in glucose tolerance that is commonly observed in elderly men.

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