PAPER

Lipolytic and nutritive blood flow response to β-adrenoceptor stimulation in situ in subcutaneous abdominal adipose tissue in obese men

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OBJECTIVE: β-Adrenoceptor-mediated whole-body lipolysis is impaired in obesity. This study investigated whether local adipocyte β-adrenergic sensitivity and changes in nutritive blood flow in subcutaneous abdominal adipose tissue contribute to this impaired response.

METHODS: Three microdialysis probes were placed in the subcutaneous abdominal adipose tissue of eight obese and nine lean men. Each probe was perfused with either 0.1, 1 and 10 μM isoprenaline; 1, 10 and 100 μM dobutamine or 1, 10 and 100 μM salbutamol, each dose for 45 min.

RESULTS: At baseline, interstitial glycerol concentrations and ethanol out/in ratios were comparable between groups. During nonselective β₁, β₁, and β₂-adrenergic stimulation, interstitial glycerol concentrations increased and ethanol out/in ratios decreased similarly in obese and lean men.

CONCLUSION: The lipolytic and nutritive blood flow response to β₁- β₂- and nonselective β-adrenergic stimulation in situ is comparable in lean and obese male subjects. The present data suggest that a blunted β-adrenergic sensitivity of the fat cell and an impaired local nutritive blood flow response do not contribute to the previously reported diminished whole-body β-adrenoceptor-mediated lipolytic response in obese males.

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Introduction

Obesity is associated with a blunted lipolytic response during increased sympathetic nervous system activity. Literature shows that whole-body lipolysis is impaired in obese subjects during i.v. epinephrine¹,² or isoprenaline (nonselective β-adrenoceptor agonist)³ infusion. Furthermore, glycerol and nonesterified fatty acids (NEFA) release from abdominal adipose tissue is blunted in obese females during epinephrine infusion.⁴ In an earlier study, we showed that this blunted β-adrenoceptor-mediated lipolytic response only occurs during selective β₂-adrenergic stimulation, whereas β₁-adrenoceptor-mediated increases in lipolysis are similar in obese and lean men.⁵

Several mechanisms may be responsible for the impaired whole-body β-adrenoceptor-mediated lipolytic response. On the one side, adipocyte β-adrenergic sensitivity for lipolysis might be diminished. In vitro studies show that glycerol release is reduced in subcutaneous abdominal fat cells from obese women after incubation with isoprenaline or terbutaline (β₂-adrenoceptor agonist), whereas glycerol release is similar in fat cells from normal weight and overweight women after incubation with dobutamine (β₁-adrenoceptor agonist).⁶ On the other hand, the release of glycerol from the interstitial fluid into the systemic circulation may be reduced because of a diminished β-adrenoceptor-mediated adipose tissue blood flow response. Adipose tissue blood flow, as measured by the ¹³³Xenon-clearance technique, is significantly lower in obese compared to lean subjects, both at rest⁷ and during i.v. epinephrine⁸ and isoprenaline infusion.⁹

The aim of the present study was to investigate subcutaneous adipose tissue lipolysis during local administration of β-agonists through a microdialysis probe to differentiate
between local tissue events and systemic blood flow effects. Local adipose tissue lipolysis was determined by the continuous dialysis of glycerol in the extracellular fluid of abdominal subcutaneous adipose tissue. Local nutritive blood flow was determined by means of the ethanol dilution technique.13-16.

Subjects and methods

Subjects
Eight obese and nine lean male volunteers participated in this study. Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volumat 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the equation of Siri.17 All subjects were in good health as assessed by medical history and physical examination. Furthermore, both obese and lean subjects spent no more than 2 h a week in organized sports activities. The study protocol was reviewed and approved by the Ethics Committee of Maastricht University, and all subjects gave informed consent before participating in the study.

Microdialysis experiments
All subjects were studied in the supine position after an overnight fast. They came to the laboratory by car or by bus. On arrival, three microdialysis probes (CMA 60, CMA Microdialysis, Solna, Sweden) were inserted percutaneously in subcutaneous abdominal adipose tissue. The skin was anesthetized by means of a crème containing lidocaine (25 mg/g) and prilocaine (25 mg/g) (Emla, Astra Pharmaceuticals, Zoetermeer, The Netherlands). Probes were placed 5-8 cm left or right from the umbilicus and the distance between probes was at least 3 cm. Probes consisted of a dialysis tubing (30 x 0.6 mm², 20 kDa cutoff) glued to the end of a double lumen polyurethane canula. The perfusion solvent entered the probe through the inner canula, passed down to the tip of the probe, streamed upwards in the space between the inner canula and the outer dialysis membrane and left the probe through the outer canula via a side arm, from which it was collected.

Study design
After insertion, all probes were perfused with Ringer solution (147 mM sodium, 4 mM potassium, 2.25 mM calcium and 156 mM chloride) supplemented with 50 mM ethanol at a flow rate of 0.5 μl/min for 20 min before the start of the experiment. During the first part of the experiment, the real interstitial glycerol concentration was determined by means of the zero flow method.18 Microdialysate was collected in two 20-min fractions at a flow rate of 0.5 μl/min and in three 10-min fractions at flow rates of 1, 2.5 and 5 μl/min. During the second part of the experiment, probes were perfused with increasing concentrations of different nonselective and selective β-adrenoceptor agonists at a flow rate of 5 μl/min. During each β-adrenoceptor agonist infusion period, one 15-min dialysate collection fraction was followed by three 10-min fractions. In all samples collected at flow rates of 0.5, 1 and 2.5 μl/min, dialysate glycerol concentrations were measured. In all other samples, both dialysate glycerol and ethanol concentrations were measured. Ethanol was determined both in the ingoing and outgoing perfusion solvent to assess the ethanol out/in ratio as indicator for nutritive blood flow (ethanol escape technique).19

Zero flow method
During the first part of the experiment, the real interstitial glycerol concentration was determined by means of the zero flow method.18 Therefore, probes were perfused at a flow rate of 0.5 μl/min for 40 min and at consecutive flow rates of 1, 2.5 and 5 μl/min for 30 min. Dialysate glycerol concentrations were log transformed and plotted against perfusion rates. Linear regression analysis was used to calculate the glycerol concentration at zero flow rate, corresponding to the real interstitial glycerol concentration. The ratio between the dialysate glycerol concentration at 5 μl/min and the calculated interstitial glycerol concentration represented the in vivo recovery rate of the probe.

β-Adrenoceptor agonists
During the second part of the experiment, each probe was perfused with a nonselective or selective β-adrenoceptor agonist to determine changes in lipolysis and blood flow. The calibration period with a flow rate of 5 μl/min was used as baseline measurement. Then one probe was perfused with 0.1, 1 and 10 μM isoprorenaline to stimulate all β-adrenoceptor subtypes, the second probe was perfused with 1, 10 and 100 μM dobutamine to stimulate β1-adrenoceptors and the third probe was perfused with 1, 10 and 100 μM salbutamol to stimulate β2-adrenoceptors. Each dose of agonist was given for 45 min at a flow rate of 5 μl/min.

Analytical methods
Glycerol and ethanol concentrations in the dialysate were determined on a Cobas Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Glycerol concentration was measured fluorimetrically using a standard glycerol kit (Boehringer, Mannheim, Germany), but with adapted concentrations of NADH, enzymes and buffer to achieve accurate fluorimetric detection. Ethanol concentration was measured spectrophotometrically at 340 nm using a standard ethanol kit (176290, Boehringer, Mannheim, Germany).

Data analysis
All data are presented as mean±1 standard error of the mean (s.e.m.). The effect of nonselective β, β1- or β2-adrenergic
stimulation between groups was analyzed with two-way repeated measurements of ANOVA. P<0.05 was regarded as statistically significant.

**Results**

Physical characteristics of the subjects are summarized in Table 1. By selection, obese men had a significantly higher body mass index, body fat %, fat-free mass and waist–hip ratio. However, groups were of similar height and age.

Baseline interstitial glycerol concentrations were similar in all probes in obese and lean men (before nonselective β-adrenergic stimulation: 218±24 vs 258±25 μM, before β₁-adrenergic stimulation: 200±28 vs 201±27 μM, before β₂-adrenergic stimulation: 265±17 vs 216±18 μM, all NS) (Figure 1). For all β agonists there was a significant increase in interstitial glycerol (P<0.001). The potency to induce lipolysis was different between β₁-adrenoceptor agonists. Isoprenaline revealed a much higher increase in interstitial glycerol concentration as compared to dobutamine or salbutamol, which were equally potent. Nonselective β₁, β₂ and β₂-adrenergic stimulation induced similar increases in interstitial glycerol concentrations in obese and lean men, expressed either as absolute values (Δ glycerol at 10 μM isoprenaline: 452±53 vs 379±35 μM, at 100 μM dobutamine: 196±36 vs 142±48 μM, at 100 μM salbutamol: 227±49 vs 207±31 μM, all NS) (Figure 1) or as percentage increase (data not shown). Increasing the salbutamol or dobutamine concentrations to 1000 μM did not lead to a higher increase in interstitial glycerol concentration (data not shown).

At baseline, ethanol out/in ratios tended to be higher in obese compared to lean men (before nonselective β-adrenergic stimulation: 0.79±0.03 vs 0.69±0.04 μM, P = 0.08; before β₁-adrenergic stimulation: 0.85±0.03 vs 0.71±0.06 μM, P = 0.05, before β₂-adrenergic stimulation: 0.83±0.02 vs 0.73±0.07 μM, P = 0.23) (Figure 2). There was no significant difference in the decrease in ethanol out/in ratio between both groups during nonselective β₁, β₂ and β₂-adrenergic stimulation (Figure 2), indicating a comparable increase in local nutritive blood flow in subcutaneous abdominal adipose tissue.

### Table 1 Physical characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>99.7 (87.9–104.7)</td>
<td>73.8 (67.3–83.0)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77 (1.70–1.82)</td>
<td>1.75 (1.68–1.84)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.6 (28.9–34.9)</td>
<td>24.2 (23.2–25.5)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>32.1 (28.1–39.0)</td>
<td>22.2 (12.8–25.2)</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>67.1 (62.8–75.1)</td>
<td>57.3 (52.0–62.5)</td>
</tr>
<tr>
<td>Waist–hip ratio</td>
<td>1.03 (0.97–1.12)</td>
<td>0.95 (0.81–1.07)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>55 (49–64)</td>
<td>57 (50–61)</td>
</tr>
</tbody>
</table>

Values are means (range) for eight obese and nine lean subjects. Unpaired t-test *P<0.05, **P<0.001.

**Discussion**

Obesity has been reported to be associated with an impaired lipolytic response during intravenous catecholamine infusion or β-adrenergic stimulation.1–4 This may be explained, on one hand, by an impaired β-adrenergic sensitivity of the fat cell, since in vitro studies have shown that glycerol release from subcutaneous adipocytes may be impaired in obese women after incubation with a β₁- or β₂-agonist.5 On the other hand, the release of glycerol from the interstitial fluid into the systemic circulation may be reduced because of a lowered adipose tissue blood flow response, as measured by...
the ethanol out/in ratios tended to be higher in obese as
compared to lean males, indicative of a blunted nutritive
blood flow in the basal state in obese subjects. Basal
interstitial glycerol concentrations were comparable in
both groups. During the local administration of nonselective β-,
β₁, and β₂-adrenoceptor agonists, interstitial glycerol
concentrations increased and ethanol out/in ratios decreased
similarly in obese and lean men. This suggests that obese and
lean males have a comparable adipocyte β-adrenergic
sensitivities and nutritive blood flow response.

Our data seem consistent with a previous microdialysis
study, which showed no difference in the increase in
interstitial glycerol concentrations with in situ isoprenaline
administration in lean and obese men. In contrast to our
findings, it has been previously shown that obese women
and obese female adolescents have an impaired increase in
interstitial glycerol levels during local β₂-adrenergic stimula-
tion, and that lipolytic sensitivity to norepinephrine was
suppressed in abdominal subcutaneous fat cells from upper
body obese women, ascribed to a 10-fold decrease in lipolytic
β₂-adrenoceptor sensitivity. A possible explanation for this
apparent discrepancy may be related to differences in gender.
It has been shown that there may be differences in
catecholamine-mediated lipolysis in subcutaneous adipose
tissue between men and women with a higher lipolysis in
women or a more pronounced difference in lipolysis
between abdominal and gluteal adipocytes in women as
compared to men. Secondly, β-adrenergically mediated
lipolysis may decrease with increasing age, but since in
the above-mentioned studies the study groups were matched
for age this does not seem to contribute to the discrepant
findings. Also, lipolytic and adipose tissue blood flow
responses to epinephrine have been shown to be blunted in
subcutaneous abdominal adipose tissue of upper body
obese women as compared to lean women. However, the
difference in gender and differences in type of catechola-
mine used between the latter study and our study (i.e. the
epinephrine effect may be because of variation in the
functional balance between β- and α₂-adrenoceptors) makes
a comparison with our data difficult.

From the present data, it can be speculated that the
previously reported impaired whole-body lipolytic response
in obese males after i.v. β-adrenoceptor agonist infusion
might be explained by a blunted adipose tissue blood flow
response rather than by a diminished β-adrenergic sensitivity
of the fat cell or local adipose tissue nutritive blood flow
effects. Indeed, adipose tissue blood flow, as measured by the
133Xenon-clearance technique, has been reported to be
significantly lower in obese compared to lean subjects, both
at rest and during i.v. epinephrine infusion. An impaired
subcutaneous abdominal adipose tissue blood flow response
may affect the delivery of hormones and transport proteins
for fatty acids to adipose tissue. Also, the release and reuptake
of fatty acids within adipose tissue may be controlled by
adipose tissue blood flow. The question remains whether the impaired
whole-

Figure 2 Effects of isoprenaline (nonselective β-adrenoceptor agonist),
dobutamine (β₁-adrenoceptor agonist) and salbutamol (β₂-adrenoceptor
agonist) on ethanol out/in ratio in subcutaneous abdominal adipose tissue
in eight obese (●) and nine lean (□) subjects. Values are mean ± t.e.m.

International Journal of Obesity
tissue blood flow response during β-adrenergic stimulation (as reported by Blaak et al.) is a cause or a consequence of obesity. Studies from our group show that during i.v. β-adrenoceptor agonist administration, the increase in subcutaneous abdominal adipose tissue blood flow partially improves after weight reduction. This suggests that a defectively sympathetically mediated blood flow response may rather be a secondary factor resulting from the obese state than a primary factor leading to the development of obesity.

In summary, nonselective β-, β1- and β2-adrenoceptor-mediated increases in interstitial glycerol concentration and local nutritive blood flow were similar in subcutaneous abdominal adipose tissue in obese and lean men. This suggests that the diminished whole-body β-adrenoceptor-mediated lipolytic response, as reported earlier by our group, is probably not for a large part explained by a blunted local adipocyte β-adrenergic sensitivity or nutritive blood flow. More likely, the impaired whole-body lipolytic response during i.v. β-adrenoceptor agonist administration is caused by a blunted adipose tissue blood flow response (as measured by 133Xenon wash-out), which results in an impaired adipose delivery and an impaired glycerol and NEFA release from the adipose tissue in obese male subjects.

Acknowledgements

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References

23 Blaak EE, van Baak MA, Saris WHM. β3-adrenoapeutically stimulated fat oxidation is diminished in middle-aged compared to young subjects. J Clin Endocrinol Metab 1999; 84: 3764-3769.