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Relationship of adipokine to insulin sensitivity and glycemic regulation in obese women – the effect of body weight reduction by caloric restriction

Veza između adipokina, insulinske osetljivosti i glikoregulacije kod gojaznih žena – uticaj sniženja telesna mase ograničavanjem kalorija

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Abstract

Bacground/Aim. Visceral fat is highly active metabolic and endocrine tissue which secretes many adipokines that act both on local and systemic level. It is believed that adipokines and "low-grade inflammatory state" represent a potential link between obesity, metabolic syndrome, insulin resistance and cardiovascular disease. Leptin and adiponectin are considered to be the most important adipokines with the potential metabolic and cardiovascular effects. Body weight loss improves insulin sensitivity and decreases risk for most complications associated with obesity. The aim of this study was to determine the effects of moderate loss of body weight on the level of leptin and adiponectin, insulin sensitivity and abnormalities of glycoregulation in obese women, to determine whether and to what extent the secretory products of adipose tissue, leptin and adiponectin contribute to insulin sensitivity, as well as to assess their relationship and influence on glycemia and insulinemia during the period of losing body weight using a calorie restricted diet. **Methods**. The study involved 90 obese female subjects (BMI $\geq$ 30 kg/m^2) of different age with weight loss no less than 5% during a six-month period by application of restricted dietary regime. The calorie range was between 1,100–1,350 kcal. Serum levels of leptin and adiponectin, fasting glucose, fasting insulinemia, and Homeostasis Model Assessment of Insulin Resistance (HOMA-R) index were determined in all the subjects initially and after weight reduction. The presence of glycemic disorders was assessed on the basis of oral glucose tolerance test – OGTT. **Results.** Applying a 6-month restrictive dietary regime the subjects achieved an average weight loss of 8.73 $\pm$ 1.98 kg and 8.64 $\pm$ 1.96%, which led to the reduction of fasting glycemia, fasting insulinemia and HOMA-R index at the maximum level of statistical significance ($p < 0.001$). The achieved reduction led to a statistically significant decrease of leptin level and increase of adiponectin level ($p < 0.001$). The correction of the established pre-diabetic disorders of glycoregulation was not statistically significant. There was a statistically significant correlation between the anthropometric parameters, leptin, adiponectin, fasting glycemia, fasting insulinemia and HOMA-R index. There was a positive correlation between leptin, fasting insulinemia and HOMA-R, as well as a statistically significant negative correlation between adiponectin, fasting insulinemia and HOMA-R index ($p < 0.01$). **Conclusion.** Body weight increase and central fat accumulation lead to changes in serum levels of leptin and adiponectin, reduction of insulin sensitivity and development of glycemic dysregulation. Secretory products of adipose tissue, leptin and adiponectin contribute to the genesis of these disorders. The obtained results show that the effect of adiponectin on insulin sensitivity is more significant. The analysis of the effects of weight loss on the investigated parameters shows that moderate weight reduction by restrictive dietary regime lead to changes of investigated parameters at the maximum level of statistical significance. Such results emphasize the importance of weight reduction in obese persons, as well as the need for consistent implementation of restricted dietary regime in the process of treatment of obesity.

Key words:
obesity; leptin; adiponectin; body mass index; women; diet, reducing; insulin resistance.

Apstrakt

Uvod/Cilj. Visceralna mast, visokoaktivno metaboličko i endokrino tkivo, sekretuje mnoge adipokine koji deluju na lokalnom i sistemskom nivou. Smatra se da su adipokini i „stanje niskog stepena inflamacije" potencijalna veza između gojaznosti, metaboličkog sindroma, insulinske rezistencije i kardiovaskularnih oboljenja. Kao najvažniji adipokini navode se leptin i adiponektin. Gubitak telesne mase poboljšava insulinsku senzitivnost i smanjuje rizik od većine komplikacija povezanih sa gojaznošću. Cilj ove studije bio je da se utvrde efekti umerenog sniženja telesne mase na nivo leptina i adiponektina, insulinsku senzitivnost i poremećaje glikoregulacije kod gojaznih žena, da se utvrdi da li i u kojoj meri se-

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kretorni produkti masnog tkiva, leptin i adiponektin, doprinose insulinskoj senzitivnosti, kao i da se proceni njihov međusobni odnos i uticaj na glikemiju i insulinemiju u toku snižavanja telesne mase primenom kalorijski ograničavajućeg načina ishrane. **Metode.** Ispitivanjem je obuhvaćeno 90 gojaznih žena (BMI ≥ 30 kg/m^2), kod kojih je u periodu od šest meseci primene ograničavajućeg dijetetskog režima došlo do gubitka telesne mase ne manjeg od 5%. Kalorijski raspon kretao se od 1 100–1 350 kcal. Kod svih ispitanica pre i posle sniženja telesne mase određivan je serumski nivo leptina i adiponektina, glikemija našte, insulinemija našte i *Homeostasis Model Assessment of Insulin Resistance* (HOMA-R) indeks. Na osnovu testa oralne tolerancije glikoze (OGTT) vršena je procena postojanja glikemijskih poremećaja. **Rezultati.** Primenom šestomesečnog restriktivnog režima ishrane kod ispitanica postignut je prosečni gubitak težine od 8,73 ± 1,98 kg ili 8,64 ± 1,96% što je uslovilo snižavanja nivoa glikemije našte, insulinemije našte i HOMA-R indeksa na maksimalni nivo statističke značajnosti, ($p < 0,001$). Postignuta redukcija dovela je i do statistički značajnog sniženja nivoa leptina i porasta nivoa adiponektina ($p < 0,001$). Korekcija ustanovljenih predijabetesnih poremećaja glikoregulacije nije bila statistički značajna. Ustanovljena je statistički značajna korelacija između antropometrijskih parametara, leptina, adiponektina, glikemije našte, insulinemije našte i HOMA-R indeksa. Ustanovljena je pozitivna korelacija između leptina, insulinemije našte i HOMA-R indeksa, kao i statistički značajna negativna korelacija između adiponektina, insulinemije našte i HOMA-R indeksa ($p < 0,01$). **Zaključak.** Gojaznost i centralna akumulacija masti dovode do izmene u serumskim nivoima leptina i adiponektina, sniženja insulinske senzitivnosti i nastanka glikemijske disregulacije. Sekretorni produkti masnog tkiva, leptin i adiponektin, doprinose nastanku ovih poremećaja, pri čemu je uticaj adiponektina na insulinsku senzitivnost, na osnovu dobijenih rezultata, značajniji. Analizom efekata gubitka telesne mase na ispitivane parametre, pokazano je da je umereno sniženje težine kod većine ispitanica uslovilo značajne promene praćenih parametara. Ovako postignuti rezultati snažno naglašavaju značaj sniženje telesne mase kod gojaznih osoba, kao i neophodnost doslednog sprovođenja ograničavajućeg načina ishrane u procesu lečenja gojaznosti.

Ključne reči:
gojaznost; leptin; adiponektin; telesna masa, indeks; žene; dijeta, redukciona; insulin, rezistencija.

Introduction

Obesity belongs to a group of most common metabolic diseases getting epidemic proportions despite public health education and initiatives to reduce it. It is one of leading causes of morbidity and mortality in contemporary society. Numerous studies, including the Framingham one as the first, clearly identify obesity as an independent cardiovascular risk factor, pointing out that its association with other known risk factors, primarily glucose dysregulation and hypertension, leads to enormous increase in the incidence of cardiovascular diseases [1].

Obesity induces insulin resistance, or "the state of reduced insulin action" in insulin-sensitive tissues with the consequent hyperinsulinemia, which is the underlying mechanism in the development of metabolic syndrome and diabetes mellitus. The prevalence of diabetes type 2 is 5 fold higher in obese men and 8.3 fold higher in obese women. The link between insulin resistance and obesity is complex, and numerous evidence suggests that adipose tissue, as hormone-active system, has effect on insulin action and glucose and lipid metabolism [2–4].

Adipose tissue produces a large number of bioactive molecules, known as "adipokines" (adipocytokine) including leptin, adiponectin, resistin, visfatin, apelin, TNF-α, IL-6, etc, that contribute significantly to the development of metabolic abnormalities associated with obesity. They participate in the regulation of appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and hemostasis. The effects of adipokines on vascular function, immune regulation and fat metabolism, make them key players in the pathogenesis of the metabolic syndrome, and thus responsible for development of diabetes and atherovascular disorders. This refers primarily to leptin and adiponectin [5–7].

Leptin is one of the first identified adipokines with numerous effects, including effects on energy homeostasis, neuroendocrine and immune function. Leptin suppresses food intake, increases energy consumption and regulates body weight. In humans, leptin levels correlate positively with body mass index and fat distribution [8]. Clinical conditions with reduced fat mass (lipodystrophy) are characterized by reduced concentrations of leptin, significant ectopic triglyceride deposition in muscle, liver and β cells and insulin resistance, whereby administration of leptin significantly improves glycemic control, reduces level of triglycerides and improves insulin sensitivity. Consequently, inadequate action of leptin due to leptin resistence, which characterizes obesity, may contribute to the development of insulin resistance and glycemic dysregulation [9, 10].

Along with the increase of fat mass there occurs an increase in the production and secretion of numerous proinflammatory/prothrombic adipokines, including TNF-α, IL-6, CRP, PAI-1, angiotensinogen. It is recognized that obesity is a condition characterized by chronic, systemic low-grade inflammation, which is significantly supported by decline of adiponectin [11–14]. The physiological role of adiponectin has been unclear yet, although it seems that it has significant anti-inflammatory, vasculoprotective and antidiabetic properties [15]. Application of recombinant adiponectin in pharmacological studies reduces serum glucose in healthy and diabetic rodents without stimulation of insulin secretion, suggesting that it functions primarily as an insulin sensitizer and then as a regulator of glucose homeostasis. Moreover, prospective studies have shown that low level of adiponectin is associated with the increased risk of onset of diabetes which implies its potential role in the pathogenesis of insulin resistance and diabetes [16]. However, some further studies are required to support this finding [17]. Body weight (BW) loss im-

proves insulin sensitivity and decreases large number of complications associated with obesity. However, the physiological factors that play a role in improving insulin sensitivity induced by body weight loss have not been fully identified yet.

The aim of this study was to: determine the effects of moderate loss of body weight on leptin and adiponectin levels, insulin sensitivity and glycoregulation in obese women to determine relationships and influence of leptin and adiponectin on glycemia, insulinemia and insulin sensitivity.

Methods

The study included 90 obese female subjects (BMI ≥ 30 kg/m^2) of different age, who had undergone restrictive hygienic-diet regime for six months and lost no less than 5% of their body weight. The body weight of the subjects had been stable for at least three months before they were included in the study.

The weight-reduction diets were prescribed by a medical specialist and a dietitian (at the Department of Dietetic Counseling, the Institute of Public Health in Niš). The diet was administered individually based on health condition, energy needs, anamnestic data on nutrition (nutritional surveys) and established nutritional status. The calorie range was between 1,100–1,350 kcal. The study did not include individuals with established endocrine cause of obesity, diagnosed diabetes, clinically significant active cardiovascular disease, including myocardial infarction within the past six months and/or heart failure, individuals with chronic renal failure, liver failure, malignant disease or those with acute or chronic disease where therapeutic regimen might have affected research results.

For each subject anthropometric measurements and biochemical analyses were done at the beginning of the stady and after six months.

Anthropometric measurements – height, weight, waist (WC) and hip (HC) circumference were measured with the subject standing. Weight was measured while they were minimally clothed without shoes, using digital scales and recorded to the nearest 100 g. Height was measured in a standing position without shoes, using a standard anthropometer to the nearest 0.1 cm. The body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared (kg/m^2). With the participant standing and breathing normally, waist circumference was measured midway between the superior iliac crest and the costal margin (at the level of the umbilicus), using a tape measure. Hip circumference was measured at the point of maximum circumference over the buttocks. Waist to hip ratio (WHR) was calculated.

Biochemical analyses – blood samples after overnight fasting were collected for determination of fasting plasma leptin, adiponectin, glucose (FPG) and insulin. Fasting plasma leptin was measured using the ELISA method (DRG leptin enzyme immunoassay kit), expressed in ng/ml. Fasting plasma adiponectin was measured using the ELISA method (DRG human adiponectin enzyme immunoassay kit), expressed in mg/mL. Fasting plasma glucose was measured using enzymatic UV test with hexokinase, expressed in mmol/L (reference range 4.1 to 6.1 mmol/L). Plasma insulinemia was measured by ELISA method (Biosource), using a test for the quantitative measurement, expressed in mU/L (reference value to 25.0 mU/L). Insulin resistance was estimated according to homeostasis model assessment (HOMA-IR) formula [fasting glucose (mmol/L) x fasting insulinemia (mU/L) / 22.5], where it is assumed that normal weight, healthy individuals under 35 years of age have an insulin resistance of 1, which correlates well with the values obtained by means of euglycemic clamp technique [18]).

The oral glucose tolerance test (OGTT) with 75 g glucose dissolved in 300 mL of water was done in all subjects in order to screen existing disorders of glycemic control according to the current World Health Organization (WHO) classification:

- Normal glucose regulation (NGR) – fasting glycemia < 6.1 mmol/L and in the second hour OGTT < 7.8 mmol/L;
- Impaired fasting glucose (IFG) – fasting glycemia ≥ 6.1 < 7.0 mmol/L and in the second hour OGTT < 7.8 mmol/L;
- Impaired glucose tolerance (IGT) – fasting glycemia < 7.0 mmol/L and in the second hour OGTT ≥ 7.8 < 11.1 mmol /L.

Diabetes mellitus (DM) - fasting glycemia ≥ 7,0 or in the second hour OGTT ≥ 11,1 mmol/L.

Examination was conducted in the Clinic for Endocrinology, Diabetes and Metabolic Diseases, Institute of Nuclear Medicine, Clinical Center Niš, Institute of Public Health in Niš and Biochemical Laboratory "NeoLab" in Niš.

Statistical analysis was performed using SPSS (version 15.0) software. The results were presented in tables. Data are presented as frequencies, mean ($\bar{x}$), standard deviations (SD) and 95% confidence interval (95% CI). The Student's *t*-test for dependent (paired) samples (with normal distributions) and Wilcoxon Signed Ranks Test (with distributions deviating from normal) were done to test the statistical significance of differences between the parameters at the beginning and the end of the study. The χ^2-test or Fisher's exact probability test were used as non-parametric tests to compare the frequency of some attributive numerical parameters. Using Spearman's correlation coefficient – ρ or the Pearson's correlation coefficient, – *r*, respectively, the correlation and the extent of its significance between the studied parameters were determined. The strength of correlation defined by Cohen was asumed as low level – 0.10 to 0.29, mid-level – 0.30 to 0.49 and high level – 0.50 to 1.00 [19].

Results

The examined group consisted of 90 female subjects, of the average age of 41.77 ± 10.51 years (the youngest being 19, and the oldest 63 years). The coefficient of age variation was 25.16, indicating group homogeneity.

Obeying a sixth-month restrictive dietary regime the subjects lost 8.3 ± 1.98 kg or 8.64 ± 1.96% of weight on av-

erage. Of the total number of subjects, 21 (23.33%) achieved body weight loss ≥ 10%.

The basic anthropometric data, values of FPG, insulin, HOMA-R indeks, leptin and adiponectin are presented in Table 1. After 6 months of restrictive diatary regime we find a statisticaly significant reduction of BMI, waist circumference, waist/hip ratio, fasting glucose and insulinemia, as well as HOMA-R index, to the maximal level of statistical significance ($p < 0.001$). The achieved average fasting glycemia decrease was 0.47 mmol/L. Achieved body weight reduction caused a change in levels of adipokines, so that after a 6-month period there was a statistically significant decrease in leptin levels and a statistically significant increase in adiponectin levels ($p < 0.01$) (Table 1).

with the level of fasting insulinemia and HOMA-R index. All these correlations were at medium level ($\rho = 0.30–0.49$), except for a high correlation between the BMI and the HOMA-R index. Before body weight reduction a significant positive correlation was determined only between FPG and WC ($p < 0.05$). After body weight reduction this correlation persisted ($p < 0.05$) and it also became significant between FPG and BMI ($p < 0.01$) (Table 3).

A statistically significant positive correlation between leptin and all anthropometric parameters ($p < 0.001$) was determined before BW reduction. A correlation with body weight and WHR was at medium level, and with WC and BMI at high level. There were no significant correlations after body weight reduction. Correlations of adiponectin with

Table 1

Anthropometric data, FPG, insulin, HOMA-R index, leptin and adiponectin before and after body weight reduction

Parameters	Before body weight reduction	After body weight reduction
Body weight (kg)	102.37 ± 17.1*** (97.7–105.96)	93.63 ± 16.58 (90.16–97.11)
BMI (kg/m^2)	36.43 ± 5.42*** (35.3–35.57)	33.34 ± 5.26 (32.24–34.44)
Waist circumference (cm)	103.12 ± 14.3*** (100.1–106.1)	93.79 ± 12.71 (91.13–96.45)
Waist/hip ratio	0.89 ± 0.08*** (0.88–0.83)	0.82 ± 0.06 (0.81–0.83)
FPG (mmol/L)	5.72 ± 0.66*** (5.58–5.85)	5.25 ± 0.69 (5.11–5.40)
Insulin (mU/L)	27.28 ± 7.36*** (25.74–28.82)	21.36 ± 5.80 (20.14–22.57)
HOMA-R	6.96 ± 2.10*** (6.52–7.40)	5.05 ± 1.74 (4.96–5.42)
Leptin (ng/mL)	54.22 ± 25.61*** (48.85–59.58)	27.09 ± 13.56 (24.25–29.93)
Adiponektin (μg/mL)	7.11 ± 3.30 (6.42–7.80)	10.79 ± 4.60*** (9.83–11.76)

Data are presented as mean ± SD (95% confidence intervals); FPG – fasting plasma glucose; BMI – body mass index; HOMA-R – homeostasis model assessment of insulin resistence; *** $p < 0.001$

The results of OGTT show that the reduction in body weight increases the number of normoglycemic subjects from 62.22% to 71.11% and reduces the number of subjects with increased fasting glycemia and impaired glucose tolerance (Table 2). These changes are not statistically significant.

the tested parameters were negative and low, both before and after body weight reduction but one should notice a that statistically significant correlation exists only between adiponectin and BMI as well as adiponectin and WC ($p < 0.05$) before weight reduction (Table 4).

Table 2

The oral glucose tolerancie test (OGTT) results before and after body weight reduction

OGTT	Before body weight reduction	After body weight reduction
NGR	56 (62.22)	64 (71.11)
IFG	9 (10.00)	7 (7.78)
IGT	25 (27.78)	19 (21.11)
Total	90 (100.00)	90 (100.00)

Data are presented as n (%)
NGR – normal glucose regulation; IFG – impaired fasting glucose; IGT – impaired glucose tolerance

Table 3

Correlation between anthropometric parameters and fasting plasma glucose (FPG), insulin and homeostasis model assessment of insulin resistance – HOMA-R index

Parameters	Before body weight reduction			After body weight reduction		
	FPG (mmol/L)	Insulin (mU/L)	HOMA-R index	FPG (mmol/L)	Insulin (mU/L)	HOMA-R index
Body weight (kg)	0.03	0.29**	0.27**	0.15	0.39***	0.35***
BMI (kg/m^2)	0.20	0.49***	0.51***	0.34**	0.60***	0.58***
Waist (cm)	0.22*	0.38***	0.42***	0.23*	0.45***	0.40***
Waist/hip	0.04	0.37***	0.33**	0.19	0.38***	0.34***

The results are expressed as the Spearman's correlation coefficient – ρ; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Before and after the body weight reduction we determined positive statistically significant correlations between anthropometric parameters (BW and BMI, WC and WHR)

Positive low correlations of leptin and a negative correlation of adiponectin with FPG, fasting insulinemia and HOMA-R index had been established. There was a statistically significant

Table 4

Correlations between anthropometric parameters and leptin and adiponectin

Parameters	Before body weight reduction				After body weight reduction			
	BW (kg)	BMI (kg/m^2)	Waist (cm)	Waist / Hip	BW (kg)	BMI (kg/m^2)	Waist (cm)	Waist / Hip
Leptin (ng/mL)	0.38***	0.61***	0.59***	0.41***	0.03	0.03	0.06	0.10
Adiponectin (μg/mL)	-0.13	-0.23*	-0.27**	-0.01	-0.06	-0.06	-0.15	-0.05

The results are expressed as the Spearman's correlation coefficient – ρ; BW – body weight; BMI – body mass index; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

negative correlation of adiponectin with fasting insulinemia and HOMA-R index before body weight reduction ($p < 0.01$). After completion of the hygienic-dietetic regimen there was no significant correlation (Table 5).

Since there is a close link between fasting glycemia and beta cell function, it is considered that the increase of FPG within the normal range has already been associated with a decrease of beta cell function in adults. The state of increased FPG threefold increases the risk of developing type 2 diabetes and may be considered a good marker of acute insulin response [28, 29]. Several previous studies identified high normal FPG as an independent risk factor for the reduction of insulin secretion and type 2 diabetes [30]. Reports from the Bogalusa Heart study showed that adults who developed IGT or type 2 diabetes had higher values of FPG in childhood and adolescence, compared with normoglycemic persons [31]. Nichols et al. [32] observed a 6% increased risk of developing type 2 diabetes with each 0.06 mmol/L increase in FPG. People with FPG levels between 5 to 5.56 mmol /L, were at a significantly higher risk of developing beta cells disfunction and diabetes, compared to those whose values were below 4.72 mmol/L [33].

Table 5

Correlation of leptin and adiponectin with fasting plasma glucose (FPG), insulin and homeostasis model assessment of insulin resistance (HOMA-R) index

Parameters	Before			After		
	FPG (mmol/L)	Insulin. (mU/L)	HOMA-R index	FPG (mmol/L)	Insulin. (mU/L)	HOMA-R indeks
Leptin (ng/mL)	0.07	0.16	0.20	0.05	0.01	0.04
Adiponectin (μg/mL)	-0.06	-0.30**	-0.29**	-0.02	-0.08	-0.05

The results are expressed as the Spearman's correlation coefficient – ρ; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Discussion

Obesity induces increase in insulin levels and insulin resistance which further aggravates by the increase in BMI and especially with the increase in visceral fat mass. The mechanisms that influence this relationship have not yet been fully explained. There are some possible mediators like high free fatty acid levels generated by overactive lipolysis in fat, and changes in adipokines levels produces by adipose tissue, such as adiponectin and leptin [20–22].

High levels of free fatty acids interfere with glucose utilization in muscles and liver and cause various functional abnormalities (lipotoxicity), including steatohepatitis, reduction in insulin secretion, and perhaps heart failure [23–25].

Obesity predisposes to type 2 diabetes primarily by causing insulin resistance, although some other associated metabolic abnormalities may contribute to β-cell dysfunction and increased glycemic values [26]. Nowadays it is considered that the β-cell function decreases significantly in the phase of normoglycemia, that is, significantly before the development of IGT and severe degree of insulin resistance. Impaired first phase of insulin release and reduced insulin sensitivity both predict the development of type 2 diabetes. In fact, insulin resistance and β-cell dysfunction are considered to be essential defects in type 2 diabetes, but undoubtedly operate to different degrees among individuals, whereby obesity contributes to the development of both disorders [27].

Since weight gain and central obesity significantly increase the risk of developing disorders of glucose metabolism, OGTT was performed in all subjects in addition to fasting glycemia. OGTT results show that a total of 37.8% subjects had prediabetes (10% IFG and 27.78% IGT) and the largest number of obese people examined had normal glucose regulation. The average value of FPG can be categorized as "high normal fasting glucose".

The achieved body weight reduction caused statistically insignificant correlation in the frequency of prediabetic disorders, and a statistically significant reduction in fasting glycemia of 0.47 mmol/L in our patients (Table 1).

Other authors also found positive effect of body weight reduction on glucose control and prevention of diabetes. In people with prediabetes, who changed their lifestyle and lost their weight moderately (~ 5%) a reduction in the risk of developing type 2 diabetes was registered by The Da Qing diabetes study over 60%. For example, a great followed 600 people with prediabetes. After six years of follow-up, the incidence of diabetes was 68% in the group of people who received no therapy, while the risk of developing diabetes was reduced to 32%–40% in the intervention group [34]. Finnish diabetes prevention programme also showed a statistically significant risk reduction of developing diabetes (by about 60%) in a group of people with prediabetes who had adequate diet and physical activity compared to non-intervention group. This study also showed the greatest impact of body weight reduction (4.2 kg), in the very first year of follow-up [35]. Similar results were obtained by U.S. diabetes prevention program, which showed advantage of body weight loss over

the use of metformin in reducing the incidence of diabetes in people with prediabetes [36]. Data obtained in Framingham study suggested that weight loss of more than 6.8 kg reduced the risk of developing type 2 diabetes by 50% [37].

Analysing the obtained results it may be observed that the value of FPG has the strongest correlation with waist circumference, thus emphasizing the importance of abdominal obesity in development of glucose control disorders. After weight reduction, there was a statistically significant medium correlation between BMI and FPG ($p < 0.01$) and WC and FPG ($p < 0.05$), which also confirms the importance of reduction of both total and visceral fat in improving of glucose control. The correlation of the average values of BMI, waist circumference and waist/hip ratio with insulinemia and HOMA-R index before and after body weight reduction was positive and statistically significant (Table 3).

The obtained results clearly indicate that body weight gain and obesity contribute to fasting glycemia increase and insulin resistance and that weight reduction significantly leads to their reduction and improvement of cardiometabolic profile. Although the available literature data highlight stronger effects of greater weight loss on insulin sensitivity and glucose control, it is clear that even a slight weight correction (5%–10%) may lead to a statistically significant improvement in insulin sensitivity and risk reduction of developing diabetes, which was actually shown by the results of our study.

It is considered that adipokines and "low-grade inflammatory state" represent a potential link between metabolic syndrome, insulin resistance and cardiovascular disease. Leptin and adiponectin as hormones with potent metabolic and cardiovascular effects are considered to be the most important adipokines. Both hormones achieve their effect by stimulation of AMP-activated protein kinase, a key enzyme in maintaining cellular energy homeostasis. After its activation, leptin and adiponectin lead to an increase of fatty-acid oxidation, thus preventing accumulation of triglycerides and lipotoxicity, as well as to an increase of glucose transport with a reduction of triglyceride synthesis, lipolysis and glyconeogenesis, thereby synergistically causing reduction in the levels of free fatty acids and improved insulin sensitivity. These hormones also reduce the secretion of important cytokines such as TNF-α and IL-6, which contribute significantly to the development of insulin resistance [38, 39].

Obesity and the increase of fat mass cause alterations of adiponectin and leptin levels, thus provoking pathogenic mechanisms that lead to the development of comorbidity and higher mortality of these people. Although leptin level rises parallel with BMI and the increase of body fat mass its effect is attenuated due to the increase of tissue insensitivity to it, the phenomenon known as "leptin resistance". On the other hand, the circulatory levels of adiponectin inversely correlates with BMI and total fat mass, which consequently leads to its significant reduction in obese persons [40, 41].

The correlation of adiponectin with anthropometric parameters before body weight reduction is negative and statistically significant in relation to BMI and WC ($p < 0.001$). On the other hand, the correlation between leptin and these parameters is statistically significant and positive (Table 4). Fat mass and its central distribution are important for leptin and adiponectin levels. Reduction of body weight resulted in a statistically significant changes in the level of leptin and adiponectin. The values of leptin was significantly reduced, while the values of adiponectin in this period was significantly increased (Table 1). Similar results were confirmed by studies of other authors [42–45].

Previous studies also indicate that changes in the relationship between leptin and adiponectin in obese subjects result in modification of insulin sensitivity and contribute to the development of insulin resistance [46–49]. For example, Matsubara et al. [50] in a study including 486 non-diabetic females find that adiponectin and leptin are significant predictors of HOMA-R and insulin levels, regardless systolic pressure, BMI and triglycerides. Zoico et al. [51] also find that leptin and adiponectin are strongly related to total fat mass and insulin resistance in both sexes with values of these hormones significantly higher in women than in men. Ebinç, et al. [22] find that the level of serum adiponectin may represent a useful marker for identifying individuals at risk of developing obesity-related diseases, primarily cardiovascular atherosclerotic disease, regarding the determined difference of its level and the degree of insulin resistance between metabolically normal obese persons/overweight persons and obese/overweight persons with associated complications. These authors suggest that the decline of serum adiponectin is followed by an increase of HOMA-R index in all groups of subjects, whereby the largest decrease in adiponectin and insulin sensitivity was established in the group of obese patients with dyslipidemia and/or type 2 diabetes. Yamauchi et al. [52] suggest that normalization of adiponectin and leptin levels in obese and diabetic lipoatrophic mice completely eliminates insulin resistance and improves insulin sensitivity.

The analysis of our results prior to weight loss shows a positive correlation of leptin and negative correlation of adiponectin with fasting glucose, fasting insulinemia and HOMA-R index. A statistically significant correlation was found only between adiponectin with fasting insulinemia and HOMA-R index ($p < 0.01$). This indicates that decreased level of adiponectin and increased level of leptin in obese persons have effect on insulin sensitivity, but the effect of adiponectin was more significant. These results lead to a conclusion that some other pathogenetic mechanisms, apart from leptin and adiponectin, may also play a role in improving metabolic disorders induced by body weight loss. This issue, however, requires some further investigations.

Conclusion

The analysis of the effects of weight loss on the investigated parameters shows that moderate weight reduction, by the restricted diet only, caused changes of these parameters at the maximum level of significance. Leptin and adiponectin levels in obese persons have effect on insulin sensitivity and effect of adiponectin is more significant. Such results emphasize the importance of weight reduction in obese persons, as well as the need for consistent implementation of restricted dietary regime in the process of treatment of obesity.

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