Physiol. Res. 54: 271-277, 2005

Effects of Different Weight Loss Protocols on Serum Leptin Levels in Obese Females

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Received January 8, 2004
Accepted July 8, 2004
On-line available December 9, 2004

Summary

We investigated the effects of different weight loss protocols on leptin levels in obese females with the aim of addressing the leptin resistance which has been found to be an aggravating factor in obesity. Twenty-four obese females enrolled to one of three 12-week weight loss protocols: orlistat-induced weight loss (OWL, n=8), exercise-induced weight loss (EWL, n=8) and orlistat plus exercise-induced weight loss (OEWL, n=8). Serum leptin levels were measured in duplicate by radioimmunoassay. There were significant reductions ($P<0.01$) in body weight and fat mass after the 12 week period in all groups: -11.4±0.5 kg and -9.8±0.5 kg (OEWL), -8.3±0.8 kg and -5.7±0.9 kg (OWL), -8.9±1.2 kg and -7.4±1.2 kg (EWL), respectively. Serum leptin levels were also decreased markedly in all groups: -59.2 % (OEWL1), -37.8 % (OWL) and -48.6 % (EWL) ($P<0.01$ all). In addition, there were marked decreases in leptin levels for each kilogram of fat mass after the 12 week period: -48.2±7.2 % (OEWL), -27.8±4.8 % (OWL) and -39.3±4.3 % (EWL) ($P<0.01$ all). Decreases in serum leptin levels expressed per kilogram of fat mass were significantly higher in the OEWL group compared to the OWL group ($P=0.03$). Consequently, an exercise training program in adjunct to pharmacotherapy provides higher weight reduction and fat mass loss in obesity treatment. It also seems to have further beneficial effects on leptin resistance, as indicated by decreases in leptin levels expressed per kilogram of fat mass.

Key words
Leptin • Exercise • Obesity • Pharmacotherapy • Weight loss

Introduction

Obesity, which is an important public health problem, can lead to serious medical problems, including increased insulin resistance, elevated blood pressure and altered lipid parameters (Pi-Sunyer 1993). The pathogenesis of obesity is complicated and not fully understood. It is believed to be mostly a disorder of energy balance, due to increased caloric intake and decreased energy expenditure (Doucet and Tremblay 1997).

Obesity is also reported to be associated with

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alterations in leptin levels. Markedly elevated leptin levels have been shown in obese human compared with non-obese humans (Considine *et al.* 1996, Rosicka *et al*. 2003, Orel *et al.* 2004). However, leptin levels severely decline in underweight human subjects compared with normal weight humans (Haluzík *et al.* 1999). The hormone leptin is known to be produced by adipocytes (Hamilton *et al.* 1995). Leptin has been thought to contribute to body weight regulation by controlling food intake and energy expenditure at the hypothalamic level (Wauters *et al.* 2000). Leptin abnormalities have been proposed to increase the propensity to obesity. Besides its role in metabolic disorders and obesity, leptin also has an important regulatory role on body hormonal (Carro *et al.* 1997, Shimabukuro *et al.* 1997) and gonadal functions (Garcia-Mayor *et al.* 1997).

The efficiency of leptin is low in obese patients, despite the fact that its levels are high in these patients. This is most likely due to an increased resistance rather than to its deficiency (Haffner *et al.* 1996). Considering the regulatory role of leptin in the energy status of the body, it is important to understand the factors that affect leptin metabolism.

Calorie restriction, exercise training and pharmacotherapy are the current protocols in obesity management. Orlistat (XenicalTM, tetrahydrolipstatin), a widely used antiobesity drug, reduces fat absorption (by approximately 30 %) from the intestinal lumen by inhibiting lipase enzymes (Krempf *et al.* 2003). Specific effects of orlistat therapy on body weight and blood lipid parameters have been shown (Rossner *et al.* 2000, Hauptman *et al.* 2000).

The interventions modifying body weight, especially the fat mass, can also have an effect on leptin levels. However, previous studies investigating the effects of weight loss secondary to a calorie-restricted diet (Considine *et al.* 1996, Wing *et al.* 1996) and exercise training (Perusse *et al.* 1997, Kraemer *et al.* 1999, Sartorio *et al.* 2003) on leptin levels yielded different results. Yet, the impact of various weight loss protocols including orlistat, exercise and the combination of exercise plus orlistat on leptin levels has not been convincingly reported and compared in the same study.

In the present study, we investigated the possible effects of different weight loss protocols (orlistat, exercise training and a combination of orlistat plus exercise) on serum leptin levels addressing the leptin resistance which is thought to be an aggravating factor in obesity.

Methods

A total of 24 sedentary obese women (body mass index, BMI >30 kg/m^2) who were treated for obesity at the Obesity Clinic, University of Firat Hospital, participated in the study. All patients underwent medical examination before enrolling to the study. Patients with electrocardiographic abnormalities, cortisol, thyroid or insulin dysfunction and those taking any medications known to affect body composition were excluded from the study. The study protocol was approved by the local Ethics Committee, and informed written consents were obtained from each patient at the start of the study.

After medical screening, patients were randomly divided into three groups as follows:

OWL (orlistat-induced weight loss): eight patients (age 38.0±3.1 y, height 157.2±2.0 cm) received oral treatment with orlistat (Xenical^{TM}) 120-mg capsules three times a day.

EWL (exercise-induced weight loss): eight patients (age 43.0±2.1 years, height 159.3±1.6 cm) enrolled in this group performed aerobic exercise training. The training intensity was set to the anaerobic threshold and performed aerobic exercises 3-4 times per week, using an electromagnetically braked-cycle ergometer (LODE, Groningen, The Netherlands), over 12 weeks. Each training session lasted 45 min. Anaerobic threshold reflects aerobic to anaerobic metabolic transition point (Wasserman *et al*. 1994) and is associated with maximal fat oxidation (Astorino 2000, Ozcelik *et al*. 2004). The anaerobic threshold was estimated from the relationships between ventilation and metabolism (Wasserman *et al.* 1994) and determined from the capillary blood lactate samples (Accutrend Lactate, Roche Diagnostics, Mannheim, Germany).

OEWL (orlistat plus exercise-induced weight loss): eight patients (age 35.3±3.1 years, height 155.7±1.8 cm) received a combination of orlistat and a regular exercise training program as described in OWL and EWL groups.

All patients in the three study groups were encouraged to consume a mild hypocaloric diet with an energy content of about 1200-1600 kcal/day. The prescribed diet contained 30 % of calories from fat, 50 % from carbohydrate, 20 % from proteins. The patients received dietary advice from a qualified dietician. Dietary controls based on self reports were carried out once or twice a week.

Body weight and height were measured to the

nearest 0.1 kg and 0.5 cm, respectively. Body composition was assessed using leg-to-leg bioelectrical impedance (Tanita Body Fat Analyser, TBF 300 M, Tanita, Tokyo, Japan). During the study period, each patient was admitted to the hospital for body composition assessment once or twice per week. Measurements of body composition were standardized and in the morning of the study visits, the patients were asked about their menstrual status and about their liquid and food intake in the morning. All measurements were done with the same equipment, each time by the same investigator. The validity of bioelectrical impedance in the measurement of body composition in obese patients has been criticized (Deurenberg 1996). However, its usefulness in assessment of changes in body composition has been documented (Utter *et al*. 1999).

Blood samples were drawn at the beginning of the study and after 12 weeks of therapy. After an overnight fast, a venous blood sample was obtained between 08:00 h and 9:00 h, always at the same time in the morning to avoid reduction in serum leptin levels during this period (Sinha *et al*. 1996). The samples were separated by centrifugation (4500 rpm for 10 min at +4 C) and stored at –20 ^{o}C and analyzed within 4 months. Serum leptin levels were measured in duplicate in the same run using a commercial radioimmunoassay kit (Human Leptin RIA, DSL-23100, Diagnostic Systems Laboratories).

Data were presented as mean ± S.E.M. The basal data and those obtained at the end of the 12-week therapy period in the same group were compared using Wilcoxon Signed Ranks Test. One way ANOVA was used to evaluate the differences between the groups. P<0.05 was considered significant.

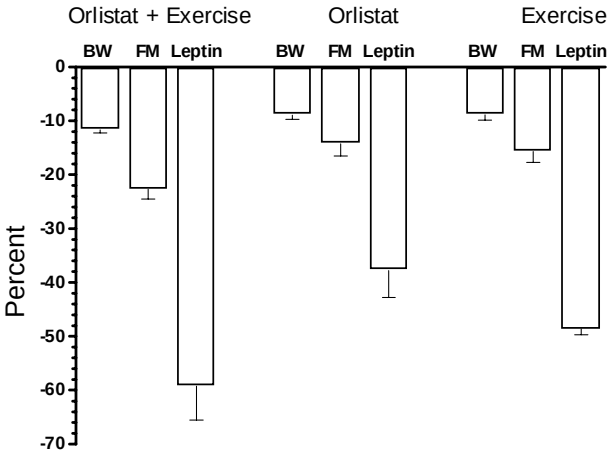

Fig. 1. The decrease in body weight (BW), fat mass (FM) and leptin levels after the 12-week therapy period with orlistat, exercise, and a combination of orlistat and exercise. Data are means ± S.E.M.

Table 1. Body mass index (BMI), body weight (BW), fat mass (FM), leptin levels and leptin levels per kilogram of fat mass (L/FM) at he onset of the study (basal) and at the end of the 12-week period (12 wk). OWL, orlistat-induced weight loss; EWL, exercise-induced weight loss; OEWL, orlistat plus exercise-induced weight loss.

	OWL		EWL		OEWL	
	Basal	**12 wk**	**Basal**	**12 wk**	**Basal**	**12 wk**
BMI (kg/m^2)	37.9±0.9	34.5±0.9	39.1±1.9	35.5±1.5	41.1±2.2	36.4±2.1
BW (kg)	94.0 ±4.3	85.6 ±4.0	99.4±5.1	90.4±4.4	99.7±5.4	88.2±5.3
FM (kg)	41.2 ±2.7	35.5±2.8	46.8±3.5	39.4±3.0	45.2±4.3	35.4±4.2
Leptin (ng/ml)	16.15 ±1.4	10.28±1.4	20.62±1.7	10.46±1.1	18.92±1.9	8.12±1.7
L/FM (ng/ml.kg)	0.396±0.03	0.286±0.03	0.440±0.02	0.266±0.03	0.436±0.04	0.225±0.04

Data are means ± S.E.M. * p<0.01 vs. basal values

Results

As shown in Figure 1, body weight, fat mass and leptin levels decreased after 12-week period in all groups. Body weight loss was –11.4±0.5 kg (P<0.01) in the OEWL group, –8.3±0.8 kg (P<0.01) in the OWL group, and –8.9±1.2 kg (P<0.01) in the EWL group. Fat mass loss in the OEWL group (–9.8± 0.5 kg) was significantly higher than in the OWL group (–5.7±0.9 kg, P=0.02) but not compared to the EWL group (–7.4±1.2 kg, P=0.4) (Table 1). The difference between the groups was significant with regard to fat mass loss and body weight reduction (P=0.02).

Serum leptin levels decreased markedly

compared with baseline levels in all patients of all groups (Table 1, Fig. 2). Leptin levels decreased from 18.92±1.9 ng/ml (baseline) to 8.12±1.7 ng/ml (12 weeks) (–59.2 %, P<0.01) in the OEWL group, from 16.15±1.4 ng/ml (baseline) to 10.28±1.4 ng/ml (12 weeks) (–37.8 %, P<0.01) in the OWL group and from 20.62±1.7 ng/ml (baseline) to 10.46±1.1 ng/ml (12 weeks) (–48.6 %, P<0.01) in the EWL group (Table 1, Fig. 2). The magnitude of change in serum leptin levels was significantly different between the groups (P=0.03). There was a greater decrease in serum leptin levels in the OEWL group compared to the OWL group (P=0.02).

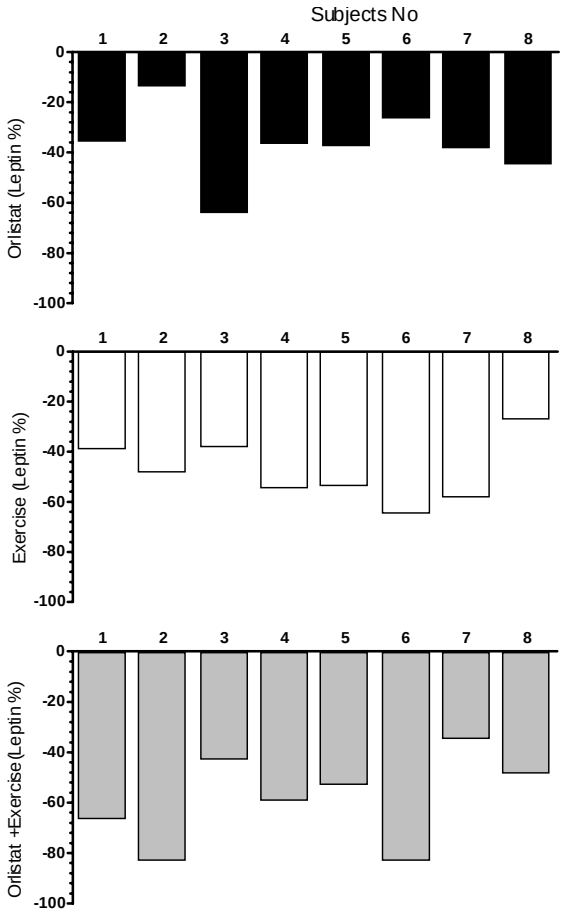

Fig. 2. The percentage decrease of leptin levels for each subject after the 12-week therapy period with orlistat (black columns), exercise (open columns) and orlistat plus exercise (gray columns).

After 12 weeks of the experimental periods, serum leptin levels per kilogram of fat mass (ng/ml/kg FM) were significantly decreased in all study groups: –48.2±7.2 % (P= 0.01, OEWL), –27.8±4.8 % (P<0.01, OWL) and –39.3±4.3 % (P<0.01, EWL) (Fig. 3). The decrease in serum leptin levels per kilogram of fat mass was significantly higher in OEWL group compared to OWL group (P=0.01). However, there was no significant difference between OWL and EWL groups (P=0.4).

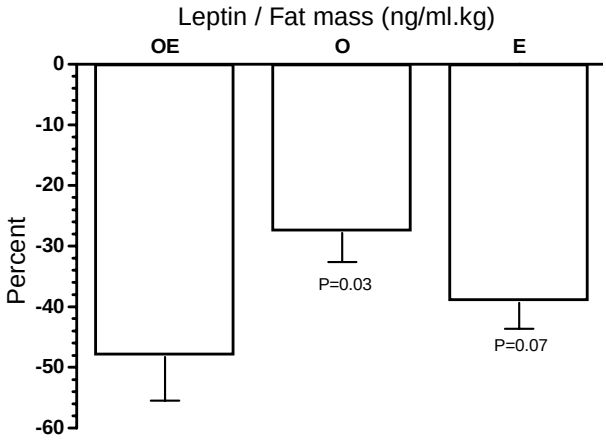

Fig. 3. The decrease in leptin levels per kilogram of fat mass after 12-week therapy period with orlistat plus exercise (OE), orlistat (O), and exercise (E). Data are means ± S.E.M.

Discussion

To our knowledge, this is the first study comparing the possible effects of weight loss induced by orlistat, exercise training and a combination of orlistat and exercise training on serum leptin levels in obese females.

This study provides data showing that a 12-week of orlistat treatment, exercise training or a combination of orlistat therapy plus exercise caused marked reductions in body weight, fat mass and serum leptin levels in obese females (Colak and Ozcelik 2004). The interesting finding of this study is that a combination of orlistat plus exercise therapy seems to have more favorable effects on serum leptin levels and as well as on leptin levels per kilogram of fat mass, which reflects leptin resistance. Leptin may be considered as one of the end-point in efficiency of obesity management protocols not only because its association with obesity but also because of its regulatory role in food intake. Although leptin appears to exert its anti-obesity effect through its central and peripheral actions, the full spectrum of its action is yet to be determined (Janečková 2001, Jequier 2002). The understanding of leptin functions and factors affecting leptin metabolism seems to be one of the important issues in obesity management (Walder and de Silva 2000). However, leptin administration has not been proven an effective method in obesity management yet. Many investigators have examined the leptin levels in response to weight loss induced by the diet, exercise and their

combinations (Considine *et al.* 1996, Perusse *et al*. 1997, Wadden *et al*. 1998, Kraemer *et al*. 1999, Sartorio *et al*. 2003). However, few studies have compared the relationships between leptin levels and pharmacotherapy or a combination of pharmacotherapy and exercise-induced weight loss.

The anti-obesity drug orlistat inhibits both gastric and pancreatic lipases. It reduces the absorption of dietary fat from intestine and may lower serum cholesterol and triglyceride levels (Rossner *et al.* 2000). It is therefore plausible that orlistat may have a decreasing effect on serum leptin levels. By examining the leptin levels in cerebrospinal fluid it has been shown that orlistat provides an advantage compared to centrally acting anti-obesity drugs (Rodrigues *et al*. 2002). In the present study, we have found a greater decrease in leptin levels (36.3 %) compared to the decreases in body weight (8.9 %) and fat mass (13.8 %) in the OWL group. However, the magnitude of decrease in serum leptin was not significantly different in the OWL and the EWL group. We have found a marked decrease in serum leptin levels (49.2 %) after a 12-week aerobic training period. Training may alter leptin levels, although reduced body fat may be responsible for such adaptations rather than direct effect of physical exercise itself (Perusse *et al*. 1997, Kraemer *et al*. 1999, Sartorio *et al*. 2003). It has been reported that aerobic exercise training without causing any weight loss did not alter leptin levels in overweight women (Kraemer *et al*. 1999). The physical activity is known to have an effect on sympathetic activity (Björntorp 1987) which is also associated with the improvements in glucose tolerance and insulin sensitivity (Bonen 1995). Factors, which cause an increase in sympathetic nerve activity and improve in glucose tolerance and insulin sensitivity, can decrease leptin levels (Shimabukuro *et al*. 1997, Slieker *et al*. 1996). A combination of orlistat and exercise training caused marked decreases in fat mass (21.6 %), serum leptin levels (57 %) and serum leptin levels expressed per kilogram of fat mass (48.3 %). The extent of decrease in fat mass, serum leptin levels and serum leptin levels expressed per kilogram of fat mass achieved by OEWL was significantly greater than those found in the OWL and EWL groups.

Calorie restriction may have an effect on acute changes in serum leptin levels (Wadden *et al*. 1998). It has been reported that acute calorie restriction can decrease leptin levels out of proportion to the decrease in fat mass (Keim *et al*. 1998). However, this was not the case in our study in which 1200-1600 kcal/day diet was given to all study groups. In previous studies, an increase in leptin resistance as a result of consuming a diet containing high fat has been reported in rodents (Frederich *et al*. 1995, van Heek *et al*. 1997). The observation of high serum leptin levels in obese patients could indicate leptin resistance. On the contrary, the high decrease in leptin levels compared to fat mass loss achieved after 12-week period could have an inverse effect on leptin resistance, i.e. normalization of leptin function. Fat mass loss triggers a decrease in serum leptin levels, and marked reduction of leptin levels per kilogram of fat mass after the 12-week therapy period may reflect restoration of leptin sensitivity (Considine *et al*. 1996, Lerario *et al*. 2001, Pilcová *et al*. 2003).

Consequently, an exercise training program in adjunct to pharmacotherapy provides higher amount of weight reduction and fat mass loss in obesity treatment. Importantly a combination of orlistat and exercise training has further beneficial effects on leptin levels which is considered as one of the end point in the efficiency of obesity management protocols. However, further studies are required to understand the factors involved in potentiating leptin metabolism, which is thought to have an important role in the development and treatment of obesity.

Acknowledgements

We are deeply grateful to Aysel Bektas, BSc, MPhil, Nurse in the Exercise Endocrine Unit, Firat Medical Center, University of Firat (Elazig, Turkey), for her assistance in blood sampling.

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