

Acute Sex Steroid Withdrawal Reduces Insulin Sensitivity in Healthy Men with Idiopathic Hypogonadotropic Hypogonadism

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Context: Evidence suggests that testosterone (T) influences insulin sensitivity in men. The mechanism of this effect is unclear but is thought to involve changes in body composition.

Objective: The aim of this study was to determine whether acute sex steroid withdrawal decreases insulin sensitivity in young, healthy men with idiopathic hypogonadotropic hypogonadism (IHH).

Design: This was a 2-wk prospective study.

Setting: The study was conducted at a General Clinical Research Center.

Patients: Twelve men with IHH (age 40.8 ± 2.8 yr) were studied: 1) on hormone replacement with normal T levels and 2) 2 wk after discontinuing therapy.

Main Outcome Measures: Each evaluation comprised a 75-g oral glucose tolerance test with assessment of insulin sensitivity (fasting insulin levels, homeostatic model assessment for insulin resistance, and Matsuda insulin sensitivity index) and insulin secretion (cor-

rected insulin response). Serum cortisol, leptin, adiponectin, free fatty acids, IL-6, C-reactive protein, and TNF- α levels were also measured.

Results: Body mass index was unchanged (27.1 ± 1.1 to 27.2 ± 1.1 kg/m²). Serum T levels decreased from 529 ± 65 to 28 ± 8 ng/dl ($P < 0.00005$). Fasting insulin levels increased from 4.9 ± 0.7 to 6.2 ± 0.6 μ U/ml ($P = 0.005$), homeostatic model assessment of insulin resistance increased from 1.07 ± 0.2 to 1.4 ± 1.01 ($P < 0.005$), and insulin sensitivity index decreased from 11.0 ± 2.3 to 7.5 ± 0.7 ($P < 0.05$). There was a trend for fasting glucose levels to increase, 86.7 ± 1.3 to 90.8 ± 1.7 mg/dl ($P = 0.09$). IL-6 levels increased from 1.2 ± 0.2 to 2.4 ± 0.5 pg/ml ($P < 0.01$), whereas TNF- α levels decreased from 1.0 ± 0.1 to 0.6 ± 0.1 pg/ml ($P < 0.05$). No other significant changes were observed.

Conclusions: 1) Acute sex steroid withdrawal reduces insulin sensitivity in young healthy IHH men. 2) The acuity of the hypogonadism and absence of changes in body mass index or leptin levels suggest that sex steroids modulate insulin sensitivity in the absence of apparent or detectable changes in body composition. (*J Clin Endocrinol Metab* 92: 4254–4259, 2007)

INSULIN RESISTANCE PLAYS a key role in the development of type 2 diabetes mellitus (T2DM) (1, 2), a disease reaching epidemic proportions and anticipated to affect 220 million people worldwide by 2010 (3). Insulin resistance is also an important risk factor for cardiovascular disease even in the absence of T2DM (4). Therefore, understanding hormonal factors that modify insulin action has important public health implications.

Both animal and human studies suggest that testosterone (T) has favorable effects on insulin sensitivity in the male. Castration of male rats results in marked insulin resistance, which is abolished by physiological T replacement (5). Similarly, androgen receptor knockout mice develop progressive insulin resistance and T2DM with age (6). In the human, a recent metaanalysis showed that T levels are significantly lower in men with T2DM than

age-matched controls (7). Furthermore, prospective studies have implicated low T levels in the pathogenesis of both T2DM (8–12) and metabolic syndrome (12, 13). However, studies designed to investigate the causality of this relationship have given conflicting results. No changes in insulin sensitivity were observed in young, healthy men given progressively increasing doses of T (14) or in older men treated with recombinant human chorionic gonadotropin (15). In contrast, T administration to obese men with low-normal T levels was associated with an improvement in insulin sensitivity (16, 17). Similarly, in men with prostate cancer, induction of hypogonadism resulted in an increase in fasting insulin levels (18–20).

To date, relatively little is known about how T influences insulin sensitivity. In prostate cancer patients undergoing androgen deprivation therapy, the increase in fasting insulin levels correlates with changes in body fat (18). However, it is not known whether T affects insulin sensitivity before changes in body composition. Inflammatory cytokines are one potential mediator of the inverse relationship between serum T levels and insulin resistance. Increased levels of TNF- α , IL-6, and C-reactive protein (CRP) have been associated with development of T2DM and cardiovascular disease (21–23). Cross-sectional studies show an inverse relationship between T and levels of proinflammatory cytokines

First Published Online August 28, 2007

Abbreviations: AUC, Area under the curve; BMI, body mass index; CIR, corrected insulin response; CRP, C-reactive protein; CV, coefficient of variation; E₂, estradiol; FFA, free fatty acids; HOMA-IR, homeostatic model assessment for insulin resistance; IHH, idiopathic hypogonadotropic hypogonadism; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; T, testosterone; T2DM, type 2 diabetes mellitus.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

(24–26); however, the causal nature of this relationship has not been clearly defined (27–30).

Thus, the aim of the present study was to determine whether acute sex steroid withdrawal reduces insulin sensitivity in young, healthy men with idiopathic hypogonadotropic hypogonadism (IHH). Previous studies used GnRH agonists to examine the impact of hypogonadism on insulin sensitivity (18–20). However, given the variable duration of the flare reaction associated with GnRH agonist therapy, this is not an optimal experimental paradigm to study the impact of acute castration. In contrast, the human model of IHH allows the acute effects of T on insulin sensitivity to be readily assessed by evaluating these patients' responses on and off hormone replacement.

Subjects and Methods

Subjects

Fourteen men (age 40.8 ± 2.8 yr) with IHH were enrolled in this study (Table 1). The diagnosis of IHH was based on the following criteria: 1) serum T level less than 100 ng/dl in association with inappropriately low gonadotropin levels, 2) absence of endogenous gonadotropin pulsations during a 12-h period of blood sampling, 3) otherwise normal reserve testing of anterior pituitary function, and 4) normal magnetic resonance imaging of the hypothalamic-pituitary region. Thirteen subjects were Caucasian, and one was African-American. Six subjects had Kallmann syndrome (IHH associated with anosmia), four had normosmic IHH, and four had adult-onset isolated IHH as previously reported by our group (31). All patients were screened for the following genes known to cause IHH: KAL1, GnRH-R, FGFR1, and GPR54. Mutations in the KAL1 gene were identified in cases 1, 2, and 4. All subjects had normal T levels after treatment with pulsatile GnRH ($n = 13$) or transdermal T ($n = 1$) (mean duration of therapy 14.8 ± 2 yr). None of the subjects was known to have a personal or family history of T2DM. Case 13 had a history of mild hypertension treated with a thiazide diuretic, whereas case 14 was receiving bupropion for depression, which was well controlled. None of the remaining subjects had any comorbid illness or was taking medication other than their hormone replacement therapy. The study was approved by the Human Research Committee at the Massachusetts General Hospital, and all subjects provided written informed consent.

Procedures

Subjects were studied twice: 1) when T levels were in the mid-normal range on hormone replacement therapy; and 2) in the hypogonadal state after therapy had been withdrawn for 2 wk.

Body composition. Height and weight were measured using standard procedures. Body mass index (BMI) was calculated from the weight in kilograms divided by the square of the height in meters. Given that

serum leptin levels are a sensitive indicator of body fat (32), we used changes in leptin levels as a surrogate for changes in body fat.

Assessment of insulin sensitivity and secretion. A 75-g oral glucose tolerance test (OGTT) was performed after a 12-h overnight fast according to World Health Organization criteria (33). Plasma glucose and insulin concentrations were measured at 0, 30, 60, 90, and 120 min. Insulin sensitivity was assessed by measuring fasting insulin levels; by the homeostatic model assessment for insulin resistance (HOMA-IR), calculated as [fasting glucose (mmol/liter) \times fasting insulin (μ U/ml)]/22.5 (34); and by the Matsuda insulin sensitivity index (ISI), calculated as [10,000/square root of (fasting glucose \times fasting insulin) \times (mean OGTT glucose \times mean OGTT insulin levels)] (35). Insulin secretion was determined by the corrected insulin response (CIR), based on the 30-min plasma glucose and insulin measurements during the OGTT: $100 \times 30\text{-min OGTT insulin}/[30\text{-min OGTT glucose} \times (30\text{-min OGTT glucose} - 70 \text{ mg/dl})]$ (36). The trapezoidal rule was used to calculate the incremental area under the curve (AUC) for glucose and insulin for the duration of the OGTT.

Hormone and adipocytokine levels. Serum levels of T, cortisol, leptin, adiponectin, free fatty acids (FFA), IL-6, CRP, and TNF- α were measured at time zero of each OGTT.

Assays

Serum T levels were measured using the Coat-A-Count RIA kit (Diagnostic Products Corp., Los Angeles, CA), which had intra- and interassay coefficients of variation (CV) of less than 10%. Estradiol (E_2) was determined by a chemiluminescent microparticle immunoassay using the automated Abbott ARCHITECT i2000 system (Abbott Laboratories, Inc., Chicago, IL), which had a functional sensitivity of 14 pg/ml and intra- and interassay CV of less than 7.4%. Immunoreactive insulin was determined by an insulin-specific, double-antibody system using human insulin standards and tracer (Linco Research, Inc., St. Charles, MO). The antiserum was raised against highly purified human insulin and does not cross-react with human proinsulin ($<0.01\%$). Glucose was measured by the hexokinase glucose-6-phosphate dehydrogenase method (Olympus Diagnostica, Melville, NY). The concentration of FFA was determined using an enzymatic colorimetric assay on the Hitachi 917 (Roche Diagnostics, Indianapolis, IN) using reagents from Wako Chemicals USA (Richmond, VA). TNF- α , IL-6, and adiponectin were measured using high-sensitivity ELISA kits (R&D Systems Inc., Minneapolis, MN) with interassay CV of less than 17%, less than 10%, and less than 7%, respectively. CRP was measured by an ELISA (American Laboratory Products Co., Windham, NH) with a sensitivity of 0.124 ng/ml and an interassay CV of less than 9%. Leptin was measured using a commercially available RIA kit (Linco) with a sensitivity of 0.5 ng/ml and intra- and interassay CV of 5 and 7.1%, respectively. Serum cortisol levels were measured using a fully automated chemiluminescent immunoassay system (Immulite 2000; Diagnostic Products).

TABLE 1. Clinical characteristics of men with IHH

Case	Current age (yr)	Dx	Age at Dx (yr)	Duration of androgen Tx (yr)	Associated phenotypes
1	29	KS	19	10	Cryptorchidism
2	34	KS	18	16	Synkinesia
3	37	KS	19	18	Short 4th metacarpal, synkinesia
4	35	KS	16	19	Synkinesia
5	40	KS	20	20	None
6	51	KS	19	32	Cryptorchidism
7	30	nIHH	21	9	None
8	38	nIHH	19	19	Microphallus
9	38	nIHH	21	11	None
10	41	nIHH	22	10	None
11	36	AHH	31	5	None
12	47	AHH	27	20	None
13	48	AHH	40	8	Hypertension
14	67	AHH	57	10	Depression

AHH, Adult-onset IHH; Dx, diagnosis; KS, Kallmann syndrome; nIHH, normosmic IHH; Tx, therapy.

Statistical methods

The data are expressed as mean \pm SE unless otherwise stated. Normality was assessed by the Kolmogorov-Smirnov test. Data demonstrating a normal distribution were compared using two-sided paired *t* tests, whereas the Wilcoxon signed-rank test was used to analyze data that were not normally distributed. The linear association between any two variables was examined by Pearson's correlation coefficient. All *P* values are two-sided with a *P* value < 0.05 considered statistically significant.

Results

Two subjects were excluded from the study, one because he met criteria for T2DM on his baseline OGTT (subject 14) and the second because his baseline samples were hemolyzed and could not be used for insulin measurements (subject 2). Data are thus presented for the 12 subjects on whom complete data are available.

Four subjects were lean (BMI < 25 kg/m²), six were overweight (BMI = 25.0–29.9 kg/m²), and two were obese (BMI ≥ 30.0 kg/m²). There was no change in either BMI (27.1 ± 1.1 to 27.2 ± 1.1 kg/m²) or leptin levels (Table 2). Serum T levels decreased from 529 ± 65 to 28 ± 8 ng/dl ($P < 0.00005$), and E₂ levels decreased from 31 ± 3 to 18 ± 2 pg/ml ($P < 0.05$).

Apart from the subject with newly diagnosed T2DM, all remaining participants had normal glucose tolerance at baseline. Mean fasting glucose increased from 86.7 ± 1.3 to 90.8 ± 1.7 mg/dl ($P = 0.09$) (Fig. 1A). One subject whose fasting glucose level increased from 83 to 103 mg/dl met criteria for impaired fasting glucose. Fasting insulin levels increased from 4.9 ± 0.7 to 6.2 ± 0.6 μ U/ml ($P = 0.005$) (Fig. 1B). No significant changes were seen in glucose or insulin levels at other time points during the OGTT or in the AUC for glucose (486 ± 21 vs. 508 ± 18 , $P = 0.22$) or AUC for insulin (149 ± 29 vs. 148 ± 20 , $P = 0.9$). HOMA-IR increased from 1.07 ± 0.2 to 1.4 ± 0.1 ($P < 0.005$), whereas the Matsuda ISI decreased from 11.0 ± 2.3 to 7.5 ± 0.7 ($P < 0.05$) (Fig. 2). No significant change was seen in the CIR (0.44 ± 0.09 vs. 0.48 ± 0.12 , $P = 0.5$).

There was no correlation between baseline BMI and the change in fasting insulin levels ($r = -0.1$; $P = 0.8$), HOMA-IR ($r = -0.1$; $P = 0.7$), or the ISI ($r = 0.01$; $P = 0.9$) after sex steroid withdrawal. Similarly, no relationship was observed between either baseline T or change in T levels and parameters of insulin sensitivity or glucose levels (data not shown).

Reducing sex steroids was associated with a significant increase in IL-6 and a modest decrease in TNF- α levels (Table 2). There was a positive correlation between IL-6 and both fasting insulin levels ($r = 0.8$; $P < 0.002$) and HOMA-IR ($r = 0.8$; $P < 0.005$) at baseline. There was a trend toward an inverse rela-

tionship between the change in T levels and the increase in IL-6 levels ($r = -0.5$; $P = 0.07$). However, the change in IL-6 levels after sex steroid withdrawal did not predict the reduction in insulin sensitivity ($r = -0.3$; $P = 0.3$). No significant changes were seen in CRP, adiponectin, FFA, or cortisol levels (Table 2).

None of the patients reported significant symptoms of hypogonadism such as hot flashes, night sweats, or mood changes during the 2 wk of the study.

Discussion

In this prospective study, we show that acute sex steroid withdrawal decreases insulin sensitivity in young healthy men with IHH as evidenced by significant changes in fasting insulin levels, HOMA-IR (34), and the Matsuda ISI (35). There was a trend for fasting glucose levels to increase, and one subject met criteria for impaired fasting glucose after only 2 wk of hypogonadism. The acuity of the hypogonadism, in conjunction with the absence of changes in BMI or leptin suggests that sex steroids modulate insulin sensitivity independent of apparent or detectable alterations in body composition.

In subjects with normal glucose tolerance, the indices of insulin sensitivity that we used in this study correlate highly ($r = 0.6$) with the gold standard measure, the hyperinsulinemic-euglycemic clamp (37). Therefore, we are confident that the changes we observed in insulin sensitivity are accurate. Interestingly, the reduction in insulin sensitivity that occurred in response to acute hypogonadism was not accompanied by a compensatory increase in insulin secretion as assessed by the CIR, suggesting that low sex steroid levels may also have a negative effect on β -cell function. This hypothesis is supported by animal experiments showing that T protects against streptozotocin-induced apoptosis of pancreatic β -cells (38). Furthermore, *in vitro* studies using isolated rat islets of Langerhans have shown that physiological T concentrations rapidly stimulate insulin secretion and calcium uptake (39). In contrast, the correlation between the CIR and the gold standard method for assessing insulin secretion, the insulin response to an iv glucose infusion, is not as robust ($r = 0.35$) (37). Therefore, caution should be exerted in interpreting the effects of low sex steroid levels on insulin secretion based on the results of the present study.

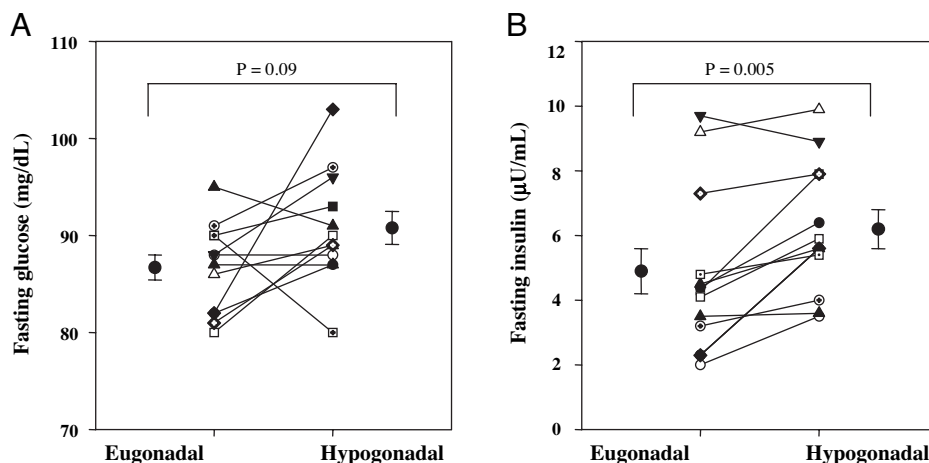
Our data are consistent with previous reports of men with prostate cancer developing insulin resistance after 3 months of GnRH analog-induced castration (18–20). However, our study differs in a number of important respects. First, we studied young, otherwise healthy men with IHH in the absence of any

TABLE 2. Change in hormone and cytokine levels after withdrawing sex steroids for 2 wk in 12 men with IHH

	Wk 0 (eugonadal)	Wk 2 (hypogonadal)	<i>P</i> value
Testosterone (ng/dl)	529 \pm 65	28 \pm 8	<0.00005
Estradiol (pg/ml)	31 \pm 3	18 \pm 2	<0.05
Leptin (ng/ml)	6.4 \pm 1.4	5.5 \pm 0.9	NS
Adiponectin (μ g/ml)	12.3 \pm 0.8	11.9 \pm 0.9	NS
IL-6 (pg/ml)	1.2 \pm 0.2	2.4 \pm 0.5	<0.01
CRP (μ g/ml)	1.3 \pm 0.3	1.3 \pm 0.4	NS
TNF- α (pg/ml)	1.0 \pm 0.1	0.6 \pm 0.1	0.05
Free fatty acids (mEq/liter)	0.81 \pm 0.1	0.83 \pm 0.2	NS
Cortisol (μ g/dl)	13.8 \pm 1.6	12 \pm 1.2	NS

Data are expressed as mean \pm SE. NS, Not significant.

FIG. 1. Impact of acute sex steroid withdrawal on fasting glucose (A) and insulin (B) levels in 12 healthy men with IHH. The various symbols represent individual patients. Each line represents data from an individual subject at baseline (eugonadal) and 2 wk after withdrawing hormone therapy (hypogonadal). The filled circles on the left and right of each graph represent mean \pm SEM for the eugonadal and hypogonadal states, respectively.



potential confounding issues related to cancer pathology. Second, the model we used allowed us to determine the impact of acute sex steroid withdrawal and is the first study to show a significant reduction in insulin sensitivity in a timeframe within which changes in body composition are unlikely to have occurred. Although it is possible that small changes in body fat may have occurred without being reflected in changes in leptin levels, our data are consistent with the results of large epidemiological studies showing that the association between low T levels and insulin resistance persists after adjusting for body fat (7, 40). Similarly, androgen receptor knockout mice develop insulin resistance and glucose intolerance before the modest increase in body fat that occurs with aging (6). In contrast to our data, Singh *et al.* (14) reported no change in insulin sensitivity in young healthy men after 20 wk of GnRH agonist-induced sex steroid ablation with graded T addback. However, subjects in that study were not rendered overtly hypogonadal in that even those who received the lowest dose of T addback had nadir T levels of 253 ng/dl compared with 31 ng/dl in our study.

The clinical significance of the metabolic changes we observed after 2 wk of hypogonadism is uncertain. Although the magnitude of the change is relatively modest in absolute terms, we believe that a 48% decrease in insulin sensitivity is significant given that oral hypoglycemic agents such as rosiglitazone cause only a 20% improvement in insulin sensitivity in men with T2DM (41). Furthermore, an accumulating body of evidence is now linking iatrogenic hypogonadism in men with prostate cancer with an increase in both T2DM (42, 43) and cardiovascular disease (43, 44). Taken together, these data suggest that men undergoing androgen-deprivation therapy may

benefit from close monitoring of glucose tolerance to permit early intervention and prevention of treatment-related diabetes and cardiovascular disease.

One potential weakness of this study is the absence of a control group, which raises the possibility that the changes observed in insulin sensitivity could be explained by inherent variability in the methods used as opposed to a true consequence of sex steroid withdrawal. However, a recent study, which examined the reproducibility of HOMA-IR by measuring fasting glucose and insulin levels 2 wk apart under identical conditions, reported a coefficient of variation between the two visits of 23% (45). In our study, the HOMA-IR increased by almost 50%, suggesting that the changes in insulin sensitivity that we observed are unlikely to be explained by methodological issues alone. However, given that our study was not randomized in terms of treatment order, the role of time or order effects cannot be fully excluded.

From a mechanistic perspective, relatively little is known about how sex steroids influence insulin action. Recent studies using genetic analysis (46, 47), functional imaging (48, 49), and rat models of differential aerobic capacity (50) have suggested an important role for mitochondrial dysfunction in inducing insulin resistance. We have previously shown a correlation between serum T levels and both genetic and functional indices of mitochondrial function, namely expression of genes involved in oxidative phosphorylation in skeletal muscle and maximal aerobic capacity (51). Studies are currently underway to determine whether these indices of mitochondrial function change in response to short-term castration.

Given the study design we used, it is not possible to deter-

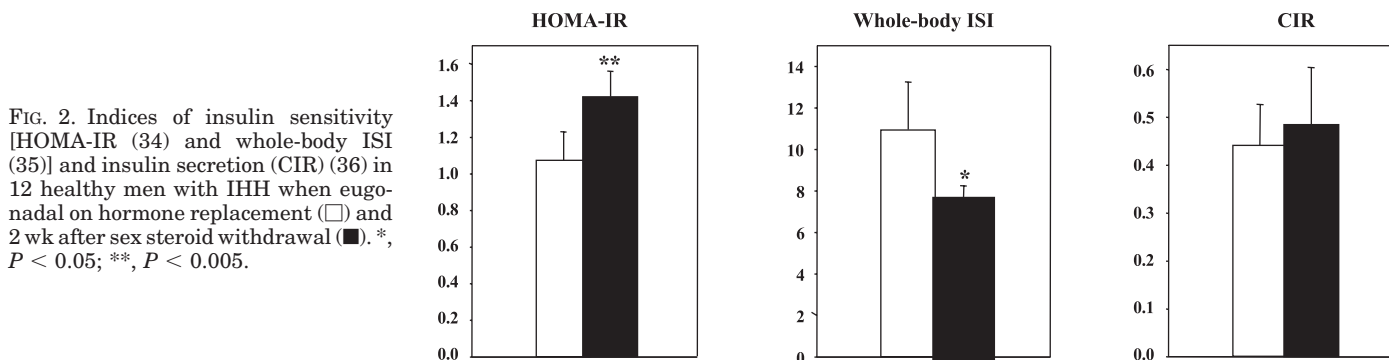


FIG. 2. Indices of insulin sensitivity [HOMA-IR (34) and whole-body ISI (35)] and insulin secretion (CIR) (36) in 12 healthy men with IHH when eugonadal on hormone replacement (\square) and 2 wk after sex steroid withdrawal (\blacksquare). *, $P < 0.05$; **, $P < 0.005$.

mine whether the changes in insulin sensitivity observed after discontinuing hormone therapy were due to withdrawal of T, E₂, or both. Several lines of evidence suggest that E₂ may play a role in mediating T's effects on insulin action. Ingestion of anabolic steroids, which are nonaromatizable 17 α -alkylated androgens, induces insulin resistance in men (52, 53). Similarly, administration of dihydrotestosterone, another nonaromatizable androgen, has no effect on insulin sensitivity in men with central obesity in contrast to the beneficial effects of T in the same population (17). In addition, insulin resistance is part of the phenotype of congenital estrogen deficiency due to mutations in either the aromatase (54–58) or estrogen receptor- α (ER α) genes (59, 60) in both human and mouse models. Moreover, estrogen therapy causes a significant improvement in insulin sensitivity in these models (55, 56, 58, 61). Therefore, additional studies employing selective suppression of T and E₂ are needed to determine their relative importance in influencing insulin action.

Previous studies have reported a significant correlation between circulating IL-6 levels and indices of insulin resistance in men after controlling for BMI and fat mass (62). We therefore explored the possibility that changes in the levels of inflammatory cytokines might underlie the reduction in insulin action in our population. However, the cytokine response to short-term hypogonadism in young healthy subjects with IHH was highly variable, characterized by an increase in IL-6, a decrease in TNF- α and no change in CRP levels. The literature on the relationship between T and markers of inflammation has produced conflicting results. Cross-sectional studies have variably reported either no association or an inverse relationship between T and inflammatory cytokines depending on the patient population and the marker studied (24–26). Similarly, although T suppression resulted in modest increases in both IL-6 and TNF- α levels in one study of elderly men (28), T replacement was associated with a decrease in TNF- α levels but no change in IL-6 in another cohort of older men (30). The biological significance of the changes in IL-6 and TNF- α that we observed is uncertain given our small sample size, the lack of correlation between the change in the levels of these cytokines and the reduction in insulin sensitivity, the rather high CV for these assays, and the many factors, other than sex steroids, that can influence cytokine levels.

Induction of hypogonadism may be associated with distressing symptoms such as hot flashes, which can, in turn, trigger a stress response. We therefore considered the possibility that increased activity of the hypothalamic-pituitary-adrenal axis could antagonize insulin action and contribute to the decrease in insulin sensitivity that we observed. However, none of our study subjects complained of hot flashes during the 2 wk of low sex steroid levels, and morning plasma cortisol levels were unchanged. Although it is clear that activation of the hypothalamic-pituitary-adrenal axis did not underlie the reduction in insulin sensitivity seen in our study, it is possible that secretion of stress hormones other than cortisol such as catecholamines or GH might have contributed to the changes observed.

In summary, these data provide evidence that sex steroids are important modulators of insulin sensitivity in young, healthy men with IHH. The short duration of hypogonadism in conjunction with the absence of changes in BMI or leptin suggests that sex steroids can modulate insulin sensitivity

independent of apparent alterations in body fat. Additional studies are needed to elucidate better both the time course and mechanism of this reduction in insulin sensitivity.

Acknowledgments

Received February 27, 2007. Accepted August 21, 2007.

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This work was supported by National Institutes of Health Grants R03 DK064276 A 01 and M01-RR-01066 and by the National Center for Research Resources, General Clinical Research Centers Program, and a Career Development Award of the American Diabetes Association (F.J.H.).

Disclosure Statement: The authors have nothing to disclose.

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