Evidence for a Major Role of Skeletal Muscle Lipolysis in the Regulation of Lipid Oxidation During Caloric Restriction In Vivo

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A lipolytic process in skeletal muscle has recently been demonstrated. However, the physiological importance of this process is unknown. We investigated the role of skeletal muscle lipolysis for lipid utilization during caloric restriction in eight obese women before and after 11 days of very low–calorie diet (VLCD) (2.2 MJ per day). Subjects were studied with indirect calorimetry and microdialysis of skeletal muscle and adipose tissue in order to analyze substrate utilization and glycerol (lipolysis index) in connection with a two-step euglycemic-hyperinsulinemic (12 and 80 mU/m2 · min) clamp. Local blood flow rates in the two tissues were determined with 133Xe-clearance. Circulating free fatty acids and glycerol decreased to a similar extent during insulin infusion before and during VLCD, and there was a less marked insulin-induced reduction in lipid oxidation during VLCD. Adipose tissue glycerol release was hampered by insulin infusion to the same extent (~40%) before and during VLCD. Skeletal muscle glycerol release was not influenced by insulin before VLCD. However, during VLCD insulin caused a marked (fivefold) (P < 0.01) increase in skeletal muscle glycerol release. The effect was accompanied by a fourfold stimulation of skeletal muscle blood flow (P < 0.01). We propose that, during short-term caloric restriction, the reduced ability of insulin to inhibit lipids, despite a preserved antilipolytic effect of the hormone in adipose tissue, is caused by an augmented mobilization of fat from skeletal muscle, and that a physiological role of muscle lipolysis provides a local source of fatty acids. Diabetes 50:1604–1611, 2001

Although adipose tissue is the major source of endogenous triglycerides (TGs), it has been shown that skeletal muscle myocytes also contain TGs (1–4). Furthermore, several groups have demonstrated marked lipolytic activity in human skeletal muscle (5–7), generating glycerol and free fatty acids (FFAs) from TGs by hydrolysis. It appears that lipolysis in skeletal muscle is regulated in a different manner than in adipose tissue. Thus, during oral glucose loading, the lipolysis rate is suppressed in fat tissue but remains unaltered in skeletal muscle (8). It is also known that muscle TG content is inversely associated with insulin sensitivity (2,3). However, the regulation and physiological role of muscle lipolysis has not been fully explored. Also, the importance of skeletal muscle TG as a source of FFAs during exercise has recently been questioned (9).

We hypothesized that a possible role for muscle lipolysis is to regulate the local supply of energy during caloric restriction. In this condition there is increased lipid utilization and decreased glucose oxidation (10). As a model, we chose to use very low–calorie diet (VLCD) instead of total fasting. VLCD offers the minimum nutrients needed to avoid the risks of total fasting while inducing effective weight-reduction (11). The major energy source during caloric restriction is stored TGs. The FFAs liberated are subsequently used for lipid oxidation, which mainly occurs in skeletal muscle. However, it has been generally accepted that FFAs derive from lipolysis in adipose tissue during caloric restriction (12).

The aim of this study was to investigate the influence of insulin on lipolysis in muscle and fat tissues and to analyze results in relation to lipid oxidation. We simultaneously monitored adipose tissue and skeletal muscle tissue lipolysis with microdialysis and performed whole-body measurements of energy expenditure and fuel utilization with indirect calorimetry before and at the end of a 2-week VLCD period in obese subjects. The effects of insulin during a two-step euglycemic-hyperinsulinemic clamp were also investigated.

## RESEARCH DESIGN AND METHODS

A total of 11 obese but otherwise healthy women aged 42 ± 4 years (mean ± SE) were recruited from the Obesity Unit at Huddinge University Hospital before starting a lifestyle intervention program to achieve weight reduction. None of the subjects were on any regular medication or had undertaken a slimming effort during the previous 6 months. Three subjects did not complete the diet program and were therefore excluded. Thus, eight subjects (aged 44 ± 4 years) completed the study and were evaluated. Their characteristics before and during VLCD, including estimation of body composition using impedance weighing (Tanita TBF 305; Tanita, Tokyo), are presented in Table 1. In addition, a total of 11 healthy nonobese volunteers were included for different methodological investigations. Informed consent was obtained from each participant, and the study protocol was approved by the Ethics Committee of Huddinge University Hospital.

**Protocol.** Each obese subject was studied twice, before and during a hypocaloric protein-rich diet (Nutrilett; Nycomed Pharma, Oslo, Norway) (2.2 MJ; 8 g fat, 60 g protein, and 50 g carbohydrate per day). The VLCD period was scheduled for 10–14 days (mean duration 11 days for the eight subjects who...
and 156 mmol/l chloride, at 0.3 analysis catheters were continuously perfused with Ringer's solution (Apoteksm- placing the subjects' head in a ventilated plastic hood, indirect calorimetry rested in a recumbent position and at an ambient temperature of 22°C. After sampling was started 120 min after insertion of the microdialysis catheters to thus indicating that almost the true tissue levels are recorded. Microdialysate catheter, a recovery of.

So¨ derta¨lje, Sweden) was used before insertion of the catheters. The microdi- molecular weight cutoff (CMA/60; CMA Microdialysis, Stockholm, Sweden), were the medial part of the gastrocnemius muscle, respectively.

the study period, and the urine was collected for analysis of nitrogen.

TABLE 1
Subject characteristics before and during 11 days of caloric restriction (VLCD)

<table>
<thead>
<tr>
<th></th>
<th>Before VLCD</th>
<th>During VLCD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total weight (kg)</td>
<td>112 ± 4.2</td>
<td>108.8 ± 4.3</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>39.5 ± 1.2</td>
<td>38.2 ± 1.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>69.0 ± 5.8</td>
<td>65.5 ± 5.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>43.2 ± 3.9</td>
<td>42.9 ± 3.9</td>
<td>0.85</td>
</tr>
<tr>
<td>S-cholesterol (mmol/l)</td>
<td>5.5 ± 0.4</td>
<td>4.0 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P-triglycerides (mmol/l)</td>
<td>1.6 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P-glucose (mmol/l)</td>
<td>5.6 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P-glyceral (μmol/l)</td>
<td>59 ± 11</td>
<td>80 ± 4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S-FFM (kg)</td>
<td>0.77 ± 0.08</td>
<td>0.97 ± 0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S-insulin (mU/l)</td>
<td>13.7 ± 1.9</td>
<td>5.7 ± 1.0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are means ± SE. P, plasma; S, serum.

during the entire study period. The last 30 min of the baseline period and each insulin infusion step were used for blood flow calculations. For determination of skeletal muscle blood flow, repeated injections were necessary because the decay curve becomes multieponential over time (17). Thus, three separate injections of 0.3 MBq of Xenon dissolved in 0.1 ml saline were carried out in the gastrocnemius muscle. The site for each injection was changed to avoid interference by residual activity. Recordings were started 5 min after the injection and continued during the last 30 min of the baseline period and each insulin step. The first 10 min of the skeletal muscle decay curve was used for calculations. This is the early and supposed linear (17) phase of isolate washout.

In one set of separate methodological experiments on four healthy control subjects, microdialysis catheters were inserted as previously described in adipose tissue and calf muscle (n = 1–2 for each tissue). The catheters were perfused with Ringer's solution with or without Dextan (60 kDa, 3%) (Braun Medical, Solna, Sweden) to exclude an effect of a colloid on sample volume a-min period. After an equilibration period of 60 min, samples were collected every 30 min during 3 h. In another set of separate methodological experiments, basal blood flow measurements in muscle were performed twice, exactly as previously described, with 4–10 months between the measure- ments. Seven nonobese subjects participated in this study.

Analytical procedures. Microdialysate glycerol was determined with an en- zymatic fluorometric method, using a tissue sample analyzer (CMA/900; CMA Microdialysis) (5). Plasma glycerol was determined by bioluminescence (18). Plasma free insulin was determined by a commercial radioimmunoassay (Phar- macia, Uppsala, Sweden) after precipitation with polyethylene glycol (19) and FFAs in serum by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). O₂ and CO₂ concentrations in the gas from the ventilated hood were measured by a paramagnetic analyzer and by an infrared analyzer, respectively. The analyzers were calibrated with air and precisely known gas concentrations (95% O₂ and 5% CO₂) before each study. Urinary nitrogen was measured by the Kjeldahl method (20). Glucose, hematocrit, O₂ satura- tion, cholesterol, and TGs were analyzed using the routine clinical laboratory.

Calculations. TBF was calculated according to the following equation:

\[ TBF = K \times \frac{Q}{100} \]

where K denotes the rate constant of the decay of the residual activity and \( Q \) represents the tissue to blood partition coefficient (10 μg/g for adipose tissue [16] and 0.7 μg/g for skeletal muscle [21]).

Glycerol release from the tissues was calculated according to Fick's principle:

\[ (V - A) \times Q \times (1 - \text{hematocrit} \times 10^{-2}) \times (\text{μmol} \cdot 100 \cdot 1 \cdot \text{g}^{-1} \cdot \text{min}^{-1}) \]

Venous (V) glycerol was calculated from interstitial (I) glycerol according to the following equation:

\[ V = (I - A) \times (1 - e^{-\text{bw}Q}) + A \]

where V denotes venous glycerol, A arterial glycerol, Q calculated plasma blood flow, and ps the permeability surface product area (adopted to be 5 ml/100 g · min) (22,23).

Average values for respiratory gas exchange were calculated over each 1-min period for O₂ consumption, CO₂ production, and respiratory quotient (RQ). The nonprotein respiratory quotient (mPQR) was calculated from calorimetric values and urinary nitrogen. Substrate oxidation was obtained with the formulae of Weir (24), which are based on mPQRs of 1.00 for 100% carbohydrate oxidation and 0.707 for 100% fat oxidation. Protein oxidation was assumed to be constant during the study period.

Statistics. Values are means ± SE. Paired comparisons of the two study occasions were performed using Student’s paired t test or Wilcoxon’s signed- rank test when applicable. For comparisons over time of a parameter (basal and 1st and 2nd insulin infusions), a one-way repeated measurement analysis of variance (ANOVA) was used. In post hoc analysis, the Scheffe’s test was considered significant at P < 0.05. Linear regression analysis was also used. Statistical calculations were made using a statistical software package (Stat View II; Abacus Concepts, Berkeley, CA).

RESULTS

Methodological experiments. When microdialysis is performed at a low perfusion speed, as in the present experiments, there might be evaporation of dialysate fluid, so that the concentration of glycerol dialyzed from the tissue becomes artificially elevated (25). If so, the addition of Dextran, an osmotic agent, to the perfusion solvent.
should decrease the glycerol level in the dialysate (25). This was investigated on four lean control subjects. When Dextran was added to the microdialysis solvent, no difference in dialysate glycerol levels between control and Dextran-containing probes were recorded in either muscle or adipose tissue. During the last hour of microdialysis dialysate, glycerol concentrations were $244 \pm 22$ and $211 \pm 50 \mu$mol/l in fat and $77 \pm 5$ and $73 \pm 5 \mu$mol/l in muscle with and without Dextran in the perfusate, respectively ($P = \text{NS}$ for both tissues). Thus, there was no evidence of evaporation under the present microdialysis experiments. In another set of experiments on seven lean subjects, the method to measure blood flow in skeletal muscle was evaluated by measuring the flow twice during baseline conditions.

The measurements were performed exactly as in the obese study group, with a 4- to 10-month interval between measurements. $R$ values for the decay curve were 0.92–0.98 for the first investigation and 0.95–0.98 for the second investigation. Blood flow (ml/100 g · min) was $2.0 \pm 0.2$ at the first occasion and $1.8 \pm 0.2$ at the second. These values did not differ significantly. The coefficient of variation for duplicate determinations was 20.6%. Thus, it is calculated that in eight subjects (the size of the present study group), a difference of $0.4 \text{ml/100 g · min}$ in skeletal muscle blood flow within an individual, i.e., $\sim 25\%$ of the blood flow at basal conditions, can be detected with $80\%$ power at a 5% significance level.

We also wanted to make certain that glycerol levels in muscle were at steady state during baseline (Fig. 1). Raw data for glycerol concentration in microdialysate of muscle are shown for three typical subjects before and during VLCD. As expected, and confirming earlier observations (14,15), there was a marked decline in muscle glycerol immediately after insertion of the microdialysis catheter. After $<120 \text{min}$, glycerol concentrations in dialysate were constant. We also analyzed the two different baseline samples (30-min period each) in fat and skeletal muscle tissue of all subjects. Almost identical values for the two samples were recorded before and during VLCD in both tissues, so baseline steady-state conditions were at hand.

**Clinical data.** Earlier studies of changes in body composition of obese women during caloric restriction, using nitrogen balance and total body water measurements with tritiated water, showed that from day 10 and thereafter, nearly half of the body weight loss during VLCD is caused by loss of fat (26). The characteristics of the obese subjects before and during caloric restriction are shown in Tables 1 and 2. A significant weight reduction was obtained ($\sim 3.5 \text{kg}$) and predominantly consisted of fat. We used bioimpedance to measure body fat content, which is a less accurate method when performed on obese subjects. The sole purpose of the measure was to discover whether there was a loss of water, proteins, and carbohydrates. Therefore, we also calculated expected fat loss based on the following assumptions: 1) average resting metabolic rate was 2,000 kcal/day (see below); 2) daily caloric intake was 500 kcal; 3) daily loss of energy caused by physical activity was estimated at 600 kcal; 4) 1 kg of fat corresponds to 9,300 kcal; and 5) the mean duration of VLCD was 11 days. Therefore, the expected average fat loss would be $11 \times 2,100/9,300 = 2.5 \text{kg}$, which suggests that the fat loss was overestimated by $\sim 1 \text{kg}$ using bioimpedance. This assumption is further strengthened by the fact that the statistical $P$ value was 0.0002 for BMI loss and 0.04 for fat loss. BMI is a direct and more accurate measurement of body fat than bioimpedance measurements of fat content. Blood lipids and glucose decreased significantly during semi-starvation, whereas circulating levels of FFAs and glycerol increased. Furthermore, there was an expected decrease in fasting plasma insulin levels from $14$ to $6 \text{mU/l}$ during VLCD ($P < 0.05$). There was no difference in the amount of glucose needed to maintain euglycemia (M) during the respective insulin steps before and during VLCD. At both insulin infusion rates, the steady-state insulin levels (I) were higher before than during VLCD, so the M to I ratio at both insulin infusion rates was elevated during VLCD compared with before ($P = 0.07$ and 0.02 at low and high insulin, respectively). However, because the fasting insulin levels decreased during VLCD, the increase in insulin above the fasting level was of the same magnitude before and during VLCD.

**Indirect calorimetry.** Data for the RQ and the energy expenditure are shown in Table 2. RQ was unchanged in the basal state after diet. Both before and during VLCD, it
TABLE 2
Glucose infusion rate, plasma insulin concentrations, RQ, and energy expenditure during a euglycemic insulin clamp before and during caloric restriction in obese women

<table>
<thead>
<tr>
<th></th>
<th>Before VLCD</th>
<th>During VLCD</th>
<th>P during vs. before VLCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Glucose infusion rate (mg/kg · min)</td>
<td>—</td>
<td>0.9 ± 0.1</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>P-insulin (nU/l)</td>
<td>26 ± 2</td>
<td>135 ± 12</td>
<td></td>
</tr>
<tr>
<td>RQ</td>
<td>0.75 ± 0.01</td>
<td>0.76 ± 0.01</td>
<td>0.83 ± 0.02</td>
</tr>
<tr>
<td>Energy expenditure</td>
<td>2,006 ± 91</td>
<td>2,028 ± 87</td>
<td>2,071 ± 78</td>
</tr>
</tbody>
</table>

Data are means ± SE.

Increased significantly during the clamp ($F = 1, P < 0.0002$ before VLCD, and $F = 6.8, P = 0.03$ during VLCD). During the highest insulin infusion rate, it increased to higher values before than during VLCD ($P = 0.001$). There was a significant increase in energy expenditure during insulin-glucose infusion both before (ANOVA $F = 8.2, P = 0.004$) and during ($F = 5.7, P = 0.02$) VLCD. However, the energy expenditure was lower during caloric restriction both in the basal state and during the two insulin infusion levels. Baseline carbohydrate or fat oxidation did not change significantly during VLCD (23.4 ± 14.7% vs. 1.3 ± 4.4% mg/min for carbohydrates and 97.3 ± 13.9% vs. 111.6 ± 3.2% mg/min for lipids, before and during VLCD). Carbohydrate oxidation (Fig. 2A) increased in response to insulin and glucose at both study occasions (before VLCD $F = 19, P = 0.0001$, and during VLCD $F = 13.3, P = 0.0006$). The increase was markedly blunted during VLCD (104 ± 14% vs. 51 ± 12% mg/min; $P = 0.01$ at the high dose) and the difference was ~50%. During insulin and glucose infusion, lipid oxidation decreased both before ($F = 14.9, P = 0.0003$) and during ($F = 9.2, P = 0.003$) VLCD (Fig. 2B). However, at the higher insulin infusion rate, the decline was much less pronounced during VLCD (39 ± 6% vs. 16 ± 5% mg/min, $P = 0.01$), and the difference was more than half. Protein oxidation was similar before and during VLCD (48.6 ± 7.7 and 41.7 ± 3.0 mg/min, respectively, NS).

Levels of glycerol and FFAs. Fig. 3A and B show the glycerol and FFA concentrations. The baseline microdialysate levels are mean glycerol values of two consecutive 30-min sampling periods. During the first 2 h after the insertion of the microdialysis catheters into muscle, there was a decline in microdialysate glycerol levels. However, in both tissues before and during VLCD, glycerol levels were in steady state during baseline conditions, as judged by the values recorded in the two baseline samples. Before VLCD, these values were 278 ± 28 and 261 ± 23 μmol/l in adipose tissue and 138 ± 23 and 125 ± 16 μmol/l in skeletal muscle. Corresponding values during VLCD were 277 ± 29 and 273 ± 21 μmol/l in adipose tissue and 151 ± 19 and 135 ± 12 μmol/l in skeletal muscle. For all conditions, there was not a significant difference between values at the two time points.

Baseline glycerol concentrations were higher in adipose tissue than in skeletal muscle, and in both tissues, the concentrations were higher than in plasma, indicating a production of glycerol in these tissues. The baseline tissue glycerol levels in adipose tissue and skeletal muscle did not change during VLCD (Fig. 3). However, there was a slight but significant increase in baseline plasma glycerol during VLCD (Table 1). Before VLCD, glycerol concentrations decreased gradually in all three compartments during the two insulin infusion periods ($P = 0.0008–0.0001$). During VLCD, there was also a significant and similar decrease in glycerol in the three compartments after insulin infusion ($P = 0.0001$). The data in Fig. 3A represent glycerol values during the last 30 min of the two 90-min infusion periods. When these values were compared with those recorded for the second 30-min period of each infusion step, no significant differences between values were detected. This was true for plasma, fat, and muscle before as well as after VLCD. Thus, the glycerol values...
recorded and shown in Fig. 3 represented steady state and could therefore be used in the calculation of substrate mobilization according to Fick’s principle (see below). Circulating FFAs decreased significantly during insulin infusion ($F = 73$, $P < 0.0001$ and $F = 95$, $P < 0.0001$) before and during VLCD, respectively. At all levels, the concentration of FFA was higher during VLCD ($P = 0.01–0.04$), but the net decrease (basal minus insulin) was similar in the two dietary states.

**Blood flow.** ATBF was similar at baseline before ($1.6 \pm 0.4 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) and during ($2.0 \pm 0.2 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) VLCD. ATBF did not change significantly during either of the insulin infusion rates, neither before nor during VLCD. Therefore, the mean ATBF for the full study period was used for the subsequent calculations. Basal skeletal muscle tissue blood flow (MTBF) was $0.7 \pm 0.3$ and $0.4 \pm 0.1 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ (NS) before and during VLCD and did not change significantly during insulin infusion before caloric restriction. However, during VLCD there was a marked increase in MTBF ($1.2 \pm 0.5$ and $1.3 \pm 0.3 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) at the low- and high-insulin infusion rates, respectively (ANOVA $F = 7.4$, $P = 0.006$). This represents a three- to fourfold stimulation of skeletal MTBF by insulin. There was no significant difference between the skeletal muscle blood flow at the low- and high-insulin infusion rates, respectively.

**Glycerol production.** We used the glycerol values recorded in Fig. 3A to calculate glycerol release according to Fick’s principle, because these values, as well as the blood flow rates during insulin infusion, were in steady state. The glycerol release from adipose and skeletal muscle tissue is shown in Fig. 4. The adipose tissue glycerol release decreased during insulin infusion before ($F < 0.001$) and during ($P < 0.001$) caloric restriction (Fig. 4A). The rate of glycerol release in the basal state and after the two insulin infusion periods was almost identical before and during VLCD ($F = 1.5$ by two-way ANOVA, $P = 0.2$). Insulin reduced the rate of glycerol release in a dose-dependent fashion by $\sim 40\%$ in both conditions.

In skeletal muscle, the calculated glycerol release (Fig. 4B) was not influenced by the insulin infusion before VLCD ($F = 0.8$, NS). During VLCD, skeletal muscle glycerol release was markedly stimulated by insulin ($F = 8.3$, $P = 0.004$). The increase was similar during the low-insulin and high-insulin infusion rates, respectively, and corresponds to a fivefold stimulation of glycerol release.

**DISCUSSION**

In this study, we investigated the possible role of skeletal muscle lipolysis in relation to the regulation of whole-body

**FIG. 3.** Glycerol (A) and FFAs (B) before VLCD (□) and during VLCD (●) in connection with a two-step insulin clamp. Values are means ± SE. ▲ $P < 0.05$ before vs. during VLCD (Wilcoxon’s signed-rank test); * $P < 0.05$ vs. basal levels (shown in text) during clamp

**FIG. 4.** Glycerol release in adipose tissue (A) and skeletal muscle (B) during low- and high-insulin infusion rates in eight obese women before VLCD (□) and during VLCD (●). Glycerol release was calculated using steady-state values of interstitial glycerol from microdialysis measurements in abdominal subcutaneous adipose tissue and the gastrocnemius muscle with blood-flow data using the Xe-clearance method. Values are means ± SE. See text for statistics.
lipid metabolism during caloric restriction. This was achieved by performing microdialysis under steady-state conditions. In accordance with previous findings, we found that VLCD for 11 days was accompanied by an overall reduction in energy expenditure (11) and a preserved or even improved whole-body insulin-stimulated glucose uptake (27). This indicates that our study model is relevant for caloric restriction.

In contrast to the findings with whole-body glucose uptake, the action of insulin on fuel oxidation shows clear signs of hormone resistance during VLCD, when the capacity of insulin to stimulate glucose oxidation and inhibit lipid oxidation was markedly reduced. Thus, it appears that the body shifts from using glucose to lipids as an energy source during insulin stimulation in a VLCD situation. This may be the major reason behind the observed loss of body fat during VLCD that occurs despite reduced energy expenditure and decreased carbohydrate oxidation. However, it should be noted that we have overestimated the actual fat loss by the bioimpedance method, as judged by calculations of actual fat loss.

An important question arises from this study: what is the mechanism behind maintenance of a high rate of lipid oxidation during insulin-glucose infusions in VLCD? It is generally accepted that FFAs derived from adipose lipolysis are the only important source for lipid oxidation during caloric restriction (12,28). On the other hand, these ideas about FFAs were conceived before the recent discovery of lipolysis in skeletal muscle. It is not currently possible to directly measure FFA release from tissues. However, direct information on in vivo lipolysis in the two tissues can be obtained by measurements of glycerol release. True rates of lipolysis in the muscle might be slightly underestimated in our subjects because some of the glycerol formed during lipolysis in the tissue could be reutilized (29,30).

As expected, in adipose tissue, insulin inhibited the rate of glycerol release. The lipolysis rate was inhibited to the same extent before and during VLCD (~40%). Hence, the cause of resistance in insulin-inhibited lipid oxidation during VLCD cannot be found in adipose tissue lipolysis. It should be stressed that this notion is based on measurements of the subcutaneous fat depot. This is by far the largest fat region in humans, constituting >80% of the total adipose tissue depot. Presently, it is not possible to directly measure lipolysis in vivo in visceral fat regions. Indirect information can be obtained by combining isotopic infusion with selective venous catheterization (31). However, for technical reasons, it was not possible to add these methods to the present experimental protocol. A unique regulation of visceral lipolysis during VLCD seems unlikely, as judged by the findings with circulating glycerol and FFAs, which were decreased by insulin infusion to the same extent before and during VLCD.

If we accept that the maintenance of a high rate of lipid oxidation during insulin-glucose infusions in VLCD cannot readily be explained by FFAs derived from adipose tissue lipolysis, then we must focus our interest on the other possible lipolytic source, namely skeletal muscle. Our study showed that before VLCD, insulin did not influence the rate of glycerol release from skeletal muscle. During VLCD, insulin caused a quite unexpected and marked stimulation of glycerol release. This effect was maximal at the lowest infusion rate (approximately fivefold stimulation of the basal rate), when there was only a moderate increase in circulating insulin. At the moment, we have no molecular mechanism to explain why insulin actually stimulates the rate of lipolysis in skeletal muscle because there are no laboratory animal or human models available for detailed investigations of lipolysis in myocytes or skeletal muscle tissue. Furthermore, it cannot be clearly stated whether the increased skeletal muscle lipolysis results from an increased activity of lipoprotein lipase in the vascular compartment or from a hormone-sensitive lipase in the myocytes.

However, in a recent study we investigated glycerol levels in adipose tissue by the techniques of venous cannulation and microdialysis (32). Lower glycerol levels were recorded with microdialysis than with venous cannulation. This might be caused by the fact that microdialysis mainly reflects glycerol derived from lipolysis of TGs within fat cells, whereas the cannulation technique measures all lipolysis, i.e., from circulating as well as adipocyte TGs. Nevertheless, in accordance with the present data, we have recently shown a lack of lipolysis suppression in skeletal muscle during another hyperinsulinemic condition, namely that of oral glucose loading, in both obese and normal-weight subjects (8). One reason for lack of an antilipolytic effect could be that human skeletal muscle contains very low levels of phosphodiesterase III, and this enzyme does not seem to be functionally active in the muscle in vivo (33). Phosphodiesterase III is believed to be the key enzyme responsible for the antilipolytic effect of insulin (34). Another factor could be the blood flow in skeletal muscle. Before VLCD, insulin did not stimulate skeletal muscle blood flow. On the other hand, insulin infusion during VLCD caused a marked increase in skeletal muscle blood flow. It is possible that this reflects a defective blood flow regulation in the obese subject (35), which is normalized during weight loss. The insulin effects on blood flow and glycerol release were of the same order of magnitude (four- to fivefold), and both were already maximally increased at the lowest infusion rate. It is likely that the increase in glycerol release in response to insulin demonstrated in skeletal muscle during VLCD is secondary to insulin stimulation of blood flow in the tissue. It should also be noted that we have measured net release of glycerol and not total production of glycerol by the muscle.

Because the stimulation of skeletal muscle blood flow during VLCD by insulin is the crux of the study, it is necessary to consider the methodological aspects. We believe that the findings with muscle blood flow are valid for the following reasons. First, the separate methodological study showed that baseline blood flow is stable over time, and the slope of the decay curve is accurate (r always >0.9). In addition, the statistical power calculation revealed that the increase in flow by insulin during VLCD was far above the detection limit for blood flow changes within an individual. Second, we recently compared, using the present experimental protocol, muscle blood flow and glycerol release in muscle from lean and obese women (E.M., E.H.-T., J.B., S. Sjöberg, unpublished observations). In lean individuals, insulin infusion stimulates blood flow markedly, whereas this study confirmed that in obese
individuals, insulin was ineffective. Third, we have measured skeletal muscle blood flow after an overnight fast in three different groups of obese subjects. The mean blood flow rates were similar in these groups: 0.7 ± 0.3 (in the present study), 0.9 ± 0.3 (for the eight women in reference 8), and 0.7 ± 0.2 ml·100 g⁻¹·min⁻¹ (in E.M. E.H-T., J.B., S. Sjöberg, unpublished observations). Based on these data, it is proposed that obese women are resistant to the stimulatory effect of insulin on blood flow in skeletal muscle. During VLCD, this feature of insulin action improves. Because of a yet unidentified mechanism, the hypocaloric state also causes insulin to paradoxically stimulate glycerol release from muscle. Interestingly, in lean subjects, insulin did not stimulate glycerol output from muscle, despite the stimulatory effect on blood flow.

It should be noted that slightly lower hormone levels during hyperinsulinemic infusion were observed during, compared with before, VLCD. Unfortunately, it is not possible to perfectly match insulin levels when infused under two conditions that differ in hormone kinetics. However, this has no bearing on the interpretation of our results. First, lipolysis in adipose tissue was equally suppressed by insulin infusions before and during VLCD. Second, a lack of effect of insulin infusion on skeletal muscle lipolysis was observed before VLCD, although hormone levels during infusion were higher than during VLCD (when insulin stimulated lipolysis).

The current data point to an important methodological issue when using the microdialysis technique for studying tissue glycerol levels. When microdialysis data are interpreted, variations in the TBF must be monitored and accounted for. Skeletal muscle blood flow was not changed by insulin infusion before dieting, a finding which is in accordance with previous studies (14,15). Interstitial muscle glycerol levels were decreased after insulin infusion in agreement with earlier findings (7,14). However, the rate of glycerol release was not influenced by insulin. There are several explanations for this discrepancy. Compartmentalization and/or reutilization of glycerol by muscle could be influenced by insulin. The hormone could also cause a small stimulation in skeletal blood flow, which could not be detected by the Xenon measurements. It has been demonstrated that tissue glycerol levels can be lowered by selective stimulation of blood flow (36). Nevertheless, the present muscle data imply that a decrease in muscle glycerol levels cannot be interpreted as an inhibition of lipolysis without concomitant knowledge regarding tissue blood-flow rates.

In this study, we used previously determined values for the permeability surface area product (22,23). However, there is no reason to believe that these values are influenced by VLCD. Even if this was the case, then only absolute values for rates of glycerol mobilization would be altered. The major novel finding in muscle would still prevail, showing a lack of insulin effect before VLCD and a stimulatory effect during VLCD.

Although the investigations of lipolysis were performed in a single muscle group (gastrocnemius) in a selected group of patients, we propose the following mechanism for regulation of lipid oxidation during VLCD. A high rate of lipid oxidation is maintained, despite a preserved insulin inhibition of adipose tissue lipolysis, because skeletal muscle lipolysis is actually stimulated by insulin during VLCD. This elevates skeletal muscle FFAs and maintains lipid oxidation, although the supply of FFAs from adipose tissue lipolysis is diminished. Muscle lipolysis might also explain the findings with glucose oxidation and circulating FFAs during VLCD. Insulin-stimulated glucose oxidation was decreased during VLCD, which might be secondary to increased local FFA availability in muscle because glucose and FFAs compete as energy substrates in muscle and because glucose storage takes priority over glucose oxidation in the refeeding state (37). It should be stressed that these mechanisms may only operate under relatively short-term periods of VLCD. Other or adaptive effects may be introduced during longer periods of reduced caloric intake.

In summary, this study provides the first demonstration of a possible physiological role of skeletal muscle lipolysis. This process seems to be an important source of fatty acids for lipid oxidation during caloric restriction, at least in obese women.

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