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The Influence of Very-Low-Calorie Diet on Serum Leptin, Soluble Leptin Receptor, Adiponectin and Resistin Levels in Obese Women

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Summary

The aim of our study was to determine whether adipocyte-derived hormones leptin, adiponectin and resistin contribute to the improvement of insulin sensitivity after very-low calorie diet (VLCD). Therefore, serum levels of these hormones were measured in fourteen obese females before and after three weeks VLCD and in seventeen age- and sex-matched healthy controls. Body mass index, HOMA index, serum insulin and leptin levels in obese women before VLCD were significantly higher than in control group (BMI 48.01±2.02 vs. 21.38±0.42 kg/m^2, HOMA 10.72±2.03 vs. 4.69±0.42, insulin 38.63±5.10 vs. 18.76±1.90 μIU/ml, leptin 77.87±8.98 vs. 8.82±1.52 ng/ml). In contrast, serum adiponectin and soluble leptin receptors levels were significantly lower in obese women before VLCD than in the control group. No differences were found in serum glucose and resistin levels between the obese group before VLCD and the control group. VLCD significantly decreased BMI, HOMA index, serum glucose, insulin and leptin levels and increased soluble leptin receptor levels. The changes in serum adiponectin and resistin levels in obese women after VLCD did not reach statistical significance. We conclude that leptin and soluble leptin receptor levels were affected by VLCD while adiponectin and resistin concentrations were not. Therefore, other mechanisms rather than changes in the endocrine function of the adipose tissue are probably involved in the VLCD-induced improvement of insulin sensitivity.

Key words

Obesity • Insulin sensitivity • Very-low-calorie diet • Leptin • Adiponectin • Resistin

Introduction

Obesity, and in particular increased deposition of intraabdominal visceral fat, is considered one of the most important risk factors of type 2 diabetes mellitus and frequently clusters with insulin resistance, arterial hypertension, dyslipidemia, impaired fibrinolysis and numerous others pathological conditions referred to as the metabolic or Reaven syndrome (Reaven 2002). Many studies have attempted to identify the mechanism linking obesity with insulin resistance and type 2 diabetes mellitus, and a number of factors have been suggested as

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Much new information about the function and possible role of adipose tissue in this process has been published over the last decade. It is now generally accepted that this tissue serves not only as an energy store but also as an endocrine organ producing numerous hormones called adipocytokines (adiponectin, leptin, resistin, TNF-α etc.) (Havel 2002). The changes in serum concentrations of adipose tissue-derived hormones can significantly affect insulin sensitivity and in some cases even directly induce insulin resistance (Kubota *et al.* 2002, Maeda *et al.* 2002). Leptin, the first discovered hormone produced almost exclusively by adipocytes, inhibits food intake, enhances energy expenditure and regulates body weight and neuroendocrine response to fasting in mice (Ahima *et al.* 1996, Zhang *et al.* 1994). In humans, leptin levels positively correlate with body mass index and body fat content (Haluzík *et al.* 1999, Maffei *et al.* 1995). The fact, that leptin levels are increased instead of decreased in most of the obese subjects and leptin administration shows only very limited or no effects on body weight, led to the concept of resistance to leptin effects in obese humans (Ravussin and Smith 2002). The direct antidiabetic effect of leptin has been demonstrated in several experimental studies and also in leptin-deficient humans with lipoatrophic diabetes (Oral *et al.* 2002, Reitman *et al.* 2000).

Adiponectin is another protein hormone produced exclusively by adipocytes. Its administration in mice and rats increases insulin sensitivity by stimulating fatty acid oxidation with subsequent reduction of circulating and intracellular triglycerides and/or other lipid metabolites in liver and skeletal muscle (for review see Haluzík *et al.* 2004). In addition, adiponectin exerts antiinflammatory and antiatherogenic properties through suppression of migration of monocytes and macrophages and their transformation into foam cells (Ouchi *et al.* 2001). In contrast to leptin, plasma adiponectin levels are reduced in obese subjects and patients with type 2 diabetes mellitus (Hotta *et al.* 2000). It has therefore been suggested that adiponectin deficiency may play a role in the etiopathogenesis of insulin resistance and diabetes.

Resistin is another fat-derived hormone that has been linked to obesity and diabetes (Steppan *et al.* 2001). It is a member of the newly discovered cysteine-rich secretory protein family referred to as RELM (resistin like molecules). Resistin is produced in adipose tissue and in peripheral blood monocytes. Initial studies in rodents suggested that resistin may represent a potential link between obesity and insulin resistance (Steppan *et al.* 2001). Later studies did not fully confirm this original hypothesis (Way *et al.* 2001).

Weight reduction improves many of the medical complications associated with obesity, including insulin resistance. However, the factors that play a physiological role in the enhanced insulin sensitivity induced by weight loss remain only partially identified. The aim of our study was to determine whether adipocyte-derived hormones contribute to the improvement of insulin sensitivity during a very-low-calorie diet (VLCD). We therefore measured leptin, resistin and adiponectin concentrations in patients with morbid obesity before and after three weeks of very-low-calorie diet and compared the results with that of control group of lean healthy women.

Methods

Study subjects

Fourteen obese female subjects (BMI 48.01±2.02 kg/m^2) and seventeen age- and sex-matched healthy controls (BMI 21.75±0.416 kg/m^2) were included into the study. Their body weight had remained stable for at least 3 months before the beginning of the study. None of the studied subjects suffered from acute infectious disease. Written informed consent was provided by all participants before being enrolled in the study**.** The study was approved by the Human Ethical Review Committee, First Faculty of Medicine and General University Hospital, Prague, Czech Republic.

Study protocol

Subjects were given a very-low-calorie diet (VLCD) for three weeks – 2200 kJ/day (550 kcal/day) energy content. Measurements of clinical and hormonal parameters were performed one day before the beginning of the diet and at the end of the third week.

Anthropometric examination and blood sampling

All patients were examined at the basal state before starting of VLCD and after 3 weeks. Control subjects underwent only one physical examination and blood withdrawal. All subjects were measured and weighted. Blood samples were withdrawn between 7:00 and 8:00 h after overnight fasting.

Hormonal and biochemical assays

Serum insulin concentrations were measured by commercial RIA kit (Cis Bio Internatinal, France).

Sensitivity was 2.0 μIU/ml, and the intra- and interassay variability were 4.2 % and 8.8 %, respectively. Serum leptin concentrations were measured by commercial ELISA kit (BioVendor, Czech Republic). Sensitivity was 0.12 ng/ml, and the intra- and interassay variability were 1.7 % and 8.0 %, respectively. Serum soluble leptin receptor concentrations were measured by commercial ELISA kit (BioVendor, Czech Republic). Sensitivity was 0.4 U/ml, and the intra- and interassay variability was 4.4 % and 7.2 %, respectively. Serum adiponectin concentrations were measured by commercial RIA kit (Linco Research, St. Charles, Missouri, USA). Sensitivity was 1.0 μg/ml, and the intra- and interassay variability were 1.78 % and 9.25 %, respectively. Serum resistin concentrations were measured by commercial ELISA kit (BioVendor, Czech Republic). Sensitivity was 0.2 ng/ml, and the intra- and interassay variability were 3.1 % and 6.5 %, respectively. Plasma glucose levels were measured by standard laboratory method in the Department of Biochemistry of the General University Hospital. HOMA (homeostasis model assessment) index was calculated using the following formula: fasting serum insulin (μIU/ml) * fasting serum glucose (mmol/l)/22.5.

Statistical analysis

The statistical analysis was performed on SigmaStat software (Jandel Scientific, USA). The results are expressed as means ± S.E.M. Data of women before and after VLCD were compared by the paired t-test. The comparison of women before and after VLCD with control subjects respectively was done by one-way analysis of variance followed by Dunnet´s test.

Results

Basal comparison between obese and control group

Body mass index, HOMA index, serum insulin and leptin concentrations in obese women before VLCD were significantly higher (Table 1), while serum adiponectin (Fig. 1) and soluble leptin receptor concentrations were significantly lower than in control group (Table 1). No differences between obese women and the control group were found in serum glucose (Table 1) and resistin levels (Fig. 2).

Influence of very-low-calorie diet on anthropometric, biochemical and hormonal parameters

VLCD induced a significant decrease in body mass index, HOMA index, serum insulin, glucose and leptin concentration and an increase in serum soluble receptor concentrations in obese women (Table 1). The changes in serum adiponectin and resistin concentrations did not reach statistical significance (Figs 1 and 2).

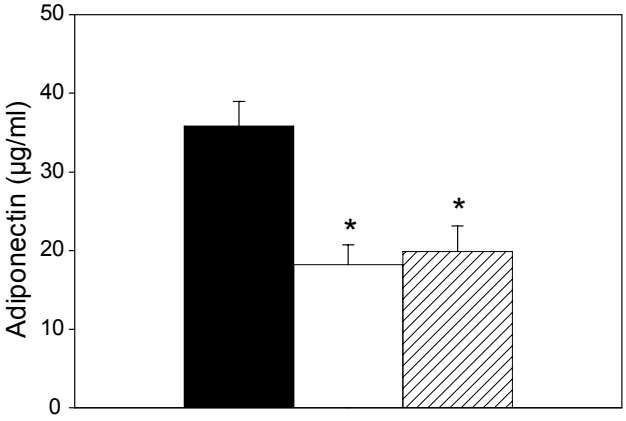

Fig. 1. Serum adiponectin concentrations in control group (black bar), obese group before (white column) and after three weeks of VLCD (hatched column). Values are means ± S.E.M. Statistical significance is from one-way ANOVA: * indicates $p<0.05$ versus control group.

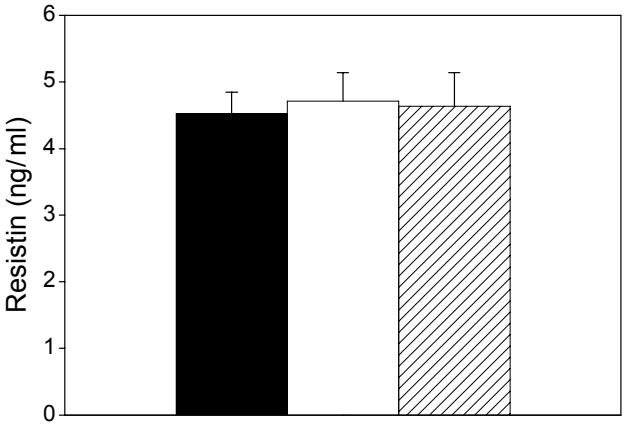

Fig. 2. Serum resistin concentrations in control group (black bar), obese group before VLCD (white column) and after three weeks of VLCD (hatched column). Values are means ± S.E.M.

Body mass index and serum leptin concentrations in obese women after VLCD were still significantly higher and serum adiponectin levels were significantly lower compared to the control group. The HOMA index, serum insulin and glucose (Table 1), adiponectin and resistin concentrations (Figs 1 and 2) in the obese group after VLCD did not differ from the control group.

Table 1. Anthropometric, biochemical and hormonal parameters of control group of healthy women and obese female subjects before (obese group 1) and after (obese group 2) three weeks of VLCD.

	Control group	Obese group 1	Obese group 2
Number of subjects	17	14	14
Body mass index (kg/m^2)	21.38±0.42	48.01±2.02*	45.63±1.88*°
Blood glucose (mmol/l)	6.26±0.55	6.24±0.50	5.11±0.25°
Insulin (μIU/l)	18.76±1.90	38.63±5,10*	25.67±2.82°
HOMA index	4.69±0.38	10.72±2.03*	5.74±0.78°
Leptin (ng/ml)	8.82±1.52	77.87±8.99*	49.27±7.70*°
Soluble leptin receptor (U/ml)	21.57±2.20	13.70±2.49*	17.86±3.23*°

Values are means ± S.E.M. Statistical significance is from one-way ANOVA and Paired t-test respectively. * $p<0.05$ vs. control group, ° $p<0.05$ obese group 1 vs. obese group 2.

Discussion

The aim of our study was to determine whether adipocyte-derived hormones contribute to the improvement of insulin sensitivity after VLCD. We found that VLCD decreased serum leptin concentrations and increased serum soluble receptor concentrations in obese women, while serum adiponectin and resistin concentrations did not change significantly after three weeks of VLCD.

A number of previous studies have demonstrated that most adipose tissue-derived hormones such as leptin, adiponectin and others are closely related to the body fat content and are therefore affected by the weight loss or gain (Laughlin and Yen 1997, Maffei *et al.* 1995, Škrha *et al.* 2005). The same was true for leptin levels in this study which were elevated in obese subjects as a result of their increased body fat content. VLCD significantly decreased body weight and lowered serum leptin levels. We have previously demonstrated that serum soluble leptin receptor – main circulating binding protein for leptin – is inversely related to serum leptin levels (Křížová *et al.* 2002, 2003). Similarly to previously published data (Wolfe *et al.* 2004), soluble leptin receptor levels in obese subjects in our study were significantly lower than in lean controls and VLCD increased its levels, albeit it still remained lower than in control group. It remains to be shown whether decreased serum soluble leptin receptor levels play a role in the proposed resistance to leptin effects in obese humans. On the other hand, both experimental and clinical studies demonstrated beneficial antidiabetic effects of leptin treatment in leptin-deficient animals and humans, respectively, but failed to do so in hyperleptinemic obese humans (Heymsfield *et al.* 1999, Oral *et al.* 2002, Reitman *et al.* 2000). In the present study, serum leptin levels were decreased rather than increased after VLCD suggesting that the change of circulating leptin was not involved in the insulin-sensitizing effect of VLCD.

Adiponectin and resistin have drawn considerable attention over the last years as possible causes in the pathophysiology of obesity-induced insulin resistance. Most of the studies have previously found that adiponectin deficiency is a typical feature of patients with obesity, diabetes and/or insulin resistance (Arita *et al.* 1999, Hotta *et al.* 2000). The same was true in our study where adiponectin levels in obese patients were twofold lower than in lean controls. Interestingly, weight loss after VLCD was not associated with a significant change in circulating adiponectin levels similarly as was previously demonstrated by Garaulet *et al.* (2004). Possible explanation of such dissociation between adiponectin levels and body fat content or insulin sensitivity might be based upon the previously described heterogeneity of circulating adiponectin. This hormone can circulate in the form of monomers, trimers and high molecular weight multimers. It has recently been demonstrated that the high molecular weight form/total adiponectin ratio rather than total adiponectin levels correlates with insulin sensitivity (Pajvani *et al.* 2004). It is therefore possible that even in our study this ratio was changed without any significant alterations of total circulating adiponectin levels. This possibility is currently being investigated in our laboratory.

Resistin was originally discovered as a potential link between obesity and insulin resistance being elevated in obese relative to lean animals (Steppan *et al.* 2001). Further studies did not fully supported this concept in all

animal models of obesity, moreover, human studies brought even more conflicting results (Savage *et al.* 2001, Way *et al.* 2001). Resistin levels were shown to be unchanged and unrelated to body fat content in some studies including our data on malnourished patients with anorexia nervosa (Housová *et al.* 2004) while others have demonstrated increased resistin levels in obesity correlating positively with body fat content (McTernan *et al.* 2002a,b). The explanation for such a contradictory data may be due to the fact that in rodents, resistin is produced predominantly by the adipocytes, while in humans, the main source of this hormone are probably monocytes and macrophages residing in the adipose tissue. Furthermore, resistin levels were markedly increased by endotoxin administration in humans suggesting that inflammatory status rather than body fat content itself may be the major regulator of serum resistin levels (Lehrke *et al.* 2004). We have shown here that serum resistin levels in morbidly obese patients were not different from those of lean subjects and were not affected by the weight loss after VLCD. Thus our data do not support the concept of resistin as a direct link between obesity and insulin resistance in humans.

In conclusion, we demonstrated that a three-week VLCD diet significantly decreased body weight and improved insulin sensitivity in morbidly obese subjects without affecting resistin and total adiponectin levels. We suggest that none of these adipose-tissue derived hormones represents a major player in the mechanism of improvement of insulin resistance after very-low-calorie diet although possible influence of changes in the high molecular weight form/total adiponectin ratio cannot be conclusively ruled out.

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