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*J Clin Invest.* 1990;**85**(6):1844-1852. <https://doi.org/10.1172/JCI114644>.

### Research Article

Obesity is characterized by decreased rates of skeletal muscle insulin-mediated glucose uptake (IMGU). Since IMGU equals the product of the arteriovenous glucose difference (AVGd) across muscle and blood flow into muscle, reduced blood flow and/or tissue activity (AVGd) can lead to decreased IMGU. To examine this issue, we studied six lean (weight 68 +/- 3 kg, mean +/- SEM) and six obese (94 +/- 3 kg) men. The insulin dose-response curves for whole body and leg IMGU were constructed using the euglycemic clamp and leg balance techniques over a large range of serum insulin concentrations. In lean and obese subjects, whole body IMGU, AVGd, blood flow, and leg IMGU increased in a dose dependent fashion and maximal rates of all parameters were reduced in obese subjects compared to lean subjects. The dose-response curves for whole body IMGU, leg IMGU, and AVGd were right-shifted in obese subjects with an ED50 two- to threefold higher than that of lean subjects for each parameter. Leg blood flow increased approximately twofold from basal 2.7 +/- 0.2 to 4.4 +/- 0.2 dl/min in lean, P less than 0.01, and from 2.5 +/- 0.3 to 4.4 +/- 0.4 dl/min in obese subjects, P less than 0.01. The ED50 for insulin's effect to increase leg blood flow was about fourfold higher for obese (957 pmol/liter) than lean [...]

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# Decreased Effect of Insulin To Stimulate Skeletal Muscle Blood Flow in Obese Man

## A Novel Mechanism for Insulin Resistance

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### Abstract

Obesity is characterized by decreased rates of skeletal muscle insulin-mediated glucose uptake (IMGU). Since IMGU equals the product of the arteriovenous glucose difference (AVGd) across muscle and blood flow into muscle, reduced blood flow and/or tissue activity (AVGd) can lead to decreased IMGU. To examine this issue, we studied six lean (weight  $68 \pm 3$  kg, mean  $\pm$  SEM) and six obese ( $94 \pm 3$  kg) men. The insulin dose-response curves for whole body and leg IMGU were constructed using the euglycemic clamp and leg balance techniques over a large range of serum insulin concentrations. In lean and obese subjects, whole body IMGU, AVGd, blood flow, and leg IMGU increased in a dose dependent fashion and maximal rates of all parameters were reduced in obese subjects compared to lean subjects. The dose-response curves for whole body IMGU, leg IMGU, and AVGd were right-shifted in obese subjects with an  $ED_{50}$  two- to threefold higher than that of lean subjects for each parameter. Leg blood flow increased approximately twofold from basal  $2.7 \pm 0.2$  to  $4.4 \pm 0.2$  dl/min in lean,  $P < 0.01$ , and from  $2.5 \pm 0.3$  to  $4.4 \pm 0.4$  dl/min in obese subjects,  $P < 0.01$ . The  $ED_{50}$  for insulin's effect to increase leg blood flow was about fourfold higher for obese ( $957$  pmol/liter) than lean subjects ( $266$  pmol/liter),  $P < 0.01$ . Therefore, decreased insulin sensitivity in human obesity is not only due to lower glucose extraction in insulin-sensitive tissues but also to lower blood flow to these tissues. Thus, in vivo insulin resistance can be due to a defect in insulin action at the tissue level and/or a defect in insulin's hemodynamic action to increase blood flow to insulin sensitive tissues. (*J. Clin. Invest.* 1990. 85:1844-1852.) glucose uptake • lactate • free fatty acids

### Introduction

Human obesity is characterized by decreased rates of insulin-mediated glucose uptake (IMGU)<sup>1</sup> (1). In accordance with this, in vivo dose-response curves for insulin's effect to stimu-

late whole body glucose uptake, constructed using the hyperinsulinemic euglycemic clamp technique have been shown to be right-shifted in obese subjects indicating a reduction in insulin sensitivity (2). Furthermore, in the most hyperinsulinemic and insulin-resistant obese subjects, glucose uptake was found to be markedly decreased at maximally effective insulin concentrations indicating a reduction in the responsiveness to insulin (2). While useful, measurements of whole body glucose uptake cannot distinguish between responses in individual insulin sensitive tissues from the whole body response. For example, although IMGU is mostly in skeletal muscle (3-6), direct quantification of reduced rates of skeletal muscle IMGU over the range of insulin concentrations have not been measured in obese man. Moreover, measurements of whole body IMGU do not allow the examination of the components of muscle glucose uptake, namely the arteriovenous glucose difference across muscle (tissue glucose extraction) and the blood flow (or the delivery rate) into muscle, which, according to the Fick principle (7), determine glucose uptake.

Recently Dohm et al. (8) have demonstrated decreased rates of glucose transport in skeletal muscle obtained from morbidly obese human subjects. Therefore, on the basis of that study one would expect to find a reduction in in vivo skeletal muscle glucose extraction. The elucidation of the role of blood flow in skeletal muscle glucose uptake is not possible on the basis of in vitro studies. Several previous in vivo studies (9-14), but not all (15-19), have documented an effect of insulin to increase plasma or blood flow to skeletal muscle in humans (11-14) and in animals (9, 10). In each of these studies except one (14), the hemodynamic response was studied at a single insulin infusion rate, which makes it impossible to elucidate the dose-response characteristics of insulin's effect on regulation of hemodynamic responses. In addition, no study has included a comparison between lean and obese subjects. Therefore, although IMGU is reduced in obesity it is not known whether glucose extraction, blood flow or both are responsible for the impairment in insulin action in these subjects.

The aim of this study was to elucidate the relationship between insulin's effects to stimulate whole body and leg muscle glucose uptake and its components (glucose extraction across and blood flow to skeletal muscle) and the alteration of this relationship in human obesity. To this end, we applied the euglycemic clamp technique to measure whole body IMGU and simultaneous femoral arterial and venous catheterization to measure leg IMGU in lean and obese subjects over a large range of insulin concentrations.

### Methods

#### Materials

Porcine monocomponent insulin was supplied by Nordisk (Rockville, MD); <sup>125</sup>I-insulin was purchased from New England Nuclear (Boston,

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Received for publication 7 July 1989 and in revised form 11 December 1989.

1. Abbreviations used in this paper: AVGd, arteriovenous glucose difference; IMGU, insulin-mediated glucose uptake.

*J. Clin. Invest.*

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0021-9738/90/06/1844/09 \$2.00

Volume 85, June 1990, 1844-1852

MA); BSA (fraction V) was obtained from Armour Pharmaceutical (Chicago, IL); guinea pig anti-insulin antibody was kindly supplied by E. Arquilla (University of California, Irvine, CA); and somatostatin (SRIF, cyclic form) was purchased from Bachem (Torrance, CA).

### Subjects

The study groups in study I consisted of six healthy lean men (age  $33 \pm 2$  years, weight  $68 \pm 3$  kg, mean  $\pm$  SEM) and six healthy obese men (age  $37 \pm 2$  years,  $P = \text{NS}$  compared with lean; weight  $94 \pm 3$  kg,  $P < 0.001$  compared with lean). All obese men had body mass index  $> 27$  kg/m<sup>2</sup> (weight [kg]/height [m]<sup>2</sup>). All lean men and four obese men had a normal oral glucose tolerance test (a 75-g load of glucose) and two obese men had impaired glucose tolerance, as defined by the National Diabetes Data Group (20). Study II included four healthy lean men (age  $35 \pm 3$  yr, weight  $67 \pm 3$  kg) with normal glucose tolerance. While hospitalized all subjects remained active to approximate their prehospital exercise level. All were chemically euthyroid, normotensive and no subject had a concurrent disease or was ingesting pharmacological agents known to affect carbohydrate or insulin metabolism. Studies were approved by the Human Subjects Research Review Committee of the University of California, San Diego.

### Diet

All subjects were fed a weight maintenance ( $\sim 32$  kcal/kg per d) diet with three divided feedings containing one, two, and two-fifths of the total daily calories, given at 0800, 1200, and 1700 h, respectively. The caloric content of the diet was 50% carbohydrate, 20% fat, and 30% protein. All subjects ate this diet for at least 48 h before any studies were performed.

### Protocol

**Study I.** Study I was designed to measure whole body and leg glucose uptake (femoral arterial and venous catheterization technique) at euglycemia ( $\sim 5$  mmol/liter) over a wide range of insulin concentrations to characterize half-maximum ( $\text{ED}_{50}$ ) and maximum effects of insulin to stimulate whole body and leg glucose uptake (blood flow  $\times$  arteriovenous blood glucose difference) in lean and obese subjects. To accomplish this, [<sup>3</sup>H-3]glucose was infused starting at 7:00 AM through a catheter inserted in an antecubital vein. At  $\sim 8:00$  AM catheters were inserted in the right femoral artery and vein (see technique below) to measure leg flow and to obtain blood samples. At least 40 min after the femoral catheters were inserted and at least 120 min after the initiation of [<sup>3</sup>H-3]glucose infusion, arterial blood was obtained at 10-min intervals over a 30-min period for the determination of basal plasma glucose specific activity. Simultaneously, femoral venous blood was obtained for the determination of serum glucose levels. Samples for arterial serum insulin and free fatty acids (FFA) and for arterial and venous serum lactate were obtained twice during the basal period and the means of these concentrations were used in statistical analyses. Basal leg blood flow was also determined (see technique below). After the basal measurements were obtained an insulin infusion was started and the serum glucose was clamped (21) at euglycemia based on samples from the femoral artery. A fall in plasma glucose level was prevented by infusing 20% dextrose at a variable rate adjusted according to glucose measurements made at 5-min intervals. Throughout the study  $\text{K}_2\text{HPO}_4$  was infused to prevent hypokalemia and hypophosphatemia. Insulin was infused initially at a rate of 10 mU/m<sup>2</sup> per min in lean subjects ( $n = 3$ ) and this rate was maintained for at least 180 min to fully express insulin's action (22). Thereafter, the insulin infusion rate was increased sequentially to 20, 40, 300, and 600 mU/m<sup>2</sup> per min. Each infusion rate was maintained at least 120 min at insulin infusion rates of 20 and 40 mU/m<sup>2</sup> per min, at least 90 min at insulin infusion rates of 300 mU/m<sup>2</sup> per min, and at least 40 min at insulin infusion rates of 600 mU/m<sup>2</sup> per min. In obese subjects insulin was infused initially at a rate of 40 mU/m<sup>2</sup> per min for at least 180 min. Thereafter, the insulin infusion rate was increased sequentially to 100, 600, and 1,200 mU/m<sup>2</sup> per min. Insulin infusion rates of 100 and 600 mU/m<sup>2</sup> per min were maintained at least 90 min and at least 40 min for the

1,200 mU/m<sup>2</sup> per min infusion. It is important to note that each insulin infusion rate was maintained for sufficient time to establish steady-state femoral arteriovenous glucose difference (AVGd) for at least 30 min during which all measurements were made. Since it takes longer to establish steady-state conditions at the lower insulin infusion rates (22), these were maintained for longer periods than the higher insulin infusion rates. Each study lasted  $\sim 10$  h. During each insulin plateau, simultaneous samples of arterial and venous blood were drawn every 5 min for serum glucose determinations. These values were meaned and taken as the representative AVGd for that plateau period. Blood flow measurements were taken at the beginning and the end of each 30-min plateau period. Samples for arterial insulin, FFA, and hematocrit and for arterial and venous lactate were drawn at the end of each plateau.

**Study II.** This study was designed to examine the time course of insulin's effect to stimulate whole body glucose uptake, femoral AVGd, and leg blood flow during a 4-h euglycemic hyperinsulinemic clamp. To accomplish this, [<sup>3</sup>H-3]glucose was infused starting at  $\sim 7:00$  AM through a catheter inserted in an antecubital vein. At  $\sim 8:00$  AM a catheter was inserted in the right femoral vein (see technique below) to measure leg flow and to obtain blood samples for venous glucose. Samples for plasma glucose specific activity and for serum glucose during basal period were obtained as in study I. Basal leg blood flow was measured as in study I (see technique below). Thereafter, a square wave insulin infusion (40 mU/m<sup>2</sup> per min) was started and the serum glucose was clamped at euglycemia. Blood samples for the determination of the arterial serum glucose concentration were taken from a retrograde cannulated hand vein, which was kept in a warming device to insure arterialization of venous blood. Blood flow measurements were taken every 30 min. Samples for hematocrit were drawn every 60 min.

### Leg glucose balance technique

With this technique catheters were inserted percutaneously in the femoral artery for the sampling of arterial blood and in the ipsilateral femoral vein for the measurement of leg blood flow and blood drawing as previously reported (23). Leg blood flow was measured by the thermodilution technique (24). Leg glucose uptake was calculated by the Fick principle (7) as the product of the arteriovenous difference for blood glucose and the leg blood flow. Serum glucose values were converted to whole blood values by multiplying the plasma value with  $1 - (0.30 \times \text{hematocrit})$  (25). The mean of 10 flow measurements at each insulin plateau (study I) and at every 30-min interval (study II) was taken as the representative value. Because we could not obtain precise quantitation of leg muscle mass in obese subjects, we chose to express the leg glucose uptake data as micromoles per leg per minute. This method probably underestimates the true difference between lean and obese subjects since leg volumes and leg skeletal muscle mass are higher in obese subjects than in lean subjects (26).

### Rates of glucose appearance (Ra) and disappearance (Rd)

**Study I.** Ra and Rd were measured in the basal state and during insulin infusions of 10 and 20 mU/m<sup>2</sup> per min using a modification of the variable tracer technique as described by Finegood et al. (27). With this technique a continuous infusion of [<sup>3</sup>H-3]glucose 15  $\mu\text{Ci}/\text{min}$  is allowed to label the glucose pool over a 2-h period. After basal measurements are obtained the insulin infusion is begun and the serum glucose level is clamped using a variable infusion of glucose that has been labeled with [<sup>3</sup>H-3]glucose so that the specific activity of the glucose infusate approximates the plasma specific activity achieved after 2 h of constant [<sup>3</sup>H-3]glucose infusion at a rate of 0.15  $\mu\text{Ci}/\text{min}$ . This technique has been validated (27) and has been shown to minimize the underestimation of Ra (and thus Rd) seen with the conventional tracer method (continuous infusion only). During the insulin infusion of 40 mU/m<sup>2</sup> per min or higher, hepatic glucose output was assumed to be completely suppressed and glucose disposal rates were estimated on the basis of glucose infusion rates.

**Study II.** Ra and Rd were measured by the variable tracer infusion technique (27). In addition to further minimize the error of estimates for Ra and Rd, insulin (40 mU/m<sup>2</sup> per min) was infused in conjunction with a SRIF infusion to suppress endogenous glucagon secretion and thus to increase insulin's ability to suppress hepatic glucose production (28).

### Analytical methods

Blood for serum glucose determinations was drawn, put in untreated polypropylene tubes, and centrifuged using a Eppendorf microcentrifuge (Brinkmann Instruments Co., Westbury, NY). The glucose concentration of the supernatant was then measured by the glucose oxidase method using a glucose analyzer (model 23A; Yellow Springs Instruments, Yellow Springs, OH). Blood for determination of serum insulin concentration was collected in untreated tubes and allowed to clot. The specimen were spun, and the supernatant was removed and stored at -20°C. Serum insulin levels were measured by double antibody RIA (29); the lower detection limit of the insulin assay was 29 pmol/liter. All values reported as < 29 pmol/liter were treated as representing 29 pmol/liter. Plasma FFA levels were measured by the method of Itaya and Vi (30).

### Data analysis

All calculations and analyses were performed using the CLINFO data base management and analysis program (Bolt Beranek and Newman, Inc., Cambridge, MA) and SPSS/PC+ programs (SPSS Inc., Chicago, IL). The data are presented as mean±SEM. Comparison between the groups was done using Mann-Whitney U test for independent samples. Steele's equations in their modified form (31) were used to calculate Ra and Rd in the basal state. Calculation of Ra and Rd by the variable tracer infusion technique during euglycemic clamp studies was based on the method of Finegood et al. (27). The dose-response curves for whole body glucose uptake, AVGd, blood flow, and leg glucose uptake vs. insulin concentration were fitted to a four-parameter logistic equation using a least mean square iterative routine (ALLFIT) (32):  $Y = [(A - D)/(1 + (I/ED_{50})^B)] + D$ , where *A* = expected maximal response, *D* = expected minimal response, *I* = insulin concentration, *ED*<sub>50</sub> = insulin concentration with expected response half way between *A* and *D*, and *B* = slope factor. The runs test was calculated both in lean and obese subjects separately to evaluate the goodness of fit. The best fit for each group is presented on the basis of the group means for whole body glucose uptake, AVGd, blood flow, leg glucose uptake vs. insulin concentration. After obtaining parameters *A*, *D*, *B*, and *ED*<sub>50</sub> for the lean and obese groups separate analyses were carried out to test if *A*, *B* or *ED*<sub>50</sub> are similar in lean and obese groups. Time course curves of insulin's effect to stimulate whole body glucose uptake, AVGd, leg blood flow, and leg glucose uptake (study II) were similarly fitted (32) to get the time point with expected response half way between *A* and *D*, where *A* = expected maximal response above basal (100%) and *D* = minimal response (0%).

## Results

### Study I

**Hormone and metabolic data.** In a 3-h oral glucose tolerance test, fasting (4.9±0.1 vs. 5.0±0.1 mmol/liter, 1-h (8.5±0.6 vs. 8.9±0.7 mmol/liter), and 2-h (5.8±0.4 vs. 8.0±0.9 mmol/liter) serum glucose levels were similar in the lean and obese subjects but 3-h glucose was higher in obese (5.3±0.7 mmol/liter) than in lean subjects (3.5±0.3 mmol/liter, *P* < 0.05). Fasting (39±8 vs. 108±46 pmol/liter), 1-h (447±116 vs. 741±247 pmol/liter), and 3-h insulin levels (54±15 vs. 324±123 pmol/liter) did not differ between the lean and obese subjects but 2-h insulin concentration was higher in the obese than in the lean subjects (694±170 vs. 177±39 pmol/liter, *P* < 0.05). Thus, while insulin concentrations were higher at all time points in the obese groups, only the 2-h point was statistically significant. During the euglycemic clamp studies the serum glucose concentration was ~ 5 mmol/liter in both groups and did not differ from the fasting serum glucose concentration at any insulin infusion rate (Table I). Coefficient of variation of glucose levels at each plateau was < 4%. Arterial insulin concentrations did not differ at basal (35±6 vs. 92±24 pmol/liter) or during 40 mU/m<sup>2</sup> per min insulin infusion (598±44 vs. 722±41 pmol/liter) and 600 mU/m<sup>2</sup> per min insulin infusion (20,951±2695 vs. 28,987±451 pmol/liter) between the lean and obese subjects (Table I).

FFA concentrations at basal and during euglycemic clamp studies did not differ between the lean and obese subjects (Table II). The suppression of FFA from basal levels at maximally effective insulin concentrations was similar in lean and in obese subjects (50% and 53%, respectively, *P* = NS). The arterial lactate concentration increased about twofold from basal in lean subjects and ~ 1.5-fold in obese subjects. During the 40 mU/m<sup>2</sup> per min insulin infusion, arterial lactate and lactate arteriovenous difference (venous lactate - arterial lactate) were significantly higher in lean subjects than in obese subjects.

**Whole body glucose uptake.** Basal rates of whole body uptake expressed as micromoles per meter squared per minute were similar in the lean and obese subjects (487±28 vs. 470±41 μmol/m<sup>2</sup> per min, respectively, Fig. 1). During insulin infusion whole body glucose uptake increased in a sigmoidal fashion in lean and obese subjects. In obese subjects the whole body glucose uptake curve as a function of insulin concentration was right-shifted and maximal whole body glucose uptake (Table III) was lower in obese subjects than in lean subjects (2,334 vs. 2,719 μmol/m<sup>2</sup> per min, *P* < 0.01). Minimum whole body glucose uptake, representing glucose uptake at 0 insulin concentration was similar in lean and obese subjects (460 vs.

Table I. Arterial Serum Glucose and Insulin Concentrations during Euglycemic Clamp Studies in Lean and Obese Subjects

	Insulin infusion rate							
	0	10	20	40	100	300	600	1,200
	mU/m <sup>2</sup> per min							
Glucose (mmol/liter)								
Lean	5.2±0.2	4.8±0.2	5.0±0.2	5.0±0.2	—	5.1±0.2	5.1±0.2	—
Obese	5.1±0.2	—	—	4.8±0.2	4.9±0.2	—	4.9±0.2	5.1±0.2
Insulin (pmol/liter)								
Lean	34.7±6.5	126.8±19.6	290.6±29.0	597.9±43.7	—	9,375±1,138	20,951±2,695	—
Obese	92.1±24.4	—	—	722.3±41.1	59±99	—	28,987±451	64,037±4,352

**Table II. Arterial Free Fatty Acid and Lactate Concentrations and Femoral Lactate Arteriovenous Difference (Venous Lactate–Arterial Lactate) during Euglycemic Clamp Studies in Lean and Obese Subjects**

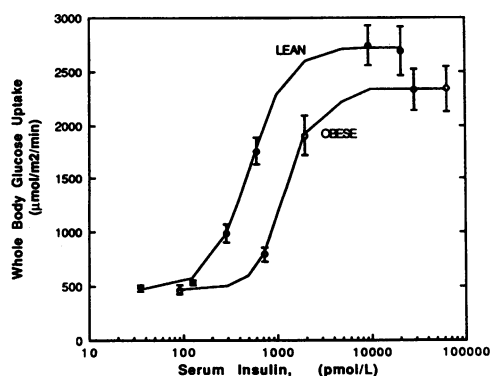
	Insulin infusion rate							
	0	10	20	40	100	300	600	1,200
	mU/m <sup>2</sup> per min							
<b>Free fatty acids (mmol/liter)</b>								
Lean	0.38±0.04	0.31±0.16	0.20±0.03	0.19±0.03	—	0.18±0.04	0.19±0.05	—
Obese	0.38±0.03	—	—	0.15±0.02	0.21±0.03	—	0.19±0.03	0.18±0.02
<b>Lactate (mmol/liter)</b>								
Lean	0.72±0.07	0.90±0.20	0.95±0.10	1.33±0.10	—	1.68±0.19	1.72±0.12	—
Obese	1.02±0.13	—	—	1.03±0.06*	1.45±0.12	—	1.63±0.08	1.55±0.10
<b>Lactate arteriovenous difference (mmol/liter)</b>								
Lean	0.17±0.05	-0.05±0.15	0.18±0.04	0.22±0.03	—	0.20±0.04	0.22±0.05	—
Obese	0.06±0.04	—	—	0.02±0.03 <sup>‡</sup>	0.15±0.03	—	0.33±0.06	0.30±0.04

\*  $P < 0.05$ ; <sup>‡</sup>  $P < 0.01$  obese vs. lean.

468  $\mu\text{mol}/\text{m}^2$  per min). The ED<sub>50</sub> for insulin's effect to stimulate whole body glucose uptake was 2.5-fold in obese subjects compared to lean subjects (1263 vs. 515 pmol/liter,  $P < 0.01$ ).

**Leg glucose uptake.** Fig. 2 A shows the blood AVGd across the leg at each insulin concentration. In the fasting state the AVGd was similar in lean and obese subjects (0.12±0.01 vs. 0.10±0.02 mmol/liter, respectively) and during hyperinsulinemia AVGd increased in a sigmoidal fashion in both groups. The maximum AVGd in obese subjects was lower than in lean subjects (1.38 vs. 1.84 mmol/liter,  $P < 0.01$ ) but the minimum AVGd (i.e., AVGd at zero insulin) was similar in lean and obese subjects (0.09 mmol/liter). The ED<sub>50</sub> for insulin's effect to increase AVGd was 2.5 times higher in obese subjects than in lean subjects (987 vs. 391 pmol/liter,  $P < 0.01$ ) (Table III).

**Leg blood flow** (Fig. 2 B) was similar in lean and obese subjects in the fasting state (2.7±0.2 vs. 2.5±0.3 dl/min, respectively) and increased about twofold in a sigmoidal fashion in both groups as a function of insulin concentration. Maximum leg blood flow rates were similar in obese and lean subjects (4.4 vs. 4.3 dl/min,  $P = \text{NS}$ ). The ED<sub>50</sub> for insulin's effect to stimulate leg blood flow was 3.6 times higher in obese subjects than in lean subjects (957 vs. 266 pmol/liter,  $P < 0.01$ ) (Table III).



**Figure 1.** Rates of whole body glucose uptake as a function of prevailing serum insulin concentration during euglycemic clamp studies in lean (●) and obese (○) subjects. Solid lines depict the fit based on a four-parameter logistic equation.

Rates of leg glucose uptake expressed, in micromoles per leg per minute (Fig. 2 C), were similar in the fasting state in the lean and obese subjects (32.7±4.4 vs. 22.9±5.2  $\mu\text{mol}/\text{leg}$  per min) and increased in a sigmoidal fashion in both groups. Leg glucose uptake was similar at 0 insulin concentration in lean and obese subjects (18 and 22  $\mu\text{mol}/\text{leg}$  per min, respectively) but maximum leg glucose uptake was significantly lower in obese than in lean subjects (605 vs. 811  $\mu\text{mol}/\text{leg}$  per min,  $P$

**Table III. Minimum and Maximum Values and ED<sub>50</sub> for Insulin's Effect to Stimulate Whole Body Glucose Uptake, Femoral Arteriovenous Glucose Difference, Leg Blood Flow, and Leg Glucose Uptake during Euglycemic Clamp Studies in Lean and Obese Subjects**

	Lean	Obese
<b>Whole body glucose uptake</b>		
Minimum ( $\mu\text{mol}/\text{M}^2$ per min)	460	468
Maximum ( $\mu\text{mol}/\text{M}^2$ per min)	2,719	2,334*
ED <sub>50</sub> for insulin (pmol/liter)	515	1,263*
<b>Arteriovenous blood glucose difference</b>		
Minimum (mmol/liter)	0.09	0.09
Maximum (mmol/liter)	1.84	1.38*
ED <sub>50</sub> for insulin (pmol/liter)	391	987*
<b>Leg blood flow</b>		
Minimum (dl/min)	2.6	2.5
Maximum (dl/min)	4.3	4.4
ED <sub>50</sub> for insulin (pmol/liter)	266	957*
<b>Leg glucose uptake</b>		
Minimum ( $\mu\text{mol}/\text{leg}$ per min)	18	22
Maximum ( $\mu\text{mol}/\text{leg}$ per min)	811	605*
ED <sub>50</sub> for insulin (pmol/liter)	420	1,153*

Minimum and maximum values represent rates of whole body glucose uptake, arteriovenous glucose difference, leg blood flow, and rates of leg glucose uptake at 0 and infinite insulin concentrations, respectively.

\*  $P < 0.01$ , obese vs. lean.

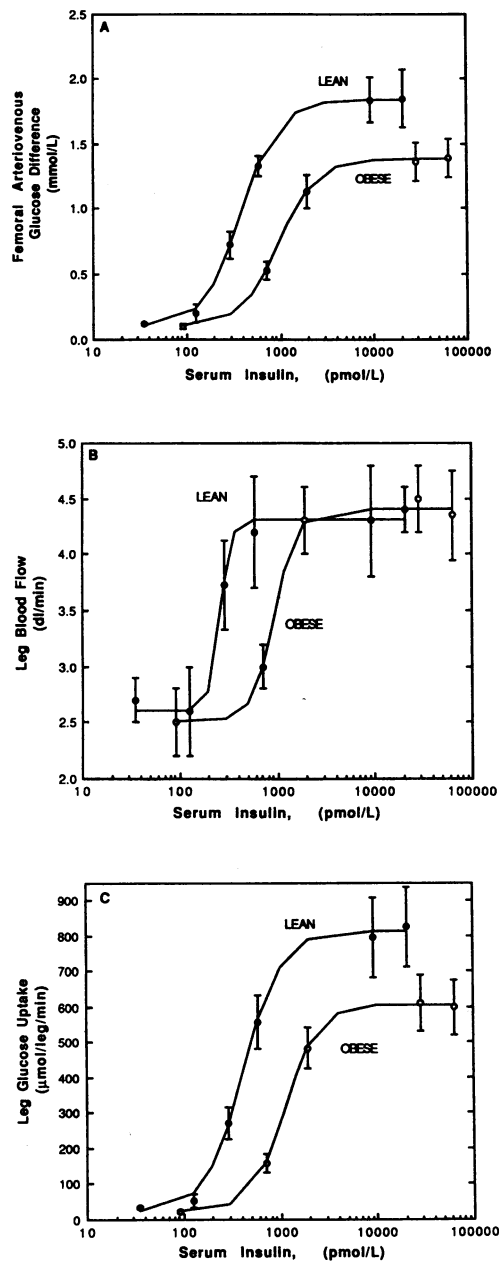


Figure 2. Femoral arteriovenous glucose difference (A), leg blood flow (B), and leg glucose uptake (C) as a function of prevailing serum insulin concentration during euglycemic clamp studies in lean (●) and obese (○) subjects. Solid lines depict the fit based on a four-parameter logistic equation.

< 0.01). The ED<sub>50</sub> for insulin's effect to stimulate leg glucose uptake was 2.7-fold in obese compared to the lean subjects (1,153 vs. 420 pmol/liter,  $P < 0.01$ ) (Table III).

### Study II

**Glucose and insulin concentrations.** The serum glucose level was clamped at  $5.0 \pm 0.1$  mmol/liter with a coefficient of variation < 4% throughout the study. The steady-state serum insulin concentrations at 60, 120, 180, and 240 min were  $524 \pm 22$ ,  $517 \pm 29$ ,  $538 \pm 29$ , and  $552 \pm 36$  pmol/liter, respectively, and did not change significantly during the clamp studies.

**Whole body and leg glucose uptake.** The basal rate of whole body glucose uptake was  $523 \pm 29$   $\mu\text{mol}/\text{m}^2$  per min and it increased by 91% during the first hour (to  $997 \pm 138$   $\mu\text{mol}/\text{m}^2$  per kg) and by 71% during the second hour of the clamp. From the second hour to the third hour whole body glucose uptake increased by 14% (from  $1,708 \pm 222$  to  $1,944 \pm 236$   $\mu\text{mol}/\text{m}^2$  per kg,  $P < 0.05$ ) but from the third hour to the fourth hour only by 4% (from  $1,944 \pm 236$  to  $2,024 \pm 217$   $\mu\text{mol}/\text{m}^2$  per kg,  $P = \text{NS}$ ). Basal blood AVGd was  $0.06 \pm 0.02$  mmol/liter. The increase in AVGd paralleled the increase in whole body glucose uptake and reached a steady-state during the third hour of the clamp (Table IV). The increase in AVGd between the third and fourth hour of the clamp was 3% (from  $1.23 \pm 0.22$  to  $1.27 \pm 0.16$  mmol/liter,  $P = \text{NS}$ ). Basal leg blood flow was  $2.5 \pm 0.3$  dl/min and increased by 36% to  $3.4 \pm 0.8$  dl/min at the first hour of the clamp, by 16% between the first and second hour of the clamp (from  $3.4 \pm 0.8$  to  $3.6 \pm 0.7$  dl/min,  $P = \text{NS}$ ), reached a steady-state during the third hour of the clamp ( $4.3 \pm 0.6$  dl/min), and remained unchanged during the fourth hour of the clamp ( $4.3 \pm 0.5$  dl/min).

To better compare the time course of blood AVGd and leg blood flow the data were expressed as the percent of the maximum increase (100%) above the basal value (no insulin infusion) (Fig. 3). AVGd was estimated on the basis of running means over four consecutive samples drawn every 5 min. Leg blood flow was measured every 30 min and running means for three consecutive measurements were calculated for each data point to smooth the biological variation in leg blood flow. The time to reach the half-maximum increase above basal was markedly shorter for AVGd ( $47 \pm 1$  min) than for leg blood flow ( $100 \pm 8$  min,  $P < 0.001$ ). Times to reach the half-maximum increase above basal for whole body and leg glucose uptake were  $57 \pm 5$  min and  $68 \pm 6$  min, respectively ( $P = \text{NS}$ ).

### Discussion

Insulin has multiple effects on target cells. Among its important in vivo effects on carbohydrate metabolism are its ability to stimulate glucose uptake, several steps of intracellular glucose metabolism, and in obesity, several of these effects have been shown to be impaired (33). In this study, we have de-

Table IV. Multiple Linear Regression Analysis of Variables Associated with Leg Blood Flow

Variable	Regression coefficient (b)	T	Significance of T
Whole body glucose uptake ( $\mu\text{mol}/\text{m}^2$ per min)	0.0007	4.74	<0.001
Free fatty acids (mmol/liter)	-2.7266	-2.46	0.017
Body mass index ( $\text{kg}/\text{m}^2$ )	-0.0049	-0.22	0.830
Lactate arteriovenous difference (mmol/liter)	0.5100	0.57	0.570

Regression analysis includes both lean and obese subjects and all data points over all insulin infusion studied.

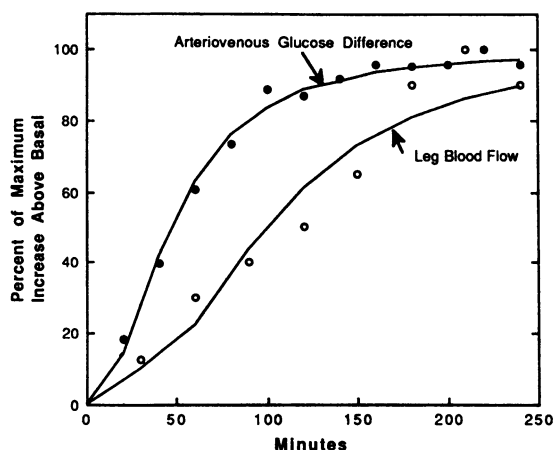


Figure 3. Time-course of femoral arteriovenous blood glucose differences (●) and leg blood flows (○) during hyperinsulinemic euglycemic clamp studies lasting 4 h. The data for AVGd and leg blood flow are expressed as percent of maximum increase (100%) above basal (no insulin infusion). Solid lines depict the fit based on a four-parameter logistic equation.

scribed a novel effect of insulin to generate increments in blood flow to insulin-sensitive tissues in a dose-responsive fashion. We have also shown that insulin's ability to generate blood flow is impaired in human obesity.

Theoretically, reduced IMGU in human obesity could be due to one or both components which determine glucose uptake in insulin sensitive tissues, namely glucose extraction (AVGd) and/or blood flow into insulin sensitive tissues. Using the glucose clamp technique various workers have observed decreased rates of whole body IMGU in human obesity (2, 6, 34). Although the precise mechanism(s) underlying this defect in insulin action is still not fully elucidated, it is generally agreed upon that skeletal muscle is the tissue responsible for decreased rates of IMGU in obese subjects (5, 6, 8, 35). Moreover, *in vivo* (2) and *in vitro* (8, 36–38) studies indicate that binding and more importantly postbinding defects in insulin action are present in obesity. Regardless of the precise abnormalities, binding and postbinding defects in insulin's action to stimulate glucose uptake in skeletal muscle would be predicted to decrease the AVGd, and have no effect on blood flow in skeletal muscle. In the current study, the decrease in rates of whole body IMGU observed in the obese group was similar to those reported in previous studies (2, 6, 34). As expected, our results obtained using the limb balance technique clearly demonstrate that skeletal muscle glucose extraction is markedly reduced in obesity. In addition, the data indicate that in obese men, insulin's physiological effect to increase blood flow to skeletal muscle is impaired. Therefore, our findings suggest that an insulin-mediated increment in skeletal muscle blood flow could be a significant determinant of *in vivo* rates of IMGU and defects in this insulin effect could be associated with *in vivo* insulin resistance.

Our analysis of the dose-response relationship between insulin's effect to increase whole body and leg glucose uptake allowed the determination of maximum values for whole body and leg glucose uptake, AVGd across the leg and leg blood flow (Table III). Rates of insulin-mediated whole body and leg glu-

cose uptake at maximally effective insulin concentrations were significantly lower in obese subjects (14% and 25%, respectively), indicating that human obesity is characterized by reduced insulin responsiveness. Maximum leg blood flow was similar in obese and lean subjects, but maximum AVGd was significantly lower in obese subjects. Therefore, in obese subjects pharmacological insulin concentrations generated near normal maximal response in leg blood flow but could not overcome the decreased ability of skeletal muscle to extract glucose.

Minimum values for whole body and leg glucose uptake, AVGd and leg blood flow were similar in lean and obese subjects (Table III). Since minimum values represent glucose uptake at 0 insulin concentration, this indicates that noninsulin-mediated glucose uptake in whole body and leg is normal in obesity, and confirms our previous findings based on the estimation of whole body glucose uptake in lean and obese subjects during SRIF-induced insulin deficiency (39).

In the current study, the  $ED_{50}$ s for insulin's effect to stimulate whole body glucose uptake, were 515 pmol/liter in lean subjects, and 1,263 pmol/ml in obese subjects. Previous similar studies of whole body insulin-mediated glucose uptake in lean subjects have resulted in similar estimates for  $ED_{50}$ s (40–42). The  $ED_{50}$  for insulin to stimulate AVGd across leg, blood flow into the leg, and leg glucose uptake were also 2.5–4-fold higher in obese than in lean subjects. Therefore, obese compared to lean subjects exhibited lower sensitivity to insulin's effect to stimulate glucose extraction, blood flow, and thus leg (skeletal muscle) glucose uptake.

If obese subjects exhibit lower sensitivity to insulin's effect to increase blood flow to skeletal muscle, how important is this impairment in insulin action with respect to skeletal muscle glucose uptake? To quantitate the impact of increases in blood flow on leg glucose uptake within the range of physiological insulin concentrations, we calculated what leg glucose uptake would have been if leg blood flow had not changed from baseline in both lean and obese groups ( $F_{fixed}$ ). These theoretical rates of leg glucose uptake were calculated by multiplying the actual blood AVGd at each insulin concentration by the blood flow rate obtained in the basal period. As seen in Fig. 4, the contribution of blood flow to leg glucose uptake was markedly different in lean and obese subjects within the physiological insulin concentration range. In lean subjects, the contribution of leg blood flow to leg glucose uptake increased exponentially up to ~ 40% at high physiological insulin levels. In contrast, in obese subjects the contribution of blood flow to leg glucose uptake was 0% up to insulin level of ~ 300 pmol/liter since no increase in leg blood flow was seen at lower insulin concentrations. At higher insulin levels, the contribution of blood flow to leg glucose uptake increased but reached only ~ 25% at insulin concentration of 800 pmol/liter. This analysis shows that the contribution of blood flow to leg glucose uptake is markedly less in obese than in lean subjects within physiological insulin range. Since the correlation of leg glucose uptake and whole body glucose uptake (individual data points) was high both in lean and obese subjects ( $r = 0.93$ ,  $P < 0.001$ ,  $r = 0.94$ ,  $P < 0.001$ , respectively) these results strongly suggest that increases in blood flow to leg skeletal muscle are likely to reflect increases in blood flow to skeletal muscle throughout the body. Therefore, it follows that the inability of insulin to increase blood flow to insulin-sensitive tissues in obese sub-

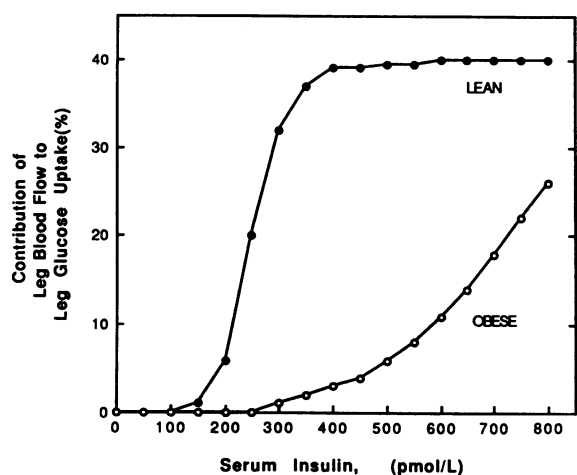


Figure 4. Contribution of leg blood flow to leg glucose uptake (in percent) in lean (●) and obese (○) subjects within physiological insulin concentrations. Contribution of blood flow to leg glucose uptake was calculated from the formula:  $(AVGd_{obs} \times F_{obs} - AVGd_{obs} \times F_{fixed}) / (AVGd_{obs} \times F_{obs}) \times 100$ , where  $AVGd_{obs}$  and  $F_{obs}$  are observed femoral arteriovenous blood glucose difference and leg blood flow, respectively, and fixed leg blood flow in the fasting state.  $AVGd_{obs} \times F_{obs}$  for lean and obese subjects at each insulin concentration were obtained by a four-parameter logistic equation (32) on the basis of data given in Table III.

jects contributes significantly to the decrease in insulin sensitivity in these subjects.

Significant differences were found in the time course of stimulation of whole body glucose uptake, blood AVGD, and leg blood flow during constant hyperinsulinemia in lean subjects (study II). The time to reach the half-maximum increase above basal for flow ( $\sim 100$  min) was substantially slower than that of whole body glucose uptake ( $\sim 60$  min) and AVGD ( $\sim 50$  min). These results have important implications. Although the increases in AVGD and leg blood flow are tightly coupled, our findings demonstrate that during the first 60 min after the initiation of the insulin infusion, the increase in the rates of whole body glucose uptake in lean subjects is determined more by the increments in AVGD than blood flow. In contrast, after 2 h of hyperinsulinemia the increase in whole body glucose uptake was more due to mechanisms associated with an increase in blood flow than to AVGD. This late increase in blood flow is likely to be an explanation for the progressive increase in rates of whole body glucose uptake during prolonged clamp studies as previously reported (43).

The most likely mechanism by which blood flow increases skeletal muscle IMGU is via the opening of previously underperfused or nonperfused capillaries (capillary recruitment) thus directly exposing more muscle cells to insulin and glucose. In the resting state most capillaries are unperfused and a large intercapillary distance determines the ability of insulin and glucose to equilibrate with muscle tissue (44, 45). This leads to a large insulin concentration (or insulin action) gradient between the capillary and its surrounding muscle tissue. As the distance between the feeding capillary decreases (capillary recruitment), the insulin concentration gradient between capillary and surrounding muscle decreases, leading to greater stimulation of glucose uptake by muscle cells. In support of

this concept, we have recently noted that an increase in leg blood flow was accompanied by commensurate changes in both vascular (capillary recruitment) and extravascular (tissue recruitment) distribution volume of glucose (46). This notion is consistent with the recent findings by others (27, 47) of an effect of insulin to increase the volume distribution of glucose.

To investigate the mechanistic link between insulin and blood flow, we studied some of the possible variables associated with leg blood flow by multiple regression analysis (Table IV). Whole body glucose uptake was the most important single factor associated with leg blood flow independently of obesity (BMI), arterial FFA levels, and lactate arteriovenous glucose uptake, and leg blood flow was similar in lean and obese subjects. In addition to the high correlation between whole body glucose uptake and leg blood flow (Fig. 5) in both lean ( $r = 0.91$ ,  $P < 0.01$ ) and obese subjects ( $r = 0.99$ ,  $P < 0.01$ ), the slope of the regression line was similar in both groups (lean subjects:  $flow = 2.6 + 0.00072 \cdot \text{glucose uptake}$ ; obese subjects:  $flow = 2.1 + 0.00103 \cdot \text{glucose uptake}$ ,  $P = \text{NS}$  between the slopes of regression lines). This indicates that similar increases in whole body glucose uptake were associated with similar increases in the rates of leg blood flow in both lean and obese subjects. Therefore, the data indicate that increments in glucose metabolism and the generation of increments in blood flow are tightly coupled. However, statistical analysis cannot distinguish whether glucose metabolism precedes or determines the generation of blood flow or vice versa. The current data demonstrating that the time course of insulin's effect to activate AVGD is substantially shorter than that for leg blood flow (study II) supports the notion that glucose metabolism precedes the generation of flow response. Thus, upon insulin stimulation one sees an initial rise in glucose extraction which could secondarily lead to a generation of a signal to increase blood flow rates. The notion that metabolic demand (as in exercise or hypoxia) is a signal or mechanism to increase blood flow in metabolically active tissue is well-described (48, 49). Thus, it is likely that the stimulation of metabolic activity in some intracellular pathway of glucose metabolism (glucose oxidation, nonoxidative glycolysis) induces directly or indirectly an increase in blood flow. We did not measure glucose oxidation or nonoxidative glycolysis, but the lactate arteriovenous difference which reflects the activity of the nonoxidative

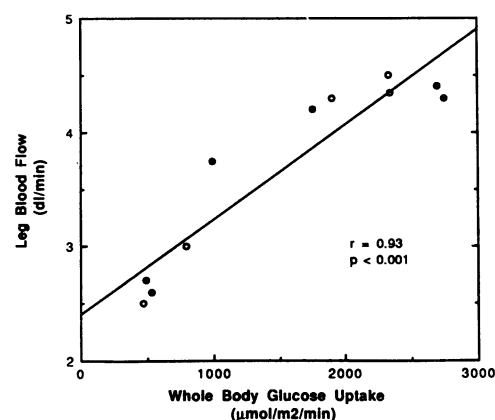


Figure 5. Correlation between whole body glucose uptake and leg blood flow in lean (●) and obese (○) subjects.



glycolytic pathway did not associate significantly with blood flow rates independently of obesity, FFA, or whole body glucose uptake (Table IV). Therefore, the activity of the glycolytic pathway is unlikely to explain our findings. That glucose oxidation is a possible modulator of blood flow within physiological insulin concentrations is supported by the study of Thibaud et al. (41). In their study, the ED<sub>50</sub> for insulin stimulation of whole body glucose uptake was remarkably similar (517 pmol/liter) to our study (515 pmol/liter), and the ED<sub>50</sub> for stimulation of glucose oxidation was 287 pmol/liter, quite similar to ED<sub>50</sub> stimulation of blood flow (266 pmol/liter) in our study. In accordance with this hypothesis is our finding that FFA concentrations were negatively and inversely associated with blood flow rates independently of obesity (Table IV). Because FFA concentrations are inversely related to glucose oxidation (50–52) it is possible that the inverse association between blood flow rates and FFA reflects indirectly the association between blood flow and glucose oxidation. Finally, it is possible that insulin has a direct vasodilatory effect thereby increasing blood flow and insulin resistance to this effect is the cause for diminished blood flow rates in human obesity.

In summary, we have shown that (a) insulin increases skeletal muscle blood flow about twofold in a dose-dependent fashion over basal both in lean and obese subjects, (b) the dose-response curve for insulin's effect to increase skeletal muscle blood flow is right-shifted in obese subjects with an ED<sub>50</sub> about fourfold that in lean subjects, and (c) differences in blood flow rates to skeletal muscle were most marked within physiological insulin range and thus contributed significantly to lower leg and whole body muscle glucose uptake in obese man. In conclusion, reduced insulin-mediated glucose uptake in human obesity is due to defects in both insulin's ability to increase glucose extraction in insulin-sensitive tissues and in its action to increase blood flow to these tissues. This latter defect is a novel mechanism of insulin resistance and may be generalized to insulin resistant states other than obesity.

## Acknowledgments

The authors thank Kate Petrey for expert secretarial assistance.

This work was supported in part by grants MO1 RR-00827 from the General Clinical Research Center Branch, National Institutes of Health; The American Diabetes Association; The Diabetes Education and Research Foundation; and a grant from The Juvenile Diabetes Foundation. Dr. Edelman was the recipient of a National Research Service Award, National Institutes of Health. Dr. Laakso was a visiting scientist from the University of Kuopio, Finland and a recipient of an International Research Fellowship grant TW 03969-01, The Fogarty International Center, National Institutes of Health.

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