ADIPOCYTOKINES, NEUROPEPTIDE Y AND INSULIN RESISTANCE IN OVERWEIGHT WOMEN WITH GYNOID AND ANDROID TYPE OF ADIPOSE TISSUE DISTRIBUTION

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ABSTRACT
The aim of the study was to compare the levels of certain adipose tissue hormones in women with the two main morphological types of obesity - android and gynoid obesity.

MATERIALS AND METHODS: The study included 2 groups of age- and weight-matched women with android (n = 32) and gynoid (n = 27) type of obesity, and a group of age-matched healthy women (n = 24) with normal weight and body constitution. Leptin, resistin, tumour necrosis factor α (TNFα), neuropeptide Y (NPY), glucose and insulin were measured. HOMA index was calculated.

RESULTS: Leptin levels in the women with gynoid obesity did not differ significantly from those in the controls and the women with android obesity. The controls had significantly lower leptin levels compared with the android obesity women. NPY was significantly higher in the control women compared to the women with android obesity and did not differ significantly between the two groups of obese women. TNFα levels in all groups were very similar. Resistin did not show significant differences between all groups but tended to have the lowest levels in the controls. In the women with android obesity, insulin was significantly higher than that in the women with gynoid obesity and the controls. Insulin resistance was found in the women with android obesity only. Basal insulin and HOMA index in the women with gynoid obesity did not differ significantly from the values in the control group.

CONCLUSION: The results from this study contribute to understanding the association of adipose tissue hormones and insulin resistance in obesity. When adipose tissue is predominantly distributed in the abdominal area at similar amount and percentage of body fats, leptin production is higher and insulin resistance develops. In the gynoid type of adipose tissue predisposition, overt insulin resistance is not found, leptin levels does not differ significantly from those in the control group.

Key words: obesity, leptin, resistin, tumour necrosis factor α, neuropeptide Y, insulin resistance

INTRODUCTION
The discovery of leptin in 1994¹ aroused interest in the adipose tissue which was no longer considered an inert tissue storing energy in the form of triglycerides but was viewed as a major endocrine organ in human body.² It is already known that adipocytes secrete a series of substances with local and distant effects³ such as leptin, resistin, TNFα, adiponectin, omentin, interleukin 6, etc.⁴ ⁵. These hormones play an important role in metabolism, reproductive processes, cardiovascular function, and immunity.⁶ Endocrine function of adipocytes...
is regulated mainly by nutritional status, and both these factors are complexly interwoven in the energy storing mechanism in the adipose tissue.\textsuperscript{7}

Adipose tissue secretory activity is influenced by the total amount of body fat as well as by its distribution. It is considered that at similar degree of obesity, the subjects with higher quantity of visceral fat are more strongly predisposed to serious diseases associated with obesity – metabolic disturbances, cardiovascular diseases, carcinomas.\textsuperscript{8,9} Sex\textsuperscript{10} and race\textsuperscript{11} also have some effect on body fat redistribution and lipid metabolism.

AIM

The aim of the study was to compare the levels of certain adipose tissue hormones (leptin, resistin, TNFα), appetite regulator NPY, and presence of insulin resistance in women with the main morphological types of obesity (android and gynoid) as well as in clinically healthy women with normal weight and body constitution.

MATERIALS AND METHODS

A comparative case-control study was performed on 2 groups of age- and weight-matched obese women: android type (n = 32), and gynoid type (n = 27). Clinically healthy age-matched women with normal weight and body composition (n = 24) served as a control group. Clinical characteristics of the groups are presented in Table 1.

A comprehensive set of hormonal tests was performed in all study subjects in order to exclude endocrine pathology leading to obesity such as Cushing’s syndrome, polycystic ovary syndrome, inherited adrenal hyperplasia, prolactinomas, hypothyroidism, hypopituitarism, hypogonadism, hormone secreting tumours, diabetes mellitus, and pregnancy. To get more precise differentiation of women with hyperandrogenia, only women with normal levels of testosterone and its precursors as well as with normal values of calculated free androgen index (FAI) were included in the study. All women gave their written informed consent to participate in the study.

The groups were formed according to the amount and redistribution of the subcutaneous fatty tissue determined by means of computation of body mass index (BMI) and anthropometric parameters including waist-to-hip ratio (WHR). Waist circumference was measured at the smallest circumference of the waist and hip circumference was measured at the widest level of the buttocks, and the average of two measurements was calculated. Obesity was specified as of gynoid type at WHR < 0.80, and as of android type at WHR ≥ 0.80. The amount and percentage of the adipose tissue and fat free tissue were determined by bioimpedance method using a professional body composition analyzer (Tanita BC-420).

Blood samples were collected from all participants in early morning at fasting stage for determination of biochemical and hormonal parameters. Leptin, resistin, TNFα, NPY, and insulin were determined in all study subjects. Basal HOMA index (basal glucose x insulin/22.5) was used as an indicator of insulin resistance. Leptin was determined by means of a solid-phase immunoenzymatic assay (Human) ELISA (commercial kit of DRG, Germany) with the following characteristics: sensitivity, 0.2 ng/ml; inter assay variation, CV% < 5.4; intra assay variation, CV% < 8.7; correlation with RIA, r = 0.95. Resistin was determined using a commercial kit of PHOENIX PHARMACEUTICAL INC, USA based on the principle of a “competitive” solid-phase immunoenzymatic method (Human) EIA with the following characteristics: sensitivity, 1.16 ng/ml; inter assay variation, CV% < 5.0; intra assay variation, CV% < 14.0. TNFα was determined using a commercial kit of DRG, Germany by means of a solid-phase immunoenzymatic kit of PHOENIX PHARMACEUTICAL INC, USA with the following characteristics: sensitivity, 0.95; inter assay variation, CV% < 10.0. NPY was determined using a commercial kit of PHOENIX PHARMACEUTICAL INC, USA based on the principle of a “competitive” solid-phase immunoenzymatic method (Human) EIA with the following characteristics: sensitivity, 0.21 ng/ml; inter assay variation, CV% < 5.0; intra assay variation, CV% < 14.0. Insulin was tested using a commercial kit for quantitative determination of immunoreactive insulin on the basis of microparticle immunoenzymatic analysis (MIEA) by means of AxSYM system (ABBOTT, USA) with the following characteristics: sensitivity, 0.8 mIU/ml; inter assay variation, CV% < 5.3; intra assay variation, CV% < 5.3.

The statistical analysis was performed by SPSS version 17.0. The results are presented as mean ± standard deviation (SD). The distribution of data was tested using the normality Kolmogorov-Smirnov test. Correlation analyses of Pearson and Spearman were used. An unpaired t-test for normally distributed data was used to compare groups. The results are presented as mean ± standard deviation (SD), p < 0.05 was considered statistically significant.
In the inter-group comparisons, the designation with same letters means absence of statistically significant differences, and different letters signify the presence of such differences.

RESULTS

All studied groups were age matched by study design. There was no significant difference in the body weight between the two groups of obese women. WHR was similar in the control group and in the group of women with gynoid obesity. As expected, the women with android obesity had the greatest WHR. The difference between the controls and obese women was undoubtedly revealed by the bioimpedance analysis of both the amount and percentage of the adipose tissue and fat free tissues. But the women with the two morphological types of obesity did not differ significantly with regard to these parameters (Table 1).

The controls had significantly lower leptin levels compared with android obesity women. Women with gynoid obesity were in between and did not differ significantly from both the controls with normal weight and the women with android obesity. Here, and also when comparing the other hormonal parameters, intra-group heterogeneity might influence the significance of the observed differences. Resistin showed only a tendency to the lowest levels in the control women. The differences in TNFα levels between all groups including the control one were negligible. NPY was significantly higher in the women with normal weight compared to the women with android obesity but the differences in respect to the women with gynoid type of obesity failed to reach statistical significance (p < 0.05). By analogy with the comparisons regarding leptin, NPY levels did not differ significantly between the two groups of obese women (Table 2).

In the obese women, leptin showed stronger positive correlation with the absolute fat mass and percentage (r = 0.80, respectively r = 0.70) and BMI (r = 0.70) than with WHR (r = 0.28). We also found positive correlation between leptin and both basal insulin and HOMA index (r = 0.30, respectively r = 0.32) while the correlation regarding NPY and resistin was negative (r = -0.46, respectively r = -0.34). TNFα did not show significant correlations with the other investigated hormones including insulin and HOMA index but had negative correlation with the percentage and amount of the adipose tissue (r = -0.36, respectively r = -0.26). Resistin did not correlate significantly with the parameters of weight, adipose tissue, insulin, and HOMA index. NPY did not correlate with the parameters of weight but was negatively correlated with WHR (r = -0.49) as well as to a lesser extent with the percentage and amount of adipose tissue (r = -0.18, r = -0.20, respectively); its correlation with insulin and HOMA index was positive (r = 0.30, r = 0.32, respectively).

Table 1. Clinical characteristics of the two groups with obesity and the control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>WHR</th>
<th>Fat (%)</th>
<th>Fat mass</th>
<th>Fat free tissue</th>
<th>Total body water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Android obesity</td>
<td>33.28 ± 7.35</td>
<td>94.97 a ± 18.48</td>
<td>34.08 a ± 6.09</td>
<td>0.87 a ± 0.04</td>
<td>42.63 a ± 6.12</td>
<td>42.94 a ± 15.11</td>
<td>54.83 a ± 6.39</td>
<td>40.44 a ± 4.75</td>
</tr>
<tr>
<td>Gynoid obesity</td>
<td>31.18 ± 8.53</td>
<td>90.63 a ± 16.79</td>
<td>33.50 a ± 6.35</td>
<td>0.72 b ± 0.05</td>
<td>41.81 a ± 4.08</td>
<td>39.33 a ± 10.22</td>
<td>53.54 a ± 6.00</td>
<td>39.10 b ± 4.88</td>
</tr>
<tr>
<td>Controls</td>
<td>30.98 ± 6.15</td>
<td>59.14 b ± 7.28</td>
<td>22.11 b ± 2.95</td>
<td>0.73 b ± 0.06</td>
<td>20.10 b ± 2.12</td>
<td>10.70 b ± 2.71</td>
<td>39.10 b ± 2.22</td>
<td>32.10 b ± 2.17</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SD. Same letters mean absence of significant differences (p < 0.05); different letters mean presence of significant differences.

Table 2. Hormonal parameters and HOMA-index in the different groups

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Leptin (ng/ml)</th>
<th>Resistin (ng/ml)</th>
<th>TNFα (pg/ml)</th>
<th>NPY (ng/ml)</th>
<th>Insulin (mIU/ml)</th>
<th>HOMA index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Android obesity</td>
<td>21.28 ± 11.14</td>
<td>2.35 ± 0.59</td>
<td>15.75 ± 6.79</td>
<td>4.59 ± 1.13</td>
<td>20.13 ± 8.17</td>
<td>4.34 ± 1.68</td>
</tr>
<tr>
<td>Gynoid obesity</td>
<td>17.14 ± 9.05</td>
<td>2.24 ± 0.76</td>
<td>18.18 ± 6.07</td>
<td>5.21 ± 1.19</td>
<td>10.47 ± 5.24</td>
<td>2.18 ± 1.34</td>
</tr>
<tr>
<td>Controls</td>
<td>10.02 ± 5.98</td>
<td>2.09 ± 1.19</td>
<td>19.17 ± 9.08</td>
<td>5.99 ± 1.18</td>
<td>8.03 ± 3.22</td>
<td>1.69 ± 0.98</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SD. Same letters mean absence of significant differences (p < 0.05); different letters mean presence of significant differences.
In the women with android obesity, insulin was significantly higher than that in the gynoid obesity women and the women with normal weight (Table 2). Insulin resistance was present in the women with android obesity as estimated by HOMA index. Basal insulin and HOMA index in the women with gynoid obesity did not differ significantly from the values in the control group (Table 2).

DISCUSSION

The adipose tissue is the body’s major energy storage depot; energy is stored there in the form of triglycerides. This energy can be readily mobilized in fasting state or other bodily needs. Until recently, adipocytes were considered as a passive participant in the energy balance maintenance. However, it is already known that these cells take active part in this process. The main adipose tissue product, leptin, is both a metabolic and a neuroendocrine hormone and plays an important role not only in the regulation of energy balance and food intake but also for the normal sexual development and reproductive processes. It is also involved in the glucose control and interacts with the hypothalamo-hypophyseal-adrenal axis, thyroid hormones, growth hormone, and it even intervenes with hemopoiesis and immune system. The role of leptin in the regulation of body weight as well as in the deposition of fats in the adipose depots is already a fact. Thus, its plasma levels closely correlate with BMI and the amount of body fat. In the circulation, leptin plays the role of a signal factor in the feedback mechanism and informs the brain about the amount of body fat.

In our group of obese women, leptin levels were higher compared to the group of women with normal weight; this was confirmed by now in most studies in the field although the differences in the levels between the distinct group with gynoid obesity and the control group did not reach statistical significance. The stronger correlation of leptin with body weight, respectively BMI and the amount of body fat compared to WHR is in accordance with the data of other researchers as well as with our preliminary data from a study with a similar design. Maybe this is the reason why there is no significance in the leptin levels between the obese women with different types of adipose tissue distribution. Such comparative studies of leptin in different morphotypes of obesity are scanty. For example, Park et al. did not find significant differences in the leptin levels in women with predominantly visceral and predominantly subcutaneous accumulation of fats assessed by means of computed tomography. One study even reported a negative correlation between leptin and WHR.

The relation between leptin and insulin resistance with all its consequences is a matter of discussions and intense research. More and more data are accumulated in favour of a direct interrelation of circulating leptin with insulin and fasting glucose levels, HOMA index, and other components of metabolic syndrome (lipoproteins, arterial pressure), which is independent or only partly dependent on obesity. Our data are consistent with the data in the literature. Thus, in a recent study of Yadav et al. in healthy subjects leptin correlated significantly with BMI (p = 0.0000), waist circumference (p = 0.007), hip circumference (p = 0.0000), fasting insulin (p = 0.002), postprandial insulin (p = 0.000) and HOMA-IR (p = 0.002). In a large randomly assembled multiethnic population Mente et al. reported significant ethnic differences in serum leptin levels. Leptin was significantly higher in South Asians (11.82 [10.72-13.04] ng/ml) and aboriginal people (11.13 [10.13-12.23] ng/ml) than in Europeans (9.21 [8.38-10.12] ng/ml) and Chinese (8.25 [7.48-9.10] ng/ml), but was positively associated with HOMA-IR (p < 0.001) in all groups. Resistin is associated with development of insulin resistance in experimental mice and is considered to play a similar role in humans. It is presumed that this newly discovered adipose tissue hormone is the link between obesity and diabetes. However, the data on its levels in the circulation and their physiological role are quite controversial. Some researchers have reported significantly higher resistin levels in patients with obesity compared to normal weight controls, while other studies (including the present study) have not found significant differences. Controversy exists in relation to the presence of significant correlations between resistin levels and the parameters of body weight, adipose tissue, insulin resistance, and their combination at basal conditions as well as following weight reduction. More recently, three large American case-control studies have indeed shown an increased risk of developing type 2 diabetes among those with elevated serum resistin at baseline. Data from the Nurses’ Health Study, and both the Women’s Health Study and the Physicians’ Health Study – which followed up previously healthy subjects for 12, 10 and 8 years, respectively – showed an increased risk even after adjusting for known diabetes risk factors. Higher levels of resistin were observed in diabetics versus controls, and the
risk of diabetes increased with resistin levels up to an odds ratio of 1.68 in subjects in the highest quintile of resistin levels.27

In the present study, significant correlations between resistin and insulin, HOMA index, BMI, WHR, etc. were not found. The absence of significant alteration in resistin levels after a 48-hour fasting and/or administration of leptin is the reason for some researchers to reject the basic role of resistin in energy homeostasis and the development of insulin resistance.26 The matter is open at present, and it should be born in mind that in general resistin levels in women are significantly higher than in men, and this fact might interfere with the data from studies where gender differentiation has not been performed.

TNFα is a cytokine produced by various cells including lymphoid cells, adipocytes, and skeletal muscles. It is believed to play a basic role in insulin resistance and obesity with the associated complications. This assumption is supported by the higher circulation levels and expression of the hormone in the adipose tissue found in obese patients, and also by their decrease with weight reduction.30,31 According to some authors, insulin resistance changes simultaneously with the change of TNFα levels30,32-34 but in one study no relationship between this cytokine and insulin resistance was found.35 We also did not find correlation between TNFα and insulin resistance in the patients from the present and the previous study.19 In principle, TNFα levels were very similar in controls and women with obesity which is consistent with the data reported by Pincelli et al.36 This suggests that circulating TNFα levels are not likely to reflect the degree of insulin resistance in obesity but it should be stressed on that this cytokine might have predominantly autocrine or paracrine action and might induce insulin resistance on tissue level. It is not clear yet how TNFα production by adipocytes contributes to its levels in the circulation.

It is considered that TNFα is involved in the regulation of the amount of body fat. In human in vitro studies, it impairs pre-adipocyte differentiation and induces pre-adipocyte and adipocyte apoptosis.37,38 Induced lipolysis in vitro39 and in vivo40 has been reported. Altogether, these findings suggest that TNFα might play a role for limiting further increase of adipose tissue amount. Probably, in such a context we could interpret the inverse correlation between TNFα levels and both the amount and percent of adipose tissue that we found using bioimpedance analysis.

Neuropeptide Y is secreted by hypothalamus and stimulates appetite inducing hyperphagia, increase of fat depots, decrease of thermogenesis, and suppression of sympathetic activity. Thus, it becomes one of the main regulators of food intake, body weight and energy expenditure.41 When leptin levels increase after food intake, the latter binds to its receptors in the hypothalamus which leads to discontinuation of NPY secretion.4 NPY levels in our obese patients were lower than those of the controls, the differences being significant when comparing the obese group as a whole and the group with android obesity only. There was negative correlation with the parameters of adipose tissue. This is in accordance with the data of Zahorska-Markiewicz et al. on 13 obese women and 6 women with normal weight.42 Notwithstanding the absence of statistically significant differences in our obese patients, we can see that at relatively highest leptin levels NPY had relatively lowest levels, and vice versa. This was supported by the established negative correlation between the two hormones. The decrease of NPY concentration in obesity may play a role of a counter-regulatory factor intended to prevent further weight gain. In the control group, significantly lower leptin levels were associated with significantly higher NPY levels as compared to the obese groups.

The studies on NPY in obesity are not many; it is interesting that significant alteration of its circulatory levels after weight reduction is not found in adults42 as well as in adolescents43 notwithstanding the progressive decrease of leptin levels. Probably, the leptin control on hypothalamic production of NPY cannot be estimated by the levels of the latter in peripheral circulation.

CONCLUSIONS

In conclusion, the results of our original study contribute to the elucidation of certain aspects of the pathogenesis of metabolic-regulatory obesity related to the role of adipose tissue as an additional “endocrine gland.” It becomes clear that when adipose tissue is predominantly distributed in the abdominal area at similar amount and percentage of body fats in the organism, leptin production is indeed higher, and insulin resistance develops. In the gynoid type of adipose tissue predisposition, overt insulin resistance is not found but certain unfavourable tendencies are present.

As to the other adipose tissue hormones and the regulators of appetite and metabolism of fats, definite conclusions cannot be drawn from our
study. The present study and a previous study with a similar design and results are pioneer studies of Bulgarian population. More extensive studies on larger groups are needed in order to help the statistical reliability of the data. At this stage, we think that the determination of insulin and leptin levels are of practical value in view of selecting therapeutic options for obesity while the study of resistin, TNFα and NPY is justified for research purposes. The study and the differentiation of the effects of insulin and leptin as well as this of the other adipose tissue hormones might clarify the etiopathogenesis of hypothalamic disturbances in general, and could elucidate some hormonal alterations and interrelations which cannot be explained only by the effects of insulin.

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АДИПОЦИТОКИНЫ, НЕЙРОПЕПТИДЫ И ИНСУЛИНОВАЯ РЕЗИСТЕНТНОСТЬ У ЖЕНЩИН С ИЗБЫТОЧНОЙ МАССОЙ ТЕЛА: АНДРОИДНОГО ТИПА

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РЕЗЮМЕ

Цель: Исследование статистически значимо сравнить уровни некоторых гормонов жировой ткани у женщин с двумя основными морфологическими типами ожирения (андроидное и гиноидное).

Материал и методы: В исследовании включено две группы женщин, близких по возрасту и по массе тела (андроидный тип ожирения (n = 32) и гиноидный (n = 27)), а также включена и контрольная группа женщин (n = 24) с нормальной массой тела и с нормальными пропорциями. Исследованы лептин, инсулин, неопеptижектины и факторы роста (TGFb, TNFα, IL-1b) в крови и жировой ткани.

Результаты: Уровни лептина у женщин гиноидного типа были выше, чем у женщин андроидного типа. Уровни инсулина также были выше у женщин гиноидного типа. Уровни TNFα также были выше у женщин гиноидного типа, чем у женщин андроидного типа. Уровни IL-1b были высокими у женщин обеих групп.

Выводы: Увеличение уровня лептина и TNFα, а также высокие уровни IL-1b в крови и жировой ткани у женщин гиноидного типа указывают на повышенную инсулинорезистентность и возможный риск развития метаболического синдрома.

REFERENCES


типа ожирения сиснификантно не различаются от уровней лептина среди женщин контрольной группы, как и среди женщин андроидного типа ожирения. Уровни лептина контрольной группы сиснификантно более низкие по сравнению с группой андроидного типа ожирения. NPY сиснификантно более высокий среди женщин контрольной группы по сравнению с группой женщин андроидного типа ожирения, но значимое различие между женщинами обоих типов ожирения не наблюдается. Уровни TNFα сходные и в трех обследованных группах. Резистин не показывает сиснификантные межгрупповые различия, но налицо тенденция к самым низким уровням у женщин с нормальной массой тела. У женщин андроидного типа ожирения наблюдаются сиснификантно более высокие базальные уровни инсулина по сравнению с женщинами гиноидного типа ожирения, а также и с женщинами с нормальным весом. Инсулиновая резистентность обнаруживается только среди женщин андроидного типа ожирения. Базальный инсулин и HOMA индекс у женщин гиноидного типа ожирения сиснификантно не различаются от тех среди контрольной группы.

ЗАКЛЮЧЕНИЕ: Данные настоящего исследования способствуют изучению взаимосвязи между гормонами жировой ткани и инсулиновой резистентностью при ожирении. У женщин с ожирением вследствие отложения жировой ткани преимущественно в области живота лептиновая продукция более высокая по сравнению с ожирением со сходной по количеству и проценту жировой тканью, но с ее распределением по бедрам, и в этом случае развивается инсулиновая резистентность. При гиноидном типе ожирения не устанавливается инсулиновая резистентность, и уровни лептина не различаются от тех при нормальном весе.