Plasma adrenaline concentration is lower in post-obese than in never-obese women in the basal state, in response to sham-feeding and after food intake

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1. Plasma pancreatic polypeptide, plasma catecholamine, blood glucose, plasma insulin and plasma peptide YY concentrations were studied to assess differences between eight formerly obese and eight never-obese control women during 25 min of sham-feeding (with the sight and smell of an English breakfast) and for 5 h after they had ingested the meal (3514 kJ, 50% fat, 35% carbohydrate). The post-obese women had maintained their normal body weight for at least 3 months before the study.

2. The plasma noradrenaline concentration was not different between the groups either during fasting (post-obese women 0.08 ± 0.01 ng/ml versus control women 0.10 ± 0.01 ng/ml) or in the significant postprandial increase (P < 0.001). The plasma adrenaline concentration increased significantly during sham-feeding in the control group from 0.024 ± 0.004 ng/ml to 0.033 ± 0.004 ng/ml (P = 0.02) in contrast with the post-obese women, who had significantly lower plasma concentrations of adrenaline in the fasting state (post-obese women 0.016 ± 0.003 ng/ml versus control women 0.024 ± 0.004 ng/ml, P = 0.003), during sham-feeding (post-obese women 0.018 ± 0.002 ng/ml versus control women 0.033 ± 0.004 ng/ml, P = 0.003) and in the postprandial increase (P = 0.003). The maximal postprandial response concentrations recorded 5 h after the meal were 0.025 ± 0.003 ng/ml in post-obese women and 0.035 ± 0.004 ng/ml in control subjects (P = 0.04). There were no significant differences in plasma pancreatic polypeptide, plasma peptide YY, plasma insulin, or blood glucose concentrations between the two groups.

3. The plasma adrenaline concentration is lower in post-obese women in the basal fasting state, during sham-feeding and in response to a meal. These results indicate that post-obese subjects respond differently to food stimulation than normal subjects.

INTRODUCTION

Sham-feeding (the sight and smell of a meal but not the taste) induces several responses in normal subjects [1–3]. Pancreatic polypeptide originates from the PP-cells of the endocrine pancreas and its secretion is primarily under vagal control. Plasma concentrations of pancreatic polypeptide increase during sham-feeding and to a large degree after a meal [4]. Peptide YY is a gastrointestinal peptide primary localized to the ileum. It increases after a meal [5]. Peptide YY has inhibitory effects in the late postprandial phase. Previous studies have suggested that gastrointestinal and hormonal responses to sham-feeding may be abnormal in obese subjects, but the results are conflicting [6–8].

The aim of the present study was to assess differences in plasma concentrations of pancreatic polypeptide, catecholamines and peptide YY between formerly obese and never-obese women during sham-feeding and after food intake.

METHODS

Eight post-obese women and eight never-obese matched control women were studied in the morning after a 10 h overnight fast following 2 days of a weight maintenance carbohydrate-rich diet (30% fat, 58% carbohydrate and 12% protein). Table 1 shows the demographic data of the two groups. Lean body mass was calculated by the whole body bio-impedance method using an Animer (HTS-Engineering, Odense, Denmark) and the equation given by Heitmann [9]. The post-obese women had maintained their normal weight for at least 3 months after a diet-induced mean weight loss of 23.5 kg.

On the day of the investigation, a cannula was inserted into a hand vein, and the subjects rested for 1 h in the supine position with 45° elevated head tilt before the study began. Blood samples were taken as arterialized venous samples, as the hand was placed in a device supplying hot air (55–60°C).
during the entire test period (Technical Department, University of Nottingham, Nottingham, U.K.). The samples were collected at times -35 and -5 min before stimulation with the sight and smell of the test meal. The meal consisted of scrambled egg, bacon, coffee, milk, juice and open sandwiches with cheese and jam to a total energy content of 3514 kJ, comprising as 15% protein, 50% fat and 35% carbohydrate. Samples were taken after 10, 15 and 20 min of sham-feeding. After 25 min of sham-feeding the subjects ingested the food (without the coffee) during 17.5 and 20 min of sham-feeding. After 25 min of sham-feeding the subjects ingested the food (without the coffee) during 17.5 and 20 min of sham-feeding. After 25 min of sham-feeding.

Blood pressure was recorded and blood samples were drawn at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 195, 210, 225, 240, 270 and 300 min after the meal. Blood samples were analysed for glucose using a standard enzymic method; plasma samples were analysed for pancreatic polypeptide, insulin and peptide YY using radioimmunoassays and for catecholamines by a radioenzymic assay.

Catecholamines. These were determined by radioenzymic assay. The blood was sampled in separate tubes and stored at -80°C. Plasma noradrenaline and adrenaline were measured by a single-isotope derivative technique [13]. The intra-assay coefficients of variation for noradrenaline and adrenaline in samples containing normal basal values were 6% and 8%, respectively (n = 10). Corresponding values of inter-assay coefficients of variation for noradrenaline and adrenaline were 7% and 11%, respectively (n = 10). The sensitivity of the assay, calculated as three times the SD of the analytical blank, was for intra-assays 0.3 pg and 0.5 pg/assay for adrenaline and noradrenaline, respectively. Corresponding values for interassays were 0.5 pg/assay for both adrenaline and noradrenaline.

Blood samples from a given test day and subject were always analysed in the same assay run to reduce the influence of interassay variation.

**Statistical analysis**

All results are presented as means ± SEM, unless stated otherwise. Statistical evaluation was performed using the Mann-Whitney U-test for comparison of basal values, of sham-feeding values and of

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**Table I. Demographic data of the eight post-obese women and the eight never-obese control women. Values are means ± SEM.**

<table>
<thead>
<tr>
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<th>Post-obese women</th>
<th>Never-obese control women</th>
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</thead>
<tbody>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>22.5 ± 1.2</td>
<td>22.8 ± 1.4</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>37.4 ± 7.6</td>
<td>37.5 ± 9.8</td>
</tr>
<tr>
<td><strong>Fat-free mass (kg)</strong></td>
<td>47.2 ± 1.5</td>
<td>47.5 ± 3.5</td>
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Plasma pancreatic polypeptide was measured using the radioimmunoassay described by Schwartz et al. [12] with the following modifications: phosphate buffer (0.04 mmol/l, pH 7.4), containing, in addition, 1 g of human plasma albumin/l and 0.24 g of thimerosal/l was used. The samples and standards were incubated in two steps: first a 50 µl sample with 100 µl of antibody at 4°C for 24 h and then after the addition of 100 µl of radioactive tracer the samples were incubated at 4°C for a further 24 h. Separation was performed with 250 µl of dextran (1.6 g/l)-coated charcoal (16 g/l). The inter-assay coefficient of variation was 13% at a mean concentration of 14 pmol/l (n = 40), 11% at 20 pmol/l (n = 37) and 8% at 50 pmol/l (n = 36). The intra-assay coefficient of variation was 11% at a mean concentration of 15 pmol/l, 14% at a mean concentration of 23 pmol/l (n = 10) and 4% at a mean concentration of 52 pmol/l. The detection limit was calculated according to RiaCalc (Wallac AS, Allerød, Denmark) to be 3 pmol/l. Unspecific binding without antibody was 0.5–2%. Anti-porcine pancreatic polypeptide serum K 5418 was used as antibody and 125I-porcine pancreatic polypeptide was used as tracer (Novo Nordisk, Bagsværd, Denmark). Human pancreatic polypeptide (PP-05-3943A, Cambridge Research Biochemicals) was used as the standard. Samples with high concentrations were diluted to the assay level.

Plasma peptide YY was measured by radioimmunoassay as described in [12a]. In this study we have shown that the increase in plasma neuropeptide Y-like immunoreactivity in response to food intake is due to an increase in the plasma peptide YY concentration, whereas the plasma neuropeptide Y concentration did not change.

Catecholamines. These were determined by radioenzymic assay. The blood was sampled in separate tubes and stored at -80°C. Plasma noradrenaline and adrenaline were measured by a single-isotope derivative technique [13]. The intra-assay coefficients of variation for noradrenaline and adrenaline in samples containing normal basal values were 6% and 8%, respectively (n = 10). Corresponding values of inter-assay coefficients of variation for noradrenaline and adrenaline were 7% and 11%, respectively (n = 10). The sensitivity of the assay, calculated as three times the SD of the analytical blank, was for intra-assays 0.3 pg and 0.5 pg/assay for adrenaline and noradrenaline, respectively. Corresponding values for interassays were 0.5 pg/assay for both adrenaline and noradrenaline.

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**Statistical analysis**

All results are presented as means ± SEM, unless stated otherwise. Statistical evaluation was performed using the Mann-Whitney U-test for comparison of basal values, of sham-feeding values and of
Plasma adrenaline and post-obesity meal

Sham

Incremental areas between the two groups. Friedman analyses were used for comparison within each group. Two-way repeated measures analysis of variance on one factor was used for comparison of the plasma concentrations and time between the groups (SigmaStat 1.0, Jandel Scientifics, GmbH, Erkrath, Germany). The level of statistical significance was set at $P < 0.05$ (two-tailed).

RESULTS

Fasting plasma noradrenaline concentrations were not statistically different in post-obese women than in never-obese control women. Both groups showed a small increase in plasma noradrenaline concentration during sham-feeding ($P = 0.06$) and a significant increase after the meal ($P < 0.01$, Fig. 1). The postprandial peak value at 90 min was not different between the groups. Two-way repeated measures analysis of variance on noradrenaline revealed no statistically significant difference between post-obese women and control women ($P = 0.1$), whereas a statistically significant difference was found in the different levels of time ($P < 0.001$). There was no statistically significant interaction between never-obese control women, post-obese women and time.

The most striking difference between the two groups was seen in the plasma adrenaline concentration. Mean plasma adrenaline concentrations were significantly higher in control women than in post-obese women ($P = 0.003$). In both groups the plasma adrenaline concentration increased with time ($P < 0.001$) with no signs of interaction ($P = 0.98$).

Comparison of the three plasma concentrations during sham-feeding and the concentration before sham-feeding (Friedman analyses) revealed a significant increase in the control group only ($P = 0.02$), whereas the plasma adrenaline concentration in the post-obese group remained unchanged during sham-feeding ($P = 0.7$) (Fig. 2).

The post-obese subjects had significantly lower plasma adrenaline concentrations both in the basal period ($P = 0.003$), in peak sham-feeding period ($P = 0.003$) and in the postprandial response evaluated by the area method and by analysis of variance ($P = 0.003$). The significant postprandial increase followed a parallel pattern in the two groups (Fig. 2). The maximal postprandial response values recorded in the post-obese group 5 h after the meal barely reached the basal level in the control group. The mean plasma adrenaline concentration in the post-obese women was approximately 40% of that in the control subjects.

There was no significant difference in the increase in plasma polypeptide concentration during sham-feeding between the two groups. After the meal the first phase response of pancreatic polypeptide was similar in the two groups, whereas the second phase
response was slightly but not significantly reduced in the post-obese women (Fig. 3).

There were no differences between the two groups in the palatability scores for aroma, palatability, appearance or taste of the meal.

Blood glucose and plasma insulin concentrations were not significantly different in post-obese women compared with control women (Fig. 4).

Blood pressure and heart rate were very similar in the two groups. Both groups showed increased heart rate and decreased diastolic blood pressure during the first 30–60 min after the meal (Fig. 5).

There was no difference in plasma concentrations of peptide YY between the post-obese women and the control women either in the fasting state or in response to the meal (Fig. 6). The significant post-prandial increase started 15 min after the beginning of food intake to peak values 2 h after the meal ($P < 0.00001$, Fig. 6). Basal values and the 2 h post-prandial response averaged $4.8 \pm 0.5$ and $16.8 \pm 2.1$ pmol/l in the post-obese women and $4.1 \pm 0.7$ and $14.0 \pm 2.8$ pmol/l in the control women. No changes were seen during sham-feeding (Fig. 6).

**DISCUSSION**

The present study shows that plasma adrenaline concentration is abnormally low at rest, during sham-feeding and in the post-prandial period in post-obese women as compared with control women. These findings are in accordance with our previous published study of the early response to a meal in post-obese women [14]. Plasma noradrenaline concentration responses were not, however, significantly different in post-obese and control women. We have in several studies observed that the plasma noradrenaline concentration is reduced in obese females [15–17] compared with normal weight control females, but it appears that this abnormality is normalized by weight reduction. Interestingly, a low plasma and/or low urinary adrenaline excretion in obese subjects has been reported in several studies [18]. The low adrenaline could be a primary abnormality in obesity in the sense that it is also present in post-obese subjects. The main metabolic role of adrenaline is to assist in the transition of the metabolism from glucose oxidation to fat oxidation by mobilization of free fatty acids from deposits and by inhibition of insulin-induced glucose oxidation. Recent studies in post-obese subjects have showed impaired suppression of carbohydrate oxidation during a high-fat diet [18a]. At present, it is unclear whether the small,
but definitely abnormal, plasma adrenaline concentrations observed in post-obese subjects contributes to this abnormality, but it deserves further investigation.

We observed another abnormality in the plasma adrenaline concentration in post-obese subjects. During sham-feeding their plasma adrenaline concentration did not increase, in contrast to the control subjects. The increase in adrenaline during sham-feeding is an arousal reaction to the sight and smell of food, and this arousal reaction was therefore smaller in post-obese subjects than in control subjects. This may indicate that subjects with weight problems cannot, to the same extent as control subjects, distinguish between situations with and without food stimulation. This abnormal arousal response may play a significant role in the greater food intake observed in obese subjects, but again further studies are required to elucidate this abnormality.

An important point in this study may be that blood samples for adrenaline measurements were obtained while the hand was placed in an air-heating box during the entire test period. In previous studies on normal subjects we have measured plasma concentrations of catecholamines during sham-feeding in venous blood samples and did not observe significant increase in adrenaline. It is well known that the extraction of adrenaline across the forearm is relatively high and increases with increasing arterial plasma adrenaline concentrations. Small changes in arterial plasma adrenaline concentrations may therefore not be detected in venous blood and some investigations require arterial or arterialized venous blood sampling [19].

Apart from the abnormality in the plasma adrenaline concentration, the metabolic and hormonal profile in post-obese subjects was very similar to that observed in control subjects. In a previous study from our laboratory using h.p.l.c. for the separation of plasma neuropeptide Y, related peptides and fragments, we found that the increase in neuropeptide Y-like immunoreactivity activity observed after food intake is due to an increase in the plasma peptide YY concentration [12a]. We found no difference in the plasma peptide YY concentration between the two groups, but the increase in the plasma peptide YY concentration observed after food intake is in accordance with previously published results [20].

In conclusion, our study indicates that the plasma adrenaline concentration is markedly abnormal in post-obese women at rest, during sham-feeding and in the postprandial period. The significance of this abnormality in the development of obesity deserves further investigation.

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