Effects of exercise on insulin distribution and action in testosterone-treated oophorectomized female rats

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Niklasson, Maria, Peter Daneryd, Peter Lönnroth, and Agneta Holmäng. Effects of exercise on insulin distribution and action in testosterone-treated oophorectomized female rats. J Appl Physiol 88: 2116–2122, 2000.—Administration of testosterone (T) to oophorectomized (Ovx) female rats is followed by severe insulin resistance, localized to postreceptor cellular events in the muscle. In this study, intervention by exercise was introduced to examine whether circulatory adaptations are involved in insulin resistance. Two groups of Ovx rats were studied: one group was given T (Ovx+T); another group had free access to running wheels (Ovx+T+Ex). In addition, one control group (sham operated) was studied. Insulin sensitivity was measured with the euglycemic hyperinsulinemic clamp technique (submaximal) for 150 min. Muscle interstitial glucose and insulin concentrations were measured by microdialysis. The measurements showed that, in Ovx+T rats, the onset of insulin action was significantly (P < 0.05) slower during the first 95 min of the clamp compared with that in Ovx+T+Ex controls. Muscle interstitial concentrations of insulin but not glucose were lower in both Ovx+T and Ovx+T+Ex rats than in controls throughout the clamp. It was concluded that physical exercise prevented the slow onset of insulin action in Ovx+T rats without changing the distribution time of muscle interstitial insulin. The results indicate that hyperandrogenicity is characterized by delayed muscle insulin action. Physical exercise reverses these defects without any beneficial effect on muscle interstitial insulin concentrations.

testosterone; insulin resistance; microdialysis; skeletal muscle

HYPERANDROGENIC CONDITIONS in women are often associated with severe insulin resistance (8, 28, 33, 37). In Swedish women selected at random, a low plasma level of sex hormone-binding globulin (SHBG) was found to be statistically associated with insulin resistance (9, 24) and with a strong risk for the development of non-insulin-dependent diabetes mellitus. In addition, a low SHBG predicts the development of hypertension and mortality from myocardial infarction in women (21, 22). There is no consensus on whether insulin resistance is the primary factor by inhibiting SHBG production from the liver, or whether androgens primar-

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tions in the oophorectomized plus testosterone treatment (Ovx+T) rat model have demonstrated that the effect of insulin on the uptake of glucose by muscle is markedly delayed as the result of a combination of muscle postreceptor defects and a time lag in the equilibration of the insulin concentration over the capillary wall (11, 14, 39). Because exercise may increase insulin sensitivity as well as muscle capillarization, it is possible that exercise shortens the time before the onset of the insulin effect.

The aim of this study was to use microdialysis measurements of insulin to study the effect of exercise and to differentiate between the effects on insulin delivery to the interstitial fluid and the effects at the postreceptor level. To meet this aim, we investigated the effects of Ovx+T on insulin sensitivity in female rats and examined whether the reduction in insulin sensitivity and the delayed insulin effect could be reversed by exercise. The euglycemic hyperinsulinemic clamp method was used simultaneously with the microdialysis technique.

MATERIALS AND METHODS

A total of 30 female Sprague-Dawley rats (B&K Universal, Sollentuna, Sweden) weighing 173 ± 2 g before treatment were used. They were fed with commercial rat chow containing 18.5% protein, 4.0% fat, 55.7% carbohydrates, and a sufficient supply of vitamins and minerals (Lactamin, Stockholm, Sweden), along with tap water ad libitum. The light cycle was 12:12 h, and the room temperature was 23°C. The study was approved by the Animal Ethics Committee of Göteborg University.

Study procedure. The rats were randomly divided into three study groups. The first group was the Ovx+T exercise group (Ovx+T+Ex, n = 10). These rats were Ovx and treated with intramuscular injections of 2 mg of testosterone enantate in sesame oil (0.1 ml) every second week. The first injection was performed in the medial femoral muscle in both legs during the clamp, as previously described (25). Two 15-mm microdialysis catheters (30 mol wt cutoff; BAS, Indianapolis, IN) were perfused with isotonic saline containing 1% bovine albumin at the rate of 1 µl/min. The catheters were inserted just before the clamp was started (time = 0 min). After 40 min of equilibration, the dialysate was collected at 55-min intervals (40–95 and 95–150 min) for measurement of inulin, insulin, and glucose.

Calculations. The interstitial glucose concentration was calculated from the in vivo probe recovery according to the internal reference calibration technique (26). The perfusate was prepared with [3H]glucose (0.05 µCi/ml), and the loss over the membrane was taken as an estimate of recovery (dialysate concentration/interstitial concentration) (26). The experiments revealed a 55.4 ± 5.8% in vivo dialysate recovery of interstitial glucose (n = 14). The insulin concentration in the interstitial fluid was calculated by means of an external reference with the use of [14C]inulin infused subcutaneously for 24 h (see Study procedure). The recoveries of both [14C]inulin and insulin in dialysates obtained in vivo were compared, and the interstitial concentration of [14C]inulin was assumed to be the same in plasma. To characterize the different diffusion properties and to correct for differences in binding of the compounds to the catheter, identical experiments were performed in which [14C]inulin and insulin were dialysed in plasma at 37°C in vitro. Results from seven such in vitro experiments demonstrated that the relative recoveries of [14C]inulin and insulin in microdialysates were 0.22 ± 0.04 and 37°C in vitro. Results from seven such in vitro experiments demonstrated that the relative recoveries of [14C]inulin and insulin in microdialysates were 0.22 ± 0.04 and 0.03 ± 0.01, respectively. The corresponding [14C]inulin recovery in experiments performed in vivo was 0.22 ± 0.04.

The interstitial insulin concentration was then calculated in the right jugular vein for blood sampling and for measurement of inulin, insulin, and glucose. The rats were anesthetized with diethyl ether, and an osmotic minipump (Alza, Palo Alto, CA) that continuously infused [14C]inulin (0.1 µCi/rat, pump rate 8 µl/h; NEN, Boston, MA) was implanted subcutaneously in the dorsal neck region 24 h before the clamp study was initiated.

Euglycemic hyperinsulinemic clamp. The euglycemic clamp technique (43) was used in experiments investigating the effect of exercise on insulin sensitivity, as previously described in detail (14). In brief, the animals (Ovx+T+Ex, Ovx+T, and C rats) were anesthetized in the morning with 170 mg/kg body wt thioubarbitral sodium salt (Inactin, RBI, Natick, MA) injected intraperitoneally. Catheters were then inserted in the left carotid artery for blood sampling and in the right jugular vein for infusion of insulin and glucose. Body temperature was maintained at 37°C with a heating blanket and a rectal probe. Measurements then started 30 min after the operation. Insulin (40 U/ml, Human Actrapid, Novo, Copenhagen, Denmark) was infused to obtain a submaximal insulin concentration (5 mU·kg⁻¹·min⁻¹) for 150 min. Euglycemia was maintained by infusion of 10% glucose solution in physiological saline. The infusion speed was guided by glucose concentration measurements in 30-µl blood samples at regular intervals (every 5 min during the first 40 min; then every 10 min). A plasma glucose level of ~7 mM was chosen to match postprandial glucose concentrations determined just before the clamp. Insulin concentrations were determined in 250-µl blood samples taken after 0, 40, 95, and 150 min of infusion. The plasma insulin concentrations in the Ovx+T+Ex, Ovx+T, and C rats at 0 min were 28 ± 3, 29 ± 5, and 26 ± 4 mU/l (not significant). Less than 2 ml of blood were used for these determinations, and the loss was compensated for by the infusion volumes. At the completion of the clamp, the rats were killed with intravenous injections of KCl, and the muscles of the lower hindlimb and the parametrical and retroperitoneal adipose tissues were dissected out and weighed.

Microdialysis. Microdialysis was performed in the medial femoral muscle in both legs during the clamp, as previously described (25). Two 15-mm microdialysis catheters (30 mol wt cutoff; BAS, Indianapolis, IN) were perfused with isotonic saline containing 1% bovine albumin at the rate of 1 µl/min. The catheters were inserted just before the clamp was started (time = 0 min). After 40 min of equilibration, the dialysate was collected at 55-min intervals (40–95 and 95–150 min) for measurement of inulin, insulin, and glucose.
each animal according to the following formulas (Eq. 1 is in vivo and Eq. 2 is in vitro)

\[
F_1 = \frac{\text{dpm (dialysate)}}{\text{dpm (plasma) · insulin concentration (dialysate)}}
\]

\[
F_2 = \frac{\text{dpm (dialysate) · insulin concentration (plasma)}}{\text{dpm (plasma) · insulin concentration (dialysate)}}
\]

\[
F_1 · F_2 = \text{interstitial insulin concentration}
\]

where \(F_1\) and \(F_2\) are formulas 1 and 2, and dpm is disintegration per min.

Because the dialysis recovery of \(^{14}\text{C}\) insulin and insulin in plasma in vitro was identical to that estimated in vivo, a recovery factor for microdialysis of insulin could be calculated.

In experiments made in vitro in plasma, microdialysis was performed with the same catheter material and perfusate but without labeled glucose (41).

Analytical methods. Blood was collected in heparinized microtubes and centrifuged immediately in a Beckman microfuge (Palo Alto, CA). Plasma and microdialysate concentrations of glucose were determined enzymatically by using 10 μl samples for simultaneous analyses in a YSI 2700 Select biochemical analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and dialysate insulin were measured with a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden). Radioactivity in \(^{14}\text{C}\) insulin in the plasma and dialysate was counted in a liquid scintillation counter by using a quenched-corrected isotope program (1217 Rackbeta, LKB, Uppsala, Sweden).

Statistical analysis. All results are expressed as means ± SE. Significance of differences was tested with Student's t-test for paired observations and, when applicable, with ANOVA with StatView software for the Macintosh. P < 0.05 was considered significant. Fisher's least significant difference test was used for post hoc analyses.

RESULTS

There were no significant differences in body weights in the three groups before treatment. Ovx+T and Ovx+T+Ex rats gained weight more rapidly than the C rats did; their body weight was significantly higher after 12 days (200 ± 3, 216 ± 3, and 217 ± 7 g for C, Ovx+T, and Ovx+T+Ex, respectively, P < 0.05). This weight difference persisted, and there was also a tendency for the Ovx+T group to gain more weight than the exercise group did (Table 1). Food intake was 5.8 ± 0.2 (C), 6.5 ± 0.2 (Ovx+T; P < 0.05 vs. C), and 8.1 ± 0.3 g·100 g body wt·1·day·1 (Ovx+T+Ex; P < 0.001 vs. C, T, and control groups).

Table 1. Total body weight and weight gain before and after 6 wk of treatment in Ovx+T+Ex, Ovx+T, and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial Body Weight, g</th>
<th>Body Weight at Clamp, g</th>
<th>Body Weight Gain, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>167 ± 3</td>
<td>245 ± 7*</td>
<td>47 ± 5*</td>
</tr>
<tr>
<td>Ovx+T</td>
<td>10</td>
<td>164 ± 2</td>
<td>301 ± 6</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>Ovx+T+Ex</td>
<td>10</td>
<td>165 ± 2</td>
<td>281 ± 9</td>
<td>71 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Ovx+T, oophorectomized, testosterone treated; Ovx+T+Ex, oophorectomized, testosterone treated, and exercised. *P < 0.05, control vs. Ovx+T and Ovx+T+Ex groups.

Figure 1 shows the glucose infusion rate during the euglycemic hyperinsulinemic clamp at submaximal insulin concentrations (5 μU·kg⁻¹·min⁻¹) for 150 min. The Ovx+T rats had a significantly lower glucose infusion rate during the first 95 min of the clamp than the other two groups did (P < 0.05). At 150 min, this difference had disappeared, and there were no longer any significant differences among the three groups during the rest of the clamp.

Figure 2A shows that there were no significant differences among the three groups in plasma insulin levels during the whole clamping period (40–150 min). The Ovx+T rats, both with and without exercise, had significantly lower interstitial insulin levels during the whole clamping period than the C rats did, as measured in medial femoral muscles (P < 0.01) (Fig. 2C). The C rats had a significantly higher interstitial level of insulin between 95 and 150 min than between 40 and 95 min of the clamping period (P < 0.01). Because of a nonphysiologically high interstitial insulin concentration measured in one C rat between 95 and 150 min, the interstitial concentration tended to be slightly higher numerically (16 ± 19 μU/ml) than in plasma without reaching statistical significance (Fig. 2). Between 40 and 95 min, the arterial-interstitial concentration ([a-i]) differences for insulin were 10 ± 13 (not significant), 41 ± 14 (P < 0.05), and 38 ± 8 μU/ml (P < 0.05), and between 95 and 150 min, they were nonsignificant in all animal groups.

Plasma glucose concentrations reached a steady state at 7 mmol/l after 40 min and were the same in all three groups throughout the insulin clamp (Fig. 2B). Figure 2D shows that there were no significant differences among the three groups in interstitial glucose concen-
Table 2.  Weights of extensor digitorum longus, soleus, and tibialis muscles and parametrial and retroperitoneal adipose tissue after 6 wk of treatment in Ovx+T+Ex, Ovx+T, and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>EDL</th>
<th>Tibialis</th>
<th>Soleus</th>
<th>Plantaris</th>
<th>Parametrial</th>
<th>Retroperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>0.50 ± 0.01</td>
<td>1.86 ± 0.03</td>
<td>0.45 ± 0.01</td>
<td>0.85 ± 0.02</td>
<td>7.0 ± 0.3</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>Ovx+T</td>
<td>10</td>
<td>0.46 ± 0.02</td>
<td>1.77 ± 0.06</td>
<td>0.42 ± 0.01†</td>
<td>0.84 ± 0.04</td>
<td>4.4 ± 0.56</td>
<td>9.6 ± 0.86</td>
</tr>
<tr>
<td>Ovx+T+Ex</td>
<td>10</td>
<td>0.48 ± 0.01</td>
<td>1.86 ± 0.03</td>
<td>0.51 ± 0.01‡</td>
<td>0.93 ± 0.02*</td>
<td>2.7 ± 0.3‡</td>
<td>3.4 ± 0.4‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. EDL, extensor digitorum longus. *P < 0.05, †P < 0.001, Ovx+T+Ex vs. Ovx+T and control; ‡P < 0.05 and §P < 0.001, Ovx+T+Ex vs. control.

The Ovx+T rat model for hyperandrogenicity and insulin resistance was previously employed to elucidate mechanisms behind insulin resistance in females with obesity and non-insulin-dependent diabetes mellitus (11, 14, 39). For the first time, it is reported here that obesity and non-insulin-dependent diabetes mellitus insulin resistance was previously employed to elucidate the action of an insulin infusion is delayed in the Ovx+T rat model for hyperandrogenicity and insulin resistance combined with hyperandrogenicity. Furthermore, it was confirmed that the increase in interstitial insulin concentration is slow. Moreover, physical exercise normalized the time before onset of insulin action without altering the interstitial insulin concentration.

The increase in interstitial levels of insulin during insulin infusion depends on the rate of transcapillary insulin delivery and the cellular insulin clearance. An increased insulin clearance, which is receptor mediated, implies an increase in receptor density. The insulin receptor number was measured in Ovx+T rats in a recent study and was found unchanged compared with that in C rats (39). Therefore, it may be hypothesized that lower interstitial insulin concentrations in Ovx+T rats might be due to altered capillary transport capacity.

Microdialysis measurements allow highly precise estimates of the interstitial concentration of any substance, provided that the microdialysis sampling catheter is calibrated in situ (26). Low-molecular-weight substances such as glucose (26), lactate (19), and glycerol (18) may be readily measured by using equilibration (26) or stop-flow (10) techniques for calibration. In contrast, peptides such as insulin that are prone to binding to plastic materials cannot be measured using conventional or standard calibration (17, 41). Therefore, we recently evaluated an external reference technique for intramuscular measurements in the rat and in humans using the continuous infusion of insulin as a reference (12). It should be noted here, however, that the technique is sensitive enough to readily measure low-molecular-weight substances such as glucose and lactate but not peptide hormones such as insulin at low physiological concentrations (26). Consequently, in the present study, data on interstitial insulin levels could not be obtained before steady-state plasma insulin concentrations were reached because of the detection limits of the analysis method presently employed. The present results were achieved under prevailing supraphysiological plasma concentrations of insulin during steady-state plasma insulin clamping conditions; therefore, tentative changes in interstitial fluid concentrations of insulin at the earlier clamping period could not be elucidated.

In the normal-to-high physiological range of insulin concentration, measured in the lean Zucker rat, the muscle interstitial insulin concentration is the same as in plasma (12), whereas the concentration of glucose in interstitial fluid is lower than in arterial plasma (12). At supraphysiological concentrations of insulin, the concentration of insulin in interstitial fluid is lower than in plasma, indicating either that transcapillary insulin transport is saturable or that insulin elimination is concentration dependent (12). However, even though an insulin concentration gradient may exist, evidence for a saturable transport of insulin has not been achieved in animal studies on hindlimb lymph representing the interstitial fluid (42). In human muscle, insulin (41) and glucose (13, 29) concentrations are lower in interstitial fluid than in plasma, even when physiological insulin concentrations are in the lower range. Hence, blood flow and transcapillary transport of insulin and/or glucose may be considered rate limiting for the insulin effect in human subjects (29, 41). Moreover, in human muscle the gradient seems independent of the concentration of insulin, conflicting with the idea that insulin transport is a saturable process (41). The importance of transcapillary delivery capacity and nutritive blood flow for the effect of insulin on the uptake of glucose by muscles in insulin resistance has recently been intensively discussed (13). Whereas insu-
Inn sensitivity correlates with muscle blood flow (2, 16), the causal relationship is not yet clear (13). Most studies in humans designed to determine this relation-ship have been performed during steady-state condi-tions, and the time kinetics of the insulin effect have been studied less often. Microdialysis sampling (17), as well as measurements of lymph (45), has demonstrated that changes in interstitial insulin concentrations do not occur until \( \sim 30 \) min after a sudden increase in the concentration of plasma insulin. In the present study, we used long (40-min) sampling intervals; thus time kinetics were not followed closely. However, it should be noted that the dialysis membrane presently used registers 100\% of the change in ambient insulin concentrations within 20 min (not shown). Therefore, a transient group difference in interstitial insulin concentrations registered at one sampling period represents at least a 20-min difference in the equilibration of insulin over the capillary wall. The Ovx\(^+\)T rats had lower concentra-tions of interstitial insulin that never reached the level of plasma insulin within the 150-min sampling period. This is in contrast to the glucose disposal rates in Ovx\(^+\)T rats showing normal insulin action already at 150 min. In the present study, it was thus confirmed that insulin action and the increase of the insulin concentration in the interstitial fluid were delayed in Ovx\(^+\)T rats (31). We propose that this latter defect may be attributed to the decrease in capillary density (14) and the resulting restricted blood flow, as well as to the longer diffusion distance between capillaries and muscle cells previously demonstrated (23). It is important to note in this context, however, that the [a-i] difference was small and that a postreceptor defect leading to a rightward shift of the dose-effect curve of insulin should, by itself, lead to a delay in the onset of the insulin effect.

Fig. 1. Glucose infusion rate during a hyperinsulinemic euglycemic clamp at submaximal insulin concentrations (5 mU·kg\(^{-1}\)·min\(^{-1}\)) for 150 min in control rats (open bars), oophorectomized, testosterone-treated rats (Ovx\(^+\)T; hatched bars), and oophorectomized, testosterone-treated, and exercised rats (Ovx\(^+\)T+Ex; solid bars). Data are expressed as means ± SE (n = 10 rats). *P < 0.05, Ovx\(^+\)T vs. control and Ovx\(^+\)T+Ex. Student's t-test was used for paired observations and ANOVA.

Fig. 2. A: plasma insulin concentrations deter-mined from mean values of plasma samples taken after 40, 95, and 150 min of a hyperinsu-linemic euglycemic clamp (5 mU·kg\(^{-1}\)·min\(^{-1}\)) in control (open bars), Ovx\(^+\)T (hatched bars), and Ovx\(^+\)T+Ex rats (solid bars). B: plasma glucose concentrations determined from mean values of plasma samples taken every 5 min. C: interstitial insulin concentrations in me-dial femoral muscles. Starting after 40 min of the clamp, samples of interstitial fluid were collected every 55 min for measurement of interstitial insulin. **P < 0.01, control vs. Ovx\(^+\)T and Ovx\(^+\)T+Ex rats; ††P < 0.01, control rats after 40–95 min vs. after 95–150 min. D: interstitial glucose concentrations. Data are expressed as means ± SE (n = 10). Student's t-test was used for paired observa-tions and ANOVA.
in the beginning of an insulin infusion. Postreceptor defects in insulin signaling and action, including defects in translocation of GLUT-4 transporter proteins to the plasma membrane and altered activation of glycogen synthase, were previously reported in studies of muscles from Ovx+T rats (39). It may, therefore, be suggested that these postreceptor defects also may contribute to the delayed onset of insulin action after Ovx+T, in addition to the lower interstitial insulin levels.

Physical exercise in Ovx+T rats prevented the development of fasting hyperinsulinemia and delayed insulin action; however, the muscle interstitial insulin concentration was lower than in C rats throughout the clamp. Thus, despite the expected effect of physical exercise to increase the number of capillaries in muscles, insulin transcapillary transport capacity was not improved enough to normalize the interstitial insulin concentration. The present data suggest that the effects of hyperinsulinemia and decreased insulin sensitivity induced by Ovx+T could be significantly improved by physical exercise by shortening the time of onset of insulin action through action on a receptor/postreceptor step in the muscle cells. Physical exercise leads to enhanced insulin effects through action on multiple postreceptor steps (5), including GLUT-4 (5) and glycogen synthase (38, 40), and also enhances the percentage of insulin-sensitive slow-twitch muscle fibers (1).

In the present study, Ovx+T resulted in elevated body weight as the result of increased amounts of retroperitoneal fat. The effect of enlargement of the abdominal fat depot on insulin sensitivity was previously demonstrated in obese women (20) with low plasma levels of SHBG. Physical exercise effectively increased muscle weight and prevented the shift in adipose tissue distribution seen after Ovx+T. Based on the data obtained in this rat model, this opens the possibility that physical exercise may decrease the risk for insulin resistance and delayed insulin action in obesity in combination with hyperandrogenicity and altered fat distribution (27, 36).

In summary, the present data obtained in the rat show that Ovx+T results in a delay in the onset of insulin action, and physical exercise prevented this time shift. The extra time needed to equilibrate insulin over the capillary wall seen in Ovx+T rats was not affected by physical exercise. The data support the view that transcapillary delivery of glucose and insulin is rate limiting for the insulin effect (12). Furthermore, the data are in agreement with the view that muscle blood flow plays a minor role in the effect of insulin on glucose uptake in resting insulin-sensitive (29) and insulin-resistant muscle (13); however, it should be noted that these data were obtained from resting animals and that transcapillary diffusion of insulin and glucose may be more critically dependent on the regulation of blood flow during exercise (6).

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