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THE INFLUENCE OF ORLISTAT, METFORMIN AND DIET ON SERUM LEVELS OF INSULIN-LIKE GROWTH FACTOR-1 IN OBESE WOMEN WITH AND WITHOUT INSULIN RESISTANCE

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A range of studies showed confusing data about the relationship between obesity, weight reduction and circulating total insulin-like growth factor -1 (IGF-1). The aim of the study was to compare the influence of orlistat (IO), metformin (IM), or calorie-restricted diet (LC) on IGF-1, with special respect to insulin-resistance status. One hundred and fourteen obese women aged from 18 to 40 years were divided into insulin sensitive (IS) and insulin resistant (IR) groups and received a low calorie diet (LC), or an isocaloric diet and 500 mg metformin twice daily (IM), or isocaloric diet with 120 mg orlistat three times daily (IO). Before and after the intervention anthropometric parameters, serum lipid profile, serum concentrations of alanine aminotransferase, aspartate aminotransferase, insulin, glucose, IGF-1, HOMA-IR (homeostatic model assessment), and visceral adiposity index (VAI), and their changes were registered. Although the reductions in weight and body fat were comparable in IS and IR groups, only women with IR showed a significant increase in IGF-1 concentration as a result of all interventions. We found significant positive correlations of ΔIGF-1 with initial and Δ values of: HOMA-IR, triglyceride/high-density cholesterol ratio, VAI. IR premenopausal women show significant increase in IGF-1 serum concentrations regardless the method of intervention. The increase in IGF-1 was parallel to the improvement of insulin resistance parameters.

Key words: *insulin-like growth factor -1, orlistat, metformin, diet, obesity, diabetes, insulin resistance, triglyceride to high-density cholesterol ratio, visceral adiposity*

INTRODUCTION

Insulin resistance (IR) and hyperinsulinemia are essential features of visceral obesity, along with type 2 diabetes (1), and increased risk for arterial hypertension (2), other cardiovascular diseases and mortality (3). In morbidly obese patients leptin resistance is also observed, along with IR (4) in obese, premenopausal women, weight reduction gives positive health consequences, including improvement of IR.

IR individuals are more prone to greater weight loss than other individuals having the same adiposity but without IR. Many behavioral studies demonstrated that high IR status resulted in greater weight loss in non-obese, middle-aged women (5, 6). In another study, the effectiveness of hypocaloric diet did not depend on initial IR (7). In our previous study, both metformin (an insulin-sensitizer) and orlistat (gastric and pancreatic lipase inhibitor) produced a comparable improvement in insulin/glucose homeostasis, but it was particularly IR women who showed improvement with treatment, irrespective of which drug was used (8-10).

Insulin-like growth factor-1 (IGF-1) is a peptide growth factor produced primarily by the liver in response to nutrient signals following stimulation by growth hormone (GH). IGF-1 regulates the growth and development of many tissues (bones, soft tissues) (11). As its receptors were found in endothelial and smooth muscle cells, its role in atherogenesis it investigated (11). Earlier studies showed confusing data about the relationship between obesity and circulating free and total IGF-1. While some investigators observed its higher concentration in obesity (12), others reported it may not change or be lower (13-15). The recent studies showed a nonlinear, inverse, U-shaped relationship between IGF-1 levels and body mass index (BMI), with the highest IGF-1 concentrations at BMI 24 – 26 kg/m^2 (16, 17). Also, in visceral obesity, IGF-1 concentrations are lower, regardless of total body fat. In the Rassmussen *et al.* study (18) obese and overweight women demonstrated an inverse association of IGF-1 levels with the abdominal sagittal diameter and with visceral adipose tissue. No significant correlations were found between IGF-1 and measures of adiposity. Data from recent studies show the association between IGF-1 deficit and obesity with further deregulation of lipid, and glucose/insulin homeostasis, clustering in metabolic syndrome (19). In most studies, low circulating IGF-1 concentration is associated with decreased insulin sensitivity. It is suggested that IGF-1 may be a link between proinflammatory

state and metabolic consequences of obesity (20). The role of IGF and IGF deficit needs further examination whether it is a potential therapeutic target in patients of increased cardiometabolic risk or only an indicator of metabolic disorders.

To our knowledge there are no studies comparing the influence of metformin (IM), orlistat (IO) or calorie-restricted diet (LC) on serum IGF-1 concentration, with special respect to initial IR status, expressed by the homeostatic model for assessment of insulin resistance (HOMA) method, visceral adiposity index (VAI) and triglyceride/high-density cholesterol ratio (TG/HDL).

MATERIALS AND METHODS

Study patients

The study and its protocol was conducted as a part of the project approved by the Ethics Committee of Poznan University of Medical Sciences, certification no. 688/09, dated 18 June 2009. The trial protocol meets the requirements of the Declaration of Helsinki with its amendments.

Part of the study data, however different than those presented in this paper, had been already published (8-10).

Of the 165 registered patients screened at the outpatient clinic of the Department of Internal Medicine, Metabolism, and Dietetics and the Department of Internal Medicine, Metabolic Disorders, and Hypertension, University of Medical Sciences, Poznan, Poland, a total of 120 obese women were enrolled into the study. The inclusion criteria were as previously (8-10): subjects written and informed consent for participation in the study; age from 18 to 40 years; simple obesity (BMI $\geq$ 30 kg/m^2); and stable body weight for one month prior to the trial (acceptable deviation was ± 1 kg) (8-10).

The exclusion criteria were: a secondary form of obesity; diabetes mellitus; poorly controlled hypertension (mean systolic blood pressure > 140 mmHg or mean diastolic blood pressure > 90 mmHg) during the month prior to the trial; coronary artery disease; clinically significant arrhythmias or conduction disorders; congestive heart failure; stroke; malignancy; history of use of any dietary supplements in the month prior to the study; clinically significant abnormal liver, kidney, or thyroid gland function; clinically significant acute or chronic inflammatory process; history of infection in the month prior to the study; nicotine, alcohol, or drug abuse; pregnancy or childbirth at enrollment or in the three months prior to enrollment; lactation at present or in the three months prior to enrollment; postmenopausal state; and any other condition that, in the opinion of the investigators, would make participation not in the best interest of the subject or could prevent, limit, or confound the efficacy of the study (8-10). The occurrence of any of the above exclusion criteria during the trial resulted in immediate cessation of participation in the study.

Study design

The study was designed as a prospective, randomized, open-label trial. All women underwent a three-month run-in period, which started with receiving dietary advice from a qualified dietitian where they were counseled to adhere to a weight-maintenance (isocaloric) diet. The intake of energy in the isocaloric diet was calculated using the Harris-Benedict equation multiplied with a factor for physical activity level (PAL). Twenty-five percent of the total energy was supplied by fat (7% by saturated fatty acids, 10% by monounsaturated fatty acids, 8% by polyunsaturated fatty acids), 20 – 25% by protein, and 50 – 55% by carbohydrates, < 10% by saccharose. Dietary intake was monitored every 14 days until the end of the trial by a qualified dietitian on the basis of dietary intake interviews and food diaries. The intake of nutrients, total calories, and caffeine was maintained at a constant level during the study. The selection of food was arranged individually with each subject. All dietary plans were prepared by a qualified dietitian using nutrient analysis software (JUMAR software, 2006, Poznan, Poland). During the trial, the consumption of dietary supplements was not permitted (8-10).

After the run-in period, all women underwent a three-month-long intervention aimed at reducing body weight. These were randomized and their data were analyzed. After the three-month period, six subjects failed to complete the study: one subject from the LC group, two from the IM group, and three from the IO group did not appear at the final meeting (*Fig. 1*).

The subjects were divided into three study groups: the LC group received a low-calorie diet for three months; this provided 60 – 70% of caloric requirements. The IM group received individual isocaloric diets and 500 mg of metformin twice a day. The IO group received individual isocaloric diets with 120 mg of orlistat three times a day. All treatments ran for three months. Compliance was monitored by counting returned medication. All subjects were instructed to maintain their current physical activity.

At the baseline and after three months, anthropometric and body composition measurements were recorded and biochemical parameters were evaluated in the women. These procedures were performed in first seven days of menstrual cycle (8-10).

Anthropometric parameters

Anthropometric parameters were measured with the subjects barefoot and wearing light clothing after a 14-hour overnight fast. Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg. BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Obesity was diagnosed with a BMI $\geq$ 30 kg/m^2. Waist circumferences were measured to the nearest 0.5 cm midway between the uppermost border of the iliac crest and the lower border of the costal margin at the end of normal expiration.

Body composition

Body composition (body fat in % and kg) was determined using bioelectrical impedance analysis with a Bodystat analyzer (1500 MDD; Bodystat Ltd., Douglas, Isle of Man, Great Britain). The analysis was performed under stable laboratory conditions after a night's rest.

Biochemical parameters

Glucose, high-density lipoprotein cholesterol (HDL-cholesterol), triglycerides (TG), serum concentrations, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) serum activities were measured using blood samples drawn after a 14-hour overnight fast. Samples of venous blood were drawn from a forearm vein. The measurement of plasma glucose concentration was performed using the Glucose HK Gen.3 enzymatic assay with hexokinase (Roche Diagnostics, Mannheim, Germany) and a Cobas Integra analyzer (Roche, Basel, Switzerland). The reference range of the assay was 0.12 – 40.0 mmol/L. HDL-C, TG, ALT, and AST were measured on a Roche Cobas Analyzer (Basel, Switzerland) *via* enzymatic colorimetric methods (Roche diagnostic, Mannheim, Germany). The plasma insulin concentrations were measured using an electrochemiluminescent immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany) with a lower limit of sensitivity of 0.20 μU/mL and intra- and interassay coefficients of variations 2.1 – 2.8% and 1.4 – 2.8%, respectively.

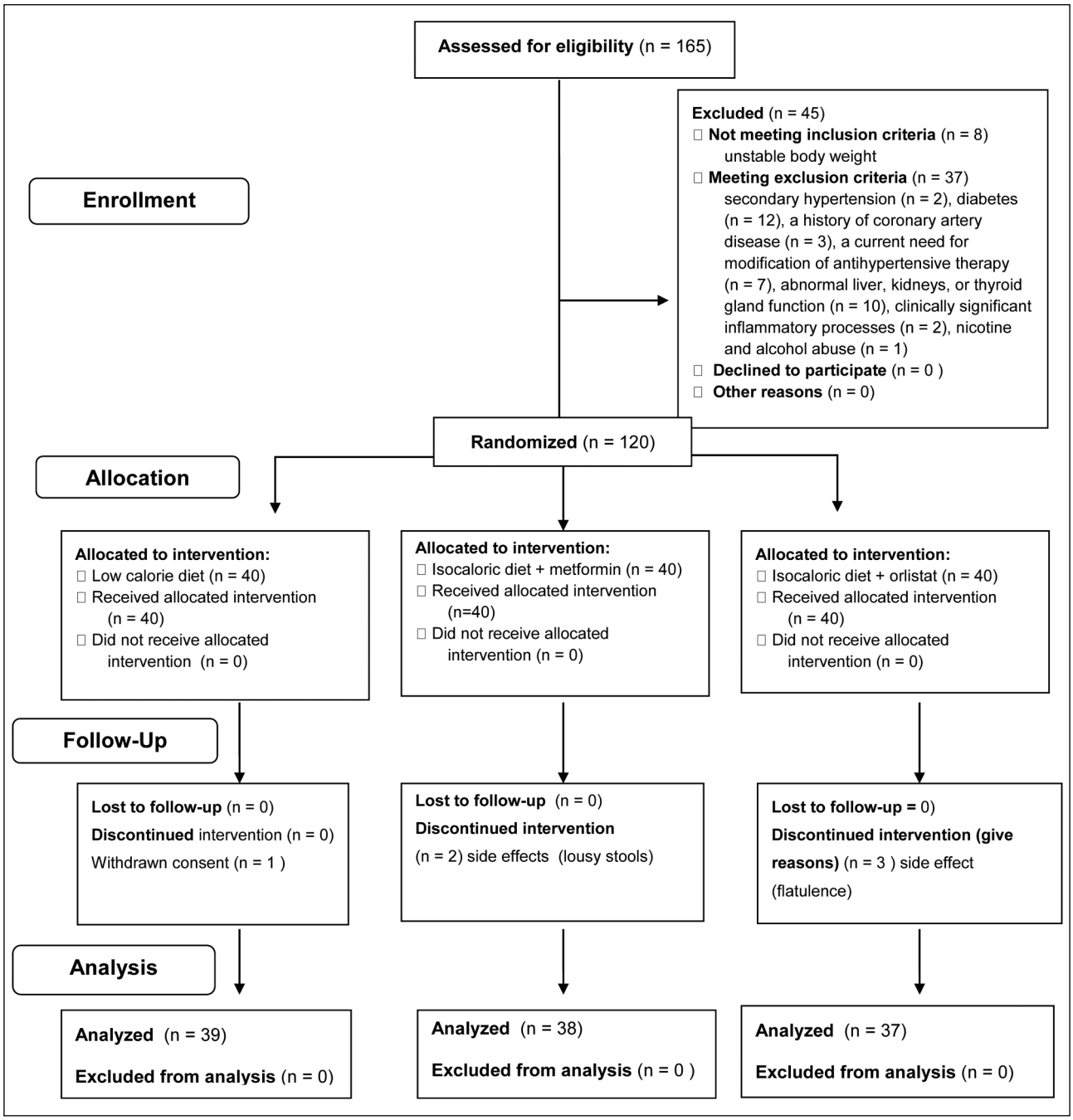

Fig. 1. Flowchart of the study.

IR was calculated using the homeostatic model for assessment of insulin resistance (HOMA) method: HOMA-IR = (fasting glucose concentration [mmol/L] × fasting insulin concentration [mU/L])/22.5 (21). Although the HOMA-IR cut-off may vary in different populations (22) we used HOMA-IR 2.5 as a cut-off value for IR. IGF-1 serum concentration measurements were estimated by SM-C-RIA-CT radioimmunoassay (DIASource ImmunoAssays S.A., Louvain-la-Neuve, Belgium) with a sensitivity of 3.4 ng/mL and assay range of 33-1529 ng/mL.

Visceral adiposity index (VAI) is thought to be a surrogate marker of adipose tissue dysfunction. VAI is significantly correlated to all metabolic syndrome factors and cardio- and cerebrovascular events (23). The VAI formula is presented below (23):

$$\text{Females: VAI} = \left(\frac{\text{WC}}{36.58 + (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right)$$

WC, waist circumference; BMI, body mass index; TG, triglycerides; HDL, high density lipoproteins.

The plasma TG/HDL cholesterol concentration ratio is shown to identify insulin resistant patients and those at significantly greater cardiometabolic risk. It is regarded by some researchers as a surrogate of IR in populations of women and men (24).

Statistical analysis

The data are presented as means ± a standard deviation (SD). Despite not a normal distribution, IGF-1 was presented as a arithmetic mean ± SD for clarity of results. All calculations were performed using the Statistica 10 software (StatSoft

Table 1. Anthropometric parameters, body composition, and hormones - baseline characteristics of examined groups.

	Insulin-sensitive women				Insulin-resistant women			
	LC-diet N = 21	**Metformin** N = 15	**Orlistat** N = 20	**P value**	**LC-diet** N = 18	**Metformin** N = 23	**Orlistat** N = 17	**P value**
Weight, kg	100.4 ± 15.4	96.2 ± 14.0	98.1 ± 16.4	0.68	100.9 ± 17.4	104.1 ± 15.9	106.4 ± 17.3	0.29
BMI, kg/m^2	36.8 ± 6.0	35.0 ± 4.7	36.0 ± 4.7	0.70	35.6 ± 5.2	39.0 ± 5.1	37.7 ± 4.6	0.15
Waist, cm	105.1 ± 11.9	10.1.8 ± 11.8	107.2 ± 12.1	0.63	104.6 ± 13.4	112.3 ± 10.9	112.5 ± 12.5	0.05
FAT, %	47.1 ± 5.3	43.7 ± 4.76	44.7 ± 4.9	0.20	46.2 ± 5.8	48.5 ± 4.8	46.1 ± 4.6	0.95
FAT, kg	46.1 ± 7.5	40.7 ± 7.8	44.4 ± 12.1	0.77	48.2 ± 13.5	49.9 ± 12.0	49.5 ± 12.3	0.83
Insulin, mU/L	6.9 ± 0.6	7.72 ± 3	7.0 ± 3.1	0.84	14.8 ± 3.6*	18.0 ± 11.1*	20.3 ± 4.1*	0.80
Glucose, mmol/l	5.1 ± 0.5	5.4 ± 0.7	4.9 ± 0.5	0.91	5.5 ± 0.6	5.6 ± 0.7	5.5±0.8	1.0
HOMA-IR	1.6 ± 0.4	1.8 ± 0.45	1.8 ± 0.5	0.87	7.7 ± 2.1*	4.4 ± 2.0*	4.4 ± 1.8*	0.61
IGF-1, ng/mL	301.7 ± 127.0	298.4 ± 118.0	347.0 ± 183.3	0.64	324.9 ± 142.7	346.5 ± 183.3	227.1 ± 113.0	0.66
VAI	2.2 ± 1.8	1.9 ± 0.9	1.6 ± 1.3	0.82	2.3 ± 1.6	3.3 ± 2.2	2.6 ± 3.0	0.93
ALT, IU	29.5 ± 11.3	29.5 ± 11.3	21.2 ± 7.5	0.78	25.4 ± 13.3	32.4 ± 16.3	26.0 ± 13.3	0.70
AST, IU	20.1 ± 4.85	21.7 ± 6.4	19.0 ± 3.6	0.87	24.0 ± 6.4	25.7 ± 9.2	20.4 ± 5.0	0.76
TG/HDL	1.21 ± 0.99	1.05 ± 0.51	0.82 ± 0.63	0.67	1.16 ± 0.7	1.78 ± 1.12	1.34 ± 1.53	0.77

Data are presented as arithmetic mean ± standard deviations. * refer to statistically significant differences ($P < 0.05$) between insulin-resistant and insulin-sensitive women within three groups (calorie-restricted diet, metformin, orlistat). ALT, alanine aminotransferase; AST, asparagine aminotransferase; BMI, body mass index; FAT, bioimpedance fat body mass; HOMA-IR, homeostatic model of assessment of insulin resistance; IGF-1, insulin-like growth factor-1; TG/HDL, triglyceride to high density cholesterol ratio; VAI, visceral adiposity index.

Polska, Sp. z o.o., Krakow, Poland). All changes before and after the intervention were calculated as differences between the final and initial values (with negative values indicating a decrease). The chi-square test was used to compare the percentage of IR individuals in the examined groups. The Shapiro-Wilk test was used to evaluate the normality of the distribution Comparisons between three groups were assessed using the ANOVA Kruskal-Wallis rank test (for IGF-1 concentrations) or the ANOVA test if the data were normally distributed. The Wilcoxon rank-sum test (for IGF-1 concentrations) and the paired *t*-test (for data with normal distribution) were used to analyze the statistical differences between the variables before and after the intervention. Simple associations between variables were calculated as the Spearman coefficient of correlation.

RESULTS

One hundred and fourteen women (aged 33.2 ± 8.1 years) were analyzed. After a 12-week run-in period, the number of IR women in the examined groups were comparable: 18 in the diet group, 23 in the metformin group, and 17 in the orlistat group. No significant changes in weight in either group of IS women (100.1 ± 14.7 versus 99.6 ± 15.0 kg) or IR women (104.9 ± 21.7 versus 105.9 ± 21.3 kg) were observed. The run-in intervention did not significantly change IGF-1 serum concentrations. The characteristics of the initial (after the run-in period) parameters are summarized in *Tables 1* and *2*. There were no statistically significant differences ($P < 0.05$) between IR and IS women as a whole nor within the three groups (LC diet, IM, IO), except for the initial HOMA-IR (which is essential for a study design).

Post-intervention changes in the examined parameters are summarized in *Tables 2* and *3*. The decrease in body mass, BMI, and body fat (% and kg) were significant and comparable in both IS and IR groups after 3 months of intervention. There was a tendency towards greater weight loss in IR women. ($P = 0.07$). As we demonstrated previously, (8) HOMA-IR reduction was significantly greater in IR women. Within three interventional groups (LC diet, IM, IO), when IS and IR groups were directly compared, a greater decrease in HOMA-IR was seen in the LC diet group, a greater reduction in body weight in the IM group, and a greater drop in body weight, body fat (% and kg), and HOMA-IR in the IO group.

Despite comparable initial concentrations of circulating IGF-1, we showed differences after the intervention. IS women showed a non-significant reduction in IGF-1 concentration whereas those with IR showed a significant increase ($P = 0.04$). There were no significant differences related to the type of intervention - within both IS and IR groups we demonstrated statistically comparable IGF-1 post-treatment changes.

Table 2. Initial values and changes in anthropometric parameters, body composition, and hormones within insulin sensitive and insulin resistant groups.

	Insulin-sensitive women N = 56		**Insulin-resistant women** N = 48		***P* value regarding Δ between groups**
	Initial value	**Δ**	**Initial value**	**Δ**	
Weight, kg	99.6 ± 15.0	–3.9 ± 6.3*	105.9 ± 21.3	–6.5 ± 6.3*	0.07
BMI, kg/m^2	37.1 ± 5.3	–1.5 ± 2.6*	39.3 ± 10.1	–2.1 ± 2.6*	0.35
Waist, cm	104.8 ± 11.8	–7.4 ±± 11.2	109.5 ± 14.7	–7.5 ± 11.7	0.97
FAT, %	45.5 ± 5.1	–3.0 ± 3.2*	47.5 ± 8.7	–3.0 ± 3.2*	0.99
FAT, kg	45.5 ± 9.5	–4.5 ± 6.6*	50.3 ± 17.4	–6.4 ± 6.6*	0.27
Insulin, mU/L	7.2 ± 2.3	1.1 ± 2.7	17.5 ± 5.6	–2.5 ± 9.6*	0.001
Glucose, mmol/l	5.2 ± 0.6	0.3 ± 0.7	5.5 ± 1.0	0.7 ± 0.8	0.92
HOMA-IR	1.6 ± 0.6	0.2 ± 0.7	5.5 ± 12.4	–0.8 ± 2.8*	0.009
IGF-1, ng/mL	322.7 ± 153.0	–50.5 ± 100.6	290.8 ± 125.6	60.2 ± 134.8*	0.04
VAI	1.9 ± 1.5	–0.1 ± 1.0	2.7 ± 2.2	–0.4 ± 1.2*	0.04
ALT, IU	25.3 ± 10.7	–2.3 ± 5.4	29.8 ± 14.0	2.6 ± 10.0	0.11
AST, IU	20.3 ± 5.1	–2.7 ± 4.8	23.9 ± 7.6	–4.5 ± 5.7*	0.02
TG/HDL	1.0 ± 0.8	0.2 ± 0.7	1.4 ± 1.1	–0.4 ± 0.6*	0.03

Data are presented as arithmetic mean ± standard deviation. * refers to statistically significant differences (P < 0.05) between changes of examined parameters in insulin sensitive and insulin-resistant groups. ALT, alanine aminotransferase; AST, asparagine aminotransferase; BMI, body mass index; FAT, bioimpedance fat body mass; HOMA-IR, homeostatic model of assessment of insulin resistance; IGF-1, insulin-like growth factor-1; TG/HDL, triglyceride to high density cholesterol ratio; VAI, visceral adiposity index; Δ, change between initial and final values.

Table 3. Changes in anthropometric parameters, body composition and hormones after the low-calorie diet, metformin and orlistat treatment in insulin sensitive and insulin resistant groups.

	Insulin-sensitive women				**Insulin-resistant women**			
	LC-Diet N=21	**Metformin N=15**	**Orlistat N=20**	**P**	**LC-Diet N=18t**	**Metformin N=23**	**Orlistat N=17**	**P**
ΔWeight, kg	–4.0 ± 5.9*	–2.1 ± 4.3	–5.5 ± 8.8*	NS	–4.5 ± 4.2*	–5.8 ± 4.4†	13.7 ± 10.2*,†	0.001
ΔBMI, kg/m^2	–1.7 ± 2.2*	–0.8 ± 1.9	–2.1 ± 3.3*	NS	–1.3 ± 2.4*	–2.0 ± 3.1*	–4.4 ± 3.4*	0.008
ΔWaist, cm	–8.9 ± 8.4	–6.3 ± 7.1	–6.1 ± 7.4	NS	–7.? ± 11.7	–4.1 ± 8.6	15.4 ± 12.2*	NS
ΔFAT, %	–3.5 ± 3.2*	–2.4 ± 2.1*	–2.9 ± 4.6*	NS	–2.3 ± 1.2*	–2.4 ± 1.7*	–6.1 ± 5.7*,†	0.002
ΔFAT, kg	–5.5 ± 7.2*	–3.1 ± 3.3*	–5.0 ± 6.6*	NS	–4.3 ± 2.4*	–3.8 ± 3.9*	11.8 ± 9.4*,†	0.009
Insulin, mU/L	0.23 ± 1.8	3.1 ± 2.7	–0.7 ± 1.5	NS	–0.04 ± 1.1	–2.3 ± 12.2	–3.9 ± 4.2*,†	0.01
Glucose, mmol/L	0.63 ± 0.7	0.14 ± 0.5	0.5 ± 0.8	NS	0.0 ± 0.5	1.1 ± 0.8	0.9 ± 1.2	NS
ΔHOMA-IR	0.1 ± 0.5	0.1 ± 0.9	–0.1 ± 0.9	NS	–0.6 ± 0.8*,†	–0.7 ± 0.8*	–1.0 ± 1.1*,†	NS
ΔIGF-1, ng/mL	–75.5 ± 40.4	–67.4 ± 115.9	–25.8 ± 70.3	NS	73.3 ± 43.3*,†	31.7 ± 67.6†	75.9 ± 80.2*,†	NS

Data are presented as mean ± standard deviation. *refers to statistically significant differences (P < 0.05) between initial and final values. †refers to statistically significant differences (P < 0.05) between insulin-resistant and insulin sensitive women within three groups (calorie-restricted diet, metformin, orlistat). BMI, body mass index; FAT, bioimpedance fat body mass; HOMA; homeostatic model of assessment of insulin resistance; IGF-1, insulin-like growth factor-1; NS, not significant; Δ, change between initial and final values.

Then, we analyzed factors potentially influencing ΔIGF-1 in the three study groups (*Table 2*, *Table 4*). IR women showed a higher VAI, TG/HDL ratio, and AST activities. We also found some associations, but only in the IR group. We demonstrated

Table 4. Correlation coefficients between changes (Δ) of circulating IGF-1 serum concentration with initial values and changes of examined parameters in the IR group.

Parameter	Initial value	P	Change (Δ)	P
Waist, cm	0.07	NS	0.01	NS
BMI, kg/m^2	0.3	NS	0.3	NS
ALT, IU	0.1	NS	0.1	NS
AST, IU	0.1	NS	0.1	NS
HOMA-IR	0.6	0.01	0.6	0.01
TG/HDL ratio	0.5	0.03	0.5	0.04
VAI	0.5	0.03	0.5	0.03

ALT, alanine aminotransferase; AST, asparagine aminotransferase; BMI, body mass index; HOMA, homeostatic model of assessment of insulin resistance; IGF-1, insulin-like growth factor-1; NS, not significant; TG/ HDL, triglyceride to high density cholesterol ratio; VAI, visceral adiposity index.

significant positive correlations of ΔIGF-1 with: initial HOMA-IR (P = 0.01); initial TG/HDL ratio (P = 0.03) and ΔTG/HDL ratio (P = 0.04); and initial VAI (P = 0.03) and ΔVAI (P = 0.03). The use of VAI, adding waist circumference to plasma TG, and HDL-cholesterol levels did not augment the correlations between ΔIGF-1 and initial TG/HDL ratio and ΔTG/HDL ratio. There was no significant relationship between ΔIGF-1 and initial values and Δ of: waist circumference, BMI, ALT, and AST.

DISCUSSION

In the present study, we elucidate for the first time that insulin-resistant, premenopausal women manifest significant improvement in IGF-1 serum concentration irrespective of the method of weight-reducing intervention (low-calorie diet, metformin, or orlistat).

In our study, initial serum IGF-1 concentrations in all examined groups are comparable and do not depend on initial IR status. Other researchers showed different results. Strong evidence came from the Framingham Heart Study (25) which demonstrated a reverse association between IGF-1 concentrations and insulin resistance measured by HOMA and number of elements of metabolic syndrome. The review provided by Akanji *et al.* (26) based on recent 10-year literature from PubMed, and Google Scholar, showed that IGF-1 levels are reduced in subjects with metabolic syndrome and its components and may be at least partly explained by a growth hormone deficiency. In the Colao *et al.* study (27) 404 normotensive normoglycemic men had significantly higher IGF-1 concentrations than those with impaired glucose tolerance or diabetes mellitus. New evidence came from the Di Somma *et al.* study (28). In a cohort of 231, apparently healthy subjects, those with IGF-I under the cut-off showed significantly higher levels of visceral adiposity index, systolic and diastolic blood pressure, glucose and insulin levels, HOMA-IR, and lower insulin sensitivity using the Matsuda index, with a concomitant worse lipid profile. Some of the healthy individuals presented a visceral adipose dysfunction associated with GH/IGF-I axis disturbances which did not meet the criteria of overt GH deficiency (29). However, the other studies are unequivocal. The relationship between blood pressure and IGF-1 was inverse in low IGF-1 conditions and direct in overtly high IGF-1 conditions (30). In Chinese, nondiabetic, obese children and adolescents, low levels of IGF-1 were associated with low levels of HDL-C, independent of IR (31). In Kowalska *et al.* study, similarly to ours, despite significantly higher insulin in PCOS women, initial serum IGF-1 did not differ between two groups of obese individuals (PCOS and non-PCOS) (32).

However, our further results are in concordance with the majority of above studies. After the three-month intervention, we demonstrated the significant increase of serum IGF-1 only in IR women. Also, taking into account the method of intervention within the IR group (low-calorie diet, isocaloric diet combined with metformin, or isocaloric diet combined with orlistat), all women benefited equally from the treatment and showed a marked rise of circulating IGF-1. Additionally, we saw significant positive correlations of ΔIGF-1 with initial values and change in HOMA-IR.

There is a small number of interventional studies investigating an influence of different pharmacological weight reducing strategies on circulating IGF-1 and our study is the first one of this type in obese women. In the Rassmussen *et al.* research (33) massive weight loss restored 24-hour growth hormone release profiles and serum insulin-like growth factor-I levels in obese subjects. In the Drent *et al.* study (34) after a four-week placebo treatment combined with an energy-restricted diet, 14 moderately obese subjects demonstrated an increase of IGF-1. Metformin treatment with respect to IFG-1 levels was investigated, mainly in polycystic ovary syndrome (PCOS). Studies applied in simple obesity showed no change or even reduction in serum IGF-1. Fifteen normal-weight men treated with metformin (850 mg twice daily) or placebo for a 15-day period in a double-blind, placebo-controlled, cross-over study demonstrated no change in body weight and body fat mass, but reduction of the concentration of plasma glucose, serum insulin, and serum IGF-1 (P = 0.013) (35). In another study (32) the metformin treatment did not change either IGF-1 or IGFBP-1 in the two groups of obese patients (PCOS and non-PCOS), although significant reductions in BMI and percentage of body fat were observed in both groups of obese patients. It has been also demonstrated that short-term treatment with metformin exerts a similar effect on markers of metabolic syndrome in women with gestational diabetes mellitus as short term insulin treatment (36). Orlistat treatment produces an increase in IGF-1 levels. It's been shown that a six-month hypocaloric diet alone or combined with orlistat produced a comparable and significant increase of plasma IGF-1 concentrations in obese women (15). In another study (37) orlistat induced a weight-independent increase in IGF-1 concentrations and IGF1/IGFBP-3 ratio compared with diet alone in obese, post-menopausal women. In the Drent *et al.* study (34) 12-week treatment with orlistat added to diet did not cause further changes in circulating IGF-1.

Further, we looked for factors correlated with ΔIGF-1 in examined women. As HOMA-IR represents overall IR, and degree of peripheral and hepatic resistance may vary between individuals, we used additional parameters such as VAI, TG/HDL ratio, and activity of aminotransferases to indicate factors influencing changes in IGF-1 more accurately. VAI, taking into account waist circumference, plasma TG, and HDL-

cholesterol levels, may be considered as a complex marker of visceral fat function (23). On the other hand, the plasma TG/HDL cholesterol ratio and serum aminotransferases' activity are markers of liver steatosis and surrogates of hepatic IR. The plasma TG/HDL cholesterol concentration ratio is regarded as a surrogate of IR (especially hepatic IR) in populations of women and men (24, 38, 39).

IR women showed higher initial VAI, TG/HDL ratio, and AST activities and significant changes of these parameters. Therefore we have found significant correlations of ΔIGF-1 only in the IR group. We showed significant positive correlations in ΔIGF-1 with initial and ΔTG/HDL ratio and significant positive correlations in ΔIGF-1 with initial and ΔVAI. These results show the beneficial effect of weight-loss intervention on surrogate indicators of insulin resistance (TG/HDL ratio, VAI) with parallel improvement of circulating IGF-1. Some authors received similar results. Kotronen *et al.* presented the inverse relationship between liver steatosis (and hepatic IR) and IGF-1 (40). Also, in the di Somma *et al.* research (28) subjects with higher VAI showed lower GH peak and lower IGF-1 (presented as IGF-1 under normal range), but unlike ours, these studies showed stable conditions, not the intervention. In our study, the use of VAI, additionally considering waist circumference to plasma TG and HDL-cholesterol levels, did not exceed the correlations of ΔIGF-1 with initial TG/HDL ratio and ΔTG/HDL ratio. It was partly supported by the study (39) which demonstrated a high correlation between the TG/HDL-C ratio and VAI (correlation coefficient = 0.99). In the authors' opinion, VAI does not identify individuals with an adverse cardiometabolic profile (IR) any better than the TG/HDL ratio. Our study did not show correlations between changes in IGF-1 with initial activities of aminotransferases as a marker for fatty liver or a surrogate of hepatic IR, but IR women were characterized by a higher activity of AST. Therefore the improvement of IGF-1 after weight loss only in the IR group could have derived from decrease in total and hepatic IR, because these factors are known to suppress production of IGF-1 in liver (40). In IS women, on the other hand, a tendency to reduce the IGF-1 after the intervention may be explained by calorie restriction in the low calorie group or differences in content of all three macronutrients in the isocaloric orlistat or metformin groups after the diet advise at the study beginning (41). In Western countries protein consumption is usually higher then recommended, also in obese women. Protein restriction during the study could be associated with the drop in serum concentration of IGF-1t in the IS group (41). The fact that baseline IGF-1 levels were not related to the presence of insulin resistance may be explained partly by differences in age and gender of studied populations. We investigated premenopausal women aged 18 – 40 (mean age 33.2 ± 8.1 years), most of above-cited studies considered older individuals and included also men.

In our previous study, both metformin and orlistat produced a comparable improvement in insulin/glucose homeostasis. In particular, IR women showed improvement after treatment, irrespective of which drug was used (8). The current study is in concordance with those results. When IS and IR groups were directly compared, a greater decrease in HOMA-IR was seen in the low-calorie diet group, a greater reduction in body weight in the metformin group, and a greater drop in body weight, body fat (% and kg), and HOMA-IR in the orlistat group. HOMA-IR reduction was significantly greater in IR women.

We conclude that IR, premenopausal women treated with orlistat combined with isocaloric diet, metformin combined with isocaloric diet or low-calorie diet show a significant rise in circulating IGF-1 serum concentration. The increase in IGF-1 serum concentration is parallel to improvement of both total and hepatic insulin resistance.

Study limitations

The major limitations of the study were the short duration of treatment and the small size of the study group. Also, a further double-blinded study should be designed.

Study impact

It was demonstrated for the first time that in obese, premenopausal women, IR status (overall and hepatic) determines IGF-1 serum concentration changes after weight-reducing intervention, irrespective of the method used (low-calorie diet, metformin, or orlistat).

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