Relationship between Skeletal Muscle Insulin Resistance, Insulin-Mediated Glucose Disposal, and Insulin Binding

Effects of Obesity and Body Fat Topography

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Abstract. Skeletal muscle sensitivity and responsiveness to insulin and their relationship to overall glucose disposal and insulin binding were determined in 89 premenopausal women of varying body fat topography (waist/hips girth ratio [WHR] 0.64–1.02) and obesity level (percentage of ideal body weight 92–230). As a marker of insulin action, the percentage of total glycogen synthase present in the I form (glucose-6-phosphate independent) was measured in quadriceps muscle biopsies. The increase in percentage of synthase I 1 h after oral glucose loading was not significantly different between nonobese and obese weight-matched subgroups of increasing WHR, but this response was maintained at the expense of increasing plasma insulin levels as the WHR rose. The increase in percentage of synthase I in response to submaximal steady state plasma insulin (SSPI) of ~100 μU/ml achieved by the infusion of somatostatin, insulin, and glucose, however, was significantly lower in obese than in nonobese subjects, and was inversely correlated with WHR. The increase in percentage of synthase I correlated inversely with the steady state plasma glucose (SSPG) concentration, which is an index of the efficiency of overall glucose disposal, and directly with insulin binding to circulating monocytes. Insulin binding also correlated inversely with WHR and with fasting plasma insulin levels. When obese subjects were separated into three weight-matched subgroups on the basis of increasing WHR, significant trends to decreased percentage of synthase I response, increased SSPG, and decreased insulin binding were found. In women with predominantly upper body obesity (WHR > 0.85), the increase in percentage of synthase in response to submaximal SSPI was diminished, but there was no impairment of percentage of synthase I responsiveness to supramaximal SSPI of ~1,000 μU/ml. At supramaximal SSPI levels, SSPG in four obese women was normal, whereas in five women, SSPG concentrations were markedly increased. Our results suggest that in premenopausal women, impaired skeletal muscle insulin sensitivity that results in decreased glucose storage capacity may contribute to the diminished efficiency of glucose disposal and insulin resistance that are associated with upper body obesity. The impairment in skeletal muscle sensitivity may be overcome in vivo at the expense of increasing plasma insulin levels, with maximal responsiveness remaining unimpaired. This defect may result from a reduction in insulin receptor number which could, in turn, be secondary to persistently elevated fasting plasma insulin levels. In some upper body segment obese women, however, an additional defect affecting other insulin-sensitive pathways may also be present.

Introduction

In obese premenopausal women, increasing localization of fat in the upper body is associated with progressively diminished glucose tolerance, hyperinsulinemia, and insulin resistance (1–3). This association is independent of the degree of obesity, the effects of body fat distribution, and obesity level being additive. Upper body fat predominance is characterized by an increase in abdominal fat cell size, which correlates with the increase in postprandial plasma glucose and insulin levels (1). Increasing adipocyte volume is known to be associated with decreasing insulin effect on glucose metabolism, which is presumably due to a decrease in the number of insulin receptors and/or a postreceptor defect (4–6). Adipose tissue,

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however, accounts for a small fraction of glucose disposal in vivo (7), and hence impaired insulin action in this organ is unlikely to be directly responsible for glucose intolerance. Nevertheless, abdominal adipocytes exhibit a high rate of lipolysis (1, 8), which could increase free fatty acid release into the plasma and result in decreased glucose utilization by other tissues (9). Compensatory hyperinsulinemia might, in turn, exacerbate the insulin resistance by down-regulating the insulin receptor in major insulin target organs (6, 10).

During an oral glucose load, glucose uptake by skeletal muscle increases by 5–10-fold, which suggests that this tissue plays an important role in glucose disposal in vivo (11, 12). That skeletal muscle may contribute to the insulin resistance and diminished glucose tolerance that is associated with obesity is suggested by forearm perfusion studies that demonstrate decreased insulin-stimulated glucose uptake by the deep tissues of the obese forearm (13, 14). In obese rodents, both diminished sensitivity and impaired responsiveness of insulin-stimulated glucose uptake, glycogen synthesis, and glycolysis have been observed (15, 16). There have been no studies, however, which directly assess insulin action in skeletal muscle of obese humans. Nor is there any information concerning the effects of body fat distributioin on insulin action in this tissue.

In the present study, we determined in vivo insulin sensitivity and responsiveness of skeletal muscle, and their relationship to overall glucose disposal in premenopausal women of varying obesity level and varying body fat distribution. As an index of insulin action, we measured basal and insulin-stimulated activities of the glucose-6-phosphate (G6P)[1] independent form of glycogen synthase (uridine diphosphate glucose [UDPG] 1,4-glucan-4 glucosyl transferase, E.C. 2.4.1.11) in quadriceps' muscle biopsies. Insulin promotes the conversion of the G6P dependent form of this enzyme, inactive in vivo, to the active G6P independent form (I) (17). The efficiency of overall glucose disposal was determined from the steady state plasma glucose (SSPG) at comparable steady state plasma insulin (SSPI) levels that were attained by the infusion of somatostatin, insulin, and glucose (18). The importance of changes in insulin binding (IB) in relation to insulin action in skeletal muscle and glucose disposal was also evaluated.

Methods

Subjects. 89 premenopausal Caucasian women were studied. All were in good health and had volunteered for a study of body fat topography in relation to metabolic aberrations, details of which have been described previously (1–3). A report from each subject's personal physician confirmed that none were known to have diabetes mellitus or other endocrine disorder, hypertension or clinical evidence of heart disease. Fasting plasma glucose was <110 mg/dl in all subjects and kidney, liver, and thyroid function tests were within the normal range. Chest X-ray and electrocardiogram were also normal. Body weight was stable for 2 mo before the study and no subject was participating in a dietary or exercise program or taking any drug, including oral contraceptives, which were known to affect carbohydrate metabolism. Ideal body weight for each subject was estimated from the midpoint for medium frame using the tables of the Metropolitan Life Insurance Co. (1959). In 45 subjects, percentage of body fat was determined from the sum of four subcutaneous skinfold thicknesses using the equations of Durnin and Womersley (19). Body fat topography was assessed by measurement of the waist/hips girth ratio (WHR), minimal waist, and maximal hips girth; these were measured in a standing position. This ratio is as effective in predicting metabolic aberrations as other, more complicated procedures which include multiple skinfold thicknesses, the diabetogenic fat mass index, and the arm: thigh adipose muscular ratio determined by computed tomography scans (20). Subjects included nonobese (percentage of ideal body weight [% IBW] < 120) and obese (% IBW > 130) groups. The latter were selected to include three subgroups of increasing WHR: <0.76, 0.76–0.85, and >0.85. Their clinical characteristics are shown in Table I. Within the obese subgroup of varying body fat distribution, there were no significant differences with respect to age, body weight, percentage of IBW or percentage of body fat. In the group as a whole, percentage of IBW and percentage of body fat were highly correlated ($r = 0.90$, $P < 0.001$).

Procedures. Before investigation, subjects were admitted to the Clinical Research Center and stabilized for 3–5 d on a weight maintenance diet that contained 40% carbohydrate, 40% fat, and 20% protein. Approval of the Human Research Review Committee of the Medical College of Wisconsin and the informed consent of each subject were obtained before the study.

Muscle biopsies were obtained in 38 subjects at 0800 h following an overnight fast and bed rest. After 30 min, an oral glucose load of 40 g/m^2 surface area was administered and a second biopsy was obtained 60 min later. This time has been found to result in maximal stimulation of percentage of glycogen synthase I (GSI) after glucose ingestion in normal subjects (21, 22). To investigate this issue further, time-course studies were performed in 17 women (six nonobese, five obese with WHR < 0.76, and six obese with WHR > 0.85). Serial muscle biopsies were obtained basally and at 30, 60, 90, and 120 min after an oral glucose load. Blood samples for determination of plasma insulin were taken before each biopsy. Muscle biopsies were obtained in another 24 subjects before and 60 min after attaining SSPI and glucose levels by the continuous intravenous infusion of somatostatin

Table I. Clinical Characteristics of the Study Groups

	Number	Age	Body weight	Body weight	Body fat*
		yr	*kg*	*% ideal*	*%*
Nonobese	29	29±1.2	59±1.2	105±1.5	24.5±1.2
Obese					
WHR < 0.76	13	34±1.9	98±2.3	174±3.9	37.4±0.8
WHR 0.76–0.85	17	34±1.8	102±3.8	179±5.0	39.1±0.6
WHR > 0.85	30	33±1.1	102±2.2	180±3.4	38.7±0.7

* Estimated from the sum of four skinfold thicknesses (19).

1. *Abbreviations used in this paper:* FPI, fasting plasma insulin; G6P, glucose-6-phosphate; GSI, glycogen synthase I; IB, insulin binding; % IBW, percentage of ideal body weight; SSPG, steady state plasma glucose; SSPI, steady state plasma insulin; UDPG, uridine diphosphate glucose; WHR, waist/hips girth ratio.

(500 μg/h), monocomponent porcine insulin (0.77 mU/kg body weight per min), and glucose (460 mg/min), according to the procedure of Nagulesparan et al. (18). This insulin dose was selected in order to achieve an insulin level that is submaximal for overall glucose disposal of ~100 μU/ml (23). In a previous report, time-course studies revealed that in both nonobese and obese women with varying body fat distribution, SSPG was achieved within 90 min from the beginning of the infusion (3). Furthermore, in a preliminary study of four nonobese and six obese women, serial muscle biopsies that were obtained at 0, 30, 60, 90, 120, and 150 min showed that maximum activation of GSI was attained at 90 min and remained constant thereafter. Infusions were initiated 30 min after obtaining the basal muscle biopsy. After 90 min, four blood samples were withdrawn at 20-min intervals for determination of glucose and insulin, with the last sample being removed before the second muscle biopsy. 14 subjects underwent repeat studies in which the insulin dose was increased to achieve supramaximal plasma insulin levels of ~1,000 μU/ml. A similar procedure using somatostatin (500 μg/h), insulin (6.16 mU/kg body weight per min), and glucose (920 mg/min) was used. Since the amount of glucose that was infused to each individual was identical, the absolute removal rate of glucose during the steady state period must be similar and equal to the amount infused. An increase in SSPG level at comparable SSPI, however, would indicate a decline in fractional removal rate and hence diminished efficiency to dispose of glucose.

Muscle biopsies were obtained from the vastus lateralis portion of the quadriceps femoris muscle under 1% lidocaine local anesthesia, using the technique developed by Bergstrom (24). There were no complications from the biopsy procedure. Any fat or connective tissue that was present was removed, and the muscle immediately froze in liquid nitrogen. Samples were stored at −70°C until assay. There was no loss of activity in frozen muscle biopsies that were stored for up to 6 mo. In preliminary studies, addition of lidocaine to skeletal muscle homogenate had no effect on total glycogen synthase or percentage of synthase I activities, which agreed with the findings of Nuttall et al. (21). Also, administration of somatostatin to rats did not affect the in vivo rise in skeletal muscle GSI in response to insulin. Furthermore, in five subjects studied by the euglycemic clamp (25), the increment of percent GSI in response to an insulin level of ~100 μU/ml with and without the simultaneous infusion of 500 μg/h of somatostatin was not significantly different (7.8±0.6 vs. 8.1±0.7%, $P > 0.05$).

The tissue was homogenized on ice in buffer (1:16, wt/vol) that contained 5 mM EDTA, 40 mM KF, and 50 mM Tris in glass homogenizers. The homogenate was then centrifuged at 10,000 *g* for 10 min at 4°C. Total glycogen synthase (expressed as nanomoles of ^{14}C-UDPG incorporated into glycogen per minute per milligram weight and per milligram of protein) and synthase I (expressed as a percentage of total synthase) activities were measured in the supernatant by the method of Thomas et al. (26). Soluble glycogen and protein were also measured in the supernatant (27, 28).

Insulin binding to circulating monocytes was determined in 37 subjects including 20 subjects who also participated in the somatostatin-insulin-glucose infusion protocol. Approximately 100 ml of blood was withdrawn in heparinized syringes after an overnight fast. Blood samples were immediately processed for the isolation of mononuclear cells using a ficoll-hypaque density gradient (29). Insulin binding to circulating monocytes was then determined using the method of Bar et al. (30). Specific binding of ^{125}I-insulin was determined by incubation of 5×10^6 monocytes/ml with 0.15 ng/ml of ^{125}I-labeled insulin in Hepes buffer (pH 8.0) for 100 min in the absence or presence of unlabeled porcine insulin (0.15–100,000 ng/ml) at 14°C. After incubation, cells were separated by centrifugation and the pellet counted. The percentage of monocytes was determined from latex bead ingestion. Cell viability as assessed by trypan blue staining was always >97%. Plasma samples for glucose were analyzed by a glucose oxidase method (31) and for insulin by a double-antibody radioimmunoassay (32).

Materials. Monocomponent porcine insulin for intravenous infusion was obtained from Novo Laboratories, Wilton, CT. Cyclic somatostain was obtained from Beckman Instruments, Inc., Palo Alto, CA. D-glucose-6-phosphate, EDTA, Tris, UDPG, and bovine liver glycogen were purchased from Sigma Chemical Co., St. Louis, MO. Uridine diphosphate glucose [glucose-^{14}C(U)] ~ 400 mCi/mmol and ^{125}I-insulin ~ 100 μCi/μg prepared by the method of Hunter and Greenwood (33) were purchased from New England Nuclear, Boston, MA. ^{125}I-insulin was purified on a Whatman cellulose column before use (34). Crystalline porcine insulin that was used for the IB studies was the kind gift of Eli Lilly & Co., Indianapolis, IN.

Statistics. Comparisons within groups were made using paired *t* test and between groups by unpaired *t* test. Analysis of variance with linear contrast model to test for trends was employed to determine significant differences between subject subgroups with increasing WHR. Simple and multiple regression and pathway analyses were performed to define the intercorrelation between the metabolic variable measured. Statistical analysis was performed using the Statistical Package for the Social Sciences Manual (35). Values presented are means±SEM.

Results

In vivo skeletal muscle insulin sensitivity. In nonobese women, total glycogen synthase activity in skeletal muscle biopsies that were obtained after an overnight fast was 13.1±0.8 nmol/mg protein per minute, of which 22±1.5% was in the I form (Table II). 1 h after oral glucose loading, percentage of synthase I increased significantly to 30.9±2.0. Total synthase activity in the obese group was 11.6±1.0 nmol/mg protein per minute, and synthase I increased from 22.7±1.8 to 30.0±2.2% after glucose administration. In both nonobese and obese groups, total synthase activity was unaltered by glucose loading. No significant difference in either basal or stimulated percentage of synthase I were observed between the two groups. Plasma insulin levels both basally and 1 h post glucose, however, were significantly higher in the obese group. Furthermore, when obese subjects were separated into three weight-matched subgroups on the basis of increasing WHR (Fig. 1), there was a significant trend to increasing fasting plasma insulin (FPI) and insulin levels after glucose loading, but no significant trend for a similar increase in either basal or stimulated percentage of synthase I as WHR increased. Although increasing WHR was associated with a normal increase in GSI response to oral glucose loading, this response was maintained in the presence of progressively rising increments in plasma insulin levels. Comparing the predominantly upper body obese (WHR > 0.85) with the weight-matched predominantly lower body obese (WHR < 0.76), subgroups revealed a 2–3-fold increase in basal and glucose-stimulated insulin levels but an equal increase in percent GSI activity after oral glucose ingestion.

Table II. Skeletal Muscle Glycogen Synthase Response to Oral Glucose Loading

	Nonobese (n = 14)		Obese (n = 24)	
	Basal	Stimulated	Basal	Stimulated
Total glycogen synthase				
Nmol/mg tissue per min	9.8±0.9	9.0±0.5	8.4±0.5	8.1±0.5
Nmol/mg protein per min	13.1±0.8	12.4±0.9	11.6±1.0	12.4±1.2
GSI (% of total)	22.0±1.5	30.9±2.0*	22.7±1.8	30.0±2.2*
Plasma insulin ($\mu U/ml$)	18.0±1.0	94.0±12.0*	38.0±3.0‡	218.0±27.0*‡

Basal glycogen synthase activity was measured in skeletal muscle biopsies that were obtained after an overnight fast and bed rest. After 30 min, an oral glucose load was administered, and a second biopsy was obtained 60 min later for measurement of stimulated activity. Blood samples were withdrawn before each biopsy for plasma insulin determination. * $P < 0.001$; compares stimulated with basal values in each group. ‡ $P < 0.001$; compares obese with nonobese values.

Fig. 2 shows time-course changes of muscle GSI and the corresponding changes of plasma insulin after the oral administration of glucose to six nonobese, five predominantly lower body obese, and six predominantly upper body obese women. In the three groups, percentage of GSI increased with glucose ingestion. Maximal activity was attained in the 60-min sample and the changes in percent GSI with time were virtually identical. At all time points, however, plasma insulin levels were increased approximately fourfold in the upper body obese and twofold in the lower body obese compared with the nonobese women.

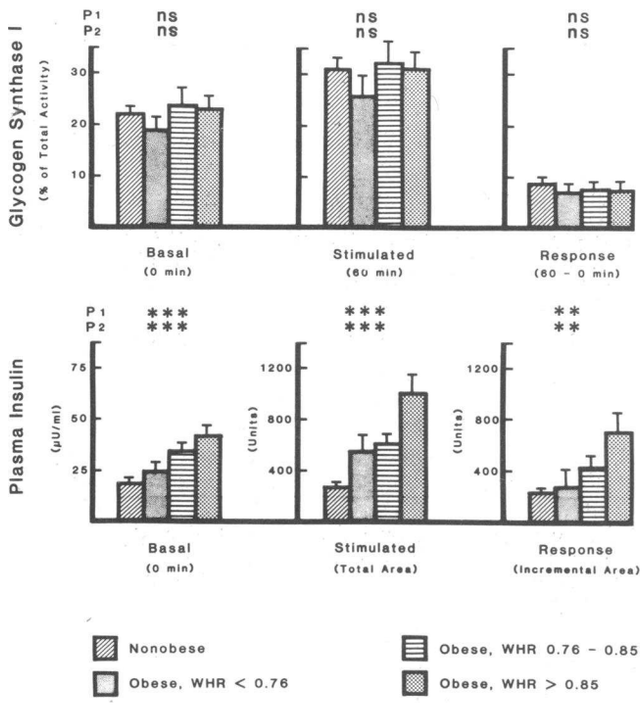

Figure 1. Relationship of body fat topography to skeletal muscle GSI and plasma insulin during oral glucose loading. Subject subgroups included: nonobese (n = 14); obese WHR < 0.76 (n = 5), % IBW 175±9; obese WHR 0.76–0.85 (n = 10), % IBW 181±7; and obese WHR > 0.85 (n = 9), % IBW 190±8. P_1, comparison between the four groups by analysis of variance with linear contrast to test for trend. P_2, Comparison between obese women with WHR > 0.85 and those with WHR < 0.76 by unpaired t test. **$P < 0.01$; ns, not significant.

Table III shows the total glycogen synthase activity and percent synthase I in muscle biopsies obtained from nonobese and obese subjects at comparable submaximal plasma insulin levels that were attained by the infusion of somatostatin, insulin, and glucose. In both groups, there was a significant increase in percentage of synthase I, but the magnitude of this increase was lower in the obese than in the nonobese group (8.8±0.9 and 12.5±1.2%, respectively; $P < 0.05$), so that the stimulated value was significantly lower in the obese subjects. Total synthase activity was unaltered during the infusion. Fig. 3 shows that there was an inverse correlation between WHR and the increase in percentage of synthase I in response to submaximal insulin levels. Furthermore, when the obese subjects were separated into three weight-matched subgroups, there was a significant trend to diminished percentage of synthase I increment as WHR increased. This was accompanied by a significant trend to decreasing efficiency of insulin-stimulated glucose disposal, as reflected by increasing SSPG at comparable plasma insulin levels (Fig. 4). Women with WHR > 0.85 had significantly lower percentage of GSI and higher SSPG than women with WHR < 0.76, and a similar degree of obesity. In the group as a whole, there was a highly significant inverse correlation between SSPG and the increase in percentage of synthase I in response to submaximal insulin levels (Fig. 5). Fasting plasma insulin also correlated inversely with the increase in percentage of synthase I ($r = -0.68$, $P < 0.001$).

Table IV shows the percentage of synthase I response to supramaximal plasma insulin levels in five normal and nine predominantly upper body obese subjects (WHR > 0.85). Per-

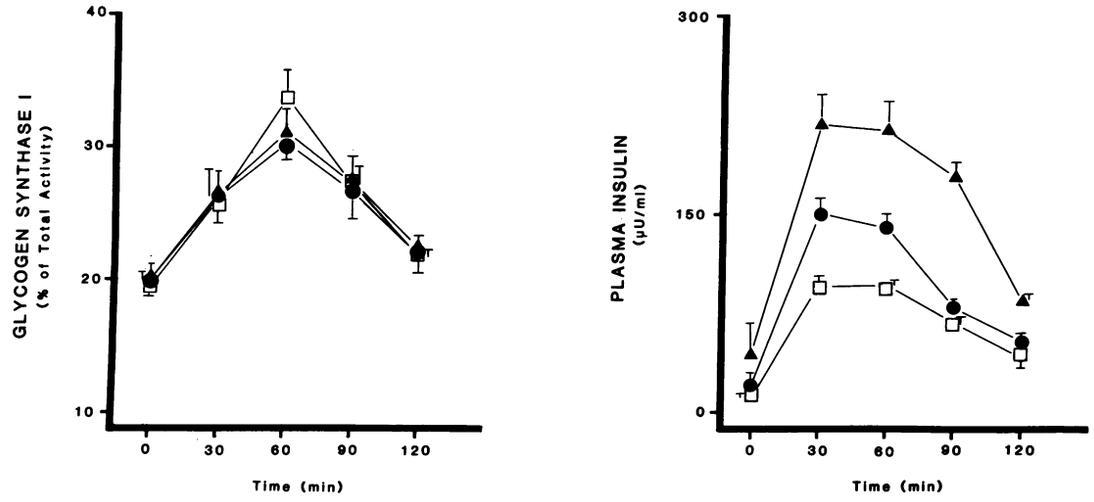

Figure 2. Time course changes in percentage of GSI and plasma insulin during oral glucose tolerance. Subject subgroups included nonobese (n = 6), predominantly lower body obese (n = 5), % IBW 174±6, and predominantly upper body obese (n = 6), % IBW 178±9. □, nonobese; ●, obese, WHR < 0.76; ▲, obese, WHR > 0.85.

centage of synthase I was markedly increased in both groups with no change in total synthase activity. The increase in percentage of synthase I above basal was significantly greater with supramaximal (~1,000 μU/ml) than with submaximal (~100 μU/ml) insulin levels in both groups (nonobese 27.8±4.8 vs. 12.5±1.2%, $P < 0.025$; obese 28.5±4.2 vs. 7.3±1.3%, $P < 0.001$). At comparable supramaximal SSPI concentrations, the increment in synthase I was not significantly different between the obese and nonobese individuals (28.5±4.2 vs. 27.8±4.8%, $P > 0.05$). At supramaximal SSPI, four obese subjects achieved normal maximum glucose disposal efficiency with SSPG concentrations <150 mg/dl (obese subgroup I), while the remaining five subjects exhibited marked reduction in maximum glucose disposal with SSPG levels of 185–288 mg/dl (obese subgroup II). The increase in percentage of synthase I of the two obese subgroups, however, was not distinctly different and was comparable to that of nonobese subjects. This is in contrast to the findings at submaximal SSPI levels, whereby the increase in percentage of synthase I was significantly diminished and SSPG was consistently increased in the upper body obese compared with the nonobese women (Fig. 6).

In 46 subjects, skeletal muscle glycogen content was determined. No significant differences between the nonobese subjects and obese subjects of varying WHR were found (nonobese, 10.7±1.3; obese WHR < 0.76, 12.5±1.7; obese WHR 0.76–0.85, 10.8±1.2; obese WHR > 0.85, 10.5±0.6 μg/mg tissue).

Relationship between insulin binding and insulin action. Fig. 7 shows that insulin binding to circulating monocytes was significantly reduced in obese subjects at all insulin concentrations. There was no significant difference in receptor affinity, with half maximal ^{125}I-insulin displacement occurring at 5.5±0.8 and 4.9±0.6 ng/ml in the nonobese and obese groups, respectively ($P > 0.1$). This suggests that the reduction in binding was due to a decreased number of insulin receptors, and was confirmed by Scatchard analysis, which revealed a mean number of receptors of 23,070/monocyte in the obese and 15,840/monocyte in the obese. Analysis of variance showed

Table III. Skeletal Muscle Glycogen Synthase Response to Submaximal SSPI Levels

	Nonobese (n = 7)		Obese (n = 17)	
	Basal	Stimulated	Basal	Stimulated
Total glycogen synthase				
Nmol/mg tissue per min	8.8±0.6	9.7±0.6	7.6±0.8	7.8±0.7
Nmol/mg protein per min	14.2±1.3	13.7±1.0	11.5±1.3	10.3±1.2
GSI (% of total)	17.7±1.6	30.2±2.4*	15.5±1.7	24.3±1.6*‡
Plasma insulin (μU/ml)	19.0±2.0	105.0±4.0*	32.0±3.0‡	112.0±6.0*

Muscle biopsies were obtained before (basal) and after (stimulated) attainment of SSPI and glucose levels. A continuous infusion of somatostatin, glucose, and insulin was performed to achieve insulin levels of ~100 U/ml. Blood was withdrawn before each biopsy for plasma insulin measurement. * $P < 0.001$; compares stimulated with basal values in each group. ‡ $P < 0.001$; compares obese with nonobese values.

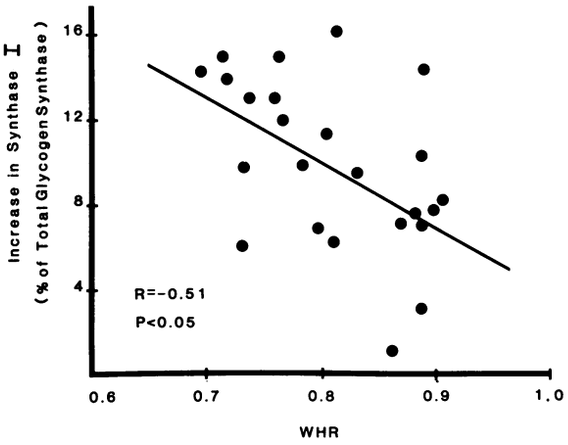

Figure 3. Correlation between WHR and the increase in skeletal muscle percentages of synthase I at submaximal insulin concentration of ~100 μU/ml.

a significant trend to decreased IB among groups with increasing WHR. Insulin binding was also significantly lower in women with predominantly upper body obesity (WHR > 0.85) than in weight-matched women with predominantly lower body fat localization (WHR < 0.76). As shown in Fig. 8, IB correlated inversely with WHR. IB correlated directly with the increase

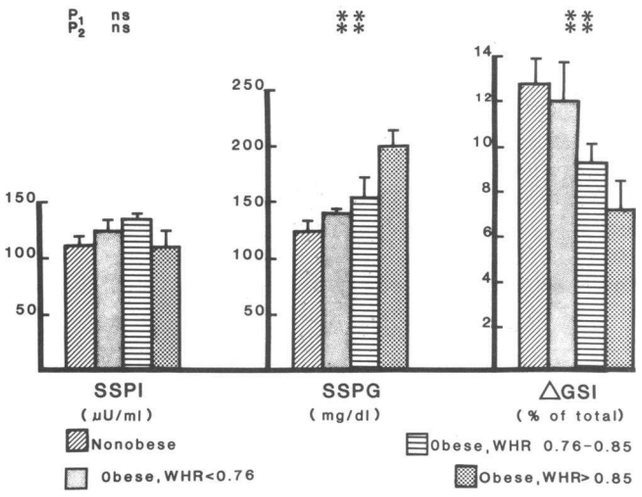

Figure 4. Relationship of body fat topography to skeletal muscle GSI and overall glucose disposal at submaximal plasma insulin levels. Subject subgroups included: nonobese (n = 7); obese WHR < 0.76 (n = 4), % IBW 178±7; obese WHR 0.76–0.85 (n = 4), % IBW 176±8; and obese WHR > 0.85 (n = 9), % IBW 167±6. GSI, increase in percent synthase I above basal; P_1, comparison between the four groups by analysis of variance with linear contrast to test for trend; P_2, comparison by unpaired t test between obese women with WHR > 0.85 and those with WHR < 0.76. **$P < 0.001$; ns, not significant.

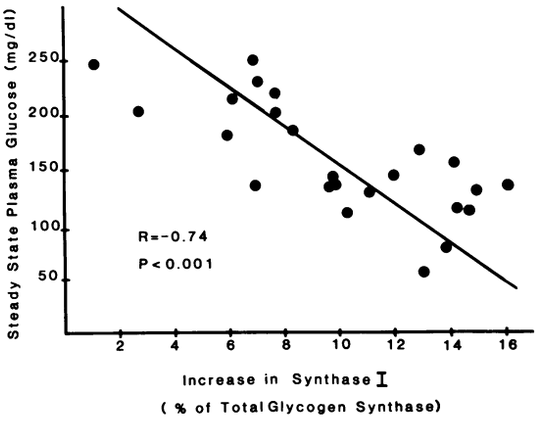

Figure 5. Correlation between SSPG level and the increase in muscle percentage of synthase I in response to submaximal insulin levels.

in skeletal muscle percentage of synthase I and inversely with SSPG at submaximal insulin levels (Fig. 9). Insulin binding also correlated inversely with fasting plasma insulin ($r = -0.43$, $P < 0.01$).

Since most of the variables measured were intercorrelated, simple and multiple regression coupled with pathway analyses were performed in order to define the metabolic cascade relating the variables to each other. Single pathway analysis revealed that the data could be fitted to at least two physiologically feasible models in which increasing WHR could be associated primarily with either an increase in FPI or a decrease in insulin sensitivity as reflected by a decrease in percent GSI response to insulin (Fig. 10, models 1 and 2, respectively). To determine the relative significance of these two models, multiple pathway analyses were performed. Both the increase in FPI and the decrease in insulin sensitivity were assumed to be primary and possibly interrelated events. Also, the decrease in IB was considered to effect a decline in overall glucose disposal efficiency (increased SSPG) via an additional mechanism that did not involve reduction in GSI activity. The path coefficient values shown in Fig. 10, model 3, indicated that the association between the decrease in percentage of synthase I activity and WHR was mediated predominantly via the increase in FPI and in turn the decrease in IB. Also, no significant direct association was found between decreased insulin binding and increased SSPG. Thus, the model generated was similar to model 1 in the single pathway analysis in which the increased FPI was considered as the primary event.

Discussion

In the present study, increasing localization of fat in the upper body of obese premenopausal women was accompanied by a progressive diminution of skeletal muscle insulin sensitivity and a progressive decline in insulin binding to circulating monocytes. The increase in percentage of GSI in response to

Table IV. Skeletal Muscle Glycogen Synthase Response to Supramaximal SSPI Levels

	Nonobese (n = 5)		Obese (n = 9)	
	Basal	Stimulated	Basal	Stimulated
Total glycogen synthase				
Nmol/mg tissue per min	9.7±1.3	10.0±3.3	7.8±0.8	7.5±0.7
Nmol/mg protein per min	13.3±2.0	15.0±2.6	12.8±1.5	12.1±1.0
GSI (% of total)	14.1±2.4	41.9±5.8*	12.6±1.1	41.1±3.8*
Plasma insulin ($\mu U/ml$)	12.2±1.0	949.0±48.0*	29.3±3.8‡	982.0±30.0*

Muscle biopsies were obtained before (basal) and after (stimulated) attainment of SSPI and glucose levels. A continuous infusion of somatostatin, glucose, and insulin was performed to achieve insulin levels of ~1000 μU/ml. Plasma insulin was measured in blood samples withdrawn before each biopsy. * $P < 0.01$ or less; compares stimulated with basal values in each group. ‡ $P < 0.01$ or less; compares obese with nonobese values.

submaximal insulin concentrations fell progressively as WHR increased. By contrast, the percentage of synthase I response to supramaximal insulin levels was unimpaired, which indicated normal maximal responsiveness. Responsiveness to rising plasma insulin levels after oral glucose loading was also unimpaired, which suggests that the decreased sensitivity could be overcome in vivo at the expense of increasing plasma insulin levels. This pattern of diminished sensitivity but normal responsiveness implies the presence of a receptor or very early postreceptor defect in insulin action. Indeed, the impairment in synthase I response to submaximal insulin levels was proportionate to the reduction in insulin-stimulated glucose disposal at submaximal insulin concentrations. Decreased insulin binding could thus contribute to the decrease in insulin action at submaximal insulin levels that was observed in the upper body obese women.

GSI activity provides a direct marker of insulin action in skeletal muscle. Insulin binding at the cell surface results in the generation of a low molecular weight mediator, which decreases the sensitivity of cAMP-dependent protein kinase to cAMP (17). Insulin also increases glucose transport into the

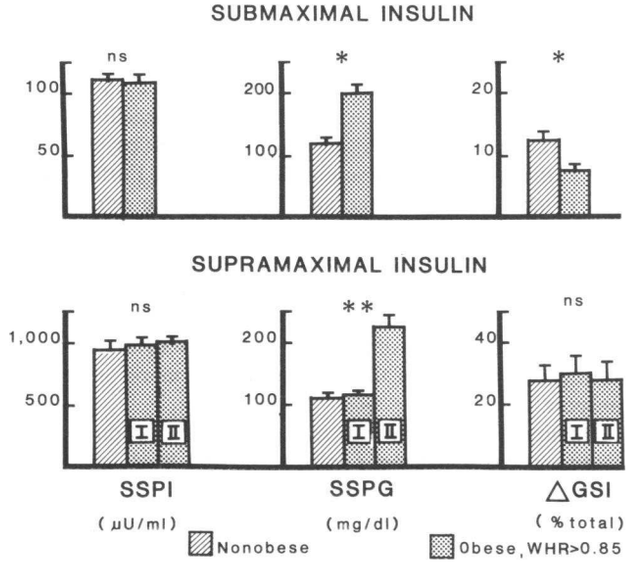

Figure 6. SSPG and insulin (SSPI) and the increase in skeletal muscle percentage of synthase I (Δ GSI) of nonobese and upper body obese (WHR > 0.85) women. (*Top*) Submaximal SSPI: nonobese (n = 7) and obese (n = 9). (*Bottom*) Supramaximal SSPI: nonobese (n = 5), obese I (n = 4), SSPG < 150 mg/dl; obese II (n = 5), SSPG > 150 mg/dl. Comparison between groups was by unpaired t test. *P < 0.05; **P < 0.01; ns, not significant.

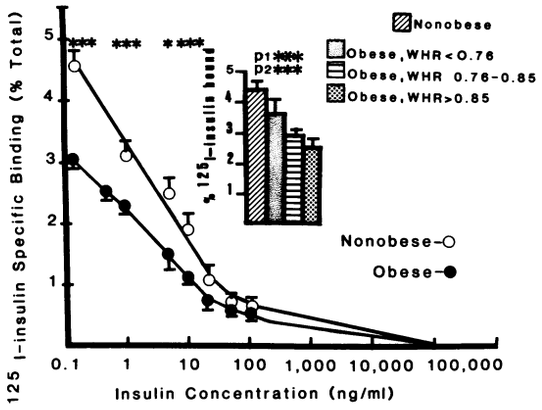

Figure 7. IB profiles to circulating monocytes. Comparison between obese and nonobese groups was by unpaired t test. The inset shows IB at trace insulin concentration in nonobese (n = 12) and in weight-obese subgroups of increasing WHR: WHR < 0.76 (n = 6), % IBW 168±5; WHR 0.76–0.85 (n = 9), % IBW 172±4; and WHR > 0.85 (n = 10), % IBW 187±8. P_1, comparison between the four groups by analysis of variance with linear contrast to test for trend. P_2, comparison between obese women with WHR > 0.85 and those with WHR < 0.76 by unpaired t test. ***P < 0.001.

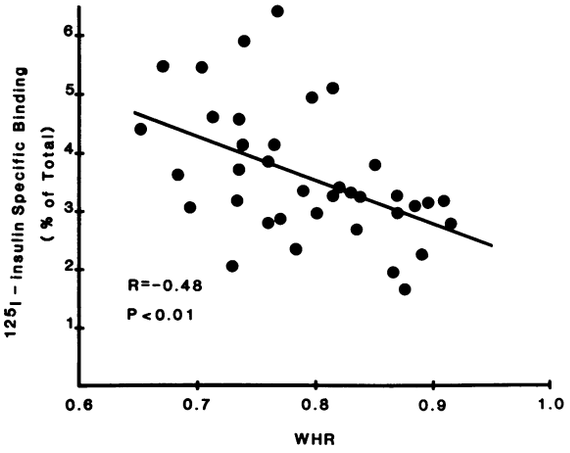

Figure 8. Correlation between WHR and insulin binding to circulating monocytes.

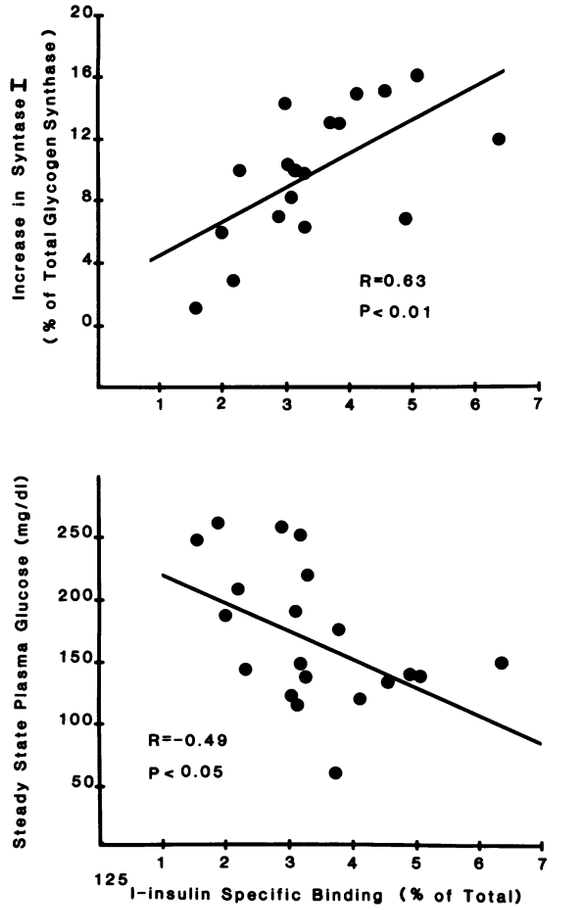

Figure 9. Relationship between IB and increase in skeletal muscle percentage of GSI (*top*) and SSPG (*bottom*) at submaximal insulin levels.

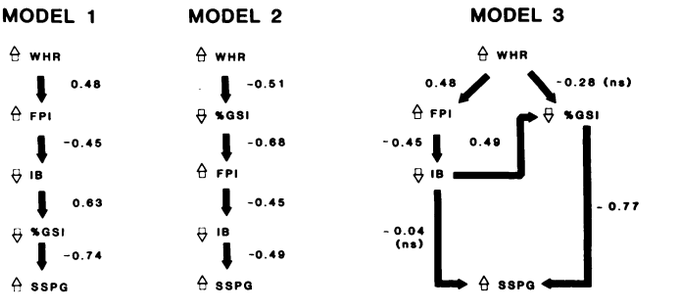

Figure 10. Simple and multiple regression and pathway analysis of intercorrelation between the metabolic variables measured. Models 1 and 2 equal single pathway analysis, and model 3 equals multiple pathway analysis. Values indicate pathway coefficients. ns, Not significant; $P > 0.05$.

cell, which results in increased G6P concentrations and, in turn, increased glycogen synthase phosphatase activity. Both of these pathways result in increased formation of the active dephosphorylated I form of glycogen synthase. Insulin provokes a dose-dependent increase in skeletal muscle percentage of GSI in vitro (36), and changes in percentage of synthase I in vivo closely parallel changes in plasma insulin levels (37). In insulin-deficient diabetic subjects there is a diminished percentage of synthase I response to glucose loading (38), and in patients with insulin resistance due to myotonic dystrophy, there is a reduction in the synthase I response to endogenous hyperinsulinemia (21). Conversely, in glycogen-depleted man, increased GSI activity correlates with insulin-mediated increased overall glucose disposal (39).

Percentage of GSI could also be influenced by a decrease in glycogen content (40) or increase in intracellular G6P concentrations (17). Among our subject subgroups, however, skeletal muscle glycogen content did not differ. Furthermore, since in all our subjects the amount of glucose infused and hence the absolute amount disposed at the steady state were identical, intracellular G6P concentrations would not be expected to differ between subgroups. The decreased percentage of synthase I response in the upper body obese subjects can thus be attributed to diminished insulin activation of the enzyme, independent of the effects of the hormone on glucose transport.

Obesity is associated with resistance to the action of both exogenous and endogenous insulin. Plasma levels of insulin are frequently elevated in obese subjects both basally and in response to glucose (41). The fall in blood glucose in response to exogenously administered insulin is attenuated in obese subjects (5), and infusion of insulin during forearm perfusion studies is associated with diminished uptake of glucose by the deep tissues (11, 13, 14). Recently, impairment of the overall efficiency of insulin-stimulated glucose disposal at comparable plasma insulin concentrations has been shown in obese subjects by both the euglycemic clamp technique (42, 43) and by measurement of SSPG at comparable plasma insulin levels

attained by infusion of insulin and glucose and either epinephrine and propranolol or somatostatin (18, 23, 44). Using the latter technique, we have demonstrated that this diminished sensitivity to insulin is a characteristic feature of upper body obesity in premenopausal women (3).

The inverse correlation between SSPG and increment in percentage of synthase I in response to submaximal insulin levels shown in the present study, suggests that diminished skeletal muscle insulin sensitivity, which results in decreased glucose storage capacity, may play an important role in the impaired efficiency of insulin-stimulated glucose disposal that is associated with upper body fat predominance. Diminished insulin sensitivity has been demonstrated in adipose tissue of obese subjects in whom insulin stimulation of glucose transport and oxidation are reduced (4, 15, 45). Impaired hepatic insulin sensitivity is also demonstrable using the insulin clamp technique (46). Insulin resistance associated with obesity thus appears to affect all the major insulin sensitive target organs.

In the present study, upper body obesity and the associated decrease in insulin-stimulated glucose disposal and skeletal muscle insulin sensitivity were accompanied by a reduction in insulin binding to circulating monocytes. This reduction correlated with the decrease in both percentage of synthase I response and overall glucose disposal, which suggests that the receptor defect may contribute to the development of the insulin resistance that is associated with upper body fat predominance. In most circumstances, changes in monocyte insulin receptors have been shown to parallel changes in other insulin sensitive organs, including muscle (10).

Previous studies have shown a correlation between diminished insulin binding to adipocytes and both glucose uptake by adipose tissue (47) and overall glucose disposal rate (23). In some insulin-resistant subjects, a postreceptor defect may also be present (43). Both receptor and postreceptor defects have been demonstrated in adipocytes and skeletal muscle of experimental animals (15, 48). Our subjects with predominantly upper body obesity exhibited impaired sensitivity of skeletal muscle glycogen synthase but normal responsiveness to supramaximal insulin levels and to increased plasma insulin levels during oral glucose loading. The higher SSPG at supramaximal insulin concentrations observed in some individuals, however, suggests that an additional defect affecting other insulin-sensitive pathways may be present. This contrasts with the situation in obese rodents in which decreased maximal responsiveness of glycogen synthase precedes the decrease of the hormone-stimulated glycolysis and total glucose metabolism in skeletal muscle (49). Whether the additional defect in our upper body obese subjects results from a receptor, postreceptor abnormality, or both, and whether this defect involves skeletal muscle or other insulin target organs, remain to be determined.

The significant intercorrelation between the metabolic variables measured is compatible with the complexity of the glucose-insulin regulatory system in which a perturbation at one point could evoke a multitude of secondary changes along the cascade describing this system. It is also difficult to distinguish which of those events is primary and which is secondary. Our multiple regression-pathway analysis suggests that increased FPI is the most likely primary abnormality that underlies the metabolic disorder of upper body obesity. Since pathway analysis can be influenced by sampling and measurement errors, conclusions based upon this approach require experimental validation. Furthermore, the pathway coefficient values obtained were modest, and thus the possible involvement of other variables at any point along the cascade should be considered. Nevertheless, our conclusion that fasting hyperinsulinemia could be a primary factor is supported by the literature. Thus, the reduction in plasma insulin levels with accompanying rise in insulin binding (30), increased efficiency of glucose disposal (44), and the increased insulin-stimulated forearm glucose uptake (14) which occur after weight reduction, suggest that the defect in insulin sensitivity in the obese state is at least partly reversible and may be secondary to the accompanying hyperinsulinemia. The development of hyperinsulinemia and decreased insulin-stimulated forearm glucose uptake in normal subjects after weight gain (14), and the development of hyperinsulinemia, decreased insulin binding, and impaired insulin action in experimentally induced obesity in rodents further supports this premise (15).

In conclusion, our results suggest that diminished skeletal muscle insulin sensitivity with impaired glucose storage capacity contributes to the insulin resistance and, in turn, the associated glucose intolerance and hyperinsulinemia of upper body obesity, and that this defect is due largely to a reduction in the number of insulin receptors, although in some individuals an additional defect affecting other insulin-sensitive pathways may also be involved. This sequence could result from persistently elevated basal plasma insulin levels which, in turn, could be secondary to the enhanced lipolysis characteristic of adipocytes in upper body obesity. The resultant increase in plasma free fatty acid that is released into the plasma could affect peripheral glucose disposal, insulin secretion, and/or hepatic uptake of insulin. The increased androgenic to estrogenic activity that is associated with upper body obesity might also influence these events.

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