

## PAPER

# Increased lipolysis in adipose tissue and lipid mobilization to natriuretic peptides during low-calorie diet in obese women

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**OBJECTIVE:** We recently demonstrated that natriuretic peptides (NP) are involved in a pathway inducing lipolysis in human adipose tissue. Atrial NP (ANP) and brain NP (BNP) operate via a cGMP-dependent pathway which does not involve phosphodiesterase-3B inhibition or cAMP. The study was performed to evaluate the effect of ANP on lipid mobilization in obese women and secondly to examine the possible effect of a low-calorie diet (LCD) on the lipolytic response of subcutaneous abdominal fat cells to NP and on the lipid mobilization induced by ANP infusion (1 µg/m<sup>2</sup> min for 60 min).

**SUBJECTS:** Ten obese women from 40.5 ± 3.4 y old were selected for this study. Their body weight was 96.4 ± 5.7 kg and their BMI was 35.3 ± 1.7 kg/m<sup>2</sup>. They received a 2.5–2.9 MJ/day formula diet for 28 days.

**DESIGN:** Before and during the LCD, an adipose tissue biopsy was performed for *in vitro* studies and, moreover, ANP was perfused *i.v.* to evaluate its lipid mobilizing action *in toto* and *in situ* in subcutaneous abdominal adipose tissue (SCAAT) using microdialysis.

**RESULTS:** The lipolytic effects of isoproterenol, ANP, BNP and bromo-cGMP (an analogue of cGMP) on fat cells increased by about 80–100% during LCD. The lipid mobilization during *i.v.* ANP infusion, assessed by plasma non-esterified fatty acids (NEFA) increase was enhanced during the LCD. However, during LCD, ANP infusion induced a biphasic effect on glycerol concentration in plasma and interstitial fluid of SCAAT; a significant increase was observed in glycerol levels during the first 30 min infusion period, followed by a steady decrease. The concentration of glycerol was lower during the post-infusion period than during the baseline period. This effect was stronger in obese subjects submitted to the LCD with a low-carbohydrate composition. Other plasma parameters were weakly increased (noradrenaline) or not modified (insulin, glucose) by ANP infusion and no difference was found before and during LCD treatment.

**CONCLUSION:** The present study shows that NP are powerful lipolytic agents in subcutaneous fat cells and that both isoproterenol- and NP-induced lipolysis increase during LCD, in obese women. These changes seem to be associated with an improvement of the lipolytic pathway at a post-receptor level. Moreover, *i.v.* administration of ANP induced a lipid mobilizing effect which was enhanced by a LCD in these objects.

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## Introduction

The treatment of obesity often includes a hypocaloric diet which promotes lipid mobilization/oxidation as the major

energy source. The hydrolysis of triglyceride stores, which releases non-esterified fatty acids (NEFA) and glycerol from adipose tissue, is a key step in the metabolic process leading to the decrease of fat mass. Catecholamines are powerful regulators of lipid mobilization in humans and are considered to stimulate lipolysis mainly through the β1- and β2-adrenoceptors (AR)<sup>1</sup> and previous data have shown that hypocaloric diets enhance catecholamine-induced lipid mobilization.<sup>2–6</sup>

We recently demonstrated that natriuretic peptides (NP) are involved in a new pathway controlling human adipose

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tissue lipolysis.<sup>7</sup> They operate via a cGMP-dependent pathway which does not involve phosphodiesterase-3B inhibition or cAMP production and atrial natriuretic peptide (ANP) was the most potent compound among the three NPs—ANP, BNP (brain-NP) and CNP (C-type NP). Moreover we have also reported in a previous clinical investigation, that ANP infusion in men promotes various metabolic effects, ie local increase of interstitial glycerol concentration in subcutaneous abdominal adipose tissue (SCAAT), increase in plasma glycerol, and NEFA, noradrenaline and insulin levels which were similar in lean and obese young healthy subjects.<sup>8</sup> A decreased lipolytic response to catecholamines has been reported in obese subjects.<sup>5,9,10</sup> Nevertheless, we have demonstrated that a very low calorie diet (VLCD) increases the *in situ* lipolytic response to isoproterenol and to dobutamine in the subcutaneous adipose tissue of obese subjects.<sup>11</sup>

The aim of the present work was to compare *in vitro*  $\beta$ -adrenergic and NP lipolytic effects on isolated adipocytes and to investigate *in situ* (microdialysis of SCAAT) and *in toto* the lipomobolizing effect of an i.v. infusion of ANP in obese subjects before and during a low-calorie diet (LCD).

## Methods

### Patients

Ten obese women from 22 to 57 y old (mean  $40.5 \pm 3.4$  y) were selected for this study. Their body weight was  $96.4 \pm 5.7$  kg (range 75.6–116.3 kg) and had been stable for at least 3 months before the beginning of the study. Their body mass index (BMI) was  $35.2 \pm 1.7$  kg/m<sup>2</sup> (range 30.2–46.3 kg/m<sup>2</sup>). All the patients received a 2.5–2.9 MJ/day formula diet (Dietifine® Lab. Bio2, Lyon, France) for 28 days. The formula included 92 g protein, 22 g carbohydrate and 16 g fat and the recommended daily allowance of vitamins and minerals. The subjects were in-patients throughout the duration of the diet and their usual medication was maintained. All subjects had given their informed consent before the study and the investigation protocol was approved by the Ethical Committee of Prague University Hospital.

### Experimental protocol

The subjects were investigated at 8 am after an overnight fast and were maintained in the supine position during the experimental period. An indwelling polyethylene catheter was inserted into the antecubital vein of each arm. At 8.30 am, a needle micro-biopsy (200–400 mg) of adipose tissue was performed under local anesthesia 10–15 cm from the umbilicus. The ANP was infused through the i.v. catheter placed in the right arm using an Auto-Syringe infusion pump. Blood samples were withdrawn from a catheter placed in the left arm. Resting baseline measurements were performed during the first 60 min. Immediately after the baseline period, ANP was administered with isotonic saline as vehicle by an infusion at a constant rate of

1  $\mu$ g/m<sup>2</sup>/min for 60 min. The total volume infused was < 40 ml. Appropriate doses of ANP were selected on the basis of changes in metabolic and cardiovascular parameters previously determined by us<sup>8</sup> and other groups.<sup>11–14</sup> During the baseline period and ANP infusion, the heart rate was continuously recorded using a standard three-lead ECG. Systolic and diastolic blood pressures were evaluated every 15 min using a Dynamap device.

Two microdialysis probes (Carnegie Medecin, Stockholm, Sweden) of 20×0.5 mm and 20 000 MW cut-off were inserted percutaneously after epidermal anesthesia (200  $\mu$ l of 1% lidocaine, Roger-Bellon, Neuilly-s-Seine, France) into the abdominal SCAAT at a distance of 10 cm to the right of the umbilicus and separated by at least 10 cm. The probes were connected to a microinjection pump (Harvard Apparatus, Les Ulis, France) and perfused with Ringer's solution (139 mmol/l sodium, 2.7 mmol/l potassium, 0.9 mmol/l calcium, 140.5 mmol/l chloride, 2.4 mmol/l bicarbonate, 5.6 mmol/l glucose). One probe was perfused with Ringer supplemented with 0.1 mmol/l propranolol ( $\beta$ -adrenergic receptor antagonist). The perfusate solutions were supplemented with ethanol (1.7 g/l). Ethanol was added to the perfusate in order to estimate changes in the blood flow, as previously described.<sup>15</sup> After a 30 min equilibration period, a 30 min fraction of dialysate was collected at a flow rate of 0.5  $\mu$ l/min. Then, the infusion was set at 2.5  $\mu$ l/min for the remaining experimental period. The calibration procedure using various infusion rates was applied for interstitial glycerol concentration determination in SCAAT as previously described by our group.<sup>11,15</sup> A simplified, but relevant and less time-consuming method was selected in this study. The estimated interstitial glycerol concentrations were calculated by plotting (after log-transformation) the concentration of glycerol in the dialysate measured at 0.5 and 2.5  $\mu$ l/min against the infusion rates. After the calibration of the probes, two 15 min fractions of the outgoing dialysate were collected and then ANP was infused i.v. for 60 min. Dialysate samples were collected for each 15 min period during infusion and during the 60 min post-infusion period. Water intake was allowed *ad libitum* during the experimental periods.

### Adipocyte preparation and lipolysis measurements

Isolated adipocytes were obtained by collagenase digestion of adipose tissue fragments in Krebs Ringer bicarbonate–Hepes buffer containing albumin (3.5 g/100 ml; KRBA) and glucose (5.6 mmol/l) at pH 7.4 and under gentle shaking at around 60 cycles/min at 37°C.<sup>7</sup> Then, the fat cells were filtered through a silk screen and washed three times with KRBA buffer to eliminate collagenase, isolated adipocytes were brought to a suitable dilution (1000–2000 cells/assay) in KRBA buffer for lipolysis assays and incubated with pharmacological agents in a final volume of 100  $\mu$ l for 90 min at 37°C as previously described.<sup>7</sup> At the end of the incubation 20–50  $\mu$ l aliquots of the infranatant were taken for glycerol

assay to determine the lipolytic index. Concentration–response assays were performed using isoproterenol (non-selective  $\beta$ -AR agonist), ANP, BNP and bromo-cGMP (an analogue of cGMP) was used at the concentration of 10 mmol/l.

### Drugs and biochemical determinations

The following reagents were used for fat cell isolation and lipolysis measurement: bovine serum albumin; fraction V, fatty-acid free; collagenase from *Clostridium histolyticum*; bromo-cGMP (Boehringer-Mannheim, Meylan, France); isoprenaline hydrochloride (Sigma, Saint Quentin Fallavier, France); human synthetic ANP (Neosystem, Strasbourg, France). ANP Clinalfa for biomedical and clinical research perfused in subjects came from France Biochem, Meudon, France. Plasma ANP concentrations were determined using a RIA kit from Phoenix Pharmaceuticals, Inc (Belmont, USA). Plasma noradrenaline and adrenaline were assayed by high performance liquid chromatography using electrochemical (amperometric) detection, as previously described.<sup>8</sup> Ethanol in dialysate and perfusate (5  $\mu$ l) was determined using an enzymatic method.<sup>16</sup> Glycerol was determined in plasma and in dialysate using an ultrasensitive radiometric method.<sup>17</sup> Plasma glucose was assayed with a glucose oxidase technique (Biotrol, Paris, France). NEFA were assayed with an enzymatic method (Unipath, Dardilly, France). Plasma insulin was measured using a Bi-insulin IRMA kit from Sanofi Diagnostics Pasteur (Marnes-La-Coquette, France).

### Statistical analysis

All the values are means  $\pm$  s.e.m. Flow-rate calibration data were analysed by linear regression, tested for goodness of fit. Statistical comparison of *in vitro* data on isolated fat cells before and during LCD was performed using two-way Anova analysis for repeated measures. During ANP infusion, plasma and interstitial response curves were calculated as the total integrated changes over baseline values (area under the curves, AUC) using the trapezoidal method. Statistical comparison of the effect of LCD was done on AUC using Student's paired *t*-test. The effects of ANP were analysed by one-way ANOVA with time as the factor of the analysis followed by a Bonferroni–Dunnett *post-hoc* test, taking basal values as control.  $P < 0.05$  was considered statistically significant.

## Results

### Changes induced by LCD

The changes in body weight and in various biological parameters observed on the 28th day of LCD for the whole population are depicted in Table 1. LCD induced a weight loss and a significant reduction of fasting plasma insulin levels. Plasma NEFA, glucose and noradrenaline concentrations were unchanged. Plasma and interstitial glycerol levels

**Table 1** Morphological, cardiovascular and biological characteristics of obese women before and after 28 days of LCD

	Before the diet	During the diet	
Weight (kg)	96.4 $\pm$ 5.7	91.2 $\pm$ 5.3	$P = 0.0001$
Body mass index (kg/m <sup>2</sup> )	35.2 $\pm$ 1.7	33.2 $\pm$ 1.6	$P = 0.004$
Percentage fat mass	34.7 $\pm$ 0.7	32.9 $\pm$ 0.5	$P = 0.001$
Waist circumference (cm)	103.6 $\pm$ 4	97.8 $\pm$ 3.2	$P = 0.001$
Systolic blood pressure (mmHg)	127 $\pm$ 4	118 $\pm$ 4	$P = 0.05$
Diastolic blood pressure (mmHg)	82 $\pm$ 3	73 $\pm$ 4	$P = 0.03$
Heart rate (beats/min)	66 $\pm$ 2	59 $\pm$ 2	$P = 0.01$
Plasma glucose (mmol/l)	4.55 $\pm$ 0.24	4.17 $\pm$ 0.20	NS
Plasma insulin ( $\mu$ U/ml)	6.45 $\pm$ 0.92	4.33 $\pm$ 1.08	$P = 0.006$
Noradrenaline (pg/ml)	196 $\pm$ 29	164 $\pm$ 29	NS
Plasma NEFA ( $\mu$ mol/l)	633 $\pm$ 52	668 $\pm$ 89	NS
Plasma glycerol ( $\mu$ mol/l)	132 $\pm$ 16	109 $\pm$ 14	NS

Data are the mean  $\pm$  s.e.m.

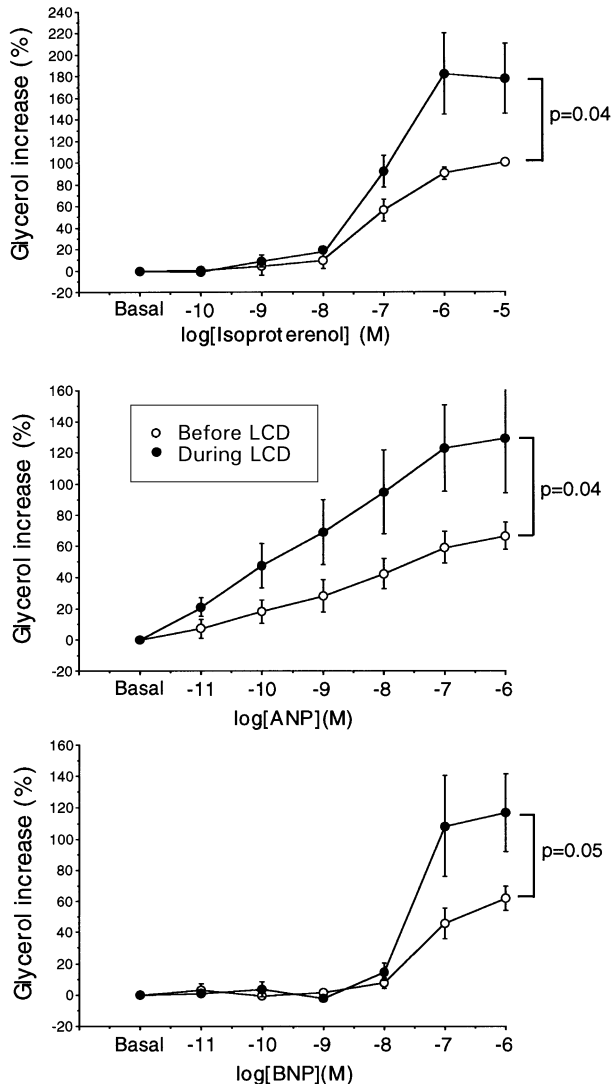
tended to be lower during LCD, but did not reach a significant difference.

### *In vitro* lipolysis of isolated fat cells

Spontaneous glycerol release (basal lipolysis) was found to be significantly increased by the LCD; values expressed in  $\mu$ mol glycerol released by 100 mg lipid for 90 min were  $0.052 \pm 0.08$  and  $0.073 \pm 0.02$  before and during LCD, respectively. The lipolytic effects of ANP, BNP and bromo-cGMP were compared to those of isoproterenol ( $\beta$ -AR agonist). The maximal lipolytic effect of isoproterenol measured before the LCD was taken as a reference for the lipolytic effect induced by all drugs before and during the LCD. The results depicted in Figure 1 show that before LCD, maximal ANP- or BNP-induced lipolysis was 60% of the isoproterenol effect and that the lipolytic effect of bromo-cGMP was 40%. The LCD induced an 80–90% increase of the maximal lipolytic effect of isoproterenol. It was also found that the LCD induced a significant increase of about 100–110% of the maximal lipolytic response initiated by ANP and BNP. Before LCD, in the presence of bromo-cGMP, glycerol production was  $0.152 \pm 0.026$   $\mu$ mol/100 mg lipid/90 min and  $0.224 \pm 0.028$  during LCD ( $P = 0.04$ ). The LCD did not modify the potency of isoproterenol or NP: calculated pD<sub>2</sub> ( $-\log EC_{50}$ ) for isoproterenol, ANP and BNP effect were similar before and during LCD ( $6.93 \pm 0.15$  vs  $7.17 \pm 0.15$ , for isoproterenol,  $8.73 \pm 0.37$  vs  $9.02 \pm 0.30$ , for ANP and  $7.41 \pm 0.11$  vs  $7.33 \pm 0.14$  for BNP).

### Plasma metabolite concentrations

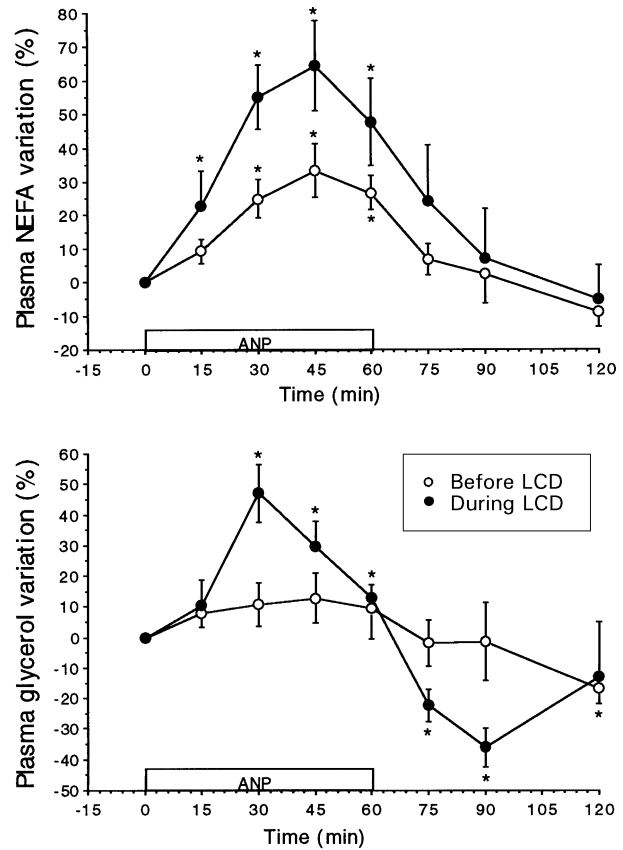
During the baseline period, plasma concentrations of ANP were similar before and during LCD ( $68.5 \pm 9.1$  vs  $87.5 \pm 14.3$  pg/ml, respectively). During the first 30 min of ANP infusion plasma ANP concentrations were similar before and during LCD ( $641.4 \pm 52.3$  vs  $646.1 \pm 31.6$  pg/ml) and



**Figure 1** Lipolytic effects of isoproterenol, ANP and BNP in abdominal subcutaneous isolated fat cells before and during LCD. Lipolysis expressed in percentage of maximal glycerol production induced by isoproterenol, before LCD (100%). Data are expressed as means  $\pm$  s.e.m. of experiments carried out on adipose tissue.

were also identical at the end of the 60 min infusion ( $658.8 \pm 42.6$  vs  $659.6 \pm 27.9$  pg/ml).

Both before and during LCD, plasma NEFA and glycerol concentrations increased after 15 min of ANP infusion and reached a maximum value at  $t=30$  min (Figure 2). Thirty minutes after stopping the infusion, plasma NEFA concentrations returned to basal values. The calculated AUC for the plasma NEFA values during the ANP infusion was different before and during LCD ( $P=0.004$ ). The time-course of changes in plasma glycerol levels differed strikingly from that of NEFA levels. Before LCD, it was observed that the ANP-induced effect was weak and did not reach a significant



**Figure 2** Changes in plasma non-esterified fatty acids (NEFA) and glycerol concentrations during the i.v. infusion of ANP in obese women before and during LCD. Values are expressed as percentage of pre-infusion values. Baseline values ( $\mu\text{mol/l}$ ) were  $633 \pm 52$  and  $668 \pm 89$  for NEFA and  $132 \pm 16$  and  $109 \pm 14$  for glycerol before and during LCD, respectively. Data are expressed as means  $\pm$  s.e.m. \* $P < 0.05$  when compared to pre-infusion values.

level whatever the infusion period. During LCD, a significant rise in plasma glycerol levels was observed, the maximal level being reached 30 min after the beginning of the infusion, then a steady decrease in plasma glycerol concentrations was noticed until the end of infusion (Figure 2). Finally, at cessation of infusion the plasma concentration of glycerol was significantly lower than during the baseline period. However, the calculated AUC for the plasma glycerol values during the ANP infusion were different before and during LCD ( $P=0.02$ ).

Other plasma metabolite concentrations are depicted in Table 2. No significant variations of plasma glucose level were observed in either period during the ANP infusion. A weak but non-significant increase in plasma insulin was observed during ANP infusion. The calculated AUC for insulin variations in the plasma during the ANP infusion did not show significant differences before or during LCD. ANP infusion also induced a slight increase in plasma

**Table 2** Effect of ANP infusion on plasma insulin, glucose, lactate and noradrenaline concentrations before and during LCD

	Basal	ANP infusion		Post ANP infusion		
		30 min	60 min	90 min	120 min	
<b>Plasma Insulin (<math>\mu\text{U}/\text{ml}</math>)</b>						
Before LCD	6.45 $\pm$ 0.92	7.05 $\pm$ 1.70	7.68 $\pm$ 1.88	—	—	NS
During LCD	4.33 $\pm$ 1.08	4.83 $\pm$ 0.87	5.39 $\pm$ 1.09*	—	—	
<b>Plasma glucose (mmol/l)</b>						
Before LCD	4.5 $\pm$ 0.2	4.6 $\pm$ 0.2	4.6 $\pm$ 0.2	4.4 $\pm$ 0.1	4.6 $\pm$ 0.2	NS
During LCD	4.2 $\pm$ 0.2	4.3 $\pm$ 0.1	4.2 $\pm$ 0.1	4.3 $\pm$ 0.1	4.8 $\pm$ 0.3	
<b>Plasma lactate (mmol/l)</b>						
Before LCD	0.75 $\pm$ 0.05	0.66 $\pm$ 0.03*	0.71 $\pm$ 0.02	0.65 $\pm$ 0.03*	0.64 $\pm$ 0.02*	$P < 0.05$
During LCD	0.66 $\pm$ 0.06	0.55 $\pm$ 0.02*	0.55 $\pm$ 0.03*	0.49 $\pm$ 0.03*	0.49 $\pm$ 0.03*	
<b>Plasma noradrenaline (pg/ml)</b>						
Before LCD	196 $\pm$ 29	253 $\pm$ 27	204 $\pm$ 28	—	—	NS
During LCD	164 $\pm$ 29	206 $\pm$ 29*	187 $\pm$ 25	—	—	

Values are means  $\pm$  s.e.m. A statistical comparison of the values was first performed by two-way ANOVA for repeated measures with diet (before and during LCD) and time as factors of the analysis. Subsequently, the effects of ANP were analysed before and during LCD by one-way ANOVA with time as the factor of the analysis followed by a Bonferroni-Dunnett *post hoc* test, taking basal values as control.

\* $P < 0.05$  when compared to corresponding basal values measured before ANP infusion.

noradrenaline levels; this effect was not significant whatever the infusion period and no difference was observed when compared to the values measured before or during LCD. Plasma lactate concentrations were decreased during the first 30 min of ANP and during the post-infusion period. The decrease was significantly higher during LCD than before.

#### Glycerol concentrations in SCAAT and ethanol changes in the dialysate

In basal conditions, the interstitial glycerol concentration in subcutaneous abdominal adipose tissue was similar in the control probe and in the probe perfused with propranolol, before LCD (180  $\pm$  7 vs 169  $\pm$  16  $\mu\text{mol}/\text{l}$ ) or during the LCD (197  $\pm$  20 vs 182  $\pm$  26  $\mu\text{mol}/\text{l}$ ). In both dialysis probes and before LCD, infusion of ANP induced a weak increase in interstitial glycerol concentrations, the maximal effect being reached after 30 min (Figure 3). Thirty minutes after stopping the infusion, interstitial glycerol concentration returned to basal values. As found for plasma glycerol, it was observed that, during LCD, the glycerol increase was significantly higher than before LCD in the control probe as well as in the probe with propranolol during the first 30 min of ANP infusion. Then, a progressive decrease in glycerol concentrations was observed during the end of the infusion period. Finally during the post-infusion period, the interstitial levels of glycerol decreased to values significantly lower than those determined during the baseline period. The AUC calculated for the interstitial glycerol concentrations during the ANP infusion period was not different before or during LCD in either probe ( $P = 0.15$  for the control probe  $P = 0.06$  for the probe perfused with propranolol).

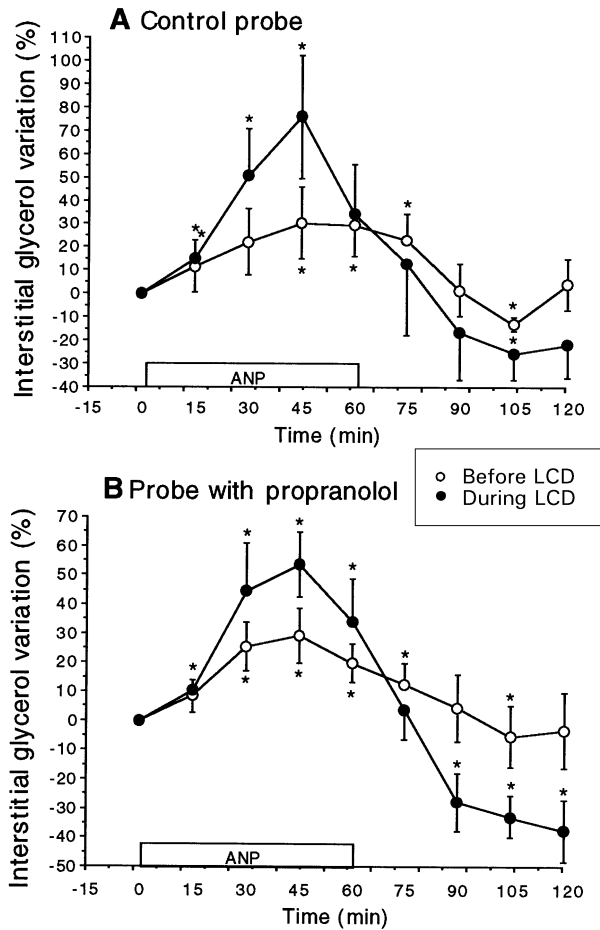
Adipose tissue blood flow was assessed using ethanol outflow/inflow ratios (ethanol concentration measured in the dialysate divided by the ethanol concentration measured in the perfusate  $\times 100$ ) from the probes and the results are depicted in Figure 4. Before and during LCD, the ethanol outflow/inflow ratio was similar in the control probe (79  $\pm$  4 vs 81  $\pm$  2, respectively) and in the probe perfused with propranolol (84  $\pm$  3 vs 83  $\pm$  3, respectively). Before LCD, a significant decrease of the ethanol outflow/inflow ratio was observed starting from  $t = 30$  min of ANP infusion in the two probes. During LCD, the decrease in ethanol ration started from  $t = 15$  min in both probes. The AUC calculated for ANP-induced vasodilation were different when comparing the corresponding AUC before and during LCD in the control probe ( $P = 0.03$ ) and in the probe perfused with propranolol ( $P = 0.05$ ).

#### Cardiovascular responses to the ANP infusion

The LCD induced a significant decrease in heart rate, systolic and diastolic blood pressure (Table 1). ANP infusion induced a discrete increase in heart rate, this effect being unchanged before and during LCD. In both situations, systolic and diastolic blood pressure were unchanged by ANP infusion (not shown).

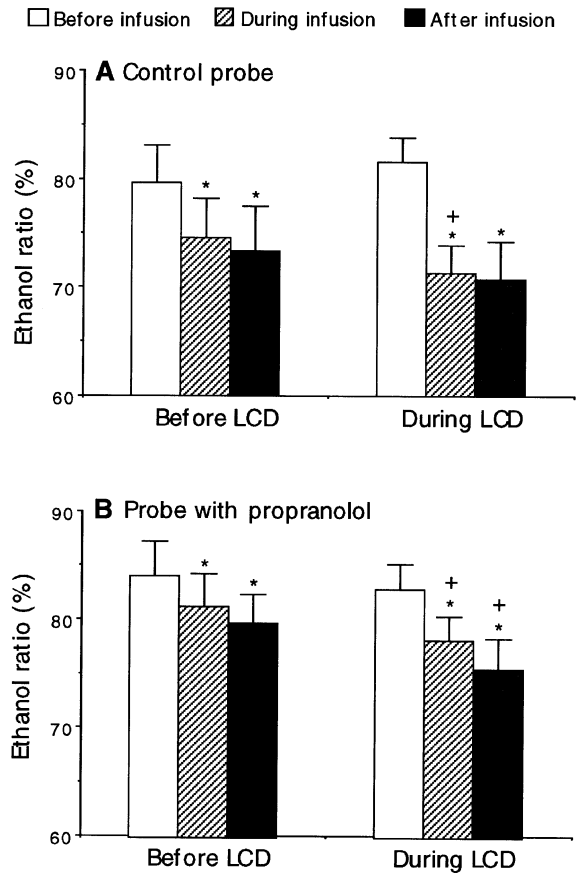
#### Discussion

The present investigation shows that NP induces a lipolytic effect in subcutaneous fat cells and ANP increases lipid mobilization in obese women. However, hypocaloric diet promoted both an increase of NP-induced lipolysis in fat cells and of ANP-mediated lipid mobilization.



**Figure 3** Changes in interstitial glycerol in subcutaneous adipose tissue during the i.v. infusion of ANP in obese women before and during LCD. One probe (A) was perfused with Ringer alone and the other (B) with Ringer plus 100  $\mu\text{mol/l}$  propranolol. Before LCD, baseline values ( $\mu\text{mol/l}$ ) were  $181 \pm 21$  and  $169 \pm 16$   $\mu\text{mol/l}$  in the control probe and in the probe with propranolol, respectively. During LCD, corresponding baseline values were  $198 \pm 20$  and  $182 \pm 26$   $\mu\text{mol/l}$  respectively. Data are expressed as means  $\pm$  s.e.m. \* $P < 0.05$  when compared to preinfusion values.

*In vitro*, isolated adipocytes spontaneously release more glycerol during hypocaloric diets, as shown previously.<sup>3,6</sup> However, our data showed that fasting plasma glycerol and NEFA concentrations did not change during the hypocaloric diet. The calculated interstitial levels of glycerol in the subcutaneous adipose tissue were also found to be quite similar before and after 28 days of LCD. In agreement with values previously reported by Jansson *et al*<sup>18</sup> in obese patients (with a different calibration method, ie no-net flux method), by us<sup>19</sup> and others<sup>18,20</sup> using the present method, the interstitial levels of glycerol were about 1.7-fold higher than in the plasma. The values were also similar to those found in the venous blood draining the SCAAT.<sup>21</sup>



**Figure 4** Changes in ethanol ratio (ethanol dialysate concentration/ethanol perfusate level  $\times 100$ ) in subcutaneous adipose tissue during the i.v. infusion of ANP in obese women before and during LCD. One probe (A) was perfused with Ringer alone and the other (B) with Ringer plus 100  $\mu\text{mol/l}$  propranolol. After a calibration period, 2.5  $\mu\text{l}$  min flow rate was maintained and dialysate fractions were collected at 15 min intervals. Data are expressed as means  $\pm$  s.e.m. \* $P < 0.05$  when compared to preinfusion values. + $P < 0.05$  when compared to corresponding values measured before LCD.

Animal studies have shown that fasting or energy restriction increase the lipolytic response promoted by catecholamines.<sup>2,22</sup> In humans, conflicting results have been reported. Spontaneous lipolysis is currently found to be enhanced by energy restriction but lipolytic responses to catecholamines have been shown to be increased,<sup>2,3,6</sup> unmodified or diminished in isolated subcutaneous fat cells from obese subjects during fasting<sup>23</sup> or after a 15-day LCD (3.3 MJ/day).<sup>24</sup> This is also in contrast with *in vivo* studies where an increased responsiveness of adipose tissue to catecholamine infusion has been demonstrated after short-term fasting.<sup>4,5</sup> A VLCD (2 MJ/day, for 4 weeks) led to an increased lipolytic response to exercise in obese subjects.<sup>25</sup> We have found that a VLCD in obese subjects improved the *in situ* lipolytic response to isoproterenol.<sup>11</sup> These discrepancies between *in vivo*, *in vitro* and *in situ* studies could reflect regional differences in the

adaptative mechanisms in adipose tissue depending how few calories were in the diet and the sex of the patients. However, our results clearly show that, in women, the  $\beta$ -adrenergic lipolytic pathway was largely increased and that, similarly, ANP- and BNP-induced lipolysis were also significantly increased during the LCD. NP represent a new pathway controlling human adipose tissue lipolysis; they operate via a cGMP-dependent mechanism which does not involve phosphodiesterase-3B inhibition or cAMP production.<sup>7</sup> It was shown in the present study that bromo-cGMP induced a higher lipolytic effect during LCD than before. A previous study carried out in women submitted to a VLCD showed that the lipolytic effect of dibutyryl-cAMP (an analog of cAMP) was also increased.<sup>6</sup> Several mechanisms have been proposed to explain the increased  $\beta$ -adrenergic sensitivity of adipose tissue after VLCD including up-regulation of  $\beta$ -AR and/or an increase in the efficiency of the coupling between  $\beta$ -AR and adenylyl cyclase and an increased expression of hormone-sensitive lipase (HSL). Previous data have shown that the increase in HSL activity during VLCD is of the same order as the increase of basal lipolysis and that an increase of protein content paralleled the increase of total activity.<sup>6</sup> In addition, unpublished data from our group have shown that the HSL is phosphorylated and activated by ANP stimulation in human fat cells. Taken together, these results suggest that the increase of both isoproterenol and NP lipolytic effects could be related to an increased expression and/or activity of the HSL induced by the LCD. The parallel improvement of both pathways suggests that the same pool of HSL is probably affected by cGMP and cAMP-dependent stimulation's.

A plasma NEFA concentration increase initiated by ANP infusion was observed before LCD and markedly raised during LCD (Figure 1). These results fit with the *in vitro* effects. Since the potency (pD<sub>2</sub> values) of ANP is not changed during LCD, the enhancement of the lipid mobilizing action of ANP, based on the plasma NEFA levels, is probably a reflection of the increased HSL activity initiated by the LCD. It is not related to an increased sensitivity of the ANP lipolytic pathway *per se*. Concerning glycerol, before LCD, ANP infusion had a weak effect on plasma or interstitial glycerol concentration and LCD promoted a significant increase in plasma and interstitial glycerol levels. The *in vivo* responsiveness to ANP infusion, assessed through the rates of appearance in the circulation of NEFA and glycerol, exhibited different profiles, particularly during the LCD. The first period of glycerol increase (lasting 30 min) was followed by a rapid and sustained decrease of its level in the plasma and in the interstitial compartment too. The post-infusion plasma levels of glycerol were found to be lower than those measured before infusion. The change in local blood flow, known to influence the concentration of the interstitial metabolites, is an important variable in microdialysis studies.<sup>26,27</sup> The ANP-mediated lipolytic response was associated with vasodilatation, which seems to be enhanced during the diet (Figure 4). Thus, the effect on local blood flow could partly mask the action of ANP on lipolysis in SCAAT since an

increment of glycerol drainage in the circulation was promoted simultaneously with the lipolytic action.

A theoretical explanation of the decrease of plasma glycerol levels can be proposed. During fasting or LCD, there may be a rapid removal of glycerol from the circulation and its re-utilization mainly by the liver and to a lesser extent by the kidney.<sup>28,29</sup> In these situations it has been shown that plasma glycerol is almost completely converted into glucose.<sup>29</sup> A higher proportion of glycerol and protein converted into glucose has been reported in obese subjects during fasting periods<sup>29</sup> and the ability of obese individuals to spare protein breakdown during acute starvation has previously been reported.<sup>30</sup> To interpret the evolution of the plasma glycerol concentration, during and after ANP infusion, it can be proposed that when lipolysis is increased under ANP infusion, a rapid uptake of glycerol for neoglucogenesis occurring in the liver contributes to the decrease in its plasma level. This effect is stronger in obese subjects submitted to LCD containing a high proportion of protein. Likewise, this hypothesis is supported by the decrease in plasma lactate concentration also observed after ANP infusion and particularly during LCD. Lactate also converted into glucose mainly by the liver.

It cannot be excluded that a part of the lipid mobilizing effect promoted by ANP is related to sympathetic nervous system activation. In fact, at the dose used, the increase in plasma noradrenaline is very weak. The present study suggests that this effect is mainly related to the direct action of ANP on fat cells because propranolol (added at high concentrations to the microdialysis perfusate) did not modify the increase in interstitial glycerol promoted by ANP infusion (Figure 3). It has been shown that at these concentrations, propranolol had no effect on basal glycerol release<sup>8,31</sup> but could blunt the lipolytic effect of noradrenaline or adrenaline infused in the microdialysis probe.<sup>32</sup>

The decrease of plasma insulin levels, described during LCD treatment,<sup>33,34</sup> could also be an important factor for the regulation of lipolytic responsiveness. The improvement of the lipolytic response to  $\beta$ -AR stimulation and to ANP could be related to the modification of insulin status particularly in subjects with an insulin resistance syndrome. In fact, we have demonstrated that the lipolytic effect of ANP on human fat cells is independent of the level of insulin.<sup>7</sup> The subjects included in the present study did not have abnormal fasting plasma insulin levels. ANP infusion moderately increased the plasma insulin level but the effect was only significant during the LCD. A significant increase of plasma insulin level has previously been noted when a five-fold higher dose of ANP than that used in the present study was infused.<sup>13</sup> Although ANP binding sites are present in rat pancreatic islets, no direct insulinotropic effect of ANP has been clearly established in pancreatic islets. In fact, ANP was able to augment cGMP levels in isolated pancreatic islets without affecting insulin secretion at various glucose concentrations.<sup>35</sup> A weak effect, dependent on the secretagogues used has been reported when working on isolated perfused

rat pancreas.<sup>36</sup> Therefore, although studies do not exist in humans, it is probable that *in vivo* elevation of plasma insulin levels in response to ANP are an indirect rather than a direct effect and represent a minor contribution to the lipid mobilizing responses reported in the present study.

Several epidemiological and clinical studies have shown an association of obesity and hypertension. It has recently been shown that short-term caloric restriction enhances ANP-induced diuresis and decreases arterial blood pressure.<sup>14</sup> Previous studies in animals help to interpret these findings and our results. Rat and human adipose tissue contain high levels of NPR messenger RNA<sup>37,38</sup> and large numbers of clearance receptor binding sites (NPr-C). A dramatic reduction of the expression of NPr-C has been reported during fasting.<sup>39</sup> This specific reduction of NPr-C gene expression in adipose tissue appeared to be accompanied by an increased biological activity of ANP. Our results are also consistent with the hypothesis that the reduction of NPr-C leads to reduced uptake of ANP allowing improved binding to biologically active NPR-A or NPR-B receptors in fat cells and higher efficiency on lipolysis during LCD.

## Conclusion

In conclusion, hypocaloric diet concomitantly promotes increased  $\beta$ -AR- and NP-induced lipolysis in fat cells and an increased lipid mobilizing effect of ANP in obese women. These changes seem to be associated to an improvement of the lipolytic pathway at the post-receptor level. Secondly, the results suggest that ANP induces an increase of glycerol metabolism in tissues other than adipose tissue during LCD.

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## References

- 1 Lafontan M, Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. *J Lipid Res* 1993; **34**: 1057–1091.
- 2 Dax E, Partilla JS, Gregerman RJ. Increased sensitivity to epinephrine-stimulated lipolysis during starvation: higher coupling of the adenylate cyclase complex. *Biochem Biophys Res Commun* 1981; **101**: 1186–1192.
- 3 Berlan M, Dang-Tran L, Lafontan M, Denard Y. Influence of hypocaloric diet on alpha-adrenergic responsiveness of obese human subcutaneous adipocytes. *Int J Obes* 1981; **5**: 145–153.

- 4 Amer P, Engfeldt P, Nowak J. In vivo observation on the lipolytic effect of noradrenaline during therapeutic fasting. *J Clin Endocr Metab* 1981; **53**: 1207–1212.
- 5 Jensen MD, Haymond MW, Gerich JE, Cryer PE, Miles JM. Lipolysis during fasting: decreased suppression by insulin and increased stimulation by epinephrine. *J Clin Invest* 1987; **87**: 207–213.
- 6 Stich V, Harant I, De Glisezinski I, Crampes F, Berlan M, Kunesova M, Hainer V, Dazats M, Riviere D, Garrigues M, Holm C, Lafontan M, Langin D. Adipose tissue lipolysis and hormone-sensitive lipase expression during very-low-calorie diet in obese female identical twins. *J Clin Endocr Metab* 1997; **82**: 63–69.
- 7 Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000; **14**: 1345–1351.
- 8 Galitzky J, Sengenès C, Thalamos C, Marques MA, Senard JM, Lafontan M, Berlan M. The lipid mobilizing effect of natriuretic peptides is unrelated to sympathetic nervous system activation or obesity in young men. *J Lipid Res* 2001; **42**: 536–544.
- 9 Wolfe RR, Peter EJ, Klein S, Holland OB, Rosenblatt J, Gary H. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am J Physiol* 1987; **252**: E189–E196.
- 10 Reynisdottir S, Langin D, Carlström K, Holm C, Rössner S, Arner P. Effects of weight reduction on the regulation of lipolysis in adipocytes of women with upper-body obesity. *Clin Sci* 1995; **89**: 421–429.
- 11 Barbe P, Stich V, Galitzky J, Kunesova M, Hainer V, Lafontan M, Berlan M. In vivo increase in  $\beta$ -adrenergic lipolytic response in subcutaneous adipose tissue of obese subjects submitted to a hypocaloric diet. *J Clin Endocr Metab* 1997; **82**: 63–69.
- 12 Weidmann P, Gnädinger MP, Ziswiler HR, Shaw S, Bachmann C, Rascher W, Uehlinger DE, Hasler L, Reubi FC. Cardiovascular, endocrine and renal effects of atrial natriuretic peptide in essential hypertension. *J Hypertension* 1986; **4**: S71–S83.
- 13 Uehlinger DE, Weidmann P, Gnädinger MP, Hasler L, Bachmann C, Shaw S, Hellmüller B, Lang RE. Increase in circulating insulin induced by atrial natriuretic peptide in normal humans. *J Cardiovasc Pharmacol* 1986; **8**: 1122–1129.
- 14 Dessi-Fulgheri P, Sarzani R, Serenelli M, Tamburrini P, Spagnolo D, Giantomassi L, Espinosa E, Rappelli A. Low calorie diet enhances renal, hemodynamic, and humoral effects of exogenous atrial natriuretic peptide in obese hypertensives. *Hypertension* 1999; **33**: 658–662.
- 15 Stich V, De Glisezinski I, Crampes F, Hejnova J, Cottet-Emard JM, Galitzky J, Lafontan M, Riviere D, Berlan M. Activation of  $\alpha$ 2-adrenergic receptors impairs exercise-induced lipolysis in subcutaneous adipose tissue of obese subjects. *Am J Physiol* 2000; **279**: R499–R504.
- 16 Bernst E, Gutman I. Determination of ethanol with alcohol dehydrogenase and NAD. In: Bergmeyer HU (ed). *Methods of enzymatic analysis*, Vol 3. Springer: Weinheim; 1974. pp 1499–1505.
- 17 Bradley DC, Kaslow HR. Radiometric assays for glycerol, glucose and glycogen. *Anal Biochem* 1989; **180**: 11–16.
- 18 Jansson P-A, Larsson A, Smith U, Lönnroth P. Glycerol production in subcutaneous adipose tissue of lean and obese humans. *J Clin Invest* 1992; **89**: 1610–1617.
- 19 Barbe P, Millet L, Galitzky J, Lafontan M, Berlan M. In situ assessment of the role of  $\beta$ 1-,  $\beta$ 2-,  $\beta$ 3-adrenoceptors in the control of lipolysis and nutritive blood flow in human subcutaneous adipose tissue. *Br J Pharmacol* 1996; **117**: 907–913.
- 20 Jansson P-A, Smith U, Lönnroth P. Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. *Am J Physiol* 1990; **258**: E918–E922.
- 21 Frayn KN, Shadid S, Hamlani R, Humphreys SM, Clark ML, Fielding BA, Boland O, Coppack SW. Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 1994; **266**: E308–E317.

- 22 Zapf J, Waldvogel M, Froesch ER. Increased sensitivity of rat adipose tissue to the lipolytic action of epinephrine during fasting and its reversal during refeeding. *FEBS Lett.* 1977; **72**: 135–138.
- 23 Kather H, Wieland E, Fisher B, Wirth A, Schlierf G. Adrenergic regulation of lipolysis in abdominal adipocytes of obese subjects during caloric restriction: reversal of catecholamine action caused by relief of endogenous inhibition. *Eur J Clin Invest* 1985; **15**: 30–37.
- 24 Crampes F, Marceron M, Beauville M, Rivière D, Garrigues M, Berlan M, Lafontan M. Platelet alpha2-adrenoceptors and adrenergic adipose tissue responsiveness after moderate hypocaloric diet in obese subjects. *Int J Obes* 1989; **13**: 99–110.
- 25 Kempen KPG, Saris WHM, Senden JMG, Menheere PP, Blaak EE, Baak MAV. Effects of energy restriction on acute adrenoceptor and metabolic response to exercise in obese subjects. *Am J Physiol* 1994; **267**: E694–E701.
- 26 Hickner RC, Rosdahl H, Borg I, Ungerstedt U, Jorfeldt L, Henriksen J. Ethanol may be used with the microdialysis technique to monitor blood flow changes in skeletal muscle: dialysate glucose concentration is blood-flow dependent. *Acta Physiol Scand* 1991; **143**: 355–356.
- 27 Enocksson S, Nordenström 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.
- 28 Landau BR, Wahren J, Previs SF, Ekberg K, Chandramouli V, Brunengraber H. Glycerol production and utilization in humans: sites and quantitation. *Am J Physiol* 1996; **271**: E1110–E1117.
- 29 Bortz WM, Paul P, Haff AC, Holmes WL. Glycerol turnover and oxidation in man. *J Clin Invest* 1972; **51**: 1537–1546.
- 30 Kekwick A, Pawan GLS, Chalmers TM. Resistance to ketosis in obese subjects. *Lancet* 1959; **ii**: 1157–1159.
- 31 Arner P, Kriegholm E, Engfeldt P, Bolinder J. Adrenergic regulation of lipolysis *in situ* at rest and during exercise. *J Clin Invest* 1990; **85**: 893–898.
- 32 Arner PE, Kriegholm E, Engfeldt P. In vivo interactions between beta-1 and beta-2 adrenoceptors regulate catecholamine tachyphylaxia in human adipose tissue. *J Pharmac Exp Ther* 1991; **259**: 317–322.
- 33 Di Biase G, Mattioli PL, Contaldo F, Mancini MA. A very-low-calorie formula diet (Cambridge diet) for the treatment of diabetic/obese patients. *Int J Obes* 1981; **5**: 319–324.
- 34 Atkinson RL, Kaiser DL. Effects of calorie restriction and weight loss on glucose and insulin levels in obese humans. *J Am Coll Nutr* 1985; **4**: 411–419.
- 35 Verspohl EJ, Ammon HP. Atrial natriuretic peptide (ANP) acts via specific binding sites on cGMP system of rat pancreatic islets without affecting insulin release. *Naunyn Schmied Arch Pharmacol* 1989; **339**: 348–353.
- 36 Fehmann HC, Noll B, Goke R, Goke B, Trautmann ME, Arnold R. Atrial natriuretic factor has a weak insulinotropic action in the isolated perfused rat pancreas. *Res Exp Med* 1990; **190**: 253–258.
- 37 Sarzani R, Paci MV, Dessi-Fulgheri P, Espinosa E, Rappelli A. Comparative analysis of atrial natriuretic peptide receptor expression in rat tissues. *J Hypertens* 1993; **11**(Suppl 5): S214–S216.
- 38 Sarzani R, Dessi-Fulgheri P, Paci MV, Espinosa E, Rappelli A. Expression of natriuretic peptide receptor expression in human adipose and other tissues. *J Endocrinol Invest* 1996; **19**: 581–585.
- 39 Sarzani R, Paci MV, Zingaretti C, Pierleoni C, Cinti S, Cola G, Rappelli A. Fasting inhibits natriuretic peptide clearance receptor expression in rat adipose tissue. *J Hypertens* 1995; **13**: 1241–1246.