In vivo human lipolytic activity in preperitoneal and subdivisions of subcutaneous abdominal adipose tissue

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Enevoldsen, Lotte H., Lene Simonsen, Bente Stallknecht, Henrik Galbo, and Jens Bülow. In vivo human lipolytic activity in preperitoneal and subdivisions of subcutaneous abdominal adipose tissue. Am J Physiol Endocrinol Metab 281: E1110–E1114, 2001.—We studied eight normal-weight male subjects to examine whether the lipolytic rate of deep subcutaneous and preperitoneal adipose tissues differs from that of superficial abdominal subcutaneous adipose tissue. The lipolytic rates in the superficial anterior and deep posterior subcutaneous abdominal adipose tissues and in the preperitoneal adipose tissue in the round ligament were measured by microdialysis and 133Xe washout under basal, postabsorptive conditions and during intravenous epinephrine infusion (0.15 nmol kg−1 min−1). Both in the basal state and during epinephrine stimulation, the superficial subcutaneous adipose tissue had higher interstitial glycerol concentrations than the two other depots. Similarly, the calculated glycerol outputs from the superficial depot were significantly higher than those from the deep subcutaneous and the preperitoneal depots. Thus, it is concluded that the lipolytic rate of the superficial subcutaneous adipose tissue on the anterior abdominal wall is higher than that of the deep subcutaneous adipose tissue on the posterior abdominal wall and that of the preperitoneal adipose tissue in the round ligament.

epinephrine; glycerol; microdialysis

IN HUMANS, MOST STUDIES OF LIPID METABOLISM in adipose tissue in vivo have been performed in the anterior abdominal, subcutaneous depot either by the microdialysis technique or by an arteriovenous catherization (direct Fick) technique (11). In a recent study (6), it was suggested that abdominal subcutaneous adipose tissue anatomically can be divided into two physiologically different layers (above and below Scarpa’s fascia). In this study, indirect evidence was found indicating that the deep abdominal, subcutaneous layer behaves metabolically like the intra-abdominal fat depots, because the amount of fat located in each of these two sites correlates similarly with insulin resistance. In another study, Misra et al. (8) showed that the subcutaneous adipose tissue on the posterior abdominal wall is more strongly associated with insulin resistance than the subcutaneous adipose tissue on the anterior abdominal wall. Seen in the light of these findings, it may be questioned whether the anterior superficial depot has a lipolytic rate comparable to that of the deep posterior depot. In an anthropometric study using ultrasonography for assessment of the ratio between the thickness of the preperitoneal fat and that of the overlying subcutaneous fat, it was shown that this ratio was positively correlated with the visceral fat mass, implying that the masses of the preperitoneal and visceral depots vary in parallel. Thus there is some indirect evidence that the preperitoneal and visceral fat depots might have common metabolic properties (13).

Many studies have linked visceral obesity with the development of non-insulin-dependent diabetes mellitus and cardiovascular disease (7). It has been proposed that a plausible mechanism behind this linkage is an increased release of nonesterified fatty acids from the visceral depots into the portal circulation. However, the metabolism of the visceral adipose tissue depots has not been studied directly in humans under physiological conditions, because the depots are not easily accessible by catheterization techniques and cannot be subjected to microdialysis even. Therefore, it would be of interest to identify an adipose tissue depot accessible for in vivo studies with metabolic characteristics like the visceral adipose tissue. Two
MATERIALS AND METHODS

Subjects. Eight healthy males participated in the study (Table 1). The subjects were given written and oral description of the study according to the Declaration of Helsinki II, and their informed consent was obtained. The study was approved by The Ethical Committee for Medical Research of Copenhagen [(KF) 01–170/00].

Experimental protocol. About 2 wk before the experimental day, subjects were scanned by dual-energy X-ray absorptiometry to determine lean body mass and fat mass (Lunar DPX-IQ, software version 4.6c, Madison, WI) (Table 1). On the day before the experiment, subjects avoided strenuous physical activity. After an overnight fast and abstinence from all drinks except water, the subjects were brought to the laboratory at 8 AM and had microdialysis, venous, and arterial catheters inserted. Then perfusion of the microdialysis probes was started, and after a 60-min equilibration period, the experiment was initiated. The experiment consisted of a 60-min basal (time 0–60 min) and a 90-min (time 60–150 min) epinephrine infusion period. Epinephrine was infused at a rate of 0.15 nmoL·kg⁻¹·min⁻¹. During the experiments, subjects wore light clothes and rested supine at a room temperature of ~24°C.

Microdialysis. Microdialysis was performed principally as described previously (12). After anesthesia (0.4 ml lidocaine, 10 mg/ml) of the skin and linea alba at the site of perforation, a microdialysis catheter (CMA 60, CMA/Microdialysis, Stockholm, Sweden) was placed in the preperitoneal adipose tissue by perforating the skin and abdominal wall ~3 cm below the processus xiphoideus and by introducing the catheter into the round ligament in the cranial direction. The catheter was inserted during ultrasound guidance using a 7.5 MHz linear-array transducer (Sonoline Elegra ultrasound system, Siemens Medical Systems, Issaquah, WA). A schematic drawing of a typical ultrasound image showing the position of the preperitoneal adipose tissue depot in relation to the other tissue types in the region is shown in Fig. 1. After anesthesia of the skin (0.1 ml lidocaine, 10 mg/ml) at the site of perforation, a microdialysis probe was placed superficially in the subcutaneous adipose tissue of the anterior abdominal wall in the periumbilical region. Another probe was inserted in the subcutaneous adipose tissue below Scapa’s fascia in the posterior abdominal wall at the level of the iliac crest in the posterior axillary line. Catheters were inserted during ultrasound guidance with care taken that they were placed above and below the Scapa’s fascia, respectively. The catheters were perfused at a rate of 2 μm/min with Ringer acetate with 2 mM glucose and 1.5 μM [3H]glycerol (specific activity 7.4 GBq/mmmol: NEN) by use of a high-precision syringe pump (CMA 100, CMA/Microdialysis). The in vivo relative recovery (RR) for glycerol was determined by the internal reference calibration technique (9). Microdialysate was collected in 200-μl capped microvials in 15-min periods and kept at ~20°C until further analysis. Dialysate sampling was delayed by 2 min relative to sampling of arterial blood to compensate for the transit time in the microdialysis probes. Dialysate glycerol concentrations were determined by a CMA 600 microdialysis analyzer (CMA/Microdialysis), each value representing a single determination. [3H]Glycerol was measured in perfusate and dialysate by liquid scintillation counting (2200CA, Packard Instruments). RR values (means ± SE) in superficial and deep subcutaneous adipose tissue and in preperitoneal adipose tissue were 0.36 ± 0.02, 0.45 ± 0.01, and 0.43 ± 0.02, respectively. The RR value in superficial subcutaneous adipose tissue was lower than RR values in preperitoneal and deep subcutaneous adipose tissue (P < 0.05).

Catheterization. In local analgesia (1 ml lidocaine, 10 mg/ml), a catheter (Ohmeda, Swindon, UK) was introduced percutaneously in the radial artery in the nondominant arm for blood sampling and continuous monitoring of blood pressure and heart rate (MX9504; Medex Medical, Lancashire, UK). The catheter was kept patent by regular flushing of isotonic saline. Additionally, the subjects had a catheter (Venflon, Viggo, Sweden) inserted in a forearm vein for infusion of epinephrine.

Blood flow. Preperitoneal and both superficial and deep subcutaneous adipose tissue blood flows were measured by the local 133Xe-washout technique, as described previously (10). About 2 MBq of 133Xe dissolved in 0.1 ml of isotonic sodium chloride were injected during ultrasound guidance into the preperitoneal and deep subcutaneous adipose tissues.
according to positions of microdialysis catheters, whereas ~1 MBq of $^{133}$Xe dissolved in 0.05 ml of isotonic sodium chloride was injected into the superficial subcutaneous adipose tissue. Washout of $^{133}$Xe was registered by a Mediscint system (Oakfield Instruments, Oxford, UK). The calculation of adipose tissue blood flow was performed, as described previously, with an average tissue-to-blood partition coefficient value ($\lambda$) of 8 ml/g in all adipose tissue depots (2).

**Blood sampling and analyses.** Blood for determination of metabolites and hormones was sampled in 0°C-cooled vials from the radial artery and immediately centrifuged. Blood was sampled at 9, 24, 39, and 54 min in the basal period and at 69, 84, 99, 114, 129, and 144 min during epinephrine infusion. Blood for determination of glycerol was measured in neutralized, deproteinized extracts of whole blood (3). Blood for determination of catecholamines was stabilized with reduced glutathione and heparin, and plasma catecholamine concentrations were determined by a single-isotope radioenzymatic assay (1).

**Calculations.** From the interstitial glycerol concentration, the concentration in venous blood water ($C_{v\text{-calc}}$) was calculated by the principle described by Intaglietta and Johnson (4). Blood water flow was calculated as \((1 - \text{hematocrit}) \cdot 0.94 + (\text{hematocrit} \cdot 0.67)\) \cdot \text{blood flow}, measured by the $^{133}$Xe washout. Adipose tissue glycerol output was calculated as the product of the blood water flow and the difference between $C_{v\text{-calc}}$ and the concentration in arterial blood water.

**Statistics.** All data are presented as means ± SE. A two-way repeated-measures ANOVA was used to test whether interstitial glycerol concentrations, glycerol outputs, and blood flows differed with time or among adipose tissues. A one-way ANOVA was used to test whether blood pressure, heart rate, hematocrit, and hormone concentrations differed with time. The Student-Newman-Keuls test was used as a post hoc test. A significance level of 0.05 in two-tailed testing was chosen a priori.

**RESULTS**

**Hormone concentrations.** Epinephrine infusion increased epinephrine concentration in arterial plasma...
from $0.31 \pm 0.05 \text{nM}$ in the basal state to $4.00 \pm 0.18 \text{nM} (P < 0.05)$. Norepinephrine concentration in arterial plasma did not change significantly with epinephrine infusion (basal state: $1.08 \pm 0.10 \text{nM}$; during epinephrine: $1.23 \pm 0.16 \text{nM}$).

Whole body. Epinephrine infusion increased heart rate ($50 \pm 2 \text{ vs. } 60 \pm 2 \text{ beats/min}$) significantly. Systolic blood pressure was increased ($126 \pm 4 \text{ vs. } 135 \pm 5 \text{ mmHg}; P < 0.05$), whereas diastolic blood pressure ($63 \pm 2 \text{ vs. } 59 \pm 3 \text{ mmHg}$) was essentially unchanged, with epinephrine infusion. Hematocrit did not change with time ($43 \pm 0 \text{ vs. } 44 \pm 0\%$).

Adipose tissue blood flow. Adipose tissue blood flows increased significantly by two- to threefold during epinephrine infusion (Fig. 2). During epinephrine infusion, adipose tissue blood flow levels were significantly higher in superficial subcutaneous adipose tissue than in preperitoneal adipose tissue.

Arterial blood and interstitial glycerol concentrations. Epinephrine infusion increased arterial and dialysate glycerol concentrations, which peaked $0.5 \text{ h}$ after start of the infusion (Fig. 3). In Fig. 3 are also given the calculated interstitial glycerol concentrations. Both in the basal state and during epinephrine infusion, the interstitial glycerol concentration was significantly higher in the superficial subcutaneous adipose tissue than in the preperitoneal and deep subcutaneous adipose tissues.

Calculated glycerol output. Epinephrine significantly increased glycerol output from all three depots ($P < 0.05$; Fig. 3). However, the output from the superficial subcutaneous adipose tissue was significantly higher than outputs from the preperitoneal and deep subcutaneous adipose tissues.

DISCUSSION

The main finding in the present study is that the increase in lipolytic activity elicited by epinephrine in a high physiological concentration is higher in the anterior abdominal, superficial subcutaneous adipose tissue layer compared with the posterior abdominal, deep subcutaneous adipose tissue layer and with the preperitoneal adipose tissue in the round ligament.

The tissue-to-blood partition coefficient ($\lambda$) for $\text{Xe}$ was not directly determined in each depot in this study. A value of 8 ml/g was used for all depots, because this has been found to be the average value in subcutaneous abdominal adipose tissue sampled randomly during abdominal surgery in a normal-weight population with an average body mass index (BMI) of 24 kg/m² (2). The subjects studied in the present experiments had an average BMI of ~25 kg/m². Jansson and Lönroth (5) studied a lean population with a BMI of ~22 kg/m² and found the average $\lambda$ to be 8.6 ml/g when estimated from needle biopsies taken from the subcutaneous, abdominal adipose tissue without distinction between superficial and deep layers. If we assume that $\lambda$ is 10 ml/g in the preperitoneal and deep subcutaneous depots, corresponding to ~5% water content and ~90% lipid content in the tissues, this would not change the overall pattern in the present findings. Thus, despite the uncertainty of the assumed average $\lambda$ value, the present findings show that the lipolytic activity in the superficial subcutaneous layer is higher than in the two other depots during epinephrine infusion. Therefore, the present results support the view that the many results published during the last decade from studies of the anterior abdominal subcutaneous adipose tissue give a reliable picture of adipose tissue metabolism and its regulation. Furthermore, the study has provided direct evidence in favor of the view that the superficial abdominal adipose tissue on the anterior abdominal wall and the deep abdominal adipose tissue on the posterior abdominal wall are not metabolically equal. An explanation for the stronger association of the deep subcutaneous adipose tissue and insulin resistance is that the major part of the subcutaneous adipose tissue on the abdominal wall is situated below Scarpa’s fascia. Thus the deep subcutaneous tissue mass is larger than the superficial mass, giving the former layer a higher metabolic impact despite a lower metabolic rate.

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REFERENCES

10. Simonsen L, Bülow J, Astrup A, Madsen J, and Christiansen NJ. Diet-induced changes in subcutaneous adipose tis-
