Neuropeptides play a pivotal role in the control of metabolic homeostasis. We aimed to evaluate the release of neuropeptides involved in the control of energy homeostasis in relation to metabolic status in aging humans. The study group consisted of 183 women: 75 centenarians (above 100 yrs old), 26 elderly women (below 70 yrs), 45 younger women (mean 26 yrs) and 37 obese women (mean 41.6 yrs). Fasting plasma concentration of leptin, adiponectin, ghrelin active, neuropeptide Y (NPY) and insulin were measured. Our results showed several differences in the metabolic and neurohormonal status in the centenarian group. The incidence of hypertension, glucose intolerance, insulin resistance and dyslipidemia was lower compared with obese women. Leptin and NPY concentrations were significantly lower than in elderly and obese subjects. Moreover, NPY level was higher than that in the younger group. Plasma adiponectin values were higher than in any of the other group. Insulin levels were significantly lower compared with the young and obese groups. Furthermore, a negative correlation was found between adiponectin and HOMA-IR, and adiponectin and insulin. Ghrelin active concentrations were significantly lower compared with the young subjects. However, ghrelin levels were higher than in obese subjects. We conclude that altered neuropeptide activity in centenarians may play a role in the mechanisms contributing to prolonged survival.

Key words: neuropeptides, longevity, obesity, adiponectin, leptin, insulin
INTRODUCTION

It has been reported that some neuropeptides play an important role in the control of food intake and expenditure of energy (1 - 3). The arcuate nucleus (ARC) in the hypothalamus has a major role in the integration of signals regulating appetite (4). One neuronal circuit stimulates food intake via expression of neuropeptide Y (NPY) and Agouti-related peptide (AgRP), while another inhibits food intake via expression of proopiomelanocortin (POMC) and cocaine-amphetamine regulated transcript (CART) (1, 5).

Many elderly present metabolic syndrome that is known to increase atherogenesis and, in consequence, to increase mortality caused by cardiovascular or cerebrovascular diseases (6). Our previous findings indicated disturbed activity of some neuropeptides in obese patients with the metabolic syndrome (7 - 9).

Besides genetic and environmental factors, it is plausible that physiological changes in the neuroendocrine system may be related to the process of aging, and some of them may also play a role in promoting longevity (10, 11). Therefore, the aim of this study was to evaluate the release of neuropeptides involved in the control of energy homeostasis in relation to metabolic status in ageing humans.

MATERIAL AND METHODS

The study group consisted of 183 women:
1. 75 centenarians aged over 100 yrs, 100-108 yrs (mean 101.1 ± 2.03 yrs)
2. 26 elderly women aged 64-67 yrs (mean 66.03 ± 0.85)
3. 45 younger women aged 20-43 yrs (mean 26.04 ± 7.6)
4. 37 obese women aged 26-54 yrs (mean 41.63 ± 12.42)

All subjects were in good health, without relevant acute or chronic disorders including cardiovascular, respiratory, hepatic or renal diseases. Informed consent was obtained from all participants and the study was approved by the local Ethics Committee.

Blood samples were taken following overnight fasting. Plasma adiponectin, leptin, ghrelin active, NPY and insulin concentrations were measured by RIA methods using commercial kits (Linco Research Inc, Linco Research Inc, Peninsula Lab, and Belmont, respectively). The sensitivity of the leptin assay was 0.5 ng/ml and interassay and intraassay coefficients of variation (cv) were 8.5% and 7.3%, respectively. The sensitivity of adiponectin assay was 1.0 ng/ml with intraassay and interassay cv of 4.8 % and 6.5 %, respectively. The sensitivity of the NPY assay was 2 pg/tube with intraassay and interassay cv of 6.2% and 8.3 %, respectively. The sensitivity of ghrelin active assay was 7.8 pg/ml with intraassay cv of 7.4% and interassay cv of 10.6%.

Biochemical measurements made using standard laboratory procedures are presented in Table 1.

Statistical analysis

Statistical analysis was performed using the ANOVA test followed by the Scheffé test.

The Spearman test was used to evaluate the correlation between neuropeptide levels and BMI, cholesterol, triglycerides, insulin, and HOMA-IR.
RESULTS

In long-lived women centenarians, we observed that the insulin resistance index (HOMA-IR), total cholesterol, LDL and TG were significantly lower (p<0.001 for all measurements) than in obese patients (Table 1).

Plasma levels of leptin, NPY, adiponectin and ghrelin active are shown in Table 2.

Compared with elderly and obese women, plasma leptin levels in the centenarian group were significantly lower (p<0.001, p<0.001, respectively).

Plasma NPY concentrations in centenarians were significantly lower than in the elderly and obese groups (p<0.01, p<0.001, respectively), but were higher than that in young subjects (p<0.001).

Table 1. Clinical and biochemical data

<table>
<thead>
<tr>
<th></th>
<th>Centenarians</th>
<th>Elderly</th>
<th>Young</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>101.1 ± 2.03</td>
<td>66.03 ± 0.85***</td>
<td>26.04 ± 7.6***</td>
<td>41.63 ± 12.42***</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.19 ± 0.49</td>
<td>26.31 ± 0.6</td>
<td>21.52 ± 0.3</td>
<td>32.71 ± 0.8***</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>129.5 ± 2.35</td>
<td>143.4 ± 3.76</td>
<td>119.8 ± 1.27</td>
<td>151.8 ± 4.2***</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>80.9 ± 1.2</td>
<td>87.6 ± 1.4</td>
<td>75.2 ± 1.3</td>
<td>93.9 ± 2.0***</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>176.4 ± 4.0</td>
<td>232.4 ± 6.6***</td>
<td>176.1 ± 1.2</td>
<td>227.8 ± 6.7***</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>61.4 ± 1.9</td>
<td>69.9 ± 3.4</td>
<td>78.7 ± 1.2***</td>
<td>58.3 ± 2.5</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>96.0 ± 3.1</td>
<td>137.7 ± 8.3***</td>
<td>68.7 ± 1.2***</td>
<td>145.9 ± 5.6***</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>94.4 ± 5.1</td>
<td>123.8 ± 12.0</td>
<td>75.5 ± 0.8</td>
<td>130.2 ± 7.1***</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>5.6 ± 0.43</td>
<td>7.8 ± 1.1</td>
<td>14.12 ± 1.6***</td>
<td>33.8 ± 4.9***</td>
</tr>
<tr>
<td>Fasting glucose mmol/l</td>
<td>5.5 ± 0.3</td>
<td>4.9 ± 0.1</td>
<td>4.05 ± 0.1*</td>
<td>6.72 ± 0.4</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.47 ± 0.2</td>
<td>1.76 ± 0.3</td>
<td>2.33 ± 0.3</td>
<td>11.18 ± 1.7***</td>
</tr>
</tbody>
</table>

BP - blood pressure, HDL - High Density Lipoproteins, LDL - Low Density Lipoproteins, TG – triglycerides. Data present Mean± SEM. * p < 0.05 between the centenarian group and investigated group, ** p < 0.01 between the centenarian group and investigated group, *** p < 0.001 between the centenarian group and investigated group.

Table 2. Plasma adiponectin, leptin, NPY and ghrelin active concentrations

<table>
<thead>
<tr>
<th></th>
<th>Centenarians</th>
<th>Elderly</th>
<th>Young</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>17.15 ± 1.1</td>
<td>10.0 ± 1.3***</td>
<td>10.78 ± 1.0 ***</td>
<td>8.21 ± 0.6 ***</td>
</tr>
<tr>
<td>Leptin ng/ml</td>
<td>8.16 ± 0.9</td>
<td>13.38 ± 1.5 ***</td>
<td>10.6 ± 2.5</td>
<td>23.6 ± 3.5 ***</td>
</tr>
<tr>
<td>NPY pg/ml</td>
<td>13.45 ± 2.3</td>
<td>25.44 ± 5.7 **</td>
<td>4.9 ± 0.3 ***</td>
<td>39.7 ± 3.5 ***</td>
</tr>
<tr>
<td>Ghrelin active pg/ml</td>
<td>58.9 ± 3.7</td>
<td>66.4 ± 6.4</td>
<td>85.6 ± 9.25*</td>
<td>49.8 ± 9.09</td>
</tr>
</tbody>
</table>

*p < 0.05 between the centenarian group and investigated group, **p < 0.01 between the centenarian group and investigated group, ***p < 0.001 between the centenarian group and investigated group
Plasma adiponectin values measured in 22 centenarians were higher than in the other groups of women (p<0.001 for all measurements).

Plasma insulin levels were lower in centenarians than in the young and obese groups (p<0.001, p<0.001).

A significant negative correlation was seen between adiponectin and HOMA-IR, and adiponectin and insulin (r = - 0.46, p<0.05 and r = - 0.48, p<0.01, respectively).

Plasma ghrelin active concentrations in centenarians were significantly lower than in young women (p<0.05), but were higher than those found in obese patients, although the difference was not significant.

In obese patients we observed an increase in plasma leptin, NPY and insulin levels compared with the other groups. However, decreased plasma concentrations of adiponectin and ghrelin active were found in this group.

**DISCUSSION**

We observed that plasma leptin concentrations in the centenarian group were lower compared with the group of women aged below 70 yrs old and obese subjects. In human obesity, high concentrations of leptin are associated with the increase of BMI and total fat mass (7 - 9). It has also been proposed that resistance to leptin may play a role in the pathogenesis of obesity and the metabolic syndrome (13). Our results indicate that factors other than nutritional status are involved in mechanisms causing the decrease in leptin release during aging, because the BMI of the centenarian group (23.2 kg/m²) did not differ from that of the younger subjects (21.5 kg/m²), and it was lower than that of elderly patients.

Leptin regulates appetite and the energy balance of the body through its suppressive effect on neuropeptide Y (NPY) that is the strongest orexigenic factor in the hypothalamic control of feeding behavior (1,14). It is also known that NPY participates in the regulation of carbohydrate ingestion (15). Moreover, NPY may be one of many factors involved in the mechanism of insulin resistance (10, 16). In previous studies we found higher plasma NPY levels in obese, diabetic and hypertensive individuals (8, 9).

The results of the present study demonstrated that long-lived female centenarians had lower NPY concentrations compared with the elderly and obese, although their NPY levels were higher than those found in young women.

We have also demonstrated that plasma adiponectin levels in centenarians were significantly higher than in young, elderly and obese subjects. Adiponectin, an adipose tissue-derived protein, has anti-diabetic, anti-atherogenic and insulin-sensitizing properties. This peptide plays an important role in lipid and glucose metabolism: it induces a decrease in circulating free fatty acids (FFA) through increasing fatty acid oxidation by skeletal muscle, decreases liver FFA influx and...
stimulates glucose uptake by adipocytes and muscles through activation of AMP-activated protein kinase (17 - 20). A decrease in triglyceride content in muscle and liver leads to increased insulin sensitivity. In addition to its metabolic effects, adiponectin possesses anti-inflammatory and atheroprotective properties that affect endothelial vascular function (21).

We also found that plasma ghrelin active concentrations in centenarians were significantly lower than those found in young women, although there were higher than in obese subjects. Ghrelin is a 28-amino acid acyl - peptide produced predominantly by the gastric mucosa of the stomach. It was found to be a natural ligand of the growth hormone secretagog receptor type 1a (GHS-R1a) (22, 23). Ghrelin activates GHS-Rs located on GH-releasing hormone containing neurons in the hypothalamic arcuate nucleus, stimulating GH release (24). GHR1a is expressed in cells of the hypothalamus and pituitary gland. The activation of GHS-Rs by ghrelin on NPY/AGRP neurons in the ARC stimulates food intake (25). Ghrelin plays an important role in the control of appetite, food intake and energy balance (23). Moreover, ghrelin decreases fat oxidation and increases fat tissue and it has local effects as a stimulator of gastric motility and gastric emptying (26). Ghrelin stimulates ileal peristalsis and inhibits cholecystokinin (CCK) - induced pancreatic protein secretion (22). A negative association between insulin and ghrelin secretion has also been shown (27 - 29). Ghrelin is recognized as a brain-gut peptide with orexigenic, adipogenic and somatotropic properties (23).

In summary, have found significant differences in the activities of neuropeptides involved in the neuroendocrine control of metabolic homeostasis in centenarians, compared with elderly (below 70 yrs), young (mean 26 yrs) and obese (41.6 yrs) women.

The lower incidence of hypertension, dyslipidemia and glucose intolerance in the centenarian group may be connected with the altered activity of these neuropeptides, especially with the decrease in the release of leptin, NPY and ghrelin and the increase in the release of adiponectin. However, in obese patients, increases in plasma concentrations of leptin, NPY and insulin, and the fall in adiponectin and ghrelin levels correlated with metabolic alterations.

In summary, our results suggest that changes in neuropeptides activities in centenarians may be important in the mechanisms of longevity.

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