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Are blood flow and lipolysis in subcutaneous adipose tissue influenced by contractions in adjacent muscles in humans?

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Aerobic exercise increases whole body adipose tissue lipolysis, but is lipolysis higher in subcutaneous adipose tissue (SCAT) adjacent to contracting muscles than in SCAT adjacent to resting muscles? Ten healthy, overnight-fasted males performed one-legged knee extension exercise at 25% of maximal workload (W\text{max}) for 30 min followed by exercise at 55% W\text{max} for 120 min with the other leg and finally exercised at 85% W\text{max} for 30 min with the first leg. Subjects rested for 30 min between exercise periods. Femoral SCAT blood flow was estimated from washout of 133Xe, and lipolysis was calculated from femoral SCAT interstitial and arterial glycerol concentrations and blood flow. In general, blood flow and lipolysis were higher in femoral SCAT adjacent to contracting than adjacent to resting muscle (time 15–30 min; blood flow: 25% W\text{max} 6.6 ± 1.0 vs. 3.9 ± 0.8 mL·100 g⁻¹·min⁻¹, P < 0.05; 55% W\text{max} 7.3 ± 0.6 vs. 5.0 ± 0.6 mL·100 g⁻¹·min⁻¹, P < 0.05; 85% W\text{max} 6.6 ± 1.3 vs. 5.9 ± 0.7 mL·100 g⁻¹·min⁻¹, P > 0.05; lipolysis: 25% W\text{max} 102±19 vs. 55±14 nmol·100 g⁻¹·min⁻¹, P = 0.06; 55% W\text{max} 86 ± 11 vs. 50 ± 20 nmol·100 g⁻¹·min⁻¹, P > 0.05; 85% W\text{max} 88 ± 31 vs. 9 ± 25 nmol·100 g⁻¹·min⁻¹, P < 0.05). In conclusion, blood flow and lipolysis are generally higher in SCAT adjacent to contracting than adjacent to resting muscle irrespective of exercise intensity. Thus specific exercises can induce “spot lipolysis” in adipose tissue.

OBESITY CAN BE DEFINED AS a condition where the amount of adipose tissue is increased to such a degree that it has consequences for the health of the person. In addition to the amount, also the distribution of fat is important. Obesity can be diminished by increased physical activity and/or reduced caloric intake (11, 21) and, at least in the short term, the size of the negative caloric balance is the primary factor determining the weight loss (22, 28). There is evidence that exercise-induced relative loss of fat is higher in visceral and abdominal subcutaneous adipose tissue (SCAT) than in femoral SCAT (23). This indicates that regional adipose tissue depots are regulated independently, and for many years it has been discussed whether specific exercises can reduce local adipose tissue depots, i.e., induce a “spot reduction” of adipose tissue, and thus modify fat distribution (19, 20).

A number of studies have examined the spot reduction theory, and conclusions have been contradictory (12, 16, 19, 20). A valid study design to test the hypothesis of spot reduction is one in which the muscles in one part of the body are trained and the muscles in the contralateral side are not, and the size of the adipose tissue depots adjacent to the trained respective sedentary muscles are measured before and after the study period. One study using this design (19) found a decrease in skinfold thickness of the trained arm, but in another study (20) the skinfold thickness decreased significantly in both the trained and the sedentary arm during the study period. The latter finding is essentially supported by a study in which the skinfold thickness of both arms was measured in tennis players and control subjects (12). The skinfold thickness did not differ between arms in tennis players or control subjects, but skinfold thickness of both arms was lower in tennis players compared with controls. Krotkiewski et al. (16) had 10 healthy middle-aged females exercise one leg for 5 wk while the other leg was resting, and ultrasound measurements revealed that the thickness of the femoral SCAT of the trained leg was significantly decreased, whereas the thickness of the SCAT of the sedentary leg was unchanged. However, a reduced thickness of the subcutaneous tissue layer could be because of a training-induced enlargement of the underlying muscle, which could result in a compression of a possibly unchanged amount of SCAT. To clarify this, Krotkiewski et al. took two biopsies of the SCAT from each thigh and found that fat cell weight decreased nonsignificantly in both biopsies from the trained leg {−7% [0.60 ± (SE) 0.07 to 0.56 ± 0.05 μg] and −26% (0.42 ± 0.07 to 0.31 ± 0.08 μg)} and increased nonsignificantly in both biopsies from the sedentary leg [+11% (0.54 ± 0.03 to 0.60 ± 0.03 μg) and +7% (0.46 ± 0.08 to 0.49 ± 0.08 μg)]. Based on the biopsy data, Krotkiewski et al. turned down the spot reduction hypothesis, but it may be speculated that their nonsignificant changes in fat cell weight could be because of a type 2 statistical error.

In the present study, we evaluated one component in the spot reduction hypothesis, namely if spot lipolysis occurs in SCAT overlying contracting skeletal muscle. Our subjects performed acute one-legged knee extension exercise, and, by use of 133Xe washout and microdialysis techniques, we estimated blood flow and lipolysis in femoral SCAT adjacent to contracting and resting skeletal muscle.

MATERIALS AND METHODS

Subjects. Ten healthy, moderately active, overnight-fasted males [age: 26 ± 2 (SE) yr, weight: 82 ± 3 kg, height: 183 ± 2 cm, BMI: 24.5 ± 0.8 kg/m², \( V\text{O₂ max:} 49 ± 2 \text{ml·kg} \cdot \text{min}⁻¹ \)] gave their written consent according to the declaration of Helsinki II to participate in the study, which was approved by the Ethics Committee for Medical Research in Copenhagen (KF 11–055/03).

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Protocol. Before the experiment, subjects were accustomed to exercise on the knee extension ergometer, and maximal work capacity (Wmax) was determined for each leg as described by Andersen et al. (1). At least 5 days before the experimental day, subjects performed a V02peak test on a bicycle ergometer using a standard progressive exercise test. Subjects were asked to refrain from vigorous physical activity for 2 days before the experiment. After a 12-h fast and abstinence from coffee, tea, alcohol, and tobacco, the subjects arrived at the laboratory at 8:00 A.M. During the experiments, subjects wore shorts and T-shirt, and the room temperature was 23 ± 1°C. A catheter (Arterial Cannula with FloSwitch; Becton-Dickinson) was inserted in the brachial or radial artery for blood sampling. Microdialysis catheters were inserted, and 133Xe was injected in SCAT of both thighs (see below). After a 90-min equilibration period, the experiment was initiated.

The experiment consisted of a 15-min resting period and three consecutive periods of one-legged knee extension exercise (Fig. 1). First subjects exercised with one leg for 30 min at 25% of Wmax, (13 ± 1 watts, heart rate 81 ± 3 beats/min). After a 30-min rest, subjects exercised with the other leg for 120 min at 55% of Wmax (26 ± 1 watts, heart rate 84 ± 4 beats/min), and after another 30-min rest they exercised with the first leg again for 30 min but now at 85% of Wmax (44 ± 2 watts, heart rate 107 ± 4 beats/min). Selection of the leg eligible for low-high respective moderate intensity was done by randomized stratification such that dominant and nondominant legs were similarly represented.

Both during the resting and the exercise periods, subjects were sitting in a chair with the torso strapped to the back of the chair. Subjects had free access to water throughout the experiment. Pulmonary oxygen uptake and carbon dioxide excretion were measured regularly during exercise using an automated on-line system (Oxycon Champion; Erich Jaeger, Hoechberg, Germany).

Microdialysis. Microdialysis was performed in principle as described previously (27). After anesthesia of the skin (0.2 ml lidocaine, 5 mg/ml) at the sites of perforation, two microdialysis catheters (CMA 60; CMA/Microdialysis, Stockholm, Sweden) were placed in the SCAT adjacent to the vastus lateralis part of the quadriceps muscle in each thigh. Catheters were placed equidistant from the skin and the muscle fascia, 13–15 cm above the patella, and with 2–3 cm between catheters. Catheters were perfused at a rate of 1.0 μl/min using high-precision syringe pumps (CMA 100; CMA/Microdialysis). The perfusate consisted of Ringer acetate with 2 mM glucose, 5 kBq/ml [14C]ethanol (NEN, Zaventem, Belgium), and 5 kBq/ml [3H]glycerol (NEN). Seven out of 40 catheters ceased to function at some point during exercise.

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The in vivo relative recovery (RR) for glycerol was determined by the internal reference calibration technique (24). This technique assumes that the relative loss of [3H]glycerol from the microdialysis catheter to the interstitial fluid equals the RR of glycerol from the interstitial fluid to the microdialysis catheter. The RR of glycerol was calculated as (dpmt − dpm0)/dpm0, where dpm0 is [3H] disintegrations per minute in 5 μl perfusate and dpmt is [3H] disintegrations per minute in 5 μl dialysate. Interstitial concentrations were calculated as (Cd − Cp)/RR + Cp, where Cd is dialysate concentration and Cp is perfusate concentration.

SCAT blood flow. Adipose tissue blood flow (ATBF) was measured by the local 133Xe washout technique (27), which has the advantage that it is a quantitative method. At least 30 min before start of the resting period, 0.5–1 MBq gaseous 133Xe (Amersham Health) in a volume of 0.05–0.1 ml was injected in SCAT adjacent to the rectus femoris part of the quadriceps muscle. The 133Xe depot was placed equidistant from the skin and the muscle fascia and 13–15 cm above the patella. The washout rate of 133Xe was measured continuously by a scintillation counter system (Oakfield Instruments, Eynsham, UK) strapped to the skin surface above the 133Xe depot. ATBF was determined in 15- or 30-min periods and calculated as −k · λ · 100 (ml/100 g · 1·min−1), where k is the rate constant of the washout and λ is the tissue-to-blood partition coefficient for 133Xe at equilibrium. λ was assumed to be 10 ml/g (15).

Changes in ATBF in areas surrounding microdialysis catheters were also estimated by the microdialysis outflow/inflow technique (13, 25). The technique was originally described using ethanol as a marker of blood flow (13), but we have demonstrated that [14C]ethanol is a valid alternative to ethanol (25). The blood flow marker is added to the perfusate (inflow), it diffuses out through the membrane, and is washed away by the blood. The higher the blood flow, the more marker is washed away, and the less marker remains in the dialysate (outflow). Accordingly, the outflow-to-inflow ratio of marker varies inversely with blood flow (9, 25). The advantage of the microdialysis outflow/inflow technique is that it estimates changes in blood flow in the same area as interstitial concentrations of metabolites are measured.

Sampling and analyses. Dialysate for analysis of glycerol was collected in 200-μl capped microvials at 15- or 30-min intervals, immediately frozen, and kept at −20°C until analysis. Dialysate sampling was delayed by 2 min relative to the rest of the experimental protocol to compensate for the transit time in the outlet tubing. Blood for determination of hormones and metabolites was sampled in iced tubes and immediately centrifuged. Blood was sampled immediately before and every 15 min during the first 30 min of the exercise periods and additionally at time 60 and 120 min during the 120-min exercise period. Blood for determination of glycerol was stabilized with EDTA, and plasma was kept at −20°C until analysis. Microdialysate and plasma glycerol concentrations were determined by a CMA 600 microdialysis analyzer (CMA/Microdialysis). Blood for determination of insulin was stabilized with trasylol and EDTA and kept at −20°C until analysis. Arterial plasma insulin concentrations were determined by a commercial ELISA (DakoCytomation). Blood for determination of epinephrine was stabilized with reduced glutathione and EDTA and kept at −80°C until analysis. Arterial plasma epinephrine concentrations were determined by a commercial RIA (High sensitive 2 CAT RIA; Labor Diagnostika, Nord, Germany).

Calculation of adipose tissue lipolysis. Adipose tissue venous glycerol concentrations were calculated based on “Ficks law of

\[
\text{ATBF} = \frac{\text{diluent concentration}}{\text{dilution ratio} \cdot \text{inflow rate}} \cdot \text{interstitial fluid volume} \cdot \text{ADTBF} \cdot \text{blood flow to the tissue}
\]

where diluent concentration is the concentration of the marker in the perfusate diluent, dilution ratio is the ratio of perfusate to blood flow, inflow rate is the rate of perfusate flow, interstitial fluid volume is the volume of the tissue, ADTBF is the adipose tissue blood flow, and blood flow to the tissue is the blood flow to the tissue as measured by the 133Xe washout technique.

Fig. 1. Experimental protocol. Ten males performed knee extension exercise with one leg (leg 1) at 25% of maximal workload (Wmax) for 30 min while the other leg (leg 2) was resting. After a short rest, subjects exercised with the other leg (leg 2) at 55% Wmax for 120 min while the first leg (leg 1) was resting. After another short rest, subjects exercised with the first leg (leg 1) at 85% Wmax for 30 min while the other leg (leg 2) was resting. Arterial blood samples were obtained as indicated by arrows, whereas microdialysis samples and 133Xe washout readings were collected across sampling periods indicated by brackets.

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RESULTS

**ATBF.** Knee extension exercise with one leg at 25, 55, and 85% $W_{\text{max}}$ increased ($P < 0.05$) femoral subcutaneous ATBF in both legs (Fig. 2A). In general, blood flow was higher in adipose tissue adjacent to working muscle than in adipose tissue adjacent to resting muscle. At 25 and 55% $W_{\text{max}}$, the ATBF difference between legs (rest vs. exercise) was significant, but this was not the case at 85% $W_{\text{max}}$. The changes in ATBF detected by the microdialysis $[^{14}\text{C}]$ethanol technique were similar to changes detected by the $^{133}\text{Xe}$ washout technique (Fig. 2, A and B).

**Adipose tissue interstitial and arterial plasma glycerol concentrations.** Knee extension exercise with one leg at 55 and 85% $W_{\text{max}}$ increased ($P < 0.05$) the femoral SCAT interstitial glycerol concentration compared with rest (Fig. 3A). At 85% $W_{\text{max}}$, the interstitial glycerol concentration was higher ($P < 0.05$) in adipose tissue adjacent to working muscle than in adipose tissue adjacent to resting muscle. At all three intensities, knee extension exercise with one leg increased ($P < 0.05$) the arterial plasma glycerol concentration compared with rest (Fig. 3B).

**Adipose tissue lipolysis.** During knee extension exercise with one leg at 25 and 85% $W_{\text{max}}$, the lipolysis was significantly higher in femoral SCAT adjacent to working muscle than in adipose tissue adjacent to resting muscle (Fig. 4). During the first 60 min of exercise with one leg at 55% $W_{\text{max}}$, the lipolysis seemed higher in adipose tissue adjacent to working muscle than in adipose tissue adjacent to resting muscle, but the difference was not significant. The difference in adipose tissue lipolysis between the two legs (lipolysis in exercising – resting leg) during time 0–30 min showed that lipolysis was larger ($P < 0.05$) than zero at 25 and 85% $W_{\text{max}}$, but not at 55% $W_{\text{max}}$ (Fig. 5). Incremental lipolysis did not change with exercise intensity ($P > 0.05$).

**Arterial plasma hormone concentrations.** Knee extension exercise with one leg at 55% and at 85% $W_{\text{max}}$ decreased ($P < 0.05$) the arterial plasma insulin concentration compared with rest (Table 1). Exercise with one leg at 85% $W_{\text{max}}$ increased ($P < 0.05$) the arterial plasma epinephrine concentration compared with rest (Table 1).

DISCUSSION

Specific exercises can induce spot lipolysis, since we found blood flow (Fig. 2) and lipolysis (Figs. 4 and 5) to be higher in SCAT adjacent to contracting than adjacent to resting muscle. Based on the present results, it cannot be foreseen if specific exercises can induce spot reduction, since triacylglycerol (TG) stores could be fully replenished or even supercompensated between exercise sessions.

During exercise, temperature increases in the contracting muscles (10), and this would increase temperature also in the tissues adjacent to the contracting muscles. An increase in adipose tissue temperature induces an increase in ATBF (9), and this mechanism could potentially explain the increased blood flow in the adipose tissue adjacent to the contracting muscle in the present study (Fig. 2). During knee extension exercise, the increase in temperature of the contracting quadriceps muscle has been found to be $\sim 2^\circ\text{C}$ (10). Felländer et al. (9) heated SCAT and increased the temperature in the tissue by 4°C, which elicited an increase in ATBF of $\sim 1.5\text{ ml} \cdot 100$...
We did not measure the temperature of the adipose tissue adjacent to the contracting muscles, but, assuming that the adipose tissue temperature increased 1°C, this could according to the data by Fellander et al. (9) at most explain an increase in ATBF of 0.4 ml·100 g⁻¹·min⁻¹.

Circulating epinephrine and norepinephrine are potent stimulators of ATBF and lipolysis (17, 26), and, in the present study, we found the plasma epinephrine concentration to increase significantly at the highest exercise intensity (Table 1). Circulating hormones influence all adipose tissue depots and not selectively adipose tissue adjacent to contracting muscles. However, because of the relatively increased blood flow in adipose tissue adjacent to contracting muscles, a larger amount of the circulating epinephrine will be delivered to this tissue.

This would increase the interstitial concentration of epinephrine in the adipose tissue, which could be one of the mechanisms behind the higher lipolysis in SCAT adjacent to contracting than adjacent to resting muscle.

Norepinephrine is not only a hormone but also a neurotransmitter in the sympathetic nervous system, and a selective stimulation of adipose tissue adjacent to contracting muscles could also be via local sympathetic nerves. In favor of this mechanism, studies in humans have demonstrated that regional differences are present in sympathetic nervous activity (5) and that direct stimulation of a cutaneous sympathetic nerve in-
creases lipolysis in the adipose tissue innervated by the stimulated nerve (6). Also, denervation of a rat retroperitoneal fat pad induces an increase in fat pad weight and adipocyte volume 1 wk after denervation, indicating that lipolysis is diminished when no sympathetic innervation is present (4).

Another mechanism to explain the increase in ATBF (Fig. 2) and lipolysis (Figs. 4 and 5) adjacent to contracting muscles could be release of paracrine substances from the contracting muscles, which could diffuse from the muscle to the adipose tissue to stimulate blood flow and lipolysis. Contracting skeletal muscle has been shown to release the myokine interleukin-6 (8), which among other things has been shown to stimulate adipose tissue lipolysis (18).

It is evident that several potential mechanisms can explain the increased ATBF (Fig. 2) and lipolysis (Figs. 4 and 5) in adipose tissue adjacent to contracting muscles, but it is more difficult to understand the rationale for the increase. The muscle and the SCAT superficial to the muscle have separate blood supplies and drainages. Accordingly, fatty acids released from adipose tissue during exercise are transported to the heart and reach all parts of the body, not specifically the muscle beneath the subcutaneous tissue from which the fatty acids were released.

In the present study, we measured changes in ATBF by two independent methods, the $^{133}$Xe washout technique (27) and the microdialysis [$^{14}$C]ethanol outflow/inflow technique (25), and findings were similar (Fig. 2). Both methods showed a general increase in ATBF during exercise and that blood flow was higher in adipose tissue adjacent to contracting muscle than in adipose tissue adjacent to resting muscle. The $^{133}$Xe washout and the microdialysis ethanol outflow/inflow techniques have previously shown comparable results in adipose tissue during external heating of the skin (9).

We found lipolysis to be higher in adipose tissue adjacent to contracting muscles than in adipose tissue adjacent to resting muscle (Figs. 4 and 5), but, in contrast to findings for ATBF (Fig. 2), we did not find a general exercise-induced increase in femoral adipose tissue lipolysis. Cycle exercise of moderate intensity increases lipolysis in abdominal SCAT (2, 14), but, in accordance with our findings, Horowitz et al. (14) found that exercise at 50% of $V_{O_{2\text{max}}}$ did not increase femoral SCAT lipolysis. We examined femoral SCAT because this tissue has a contralateral tissue site that can be used as a control, which abdominal SCAT clearly do not.

Reliability of techniques used in the study should be considered. We placed the microdialysis probes and the $^{133}$Xe depot in the femoral SCAT equidistant from skin and muscle fascia. We did not measure the thickness of the femoral SCAT, but, in studies of similar subjects, we found the femoral subcutaneous skinfold to be $\sim$10–30 mm (unpublished results). We believe that measurements of lipolysis and blood flow in adipose tissue depots of this thickness are reliable. Theoretical predictions (7) suggest that a microdialysis probe estimates the metabolite concentration in a radius of a few millimeters from the probe, and probes thus should mirror adipose tissue metabolism and not metabolism in skin or muscle. In theory, also the $^{133}$Xe depot could come in contact with skin or muscle, but, if this had occurred, the washout curve for $^{133}$Xe would have become multiexponential, and this was not the case.

More calories are expended during aerobic, whole body exercise than by exercise with local muscle groups, and, accordingly, a person seeking to lose fat must be advised to perform whole body exercise. However, the present study has shown that blood flow (Fig. 2) and lipolysis (Figs. 4 and 5) are stimulated more in adipose tissue adjacent to contracting muscles than in adipose tissue adjacent to resting muscles. The incremental femoral SCAT lipolysis (Fig. 5) showed no clear connection with exercise intensity and amounted to 22–80 nmol·100 g$^{-1}$·min$^{-1}$ during the first 30 min of the exercise bout. Assuming a molecular weight of 860 g/mol for TG, this corresponds to an extra breakdown of 0.6–2.1 mg of TG in 30 min/100 g of adipose tissue adjacent to contracting muscles. These figures are comparable to the increase in lipolysis induced by cycle exercise at 50% of $V_{O_{2\text{max}}}$, which was found to be $\sim$30 nmol·100 g$^{-1}$·min$^{-1}$ in femoral SCAT and 200 nmol·100 g$^{-1}$·min$^{-1}$ in abdominal SCAT (time 15–40 min; see Ref. 14), corresponding to a breakdown of 1.3 and 5.2 mg of TG in 30 min/100 g of femoral and abdominal adipose tissue, respectively.

In conclusion, an acute bout of exercise can induce spot lipolysis and increased blood flow in adipose tissue adjacent to contracting skeletal muscle.

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