Relation of Steroid Hormones to Glucose Tolerance and Plasma Insulin Levels in Men

Importance of visceral adipose tissue

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OBJECTIVE — Low plasma testosterone levels are associated with hyperinsulinemia and glucose intolerance in men. However, it is unclear whether these abnormalities are related to the concomitant alteration in regional adipose tissue (AT) accumulation associated with reduced androgen levels.

RESEARCH DESIGN AND METHODS — We measured plasma steroid levels in a sample of 79 men, ranging from lean to obese (aged 29–42 years), for whom an oral glucose tolerance test (OGTT), anthropometric and computed tomography (CT) measurements of body fatness, and AT distribution were performed. Sex hormone binding globulin (SHBG) and the following steroids were measured after extraction from plasma and chromatography: dehydroepiandrosterone, androstenedione, androst-5-ene-3β,17β-diol, testosterone, estrone, and estradiol (E_2).

RESULTS — Several significant negative correlations were found between adrenal C_{19} steroid precursors, testosterone, SHBG, and fasting insulin levels, as well as between plasma glucose and insulin concentrations measured during the OGTT ($-0.25 \leq r \leq -0.35$, $0.05 \geq P \geq 0.001$). The best steroid correlate of plasma glucose and insulin homeostasis indexes was the E_2:testosterone ratio ($0.34 \leq r \leq 0.42$, $0.005 \geq P \geq 0.001$). However, after correction of steroid levels for either fat mass, body mass index (BMI), or visceral AT area, as measured by CT, no significant residual associations were noted between testosterone, adrenal C_{19} steroid, SHBG, and estrogen levels and indexes of plasma glucose–insulin homeostasis, although the positive association between the E_2:testosterone ratio and glucose area remained significant after adjustment for total body fat mass and BMI. Furthermore, 15 pairs of obese subjects, matched for visceral AT area, showing either low or high levels of the steroids studied, did not differ in fasting insulin and postglucose plasma insulin levels or in glucose tolerance.

CONCLUSIONS — These results suggest that the previously reported relationships between androgen levels and indexes of plasma glucose–insulin homeostasis are mediated, to a large extent, by concomitant alterations in levels of total body fat and visceral AT in men.

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AT, adipose tissue; BMI, body mass index; CT, computed tomography; DHEA, dehydroepiandrosterone; Δ^5-DIOL, androst-5-ene-3β,17β-diol; Δ^4-DIONE, androstenedione; E_1, estrone; E_2, estradiol; OGTT, oral glucose tolerance test; SHBG, sex hormone binding globulin.

Relationships between obesity, regional distribution of body fat, and indexes of plasma glucose–insulin homeostasis have been widely studied, and many reports have shown that abdominal obesity, especially when high levels of visceral adipose tissue (AT) are present, is associated with insulin resistance, glucose intolerance, and hyperinsulinemia in both men and women (1–7). In this regard, Pouliot et al. (7) reported that obese men with high levels of visceral AT, as measured by computed tomography (CT), had higher plasma glucose and insulin responses during an oral glucose tolerance test (OGTT) than did men with low levels of visceral AT, despite the fact that both groups were closely matched for levels of total body fat.

On the other hand, both abdominal obesity and insulin resistance have been associated with alterations in steroid hormone levels. Indeed, in pre- and postmenopausal women, it has been shown that high androgen concentrations are associated with abdominal obesity, altered glucose tolerance, insulin resistance, and hyperinsulinemia (8–11). In men, however, the few reports available suggest that abdominal obesity and hyperinsulinemia may rather be associated with low androgen levels (12–16). Furthermore, it has been suggested that obese men show increased testosterone-to-estrogen conversion, presumably in AT (17). Accordingly, a higher estradiol (E_2):testosterone ratio has been associated with increased glucose and insulin areas during an OGTT in a sample of men, including myocardial infarction patients and control subjects (16).

Changes in sex hormone binding globulin (SHBG) levels have also been associated with alterations in insulin sensitivity in men and women. In pre- and postmenopausal women, low levels of SHBG have been associated with hyperinsulinemia and insulin resistance (18). In addition, prospective data in women have suggested that a low SHBG level is associated with an increased incidence of diabe-

tes (19). In men, a negative association has been noted between SHBG and insulin levels (13,21), whereas SHBG concentrations have been shown to be positively correlated with in vivo insulin sensitivity (20), although the relationship between androgens and SHBG levels remains unclear (22).

However, the independent contributions of visceral fat and steroid levels to the association between abdominal obesity and alterations in indexes of plasma glucose–insulin homeostasis have not been quantified. Results from Pasquali et al. (14) and Seidell et al. (23) suggested that, in men, the negative correlations between androgen levels and plasma insulin levels were independent from the pattern of fat distribution. To further examine this issue, we have assessed visceral AT by CT and have measured plasma steroid levels and indexes of plasma glucose–insulin homeostasis in a sample of 79 men.

RESEARCH DESIGN AND METHODS

The study sample, which included 79 men aged 29–42 years, was recruited by solicitation through the media in an attempt to cover a wide range of body fatness values (from lean to obese). All subjects underwent a complete physical examination and were required to be nonsmokers and healthy to be included in the study; those with diabetes, endocrinopathy, genetic dyslipidemias, or coronary heart disease were excluded. Subjects signed an informed consent document, and the study was approved by the medical ethics committee of Laval University.

Measurement of body fatness and AT distribution

Body density was measured by the hydrostatic weighing technique (24), in which pulmonary residual volume is measured by the helium dilution method before immersion in a hydrostatic tank (25). The mean of six body-density measurements was used, and fat mass was derived from body density using the equation of Siri (26).

Measurements of cross-sectional abdominal AT areas were performed by CT with a Siemens Somatom DHR scanner (Erlangen, Germany) according to previously described procedures (27,28). Briefly, subjects were examined in the supine position with both arms stretched above the head. The measurement was made between the L4 and L5 vertebrae while visceral AT area was obtained by drawing a line within the muscle wall surrounding the abdominal cavity using a graph pen and an attenuation range of −190 to −30 HU (29,30).

OGTT

An OGTT was performed the morning after an overnight fast, as described previously (7). Briefly, blood samples were collected in vacutainer tubes containing EDTA and Trasylol (Miles, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, and 180 min after ingestion of 75 g glucose. Insulin levels were determined by radioimmunoassay using polyethylene glycol separation (31), while plasma glucose concentrations were measured enzymatically (32). The glucose and insulin total areas under the curves were determined using the trapezoid method.

Plasma steroid measurements

After a 12-h fast, blood samples were collected into vacutainer tubes to measure plasma levels of testosterone, androstenedione (Δ^4-DIONE), androst-5-ene-3β,17β-diol (Δ^5-DIOL), dehydroepiandrosterone (DHEA), estrone (E_1), and E_2. Plasma was obtained after centrifugation at 2,000*g* for 15 min, and then samples were frozen at −80°C until steroid measurements were performed. Steroids were extracted from plasma with ethanol, as previously described (33), and then centrifuged at 2,200*g* for 15 min. The resulting pellet was resuspended in ethanol before recentrifugation. The two extracts were then combined, and ethanol was evaporated under nitrogen. The residue was suspended in water:methanol (95:5) and chromatographed on C_{18} columns (Amersham, Oakville, Ontario, Canada). Unconjugated steroids were isolated by elution with water:methanol (15:85). The organic solvent was evaporated from the fraction containing the steroids with a Speed Vac rotary concentrator (Savant, Armingdale, NY).

The aim of chromatography on LH-20 (34) was to separate unconjugated steroids. The unconjugated steroids were solubilized in 1 ml isooctane:toluene:methanol (90:5:5) and deposited on Sephadex LH-20 columns (Pharmacia, Uppsala, Sweden). Elution was performed by increasing the polarity of the organic solvent mixture, and four fractions were collected. After deposition of steroids, 15 ml of isooctane:toluene:methanol (90:5:5) was eluted and discarded. After addition of 20 ml of isooctane:toluene:methanol (90:5:5), Δ^4-DIONE and DHEA were collected. Isolation of testosterone was achieved by elution of another 20 ml of the solvent. Addition of 15 ml of the solvent mixture isooctane:toluene:methanol (70:15:15) caused the elution of Δ^5-DIOL and E_1. E_2 was obtained after elution with isooctane:toluene:methanol (60:20:20). Charcoal-treated plasma samples were used as blanks for the elution on LH-20, and background levels in these blanks were generally found to be lower than the limit of detection. Steroids were measured by radioimmunoassay as previously described (33,34). Intra- and interassay coefficients of variation for the various steroid measurements were always below 9 and 15%, respectively.

Statistical analysis

Pearson correlation coefficients were computed to quantify the relationships between fasting insulin levels, insulin and glucose areas measured during the OGTT versus steroids, SHBG levels, and body fatness and visceral AT areas measured by CT. The comparison of plasma insulin and glucose responses during the OGTT of subjects individually matched for

Table 1—*Morphological, anthropometric, metabolic, and hormonal characteristics of the sample of 79 men*

Variables	Mean ± SD	(Range)
Age (years)	36.4 ± 3.2	(29.7–41.8)
BMI (kg/m^2)	27.2 ± 3.8	(19.4–34.2)
Weight (kg)	82.1 ± 12.4	(59.5–108.1)
Fat mass (kg)	21.9 ± 7.9	(6.4–37.3)
Visceral AT area (cm^2)	122.6 ± 51.3	(28.0–253.0)
Fasting insulin (pmol/l)	80.5 ± 34.4	(26.0–224.5)
Fasting glucose (mmol/l)	5.2 ± 3.4	(3.4–8.3)
OGTT		
Glucose area ($\times 10^{-3}$ mmol/l^{-1} · min^{-1})	1.20 ± 0.29	(0.75–2.71)
Insulin area ($\times 10^{-3}$ pmol/l^{-1} · min^{-1})	73.70 ± 35.17	(20.37–175.62)
Plasma steroid levels		
Testosterone (nmol/l)	12.2 ± 3.2	(6.8–22.2)
DHEA (nmol/l)	13.2 ± 6.1	(4.5–33.3)
Δ^5-DIOL (nmol/l)	3.8 ± 1.3	(1.4–8.1)
Δ^4-DIONE (nmol/l)	1.7 ± 0.6	(0.9–3.5)
E_1 (pmol/l)	488.6 ± 120.9	(201.1–928.7)
E_2 (pmol/l)	142.0 ± 63.9	(61.0–528.5)
SHBG levels (nmol/l)	30.0 ± 10.8	(12.0–61.1)

Data are means ± SD.

plasma steroid levels, but having either high and low visceral AT areas, or of men matched for visceral fat deposition, but having either high or low steroid levels, was performed by the paired Student's *t* test procedure. Partial correlation and multiple regression analyses were performed to estimate the independent contributions of each variable to the variation of indexes of glucose–insulin homeostasis. Fasting insulin and insulin and glucose areas during OGTT were used as dependent variables, whereas steroid levels, SHBG concentrations, total body fat mass, and visceral AT area measured by CT were used as independent variables. All statistical analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS — Table 1 shows the morphological, anthropometric, and hormonal characteristics of the study group. The study sample included individuals ranging from lean to obese. Table 2 presents correlations between testosterone and adrenal C_{19} steroid concentrations (Δ^5-DIOL, Δ^4-DIONE, and DHEA) versus fasting plasma insulin levels and insulin and glucose areas measured during the OGTT. Fasting insulin levels were correlated negatively with Δ^5-DIOL and testosterone (−0.29 and −0.25; $P < 0.01$ and $P < 0.05$, respectively), whereas significant negative correlations were found between insulin area during the OGTT and DHEA, Δ^5-DIOL, and testosterone levels. However, after adjustment for either body fat mass, body mass index (BMI), or visceral AT area measured by CT, no significant correlations were noted between steroids and indexes of plasma glucose–insulin homeostasis, suggesting that these associations were largely mediated by concomitant variations in total fatness and abdominal AT deposition. Similarly, SHBG levels were negatively correlated with insulin and glucose areas, but these associations were no longer significant af-

Table 2—*Pearson correlation coefficients between plasma steroid and SHBG levels and indexes of plasma glucose–insulin homeostasis, before and after adjustments for fat mass, BMI, or visceral AT area*

		OGTT	
Independent variables	Fasting insulin	Insulin area	Glucose area
Δ^5-DIOL	−0.29†	−0.35‡	−0.35§
Adjusted for fat mass	−0.13	−0.14	−0.16
Adjusted for BMI	−0.05	−0.04	−0.11
Adjusted for visceral AT area	−0.07	−0.10	−0.15
Δ^4-DIONE	−0.02	−0.18	−0.15
Adjusted for fat mass	−0.11	−0.06	0.05
Adjusted for BMI	0.13	−0.04	−0.04
Adjusted for visceral AT area	0.15	−0.02	−0.02
DHEA	−0.14	−0.28†	−0.16
Adjusted for fat mass	0.01	−0.09	0.02
Adjusted for BMI	0.05	−0.04	0.04
Adjusted for visceral AT area	0.04	−0.07	0.02
Testosterone	−0.25*	−0.32‡	−0.31‡
Adjusted for fat mass	−0.12	−0.16	−0.18
Adjusted for BMI	−0.07	−0.11	−0.16
Adjusted for visceral AT area	−0.05	−0.10	−0.13
SHBG	−0.14	−0.29†	−0.28*
Adjusted for fat mass	−0.21	−0.15	−0.12
Adjusted for BMI	−0.15	−0.04	−0.11
Adjusted for visceral AT area	−0.16	−0.08	−0.14

* $P < 0.05$. † $P < 0.01$. ‡ $P < 0.005$. § $P < 0.001$.

Table 3—*Pearson correlation coefficients between plasma estrogen levels, the estrogen:androgen ratio, and indexes of plasma glucose–insulin homeostasis, before and after adjustments for fat mass, BMI, or visceral AT area*

Independent variables	Fasting insulin	OGTT	
		Insulin area	Glucose area
E_1	0.23*	0.26*	0.20
Adjusted for fat mass	0.14	0.13	0.08
Adjusted for BMI	0.08	0.06	0.03
Adjusted for visceral AT area	0.08	0.08	0.05
E_2	0.17	0.13	0.23*
Adjusted for fat mass	0.09	0.01	0.12
Adjusted for BMI	0.11	0.04	0.15
Adjusted for visceral AT area	0.10	0.04	0.15
E_2:T	0.34‡	0.33‡	0.42§
Adjusted for fat mass	0.22	0.14	0.27*
Adjusted for BMI	0.21	0.14	0.28*
Adjusted for visceral AT area	0.17	0.12	0.26

* $P < 0.05$. ‡ $P < 0.005$. § $P < 0.001$.

ter correction for either total body fat mass or visceral AT area. Finally, the concentration of Δ^4-DIONE was not significantly correlated with fasting insulin levels, insulin area, or glucose area.

The associations of E_1 levels and E_2 concentrations, as well as the E_2:testosterone ratio versus glucose and insulin homeostasis indexes, were examined (Table 3). Significant positive correlations were noted between E_1 levels and fasting insulin ($r = 0.23$, $P < 0.05$) and insulin area ($r = 0.26$, $P < 0.05$). The E_2:testosterone ratio was the best steroid correlate of indexes of plasma glucose and insulin homeostasis, showing significant positive correlations ($0.33 \leq r \leq 0.42$; $0.005 \geq P \geq 0.001$). Adjustment for total body fat mass, BMI, or visceral AT area eliminated most of the significant correlations. However, after adjustment of E_2:testosterone for fat mass or BMI, the correlations between this steroid ratio and glucose area remained significant.

We then examined the correlations between fat mass, visceral AT areas, and indexes of glucose-insulin homeostasis, before and after correction for concomitant variations in plasma steroid and SHBG levels. As shown in Table 4, the highly significant correlations between body fat mass, visceral AT area measured by CT, and plasma glucose and insulin levels were essentially unaffected by adjustment for steroid and SHBG concentrations.

In another attempt to examine the respective contribution of body fat distribution and steroids to the variation in indexes of plasma glucose–insulin homeostasis, subjects were matched either for levels of steroid hormones or for visceral AT areas, and responses of these subgroups to OGTT were compared. Figure 1 shows the results obtained when two subgroups of men individually matched for plasma testosterone levels, but with either high or low accumulation of visceral AT (Fig. 1*A*), were compared or when two subgroups of men individually matched for levels of visceral AT, but with either high or low levels of testosterone (Fig. 1*B*), were compared. No substantial differences in glucose and insulin areas were noted between men with low versus high testosterone levels when groups were matched for levels of visceral fat. However, despite a close pairing procedure for plasma testosterone levels, men with high levels of visceral AT showed greater insulin and glucose responses than did men with low levels of

Table 4—*Correlation coefficients between body fat (fat mass), visceral AT area, and indexes of plasma glucose–insulin homeostasis, before and after adjustments for plasma steroid and SHBG levels*

Independent variables	Fasting insulin	OGTT	
		Insulin area	Glucose area
Fat mass	0.44§	0.48§	0.44§
Adjusted for Δ^5-DIOL	0.26*	0.36*	0.31‡
Adjusted for Δ^4-DIONE	0.36§	0.46§	0.42§
Adjusted for DHEA	0.33‡	0.41§	0.41§
Adjusted for T	0.30*	0.40§	0.36‡
Adjusted for E_2:T	0.25*	0.39‡	0.30†
Adjusted for SHBG	0.27*	0.42§	0.36‡
Visceral AT area	0.53§	0.66§	0.53§
Adjusted for Δ^5-DIOL	0.49§	0.47§	0.43§
Adjusted for Δ^4-DIONE	0.58§	0.64§	0.51§
Adjusted for DHEA	0.54§	0.60§	0.51§
Adjusted for T	0.50§	0.58§	0.45§
Adjusted for E_2:T	0.47§	0.59§	0.42§
Adjusted for SHBG	0.49§	0.61§	0.46§

* $P < 0.05$. † $P < 0.01$. ‡ $P < 0.005$. § $P < 0.001$.

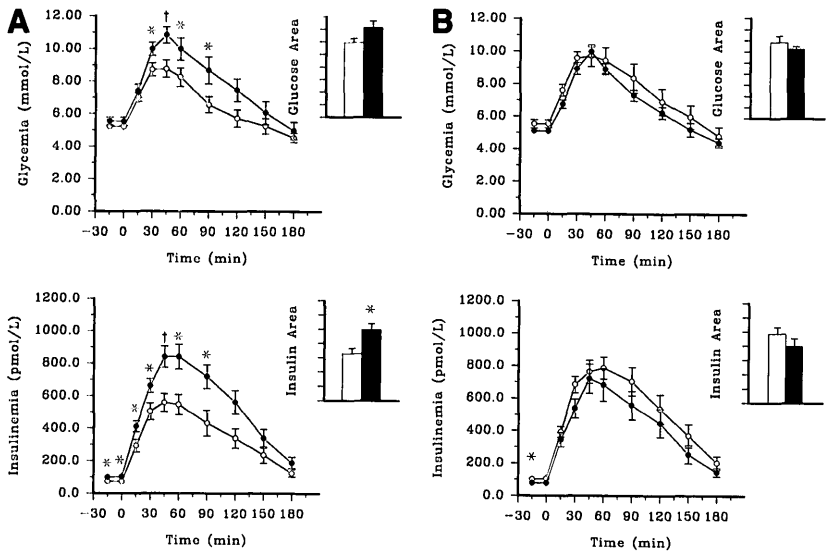

Figure 1—*Comparison of plasma glucose and insulin levels during a 75-g OGTT between* A: *two groups of 15 obese men individually matched for plasma testosterone concentrations (12.1 ± 0.9 nmol/l vs. 12.0 ± 0.9 nmol/l; NS) but showing either low (○) or high (●) visceral AT accumulation (104.3 ± 4.5 cm^2 vs. 188.1 ± 9.8 cm^2; P < 0.0001) and between* B: *two groups of 15 obese men individually matched for visceral AT area (156.1 ± 13.1 cm^2 vs. 148.5 ± 13.0 cm^2; NS) but showing either low or high plasma testosterone levels (8.9 ± 0.3 nmol/l vs. 14.0 ± 1.0 nmol/l; P < 0.0001). Bar charts represent glucose and insulin areas and are expressed as mmol · l^{-1} · min^{-1} and pmol · l^{-1} · min^{-1}, respectively.* *P < 0.05; †P < 0.01.

visceral fat. Figures 2 and 3 show the results obtained when similar matching procedures were performed for the adrenal C_{19} steroid Δ^5-DIOL and SHBG, further emphasizing the important role of visceral fat and the apparently trivial influence of Δ^5-DIOL or SHBG levels.

Finally, multivariate regression analyses were performed to evaluate the independent contributions of steroids, SHBG levels, E_2:testosterone ratio, body fatness, and AT distribution to the variance of plasma insulin and glucose levels. Table 5 indicates that variations in steroid and SHBG levels are unlikely to explain the insulin-resistant state observed in viscerally obese men, although E_2:testosterone contributed significantly to the variance of glucose area during the OGTT.

CONCLUSIONS — The present study was an attempt to better understand the associations between steroid hormones, plasma insulin, and glucose tolerance in men by adding an estimate of the contribution of the concomitant variation in the level of visceral AT. Plasma testosterone levels and Δ^5-DIOL showed the highest negative correlations with fasting insulin levels as well as with glucose and insulin areas measured during OGTT, suggesting that high circulating levels of these steroids in men were associated with improved insulin action. In addition, SHBG levels were negatively correlated with glucose and insulin areas measured during the OGTT. The negative correlations between testosterone, insulin levels, and the glycemic response to the glucose load were in agreement with results of Seidell et al. (13), Pasquali et al. (14), Simon et al. (15), and Phillips (16), who reported negative associations between androgenic steroid concentrations and plasma insulin levels and body fatness indexes in men. We also previously observed negative correlations between adiposity variables and plasma testosterone as well as adrenal C_{19} steroid levels in men (35). Since excess fatness, especially ab-

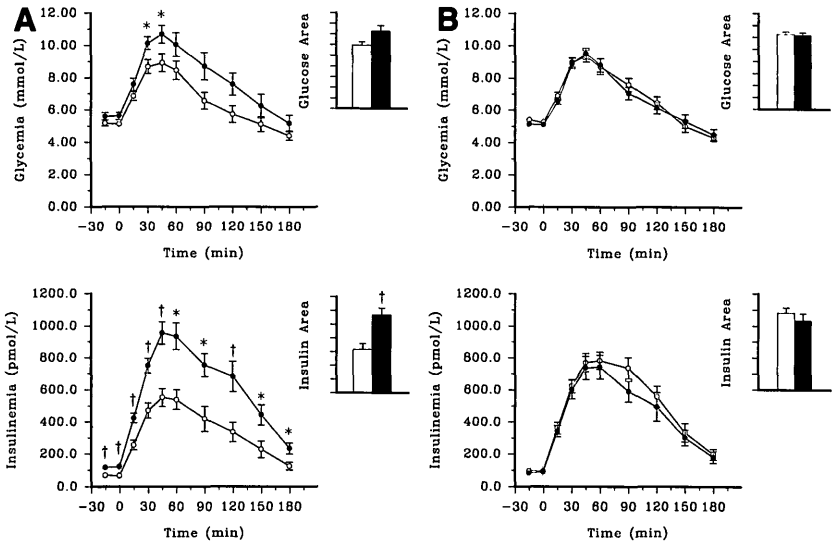

Figure 2—*Comparison of plasma glucose and insulin levels during a 75-g OGTT between* A: *two groups of 15 obese men individually matched for plasma Δ^5-DIOL concentrations (3.4 ± 0.2 nmol/l vs. 3.4 ± 0.2 nmol/l; NS) but showing either low (○) or high (●) visceral AT accumulation (103.5 ± 4.3 cm^2 vs. 203.3 ± 7.5 cm^2; P < 0.0001) and between* B: *two groups of 15 obese men individually matched for visceral AT area (141.5 ± 8.0 cm^2 vs. 147.1 ± 9.5 cm^2; NS) but showing either low or high plasma Δ^5-DIOL levels (2.4 ± 0.1 nmol/l vs. 4.2 ± 0.1 nmol/l; P < 0.0005). Bar charts represent glucose and insulin areas and are expressed as mmol · l^{-1} · min^{-1} and pmol · l^{-1} · min^{-1}, respectively.* *P < 0.05; †P < 0.01.

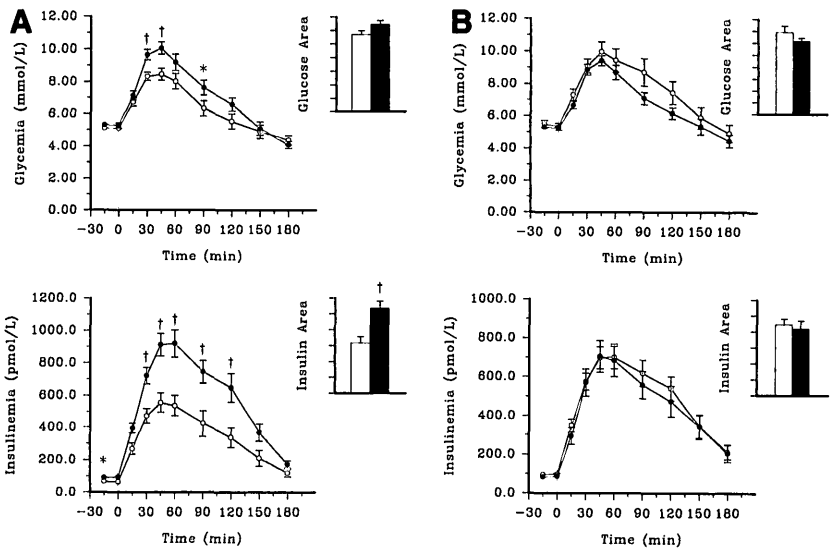

Figure 3—*Comparison of plasma glucose and insulin levels during a 75-g OGTT between* A: *two groups of 15 obese men individually matched for plasma SHBG concentrations (27.3 ± 2.5 nmol/l vs. 27.5 ± 2.4 nmol/l; NS) but showing either low (○) or high (●) visceral AT accumulation (104.7 ± 4.7 cm^2 vs. 175.7 ± 7.6 cm^2; P < 0.0001) and between* B: *two groups of 15 obese men individually matched for visceral AT area (147.4 ± 10.9 cm^2 vs. 146.6 ± 11.4 cm^2; NS) but showing either low or high plasma SHBG levels (18.4 ± 1.0 nmol/l vs. 35.7 ± 1.8 nmol/l; P < 0.0005). Bar charts represent glucose and insulin areas and are expressed as mmol · l^{-1} · min^{-1} and pmol · l^{-1} · min^{-1}, respectively. *P < 0.05; †P < 0.01.*

dominal obesity, generally has been associated with hyperinsulinemia and insulin resistance (1–7), the negative associations between androgenic steroids (such as testosterone), androgenic precursors (such as adrenal C_{19} steroids), and indexes of plasma glucose–insulin homeostasis were expected findings.

However, to the best of our knowledge, only one study has investigated the interrelationships among CT-measured visceral AT accumulation, steroid levels, and indexes of plasma glucose–insulin homeostasis in men, and these observations were obtained with a small sample of 23 subjects (13). It was found that men with relatively low testosterone levels had higher fasting plasma insulin levels and showed increased insulin secretion after an oral glucose load (13). Although visceral fat–adjusted relationships have not been extensively examined in this previous study, it was reported that free testosterone displayed negative relationships with concentrations of insulin and C-peptide that were independent of the amount of visceral fat. Results of the present study indicate that the amount of visceral fat seems to be the critical correlate of the disturbances in indexes of plasma glucose–insulin homeostasis associated with an altered plasma steroid profile. Indeed, after statistical control for concomitant differences in the amount of visceral fat, variations in the levels of steroid hormones were not related to glucose intolerance and hyperinsulinemia in this sample. Furthermore, among men matched for visceral AT areas, differences in steroid levels were not associated with differences in metabolic responses to the OGTT.

However, multiple regression analyses showed that the E_2:testosterone ratio accounted for an additional 7.4% of the variance of the glucose area. These results suggest that the balance between plasma E_2 and testosterone levels may, to some extent, be independently related to indexes of plasma glucose–insulin homeostasis. Accordingly, intervention studies from Mårin et al. (36,37) and Rebuffé-Scrive et al. (38) showed that testosterone treatment of men induced an increase in the lipolytic responsiveness of abdominal AT to norepinephrine, a decrease in abdominal AT lipoprotein lipase activity, a reduction in the level of abdominal fat, and some improvement in insulin sensitivity.

Peripheral intracrine conversion of steroids in AT could, at least in part, account for such associations. Indeed, a high deposition of visceral AT may lead to an increased conversion of adrenal C_{19} steroids to androgens or estrogens and, therefore, to reduced plasma levels of these precursors. In addition, as found in the present study, an excess accumulation of visceral AT may account for the impaired insulin action noted in men with low plasma testosterone levels (6,7).

Table 5—***Multivariate regression analyses showing the independent contributions of visceral fat (visceral AT area), plasma steroids (T, Δ^5-DIOL, Δ^4-DIONE, DHEA, E_1, E_2, and E_2:testosterone) and SHBG to the variation in fasting insulin as well as in integrated glucose (glucose area) and insulin (insulin area) levels measured during the OGTT***

Dependent variable	Independent variable	Partial (R^2 × 100)	Total (R^2 × 100)	$P \leq$
Fasting insulin	Visceral AT area	30.7	30.7	0.0001
Glucose area	Visceral AT area	27.1	34.5	0.0001
	E_2:T	7.4		0.005
Insulin area	Visceral AT area	43.4	43.4	0.0001

Many studies have provided evidence emphasizing the importance of peripheral conversion of steroids in AT. Indeed, Schneider et al. (17) suggested that the metabolism of androgens to estrogens is enhanced in obese men. We also have found positive correlations between E_2 and E_1 levels and body fatness indexes (35). Furthermore, it has been shown that messenger RNAs of the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase/$\Delta^5\Delta^4$isomerase and 17β-hydroxysteroid dehydrogenase are expressed in human AT (39). Other studies also suggested that AT stores and metabolizes steroids (40–42). Thus, it is likely that peripheral conversion of steroids in AT could explain the AT-dependent relationship between steroids and indexes of plasma glucose–insulin homeostasis found in this study.

On the other hand, there is increasing evidence suggesting that insulin could act as a modulator of ovarian and adrenal steroid hormone production. Studies in women with polycystic ovary syndrome, characterized by a hyperandrogenic state, insulin resistance, and hyperinsulinemia, provide indirect evidence supporting this model (43). Additional studies in men have also suggested that hyperinsulinemia could be the cause, rather than the result, of low levels of precursors of androgenic steroids, particularly DHEA, possibly through the inhibition of adrenal 17,20-lyase activity (43,44). Accordingly, studies from Nestler et al. (45) and Usiskin et al. (46) have shown that DHEA treatment had no short-term effect on body weight, fat mass, BMI, or waist-to-hip ratio in obese men and did not alter insulin sensitivity in normal men, suggesting further that insulin may be the primary modulator of androgen levels in men. Obviously, the design of the present study cannot address the issue of causality. Moreover, our data cannot exclude the possibility of a direct effect of insulin on the production of steroid hormones. However, in this study, the close association of visceral AT to indexes of plasma glucose–insulin homeostasis seemed to overwhelm the relationships between steroid hormones, glucose tolerance, and plasma insulin levels, thus suggesting that the association between androgenic steroid hormones and glucose-insulin metabolism is largely mediated by concomitant variation in visceral AT accumulation.

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