Catecholamine effects on lipolysis and blood flow in human abdominal and femoral adipose tissue

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Millet, L., P. Barbe, M. Lafontan, M. Berlan, and J. Galitzky. Catecholamine effects on lipolysis and blood flow in human abdominal and femoral adipose tissue. J. Appl. Physiol. 85(1): 181–188, 1998.—With the use of the microdialysis method, the present study, performed on young, healthy, nonobese subjects of both genders, compares the effects of locally infused catecholamines on glycerol concentration and blood flow in abdominal (Abd) and femoral (Fem) adipose tissue. Physiological activation of the sympathetic nervous system through active tilt was also investigated. In both genders, extracellular glycerol concentration was higher in Fem than in Abd adipose tissue. Local blood flow was lower in Fem than in Abd adipose tissue. Isoproterenol perfusion increased extracellular glycerol levels, but no differences were found by gender or fat-deposit site. Isoproterenol induced a greater increase in local blood flow in Fem adipose tissue than in Abd adipose tissue. Epinephrine and norepinephrine perfusion increased extracellular glycerol and reduced blood flow. No major differences were found according to gender and fat-deposit site. Active tilt increased plasma glycerol, free fatty acid, norepinephrine levels, and extracellular glycerol concentration to the same extent whatever the gender and fat deposit size. Thus, Fem adipose tissue is characterized by a higher extracellular glycerol concentration and a lower blood flow than is Abd tissue in men and women. In these tissues, in situ lipolysis and local blood flow were similar in response to adrenergic stimulation.

SYMPATHETIC NERVOUS SYSTEM (SNS) activation stimulates lipid mobilization in adipose tissue, which is the most important tissue for triglyceride storage and mobilization. The modulatory effects of physiological catecholamines on fat cell function involve various adrenergic-receptor (AR) subtypes (17). The coexistence of numerous ARs that increase (β1-, β2-, and β3-ARs) or decrease (α2-AR) the rate of lipolysis through activation or inhibition of the adenylyl cyclase of the adipocyte membrane still raises questions about their physiological relevance. The presence of ARs has clearly been established by performing functional in vitro assays in human isolated fat cells and binding studies with selective radioligands (17, 20). In human fat cells, in which α2-ARs numerically predominate over β-ARs, the preferential recruitment of the α2-ARs in vitro, leading to an inhibition of lipolysis at lower catecholamine concentrations, has been described (20). The β-AR-dependent lipolysis, initiated by the physiological catecholamines, occurs at the highest concentrations of the amines. This dual effect of catecholamines on isolated human fat cells (i.e., antilipolytic and then lipolytic, according to the concentration of the amine) was particularly striking in femoral adipocytes from overweight women (20) and has also been observed more recently in subcutaneous adipocytes from obese men (19). The physiological relevance of such in vitro responsiveness to catecholamines remains in doubt, although some in vivo approaches have given some interesting results with the use of in situ microdialysis. This technique appears valuable to the study of hormonal responses in vivo in various tissues (2, 4). It has been clearly shown that physical activity (3) and mental stress (12) stimulate glycerol output in adipose tissue through β-AR activation. However, an α2-AR component was not revealed. Gender-dependent and adipose tissue location-dependent differences in glycerol output during exercise have also been reported in vivo (3). In these experiments, the rate of lipolysis was assessed through measurement of glycerol output. No estimation was made of local blood flow, which is now known to strongly modify the apparent rate of glycerol recovery in microdialysis assays (4, 11, 16). Although the approaches based on the utilization of the microdialysis method have been considerably extended during the last years, studies concerning the local action of the physiological amines in human subcutaneous adipose tissues have been more limited and less well documented in young healthy subjects.

The aim of the present study, performed on young, healthy, nonobese subjects of both genders with the use of the microdialysis method, was first to compare the effects of various concentrations of isoproterenol, epinephrine (Epi), and norepinephrine (NE), directly infused in the microdialysis probe, on extracellular glycerol concentration and local blood flow in abdominal (Abd) and femoral (Fem) adipose tissue. Second, the modifications of extracellular glycerol concentration and local blood flow, promoted by a physiological activation of the SNS through an active tilt from the supine to the upright position, were also evaluated in these two fat deposits.

METHODS

Subjects. The experimental procedure was approved by the Ethical Committee of the Hospital. Fourteen healthy young adults (7 men, 7 women), who had not been submitted to any nutritional or pharmacological protocol before the study, gave their informed consent to participate to this clinical protocol. All were drug free and had stable weight during the previous 3 mo. All of the women were taking oral contraceptive drugs. The characteristics of the subjects are depicted in Table 1.

Experimental sequences. The subjects entered the room at 8:00 AM and were maintained in a supine position for 60 min. In all investigations, two microdialysis probes (20 × 0.5 mm, 20,000 Da molecular mass cutoff; Carnegie Medicine, Stock-
effect of a 20-min active tilt (passing from the supine to the upright position) on extracellular glycerol concentration and local blood flow was evaluated. The probes were continuously perfused with Ringer solution, and 10-min fractions were collected over the whole experimental period. Then the subjects were returned to the supine position for 30 min. Blood samples were drawn before and at the end of the tilt. All the experimental sessions were performed for each subject, with a washout period of at least 1 wk between sessions. Glycerol was assayed in each 10-min fraction collected, and glycerol was taken as the lipolysis index. The ethanol levels were measured in the perfusate and in the outgoing dialysate to determine the ethanol outflow-to-inflow ratio, indicating the changes of nutritive blood flow around the probes (4, 10, 11, 13).

Biochemical determinations. Glycerol in dialysate was determined by using an ultrasensitive radiometric method (7). The intra-assay and interassay variabilities were 5.0 and 9.2%, respectively. The ethanol concentration in 5-µl fractions of dialysate and perfusate was determined with an enzymatic method (6). The intra-assay and interassay variabilities were 3.0 and 4.5%, respectively. NE was assayed by high-pressure liquid chromatography with the use of electrochemical (amperometric) detection, as previously described (8).

Statistical analysis. All the values are given as means ± SE. ANOVA for repeated measures, with Scheffé’s post hoc test, and Wilcoxon’s paired test were used for comparison when appropriate. P < 0.05 was considered statistically significant. All statistical comparisons were performed by using a statistical software package (Statview 4.5, Abacus Concepts, Berkeley, CA).

**RESULTS**

Characteristics of the subjects, extracellular glycerol concentration, and ethanol outflow-to-inflow ratio in Fem and Abd subcutaneous adipose tissue. Table 1 shows the characteristics of the subjects. The subjects were of similar age and body mass index. As expected, women had a significantly higher fat mass and Fem skinfold and had a lower waist-to-hip ratio. The values of the baseline ethanol ratio (the averaged values obtained with four 10-min dialysate fractions collected before infusion of drugs for each experiment) were significantly higher in the Fem than in the Abd tissue (P < 0.003). This difference was observed in subjects of both genders (Table 2) but was not gender related. The in situ recovery rate of glycerol, at the perfusion rate of 2.5 µl/min, was significantly different in the Fem and Abd probes (29 ± 2 and 36 ± 2%, respectively; P < 0.001).
The calculated extracellular glycerol concentration (the mean of four 10-min dialysate fractions collected before drug infusion, for each experiment) was significantly higher in the Fem than in the Abd tissue (P < 0.005). This difference was observed in subjects of both genders (Table 2) but was not gender related.

Effect of isoproterenol on extracellular glycerol concentration and local blood flow in Fem and Abd subcutaneous adipose tissue. The effect of increasing concentrations of isoproterenol on extracellular glycerol concentration and ethanol ratios in women and men is depicted in Fig. 1. The addition of 0.01 µM isoproterenol into the perfusate did not modify the extracellular glycerol levels in Fem or in Abd adipose tissue in either group of subjects. Higher isoproterenol concentrations (0.1 and 1 µM) significantly increased the extracellular glycerol concentrations over the baseline (to 65 and 151% for Abd and 73 and 133% for Fem adipose tissue, respectively, when the last fraction for a given concentration is used for comparison). The isoproterenol-induced glycerol increase was not significantly different when the adipose tissue sites and gender were taken as factors in the ANOVA analysis (see Fig. 1 legend).

The perfusion of isoproterenol induced a concentration-dependent decrease in the ethanol ratio, indicating the occurrence of vasodilatation in the tissue. A significant decrease (6.8 and 10.5% for Abd and Fem, respectively) was observed 40 min after 0.01 µM isoproterenol infusion. The decrease in ethanol ratio with 1 µM isoproterenol was 21 and 28% for Abd and Fem, respectively. Although this ratio was similar in both genders, the initial ethanol ratio was significantly higher in Fem than in Abd adipose tissue. The isoproterenol-induced decrease in ethanol ratios was not significantly different according to gender but was significantly more marked in Fem adipose tissue when both the adipose tissue sites and gender were taken as factors in the ANOVA analysis (see Fig. 1 legend).

Effect of NE on extracellular glycerol concentration and local blood flow in Fem and Abd subcutaneous adipose tissue. The effect of increasing concentrations of NE on glycerol concentration and values of ethanol ratio is shown in Fig. 2. The addition of 0.001 µM NE into the perfusate did not modify extracellular glycerol levels in Fem or in Abd adipose tissue. Higher concentrations (0.1 and 10 µM) significantly increased the extracellular glycerol concentrations (to 52 and 239% and 59 and 205% over baseline in Abd and Fem adipose tissue, respectively). NE-induced glycerol increase was not significantly different when the gender was used as a factor in the ANOVA analysis. However, the NE-induced increment in extracellular glycerol was significantly higher in Abd adipose tissue (see Fig. 2 legend).

The lowest NE concentrations (0.001 and 0.1 µM) did not modify the values of the ethanol ratio, whereas the highest concentration (10 µM) did increase it (10 and 5% for Abd and Fem adipose tissue, respectively). This increase, which represents an indirect evaluation of lipolytic activity, was not different when the gender was used as a factor in the ANOVA analysis (see Fig. 2 legend).
NE-induced vasoconstriction, did not differ according to gender but was significantly higher in Abd adipose tissue (see Fig. 2 legend). These data indicated a reduction of the escape of ethanol from the dialysis solvent and a NE-dependent decrease of nutritive blood flow in adipose tissue.

Effect of Epi on extracellular glycerol concentration and local blood flow in Fem and Abd subcutaneous adipose tissue. The effect of increasing concentrations of Epi on extracellular glycerol concentration and ethanol ratio is shown in Fig. 3. The addition of 0.001 µM Epi into the perfusate did not modify extracellular glycerol levels in Fem or in Abd adipose tissue. Higher concentrations (0.1 and 10 µM) significantly increased the extracellular glycerol concentrations (to 54 and 244% and 76 and 227% over baseline for Abd and Fem adipose tissue, respectively). The Epi-induced glycerol increase was not significantly different when the adipose tissue sites and gender were taken as factors in the ANOVA analysis (see Fig. 3 legend).

Effect of 20-min active tilt on extracellular glycerol concentration and local blood flow in Fem and Abd subcutaneous adipose tissue. As we expected, change from supine to head-up posture (active tilt) induced a significant activation of the SNS, as assessed by the increase in plasma NE levels (Table 3). Nonesterified fatty acids and glycerol plasma levels rose significantly and concomitantly with the increment of plasma NE levels (Table 3).

DISCUSSION
The present study was performed to obtain new insights into the local action of physiological amines on human adipose tissue in vivo in normal, young, healthy individuals.
The first objective was to evaluate the baseline values of extracellular glycerol concentration and local blood flow parameters in two subcutaneous fat deposits of subjects of both genders to search for the existence of putative adipose site- or gender-specific differences and to check the homogeneity of results obtained with the microdialysis assays. The second objective was comparison of the in situ effects of isoproterenol and physiological catecholamines (infused in the microdialysis probe or physiologically released after an active tilt) on glycerol concentration and local blood flow changes according to the location of the fat deposits and the gender of the subjects. In this second step, we compared the data from the present study with previously published results on isolated adipocytes in vitro.

The microdialysis method has been used several times for studying the composition of the extracellular fluid in adipose tissue and muscle. Metabolites of interest have a size <10% of the cutoff point of the dialysis probes (20,000 Da in the present study) and therefore can easily pass through the membrane of the probes. This is the case for example for glycerol, glucose, lactate, or catecholamines (16). Depending on the perfusion flow rate of the probe and according to the nature of the tissue, the recovery rate can be different for a given metabolite. As suggested by Arner et al. (1), the metabolite recovery problems can be overcome at least partly by reducing the perfusion speed and by increasing the length of the dialysis probes. The concentration of a given metabolite in the extracellular fluid can be estimated, for each probe and in each experimental protocol, by using a calibration method based on the measurement of dialysate concentrations of the metabolite at various perfusion rates, as previously described (see Refs. 4 and 23 and the present study). The results of the present study indicate that the mean recovery rate was higher when the probes were inserted in Abd than in Fem adipose tissue. However, as in previous studies (4, 14, 15), the extracellular glycerol concentrations were two- to threefold higher in adipose tissue than in blood; this suggests that net release of glycerol from subcutaneous adipose tissue occurs at rest in the fasting condition. Conversely, using the same calibration method, we found that the levels of glucose and urea were in the same range in blood and in adipose tissue (unpublished observations).

Table 3. Effect of a 20-min active tilt (passing from supine to upright position) on plasma glycerol, NEFA, and norepinephrine concentrations in 10 subjects

<table>
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<tr>
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<th>Supine (60 min)</th>
<th>Upright (20 min)</th>
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<tbody>
<tr>
<td>Glycerol, µM</td>
<td>66.4 ± 8.8</td>
<td>105.0 ± 21*</td>
</tr>
<tr>
<td>NEFA, µM</td>
<td>462 ± 32</td>
<td>586 ± 48*</td>
</tr>
<tr>
<td>Norepinephrine, nM</td>
<td>1.33 ± 0.14</td>
<td>2.89 ± 0.46*</td>
</tr>
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Values are means ± SE. NEFA, nonesterified fatty acids. *P < 0.05 compared with supine values.
The results indicated that, whatever the gender of the subjects, extracellular glycerol concentration is slightly but significantly higher in Fem than in Abd adipose tissue and that no differences were observed according to the gender of the subjects. Such a difference was not previously reported by Jansson et al. (15) in their studies on a more limited number of subjects. In the present study, extracellular glycerol concentrations were determined in a larger number of experiments, and the significance of differences is clear (Table 2).

To study the functional changes occurring in adipose tissue microcirculation, a method was applied [as previously descibed for muscle and adipose tissue (4, 11, 13)] based on the evaluation of ethanol escape from the microdialysis probes (determination of ethanol outflow-to-inflow ratio values) for indirect evaluation of tissue drainage. On the basis of data from the present study, two observations can be made. First, the ethanol ratio obtained in the different experiments is reproducible from one experiment to the other, as assessed by the reasonable SE values. Second, in resting basal conditions, the ethanol ratio was significantly higher in Fem than in Abd adipose tissue, although no difference was observed according to the gender of the subjects. This is a notable observation for validation of the ethanol escape method for evaluation of blood flow changes. Although the microdialysis probes are randomly implanted in the fat deposits, the ethanol ratio determined is a reproducible characteristic for a given adipose tissue deposit. The fact that ethanol escape from the dialysis probe was higher in Abd than in Fem adipose tissue could reflect the existence of reduced fluid drainage in Fem adipose tissue. Reduction of spontaneous fluid circulation could depend on various tissue parameters, such as the larger size of the adipocytes in Fem vs. Abd tissues (14, 19, 22), differences in the organization and permeability of the connective web, and the density of microvessels surrounding the microdialysis probe. It has been demonstrated by using a similar microdialysis method that the glyceral level in human adipose tissue is influenced by the local blood flow, i.e., increased ethanol escape (obtained with the infusion of vasodilating agents) induced a decrease in extracellular glycerol concentration (9). Taking these observations together, we propose that the higher glycerol concentration we found in Fem adipose tissue may caused by a reduced local blood flow, although an increase in basal lipolysis rate could also play a role. Because basal lipolysis, explored in vitro, was found to be at least similar or even lower in Fem vs. Abd tissues in men and women (19, 22), it can be concluded that basal local blood flow plays an important role in the actual extracellular glycerol levels in adipose tissue.

In previous microdialysis experiments, we have shown that isoproterenol infusion increased extracellular glycerol levels in Abd adipose tissue simultaneously with an increase in ethanol escape (4). The present study shows (Fig. 1) that these effects are concentration-dependent in both tissue sites. No difference was seen...
in the isoproterenol-induced glycerol output in Abd and Fem tissues, whereas the initial difference noticed in the value of the ethanol outflow-to-inflow ratio progressively disappeared, and no difference was seen when 0.1 or 1 µM isoproterenol was infused. This result suggests that the lipolytic response initiated by isoproterenol and β-AR activation in Fem adipose tissue is at least similar (and even higher, because modulation of blood flow is stronger in Fem tissue) to that in Abd adipose tissue. This in vivo result agrees with the fact that a similar β-AR level and lipolytic response to isoproterenol was found in isolated adipocytes from the two deposits in humans (20, 21). Thus in vitro and in vivo studies correlate.

The infusion of 0.1 µM NE or Epi increased extracellular glycerol levels in both tissues without changing the ethanol outflow-to-inflow ratio. Only the highest concentration used (10 µM) increased the ethanol outflow-to-inflow ratio. Thus the large increase in extracellular glycerol observed at this catecholamine concentration may not be completely due to increased lipolysis but may, in part, be caused by reduced blood flow. However, mild differences in the effects of NE and Epi were found regardless of the gender of the subjects or the site of the fat deposit.

An apparent contradiction exists between results from microdialysis and in vitro assays. In vitro studies have shown that the lipolytic response of human adipocytes to physiological catecholamines was rather weak compared with response to isoproterenol. The difference is explainable, first, by the lower affinity of Epi and NE for β-ARs (21) and, second, by the stimulation of the α2-ARs located on adipocytes, the stimulation of which induces antilipolysis (20). According to previous in vitro data, Fem adipocytes possess larger amounts of α2-ARs and functional in vitro studies have shown that the impairment of Epi responsiveness was attributable to this higher number of α2-ARs (19, 20, 22). This result conflicts with the present study results that show the lipomobilizing effects of Epi or NE were quite similar in the two fat deposits. In fact, most of the in vitro studies were carried out on fat cells from older subjects, some of whom were overweight and even obese (19, 20, 22). Because of ethical and experimental limitations, nothing is known about the α2-β-AR balance in the fat deposits of the young, healthy subjects. However, the present in vivo data fit more suitably with recently published in vitro data (19, 22) that indicate that, in lean subjects (age 35 ± 5 and 36 ± 3 yr for women and men, respectively), there were no regional differences in the antilipolytic effect of Epi or UK-4304, a selective α2-AR agonist. Thus, it can be concluded that, in healthy young humans, when physiological amines are infused in microdialysis probes, no clear differences are revealed concerning the interplay of fat cell β- and α2-ARs in the regulation of fat-cell function, according to the site of fat or gender of subjects.

To complete the initial microdialysis approach, a physiological activation of the SNS was evaluated. This activation can easily be achieved through orthostatism. As expected, passing from the supine to the upright position increased subjects’ plasma NE levels. General stimulation of lipolysis was assessed by the increase in plasma glycerol and nonesterified fatty acids (Table 3). Interstitial glycerol levels increased during the whole upright period (Fig. 4). The results indicate that no striking statistical differences were found according to either the adipose tissue location or the gender of the subjects and fit with those obtained when catecholamines are directly infused in the microdialysis probes.

In summary, our study shows that, in young adult subjects, extracellular fluid circulation was the sole regional difference observed in adipose tissue, and the circulation of extracellular fluid was weaker in Fem than in Abd fat deposits. This reduction of fluid circulation may explain the higher extracellular glycerol concentration in Fem adipose tissue. It is noticeable that lipolytic responses to adrenergic stimulation are similar in the two sites, and no differences based on gender were observed. Further in vivo studies are needed to assess whether, as suggested by in vitro studies, obesity modifies the response to physiological catecholamines in relation with increased α2-ARs in fat cells.

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