Failure of Short-Term Hyperinsulinemia to Affect Testosterone Production Rates in Healthy Men

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Production rates for testosterone were determined in seven healthy men before and during a euglycemic clamp using a stable labeled tracer (1α,2α-D-testosterone 0.13 ± 0.04 mg/h) and analysis by gas chromatography–mass spectrometry (GC-MS). Following an equilibration period of 12 hours (8 PM to 8 AM), blood samples obtained at 20-minute intervals from 8 AM until 2 PM were subsequently pooled and evaluated for three 2-hour periods. Following determination of basal endogenous production (8 AM to 10 AM), the investigation was continued during two 2-hour periods of induced euglycemic hyperinsulinemia (10 AM to 2 PM). Production rates for testosterone (basal, 245 ± 91 μg/h) remained unchanged during the first (234 ± 87 μg/h) and second (207 ± 94 μg/h) period of this euglycemic clamp. These results suggest that short-term hyperinsulinemia has no effect on endogenous production rates of testosterone in healthy men.

HYPERINSULINEMIA is common in hyperandrogenic women, but a definite cause-effect relationship between insulin resistance and hyperandrogenism remains to be established.[1,2] Several, albeit not all, studies[1] on the effect of sex steroids on insulin sensitivity have suggested that hyperandrogenism in women may induce insulin resistance. Insulin itself directly or indirectly influences plasma concentrations of various steroid hormones,[3-5] although it is unknown whether it has any direct influence on the production of testosterone in males. Since the stable-label isotope dilution technique and gas chromatography–mass spectrometry (GC-MS) offer a tool to directly and accurately evaluate production rates of steroid hormones in vivo,[6-8] we used this approach to study the effect of induced hyperinsulinemia on the production rate of testosterone in healthy men.

SUBJECTS AND METHODS

Experimental Protocol

Seven healthy, non-obese men aged 23 to 26 years who had been carefully informed about the aims and possible risks of the study provided written consent to participate. On the day before the experiments, an indwelling catheter was inserted into an antecubital vein at 8 PM and 1α,2α-D-testosterone (in 500 mL 0.9% saline also containing 2 mL of the individual's own blood) was infused intravenously and continuously (21 mL/h, Infusomat; Braun, Melsungen, Germany) until the end of the investigation. At the beginning and end of each infusion, a sample of the infusate was obtained from the end of the infusion line to determine loss by adsorption. Actual infusion rates as determined retrospectively by GC-MS analysis were 130 ± 40 μg/h. After an equilibration period of 12 hours (ie, at 8 AM the following morning), the subjects assumed the supine position and a second indwelling catheter was placed in a retrograde direction in a wrist vein of the contralateral arm. This hand was kept in a heated box at 75°C to ensure arterialization of venous blood. Subsequently, blood samples were obtained at 20-minute intervals and pooled for three 2-hour periods (8 to 10 AM, 10 AM to 12 noon, and 12 noon to 2 PM). At 10 AM, a primed-continuous (1.0 mU/kg · min) infusion of biosynthetic human insulin (Actrapid HM; Novo Industri, Wien, Austria) was administered to acutely increase and maintain plasma insulin at approximately 60 μU/mL until the end of the experiment. Plasma glucose was maintained constant at the fasting level by determination of plasma glucose every 5 minutes (Glucostat II; Beckman, Fullerton, CA) and appropriate adjustment of the infusion rate of a 20% dextrose solution.

Materials

All organic solvents were of high-performance liquid chromatography–grade and were purchased from Baker Chemicals (Phillipsburg, NJ). Nonactive testosterone (17β-hydroxy-4-androsten-3-one) was obtained from Steraloids (Wilton, NH). Radioactive [^{3}H]1,2,6,7-testosterone (specific activity, 100 Ci/mmol) and stable-label 1,2-D-testosterone (isotopic enrichment, 99.0%) were purchased from New England Nuclear (Boston, MA) and CIL (Andover, MA), respectively. Plasma concentrations of insulin were determined by radioimmunoassay as described previously.[9]

Sample Preparation and Analysis by GC-MS

Plasma samples (5.0 mL) supplemented with 20,000 dpm ^{3}H-testosterone for later control of recovery and with 20 mL 0.5% trifluoroacetic acid (TFA) were applied to Sep-Pak C-18 cartridges (500 mg; Waters/Millipore, Milford, MA) pretreated with successive applications of 5.0 mL methanol, 5.0 mL ethyl acetate, 20 mL water, and 5.0 mL TFA (0.5% wt/vol). Following sample application, the cartridges were first treated three times with 5.0 mL TFA (0.5% wt/vol). Testosterone was subsequently eluted by ethyl acetate (2 × 1.0 mL), dried under a stream of nitrogen at 37°C, reconstituted in 100 μL CH_2Cl_2, and further prepurified by thin-layer chromatography (chloroform:acetone, 70:30). The zone containing testosterone was eluted (2 × 2.5 mL methanol) and supplemented with 10 ng dehydrotestosterone (1,4-androstadien-17β-ol-3-one) as an internal standard for GC-MS analysis. Derivatization was subsequently performed with heptafluorobutyric anhydride:acetone (1:4, t = 60 minutes) at room temperature. Recovery of ^{3}H-testosterone from the derivatized samples was 38.5% ± 5.0% (N = 40). Analysis by GC-MS (Finnigan MAT95 [Bremen, Germany] equipped with a 25-m CB5 fused-silica column) was then performed using selective ion monitoring and electric ionization (resolution, 6,000). The tracer ions were *m/e* 678 (dehydrotestosterone, internal standard), *m/e* 680 (native testosterone), and *m/e* 682 (1,2-D-testosterone). The sensitivity at a peak to noise ratio of 10:1 was less than 100 fg.

Calculation of Testosterone Production Rate

Production rates for testosterone (PR[T]) were calculated from the product of the known infusion rate (Rt) and the ratio of tracer infusate

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enrichment (Et) to tracer dilution in the plasma (Es): PR[T] = Rt × (Et/Es − 1).[10]

Statistics

Results are expressed as the mean ± SD. Student's *t* test for paired data was used for statistical evaluation.

RESULTS

Mean serum concentrations of insulin throughout the 6-hour observation period were 57 ± 15 μU/mL. Calculated production rates for testosterone in the seven men were 245 ± 91 μg/h during basal conditions, and remained unchanged during the first (234 ± 87 μg/h) and second (207 ± 94 μg/h) period of induced hyperinsulinemia.

DISCUSSION

The traditional view that sex steroids increase insulin resistance[1,2,11] has recently been extended by evidence that insulin may induce hyperandrogenemia. The decrease in plasma testosterone concentrations observed in normal and obese men following treatment with diazoxide has led to the conclusion that insulin stimulates testosterone production in vivo.[4] Insulin-sensitizing drugs have been used clinically in hyperandrogenemic women.[12,13] On the other hand, an increase in the molar ratio of 17α-hydroxyprogesterone/androstenedione during exogenous insulin administration may indicate that insulin selectively inhibits adrenal 17,20-lyase in men[3] and women.[5]

In the present report, we have directly evaluated the effect of induced hyperinsulinemia on testosterone production rates in healthy men using the stable-label isotope dilution technique and GC-MC analysis. Since both labeled and endogenous materials are simultaneously analyzed using identical technology, incomplete recovery of the tracer or tracee from biological materials does not present a problem.[6-8] Furthermore, unlike radioactive tracers, stable-label materials may be infused long enough to achieve steady-state conditions. Following 6 hours of preequilibration, we studied testosterone production rates during another 6-hour period. Since the insulin-free period always preceded the period of hyperinsulinemia, it may be argued that the diurnal rhythmicity[14,15] of testosterone production could have influenced the outcome of our study, since a physiological decline in testosterone production might have been counteracted by a potential effect of hyperinsulinemia. The design of this study does not permit us to rule out this possibility with certainty. However, using a similar experimental protocol and identical technology, we have recently found only minor diurnal changes in testosterone production rates in healthy young men,[16] implying that a potential effect of insulin would have been of a similar minor magnitude. Thus, the obtained results exclude a major impact of induced hyperinsulinemia on testosterone production in healthy men, providing supplementory evidence to data reported by others who had to base their conclusions on this hitherto unproven assumption.[3,5] This does not exclude the possibility that prolonged endogenous hyperinsulinemia as seen in patients with obesity may influence the production of testosterone. Furthermore, whether results obtained in healthy men might also apply to healthy women remains to be determined. In hyperandrogenemic women, where insulin-sensitizing agents such as metformin[12,13,17] have been used to ameliorate hyperandrogenism, this would be of practical clinical relevance.

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