No apparent suppression by insulin of in vivo skeletal muscle lipolysis in nonobese women

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Moberg, Erik, Stefan Sjöberg, Eva Hagström-Toft, and Jan Bolinder. No apparent suppression by insulin of in vivo skeletal muscle lipolysis in nonobese women. Am J Physiol Endocrinol Metab 283: E295–E301, 2002; 10.1152/ajpendo.00339.2001.—To investigate the antilipolytic effect of insulin in skeletal muscle and adipose tissue in vivo, the rates of glycerol release from the two tissues were compared in 10 nonobese women during a two-step euglycemic hyperinsulinemic clamp. Tissue interstitial glycerol levels were determined by microdialysis, and tissue blood flow was assessed with the \(^{133}\)Xe clearance technique. Absolute rates of glycerol release were estimated according to Fick’s principle. In both adipose tissue and muscle, glycerol levels decreased significantly already during the low insulin infusion rate. The fractional release of glycerol (difference between interstitial glycerol and arterialized venous plasma glycerol) was reduced by more than one-half in adipose tissue (\(P<0.0001\)) in response to insulin, whereas it remained unaltered in skeletal muscle. Muscle blood flow rates increased by 60% (\(P<0.02\)) during insulin infusion; in adipose tissue, blood flow rates did not change significantly in response to insulin. The basal rate of glycerol release from skeletal muscle amounted to \(15\%\) of that from adipose tissue. After insulin infusion, the rate of adipose tissue glycerol release was markedly suppressed, whereas in skeletal muscle the rate of glycerol mobilization did not change significantly in response to insulin. It is concluded that insulin does not inhibit the rate of lipolysis in skeletal muscle of nonobese women.

glycerol; microdialysis; blood flow

RESISTANCE TO INSULIN-STIMULATED glucose disposal in skeletal muscle is a key finding in type 2 diabetes mellitus as well as in other clinical manifestations of the metabolic syndrome such as hypertension, dyslipidemia, and obesity. Although the exact mechanism underlying peripheral insulin resistance is not clarified, competition between free fatty acids (FFA) and glucose as metabolic fuels, the so-called Randle cycle, may play an important role (37). Accordingly, several studies have demonstrated that high levels of FFA in the circulation induce an impaired glucose utilization in muscle (2, 38–40, 42, 48, 49). FFA liberated from intramuscular triglycerides may also be important, however, since an inverse relationship between intramuscular lipid content and insulin sensitivity has recently been reported in various insulin-resistant conditions (13, 18, 23, 26, 34, 35). This may indicate that disturbed hydrolysis of intramuscular triglycerides (lipolysis) is important for the development of insulin resistance.

In vivo intramuscular lipolysis can be determined by measuring interstitial glycerol levels by use of the microdialysis technique. With this technique, we (17) and others (22, 44) have reported decreased intramuscular glycerol levels in response to insulin infusion in normal subjects, indicative of an inhibition of muscle lipolysis by insulin. However, glycerol concentrations in the tissue interstitial compartments are the result not only of the production and possible reutilization of the metabolite by myocytes but also of the transport to and from the tissue via local blood flow. Therefore, for a correct interpretation of variations in tissue glycerol concentrations, it is important to simultaneously assess local blood flow rates. Recently, we reported a methodology estimating the absolute rate of in vivo glycerol release from skeletal muscle that combines microdialysis with determinations of local blood flow by use of the \(^{133}\)Xe clearance method (3). Unexpectedly, we found that the rate of muscle glycerol release remained unchanged during an oral glucose load in both nonobese and obese subjects. In adipose tissue, on the other hand, the lipolysis rate was clearly suppressed (3). This may indicate that, in absolute terms, the rate of lipid mobilization in muscle is unresponsive to insulin stimulation. To examine the effect of insulin in more detail, in the present study, we evaluated the rate of glycerol release from skeletal muscle during a two-step euglycemic hyperinsulinemic clamp in nonobese women. For this purpose, we used the microdialysis method for measuring the true tissue glycerol concentration in combination with simultaneous determinations of local blood flow using the \(^{133}\)Xe clearance technique. In addition, by use of the same combination of microdialysis and \(^{133}\)Xe clearance, the effect of insulin on rates of adipose tissue glycerol release was assessed at the same time for comparative purposes.

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MATERIALS AND METHODS

Subjects. Ten nonobese women (age 45 ± 2 yr, body mass index 23.8 ± 0.4 kg/m²) were studied. All subjects were free of illness, and none had taken any medication. The Ethics Committee of Huddinge Hospital approved the study. The subjects were given a detailed description of the experiment and their consent obtained.

Microdialysis device. The microdialysis probe (CMA/60; CMA Microdialysis, Stockholm, Sweden) has been described in detail (46). Briefly, a dialysis tube (30 × 0.5 mm, 20,000 mol wt cut-off) was glued to the end of a double-lumen polyurethane tubing. The inlet lumen of the probe was connected to a high-precision perfusion pump (CMA/100 microinjection pump; CMA Microdialysis) and the probe continuously perfused with a sterile solution. The perfusion solvent entered the probe through the outer lumen, streamed to the tip of the probe, and left the probe through the inner lumen, from which it was collected.

Study protocol. The subjects were studied in the morning after an overnight fast. Body composition (fat and lean body mass) was determined with bioelectrical impedance analysis (28). 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 placed in a heated box (63°C) for sampling of arterialized venous plasma. One microdialysis probe was inserted percutaneously into the medial part of the gastrocnemius muscle, and another microdialysis probe was placed into the abdominal subcutaneous adipose tissue 8 cm lateral to the umbilicus, as described in detail previously (3). Both probes were continuously perfused at a low rate (0.3 μl/min), and the dialysate was collected in 30-min fractions. We have shown in previous studies (17) that the microdialysis recovery of glycerol in skeletal muscle and adipose tissue is almost 100% under these conditions. Therefore, the dialysate level mirrors the true interstitial concentration of glycerol. To ensure steady-state conditions, the 60-min period of basal sampling was started 120 min after probe insertion (17, 41). Thereafter, a two-step euglycemic hyperinsulinemic clamp, 12 and 80 mU·m−2·min−1, 90 min on each insulin dose, was performed. Arterialized plasma samples were drawn in the middle of each 30-min dialysate sampling period for the determination of glycerol and free insulin. The plasma and dialysate collected during the last 30-min period, at baseline, and at each insulin infusion step, respectively, were used in the calculations.

Muscle blood flow was measured using the 133Xe clearance method (29), whereby 133Xe (0.3 MBq in 0.1 ml of saline (Mallinekrodt, Petten, the Netherlands)) was injected into the medial part of the gastrocnemius muscle, and, after a 5-min equilibration period, the residual activity was recorded by a scintillation detector (Mediscint; Oakfield Instruments, Oxford, UK). It is not possible to use 133Xe clearance for estimating skeletal muscle blood flow over extended time periods, because the 133Xe decay curve in muscle gradually becomes multieponential. However, it has been demonstrated that skeletal muscle blood flow can be correctly assessed from the initial part of the 133Xe washout curve (43). Therefore, in the present study, 133Xe was injected three times during the experiment, i.e., during the last 30 min of the baseline period and during the last 30 min of each insulin infusion step. Muscle blood flow was calculated over the 10-min period during the initial decay of the curve. In adipose tissue, 133Xe (1 MBq) was injected percutaneously into the subcutaneous adipose tissue in the contralateral side of the umbilicus. After a 30-min equilibration period, the residual activity was continuously monitored and the fractional decay per minute was assessed for consecutive 30-min periods throughout the study period. Adipose tissue and muscle blood flow (TBF) was calculated according to the equation: TBF = K × λ × 100 (ml·min⁻¹·100 g tissue⁻¹), where K denotes the rate constant of the decay of the residual activity and λ the tissue-to-blood partition coefficient. The values for λ were set at 0.7 for muscle and 10 ml/g for adipose tissue, respectively (29, 30).

In methodological experiments to be published elsewhere, basal blood flow measurements in skeletal muscle were performed twice in seven nonobese subjects with 4–10 min between the measurements. The coefficient of variation (CV) for duplicate measurements was 20.6%. On this basis, it was calculated that, in 10 subjects, a difference in blood flow of 0.4 ml·100 g⁻¹·min⁻¹, i.e., ∼24% of the blood flow at basal conditions, could be detected with 80% power at the 5% significance level.

The absolute rates of tissue glycerol release were calculated according to Fick’s principle, where arterialized venous plasma (A) and capillary venous plasma (V) concentrations of glycerol and plasma flow rate (Q) were entered into the formula: (V - A) × Q × (1 - hematocrit × 10⁻²) (μmol·100 g⁻¹·min⁻¹). Conversion of interstitial (I) to venous (V) glycerol concentrations was made according to the equation: V = (I - A) × (1 - e⁻¹⁰⁻²·PS) + A, where PS denotes the permeability surface product area (approximated to 5 ml·100 g⁻¹·min⁻¹) (32, 33).

Analytical methods. Dialysate glycerol concentrations were determined with enzymatic fluorometric methods, using a tissue sample analyzer that allows very small sample volumes (CMA/600; CMA Microdialysis). Plasma glycerol was determined by bioluminescence (19). Plasma-free insulin was determined by radioimmunoassay using a commercial kit (Pharmacia, Uppsala, Sweden).

Statistics. Values are means ± SE. Variations of data over time in the same individuals were calculated by one-factor analysis of variance (ANOVA) for repeated measurements. Post hoc analyses were performed by Scheffe’s F-test. Comparisons of plasma, muscle, and adipose tissue data were performed using factorial ANOVA. A value of P < 0.05 was considered statistically significant.

RESULTS

Total body fat mass was 23.2 ± 1.3 kg, and lean body mass was 44.8 ± 0.9 kg in the women. Serum levels of free insulin were 39 ± 2 pmol/l at baseline, 115 ± 5 pmol/l during the low insulin infusion rate, and 754 ± 16 pmol/l during the high insulin infusion rate. Glucose infusion rates during the clamps (M values) were 1.9 ± 0.2 mg·kg⁻¹·min⁻¹ during the low insulin infusion rate and 8.2 ± 0.5 mg·kg⁻¹·min⁻¹ during the high insulin infusion rate (P = 0.0001).

In the fasting state, glycerol levels were about four times higher in adipose tissue and 1.4 times higher in muscle compared with the arterialized venous plasma glycerol concentration (P < 0.001 by factorial ANOVA; Fig. 1A). In response to insulin, glycerol in adipose tissue, muscle, and plasma were significantly reduced (P < 0.0001 by ANOVA); already at the low insulin infusion rate, glycerol levels were maximally decreased, with no further significant reduction at the high insulin infusion rate (Fig. 1A). The maximum
decrease in glycerol concentrations, however, was more apparent in adipose tissue and plasma (60%) than in muscle (40%). Figure 1B depicts the effect of insulin on the fractional release of glycerol from adipose tissue and skeletal muscle, as calculated by the difference between the interstitial tissue glycerol concentration and the arterialized venous plasma glycerol level (I-A difference; B). Values are means ± SE.

Determinations of adipose tissue and skeletal muscle blood flow rates are shown in Fig. 2. Tissue blood flow at baseline was similar in the two tissues. During insulin infusion, muscle blood flow increased significantly by ~60% already during the low insulin infusion rate (P < 0.05), whereas at both the low and the high insulin infusion rates it remained unchanged in adipose tissue.

The Fick principle for the calculation of glycerol release is only applicable during steady-state conditions. Therefore, glycerol levels during the last 30 min of the 90-min period of each clamp step were used in the calculations. There were no significant changes between the glycerol levels during the second and last 30-min periods of the low-dose and high-dose clamp in plasma, adipose tissue, or muscle (ANOVA, repeated measurements).

The rates of glycerol release from adipose tissue and skeletal muscle are shown in Fig. 3. At baseline, the absolute rate of glycerol release from muscle was ~13% of that from adipose tissue. In adipose tissue, the rate of glycerol release was significantly reduced by almost one-half at the low insulin infusion rate (P < 0.0001 by ANOVA), and no further decrease was observed at the high insulin infusion rate. In skeletal muscle, no significant change in glycerol release was observed in response to insulin.
DISCUSSION

Several investigators have documented a decreased muscle glycerol concentration during insulin infusion in humans (17, 22, 44), suggesting an inhibition of muscle lipolysis by insulin. Accordingly, in the present study, the interstitial concentration of glycerol in skeletal muscle was found to be maximally reduced already at the low insulin infusion rate in the same way as that in adipose tissue in healthy nonobese women, suggesting a high sensitivity to insulin-induced antilipolysis in skeletal muscle in the normal state. It is important to note, however, that the glycerol concentration in the tissue interstitial compartment is the result of several events. Thus, not only the rate of triglyceride hydrolysis within the myocytes by hormone-sensitive lipase and in the vascular bed by lipoprotein lipase (LPL) but also the transport of glycerol to and from the tissue via the local microcirculation and the possible reutilization of glycerol by the tissue have to be considered. It is generally believed that circulating glycerol is derived mainly from triglyceride hydrolysis in adipose tissue, a process that is clearly inhibited by relatively small increments in plasma insulin levels (14, 24, 31, 36). In consonance with this notion, we found that, already during the low-dose insulin infusion, glycerol levels in adipose tissue and in plasma were maximally reduced, the reduction in the two compartments being comparable in relative terms. In skeletal muscle, on the other hand, the relative decrease in interstitial glycerol in response to insulin was less pronounced. More important, whereas the fractional release of glycerol [i.e., the difference between the interstitial tissue glycerol concentration and the arterialized venous plasma glycerol level (I-A difference)] was markedly suppressed in response to insulin in adipose tissue, no significant change of this I-A difference was registered in skeletal muscle. This finding strongly indicates that the decrease in interstitial glycerol levels in skeletal muscle after insulin infusion was mainly the result of reduced arterial inflow of glycerol to the tissue because of insulin-induced antilipolysis in adipose tissue, rather than being a reflection of suppression of skeletal muscle lipolysis by insulin.

In keeping with previous data (1), the present study showed that muscle blood flow increased significantly by ~60% in response to insulin, whereas adipose tissue blood flow remained unaltered. Therefore, the decreased muscle glycerol levels seen during insulin stimulation may also to some extent be explained by increased clearance of glycerol from the tissue interstitial compartment via the microcirculation. In the aforementioned reports, in which the influence of insulin on muscle glycerol was investigated in vivo using microdialysis (17, 22, 44), no quantitative determination of muscle blood flow was carried out. Instead, the ethanol clearance technique (20) was used as a qualitative measure of muscle blood flow. It has been shown, however, that the latter technique can detect only major changes (i.e., >50%) in tissue blood flow rates (10, 21). It is therefore probable that the relatively minor increase in muscle blood flow in response to insulin was overlooked in the previous investigations (17, 22, 44). In this study, we used the $^{133}$Xe clearance method (29) for measurements of absolute blood flow rates in adipose tissue and muscle. For many years, this technique has been used for determination of adipose tissue blood flow, and it has also been validated for measurements of muscle blood flow (43). Accordingly, we found the reproducibility and precision of the method for assessment of skeletal muscle blood flow rates to be satisfactory (CV ~20%). The findings with blood flow rates in skeletal muscle and adipose tissue in this study are in keeping with previously reported data from our own group (3) as well as from other researchers (12) using the $^{133}$Xe clearance technique for simultaneous determinations of skeletal muscle and adipose tissue blood flow rates. Others have used strain gauge plethysmography to measure forearm blood flow and $^{133}$Xe clearance to determine abdominal subcutaneous adipose tissue blood flow at the same time. For example, by so doing, Coppack et al. (4) showed higher blood flow rates in adipose tissue than in the forearm, whereas Evans et al. (9) found similar blood flow rates in the two tissue beds. The strain gauge plethysmography technique, however, measures blood flow within both the larger vessels and the microvasculature. The same is true also for the positron emission tomography method, which has been used for quantitative measurements of skeletal blood flow. The $^{133}$Xe washout method, on the other hand, preferentially determines the nutritive blood flow in the tissue. Hence, comparison of blood flow data obtained with different techniques should be done with caution.

When blood flow was included in the calculations, it was observed that the absolute rate of glycerol release from skeletal muscle was ~15% of that from adipose tissue in the postabsorptive state. This is comparable to that previously reported in nonobese subjects (3). As expected, the net rate of glycerol release from adipose tissue was markedly suppressed by insulin infusion, showing a clear antilipolytic effect of insulin in adipose tissue. In contrast, in skeletal muscle, the absolute rate of glycerol release did not change significantly in response to insulin. Again, this strongly argues against a significant inhibitory effect of insulin on human skeletal muscle lipolysis rates in vivo.

The molecular basis for this tissue-specific variation in lipolysis regulation by insulin is not known. It may be coupled to differences in phosphodiesterase subtypes in adipose tissue and muscle (8, 16), or to the two insulin-sensitive lipases: hormone-sensitive lipase and LPL. Although it is not possible with microdialysis to specifically separate tissue lipolysis and intravascular (LPL-mediated) triglyceride hydrolysis, the findings of a recent study in humans (45), where adipose tissue glycerol kinetics were investigated simultaneously with microdialysis and the arterial-venous (a-v) difference technique, indicated that microdialysis measurements of interstitial glycerol more closely reflected fat cell lipolysis, whereas intravascular, LPL-induced triglyceride hydrolysis was better determined by the a-v...
difference method. This may suggest that most glycerol derived from hydrolysis of circulating triglycerides remains in the vascular compartment, with little transfer across the capillary wall into the interstitial fluid. Moreover, with regard to the quantitative impact of LPL-mediated hydrolysis of circulating triglycerides on tissue glycerol release, it has been shown in adipose tissue that this process contributes only to a minor extent to the glycerol released locally in the postabsorptive state, whereas it increases markedly in response to glucose infusion and hyperinsulinemia (4). Thus, if this resulted in an increase in LPL-derived glycerol in the tissue interstitial compartment, we might even have underestimated the true antilipolytic effect of insulin in adipose tissue in the present study. LPL activity in adipose tissue and skeletal muscle is regulated in a reciprocal way, however, and during a hyperinsulinemic clamp there is a decrease in skeletal muscle LPL activity (6). Therefore, it seems improbable that a suppressive effect of insulin on skeletal muscle lipolysis should have been masked by a corresponding increase in LPL-mediated hydrolysis of circulating triglycerides, resulting in unchanged I-A differences in glycerol levels and rates of glycerol release from the muscle tissue in response to insulin stimulation.

The extracellular concentration of glycerol in skeletal muscle may also be influenced by uptake and reutilization of glycerol by the myocytes. Experiments in rodents have indeed demonstrated that glycerol may be taken up by skeletal muscle and used for intramyocellular triglyceride synthesis, the uptake of glycerol apparently being of varying magnitude in different muscle groups and most prominent after prolonged fasting (15). In humans, direct demonstration of skeletal muscle glycerol uptake is still lacking, and a-v difference studies across the human forearm have yielded contradictory results (11). However, data from more recent studies, using a combination of a-v difference and isotope tracer techniques, have suggested the occurrence of glycerol uptake across the forearm (4, 7, 27) and leg (25) tissue beds. Hence, we cannot rule out the possibility that the effect of insulin on the rate of skeletal muscle lipolysis may have been underestimated to some extent in the present study, owing to the presence of myocellular glycerol uptake and reutilization, because in the aforementioned reports the uptake of glycerol was most apparent in the postabsorptive state and decreased in response to insulin stimulation (4, 25). However, the estimation of the rate of glycerol uptake was substantially lower than the corresponding assessment of glycerol release across the tissue beds. Therefore, it is unlikely that our main conclusion, that insulin exerts no inhibitory effect on skeletal muscle lipolysis, should have been obscured in a major way by the possible uptake of glycerol in the tissue. It is important to note also that there may be differences in lipolytic activity and its regulation in different muscle groups. Therefore, our results do not exclude an antilipolytic action of insulin in other muscle sites.

We calculated the rates of adipose tissue and skeletal muscle glycerol release according to Fick’s principle that is strictly applicable only during steady-state conditions. Notably, in the present study, data obtained during the last 30 min of the 90-min clamp periods were used in the calculations. During these periods, glycerol levels in adipose tissue, muscle, and plasma, as well as the blood flow rates in the two tissues, were shown to be stable.

In the present study, we used the same numerical value (5 ml·100 g⁻¹·min⁻¹) for the PS in adipose tissue and skeletal muscle, as previously suggested (32, 33). Note that the main conclusion of the study, that there was no insulin-induced antilipolysis in skeletal muscle, would still be valid whether or not similar PS values had been used. As extensively reviewed by Crone and Levitt (5), however, it has been experimentally shown that the permeability values for low-molecular-weight substances are comparable in most organs with continuous capillaries, including skeletal muscle and adipose tissue. Moreover, the permeability for hydrophilic molecules, such as glycerol, is not influenced by blood flow rates. Just recently, on the other hand, data have been presented that indicate that the insulin-induced increase in skeletal muscle blood flow may involve the recruitment of more capillaries (47). If this is the case, the capillary surface area and, hence, the PS value may increase. Therefore, to evaluate to what extent different PS values would influence the data, we recalculated the basal rate of skeletal muscle glycerol release using different PS values, the results showing an increase of <5% in the absolute rate of glycerol release when the PS value was increased up to 10 times. Hence, in absolute terms, the data are only marginally changed when different PS are used in the calculations.

In conclusion, the results of this study demonstrate that insulin exerts no apparent antilipolytic effect in human skeletal muscle in vivo. Although the interstitial glycerol concentration is clearly reduced in response to small increments in circulating insulin levels, this is due to suppression of adipose tissue lipolysis and decreased glycerol transport to the muscle interstitial compartment via the arterial blood flow, in combination with enhanced local blood flow rates, in response to insulin stimulation. These findings emphasize the necessity of combining the microdialysis technique with measurements of blood flow rates to correctly interpret changes in tissue glycerol levels and thus the regulation of the tissue lipolytic activity.

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