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Research Article

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Splanchnic Insulin Metabolism in Obesity

Influence of Body Fat Distribution

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Abstract

The effects of obesity and body fat distribution on splanchnic insulin metabolism and the relationship to peripheral insulin sensitivity were assessed in 6 nonobese and 16 obese premenopausal women.

When compared with the nonobese women, obese women had significantly greater prehepatic production and portal vein levels of insulin both basally and following glucose stimulation. This increase correlated with the degree of adiposity but not with waist-to-hip girth ratio (WHR). WHR, however, correlated inversely with the hepatic extraction fraction and directly with the posthepatic delivery of insulin. The latter correlated with the degree of peripheral insulinemia. The decline in hepatic insulin extraction with increasing WHR also correlated with the accompanying diminution in peripheral insulin sensitivity.

Increasing adiposity is thus associated with insulin hypersecretion. The pronounced hyperinsulinemia of upper body fat localization, however, is due to an additional defect in hepatic insulin extraction. This defect is closely allied with the decline in peripheral insulin sensitivity.

Introduction

Previous studies have shown that in healthy, premenopausal women, localization of fat in the upper body is associated with diminished glucose tolerance, insulin resistance and hyperinsulinemia. This association is independent of and additive to that due to obesity level (1–4). It has also been demonstrated that diminished skeletal muscle insulin sensitivity resulting in impaired glucose utilization contributes to the insulin resistance and the abnormal metabolic profile (5). This defect is largely due to a reduction in the number of insulin receptors although in some individuals, an additional postreceptor defect is also involved. This sequence could result from, or at least be exacerbated by, the persistently elevated plasma insulin levels characteristic of upper body obesity.

The mechanism of hyperinsulinemia in obesity remains controversial. Previous studies have suggested both an increase in pancreatic production (6–9) and/or a decrease in hepatic extraction of insulin (10–14) as the primary abnormality. A major difficulty in interpreting these studies is their reliance on methodologies, the validity of which is unproven (15). In addition, no information is currently available concerning the effects of body fat topography on splanchnic insulin metabolism. The present study was undertaken to unravel the pathophysiologic mechanisms responsible for the hyperinsulinemia of obesity and to establish whether the increase in plasma insulin levels with upper body fat localization is the result of an increase in pancreatic production, a decrease in the hepatic removal of insulin, or both.

Since insulin and its connecting peptide (c-peptide) are secreted from the pancreas on an equimolar basis (16) and since hepatic extraction of c-peptide in contrast to insulin is negligible (17, 18), measurement of peripheral c-peptide turnover estimates the prehepatic insulin production. Peripheral insulin turnover, on the other hand, estimates the total body flux excluding the amount retained by the liver during the first portal passage (posthepatic insulin delivery rate). The difference between prehepatic production and posthepatic delivery quantifies the hepatic insulin extraction. Turnover kinetics of plasma c-peptide and peripheral insulin and estimates of prehepatic production, posthepatic delivery rates, and hepatic extraction fraction of insulin were therefore determined in groups of normal weight and obese premenopausal women with varying body fat distribution patterns. The influence of obesity level, body fat distribution, and the degree of peripheral insulin sensitivity upon the kinetic parameters of splanchnic insulin metabolism were assessed.

Methods

Subjects

22 premenopausal Caucasian women were recruited for the study. Subjects were selected to be healthy, without significant history of hirsutism, major gynecological disorder, or clinical evidence suggestive of endocrine disorders, diabetes mellitus, hypertension, or heart disease. These disorders were excluded by a report from the subject's personal physician, thorough physical examination, electrocardiogram, and chest x-ray. Normal kidney, liver, and thyroid functions were ascertained by laboratory studies. Body weight was stable for at least 2 mo prior to the study. Subjects participating in dietary or exercise programs for weight reduction or taking any drug known to influence carbohydrate metabolism were excluded.

Obesity level was determined from body weight and height expressed as body mass index (BMI)[1] and from the percentage of ideal body weight (%IBW) estimated according to the 1983 Metropolitan Life Insurance Company tables. An estimate of the percentage of body fat was deter-

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1. *Abbreviations used in this paper:* BMI, body mass index; c-peptide, connecting peptide; HEF, hepatic extraction fraction; IBW, ideal body weight; MCR, metabolic clearance rate; M/I, metabolized glucose/insulin concentration; PostHDR, post-hepatic delivery rate; PreHPR, prehepatic production rate; WHR, waist-to-hip girth ratio.

mined from the sum of four skinfold thicknesses using the equations of Durnin and Womersley (19). Body fat distribution was assessed by measurements of the waist-to-hip girth ratio (WHR) as described previously (1–5). This index has been shown to correlate with more complicated procedures including multiple skinfold thickness measurements, arm-to-thigh adipose muscular ratio, and the intra- to extra-abdominal fat distribution determined by computed tomography (CT) scans, and is equally effective in predicting changes in glucose and insulin homeostasis.

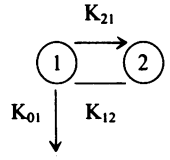
6 normal weight and 16 moderately obese women were admitted to the study. Obese women were subdivided to represent three age- and weight-matched subgroups: predominantly upper body obesity (WHR > 0.85), predominantly lower body obesity (WHR < 0.76), and an intermediate pattern of body fat distribution (WHR 0.76 to 0.85). We based this subdivision upon the fact that our previous research in a larger population demonstrated that the three subgroups exhibit significant trends of increasing plasma insulin and glucose levels (1–5). The clinical characteristics of the study groups are shown in Table I.

Studies were performed after admission to the Medical College of Wisconsin Clinical Research Center. A weight maintenance diet providing 30 kcal/kg per d (composed of 40% carbohydrate, 40% fat, and 20% protein), 300 mg cholesterol, and a polyunsaturated/saturated fat ratio of 0.3 was prescribed for 4–7 d prior to investigation. The study protocol was approved by the Clinical Research Center and the Medical College of Wisconsin's Human Research Review Committee. Informed consent was obtained from each subject prior to investigation.

Procedures: principle and rationale

MEASUREMENT OF SPLANCHNIC INSULIN METABOLISM. *C-peptide kinetics and prehepatic insulin production.* Because of the unavailability of c-peptide for human use, endogenous c-peptide secretion was stimulated in each individual to a new steady state during 150 min of hyperglycemia (100–125 mg/100 ml above basal) using the hyperglycemic clamp technique (20). Somatostatin (0.5 mg/h) was then infused to suppress pancreatic c-peptide secretion and plasma samples were collected at intervals for another 120 min during which plasma glucose concentration was maintained at the same level as prior to the somatostatin infusion. Plasma c-peptide levels were measured and the disappearance curve was fitted to a two-compartment model from which the equilibration kinetic constants and the fractional removal rate of c-peptide were determined.

Published studies in which synthetic c-peptide was given by pulse injection to either animals (21) or humans (22, 23) demonstrate that the disappearance of c-peptide from plasma follows a multiexponential decay and that a two-compartment mathematical model approximates the kinetic behavior of c-peptide metabolism. In this model, as depicted schematically below, we assumed that compartment 1 represents the c-peptide mass in the plasma and compartment 2 represents the c-peptide in all extravascular pool(s); k_{01} is the fractional removal rate or the irreversible loss from compartment 1, and k_{21} and k_{12} are the equilibration kinetic constants between the two compartments. This is just one of many models that may be appropriate for the analysis of c-peptide kinetics.



In the 6 normal weight and the 16 obese women studied, the plasma c-peptide data exhibited a biexponential decay and, thus, were consistent with the two-compartment model. Good agreement between the observed and model-generated data as well as a uniformly distributed residual error over the entire 120 min of observation were noted. The fitting criterion used was that of an iterative, nonlinear weighted least squares with weights defined as the fractional standard deviation of each datum. Compartmental analysis was performed using the SAAM (simulation, analysis and modeling) computer program (24). The intravascular space was considered to represent 30% of the total extracellular volume (25) and was calculated based upon reported measurement of plasma volumes in obese individuals as 4.1% of the ideal body weight plus 1.0% of excess body weight (26).

In all individuals, a quasisteady state (representing 4–10% of the initial plasma c-peptide concentration prior to the introduction of somatostatin) was observed at the tail portion of the c-peptide decay curve. Since previous studies (27, 28) have shown that a fraction of basal insulin production may remain uninhibited during somatostatin infusion, we assumed that this quasistate represents residual endogenous insulin secretion during the entire observation period. In the analysis procedure, a constant was, therefore, allowed to be fitted using the method of least squares and was subtracted from the compartmental data. The magnitude of the constant in the nonobese and the obese groups were 8±0.9% (range 5–10) and 7±0.7% (range 4–10) of the initial plasma c-peptide concentration, respectively ($P < 0.1$).

Steady state basal and intravenous glucose-stimulated prehepatic insulin production rates were determined from the subject's c-peptide fractional removal rate and from the mean plasma c-peptide levels obtained after an overnight fast and during the last 30 min of the hyperglycemic clamp prior to the introduction of somatostatin, respectively. In all subjects, an apparent steady-state of plasma c-peptide was achieved during these two phases. The mean coefficient of variation in plasma c-peptide concentration was 7±0.9% (range 2–10). The prehepatic production rate (PreHPR) thus was calculated as follows: PreHPR (mU/min per m^2) = [mean steady state arterial c-peptide concentration (ng/ml) × 0.045* × plasma volume (ml) × fractional removal rate of c-peptide (k_{01})]/surface area (m^2), where * is a conversion factor for c-peptide (ng/ml) to insulin (mU/ml) based upon a molecular weight of 3,300 for c-peptide and 6,000 for insulin and a relationship of 40 μg for each mU of insulin; estimated plasma volume equals 4.1% of ideal body weight plus 1.0% of excess body wt, and k_{01} is determined from the two-compartment model of c-peptide decay.

The nonsteady state prehepatic insulin production rate during oral glucose stimulation was determined from the variable plasma c-peptide levels at time intervals following glucose ingestion and the kinetic con-

Table I. Clinical Characteristics of Study Groups

	Number	Age	Weight	Relative weight		
				%IBW	BMI	%BF
		yr	*kg*		*kg/ht · m²*	
Nonobese	6	31.8±1.9	57.2±2.6	92.4±4.0	21.2±1.0	26.9±0.9
Obese	16	33.8±0.8	99.6±3.3	159.9±4.8	36.7±1.1	43.9±0.8
WHR < 0.76	7	31.7±1.2	98.8±4.4	158.2±6.9	36.4±1.6	43.2±0.9
WHR 0.76–0.85	3	35.0±1.0	92.9±10.4	152.8±11.2	35.3±2.0	43.2±3.3
WHR > 0.85	6	35.7±1.1	103.9±5.2	165.3±9.0	37.7±2.2	45.1±1.1

stants of c-peptide removal and equilibration (k_{01}, k_{21}, and k_{12}) determined in each individual as described above. If at time t_1 the intravascular and extravascular c-peptide pools are in equilibrium, then the mass of c-peptide in the extravascular compartment (Q_2) can be defined in terms of the plasma c-peptide mass (Q_1) from the relationship $k_{21}Q_1(t_1) = k_{12}Q_2(t_1)$. A spline fit was first used as the smooth analytic function approximating the discrete plasma c-peptide levels. An integro-differential solution was then used to calculate the c-peptide and, consequently, the prehepatic insulin production rate at each time interval. The mathematical derivations and the calculation procedures are similar to that described by Eaton et al. (23).

Basal, intravenous glucose and oral glucose-stimulated portal vein insulin concentrations were calculated from the corresponding prehepatic insulin production rate and the splanchnic plasma flow as follows: Portal vein insulin concentration (μU/ml) = Prehepatic insulin production rate (μU/min)/Splanchnic plasma flow (ml/min).

In calculating the turnover rate of c-peptide and consequently prehepatic insulin production rate, it is assumed that the metabolic clearance rate of c-peptide remains constant over the range of plasma levels measured. Evidence to support the validity of this assumption has been published (21, 22, 29). It is also assumed that somatostatin does not alter the c-peptide removal kinetics, an assumption that is also likely to be valid since c-peptide removal occurs primarily via the kidney and somatostatin does not alter renal plasma flow (28). Moreover, somatostatin does not influence the removal kinetics of intravenously administered human c-peptide (29).

Peripheral insulin metabolism and posthepatic delivery. Kinetics of peripheral insulin metabolism were assessed by measurement of the metabolic clearance rate (MCR) of exogenously administered insulin using the euglycemic insulin clamp as described previously (20). MCR was calculated according to the dilution principle as the ratio of the insulin infusion rate to the steady state arterial concentration. Since the contribution of endogenous insulin to the arterial steady state concentration could not be determined directly, this was corrected for by determining fasting plasma insulin and the ratio of steady-state to fasting c-peptide concentrations: MCR (ml/min per m^2) = IR (μU/min per m^2)/[IRIa − IRIe (μU/ml)], where IR = insulin infusion rate during the insulin euglycemic clamp; IRIa = arterial steady state total insulin concentration; and IRIe = arterial steady state endogenous insulin concentration determined from fasting arterial insulin concentration × (steady state/fasting arterial c-peptide).

Since the metabolic fate of exogenous and endogenous insulin is the same, the MCR of exogenous insulin should equal the clearance rate of endogenous insulin delivered into the systemic circulation from the hepatic veins or the plasma clearance of insulin by all body tissues, excluding the first portal passage. The steady state basal and intravenous glucose-stimulated posthepatic delivery rate (postHDR) were thus determined from the MCR, and the steady-state plasma insulin concentration attained after an overnight fast and after 150 min of sustained hyperglycemia induced by the hyperglycemic clamp, calculated as follows: PostHDR (mU/min per m^2) = mean steady state arterial plasma insulin concentration (mU/ml) × MCR (ml/min per m^2).

Published kinetic data (30) indicates that equilibration between the intra- and extravascular insulin compartments occurs at a rate of at least one order of magnitude slower than the rate of its removal and that plasma insulin level is influenced only to a minor degree by variations in the equilibration coefficients. Cumulative posthepatic insulin delivery during the time-course of the oral glucose tolerance test was, therefore, estimated by multiplying the insulin MCR by the plasma concentration curve integrated for the 300-min period of the test as described previously (28).

In calculating the posthepatic insulin delivery rate, insulin MCR was assumed to be constant over the range of plasma levels achieved, an assumption that has been validated by previous studies (28). To further ascertain the validity of this assumption, insulin MCR was measuring during euglycemic insulin clamps at increasing plasma insulin levels between 25 and 400 μU/ml. In four nonobese subjects the insulin MCR measured at 25 and 100 μU/ml were 614±27 and 511±57 ml/min per m^2 ($P > 0.05$). At comparable insulin levels, the MCR in four obese subjects were 462±75 and 511±94 ml/min per m^2 ($P > 0.05$), and in another four the MCR at plasma levels of 100 and 400 μU/ml were 488±28 and 501±7 ml/min per m^2, respectively ($P > 0.05$).

From the prehepatic production rate (PreHPR) and the posthepatic delivery rate (postHDR) determined in the same individual, the percentage hepatic extraction fraction (HEF) was estimated as follows: HEF (%) = PreHPR − PostHDR/PreHPR × 100.

MEASUREMENT OF PERIPHERAL INSULIN SENSITIVITY. Insulin-stimulated glucose disposal was determined using the euglycemic clamp procedure as described previously (20). A primed continuous infusion of insulin (40 mU/min per m^2) was administered while plasma glucose was maintained constant via a variable glucose infusion rate. The exogenous glucose infusion rate required to maintain the euglycemic state enabled the calculation of the total amount of glucose metabolized by the body (M). Because of differences in the MCR of insulin and, consequently, in the steady state insulin level achieved in each individual (I), insulin sensitivity was expressed as the ratio of the amount of glucose metabolized to the prevailing plasma insulin level (M/I). Since at the level of insulinemia achieved hepatic glucose production determined using a [^{3}H]glucose infusion accounted for only 8–17% of glucose utilization (unpublished results), its contribution to the total glucose metabolized was not considered in this calculation.

Experimental protocol: oral glucose tolerance tests

After an overnight fast, an oral glucose tolerance test was performed. Following a 30-min stabilization period, three basal blood samples were withdrawn. After ingestion of glucose (40 g/m^2 body surface area), samples were obtained at 15, 30, 60, 90, 120, 180, 240, and 300 min. Samples were analyzed for glucose, immunoreactive insulin, and immunoreactive c-peptide levels.

HYPERGLYCEMIC CLAMP. The principles and details of the hyperglycemic clamp have been described previously (20). At 0800, after an overnight fast, arterialized blood samples were obtained via a polyethylene catheter inserted retrogradely into an arm vein which kept at 66°C via a warming box. A second catheter for infusing test substances was inserted into an antecubital vein. After a 30–45-min stabilization period and collection of four basal samples, a primed continuous infusion of glucose was administered to acutely raise and maintain plasma glucose by 100–125 mg/100 ml above basal values. This was accomplished by infusing 20% glucose in water in two phases. A 13-min priming dose was first infused to raise the glucose level in the plasma and the extravascular glucose compartment to the desired plateau and then a maintenance dose was infused and computed at 5-min intervals throughout the study. The computation for the periodic adjustment in the glucose infusion is based on the negative feedback principle. During the last 30 min of the glucose infusion phase, blood samples were obtained at 5-min intervals for determination of glucose, insulin, and c-peptide concentrations. Somatostatin (Peninsula Laboratories, Inc., Belmont, CA) was prepared in sterile normal saline containing 1% human serum albumin and infused at a rate of 0.5 mg/h for an additional 120-min period. Plasma glucose was maintained at the presomatostatin level. Blood samples were obtained every 1–2 min for the first 30 min, every 5 min for the next 30 min, and then every 10 min for the remaining period. Plasma samples were analyzed for c-peptide levels.

EUGLYCEMIC CLAMP. The procedure for the euglycemic clamp is essentially similar to that described previously (20). Crystalline porcine insulin (Eli Lilly Co., Indianapolis, IN) was diluted in normal saline to a concentration of 300 mU/ml. 2 ml of the patient's blood per 50 ml of infusate was added. Three plasma samples were taken at 10-min intervals during a basal period following which a primed continuous infusion of insulin was given at the rate of 40 mU/min per m^2 for 120 min. Plasma glucose was maintained at the basal level by determining the plasma glucose concentration every 5 min and periodically adjusting the glucose infusion rate. Another three plasma samples were obtained during the last 30 min of the clamp procedure. Samples were analyzed for glucose, insulin, and c-peptide concentrations.

HEPATIC PLASMA FLOW. To account for the possible effects of intravenously or orally administered glucose on portal plasma flow and consequently on determination of prehepatic insulin production rate, an estimate of portal plasma flow was obtained by measurement of the systemic clearance of indocyanine green given by intravenous infusion during the basal state, the hyperglycemic clamp and the oral glucose tolerance test. Previous studies have shown that hepatic plasma flow assessed by this technique is comparable to that determined through hepatic vein catheterization procedures (31). The method assumes that indocyanine green extraction occurs predominantly in the liver. Indocyanine green was diluted in normal saline containing 5% human serum albumin and infused at a rate of 0.3 mg/min per m^2. The clearance rate was estimated from the infusion rate and the concentration of dye in the arterialized plasma.

MEASUREMENT OF SERUM INSULIN AND C-PEPTIDE. Radioimmunoassay for c-peptide was performed using an antibody specific for human c-peptide and ^{125}I-*n*-hydroxyphenyl-propionyl c-peptide tracer. Bound antigen was separated from free antigen by addition of polyethylene glycol solution. Reagents were obtained from Immunex, San Diego, CA. The lower limit of detection of c-peptide was 0.10 ng/ml and the assay recovery showed a coefficient of variation of 6.8% at c-peptide levels between 0.5 and 20 ng/ml. No crossreactivity was found with human insulin. Human proinsulin showed a maximum crossreactivity of 5% at levels > 10 ng/ml.

A solid-phase ^{125}I radioimmunoassay was used for quantitative measurement of serum insulin. Reagents were obtained from Diagnostic Products Corp., Los Angeles, CA. The assay could detect levels as low as 1 μU/ml. The coefficient of variation was 6.5% at insulin concentrations of 1–20 μU/ml and 3.7% at 100–300 μU/ml. A conventional data reduction program was used to calculate serum c-peptide and insulin concentrations.

Statistical methods

Values are presented as means±SEM. Comparisons between groups were made using the unpaired Student's *t* test. Correlation coefficients were determined by regression analysis.

Results

C-peptide kinetics and prehepatic insulin production. The kinetic parameters of c-peptide metabolism in nonobese and obese women are shown in Table II. Large individual variability was observed, fractional removal rate (k_{01}) ranging from 0.092 to 0.196 in the nonobese and from 0.133 to 0.285 min^{-1} in the obese subjects. No significant association was found between k_{01} and the steady state plasma c-peptide concentration preceding suppression of endogenous insulin secretion with somatostatin ($r = 0.29$, $P > 0.05$).

Large individual variability was also found in the equilibration coefficients k_{21} and k_{12}. The close agreement between k_{01} and k_{21} in all subjects, however, suggests that the processes involved in the irreversible removal of c-peptide from the plasma and its translocation to the extravascular space are similar. Moreover, the relative magnitude of k_{21} to k_{01} negates the utilization of a single compartment model for determination of the nonsteady state kinetics of c-peptide metabolism, thus emphasizing the importance of characterizing individual variability in all of the kinetic parameters in order to obtain a precise estimate of plasma c-peptide flux.

The mean fractional removal rate in the obese group (0.188±0.013) was not significantly different from that of the nonobese subjects (0.153±0.014). Furthermore, within the obese group there was no significant correlation between WHR and k_{01} ($r = 0.08$, $P > 0.05$) or any significant difference in the mean k_{01} value between the upper and lower body obese women (0.205±0.020 vs. 0.188±0.013 min^{-1}, $P > 0.05$).

Table II. Plasma C-Peptide Kinetic Parameters in Nonobese and Obese Women

Subject No.	Kinetic constants (min^{-1})* k_{01}	k_{21}	k_{12}
Nonobese			
1	0.164	0.087	0.037
2	0.168	0.216	0.097
3	0.145	0.167	0.072
4	0.151	0.146	0.062
5	0.196	0.203	0.100
6	0.092	0.113	0.048
Mean±SEM	0.153±0.014	0.155±0.020	0.069±0.010
Obese			
WHR < 0.76			
7	0.118	0.089	0.038
8	0.125	0.114	0.049
9	0.285	0.210	0.090
10	0.204	0.216	0.092
11	0.213	0.218	0.093
12	0.155	0.140	0.060
13	0.160	0.184	0.079
WHR 0.76–0.85			
14	0.134	0.100	0.045
15	0.211	0.093	0.040
16	0.169	0.204	0.088
WHR > 0.85			
17	0.167	0.203	0.100
18	0.143	0.131	0.056
19	0.216	0.124	0.052
20	0.235	0.141	0.061
21	0.281	0.216	0.091
22	0.185	0.167	0.071
Mean±SEM	0.188±0.013	0.159±0.048	0.069±0.005

* Determined from the kinetic data of plasma c-peptide decay following inhibition of endogenous secretion by infusion of somatostatin during the hyperglycemic clamp. Compartmental analysis was performed using the SAAM Program and a 2-compartment model as depicted in Fig. 1. The fitting criterion used was that of an iterative, nonlinear weighted least squares as described in Methods.

Basal steady state prehepatic insulin production rates and portal vein insulin levels after an overnight fast are shown in Table III. In the nonobese subjects, fasting plasma c-peptide levels averaged 0.86±0.10 ng/ml with an estimated basal prehepatic insulin production of 8.9±1.4 mU/min per m^2. In the obese subjects, the mean basal c-peptide level (2.4±0.3 ng/ml) was significantly greater than in the nonobese, and the prehepatic insulin production (35.4±5.6 mU/min/m^2) was also significantly greater in the obese. Basal splanchnic plasma flow averaged 438±18.0 ml/min in the nonobese and 741±55.0 ml/min in the obese subjects ($P < 0.01$). Portal vein plasma insulin concentration therefore was three to fourfold higher in the obese group.

Table IV shows the steady state prehepatic insulin production rate and portal vein insulin concentration attained in response to the intravenous administration of glucose during the hyperglycemic clamp. In nonobese subjects, the plasma c-peptide in-

Table III. Steady State Plasma C-Peptide Concentration, Prehepatic Insulin Production and Portal Vein Insulin Concentration After Overnight Fast

	Plasma c-peptide concentration	Prehepatic insulin production	Portal vein insulin concentration
	ng/ml	*mU/min per m²*	*μU/ml*
Nonobese	0.86±0.1	8.9±1.4	33±5
Obese	2.4±0.3*	35.4±5.6*	97±16*
WHR < 0.76	2.1±0.3	39.7±9.4	110±28
WHR > 0.85	2.9±0.6	36.9±7.6	107±25

* $P < 0.05$ or less, compared with nonobese subjects.

creased to a steady state concentration of 6.1±0.9 ng/ml with an increase in prehepatic insulin production to a mean of 60.0±6.6 mU/min per m². The intravenous administration of glucose during the clamp procedure did not significantly influence splanchnic plasma flow. Portal vein insulin concentration in the nonobese group averaged 222±25 μU/ml. On the other hand, plasma c-peptide concentration (11.0±1.0 ng/ml), prehepatic insulin production (166.3±25.6 mU/min per m²) and portal vein insulin level (466±72 μU/ml) achieved during the steady state glucose-stimulated phase of the hyperglycemic clamp were significantly higher in the obese subjects.

Among the obese women, there was no significant association between the WHR and prehepatic insulin production rate in either the basal state ($r = 0.23$, $P > 0.05$) or in the glucose-stimulated phase of the hyperglycemic clamp ($r = 0.01$, $P > 0.05$). Furthermore, in the two age- and weight-matched subgroups of upper and lower body obese subjects, both basal and glucose-stimulated prehepatic insulin production and portal vein insulin concentration were increased to a comparable extent (Tables III and IV). Within the obese group, however, the degree of adiposity as assessed by percentage body fat correlated significantly with prehepatic insulin production rate basally ($r = 0.52$, $P < 0.05$) and during intravenous glucose stimulation ($r = 0.50$, $P < 0.05$).

Fig. 1 shows the mean plasma insulin and c-peptide levels during an oral glucose tolerance test in nonobese and obese individuals. The plasma c-peptide level was significantly higher in the obese individuals both before and after glucose loading. Basal and glucose-stimulated plasma insulin levels were also higher in the obese group.

Table IV. Steady-state Plasma C-Peptide Concentration, Prehepatic Insulin Production, and Portal Vein Insulin Concentration in Response to Intravenous Glucose Stimulation During the Hyperglycemic Clamp

	Plasma c-peptide concentration	Prehepatic insulin production	Portal vein insulin concentration
	ng/ml	*mU/min per m²*	*μU/ml*
Nonobese	6.1±0.9	60.0±6.6	222±25
Obese	11.0±1.0*	166.3±25.6*	466±72*
WHR < 0.76	9.8±1.2	184.7±49.9	508±135
WHR > 0.85	13.5±1.9	177.4±31.6	511±98

* $P < 0.05$ or less, compared with nonobese subjects.

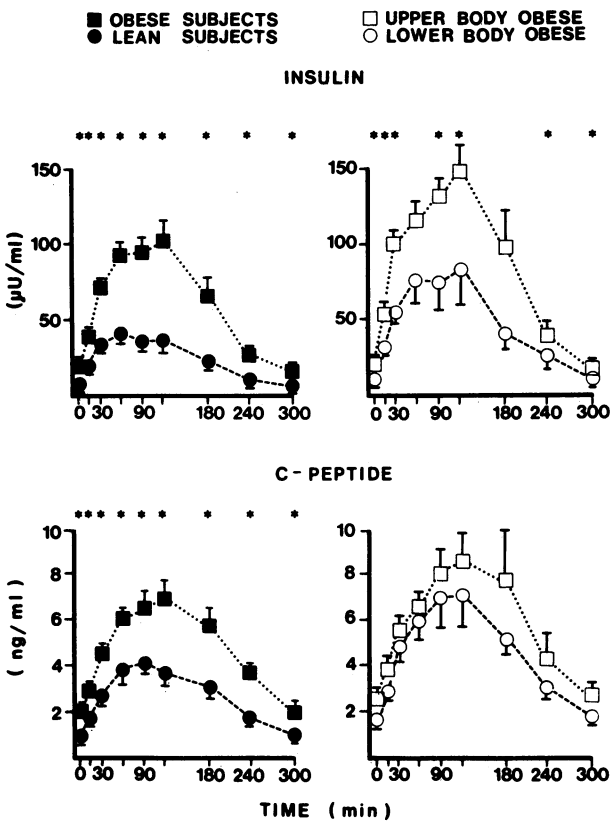


Figure 1. Plasma insulin and c-peptide concentration during oral glucose tolerance testing. (*Right panel*) Comparison between the nonobese ($n = 6$) and the obese ($n = 16$) groups. (*Left panel*) Comparison between the age- and weight-matched upper ($n = 6$) and lower ($n = 7$) body obese subgroups. * Statistically significant, $P < 0.05$ or less.

In obese women subdivided into age- and weight-matched subgroups of predominantly upper or lower body fat patterns, plasma c-peptide excursions during the oral glucose tolerance test were increased to a similar degree. Upper body obese women, on the other hand, displayed significantly greater insulin response at all time points during the oral glucose challenge.

Fig. 2 shows the time course changes in prehepatic insulin production in nonobese and obese women during the glucose tolerance test. At all time intervals, the prehepatic insulin production rate was significantly greater in the obese group. The cumulative insulin production during the 300-min period of the glucose tolerance test was 8.7±1.7 U/m² in the nonobese and 21.8±2.4 U/m² in the obese ($P < 0.01$). During the oral glucose loading, the splanchnic plasma flow increased above basal by 10–15% in both the nonobese and obese subjects. The calculated portal vein plasma insulin concentration during oral glucose stimulation was also significantly greater in the obese group.

As shown in Fig. 2, prehepatic insulin production and portal vein insulin levels were increased to the same extent in the two age- and weight-matched subgroups of upper or lower body obese women. The cumulative insulin production in the upper body obese (23.3±3.9 U/m²) was not significantly different from that of lower body obese subjects (19.7±3.7 U/m²). Portal vein insulin levels were also increased to the same extent (Table V). Furthermore, within the obese group, no significant correlation was observed between the WHR and the cumulative prehepatic insulin production ($r = 0.13$, $P > 0.05$) or the portal vein insulin concentration ($r = 0.14$, $P > 0.05$).

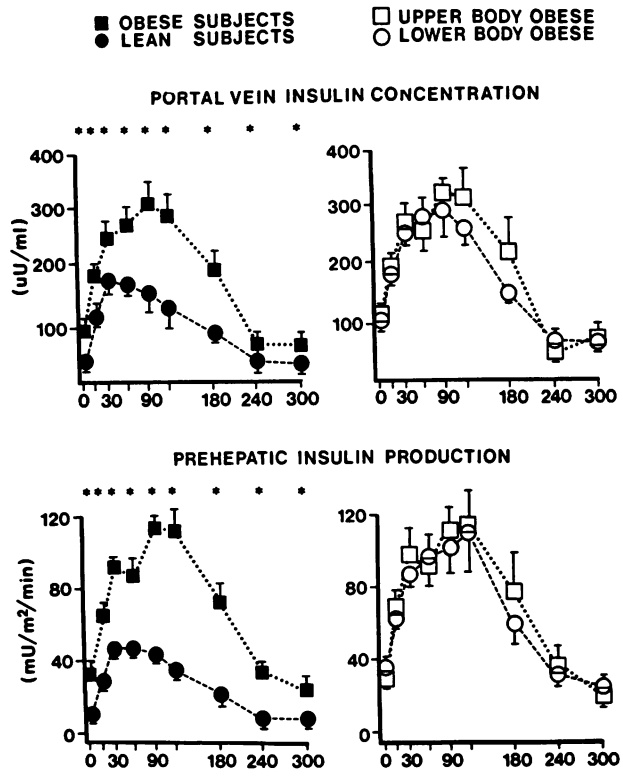


Figure 2. Prehepatic insulin production rate and portal vein insulin concentration during oral glucose testing. (*Right panel*) Comparison between the nonobese (n = 6) and the obese (n = 16) groups. (*Left panel*) Comparison between the age- and weight-matched upper (n = 6) and lower (n = 7) body obese subgroups. *Statistically significant, $P < 0.05$ or less.

Peripheral metabolism and posthepatic delivery of insulin. The metabolic clearance rate of exogenously administered insulin determined during the euglycemic clamp averaged 579±31 ml/min per m^2 in the nonobese subjects. In obese women, the MCR averaged 661±56 ml/min per m^2 and was not significantly different from that of the nonobese group.

Table VI shows the steady state basal and intravenous glucose-stimulated posthepatic insulin delivery and the corresponding peripheral plasma insulin levels in the nonobese and obese groups. In the nonobese subjects, the posthepatic insulin delivery after overnight fasting averaged 2.6±0.3 mU/min per m^2 and increased to a new steady state of 30.3±4.1 mU/min per m^2 with the increase in plasma glucose during the hyperglycemic clamp studies. In obese subjects, both the basal and the glucose-stimulated insulin delivery rates (8.6±1.0 and 104.6±16.1 mU/min per m^2, respectively) were significantly higher than in the nonobese individuals. In all subjects, peripheral plasma insulin levels correlated closely with the posthepatic delivery rate both basally (r = 0.85, $P < 0.01$) and during glucose stimulation (r = 0.93, $P < 0.01$). Fig. 3 shows that among obese subjects, both basal and glucose-stimulated posthepatic insulin delivery rates correlated significantly with WHR.

Table V. Cumulative‡ Plasma C-Peptide Concentration, Prehepatic Insulin Production, and Portal Vein Insulin Concentration During Oral Glucose Tolerance Stimulation

	Plasma c-peptide concentration	Prehepatic insulin production	Portal vein insulin concentration
	ng/ml × 10^{-3}	*U/300 min/m^2*	*μU/ml × 10^{-3}*
Nonobese	0.751±0.133	8.7±1.7	30.3±2.8
Obese	1.581±0.196*	21.8±2.4*	56.1±6.5*
WHR < 0.76	1.418±0.285	19.7±3.7	52.9±6.6
WHR > 0.85	1.730±0.340	23.3±3.9	59.1±12.3

* $P < 0.05$ or less, compared with nonobese subjects.
‡ Integrated for the 300-min values of the oral glucose tolerance test.

Cumulative posthepatic insulin delivery rate during the oral glucose tolerance test in obese subjects (11.3±1.5 U/m^2) was significantly greater than that of the nonobese individuals (3.1±0.16 U/m^2). The cumulative portal vein insulin concentration was also higher in the obese group. Significantly greater posthepatic delivery and peripheral plasma levels of insulin were observed in the upper body obese compared with age- and weight-matched lower body obese women. Furthermore, within the obese group, WHR correlated significantly with the cumulative insulin delivery rate (Fig. 4).

In nonobese women, the estimated hepatic insulin extraction fraction during the first portal passage averaged 70±3% after an overnight fast, and decreased to 49±4% with the rise in prehepatic insulin production during the steady state phase of insulinemia achieved by the hyperglycemic clamp. In the obese group, the hepatic insulin extraction fraction varied from 94 to 53% in the basal state and from 87 to 5% after glucose stimulation. As shown in Fig. 5, increasing WHR was associated with a progressive decline in the hepatic extraction fraction of insulin in both the basal and the intravenous glucose-stimulated states.

Cumulative hepatic insulin extraction during the oral glucose tolerance test averaged 59±6% in the nonobese group and 48±5% in the obese. Within the obese individuals, the cumulative extraction fraction varied from 72 to 12% and was inversely correlated with WHR (Fig. 3).

Compared with nonobese women, lower body obese subjects had a higher insulin extraction fraction in the basal state and a similar degree of extraction capacity during the intravenous glucose and the oral glucose stimulation of prehepatic insulin production. Upper body obese women, on the other hand, had a normal rate of extraction in the basal state but a remarkable reduction in hepatic insulin uptake during the hyperglycemic clamp and oral glucose ingestion (Fig. 5).

Relationship of peripheral insulin sensitivity to splanchnic insulin metabolism. Peripheral glucose metabolism (M) assessed during the euglycemic insulin clamp (40 mU/min per m^2) averaged 205±21 in the nonobese and 126±16 mg/min per m^2 in the obese women ($P < 0.01$). When corrected for the prevailing steady-state plasma insulin levels (M/I), the mean value for the nonobese was 2.86±0.4 and for the obese 1.62±0.3 mg/min/m^2 per μU per ml ($P < 0.05$).

Fig. 6 shows that among obese women, the degree of peripheral insulin sensitivity assessed from M/I was inversely correlated with WHR. Women with upper body obesity had the greatest decline in insulin-mediated glucose disposal. This decline was correlated with the reduction in hepatic extraction fraction and the rise in the posthepatic delivery of insulin during the glucose-stimulated phase of insulin secretion. No correlation was found between M/I and either the basal or the stimulated prehepatic insulin production rates (r = 0.024 and 0.03, respectively).

Table VI. Peripheral Insulin Metabolism in Nonobese and Obese Women: Basal, Intravenous Glucose, and Oral Glucose-stimulated Posthepatic Insulin Delivery Rates and Arterial Plasma Insulin Levels

	Posthepatic delivery rate			Arterial concentration		
	Basal	Intravenous glucose stimulated	Oral glucose§ stimulated	Basal	iv Glucose stimulated	Oral glucose§ stimulated
	mU/min per m²	*mU/min per m²*	*U/300 min per m²*	*μU/ml*	*μU/ml*	*μU/ml × 10⁻³*
Nonobese	2.6±0.3	30.3±4.1	3.07±0.16	5±0.6	56±8	4.84±0.56
Obese	8.6±1.0*	104.6±6.0*	11.3±1.5*	14±2.3*	186±40*	18.79±2.77*
WHR < 0.76	6.7±1.1	76.6±10.7	7.3±1.2	11±1.7	123±15	13.62±2.82
WHR > 0.85	11.6±1.8‡	160.4±27.8‡	16.6±2.1‡	20±4.2‡	306±30‡	25.25±5.25‡

* $P < 0.05$ or less, compared with nonobese subjects. ‡ $P < 0.05$ or less, compared to age- and weight-matched lower body obese subjects. § Integrated for the 300-min values of the oral glucose tolerance test.

Discussion

This study demonstrates that the degree of adiposity and the site of body fat localization could influence splanchnic insulin metabolism via two independent and possibly additive mechanisms. In premenopausal women, obesity thus was associated with increased pancreatic insulin secretion both in the basal state and following stimulation with intravenous or oral glucose loading, this association being related to the magnitude of adiposity but uninfluenced by the site of body fat predominance. Increasing localization of fat in the upper body, on the other hand, was accompanied by progressive diminution in the hepatic insulin extraction fraction and consequently increased posthepatic or systemic delivery of the hormone. Together with the increase in prehepatic insulin production, this defect accounted for the pronounced peripheral hyperinsulinemia characteristic of upper body obesity.

The presence of insulin hypersecretion in obesity receives credence from the postmortem demonstration of islet cell hyperplasia in obese subjects (6). Animal models of obesity and hyperinsulinemia have also shown beta cell hyperplasia and/or increased insulin content (32, 33). Several human studies have demonstrated higher c-peptide values in the basal state and following glucose stimulation (7–9). This difference persisted when the integrated area under the time curve of total daily secretion rates was assessed (11). More importantly, invasive studies in obese subjects have confirmed the presence of portal hyperinsulinemia both in the basal state and following stimulation with a variety of insulin secretogogues (34, 35).

To our knowledge, the present study is the first to utilize noninvasive techniques to determine total body c-peptide turnover and consequently quantify the prehepatic insulin production rate in nonobese and moderately obese women with varying body fat distribution patterns. The method accounted for the potential variability in the metabolic removal rate and the distribution kinetics of c-peptide in different subjects and between the groups being compared to each other. Prehepatic insulin production was determined during the steady states of basal and

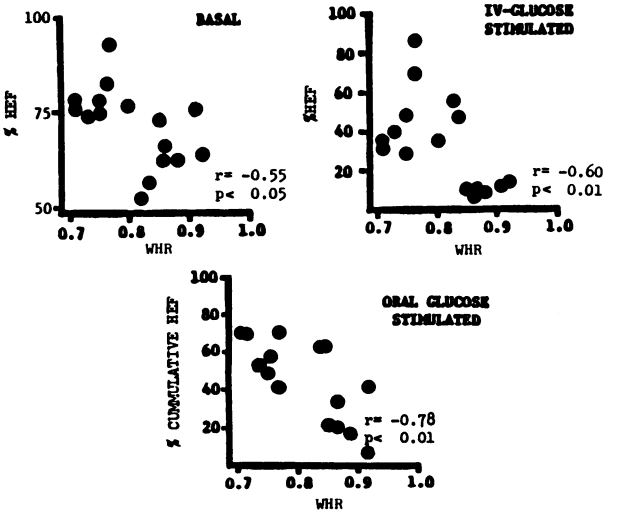


Figure 3. Relationship between WHR and the HEF of insulin during the basal state and following intravenous and oral glucose stimulation of insulin secretion in obese women.

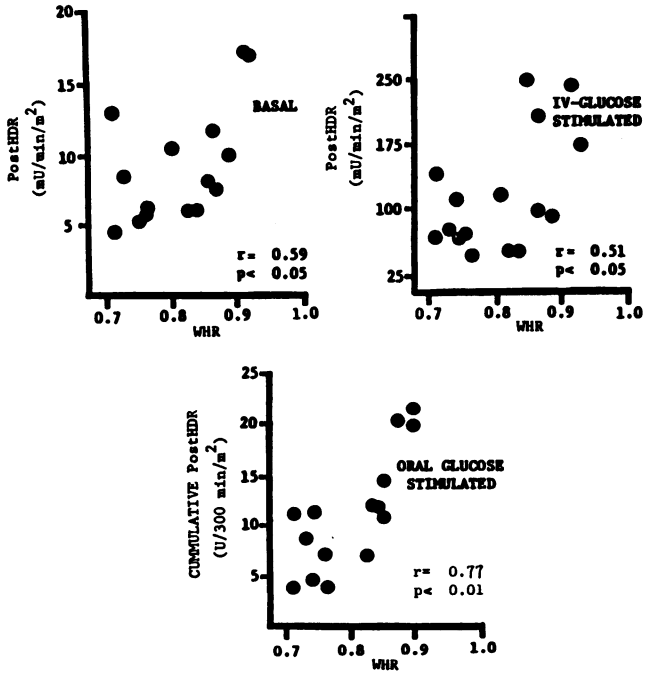


Figure 4. Relationship between WHR and posthepatic delivery rate (postHDR) of insulin during the basal state and following intravenous and oral glucose stimulation in obese women.

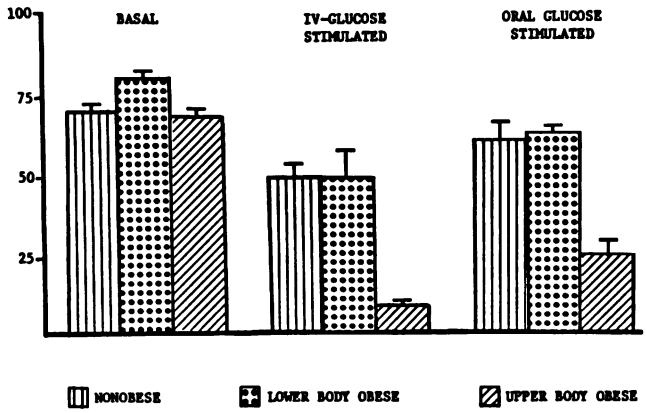


Figure 5. Mean hepatic insulin extraction fraction in the basal state and during intravenous and oral glucose administration to women with predominantly upper and lower body obesity compared to nonobese subjects.

intravenous glucose-stimulated insulin secretion induced by the hyperglycemic clamp technique. A compartmental mathematical model was also used to calculate the variable insulin secretion rate at various time intervals following an oral glucose load. Evidence to support the assumptions upon which this method is based have been published (16, 17, 21, 29) and the estimates of prehepatic insulin production and portal vein insulin concentration were concordant with those determined by portal vein and hepatic vein catheterization studies (36–39).

It is not known whether the pancreatic hypersecretion of insulin is a primary event or an adaptive response to the diminished insulin sensitivity of the obese state. Our results suggest that the increase in basal and glucose-stimulated insulin production was independent of the degree of peripheral insulin insensitivity, thus supporting the contention that the hypersecretion could be a primary defect. This conclusion is supported by several animal models of obesity. In the homozygous ob/ob mouse, marked islet cell hypertrophy in association with extremely high plasma insulin levels is seen with increasing adiposity while the blood sugar is normal and peripheral tissue responsivity to insulin is preserved and may, in fact, be increased (32, 33). Suppression of pancreatic islet hyperplasia with alloxan restores the in vivo insulin sensitivity in the obese mice (40). Similarly, hyperinsulinemia has been demonstrated in the obese Zucker rat prior to the onset of peripheral insulin resistance (41).

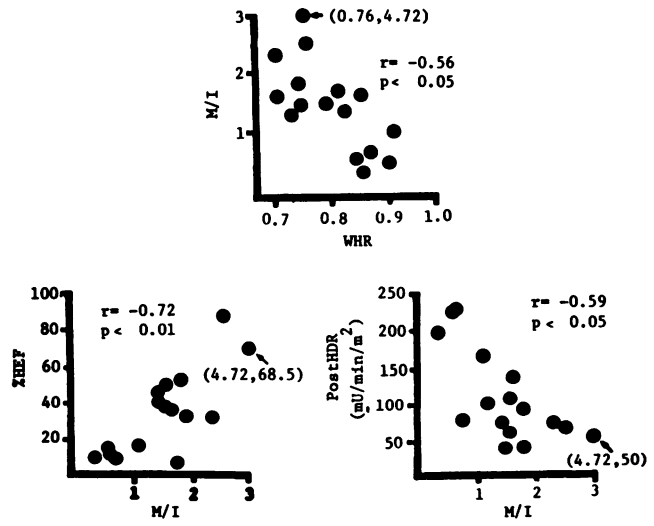


Figure 6. Relationship of the peripheral insulin sensitivity index, M/I (metabolized glucose in mg/min per m^2 per plasma insulin concentration in uU/ml) to the WHR, the HEF, and the posthepatic delivery rate (postHDR) of insulin during intravenous glucose stimulation in obese women.

Human studies provide additional evidence of an independent relationship between the increase in pancreatic insulin production and the diminution of insulin sensitivity in obese individuals. Bogardus et al. (42) reported no relationship between insulin-stimulated glucose metabolism and %IBW, whereas in the present study prehepatic insulin production rate correlated with the degree of adiposity. Furthermore, the improvement in insulin sensitivity after marked weight reduction achieved with gastric bypass surgery is associated with persistently elevated plasma c-peptide levels in both the basal state and during oral glucose stimulation (43).

It is well established that the liver is the major site of degradation of circulating insulin (44). A large body of in vitro and in vivo (45–48) work has confirmed the importance of this pathway and elucidated the kinetic and enzymatic characteristics of this function. Interposed between pancreatic insulin output and systemic circulation, the liver is exposed to higher insulin concentrations than any other organ. As a result, the liver operates as a prime regulator of peripheral insulin levels. Indeed, our results suggest that in normal weight healthy subjects, ~40–70% of the insulin secreted into the portal system is removed by the liver during the first passage. This is consistent with the estimated hepatic removal fraction determined by hepatic catheterization technique in humans (36, 49) and portal-hepatic vein concentration differences in animals (50).

Despite markedly enhanced prehepatic insulin production rate and increased portal vein insulin level in premenopausal women with lower body obesity, peripheral plasma insulin concentration was only moderately elevated, suggesting that the liver maintains its protective function. In fact, in some individuals, the hepatic insulin extraction exceeded 90% of the pancreatic production rate. Increasing localization of fat in the upper body as indexed by increasing WHR, however, was associated with progressive diminution in the hepatic extraction capacity and increasing posthepatic or systemic delivery of insulin. Consequently, increasing WHR was associated with increasing peripheral plasma insulin levels that correlated closely with the increase in posthepatic delivery of the hormone. Despite an equal degree of prehepatic insulin production and portal vein insulin levels, the upper body obese women thus maintained a higher peripheral plasma insulin than lower body obese subjects both basally and following stimulation with intravenous or oral glucose loading.

The association between diminished peripheral insulin sensitivity and increased posthepatic insulin delivery among obese women suggests several possible mechanisms. First, the decrease in hepatic extraction and resultant increase in posthepatic insulin delivery could initiate the decline in peripheral insulin sensitivity via a receptor or postreceptor mechanism (51, 52). Secondly, the defect in insulin sensitivity in the peripheral tissue was also expressed in the liver and resulted in diminished capacity of the liver to extract insulin from portal blood. This assumes that insulin action and degradation follow similar or related pathways that possibly involve insulin receptors (53, 54). Finally, the cor-

relation between peripheral insulin sensitivity and hepatic insulin extraction might be mediated by a third variable that could independently influence the hepatic insulin metabolism and peripheral insulin actions. Previous studies have demonstrated that upper body obese premenopausal women exhibit an increase in androgenic to estrogenic activity correlating with the degree of impairment in insulin-mediated glucose disposal and the elevation in plasma insulin levels (3). Our preliminary studies also indicate a close correlation between the degree of androgenicity and the decline in hepatic insulin extraction in premenopausal women (55). Whether this increased androgenicity could affect hepatic insulin extraction capacity directly or via deposition of highly lipolytic adipocytes intraabdominally and, in turn, the increased hepatic exposure to free fatty acids remains to be explored.

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