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

International Journal of Obesity (2003) 27, 227–231

0307-0565/03 $25.00
www.nature.com/ijo

PAPER

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

SLH Schiffelers[1], JAP Akkermans[1], WHM Saris[1] and EE Blaak[1]*

[1]*Department of Human Biology, Nutrition Toxicology and Environment Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands*

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 β-, β_1- and β_2-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 β_1- β_2- 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.
International Journal of Obesity (2003) **27**, 227–231. doi:10.1038/sj.ijo.802230

Keywords: lipolysis; blood flow; β-adrenoceptor; obesity

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. epinephrine[1,2] 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 β_2-adrenergic stimulation, whereas β_1-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 (β_2-adrenoceptor agonist), whereas glycerol release is similar in fat cells from normal weight and overweight women after incubation with dobutamine (β_1-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 ^{133}xenon-clearance technique, is significantly lower in obese compared to lean subjects, both at rest[7–11] and during i.v. epinephrine[4,12] and isoprenaline infusion.[8]

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

*Correspondence: Dr EE Blaak, Department of Human Biology, Maastricht University, PO Box 616, NL-6200 MD Maastricht, The Netherlands.
E-mail: e.blaak@hb.unimaas.nl
Received 1 July 2002; revised 23 September 2002; accepted 7 October 2002

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 (Volugraph 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 prolocaine (25 mg/g) (Emla, Astra Pharmaceutica, 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 ($30 \times 0.6\,mm^2$, 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 asses 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 isoprenaline to stimulate all β-adrenoceptor subtypes, the second probe was perfused with 1, 10 and 100 µM dobutamine to stimulate β_1-adrenoceptors and the third probe was perfused with 1, 10 and 100 µM salbutamol to stimulate β_2-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 ± standard error of the mean (s.e.m.). The effect of nonselective β, β_1- or β_2-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 β_1-adrenergic stimulation: 200±28 *vs* 201±27 μM, before β_2-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 β-adrenoceptor agonists. Isoprenaline revealed a much higher increase in interstitial glycerol concentration as compared to dobutamine or salbutamol, which were equally potent. Nonselective β-, β_1- and β_2-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 β_1-adrenergic stimulation: 0.85±0.03 *vs* 0.71±0.06 μM, $P=0.05$, before β_2-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 β-, β_1- and β_2-adrenergic stimulation (Figure 2), indicating a comparable increase in local nutritive blood flow in subcutaneous abdominal adipose tissue.

Table 1 Physical characteristics

Parameter	*Obese*		*Lean*
Body weight (kg)	99.7 (87.9–104.7)	***	73.8 (67.3–83.0)
Height (m)	1.77 (1.70–1.82)		1.75 (1.68–1.84)
Body mass index (kg/m^2)	31.6 (28.9–34.9)	***	24.2 (23.2–25.5)
Body fat (%)	32.1 (28.1–39.0)	***	22.2 (12.8–25.2)
Fat-free mass (kg)	67.1 (62.8–75.1)	***	57.3 (52.0–62.5)
Waist–hip ratio	1.03 (0.97–1.12)	*	0.95 (0.81–1.07)
Age (y)	55 (49–64)		57 (50–61)

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

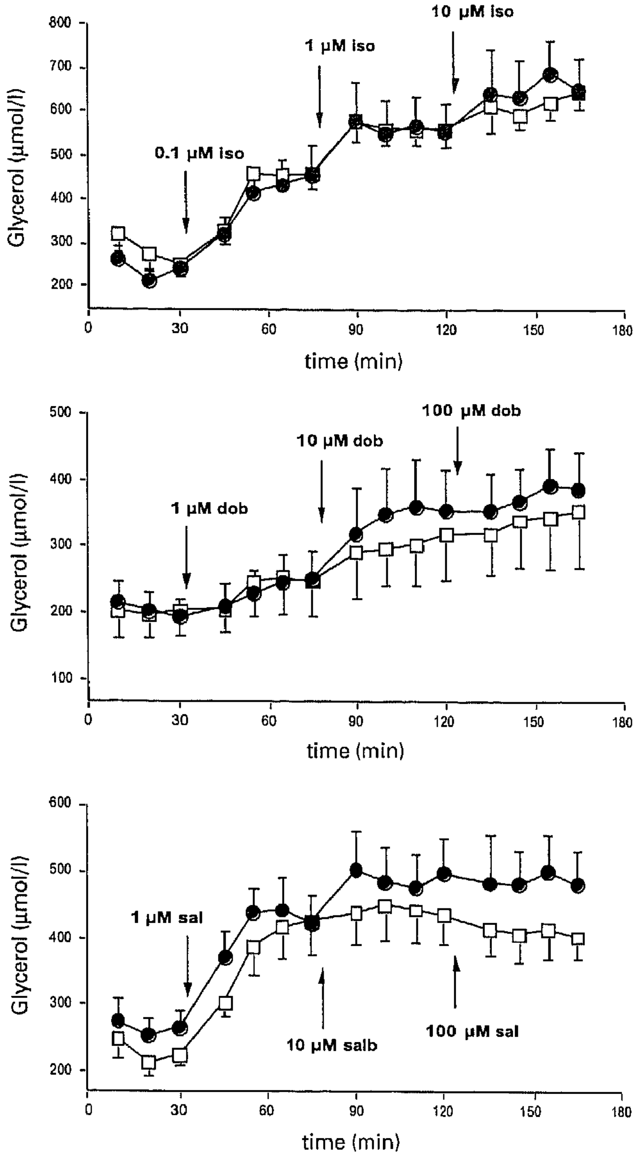

Figure 1 Effects of isoprenaline (nonselective β-adrenoceptor agonist), dobutamine (β_1-adrenoceptor agonist) and salbutamol (β_2-adrenoceptor agonist) on interstitial glycerol concentrations in subcutaneous abdominal adipose tissue in eight obese (●) and nine lean (□) subjects. Values are mean±s.e.m.

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 β- or β_2-agonist.[6] 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

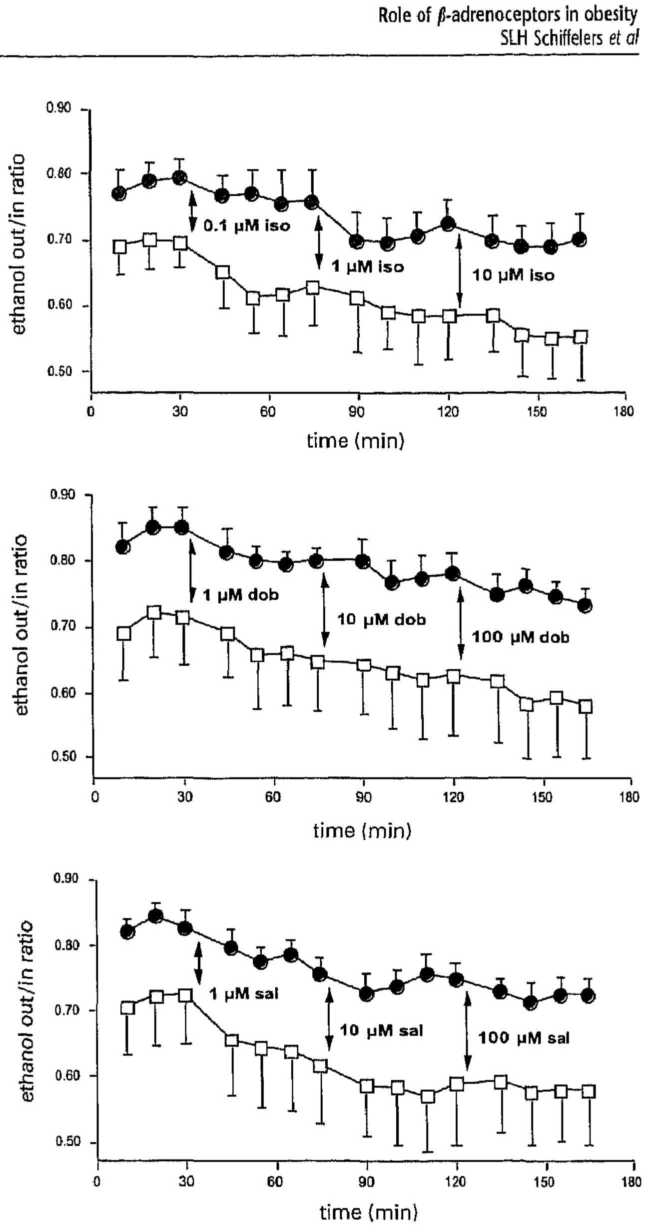

Figure 2 Effects of isoprenaline (nonselective β-adrenoceptor agonist), dobutamine (β_1-adrenoceptor agonist) and salbutamol (β_2-adrenoceptor agonist) on ethanol out/in ratio in subcutaneous abdominal adipose tissue in eight obese (●) and nine lean (□) subjects. Values are mean±s.e.m.

the ^{133}xenon-clearance technique.[4,8,12] Local nutritive blood flow, as assessed by the microdialysis technique by determining the ethanol out/in ratio, increases in lean subjects during local administration of isoprenaline,[13–15] terbutaline[13,16] or dobutamine.[13] However, little is known on whether β-adrenoceptor changes in local nutritive blood flow are altered in obesity.

The aim of the present study was to investigate subcutaneous adipose tissue lipolysis during local administration of β-agonists through a microdialysis probe to study whether the β-adrenergic sensitivity for lipolysis and the nutritive blood flow response were altered in obese males . At baseline, 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-, β_1- and β_2-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 sensitivity and nutritive blood flow response.

Our data seem consistent with a previous microdialysis study,[20] 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 β_2-adrenergic stimulation,[21] 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 β_2-adrenoceptor sensitivity.[6] 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 women and men with a higher lipolysis in women or a more pronounced difference in lipolysis between abdominal and gluteal adipocytes in women as compared to men.[22] Secondly, β-adrenergically mediated lipolysis may decrease with increasing age,[23,24] but since in the above-indicated 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.[4] However, the difference in gender and differences in type of catecholamine used between the latter study and our study (ie the epinephrine effect may be because of variation in the functional balance between β- and α_2-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 ^{133}xenon-clearance technique, has been reported to be significantly lower in obese compared to lean subjects, both at rest[7–11] and during i.v. epinephrine[4,12] and isoprenaline infusion.[8] 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-tissue blood flow response during β-adrenergic stimulation (as reported by Blaak *et al*[8]) 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.[8] This suggests that a defective 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-, β_1- and β_2-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,[3,5] 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 ^{133}xenon wash-out), which results in an impaired agonist delivery and an impaired glycerol and NEFA release from the adipose tissue in obese male subjects.

Acknowledgements
The authors wish to thank Jos Stegen for his support during the analysis of dialysate samples.

References

1 Wolfe RR, Peters EJ, Klein S, Holland OB, Rosenblatt J, Gary HJ. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am J Physiol* 1987; **252**: E189–E196.
2 Connacher AA, Bennet WM, Jung RT *et al*. Effect of adrenaline infusion on fatty acid and glucose turnover in lean and obese human subjects in the post-absorptive and fed states. *Clin Sci* 1991; **81**: 635–644.
3 Blaak EE, van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA, Saris WH. β-Adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. *Am J Physiol* 1994; **267**: E306–E315.
4 Horowitz JF, Klein S. Whole body and abdominal lipolytic sensitivity to epinephrine is suppressed in upper body obese women. *Am J Physiol* 2000; **278**: E1144–E1152.
5 Schiffelers SLH, Saris WHM, Boomsma F, van Baak MA. β_1- and β_2-adrenoceptor-mediated thermogenesis and lipid utilization in obese and lean men. *J Clin Endocrinol Metab* 2001; **86**: 2191–2199.
6 Reynisdottir S, Wahrenberg H, Carlstrom K, Rössner S, Arner P. Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of β_2-adrenoceptors. *Diabetologia* 1994; **37**: 428–435.
7 Engfeldt P, Linde B. Subcutaneous adipose tissue blood flow in the abdominal and femoral regions in obese women: effect of fasting. *Int J Obes Relat Metab Disord* 1992; **16**: 875–879.
8 Blaak EE, van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA, Saris WH. β-Adrenergic stimulation and abdominal subcutaneous fat blood flow in lean, obese, and reduced-obese subjects. *Metabolism* 1995; **44**: 183–187.
9 Jansson PA, Larsson A, Smith U, Lönnroth P. Glycerol production in subcutaneous adipose tissue in lean and obese humans. *J Clin Invest* 1992; **89**: 1610–1617.
10 Summers LK, Samra JS, Humphreys SM, Morris RJ, Frayn KN. Subcutaneous abdominal adipose tissue blood flow: variation within and between subjects and relationship to obesity. *Clin Sci Colch* 1996; **91**: 679–683.
11 Bolinder J, Kerckhoffs DA, Moberg E, Hagstrom-Toft E, Arner P. Rates of skeletal muscle and adipose tissue glycerol release in nonobese and obese subjects. *Diabetes* 2000; **49**: 797–802.
12 Hjemdahl P, Linde B. Influence of circulating NE and Epi on adipose tissue vascular resistance and lipolysis in humans. *Am J Physiol* 1983; **245**: H447-H452.
13 Barbe P, Millet L, Galitzky J, Lafontan M, Berlan M. *In situ* assessment of the role of the β_1-, β_2- and β_3-adrenoceptors in the control of lipolysis and nutritive blood flow in human subcutaneous adipose tissue. *Br J Pharmacol* 1996; **117**: 907–913.
14 Enoksson S, Nordenstrom J, Bolinder J, Arner P. Influence of local blood flow on glycerol levels in human adipose tissue. *Int J Obes Relat Metab Disord* 1995; **19**: 350–354.
15 Millet L, Barbe P, Lafontan M, Berlan M, Galitzky J. Catecholamine effects on lipolysis and blood flow in human abdominal and femoral adipose tissue. *J Appl Physiol* 1998; **85**: 181–188.
16 Enocksson S, Shimizu M, Lönnqvist F, Nordenström J, Arner P. Demonstration of an *in vivo* functional β_3-adrenoceptor in man. *J Clin Invest* 1995; **95**: 2239–2245.
17 Siri WE. The gross composition of the body. *Adv Biol Med Physiol* 1956; **4**: 239–280.
18 Stahle L, Segersvard S, Ungerstedt U. A comparison between three methods for estimation of extracellular concentrations of exogenous and endogenous compounds by microdialysis. *J Pharmacol Methods* 1991; **25**: 41–52.
19 Arner P, Bülow J. Assessment of adipose tissue metabolism in man: comparison of Fick and microdialysis techniques. *Clin Sci* 1993; **85**: 247–256.
20 Borsheim E, Lonnroth P, Knardahl S, Jansson PA. No differences in the lipolytic response to beta-adrenoceptor stimulation *in situ* but a delayed increase in adipose tissue blood flow in moderately obese compared with lean men in the postexercise period. *Metabolism* 2000; **49**: 579–587.
21 Enoksson S, Talbot M, Rife F, Tamborlane WV, Sherwin RS, Caprio S. Impaired *in vivo* stimulation of lipolysis in adipose tissue by selective β_2-adrenergic agonist in obese adolescent girls. *Diabetes* 2000; **49**: 2149–2153.
22 Blaak EE. Gender differences in fat metabolism. *Curr Opin Clin Nutr Metab Care* 2001; **4**: 499–502.
23 Blaak EE, van Baak MA, Saris WHM. Beta-adrenergically stimulated fat oxidation is diminished in middle-aged compared to young subjects. *J Clin Endocrinol Metab* 1999; **84**: 3764–3769.
24 Lönnqvist F, Nyberg B, Wahrenberg H, Arner P. Catecholamine-induced lipolysis in adipose tissue of the elderly. *J Clin Invest* 1990; **85**: 1614–1621.