

Rates of Skeletal Muscle and Adipose Tissue Glycerol Release in Nonobese and Obese Subjects

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Skeletal muscle and adipose tissue lipolysis rates were quantitatively compared in 12 healthy nonobese and 14 insulin-resistant obese subjects for 3.5 h after an oral glucose load using microdialysis measurements of interstitial glycerol concentrations and determinations of local blood flow with ^{133}Xe clearance in the gastrocnemius muscle and in abdominal subcutaneous adipose tissue. Together with measurements of arterialized venous plasma glycerol, the absolute rates of glycerol mobilization were estimated. In the basal state, skeletal muscle and adipose tissue glycerol levels were 50% higher ($P < 0.05$ – 0.01) and adipose tissue blood flow (ATBF) and muscle blood flow (MBF) rates were 30–40% lower ($P < 0.02$ – 0.05) in obese versus nonobese subjects. After glucose ingestion, adipose tissue glycerol levels were rapidly and transiently reduced, whereas in muscle, a progressive and less pronounced fall in glycerol levels was evident. MBF remained unchanged in both study groups, whereas ATBF increased more markedly ($P < 0.01$) in the nonobese versus obese subjects after the oral glucose load. The fasting rates of glycerol release per unit of tissue weight from skeletal muscle were between 20 and 25% of that from adipose tissue in both groups. After glucose ingestion, the rates of glycerol release from skeletal muscle and from adipose tissue were almost identical in nonobese and obese subjects. However, the kinetic patterns differed markedly between tissues; in adipose tissue, the rate of glycerol mobilization was suppressed by 25–30% ($P < 0.05$) after glucose ingestion, whereas no significant reduction was registered in skeletal muscle. We conclude that significant amounts of glycerol are released from skeletal muscle, which suggests that muscle lipolysis provides an important endogenous energy source in humans. In response to glucose ingestion, the regulation of skeletal muscle glycerol release differs from that in adipose tissue; although the rate of glycerol release from adipose tissue is clearly suppressed, the rate of glycerol mobilization from skeletal muscle remains unaltered. In quantitative terms, the rate of glycerol release per unit of tissue weight in adipose tissue and in skeletal muscle is similar in nonobese and obese subjects in both the postabsorptive state and after glucose ingestion. *Diabetes* 49:797–802, 2000

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ANOVA, analysis of variance; ATBF, adipose tissue blood flow; FFA, free fatty acid; MBF, muscle blood flow; TG, triglyceride.

Skeletal muscle insulin resistance, as reflected by attenuated insulin-mediated glucose disposal, is a key finding of the various clinical components of the metabolic syndrome (e.g., obesity, dyslipidemia, hypertension, and type 2 diabetes) and thus is a predisposing factor in the pathogenesis of premature atherosclerotic cardiovascular disease (1). Among the putative mechanisms underlying insulin resistance, increased attention has been paid to elevated circulating concentrations of free fatty acids (FFAs). We have known for many years that an inverse relationship exists regarding the use of lipids and carbohydrates as metabolic fuels and that inappropriate elevation of FFA levels mediates an impairment of muscle glucose utilization (2); more recently, this finding has been verified in several experimental studies in humans (3–9).

In humans, the deposition of triglycerides (TGs) as energy stores and the liberation of FFAs (and glycerol) via hydrolysis (lipolysis) of TGs during increased energy demands have traditionally been thought to occur only in adipose tissue. Recently, however, a study showed that TGs are also deposited in skeletal muscle in humans (10). Moreover, very high concentrations of glycerol have been demonstrated in muscle *in vivo* (11), which are indicative of skeletal muscle lipolytic activity.

The physiological significance of human skeletal muscle lipolysis is not known, although estimates indicate that as much as 50% of the FFAs oxidized in muscle during exercise originate from intramuscular lipolysis (12,13). Furthermore, an inverse relationship between the amount of TG deposited in muscle and sensitivity to insulin-induced glucose uptake has been recently observed in the normal state and in various insulin-resistant conditions (14–18). These findings suggest that FFAs derived from skeletal muscle lipolysis may be an important endogenous energy source and indicate that increased TG deposition and enhanced lipolysis and FFA mobilization/oxidation in skeletal muscle are important in the pathogenesis of peripheral insulin resistance via interactions between glucose and FFA utilization in muscle cells. However, the exact role of skeletal muscle lipolysis in insulin-resistant conditions remains to be elucidated. In the present study, we investigated *in vivo* lipolysis rates in skeletal muscle and subcutaneous adipose tissue in nonobese and obese subjects using microdialysis measurements of glycerol concentrations *in situ* and simultaneous determinations of local blood flow with the ^{133}Xe clearance technique in the 2 tissues. With this combined methodology, determining the absolute rate of glycerol release is possible, which reflects lipolytic activity in the tissue (19). The recordings were made both in

the postabsorptive state and after an oral glucose load. Thus, the aim was to quantitatively compare lipolysis rates in the 2 tissues in the fasting and postprandial states in healthy nonobese and insulin-resistant obese subjects.

RESEARCH DESIGN AND METHODS

Subjects. The study consisted of 12 nonobese subjects (5 men and 7 women, 40 ± 2 years of age, BMI 24.0 ± 0.5 kg/m²), and 14 obese subjects (6 men and 8 women, 36 ± 3 years of age, BMI 37.1 ± 1.5 kg/m²). All subjects were drug free. They all performed regular physical activity but not at an athletic level. The study was approved by the Ethics Committee of the Karolinska Institute. The subjects were given a detailed description of the study before they gave their informed consent.

Study protocol and microdialysis procedures. The subjects were investigated at 8:00 A.M. after an overnight fast. First, body composition (fat and lean body masses) was determined with bioelectrical impedance analysis as previously described (20). Thereafter, experiments were carried out in a quiet room with a constant temperature of 23°C. The subjects remained in a supine position throughout the study period.

A retrograde catheter was inserted in a dorsal vein in the hand that was then placed in a heated box (63°C) for sampling of arterialized venous blood (21).

The principles of the microdialysis technique (19) and the microdialysis device (22) have been described in detail previously. After superficial skin anesthesia (EMLA; Astra, Södertälje, Sweden), a microdialysis catheter (CMA/60; CMA/Microdialysis AB, Stockholm, Sweden) with a semipermeable polyamide membrane (30×0.62 mm, molecular mass cutoff of 20 kDa) was inserted with a steel guide cannula into the periumbilical subcutaneous adipose tissue ~8 cm lateral to the umbilicus. A second microdialysis catheter was inserted percutaneously (after skin anesthesia) in the medial portion of the gastrocnemius muscle. Penetration of the muscle fascia was easily recognized during the insertion procedure, and the intramuscular placement of the microdialysis catheter was ascertained by the occurrence of involuntary muscle twitches. The catheters were connected to microinfusion pumps (CMA/106) and were continuously perfused with Ringer's solution (147 mmol/l Na⁺, 4 mmol/l K⁺, 2.20 mmol/l Ca²⁺, 156 mmol/l Cl⁻; Apoteksbolaget, Umeå, Sweden) at a flow rate of 0.3 µl/min. With an isotope method for estimating microdialysis recovery, we have previously shown that glycerol recovery is almost complete (~95%) and is similar in adipose tissue and skeletal muscle at this flow rate (23). After a 60-min equilibration period, dialysate samples were collected every 30 min for glycerol determinations. Arterialized venous blood was drawn in the middle of each 30-min period for determinations of hematocrit and plasma glycerol. Arterialization was confirmed by blood gas analysis (>95% O₂ saturation). After a 60-min baseline sampling period, the subjects were given a 75-g oral glucose load. Thereafter, dialysate and plasma samples were collected during a 210-min period.

Blood flow measurements. Adipose tissue blood flow (ATBF) and skeletal muscle blood flow (MBF) rates were determined before and after the oral glucose load with the ¹³³Xe clearance technique (24). In adipose tissue, ¹³³Xe (1 MBq in 0.1 ml saline containing no preservatives; Mallinckrodt, Petten, the Netherlands) was injected percutaneously into the subcutaneous tissue in the contralateral abdominal side (i.e., the side not containing a microdialysis catheter) 90 min before glucose administration. The injection needle was kept in position for 30 s after ¹³³Xe injection to avoid retrograde leakage of the isotope. After a 30-min equilibration period, the residual activity was continuously monitored externally during the study period with a portable scintillation detector (Mediscint; Oakfield Instruments, Eynsham, U.K.) as previously described by Samra et al. (25). In skeletal muscle, using ¹³³Xe clearance for estimating blood flow for extended periods is not possible because the ¹³³Xe decay curve in muscle gradually becomes multiexponential, probably as a result of venoarteriolar shunting by diffusion of ¹³³Xe (26). However, studies have shown that skeletal MBF can be correctly assessed from the initial part of the ¹³³Xe washout curve (26). Consequently, repeated intramuscular depositions of ¹³³Xe must be performed to estimate skeletal MBF for longer periods. In the present study, ¹³³Xe (0.3 MBq in 0.1 ml saline) was injected in the medial portion of the gastrocnemius muscle (contralateral to the microdialyzed muscle) 35 min before the oral glucose load. At 55 min after glucose ingestion, another injection of ¹³³Xe (0.3 MBq) was given in the contralateral gastrocnemius muscle. In addition, in 5 of the nonobese and in 8 of the obese subjects, a third intramuscular ¹³³Xe injection was deposited at 115 min after the glucose load. The latter deposition of ¹³³Xe was given in the same muscle as the first ¹³³Xe injection; the residual activity in the muscle before the repeated isotope injection was negligible. After each intramuscular deposition of ¹³³Xe, 5 min were allowed to elapse for equilibration. Thereafter, residual activity was monitored for 10 min as described above.

Counts were accumulated during consecutive 60-s intervals and were plotted on a semilogarithmic diagram as a function of time. The first order rate constants (*k*) were calculated by linear regression analysis. In adipose tissue, rate constants were assessed for consecutive 30-min periods throughout the experiment and were expressed as the fractional decay per min. In skeletal muscle, the corresponding calculation was made during a 10-min period after each ¹³³Xe injection.

ATBF and skeletal MBF rates were thereafter calculated according to the following formula: $k \times \lambda \times 100$ (ml · 100 g⁻¹ · min⁻¹), where λ denotes the tissue-to-blood partition coefficient. The values for λ in adipose tissue and skeletal muscle were set at 10 and 0.7 ml/g, respectively (24,27).

Estimation of glycerol release. The absolute rates of glycerol release from adipose tissue and skeletal muscle were calculated according to Fick's principle in which arterialized venous plasma (*A*) and capillary venous plasma (*V*) concentrations of glycerol and plasma flow rate (*Q*) are entered into the following formula: $(V - A) \times Q \times (1 - \text{hematocrit} \times 10^{-2})$ (µmol · 100 g⁻¹ · min⁻¹). Conversion of interstitial (*I*) to venous glycerol (*V*) concentrations were made according to the following equation: $V = (I - A) \times (1 - e^{-PS/Q}) + A$, where *PS* denotes the permeability surface product area (approximated to 5 ml · 100 g⁻¹ · min⁻¹ [28,29]). In adipose tissue, the rate of glycerol release was calculated for each 30-min period during the study using the corresponding ATBF measurement. In skeletal muscle, the mean value of the repetitive MBF determinations was used in the calculations because no change was evident in MBF before and after the glucose challenge (see RESULTS).

Biochemical analyses. Dialysate glycerol was measured with an enzymatic fluorometric method with an automatic tissue dialysate sample analyzer (CMA/600) as described previously (30). Plasma glycerol was determined with bioluminescence (31). Plasma insulin was measured with a commercial radioimmunoassay (Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol (32). Hematocrit and O₂ saturation were determined by the hospital's routine clinical chemistry department.

Statistics. Data are means ± SE. Baseline and steady-state values were compared with Student's *t* test with the paired or unpaired *t* test when applicable. Variations over time in the same compartment were evaluated by one-factor analysis of variance (ANOVA) for repeated measurements. Comparisons over time between groups were analyzed by 2-factor ANOVA corrected for repeated measurements. Post hoc analysis by Fisher's protected least significant difference test was used to compare different time points. When comparisons did not involve time, differences between more than 2 groups were calculated with factorial ANOVA. All statistical calculations were made with a statistical software package (StatView II; Abacus Concepts, Berkeley, CA). A value of *P* < 0.05 was considered statistically significant.

RESULTS

The total body fat masses for the nonobese and obese subjects were 19.4 ± 1.5 and 53.9 ± 5.6 kg, respectively (*P* < 0.0001). The corresponding values for lean body mass were 52.6 ± 3.4 and 54.0 ± 3.6 kg (NS).

Plasma glucose and insulin levels in the postabsorptive state and after oral glucose administration for both the nonobese and obese subjects are shown in Fig. 1. Baseline plasma glucose concentrations and the changes in plasma glucose after the oral glucose challenge were almost identical in the 2 study groups. However, basal plasma insulin levels were twice as high in the obese subjects compared with the nonobese subjects (*P* < 0.01). Moreover, the increase in insulin levels after glucose ingestion was much more pronounced (*P* < 0.001 by ANOVA) in the former group, which indicates insulin resistance on glucose metabolism in the obese subjects.

The glycerol concentrations in arterialized venous plasma, adipose tissue, and skeletal muscle before and after the oral glucose load are shown in Fig. 2. In the fasting state, glycerol levels in all 3 compartments were ~50% higher in the obese compared with the nonobese subjects (*P* < 0.05–0.01). In both study groups, significant differences were evident among the basal glycerol levels in the 3 compartments. The concentrations of glycerol were 4–5 times higher in adipose tissue and were twice as high in muscle compared with

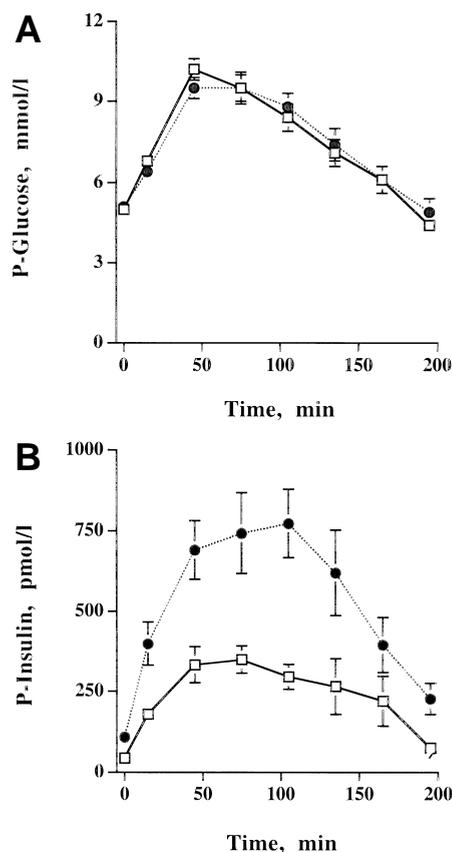


FIG. 1. Glucose (P-Glucose; **A**) and insulin (P-Insulin; **B**) concentrations in arterialized venous plasma before and after a 75-g oral glucose load in 12 nonobese (□) and 14 obese subjects (●). Data are means \pm SE.

plasma ($P < 0.001$ by factorial ANOVA). After glucose ingestion, both groups showed a marked and parallel decrease in glycerol levels in adipose tissue and arterialized venous plasma to a plateau of approximately half of the baseline concentrations. During the last hour of the experimental period, both plasma and adipose tissue glycerol gradually increased toward basal levels. In contrast, skeletal muscle experienced a less pronounced and more protracted decrease in glycerol concentrations, which did not level off until the last 90 min of the study period. At the end of the experiment, muscle glycerol levels were still markedly suppressed. Throughout the experiment, glycerol levels in the 3 compartments were significantly higher in the obese versus the nonobese subjects ($P < 0.05$ by ANOVA).

Figure 3 depicts the blood flow determinations in adipose tissue and skeletal muscle. In the fasting state, ATBF and skeletal MBF rates were 60–70% higher in the nonobese versus the obese subjects ($P < 0.05$ for ATBF; $P < 0.02$ for MBF). No significant differences were evident between basal ATBF and MBF within each study group. In the nonobese subjects, ATBF increased rapidly after glucose ingestion and remained significantly elevated between 45 and 135 min after the oral glucose load ($P < 0.0001$ by ANOVA). In the obese subjects, the corresponding increase in ATBF was only significant between 45 and 105 min after glucose ingestion ($P < 0.05$ by ANOVA). After the oral glucose load, ATBF remained higher in the nonobese group compared with the obese group ($P <$

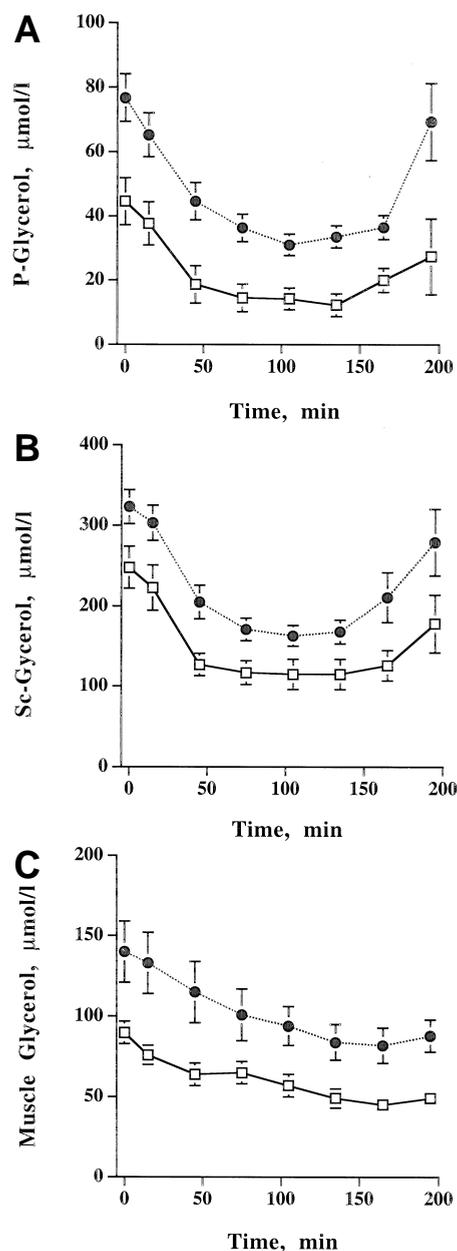


FIG. 2. Glycerol concentrations in arterialized venous plasma (P-Glycerol; **A**), subcutaneous adipose tissue (Sc-Glycerol; **B**), and skeletal muscle (Muscle Glycerol; **C**) before and after glucose ingestion in 12 nonobese (□) and 14 obese subjects (●). See the legend for Fig. 1 for further details.

0.01 by ANOVA). In both study groups, MBF was similar before and at 60 min after the oral glucose load. Likewise, in the 5 nonobese and 8 obese subjects in whom MBF was also recorded 120 min after glucose ingestion, no significant change was evident in blood flow rates (data not shown).

The rates of glycerol mobilization from adipose tissue and skeletal muscle are shown in Fig. 4. In the fasting state and after the oral glucose challenge, no significant differences were evident in the rates of glycerol release between nonobese and obese subjects in either adipose tissue or skeletal muscle. In quantitative terms, glycerol release from skeletal muscle amounted to 20–25% of that from adipose tissue in the 2 groups ($P < 0.001$). After glucose ingestion, glycerol

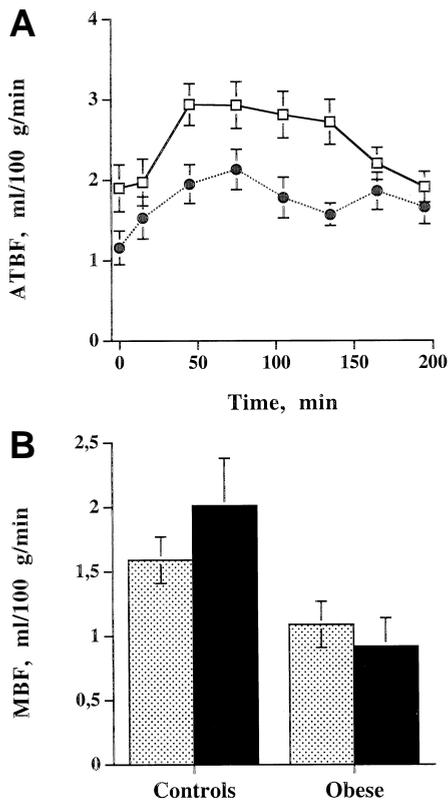


FIG. 3. ATBF (**A**) and MBF (**B**) rates before and after glucose ingestion in 12 nonobese (□) and 14 obese subjects (●). ATBF was continuously monitored during the experiment. MBF was recorded in the basal state (▨) and at 60 min after the glucose challenge (■). See the legend for Fig. 1 for further details.

release in adipose tissue gradually decreased to a plateau of ~65% of the fasting rate (nonobese subjects, $P < 0.02$; obese subjects, $P < 0.05$ by ANOVA); during the last hour of the experimental period, glycerol release increased toward basal levels. In skeletal muscle, no significant decrease was evident in the rate of glycerol release during the first 2 h after glucose ingestion. Thereafter, glycerol release was gradually reduced by approximately half during the last hour of the experiment ($P < 0.0001$ in both groups by ANOVA).

The rates of glycerol release from adipose tissue and skeletal muscle were also calculated during apparent steady-state conditions (i.e., in the basal state and during specific time periods after glucose ingestion during which no statistically significant changes were evident in blood flow or in arterialized venous plasma and interstitial glycerol concentrations in the 2 respective tissue compartments) (Table 1). In adipose tissue, all of these parameters remained unchanged at 75–105 min after the oral glucose load in both study groups. During this time, the average rates of glycerol release were reduced by ~30 and 25% compared with postabsorptive values in the nonobese and obese subjects, respectively ($P < 0.05$ within both groups, NS between groups). In skeletal muscle, a corresponding steady-state period was ascertained at 135–165 min after the oral glucose load. In both study groups, no significant differences were evident between the mean rates of skeletal muscle glycerol release in the fasting state and during steady-state conditions after the oral glucose load (Table 1).

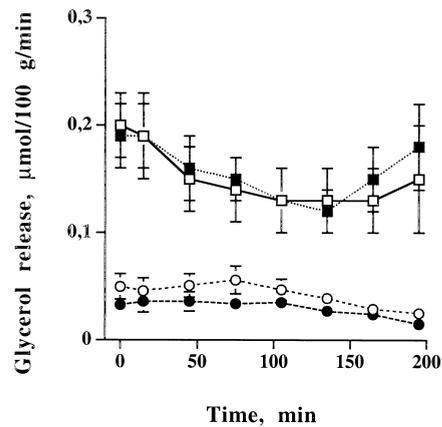


FIG. 4. Rates of glycerol release from skeletal muscle (●, ○) and adipose tissue (■, □) before and after glucose ingestion in 12 nonobese (□, ○) and 14 obese subjects (■, ●). See the legend for Fig. 1 for further details.

DISCUSSION

In the present study, we have for the first time quantified the rate of glycerol release from skeletal muscle in vivo in healthy nonobese subjects and in insulin-resistant obese subjects. A comparison was also made with adipose tissue lipolysis rates. This was accomplished by combining microdialysis measurements of absolute tissue interstitial glycerol concentrations (23) with determinations of local blood flow rates using the ^{133}Xe washout technique. Together with measurements of arterial (i.e., arterialized venous) plasma glycerol, the mobilization of glycerol was then calculated according to Fick's principle. This methodology was introduced by Jansson et al. (33) for estimating glycerol production in adipose tissue in humans. Our study shows that combining the techniques for assessment of skeletal muscle lipolysis is also feasible.

For many years, the ^{133}Xe clearance technique has been used for determining blood flow rates in adipose tissue (24) and skeletal muscle (27) in humans. However, in skeletal muscle, blood flow can only be correctly recorded shortly after ^{133}Xe deposition. Thereafter, the isotope decay curves in muscle gradually become multiexponential, which leads to underestimation of blood flow rates (26). Consequently, continuously monitoring MBF in the same way as ATBF throughout the experiments was not possible. However, when MBF was determined before and at 1 h (and in a subset of the nonobese and obese subjects also at 2 h) after glucose ingestion, no differences in blood flow rates over time were registered. Consistent with previous observations (34,35), this finding suggests that no change occurs in MBF in response to an oral glucose challenge. In contrast, in adipose tissue, a marked increase was evident in blood flow rates in the nonobese subjects that paralleled the elevation in circulating insulin levels. This was an expected finding because of the well-recognized fact that ATBF in humans is stimulated by carbohydrate intake (33–36). In the obese subjects, on the other hand, basal MBF and ATBF rates were significantly lower than in the nonobese study group. The increase in ATBF after glucose ingestion was also less pronounced. Similar observations have been reported by others (24,33,37–39), which may indicate an association between obesity and defective blood flow regulation in adipose tissue and skeletal muscle in humans.

TABLE 1
Rates of adipose tissue and skeletal muscle glycerol release during steady-state conditions

Subjects	Adipose tissue ($\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$)			Skeletal muscle ($\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$)		
	Basal state	75–105 min after glucose	<i>P</i>	Basal state	135–165 min after glucose	<i>P</i>
Nonobese	0.196 ± 0.035	0.138 ± 0.028	<0.05	0.050 ± 0.012	0.046 ± 0.012	NS
Obese	0.171 ± 0.031	0.130 ± 0.023	<0.05	0.033 ± 0.008	0.025 ± 0.006	NS
<i>P</i>	NS	NS	—	NS	NS	—

Data are means ± SE. Mean rates of adipose tissue and skeletal muscle glycerol release were calculated in 12 nonobese and 14 obese subjects in the basal state (–60 to 0 min) and over the specified time periods after glucose ingestion during which no statistically significant changes were evident in blood flow or arterialized venous plasma and interstitial glycerol concentrations in the 2 tissue compartments.

In the obese subjects, the interstitial levels of glycerol in both skeletal muscle and adipose tissue were ~50% higher than the corresponding concentrations for the nonobese subjects. Whether this was mainly because of the lower tissue blood flow rates that resulted in decreased removal of glycerol or the enhanced lipolytic activity in the 2 tissue compartments in the obese subjects is not known. Nevertheless, in absolute terms, glycerol release from both adipose tissue and skeletal muscle was almost identical in the nonobese and obese subjects in the fasting state and after glucose ingestion. In this context, note that the Fick principle for estimating rates of glycerol mobilization is only strictly applicable when no changes are evident in blood flow or substrate concentrations across the sampling bed during the observation period. Therefore, we also calculated rates of glycerol release from the 2 tissue compartments during apparent steady-state conditions (Table 1). Findings from the latter estimations confirmed comparable rates of glycerol release from adipose tissue and from skeletal muscle in the 2 study groups in the postabsorptive state and after glucose ingestion. Our data support previous findings that glycerol production in adipose tissue per unit of tissue weight is comparable in nonobese and obese subjects (33), and our data demonstrate for the first time that the same is also true for skeletal muscle glycerol release. This study also revealed that the fasting rate of glycerol release in terms of tissue weight from skeletal muscle *in vivo* amounts to as much as 20–25% of that from subcutaneous adipose tissue. That differences exist in the rate of glycerol mobilization from various fat deposits is well known (40). Whether the same is true for skeletal muscle lipolysis is not known. Although the hazards of extrapolating data must be taken into account, our findings imply an even greater effect of skeletal muscle lipolysis for total-body glycerol production in the normal state when muscle and adipose tissue masses are considered. In a normal-weight subject, skeletal muscle mass equals ~30 kg (41). This is ~50% more than the presently determined fat mass in the nonobese study group. In the obese subjects, on the other hand, adipose tissue is probably much more important than skeletal muscle for the total rate of glycerol release because of the pronounced increase in total fat mass (in this study, ~54 kg). Accordingly, increased adipose tissue mass provides the most likely explanation for the increased circulating plasma glycerol levels that we observed in the obese subjects in the fasting state and after the oral glucose load.

Our results also strongly indicate that the regulation of skeletal muscle glycerol release differs from that in adipose tissue. In muscle, a slow and gradual decrease was evident in glycerol levels after glucose ingestion but not a significant sup-

pression of the rate of skeletal muscle glycerol release in steady-state conditions after the oral glucose load. In contrast, in adipose tissue, we observed a transient reduction of glycerol concentrations and a significant 25–30% reduction in the rate of glycerol mobilization that paralleled changes in circulating insulin. We recently reported similar differences between skeletal muscle and adipose tissue glycerol kinetics after an oral glucose challenge in nonobese subjects (35). The present study shows that the same phenomenon exists in obese subjects. Recent data have shown that different phosphodiesterase subtypes mediate insulin-induced antilipolysis in muscle and fat tissue *in vivo* (42), which may indicate tissue-specific variations in the inhibition of lipolysis between skeletal muscle and adipose tissue. Oral glucose ingestion apparently suppresses the lipolysis rate in adipose tissue, although skeletal muscle seems unresponsive in this regard. We do not know the molecular basis for this tissue difference; it may be coupled to the 2 insulin-sensitive lipases, hormone-sensitive lipase and lipoprotein lipase. Unfortunately, separating the effects of these enzymes on extra- and intracellular TG hydrolysis by means of microdialysis is not possible. Differences between skeletal muscle and adipose tissue glycerol utilization do not likely explain the observations. Although fat tissue cannot reutilize glycerol, a minor uptake of glycerol in muscle cannot be completely disregarded (43,44). However, enhanced glycerol utilization by muscle should cause a more rapid (rather than slower) reduction in skeletal muscle interstitial glycerol levels.

The results of this study clearly demonstrate the necessity to combine the microdialysis method with determinations of local blood flow to correctly interpret changes in tissue metabolite levels. If we had considered only plasma and tissue glycerol concentrations, then our data would have implied that the rate of lipolysis is increased in adipose tissue and skeletal muscle and that the lipolytic activities in these tissues are not regulated in a normal way after glucose ingestion in human obesity. When tissue blood flow rates are included in the analysis, the rates of glycerol mobilization from both tissues are essentially normal, and glycerol release is regulated in a normal way in response to glucose ingestion in insulin-resistant obese subjects.

In summary, the results of this study show that significant amounts of glycerol are released from skeletal muscle, which suggests that muscle lipolysis constitutes an important endogenous energy source in humans. Although muscle lipolysis rates appear to be of similar magnitude in nonobese and obese subjects in the fasting state and after glucose ingestion, glycerol mobilization from adipose tissue pre-

dominates in obesity as a result of increased total fat mass. The data also indicate that, in both nonobese and obese subjects, lipolysis in skeletal muscle and adipose tissue is regulated in different ways in response to glucose ingestion and enhanced endogenous insulin secretion. In the latter situation, glycerol release from adipose tissue is clearly suppressed, whereas the rate of glycerol mobilization from skeletal muscle remains unaffected.

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