Title: Abrupt decrease in serum testosterone levels after an oral glucose load in men: implications for screening for hypogonadism.

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1. Title
Abrupt Decrease in Serum Testosterone Levels After an Oral Glucose Load in Men: Implications for Screening for Hypogonadism

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Decrease in serum testosterone after glucose

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Abstract

Objective: This study examines the physiologic impact of a glucose load on serum testosterone (T) levels in men with varying glucose tolerance (GT).

Design: Cross-sectional study

Patients and Methods: 74 men (19-74 years, mean 51.4 ± 1.4 years) underwent a standard 75-g oral glucose tolerance test with blood draws at 0, 30, 60, 90, and 120 min. **Fasting serum** glucose, insulin, total T (and calculated free T), LH, SHBG, leptin and cortisol were measured.

Results: 57% of the men had normal GT, 30% had impaired GT, and 13% had newly diagnosed type 2 diabetes. Glucose ingestion was associated with a 25% decrease in mean T levels (delta = –4.2 ± 0.3 nmol/L, $p < 0.0001$). T levels remained suppressed at 120 minutes compared to baseline (13.7 ± 0.6 vs. 16.5 ± 0.7 nmol/L, $p < 0.0001$) and did not differ across GT or BMI. Of the 66 men with normal T levels at baseline, 10 (15%) had levels which decreased to the hypogonadal range (<9.7 nmol/L) at one or more time points. SHBG, LH, and cortisol levels were unchanged. Leptin levels decreased from baseline at all time points ($p < 0.0001$).

Conclusions: Glucose ingestion induces a significant reduction in total and free T levels in men, which is similar across the spectrum of glucose tolerance. This decrease in T appears to be due to a direct testicular defect but the absence of compensatory changes in LH suggests an additional central component. Men found to have low **non-fasting** T levels should be reevaluated in the fasting state.

INTRODUCTION

More than two thirds of American adults are overweight or obese. This trend creates significant public health concerns related to the growing numbers of individuals with insulin resistance, metabolic syndrome, and type 2 diabetes [1]. We, and others, have demonstrated an inverse relationship between testosterone (T) levels and insulin resistance in men with type 2 diabetes and the metabolic syndrome [2-5]. The combination of an aging American public and an obesity epidemic has produced a growing awareness of hypogonadism reflected in a significant increase in the annual number of T prescriptions [6]. The decision to prescribe T is often based on a single blood sample, despite well documented diurnal and day-to-day variability in men [7, 8] and clinical recommendations by expert panels that two or more morning samples be drawn for assessment of T levels [9]. However, no emphasis has been placed on when a sample for T measurement should be drawn in relation to food intake or if it should be obtained in the fasting state.

To date, three studies have investigated the T response in men to different caloric and macronutrient (fat) content in meals. Depending on the meal composition, these studies demonstrated either a postprandial decrease in T ranging from 15 to 40%, or no change in T levels [10-12]. Yet, the differences in meal size and macronutrient proportions create ambiguity regarding what caused, or failed to cause, a decrease in T levels. In addition, three previous studies have reported that in men given an oral glucose load, serum T levels decreased by approximately 10% [13], 14% [14] and 30% [15], respectively. However, conclusions from these studies are limited by both small sample size and the fact that all subjects had normal glucose tolerance.

Nutrient ingestion initiates a complex cascade of physiological processes, including the secretion of a variety of peripheral signals that act at the hypothalamus. Interestingly, many of the signals implicated in energy homeostasis have also been demonstrated to affect the reproductive axis [16]. In contrast to the inverse relationship between T and insulin resistance in men, *in vitro* studies demonstrate the stimulatory effect of insulin on the reproductive axis. Insulin has been shown to promote gonadotropin-releasing hormone secretion in a hypothalamic neuronal cell line [17], stimulate gonadotropin secretion from pituitary cell cultures [18], and stimulate T secretion from cultured Leydig cells [19]. Further, pharmacologic doses of insulin have been shown to significantly increase serum T levels in healthy men during hyperinsulinemic-euglycemic clamp studies [4]. To date, the mechanism underlying these changes in T has not been defined.

Thus, the aims of this study were to: i) examine the physiologic impact of an oral glucose load on serum T levels in a cohort of men diverse in terms of age and BMI; ii) determine if the T response varies with glucose tolerance; iii) establish if changes in T levels have a neuroendocrine basis or result from a direct testicular effect; and iv) explore mechanisms that might account for any change.

PATIENTS AND METHODS

A total of 74 healthy, adult males (range: 19-74 years, mean: 51.4 ± 1.4) were included in the study. This community dwelling group included fifty-nine (80%) Caucasian, 12 (16%) African American, 1 (1%) Asian, and 2 (3%) Hispanic/Latino men. Subjects were excluded if they had a history of chronic illness, reproductive disorders, prostate cancer, type 2 diabetes, or use of medications known to interfere with androgen synthesis/action or glucose homeostasis. All

subjects had undergone a normal puberty and had normal serum prolactin and thyroid stimulating hormone levels. The study was approved by the Human Research Committee of the Massachusetts General Hospital and all subjects provided written informed consent prior to the initiation of study procedures.

Study subjects underwent a full physical examination including calculation of body mass index (BMI) from the measurement of weight in kilograms divided by the square of height in meters and were classified as normal weight (< 25 kg/m^2), overweight (25.0-29.9 kg/ m^2), or obese (≥ 30.0 kg/ m^2). A standardized 2-hour oral glucose tolerance test (OGTT) using 75g of glucose was administered following a 12-hour overnight fast. **All subjects began testing between the hours of 08:00-09:00 AM with blood samples drawn at baseline (time 0) and at +30, +60, +90, and +120 minutes following glucose ingestion**. Subjects were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or type 2 diabetes according to American Diabetes Association criteria [20]. Blood samples were analyzed to measure glucose, insulin, T, sex hormone binding globulin (SHBG), luteinizing hormone (LH), and leptin. Free T concentrations were calculated from total T and SHBG using the Vermeulen equation [21]. Based on their total serum T level at baseline (time 0) and the normal range for our T assay, subjects were classified as having normal (≥9.7 nmol/L) or low (<9.7 nmol/L) T levels. Serum cortisol levels were assessed in a subset of subjects (n=40) at 0, 30, and 60 minutes.

Assays

All assay methods used in this study have been previously described [2, 22]. Serum T levels were measured using the DPC Coat-A-Count RIA kit (Diagnostics Products Corporation, Los

Angeles, CA) with intra- and interassay coefficients of variation (CV) of less than 10%. Serum LH concentrations were determined by microparticle enzyme immunoassay using the automated Abbott AxSYM system (Abbott Laboratories, Chicago, IL), which had an intraassay CV of less than 7% and an interassay CV of less than 7.4%. SHBG was measured using a fully automated system (Immulite; Siemens) with an intra and interassay CV of less than 7%. Glucose was measured by the hexokinase glucose-6-phosphate dehydrogenase method (Olympus Diagnostica, Melville, NY). Leptin was measured using a commercially available RIA kit (Linco Inc., St. Charles, MO), which had an intra- and interassay CV of less than 6%. Immunoreactive insulin was determined by using a commercially available RIA kit (Linco Inc., St. Charles, MO) using ^{125}I-labeled human insulin and human insulin antiserum. Serum cortisol levels were measured using a fully automated chemiluminescent immunoassay system [Immulite 2000, Diagnostics Products Corp. Inc. Los Angeles CA]. The inter-assay CV range was 11.8, 7.3, and 5.8 % for control sera containing from 125, 335, and 965 nmol/L respectively.

Statistical Analysis

Data are presented as mean ± SEM unless otherwise stated. Baseline characteristics were compared across glucose tolerance groups using one-way ANOVA. Repeated measures ANOVA was used to assess the overall time effect and test for an association between T and covariates at the previous time point. A Dunnet's test was employed to compare each time point to baseline levels while correctly accounting for multiple comparisons. A random slopes model was used for each covariate in a separate regression model to test for an association between T and other covariates. Linear association between any two variables was assessed using the Pearson Product Moment Correlation. A p value < 0.05 was considered statistically significant.

RESULTS

Baseline clinical and biochemical characteristics of the study population are presented in Table 1. Subjects' BMI ranged from 20.4-49.4 kg/m^2 including 8 normal weight, 29 overweight, and 37 obese subjects. In terms of glucose tolerance, 42 subjects (57%) had NGT, 22 (30%) had IGT, and 10 (13%) had newly diagnosed type 2 diabetes. At baseline (time 0), 8 men had T levels below the normal range (<9.7 nmol/L), while the remainder were eugonadal (n=66).

In the group as a whole, serum T levels were significantly lower than baseline ($p<0.0001$) at all time points following the 75g oral glucose load (Figure 1). Free T levels mirrored the decrease in total T (Fig. 1) as serum SHBG levels did not change during testing (Table 2). Interestingly, only one subject did not have a serum T level fall below baseline during the 2 hours of testing. No significant changes in LH were observed and repeated measures analysis revealed that LH neither exerted an effect on T levels at the same time point, nor 30 minutes earlier. Leptin levels were significantly decreased at 30 minutes and the lowered level persisted throughout testing ($p< 0.0001$). No changes in plasma cortisol levels were observed (Table 2).

Notably, 10 of the 66 subjects (15%) with normal T levels at baseline had a T level that fell below the normal range during at least one time point during the testing. Of those 10 men, 5 had low T levels at +30 minutes, 7 at +60 minutes, 6 at +90 minutes, and 8 at +120 minutes. Across the entire cohort, the time to reach nadir T level varied; 8 men (10.1%) exhibiting their lowest serum T at 30 minutes, 21 (28.4%) at 60 minutes, 22 (29.7%) at 90 minutes, and 23

(31.1%) at 120 minutes. The mean maximum intra-individual decrease in serum T was 4.2 ± 0.3 nmol/L, a reduction of 24.7% from baseline.

When the cohort was stratified according to glucose tolerance, T levels were not different across the three groups during testing (Fig. 2). Significantly decreased T levels were observed in the NGT group at all time points compared to baseline ($p<0.0001$) while the type 2 diabetes group was decreased ($p<0.005$) at 60, 90, and 120 minutes, and the IGT group showed T levels significantly lower than baseline only at 60 and 90 minutes ($p <0.01$). Neither mean values at each time point, nor changes from baseline in LH, leptin, free T, or SHBG in response to glucose differed significantly across the three groups. Changes in T were not different between BMI groups (normal weight, overweight, or obese). Further, no associations were identified between changes in total T levels and changes in any of the covariates examined.

DISCUSSION

The Endocrine Society guidelines for the evaluation and treatment of androgen deficiency syndromes in adult men recommend that a diagnosis be made only in men with consistent signs and symptoms as well as unequivocally low, repeated morning serum T levels [9]. To date, no recommendations exist regarding T measurement in relation to food intake. Herein, we report a significant decrease in serum T following a standard 75g glucose load in a diverse cohort of adult men across a spectrum of glucose tolerance. Further, T levels decreased by as much as 47% from fasting baseline levels in eugonadal men following glucose ingestion and 15% of men with normal T levels at baseline developed low T levels at one or more time point during the OGTT. **Recently, a large study of over 300 men (40-97 years of age) showed that compared to non-**

fasting levels, fasting men havesignificantly higher serum T levels [23]. These data raise an important issue with regard to the optimal conditions under which hypogonadism should be assessed in men. Based on these data we propose that men who present with signs and symptoms of hypogonadism and who display borderline low serum T levels should have morning measurements taken in a fasting state to get a clearer picture of total T levels. This is particularly important as serum T levels have been shown to transiently fall well below the normal range in approximately 15% of healthy males over the course of a day [7]. Thus, careful assessment of hypogonadism in men is critical to ensure that appropriate therapeutic decisions are made.

However, while the clinical implications for these findings may be evident, the mechanism is less clear. In the present study, men exhibited on average a 25% decrease in serum T level from fasting levels and no differences were observed according to glucose tolerance. These data are in line with previous studies reporting changes in T levels ranging from 10-30% in men with normal glucose tolerance [13-15]. While our data are bolstered by the diverse population studied, we also sought to elucidate the mechanism of these hormonal dynamics. The first, and perhaps most obvious, explanation for the observed changes is a technical issue, that the measurements were affected by subjects' hydration status or dilution issues. **While these may have been contributing factors, there are several reasons why it they are unlikely to fully explain the observations. First, while this is a rather crude measure of hydration status, hematocrit was normal in all subjects. Second, all but 1 subject showed the same pattern of decrease. Third, the sheer magnitude of change makes it highly unlikely that changes could be explained by hydration status of dilution issues.** Another explanation for the observed

changes was that the stress of fasting, having an i.v. cannula inserted and being subjected to an OGTT stimulated cortisol levels which could, in turn, suppress T levels. However, cortisol levels were unchanged in the present study, and actually decreased in prior reports [24, 25], thus ruling this out as a possible cause.

An alternate mechanism underlying the decrease in T is that peripheral signals to the central nervous system such as leptin might be influencing the HPG axis. Both insulin and leptin play important roles in transmitting messages to the brain on energy stores, which in turn affect appetite and energy expenditure [26]. **Prior studies have shown that leptin levels decline following fasting-induced decreases in insulin [27] and are restored with refeeding [28]. Interestingly, elevated leptin levels are associated with insulin resistance independent of body fat mass [29] yet insulin does not acutely stimulate leptin production [30]. Others have reported decreases in leptin following a meal or at 2-hours of an OGTT [31]. In contrast, the study reporting a 14% decrease in T with OGTT at 2 hours found no change in leptin levels compared to fasting baseline levels [14]. We observed a significant and sustained suppression of leptin following glucose ingestion similar to the observed T suppression. Epidemiologic studies have shown a negative correlation between T and leptin and when a GnRH analog is used to suppress serum T levels, both insulin and leptin levels increase [32]. However, the changes observed in the present study were acute and therefore, it may be that the observed decrease in leptin is related to its diurnal secretion pattern as others have postulated [31]. Alternatively, it may suggest a more complex relationship between metabolism and reproduction in men.**

We hypothesized that the diminished T levels could have resulted from an inhibitory effect of insulin on hepatic SHBG production [33]. However, SHBG levels were neither different across the spectrum of glucose tolerance nor did they change during the OGTT and accordingly the decrease in calculated free T levels mirrored that of total T. Lastly, we considered that the decrease in T levels might be due to a central defect in the HPG axis. The prior studies are conflicting reporting either no change in LH levels [14] or increased levels at 2-hours [13]. However, given the pulsatile nature of LH secretion, a single measurement of LH is often insufficient to characterize dynamic levels, thus limiting the inferences that can be drawn from these studies. Recently, a study of more than 50 men, nearly all of whom had normal glucose tolerance, utilized frequent blood sampling to demonstrate that glucose ingestion induced a decrease in pulsatile LH secretion over a 6-hour period [34]. In the current study, we evaluated LH levels at each of the 5 time-points during the OGTT revealing normal levels that were unchanged throughout. The lack of compensatory increase in LH levels in response to a fall in serum T level is particularly noteworthy as one would anticipate that the decreased negative feedback of T would lead to increased LH levels [35]. Thus, these data appear to be consistent with a central defect.

In summary, we demonstrate a significant decrease in serum total T and calculated free T during a standard 75g oral glucose tolerance test in men across a spectrum of glucose tolerance. On average, T levels decreased by 25%. Importantly, 15% of the men who were eugonadal at baseline developed low T levels following glucose ingestion. The decrease in T appears to be due to a direct testicular defect but the absence of compensatory changes in LH suggests an additional central component. Based on these data we suggest that evaluating T levels in a

fasting state may aid in the clinical decision making regarding testosterone supplementation for hypogonadism in men.

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Table 1. Clinical and biochemical characteristics at baseline (mean ± SEM)

	All subjects (n=74)	Normal glucose tolerance (n=42)	Impaired glucose tolerance (n=22)	Type 2 diabetes (n=10)
Age (years)	51.4 ± 1.4	48.8 ± 1.2	53.8 ± 1.2	57.7 ± 2.8
BMI (kg/m^2)	30.8 ± 0.7	29.6 ± 0.6	32.6 ± 0.6	31.7 ± 1.1
Testosterone (nmol/L)	16.5 ± 0.7	16.9 ± 0.9	14.9 ± 1.3	18.4 ± 1.6
Subjects with low testosterone levels (%)	8 (10.8%)	4 (9.5%)	3 (13.0%)	1 (11.1%)
Glucose (mmol/L)	6.4 ± 0.4	4.9 ± 0.1	**5.4 ± 0.1***	**7.4 ± 1.1*,†**
Insulin (pmol/L)	78.7 ± 6.7	58.0 ± 6.7	**106.3 ± 13.1***	**102.9 ± 22.0***

* P<0.05 compared to NGT, † P<0.05 compared to IGT

Table 2. Biochemical response to a 75g oral glucose load

Time	0	30	60	90	120
Glucose (mmol/L)	5.4 ± 0.2	**8.6 ± 0.3***	**9.4 ± 0.4***	**8.8 ± 0.5***	**7.6 ± 0.4***
Insulin (pmol/L)	78.7 ± 6.7	**403.6 ± 40.1***	**452.2 ± 39.0***	**486.9 ± 47.1***	**422.4 ± 50.0***
SHBG (nmol/L)	37.5 ± 2.4	38.4 ± 2.6	36.9 ± 2.5	38.6 ± 2.6	37.5 ± 2.4
LH (IU/L)	11.1 ± 0.8	11.5 ± 0.8	11.5 ± 0.8	12.1 ± 0.8	12.0 ± 0.9
Leptin (ug/L)	11.2 ± 1.7	**6.6 ± 0.8***	**6.7 ± 0.8***	**6.9 ± 0.8***	**6.6 ± 0.8***
Cortisol† (nmol/L)	248.3 ± 19.0	231.2 ± 15.7	225.1 ± 13.8	NA	NA

* P< 0.0001 compared to Time 0; † Data obtained on random subset of 40 subjects; NA = not assessed

Figure 1. Changes in total testosterone (circles) and calculated free testosterone (squares) in response to a 75g oral glucose load (n=74). Each time point represents mean ± SEM, asterisks (*) denote change from baseline ($p<0.0001$).

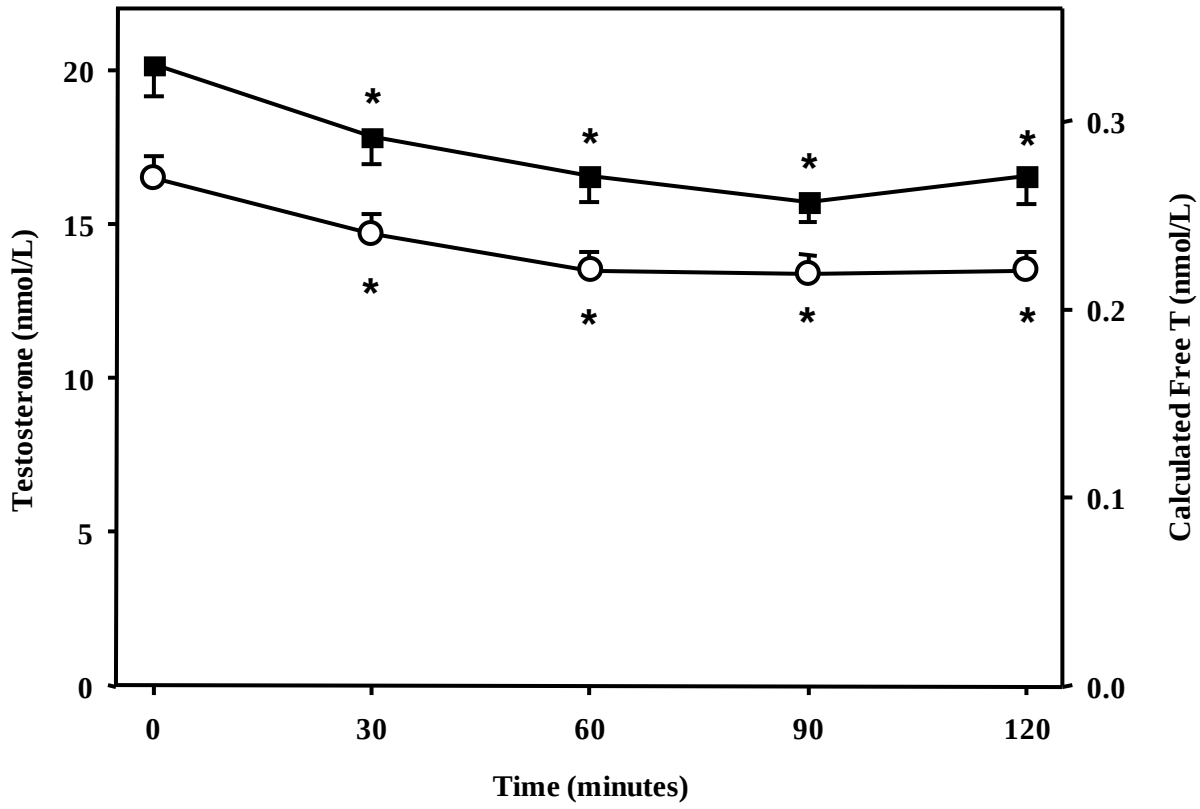

Figure 2. Changes in serum testosterone levels in men with normal glucose tolerance ([circles], n= 42, p< 0.0001[‡]), impaired glucose tolerance ([squares], n= 22, p <0.01 [*]), and type 2 diabetes ([triangles], n= 10, p<0.005 [†]) in response to a 75g oral glucose load. Each time point represents the mean ± SEM for the group. P values indicate change from baseline, changes across groups were not significant.