Lipid mobilization in subcutaneous adipose tissue during exercise in lean and obese humans. Roles of insulin and natriuretic peptides

Katrien Koppo,1,2 Dominique Larrouy,3,4 Marie A. Marques,3,4 Michel Berlan,3,4 Magda Bajzova,1,4 Jan Polak,1,4 Johan Van de Voorde,2 Jens Büllo,5 Max Lafontan,3 François Crampes,3,4 Dominique Langin,3,4,6 Vladimir Stich,1,4 and Isabelle de Glisezinski3,4,7

1Department of Sports Medicine, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 2Department of Pharmacology, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; 3INSERM, U858, Laboratoire de Recherches sur les Obésités, Institut de Médecine Moléculaire de Rangueil, IFR31 and Paul Sabatier University, Toulouse, France; 4Franco-Czech Laboratory of Clinical Research on Obesity, INSERM and Third Faculty of Medicine of the Charles University, Prague, Czech Republic; 5Department of Clinical Physiology, Bispebjerg Hospital, Copenhagen, Denmark; and Departments of 6Clinical Biochemistry and 7Sports Medicine, CHU, Toulouse, France

Submitted 22 December 2009; accepted in final form 13 May 2010

Koppo K, Larrouy D, Marques MA, Berlan M, Bajzova M, Polak J, Van de Voorde J, Bulow J, Lafontan M, Crampes F, Langin D, Stich V, de Glisezinski I. Lipid mobilization in subcutaneous adipose tissue during exercise in lean and obese humans. Roles of insulin and natriuretic peptides. Am J Physiol Endocrinol Metab 299: E258–E265, 2010. First published May 18, 2010; doi:10.1152/ajpendo.00767.2009.—The aim of this study was to evaluate the relative contributions of various hormones involved in the regulation of lipid mobilization in subcutaneous adipose tissue (SCAT) during exercise and to assess the impact of obesity on this regulation. Eight lean and eight obese men performed a 60-min cycle exercise bout at 50% of their peak oxygen uptake on two occasions: during intravenous infusion of octreotide (a somatostatin analog) or physiological saline (control condition). Lipolysis in SCAT was evaluated using in situ microdialysis. One microdialysis probe was perfused with the adrenergic blockers phentolamine and propranolol while another probe was perfused with the phosphodiesterase and adenosine receptor inhibitor aminophylline. Compared with the control condition, infusion of octreotide reduced plasma insulin levels in lean (from 3.5 to 0.5 µU/ml) and in obese (from 3.9 to 2 µU/ml), blunted the exercise-induced rise in plasma GH and epinephrine levels in both groups, and enhanced the exercise-induced natriuretic peptide (NP) levels in lean but not in obese subjects. In both groups, octreotide infusion resulted in higher exercise-induced increases in dialysate glycerol concentrations in the phentolamine-containing probe while no difference in lipolytic response was found in the aminophylline-containing probe. The results suggest that insulin antilipolytic action plays a role in the regulation of lipolysis during exercise in lean as well as in obese subjects. The octreotide-induced enhancement of exercise lipolysis in lean subjects was associated with an increased exercise-induced plasma NP response. Adenosine may contribute to the inhibition of basal lipolysis in both subject groups.

octreotide; adenosine; catecholamines; microdialysis; exercise-induced lipolysis

REGULATION OF LIPOLYSIS. In agreement with rapidly changing demands, is a basic metabolic necessity that, seen from an evolutionary point of view, can hardly be assigned to a single hormone stimulus. The diversity of inhibitory and stimulatory pathways controlling lipolysis has clearly been revealed in vitro studies (23). Nevertheless, in humans, the nature and physiological roles of activators/inhibitors are not fully understood. Subcutaneous adipose tissue (SCAT) lipolysis is regulated by an interplay of inhibitory and stimulatory pathways that controls the activity of lipases. Insulin, catecholamines, and natriuretic peptides (NPs) are considered to be the key players in the regulation of lipolysis. Insulin is the most important antilipolytic hormone in vivo (5, 21). Adenosine (a degradation product of cAMP) is another potential inhibitor of lipolysis. It has been shown that lipolysis in isolated human adipocytes decreased when incubated with adenosine concentrations that were similar to those measured in vivo in subcutaneous periumbilical tissue of healthy humans (24). Among the factors that stimulate SCAT lipolysis, catecholamines are the most powerful. Factors such as diet, physical activity level, and adiposity affect the interplay between the lipolytic β-adrenergic effect and the antilipolytic α2-adrenergic effect, although in general it is the lipolytic β-adrenergic effect that dominates (for a review see Ref. 23). Besides catecholamines, NPs [atrial NP (ANP) and B-type NP (BNP)] have a powerful stimulatory effect on SCAT lipolysis (31). Whereas the effects of insulin, adenosine, and catecholamines are mediated by a cAMP-dependent pathway and modulation of protein-kinase A activity, NPs act through a cyclic GMP (cGMP)-dependent pathway that, via protein kinase G (PKG), phosphorylates hormone-sensitive lipase and perilipin A, leading to the stimulation of lipolysis (33). Other lipolytic hormones are growth hormone (GH) (16, 18) and glucocorticoids (9, 10), although their effect is delayed and their exact mechanism of action is not fully established.

The tuning of SCAT lipolysis depends on the energy needs. In postabsorptive resting states, it appears that lipolysis is set by plasma insulin levels (27). During moderate-intensity exercise, the rise in lipolysis was assumed to be the result of an increase in β-adrenergic receptor (AR) stimulation (2). It was shown that sympathoadrenergic activity increases lipolysis through an exercise-induced rise in circulating catecholamines rather than through changes in sympathetic nerve activity (6, 34). However, the prominent role of β-adrenergic stimulation has been questioned by studies that showed a significant remaining lipolytic rate during exercise performed under β-blockade (19, 25, 28). Therefore, NPs have been put forward as another likely candidate contributing to the increase in exercise-induced lipolysis (25). However, some of the increase in lipolysis during exer-
Lipid mobilization in abdominal SCAT of lean and obese subjects was evaluated using glycerol release measured with SCAT microdialysis. The somatostatin analog octreotide was intravenously infused during rest and moderate-intensity exercise. In addition, pharmacological compounds were directly infused into the microdialysis probes. In one probe, the ARs of the surrounding fat cells were blocked by infusion of β- and α2-AR antagonists (respectively, propranolol and phentolamine) to eliminate the adrenergic part of lipolysis regulation. In another probe, aminophylline, a theophylline-ethylene diamine complex where theophylline is the main component, was infused. Theophylline is a nonselective adenosine receptor antagonist and is also known to block phosphodiesterase 3B (PDE-3B) action, which allowed us to further characterize the insulin-mediated effect on lipolysis.

### MATERIALS AND METHODS

#### Subjects

Eight lean and eight obese healthy young men who had not been enrolled in any pharmacological or nutritional protocol prior to the study were recruited. All subjects had a stable weight during the 6 mo that preceded the study. The selection of the subjects was based on a screening evaluation of a detailed medical history, a physical examination, and blood chemistry analyses (Table 1). All subjects were fully informed about the aim and the protocol of the study and signed an informed consent approved by the ethics committee of the third Medical Faculty Hospital of the Charles University of Prague.

#### Experimental Protocol

One week before the experiments, the subjects performed an incremental exercise test on an electromagnetically braked bicycle ergometer (Ergometers 800s Ergoline) to determine their peak oxygen uptake ($V_{\text{O}_2\text{peak}}$). Work rate was increased by 30 W every 3 min, from an initial work rate of 50 W, until the subjects could no longer maintain the instructed pedal rate of ~65 rpm despite strong verbal encouragement. For all subjects, work rates equivalent to 50% $V_{\text{O}_2\text{peak}}$ were calculated (i.e., 100 ± 7.6 W for the lean and 80 ± 28 W for the obese subjects).

Subsequently, the subjects were randomly assigned to receive either physiological saline (control condition) or octreotide infusion in a crossover design, with a 7-day washout period between the two conditions. Somatostatin is known to inhibit the secretion of insulin (1), GH (8, 13), and the release of epinephrine (6, 13). A schematic representation of the protocol is shown in Fig. 1. The subjects entered the laboratory at 8:00 AM after an overnight fast and were maintained in a semirecumbent position. Two indwelling polyethylene catheters were inserted into antecubital veins, one for blood sampling and the other for saline or octreotide (Sandostatine; 30 ng kg⁻¹ min⁻¹) infusion. At 8:30 AM, microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20 × 0.5 mm and 20,000-MW cutoff were inserted...
percutaneously after epidermal anesthesia (200 μl of 1% Lidocaine; Roger-Bellon, Neuilly-sur-Seine, France) into the SCAT at a distance of 10 cm from the umbilicus. The probes were connected to a microperfusion pump (Harvard apparatus, S.A.R.L., Les Ulis, France) and perfused at a rate of 2.5 μl/min. One probe (PhentoPro probe) was perfused with 100 μmol/l phenolamine (an α₁₂-AR antagonist) plus 100 μmol/l propranolol (a nonselective β-AR antagonist). The other probe (Amino probe) was perfused with 10 mmol/l aminophylline [an A1 adenosine receptor (A1AR) antagonist and a PDE-3B inhibitor]. Ethanol (1.7 g/l) was added to the perfusate to estimate changes in the adipose tissue blood flow (ATBF), as previously described (14). The ethanol ratio was calculated in percent ratio of the ethanol concentration measured in the dialysate divided by the ethanol concentration measured in the perfusate × 100 and taken as an index of ethanol washout. A period of 60-min equilibration was performed; then, for 60 min, four 15-min fractions of the outgoing dialysate were collected in the probes. Thereafter, the subjects received an infusion of saline or octreotide for 150 min. After 60 min of infusion at rest, the subjects performed a 60-min exercise bout at 50% V̇O₂peak followed by 30 min of rest, as shown in Fig. 1. At rest and during exercise and recovery, dialysate was collected every 15 min. Blood samples were taken every 30 min during rest and recovery and every 15 min during exercise. Water intake was allowed ad libitum during the entire experiment. The recovery of the dialysate probes was not assessed in the present study; we demonstrated in our previous work (3, 35) that the concentration of glycerol in the dialysate reflects the true interstitial concentration with a recovery rate between 28 and 33% in lean as well as in obese subjects, with similar microdialysis probes as in the present study.

Measurements

Insulin sensitivity of the subjects (Table 1) was assessed by HOMA-IR ( fasting glucose (mg/dl) × fasting insulin (U/ml)/22.5) (22) and by the revised QUICKI {= 1/log [fasting glucose (mg/dl) × fasting insulin (μU/ml) × fasting NEFA (mmol/l)]} (29). At rest and during exercise, heart rate was continuously monitored with a Polar Accurex Plus cardiometer. Before and during exercise, blood was collected from a catheter for hormonal and metabolic parameter determination. Three milliliters of supplementary blood was collected in 50 μl of an anticoagulant and antioxidant cocktail (Immunotech, Marseilles, France), for determination of catecholamines and was immediately centrifuged in a refrigerated centrifuge. The plasma was stored at −80°C until analysis. Glycerol in dialysate and in plasma was analyzed by an enzymatic method (Sigma, St. Louis, MO). The intra-assay CV was 7.6–8.0% and the interassay CV was 8.8–9.6%. Ethanol in dialysate and perfusate (5 μl) was determined with an enzymatic method (4). The intra- and interassay CVs were 3.0 and 4.5%, respectively. Plasma glucose and NEFA were determined with a glucose oxidase technique (glucose RTU; Biomerieux, Marcy l’Étoile, France; CV 1.1–2.0%) and an enzymatic procedure (Wako kit; Unipath, Dardilly, France; CV 1.1–2.0%) and an enzymatic procedure (Wako kit; Unipath, Dardilly, France; CV 1.1–2.0%) and an enzymatic procedure (Wako kit; Unipath, Dardilly, France; CV 1.1–2.0%) and an enzymatic procedure (Wako kit; Unipath, Dardilly, France; CV 1.1–2.0%) and an enzymatic procedure (Wako kit; Unipath, Dardilly, France; CV 1.1–2.0%). Plasma triglycerides were determined using EIA kits from Mercodia (Uppsala, Sweden; CV 2.8–4.4%). Plasma epinephrine and norepinephrine were assayed in 1-ml aliquots of plasma by high-pressure liquid chromatography using electrochemical (amperometric) detection. Plasma lactate was evaluated using an enzymatic method (BioMerieux). Plasma ANP, BNP, and GH, collected on EDTA and aprotinin, were determined using radioimmunoassay kits from Peninsula Laboratories (San Carlos, CA) and EIA kits (R&D systems, Lille, France), respectively. C-peptide was determined using the C-peptide ELISA kit from Merckodia (CV 4.8–4.4%). Octreotide (Sandostatine) was provided by Zeneca Pharma (Cergy, France). Phentolamine methanesulfonate (Regitine) was obtained from Novartis Pharma (Rueil-Malmaison, France), propranolol (Avlocardyl) from Therva Medical (Neuilly-sur-Seine, France) and aminophylline (Aminophylline Renaudin) from Laboratoire Renaudin (Ixassou, France).

Statistical Analysis

All values are presented as means ± SE. Statistical evaluation of the data was performed using a repeated-measures ANOVA (SPSS statistical software, SPSS, Chicago, IL). To quantify the magnitude of the exercise-induced SCAT lipolysis, the area under the curve (AUC, i.e., the integral of the total increase above the baseline value) of the dialysate glycerol was calculated. Baseline levels for ANP/BNP, insulin, and dialysate glycerol were calculated as the average value measured between 60 and 120 s. Differences in these baseline levels and in AUC between the control and octreotide conditions were analyzed with a two-tailed paired-samples t-test. Where appropriate, comparisons were made between lean and obese, and differences were tested for statistical significance using a two-tailed independent-samples t-test. Significance was determined at P < 0.05.

RESULTS

Effect of Octreotide Infusion on Plasma Values at Rest and During Exercise in Lean Subjects

Rest. During octreotide infusion, the baseline levels of plasma insulin were markedly lower compared with the control condition (0.33 ± 0.12 vs. 3.35 ± 0.62 μU/ml; Fig. 2A). Also, the baseline levels of C-peptide (Fig. 2B) and plasma glucose were significantly lower under octreotide infusion, whereas those of plasma glycerol and NEFA were significantly higher (Table 2). No differences in baseline levels were found for plasma GH, epinephrine, norepinephrine, lactate (Table 2), ANP, and BNP (Fig. 2C).

Exercise. Throughout the exercise, plasma insulin and C-peptide levels remained markedly lower in the octreotide condition than in the control condition (Fig. 2, A and B). The exercise-induced rises in plasma levels of GH and epinephrine were lower in the octreotide than in the control condition. Norepinephrine, however, increased similarly during exercise under saline or octreotide infusion. The plasma concentrations of glucose, glycerol, NEFA (Table 2), ANP, and BNP (Fig. 2C) were significantly higher during exercise under octreotide than under saline infusion. The increase in plasma lactate concentration during exercise was similar in both conditions (Table 2).

Effect of Octreotide Infusion on Plasma Values at Rest and During Exercise in Obese Subjects

Rest. Similar to the lean subjects, the baseline level of plasma insulin was significantly lower during octreotide infusion (0.59 ± 0.29 vs. 8.98 ± 1.86 μU/ml; Fig. 2D). Also, the baseline level of C-peptide was significantly lower during octreotide infusion (Fig. 2E), whereas those of plasma glycerol and NEFA were significantly higher compared with the control condition (Table 3). No differences in baseline levels were found for plasma GH, epinephrine, norepinephrine, lactate (Table 3), ANP, and BNP (Fig. 2F). Unlike in lean subjects, where baseline plasma glucose was significantly lower in the octreotide condition, only minor changes occurred under octreotide infusion in the obese subjects (i.e., a small decrease at rest followed by a mild increase at the end of exercise; Table 3). Interesting to note is that the baseline plasma ANP and BNP levels were higher in the lean than in the obese subjects both in

Statistical Analysis

All values are presented as means ± SE. Statistical evaluation of the data was performed using a repeated-measures ANOVA (SPSS statistical software, SPSS, Chicago, IL). To quantify the magnitude of the exercise-induced SCAT lipolysis, the area under the curve (AUC, i.e., the integral of the total increase above the baseline value) of the dialysate glycerol was calculated. Baseline levels for ANP/BNP, insulin, and dialysate glycerol were calculated as the average value measured between 60 and 120 s. Differences in these baseline levels and in AUC between the control and octreotide conditions were analyzed with a two-tailed paired-samples t-test. Where appropriate, comparisons were made between lean and obese, and differences were tested for statistical significance using a two-tailed independent-samples t-test. Significance was determined at P < 0.05.

RESULTS

Effect of Octreotide Infusion on Plasma Values at Rest and During Exercise in Lean Subjects

Rest. During octreotide infusion, the baseline levels of plasma insulin were markedly lower compared with the control condition (0.33 ± 0.12 vs. 3.35 ± 0.62 μU/ml; Fig. 2A). Also, the baseline levels of C-peptide (Fig. 2B) and plasma glucose were significantly lower under octreotide infusion, whereas those of plasma glycerol and NEFA were significantly higher (Table 2). No differences in baseline levels were found for plasma GH, epinephrine, norepinephrine, lactate (Table 2), ANP, and BNP (Fig. 2C).

Exercise. Throughout the exercise, plasma insulin and C-peptide levels remained markedly lower in the octreotide condition than in the control condition (Fig. 2, A and B). The exercise-induced rises in plasma levels of GH and epinephrine were lower in the octreotide than in the control condition. Norepinephrine, however, increased similarly during exercise under saline or octreotide infusion. The plasma concentrations of glucose, glycerol, NEFA (Table 2), ANP, and BNP (Fig. 2C) were significantly higher during exercise under octreotide than under saline infusion. The increase in plasma lactate concentration during exercise was similar in both conditions (Table 2).

Effect of Octreotide Infusion on Plasma Values at Rest and During Exercise in Obese Subjects

Rest. Similar to the lean subjects, the baseline level of plasma insulin was significantly lower during octreotide infusion (0.59 ± 0.29 vs. 8.98 ± 1.86 μU/ml; Fig. 2D). Also, the baseline level of C-peptide was significantly lower during octreotide infusion (Fig. 2E), whereas those of plasma glycerol and NEFA were significantly higher compared with the control condition (Table 3). No differences in baseline levels were found for plasma GH, epinephrine, norepinephrine, lactate (Table 3), ANP, and BNP (Fig. 2F). Unlike in lean subjects, where baseline plasma glucose was significantly lower in the octreotide condition, only minor changes occurred under octreotide infusion in the obese subjects (i.e., a small decrease at rest followed by a mild increase at the end of exercise; Table 3). Interesting to note is that the baseline plasma ANP and BNP levels were higher in the lean than in the obese subjects both in
During exercise, the obese subjects showed a similar pattern to the lean ones, i.e., lower plasma insulin and C-peptide levels (Fig. 2, D and E) and lower exercise-induced rises in plasma GH and epinephrine in the octreotide condition. As in the lean subjects, norepinephrine increased similarly during exercise under saline or octreotide infusion. The plasma concentrations of glucose, glycerol, and NEFA (Table 3) were significantly higher during exercise under octreotide than under saline infusion. Unlike in the lean subjects, the exercise-induced rise in plasma ANP was similar in the control and octreotide condition, and plasma BNP levels did not rise at all during exercise, irrespective of whether saline or octreotide was infused (Fig. 2F).

**Effect of Octreotide Infusion on Dialysate Glycerol from SCAT at Rest and During Exercise in Lean and Obese Subjects**

**PhentoPro probe.** At rest, infusion of octreotide did not increase dialysate glycerol concentration either in lean (P = 0.949) or in obese (P = 0.163) subjects (Fig. 3, A and C). However, during exercise, dialysate glycerol concentration was higher under octreotide than under saline infusion both in lean (P = 0.003) and in obese (P = 0.003) subjects (Fig. 3, A and C). When lean and obese were compared, baseline dialysate glycerol concentrations were not significantly different either under saline (P = 0.297) or under octreotide infusion (P = 0.920). Also, their exercise-induced lipolysis was comparable in the control (P = 0.850) as well as in the octreotide condition (P = 0.489).

**Amino probe.** As in the PhentoPro probe, infusion of octreotide did not increase dialysate glycerol concentration at rest either in lean (P = 0.794) or in obese (P = 0.155) subjects (Fig. 3, B and D). Also during exercise, dialysate glycerol concentration was similar under octreotide and saline infusion both in lean (P = 0.858) and in obese subjects (P = 0.658) (Fig. 3, B and D).

**Amino vs. PhentoPro probe.** In the octreotide condition, where insulin levels and PDE-3B activity are low, and where aminophylline acts mainly through blocking the A1ARs, the effect of adenosine on the lipolysis can be isolated. When the Amino probe was compared with the PhentoPro probe, a significantly higher dialysate glycerol concentration was observed in the Amino probe throughout the entire protocol both
Table 2. Effect of octreotide and saline infusion on plasma catecholamines (pg/ml), GH (ng/ml), glycerol (μmol/l), NEFA (μmol/l), glucose (mmol/l), and lactate (mmol/l) in lean men

<table>
<thead>
<tr>
<th>Sampling Time, min</th>
<th>Control (saline)</th>
<th>Exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>107 ± 46</td>
<td>103 ± 21</td>
<td>622* ± 181</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>57 ± 23</td>
<td>54 ± 19</td>
<td>73* ± 22</td>
</tr>
<tr>
<td>GH</td>
<td>0.14 ± 0.04</td>
<td>0.13 ± 0.04</td>
<td>0.61 ± 0.30</td>
</tr>
<tr>
<td>Glycerol</td>
<td>92 ± 23</td>
<td>76 ± 8</td>
<td>142* ± 24</td>
</tr>
<tr>
<td>NEFA</td>
<td>121 ± 13</td>
<td>131 ± 15</td>
<td>108 ± 11</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.74 ± 0.08</td>
<td>4.55 ± 0.14</td>
<td>4.79 ± 0.08</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.89 ± 0.07</td>
<td>0.98 ± 0.07</td>
<td>2.01* ± 0.29</td>
</tr>
</tbody>
</table>

Octreotide

<table>
<thead>
<tr>
<th>Sampling Time, min</th>
<th>Control (saline)</th>
<th>Exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>93 ± 5</td>
<td>78 ± 13</td>
<td>647* ± 166</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>58 ± 15</td>
<td>64 ± 24</td>
<td>69 ± 22</td>
</tr>
<tr>
<td>GH</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.06* ± 0.01</td>
</tr>
<tr>
<td>Glycerol</td>
<td>97 ± 14</td>
<td>124* ± 14</td>
<td>227* ± 56</td>
</tr>
<tr>
<td>NEFA</td>
<td>132 ± 22</td>
<td>274* ± 41</td>
<td>215* ± 27</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.96 ± 0.12</td>
<td>4.07 ± 0.14</td>
<td>5.12* ± 0.16</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.98 ± 0.13</td>
<td>0.97 ± 0.13</td>
<td>2.24* ± 0.50</td>
</tr>
</tbody>
</table>

Values are means ± SE. Octreotide or saline was infused from 60 until 210 min. Exercise was performed from 120 until 180 min. *P < 0.05 vs. baseline values; †P < 0.05 vs. saline infusion.

DISCUSSION

The principal finding of this study is that intravenous octreotide infusion enhanced the exercise-induced rise in SCAT lipolysis compared with the control condition in lean as well as obese subjects. Octreotide infusion induced lower levels of plasma insulin, epinephrine, and GH and, in lean subjects, higher levels of plasma ANP and BNP. Another finding was that the dialysate glycerol concentration was higher in the Amino probe than in the PhentoPro probe both at rest and during exercise, suggesting a tonic suppression of SCAT lipolysis by adenosine. However, in the Amino probe, exercise-induced lipid mobilization was similar between the control and octreotide conditions in both lean and obese subjects, suggest-

Table 3. Effect of octreotide and saline infusion on plasma catecholamines (pg/ml), GH (ng/ml), glycerol (μmol/l), NEFA (μmol/l), glucose (mmol/l), and lactate (mmol/l) in obese men

<table>
<thead>
<tr>
<th>Sampling Time, min</th>
<th>Control (saline)</th>
<th>Exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>121 ± 15</td>
<td>119 ± 14</td>
<td>624* ± 126</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>47 ± 3</td>
<td>46 ± 7</td>
<td>67* ± 7</td>
</tr>
<tr>
<td>GH</td>
<td>0.14 ± 0.07</td>
<td>0.19 ± 0.10</td>
<td>0.33 ± 0.18</td>
</tr>
<tr>
<td>Glycerol</td>
<td>88 ± 11</td>
<td>86 ± 8</td>
<td>144* ± 16</td>
</tr>
<tr>
<td>NEFA</td>
<td>299 ± 68</td>
<td>309 ± 72</td>
<td>215* ± 54</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.45 ± 0.12</td>
<td>4.49 ± 0.13</td>
<td>4.66* ± 0.14</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.25 ± 0.12</td>
<td>1.32 ± 0.13</td>
<td>2.38* ± 0.33</td>
</tr>
</tbody>
</table>

Octreotide

<table>
<thead>
<tr>
<th>Sampling Time, min</th>
<th>Control (saline)</th>
<th>Exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>104 ± 17</td>
<td>108 ± 20</td>
<td>556* ± 85</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>52 ± 3</td>
<td>49 ± 4</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>GH</td>
<td>0.09 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Glycerol</td>
<td>184* ± 42</td>
<td>201* ± 35</td>
<td>251* ± 36</td>
</tr>
<tr>
<td>NEFA</td>
<td>316 ± 50</td>
<td>542* ± 109</td>
<td>465* ± 101</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.11* ± 0.09</td>
<td>4.06 ± 0.19</td>
<td>4.80* ± 0.27</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.23 ± 0.10</td>
<td>1.36 ± 0.13</td>
<td>2.13* ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SE. Octreotide or saline was infused from 60 until 210 min. Exercise was performed from 120 until 180 min. *P < 0.05 vs. baseline values; †P < 0.05 vs. saline infusion.
ing that adenosine does not contribute to changes in lipid mobilization during exercise under octreotide infusion.

As mentioned in the introduction, insulin has been shown to play an important role in the regulation of SCAT lipolysis during rest but its contribution to the regulation during exercise is still unclear. During exercise, when changes in several hormone levels (such as insulin, catecholamines, and NPs) occur, it is less obvious to assess the contribution of a change in a single hormone level to the regulation of exercise-induced SCAT lipolysis. In the present study, the contribution of the adrenergic system to the regulation of SCAT lipolysis was eliminated by direct perfusion of phentolamine (an α1,2-AR antagonist) and propranolol (a nonselective β-AR antagonist) in the microdialysis probe. Because phentolamine was perfused into the probe, the greater involvement of the α2-AR activity in the obese subjects, previously reported as being partly responsible for their impaired exercise-induced lipolysis (36), was eliminated. The results of the PhentoPro probe revealed that, in the control condition, even though the adrenergic system efficacy at the fat cell level was blocked, dialysate glycerol increased during exercise, and the exercise-induced lipolysis was comparable in lean and obese subjects (Fig. 3, A and C). The moderate decrease in plasma insulin levels observed during exercise might have been a contributing factor.

It is unlikely that exercise-dependent GH secretion was one of the factors that underlay the rise in lipolysis observed during exercise in the control condition, since previous studies showed that the lipolytic effect of a GH injection appeared after at least 2 h (11, 17) and that GH-induced lipid mobilization occurred only after 60 min of exercise (15). Also, it is difficult to argue that the observed enhancement of lipid mobilization during exercise under octreotide infusion might have been related to differences in circulating GH. As mentioned above, it was shown that octreotide administration inhibits the exercise-dependent GH secretion (8), and it totally suppressed the GH-induced lipid mobilization after a short-term exercise (13). If GH plays a role in the control of exercise-induced lipolysis, this would mean that, in the present study, octreotide infusion should have led to a decrease in exercise-induced lipolysis, which was completely the opposite of what we observed.

Another pathway that could mediate the octreotide effect on exercise-induced lipolysis is the cGMP-dependent pathway stimulated by increased plasma ANP and BNP levels. It was shown that ANP released by the heart during exercise (25) or during head-down bed rest (26) increased lipolysis. Also, in the present study the rise in ANP/BNP levels observed during exercise in the control condition was possibly involved in the exercise-induced lipolysis. In the lean subjects, infusion of octreotide induced even higher levels of ANP/BNP during exercise-induced lipolysis to higher levels than those observed in the control condition. This suggests that insulin may play an inhibitory role in the physiological control of lipolysis regulation in lean as well as obese subjects, although other factors might also have been involved.

It is unlikely that exercise-dependent GH secretion was one of the factors that underlay the rise in lipolysis observed during exercise in the control condition, since previous studies showed that the lipolytic effect of a GH injection appeared after at least 2 h (11, 17) and that GH-induced lipid mobilization occurred only after 60 min of exercise (15). Also, it is difficult to argue that the observed enhancement of lipid mobilization during exercise under octreotide infusion might have been related to differences in circulating GH. As mentioned above, it was shown that octreotide administration inhibits the exercise-dependent GH secretion (8), and it totally suppressed the GH-induced lipid mobilization after a short-term exercise (13). If GH plays a role in the control of exercise-induced lipolysis, this would mean that, in the present study, octreotide infusion should have led to a decrease in exercise-induced lipolysis, which was completely the opposite of what we observed.

Another pathway that could mediate the octreotide effect on exercise-induced lipolysis is the cGMP-dependent pathway stimulated by increased plasma ANP and BNP levels. It was shown that ANP released by the heart during exercise (25) or during head-down bed rest (26) increased lipolysis. Also, in the present study the rise in ANP/BNP levels observed during exercise in the control condition was possibly involved in the exercise-induced lipolysis. In the lean subjects, infusion of octreotide induced even higher levels of ANP/BNP during exercise-induced lipolysis to higher levels than those observed in the control condition. This suggests that insulin may play an inhibitory role in the physiological control of lipolysis regulation in lean as well as obese subjects, although other factors might also have been involved.

It is unlikely that exercise-dependent GH secretion was one of the factors that underlay the rise in lipolysis observed during exercise in the control condition, since previous studies showed that the lipolytic effect of a GH injection appeared after at least 2 h (11, 17) and that GH-induced lipid mobilization occurred only after 60 min of exercise (15). Also, it is difficult to argue that the observed enhancement of lipid mobilization during exercise under octreotide infusion might have been related to differences in circulating GH. As mentioned above, it was shown that octreotide administration inhibits the exercise-dependent GH secretion (8), and it totally suppressed the GH-induced lipid mobilization after a short-term exercise (13). If GH plays a role in the control of exercise-induced lipolysis, this would mean that, in the present study, octreotide infusion should have led to a decrease in exercise-induced lipolysis, which was completely the opposite of what we observed.

Another pathway that could mediate the octreotide effect on exercise-induced lipolysis is the cGMP-dependent pathway stimulated by increased plasma ANP and BNP levels. It was shown that ANP released by the heart during exercise (25) or during head-down bed rest (26) increased lipolysis. Also, in the present study the rise in ANP/BNP levels observed during exercise in the control condition was possibly involved in the exercise-induced lipolysis. In the lean subjects, infusion of octreotide induced even higher levels of ANP/BNP during exercise-induced lipolysis to higher levels than those observed in the control condition. This suggests that insulin may play an inhibitory role in the physiological control of lipolysis regulation in lean as well as obese subjects, although other factors might also have been involved.
exercise than those observed during exercise in the control condition (Fig. 2C). Together with decreased insulin levels under octreotide infusion, this might have been responsible for their higher exercise-induced lipolysis in the octreotide than in the control condition (Fig. 3A). The obese subjects had lower baseline levels of plasma ANP/BNP than the lean, and, unlike in the lean subjects, their plasma ANP/BNP levels did not increase under octreotide infusion. Different baseline ANP/BNP levels between lean and obese subjects were previously reported. For example, Wang et al. (37) reported significantly lower N-ANP and BNP levels in overweight and obese men and women than in their lean counterparts (n = 3,389). They hypothesized that the lower plasma NP levels in the obese might have been related to a reduced secretion of NPs (from diminished myocardial hormone release or impaired synthesis) or to an increased clearance of NPs by the NP clearance receptors (NPR-C), which are abundant in adipose tissue. Furthermore, it has been proposed that the increase in adipose tissue mass could lead to enhanced expression of NPR-C in adipocytes that, in turn, would diminish the local actions of ANP on adipocytes (30). These mechanisms could also explain the attenuated exercise-induced rise of ANP/BNP in the obese subjects in the control condition, although further studies are required to thoroughly investigate this topic.

Since in the obese subjects the rise in plasma ANP/BNP levels was blunted under octreotide infusion, of all the factors that were discussed so far, only insulin seems to be a likely candidate responsible for the difference in exercise-induced lipid mobilization between the control and octreotide conditions. This would mean that obese subjects have a preserved sensitivity to the antilipolytic action of insulin at the level of SCAT despite being less sensitive to insulin at the level of whole body carbohydrate metabolism (see HOMA-IR and revised QUICKI results in Table 1). Our results are in line with the study of Hickner et al. (20), which demonstrated that, in a group of obese women, the site of resistance seemed to be located at the intra-abdominal adipose tissue rather than at the SCAT. Despite the importance of insulin during exercise, our results question the major effect of insulin during rest. In the octreotide condition, where insulin was suppressed, dialysate glycerol concentration was similar to the concentration observed in the control condition. However, in plasma, glycerol levels were more elevated under octreotide infusion compared with the control condition. Furthermore, using an arteriovenous catheterization technique, Enevoldsen et al. (13) found that the SCAT net release of glycerol increased significantly during rest when an octreotide infusion was initiated. These latter findings might be the result of the mass effect of the SCAT. A small variation cannot be revealed in the small SCAT volume surrounding the microdialysis probe.

Utilization of an aminophylline probe revealed the existence of a potent tonic inhibition of lipolysis. In the control condition, a significantly higher dialysate glycerol release was observed during rest and exercise in the Amino probe than in the PhentOPro probe. However, since aminophylline is not only an A1AR antagonist but also a PDE-3B inhibitor, the antilipolytic share of insulin and adenosine could not be determined in this condition. In the octreotide condition, where insulin levels were low and had a minimal impact on the PDE-3B activity, a similar upward shift of the dialysate glycerol curve was observed after aminophylline treatment. These findings suggest that the tonic inhibition of the SCAT lipolysis can be attributed mainly to adenosine, whatever the insulin levels. Moreover, the antilipolytic effect of an adenosine receptor agonist appears to be independent of insulin resistance in animal models (7). Our results are in line with the study of Lönnroth et al. (24), in which it was suggested that basal levels of adenosine are sufficient to cause inhibition of lipolysis. The similarity of the aminophylline action in placebo- and octreotide-treated lean and obese subjects during exercise suggest that, although adenosine is an efficient antilipolytic factor, it is not contributing differently in the exercise-induced lipid mobilizing response in lean and obese.

In conclusion, this study demonstrated an enhancement of exercise-induced lipolysis in the presence of octreotide infusion. As the octreotide administration induced a marked decrease of plasma insulin levels in both groups, these results suggest that the antilipolytic action of insulin is involved in the regulation of exercise-induced lipolysis in lean as well as obese subjects. Furthermore, in lean subjects, the octreotide-induced enhancement of exercise lipolysis was associated with an enhanced exercise-induced response of plasma NPs. In addition, an antilipolytic action of adenosine in the regulation of basal and exercise-induced lipolysis was suggested in both subject groups.

**ACKNOWLEDGMENTS**

We thank Z. Parizkova for excellent technical assistance.

**GRANTS**

This study was supported by a grant from the Direction Générale de la Coopération Internationale et du Développement (Programme d’Action Intégré Franco-Tchèque), from Novartis Pharma S.A.S. by the FP6 project Hapidip, by the grant of the Ministry of Education of Czech Republic MSM 0021620814, and by grant 303/07/0840 of the Grant Agency of the Czech Republic. K. Koppo was supported by a postdoctoral research grant from the Special Research Fund of Ghent University.

**DISCLOSURES**

No conflicts of interest are reported by the authors.

**REFERENCES**


