Lactate release from adipose tissue and skeletal muscle in vivo: defective insulin regulation in insulin-resistant obese women

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Qvisth V, Hagström-Toft E, Moberg E, Sjöberg S, Bolinder J. Lactate release from adipose tissue and skeletal muscle in vivo: defective insulin regulation in insulin-resistant obese women. Am J Physiol Endocrinol Metab 292: E709–E714, 2007. First published October 31, 2006; doi:10.1152/ajpendo.00104.2006.—To study the local tissue lactate production in the normal state and its possible disturbances in insulin resistance, rates of lactate release from adipose tissue (AT) and skeletal muscle (SM) were compared postabsorptively and during a hyperinsulinemic euglycemic clamp in 11 healthy nonobese and 11 insulin-resistant obese women. A combination of microdialysis, to measure interstitial lactate, and the 133Xe clearance technique, to determine local blood flow, were used. In the controls, local blood flow increased by 40% in SM (P < 0.05) and remained unchanged in AT, whereas the interstitial-plasma difference in lactate doubled in AT (P < 0.005) and was unaffected in SM during hyperinsulinemia. In the obese, blood flow and interstitial-plasma difference in lactate remained unchanged in both tissues during hyperinsulinemia. The lactate release (μmol·100 g−1·min−1) was 1.17 ± 0.22 in SM and 0.43 ± 0.11 in AT among the controls (P < 0.01) and 0.86 ± 0.23 in SM and 0.83 ± 0.25 in AT among the obese women in the postabsorptive state. During insulin infusion, lactate release in the controls increased to 1.92 ± 0.26 in SM (P < 0.005) and to 1.14 ± 0.22 in AT (P < 0.005) but remained unchanged in the obese women. It is concluded that AT and SM are both significant sources of lactate release postabsorptively, and AT is at least as responsive to insulin as SM. The ability to increase lactate release in response to insulin is impaired in AT and SM in insulin-resistant obese women, involving defective insulin regulation of both tissue lactate metabolism and local blood flow.

Microdialysis; blood flow

Disturbances in whole body lactate turnover appear to be of importance in the development of insulin resistance. Elevated fasting plasma levels of lactate have been reported in both obese and type 2 diabetic subjects (29, 36). Moreover, increased fasting plasma lactate is suggested to be an independent risk factor for development of type 2 diabetes (45). It is postulated that increased lactate levels in type 2 diabetes reflect the rate of Cori cycling of lactate, with accelerated hepatic gluconeogenesis and elevated fasting glucose as a consequence (9, 33).

Lactate is produced from glucose and glycogen through glycolysis, by the conversion of pyruvate by lactate dehydrogenase, in virtually all human organs and tissues. The production of lactate is not only an anaerobic energy source and a precursor for gluconeogenesis and glycogen synthesis in the liver but also an energy substrate to compete with glucose for aerobic oxidation in peripheral tissues (24). Furthermore, it is suggested that there is an intracellular lactate shuttle where the lactate produced directly within the cell is transported in the mitochondria for oxidation (4).

Skeletal muscle is considered to be the major site of lactate production. The regulation of lactate metabolism in muscle during exercise is well studied, whereas lactate production at rest is less well characterized. During exercise, the muscle produces lactate to yield energy from anaerobic glycolysis; however, there is also a significant lactate production in the fully oxygenated contracting muscle (40). Likewise, at rest, skeletal muscle would seem to be an important tissue for lactate production during insulin stimulation, since insulin-mediated glucose uptake takes place mainly in the muscle. However, in arteriovenous balance studies, only a small or no net release from the limb was registered postabsorptively (12, 44), and no increase in lactate release was seen over the forearm during hyperinsulinemia or after a meal (20, 35, 44). Conversely, studies with lactate isotopes have demonstrated that there is a continuous uptake and release of lactate in the skeletal muscle (8, 40) and that the lactate release increases during hyperinsulinemia (8, 32). Furthermore, studies using microdialysis have shown higher lactate levels in skeletal muscle than in plasma in the fasting state and that interstitial lactate levels in muscle increase in response to oral glucose ingestion (23) and hyperinsulinemia (17), indicating lactate release.

Many studies, using different techniques, have shown that adipose tissue is also a significant source of lactate release (11, 14, 16, 22). The lactate release from adipose tissue is demonstrated to increase in response to oral glucose ingestion (16, 19, 21) and hyperinsulinemia (11, 14, 17, 19).

In insulin-resistant conditions, the regulation of lactate production from skeletal muscle and adipose tissue is poorly elucidated. In skeletal muscle, studies using arteriovenous balance and isotope techniques have registered both increased and similar rates of skeletal muscle lactate release in type 2 diabetics compared with controls (1, 5, 9). In adipose tissue, comparison of lactate release with microdialysis in lean and obese men has shown impaired ability to increase lactate production following oral glucose in obese subjects (21). In contrast, first-degree relatives of type 2 diabetics appear to have increased lactate production from adipose tissue during hyperinsulinemia (37).

Thus the regulation and possible disturbances in insulin-resistant conditions of lactate production in human skeletal muscle and adipose tissue at rest remain largely unknown. The aims of the present study were, first, to compare the basal rates

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of lactate release in human adipose tissue and skeletal muscle and, second, to evaluate the effects of insulin on lactate release in these two tissues in both healthy normal-weight and in insulin-resistant obese women. The effects of insulin were studied during a hyperinsulinemic euglycemic clamp. The rates of net lactate release in adipose tissue and skeletal muscle were assessed using the combination of microdialysis, to measure interstitial lactate, and the $^{133}$Xe clearance technique, to determine local blood flow.

**MATERIALS AND METHODS**

**Subjects.** The study was comprised of 11 nonobese [age 42 ± 2 yr, body mass index (BMI) 22.8 ± 0.6 kg/m$^2$] and 11 obese (age 37 ± 1 yr, BMI 39.9 ± 0.4 kg/m$^2$) women. They were all healthy and did not take any regular medication. The subjects gave their informed consent, and the study was approved by the Ethics Committee of the Karolinska Institute.

**Microdialysis.** The principle of microdialysis has been described in detail previously (41). The microdialysis catheter (CMA/60; CMA Microdialysis, Stockholm, Sweden) consists of a semipermeable membrane (30 × 0.62 mm, molecular mass cutoff 20 kDa) connected to the end of a double-lumen polyurethane tube. The probe was inserted in the tissue and was continuously perfused using a precision pump (CMA/100 microinjection pump; CMA Microdialysis) with a sterile solution. An exchange of metabolites then takes place over the microdialysis membrane, and the composition of the outflow solution reflects the extracellular fluid.

**Study protocol.** All subjects were investigated in the supine position after an overnight fast. The experiment was started in the morning (7:30 AM). Body composition (fat and lean body mass) was determined with bioelectrical impedance analysis, as previously described (25). The validity of body fat measurements with this method has been demonstrated by others to have good correlation with the reference method (dual-energy X-ray absorptiometry; see Refs. 28 and 43).

A retrograde catheter (Venflon) was inserted in a dorsal hand vein. The hand was placed in an air-heated box (63°C) for sampling of lactate, glucose, and free insulin. After a 60-min equilibration period, the first injection was given after 120 min of basal sampling. The second injection was given after 30 min of insulin infusion. Recordings were started 5 min after the injection and continued for 30 min. The first 10 min of the skeletal muscle decay curve was used for calculations.

Adipose tissue and muscle blood flow was calculated according to the following equation:

$$\text{TBF} = k \times \lambda \times 100 \text{ (ml·100 g}^{-1} \text{·min}^{-1})$$

where TBF denotes tissue blood flow, $k$ denotes the rate constant of the decay of the residual activity, and $\lambda$ the tissue-to-blood partition coefficient. The values for $\lambda$ were set at 10 ml/g for adipose tissue and 0.7 ml/g for muscle (26, 27).

The reproducibility of the $^{133}$Xe clearance technique has previously been evaluated in methodological experiments. The coefficient of variation for duplicate measurements of basal blood flow in skeletal muscle was 20.6% when the blood flow was measured in the same subject with 4–10 mo between the measurements. Based on this finding, it was calculated that, in 10 subjects, a difference of blood flow of 0.4 ml·100 g$^{-1}$·min$^{-1}$, i.e., ~23% of the blood flow at basal conditions, could be detected with 80% power at the 5% significance level (32).

**Calculations.** The absolute rates of lactate release from adipose tissue and skeletal muscle were calculated according to Fick’s principle:

$$V = (V - A) \times Q \times (1 - \text{hematocrit×} 10^{-2}) \text{ (mmol·100 g}^{-1} \text{·min}^{-1})$$

where $V$ denotes venous lactate, $A$ arterial lactate, and $Q$ calculated plasma blood flow.

Conversion of interstitial (I) to venous (V) lactate concentrations was made according to the following equation:

$$V = (I - A) \times (1 - e^{-kPS})$$

where $PS$ is the permeability surface product area (adopted to be 4 ml·100 g$^{-1}$·min$^{-1}$ for lactate; see Ref. 13).

**Biochemical analysis.** Microdialysate lactate was determined with an enzymatic fluorometric method, using a tissue sample analyzer (CMA/600; CMA Microdialysis). In agreement with earlier findings, the coefficient of variation for duplicate analyses of lactate during the basal period was 6.1 ± 0.1% in skeletal muscle and 6.0 ± 0.1% in adipose tissue. Plasma lactate was determined by an enzymatic fluorometric method (34). Plasma free insulin was determined by a commercial RIA (Pharmacia, Uppsala, Sweden).

**Statistics.** The results are expressed as means ± SE. Statistical analyses were performed with repeated-measures ANOVA followed by post hoc analyses with Fischer’s protected least-significant difference test. Because of nonhomogenous correlations, a general linear model for mixed effects was used when the interstitial-arterial difference lactate values were compared. Student’s paired $t$-test was used for the comparison of fasting glucose between the study groups. A value of $P < 0.05$ was considered statistically significant. A statistical software package (StatisticaVersion 7.1 for personal computer; Statsoft) was used for all statistical calculations.

**RESULTS**

The total body fat masses were 67.4 ± 1.3 kg for the obese women and 20.4 ± 1.8 kg for the nonobese women ($P < 0.001$). The corresponding lean body masses were 43.1 ± 0.7 and 46.3 ± 1.5 kg (not significant).

Plasma glucose, lactate, insulin, insulin sensitivity [metabolized glucose per unit of insulin (M/I) ratio], and local blood
flow rates and fractional release of lactate in adipose tissue and skeletal muscle are presented in Table 1.

**Basal conditions.** In the fasting state, plasma glucose and lactate did not differ between the obese and nonobese women. In contrast, fasting plasma insulin levels were 2.5 times higher in the obese women than in the nonobese women, implying insulin resistance ($P < 0.001$).

The fractional release of lactate from adipose tissue and skeletal muscle was calculated as the difference between the interstitial lactate concentration in the two respective tissues and the arterialized venous plasma lactate level. The interstitial-plasma difference in lactate was $\sim 3.5$ times higher in skeletal muscle compared with adipose tissue postabsorptively in the lean women ($P < 0.001$, mixed model) and about two times as high in the obese women ($P < 0.001$, mixed model). The basal interstitial-plasma lactate difference in adipose tissue was significantly higher in the obese women compared with the nonobese women ($P < 0.05$, mixed model), whereas in skeletal muscle it did not differ between the study groups.

There were no significant differences in blood flow rates between the controls and the obese women in the fasting state in either adipose tissue or skeletal muscle. Basal blood flow rates in adipose tissue were higher than in skeletal muscle in both the controls and obese subjects ($P < 0.05$).

The rates of lactate release in adipose tissue and skeletal muscle are shown in Fig. 1. Fick’s principle for calculation of rates in adipose tissue were higher than in skeletal muscle in the obese women. The rates of lactate release at baseline did not differ between the study groups in either adipose tissue or skeletal muscle.

In the controls in the postabsorptive state, the rate of lactate release from either skeletal muscle or adipose tissue were seen in response to insulin. There were no significant differences in basal lactate release between the two tissues in the obese women. The rates of lactate release at baseline did not differ between the study groups.

There were no significant differences in blood flow rates between the controls and the obese women in the fasting state in either adipose tissue or skeletal muscle. Basal blood flow rates in adipose tissue were higher than in skeletal muscle in both the controls and obese subjects ($P < 0.05$).

The rates of lactate release in adipose tissue and skeletal muscle are shown in Fig. 1. Fick’s principle for calculation of rate of lactate release is applicable only during steady-state conditions. The lactate release from adipose tissue and skeletal muscle was calculated from the last 30-min steady-state periods.

### Table 1. Glucose, insulin, and lactate concentrations in P, M/I ratio, I-A difference, and lactate and blood flow rates in skeletal muscle during basal state and insulin infusion in obese and nonobese subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Lean</th>
<th>Obese</th>
<th>P Level (lean vs. obese)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP glucose, mmol/l</td>
<td>4.9 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>P insulin, pmol/l</td>
<td>38.5 ± 2.3</td>
<td>97.4 ± 13.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>P lactate, mmol/l</td>
<td>1.97 ± 0.06*</td>
<td>0.99 ± 0.02*</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>M/I ratio, mg·kg LBM$^{-1}$. min$^{-1}$</td>
<td>0.011 ± 0.001</td>
<td>0.004 ± 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

| I-A difference lactate, mmol/l | 0.39 ± 0.11 | 0.98 ± 0.24 | < 0.05 |
| Adipose tissue | 0.88 ± 0.18* | 0.86 ± 0.12 | NS |
| Skeletal muscle | 1.5 ± 0.20 | 1.79 ± 0.19 | NS |
| Blood flow rates, ml·100 g$^{-1}$·min$^{-1}$ | 2.78 ± 0.41 | 1.80 ± 0.29 | NS |

| Adipose tissue | 2.74 ± 0.20 | 2.12 ± 0.31 | NS |
| Skeletal muscle | 1.59 ± 0.20 | 1.05 ± 0.29 | NS |
| iv Insulin | 2.56 ± 0.31* | 1.09 ± 0.27 | < 0.05 |

Values are means ± SE. *P < 0.05, basal vs. insulin-stimulated samples within the tissue. NS, nonsignificant; IP, fasting plasma; P, plasma; M/I, metabolized glucose per unit of serum insulin; I-A, interstitial-arterial.

### DISCUSSION

In the present study, the rates of lactate release from both adipose tissue and skeletal muscle have for the first time been compared in healthy nonobese subjects and in insulin-resistant obese subjects in vivo.

In corroboration with previous studies in healthy subjects, we found that both skeletal muscle and adipose tissue are significant sources of lactate production in the postabsorptive state and that insulin stimulates net lactate release in both tissues (1, 5, 11, 14, 16, 17, 19, 21–23). The basal lactate release was, as expected, higher in skeletal muscle than in adipose tissue. However, in response to insulin infusion, the relative increase in net lactate release was clearly more pronounced in adipose tissue compared with muscle tissue. This is in keeping with previous findings indicating that plasma lactate is mainly determined by the rate of glucose disposal in extra-muscular tissues such as adipose tissue rather than skeletal muscle during hyperinsulinemia (44).

By contrast, our results showed impaired insulin-stimulated lactate release in both skeletal muscle and adipose tissue in the insulin-resistant obese women. This is in accordance with previous data where the rates of lactate release from adipose tissue increased in lean but not in obese subjects following an
oral glucose load (21). It is well known that there are regional differences in the intermediate metabolism, both in various fat deposits and skeletal muscles. We believe this is important to take into consideration in the context of extrapolating data from a local fat deposit or skeletal muscle to the whole body. However, our findings may suggest that, when muscle and adipose tissue masses are considered, the adipose tissue in the obese subjects probably is much more important than skeletal muscle for the total rate of lactate release at least postabsorptively because of the pronounced increase in total fat mass (in this study ~47 kg). This may imply that the increased adipose tissue mass in obesity is the main contributor to the enhanced lactate turnover and gluconeogenesis seen in type 2 diabetes in the postabsorptive state (9).

Contradictory, in this study, the concentrations of fasting plasma lactate did not differ between the controls and obese women. Increased levels of fasting plasma lactate have previously been registered in both type 2 diabetes (1, 36) and obesity (21). However, Lovejoy et al. (29) found no difference in basal plasma lactate between obese and lean subjects, whereas a negative correlation between basal plasma lactate and the degree of insulin sensitivity was demonstrated. Furthermore, it is well known that it is the visceral fat deposits that are associated with insulin resistance and type 2 diabetes. Consequently, it could be increased lactate release from intra-abdominal fat masses via the portal system, which do not reach the systemic circulation that contributes to glucose production in the liver. This is supported by earlier studies with isolated adipocytes in vitro that showed higher lactate production in visceral fat cells than in subcutaneous cells (31).

The impaired net lactate release from skeletal muscle and adipose tissue in response to insulin may explain the lower plasma lactate levels that were seen in the obese women during hyperinsulinenia in the present study, as well as in earlier investigations of obesity (28) and type 2 diabetics (39).

Our findings with local blood flow rates in the control group are consistent with previous studies showing that insulin stimulates the local blood flow in skeletal muscle (2, 6) probably via insulin-mediated capillary recruitment (38). In adipose tissue, the blood flow remained unchanged, and earlier studies have shown both stimulation and no effect by insulin on the nutritive blood flow in adipose tissue (17, 32). The fractional release of lactate, on the other hand, increased only in adipose tissue during insulin infusion. This may suggest that the stimulatory effect of insulin on net lactate release in skeletal muscle was mainly the result of an insulin-induced increase in blood flow rates, whereas in adipose tissue insulin predominantly stimulates the production of lactate.

However, given the coefficient of variance of the $^{133}$Xe-clearance washout technique, detecting small differences in the calculated lactate release may be difficult.

Nevertheless, the diminished rise in lactate release from skeletal muscle seen in the obese women may, at least to some extent, have been a result of the impaired blood flow response to insulin and may indicate an association between obesity and defective blood flow regulation in adipose tissue and skeletal muscle. Accordingly, an impaired ability of insulin to increase skeletal muscle blood flow has previously been reported in states of insulin resistance, and it has been suggested that diminished tissue perfusion could contribute to insulin resistance (3, 6).

When estimating the lactate release, we used the same $PS$ value (4 ml·100 g$^{-1}$·min$^{-1}$) in all calculations, as previously suggested (13). The $PS$ value, the product of permeability and surface area, is considered to be comparable in skeletal muscle and adipose tissue (13). However, recent studies have indicated that the calculated $PS$ value for various substances may differ between lean and insulin-resistant obese subjects. Coppack et al. (10) found that the $PS$ value for glycerol in subcutaneous abdominal adipose tissue declined with increasing adiposity. Likewise, Gudbjornsottir et al. (15) have demonstrated reduced $PS$ values for glucose and insulin in skeletal muscle in insulin-resistant type 2 diabetic subjects. In both studies, the $PS$ values in the obese subjects were approximately one-half that of the controls. To evaluate to what extent reduced $PS$ values in the obese subjects would influence the results in the present study, we recalculated our data with the $PS$ value set to 2 ml·100 g$^{-1}$·min$^{-1}$ for the obese subjects. The rates of lactate release from adipose tissue and skeletal muscle then declined...
by 10–20% in these obese subjects; however, the comparison between lean and obese subjects did not change.

In the present study, the true lactate production in skeletal muscle may have been underestimated because of simultaneous lactate uptake (8, 40). In adipose tissue, on the other hand, lactate uptake has not been shown. However, our main aim was to study the net lactate release, and the relative contributions, of adipose tissue and skeletal muscle to whole body lactate turnover in lean and obese subjects, since the net tissue outflow of lactate is probably most important in the development of insulin resistance by delivery of lactate for gluconeogenesis and glycogen formation in the liver.

Lactate production in skeletal muscle is traditionally considered to be higher in glycolytic type 2 fibers than in oxidative type 1 fibers (42). In the present study, the gastrocnemius muscle was investigated, which has a high proportion of oxidative type 1 fibers (18). Hence, the rates of lactate release in other more glycolytic muscle groups may differ from our results.

In summary, this study confirmed that adipose tissue is a significant source of lactate release in both the postabsorptive state and during hyperinsulinemia. Insulin-resistant obese subjects show an impaired ability to increase lactate release in response to insulin in tissues involved in both blood flow and tissue production. Because of the increased fat mass in obesity, lactate release from adipose tissue would seem to be of greater importance than lactate release from skeletal muscle and could possibly contribute to the increased lactate turnover seen in insulin resistance and type 2 diabetes.

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