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Exercise Training as an Adjunct to Orlistat Therapy Reduces Oxidative Stress in Obese Subjects

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OZCELIK, O., OZKAN, Y., KARATAS, F. and KELESTIMUR, H. *Exercise Training as an Adjunct to Orlistat Therapy Reduces Oxidative Stress in Obese Subjects*. Tohoku J. Exp. Med., 2005, **206** (4), 313-318 —— The anti-obesity drug orlistat promotes weight loss and improves obesity-related risk factors, but its effect on oxidative stress is not clear yet. Orlistat reduces dietary fat absorption, which may have effects on fat soluble vitamins especially the antioxidant vitamins A and E. The aim of this study was to determine and compare the effects of weight loss achieved by orlistat therapy and a combination of orlistat with aerobic exercise training on lipid peroxidation and antioxidative defense in obese subjects. Total of 24 obese subjects were randomly assigned to receive 12-week treatment with hypocaloric diet-orlistat (120 mg three times daily) (DO group) or diet-orlistat-exercise (DOE group). Serum levels of malondialdehyde (MDA), a marker for lipid peroxidation, and vitamins A and E were measured by high performance liquid chromatography at baseline and at the end of the treatment. Body weight and fat mass were significantly reduced in the two groups ($p < 0.001$). In the DO group, the MDA levels remained unchanged ($p = 0.59$), while vitamins A ($p < 0.01$) and E ($p < 0.001$) were significantly decreased. In contrast, the subjects treated with DOE exhibited marked decreases in MDA ($p = 0.002$) and a small but significant decrease in vitamins A ($p = 0.003$) and E ($p = 0.003$). Thus, orlistat therapy alone caused a significant reduction in antioxidative capacity without affecting oxidative stress, whereas orlistat in combination with exercise training provided a significant decrease in MDA levels. The beneficial effect of aerobic exercise as an adjunct to the orlistat therapy is of importance with regard to the obesity-associated risk factors. ———— obesity; body mass index; MDA; orlistat; exercise

Obesity increases risk for many common diseases, including type 2 diabetes mellitus, systemic hypertension, dyslipidemia and coronary artery diseases (Calle et al. 1999). Although the etiology of obesity is complicated and not well understood, various factors, including interactions of genetic, metabolic, nutritional, cultural and psychosocial are thought to be the major determinants in obesity etiology. Current strategies for preventing and treating obesity involve diet, exercise, pharmacotherapy and their combinations (Bray and Greenway 1999). The commonly accepted primary target for the obesity management program is based on the achievement of 5-10%

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weight loss from the initial body weight, which is often sufficient to reduce obesity related risk factors (Lindgarde 2000; Mertens and Van Gaal 2000).

Orlistat (tetrahydrolipstatin, Xenical™) is a widely used peripherally acting anti-obesity drug. It promotes weight loss by reducing 30% of fat absorption from the intestine through a gastric and pancreatic lipases inactivation (Curran and Scott 2004). The specific effects of orlistat on body weight and blood lipid parameters have been shown in previous studies (Van Gaal et al. 1998; Lindgarde 2000). However, the oxidative stress, which is a normal product of body processes, is another important point to be considered in obesity management. It has been reported that oxidative stress is markedly high in obese subjects compared to normal weight subjects (Keaney et al. 2003; Mutlu-Turkoglu et al. 2003). Yet an increased oxidative stress has been shown to be associated with many chronic progressive diseases (Cerutti 1994; Singal et al. 2001).

Effect of weight loss induced by orlistat therapy on oxidative stress and antioxidant system has not been well documented yet. Since orlistat reduces fat absorption from intestinal system, it may have effects on fat soluble vitamins including the antioxidant vitamins A and E (Melia et al. 1996; McDuffie et al. 2002). Therefore, the important issue that needs to be addressed in obesity management is whether oxidative stress is influenced by the weight loss induced by orlistat to reduce mortality and morbidity.

In the present study, we aimed to determine and compare the effects of weight loss achieved by orlistat therapy alone and a combination of orlistat therapy with aerobic exercise training program on lipid peroxidation and antioxidative defence in obese subjects.

Methods

The protocol of this study, designed according to Helsinki Declaration on human subjects, was approved by Institutional Ethics Committee. Informed written consents were obtained from each patient at the start of the study. The study was carried out on an out-patient basis with subjects returning to our center at weekly intervals to be weighed.

This study was conducted over a 12-week period by utilizing the weight loss program with orlistat alone or in combination with aerobic exercise training. Following a complete medical history, physical examination and basic laboratory studies conducted at the beginning of the study, only those subjects found to be free of severe medical problems were chosen for participation. A total of 24 obese patients (22 females and 2 males) who were treated for obesity at the Obesity Clinic, University of Firat Hospital, participated in the study. Patients with cortisol, thyroid, or insulin hormonal dysfunction, cardiovascular diseases, and those taking any medications known to affect body composition were excluded from the study.

During the 12-week study period, the patients were maintained on a nutritionally balanced mildly hypocaloric diet (1,200-1,600 kcal/day). The prescribed diet contained approximately 30% of calories from fat, 50% from carbohydrate, 20% from protein. The patients received dietary advice from a qualified dietitian. No vitamins were provided, and patients were specifically asked not to take any vitamin preparations, especially vitamins A, C, and E and any antioxidant therapy.

The patients were randomly divided into two groups (eleven females and a male patient for each group). One group (DO group 40.0 ± 3.1 yr, and 159.5 ± 1.5 cm) received orlistat 120-mg capsule tree times per day (120 mg prior to each meal), the dosage that has been shown to be the most effective (Van Gaal et al. 1998). The subjects in DO group were not given any instruction regarding exercise. The other group (DOE group 37.3 ± 2.4 yr and 159.0 ± 3.0 cm) performed a regular exercise training in addition to orlistat therapy. Each patient in DOE group performed an incremental ramp test using an electromagnetically braked cycle ergometer (LODE, Groningen, The Netherlands) to determine aerobic to anaerobic metabolic transition point (i.e., the anaerobic threshold). The training work rate was set to the anaerobic threshold and performed three times per week, over a 12-week period. Each training session lasted for approximately 45 min. Heart rate was monitored electrocardiograpically throughout the test.

Body composition was assessed using leg-to-leg bioelectrical impedance method (Body Fat Analyser, TBF 300 M, Tanita, Tokyo). The validity of this method in the measurement of body composition in obese patients has been documented (Utter et al. 1999).

Blood samples were drawn at study entry and at the

end of the 12-week therapy period. After an overnight fasting, venous blood sample was obtained from forearm in resting condition between 08:00 to 09:00 always approximately at the same time in the morning. The samples were separated by centrifugation and stored at –70°C until analyzed for malondialdehyde (MDA), and vitamins A and E.

The quantification was performed according to the method of Miller et al. (1984) utilizing High Performance Liquid Chromatography (HPLC). Separations were accomplished at room temperature with a Cecil liquid chromatography system (Series 1100) consisting a sample injection valve (Cotati 7125) with a 20 μl sample loop, an ultra-violet (UV) spectrophotometric detector (Cecil 68174), 326 and 296 nm for vitamins A and E, respectively. Integrator (Hewlett Packard 3395) and a Techsphere Octadecyl Silane-2 packed (5 μm particle and 80Å pore size) column (250 × 4.6 Inner diameter) with a methanol: acetonitril: chloroform (47: 42: 11, volume/volume) as a mobile phase at 1.0 ml/min flow rate.

The extraction of MDA was performed according to Cerhata et al. (1994). The supernatant was filtered and the free MDA level was determined by the method of Karatas et al. (2002). The Tecopak C18 HPLC reversed-phase column (10 μm particle size and 250 × 3.9 ID) was used for the detection of MDA levels. The MDA level was determined with mobil phase 30 mM potassium dihydrogen phosphate buffer, pH = 4 with phosphoric acid and methanol (65%-35% volume/volume) at 1.5 ml/min flow rate.

Data are presented as mean ± S.E.M. Mann Whitney's U-test was used to evaluate the statistical significance on differences between the groups. In addition, Wilcoxon Rank Test was used to evaluate the data in same group for the basal and 12-week values. The statistical differences were considered significant at $p < 0.05$.

Results

The effects of the 12-week weight loss protocols, orlistat alone and its combination with exercise training, on body weight and fat mass are given in Table 1. Total body weight loss was significant in both groups: 8.5% in DO ($p < 0.001$)

Table 1. *Body mass index (BMI), body weight (BW) and fat mass (FM) at baseline and at the end of the 12-week treatment with diet-orlistat (DO) and diet-orlistat-exercise (DOE)*

	DO (n = 12)		DOE (n = 12)	
	Baseline	12 weeks	Baseline	12 weeks
BMI (kg/m^2)	37.9 ± 1.2	34.7 ± 1.1*	39.1 ± 1.3	35.0 ± 1.2*
BW (kg)	96.9 ± 4.3	88.6 ± 4.1*	98.1 ± 4.5	88.0 ± 4.2*
FM (kg)	42.2 ± 2.9	36.6 ± 2.7*	42.7 ± 2.2	34.6 ± 2.2*

* Statistically significant compared to baseline ($p < 0.001$).

Table 2. *Serum levels of malondialdehyde (MDA) and vitamins A and E at baseline and at the end of the 12-week treatment with diet-orlistat (DO) and diet-oristat-exercise (DOE)*

	DO (n = 12)			DOE (n = 12)		
	Baseline	12 weeks	% change (p)	Baseline	12 weeks	% change (p)
MDA (μg/ml)	1.73 ± 0.1	1.67 ± 0.1^{NS}	-3.4 $p = 0.59$	1.79 ± 0.08	1.20 ± 0.08*	-33.3 $p = 0.002$
Vit A (μg/ml)	0.67 ± 0.09	0.37 ± 0.04	-44.7 $p < 0.001$	0.63 ± 0.08	0.43 ± 0.05*	-31.7 $p = 0.003$
Vit E (μg/ml)	6.13 ± 0.9	2.60 ± 0.2	-57.6 $p < 0.001$	5.38 ± 0.5	3.83 ± 0.4*	-28.8 $p = 0.003$

* Statistically significant differences between the two groups.
NS, not significant.

and 10.2% ($p < 0.001$) in DOE (Table 1). In addition, there was a marked decrease in fat mass in both groups: 13.2% ($p < 0.001$) in the DO group and 18.9% ($p < 0.001$) in DOE group (Table 1).

After the 12-week treatment, serum levels of MDA were decreased markedly (33.3%) compared with its baseline level in DOE group ($p = 0.002$). In contrast, there was no significant decrease (3.4%) in MDA levels in DO group ($p = 0.59$) (Table 2). Thus, the decrease in serum MDA levels was significantly different between the two groups ($p = 0.01$).

On the other hand, serum levels of vitamins A and E were decreased in DO group (44.7%, $p < 0.001$ and 57.6%, $p < 0.001$, respectively). There was a small but significant decrease in vitamin A (31.7%, $p = 0.003$) and E levels (28.8%, $p = 0.003$) in DOE group (Table 2). The decrease in vitamin A ($p = 0.04$) and E ($p = 0.04$) levels was significantly different between the two groups.

Discussion

Although much research has been conducted to investigate obesity and lipid peroxidation relationships, to our knowledge there are no satisfactory results concerning the effects of weight loss induced by orlistat therapy on lipid peroxidation in obese patients. The effective and successful treatment of obesity has to be one of the most difficult and complex task to challenge in clinical medicine. Reduction of body weight, preventing further weight gain and maintaining a lower body weight over the long term are the general goals of obesity management (Glazer 2001).

Orlistat in conjunction with diet has been proven effective and safe in long-term of obesity management (Lindgarde 2000; Mertens and Van Gaal 2000). In the present study, orlistat therapy combined with a dietary prescription helped patients to loss 8.5% of body weight and 13.2% of fat mass, which are comparable with report of previous short-term studies (Van Gaal et al. 1998; Aydin et al. 2004). One may expect that the achieved amount of weight loss during study period is in medically accepted optimal ranges and is often sufficient to reduce obesity related risk factors (Lindgarde 2000; Mertens and Van Gaal 2000). Despite proven success in providing weight loss, most of the current obesity management protocols have not been evaluated with respect to oxidative stress and lipid peroxidation, which are secondary but significant end points. In the present study, the weight loss induced by a short-term orlistat therapy combined with energy restricted diet did not have a significant effect on MDA, which is one of the most frequently used indicators of lipid peroxidation (Nielsen et al. 1997) (Table 2). This result is in contrary to the finding of Yesilbursa et al. (2005) who observed marked decreases in MDA in association with orlistat induced weight loss.

The high level of oxidative stress associated with increased lipid peroxidation may be one of the reasons why those who are overweight are at greater risk for developing heart diseases (Rumley et al. 2004). Considering abnormally high lipid peroxidation and its damage to the body cells, obesity management, providing reduction in MDA level, serves a significant advantage in obese patients. Decreases in lipid peroxidation following weight loss induced by dietary restriction in obese patients over a short period has been reported (Dandona et al. 2002). The present study provides data showing that weight loss induced by a short-term orlistat therapy may not provide significant beneficial effects on oxidative stress and even impair the expected beneficial effect of concurrently prescribed diet restriction. Orlistat may reduce the absorption of fat soluble vitamins including vitamins A and E, which contributes to the antioxidative capacity of the body (Melia et al. 1996; McDuffie et al. 2002). Indeed, employing a short term orlistat therapy, we observed a dramatic decrease in levels of vitamins A and E. Fat soluble antioxidant vitamins inactivate the toxic lipid peroxidation (Granado et al. 1998) and prevent the damage to body cell membranes (Diplock 1991).

Exercise training has been recognized as an important component of obesity treatment regimen. Our results confirmed that a combination of orlistat and aerobic exercise training appears to significantly reduce fat mass, reflecting increases in fat utilization (Tremblay et al. 1991). There

are several lines of evidence suggesting that exercise training is associated with activation of antioxidant enzymes in specific tissues, reduced oxidative stress and lipid peroxidation tissues (Spina et al. 1996; Marzatico et al. 1997; Miyazaki et al. 2001; Gunduz et al. 2004). That is, trained subject can perform higher work rate exercise with less oxidative stress (Leaf et al. 1999). Our finding showed that aerobic exercise training as an adjunct to the orlistat therapy aids in better coping with oxidative stress in obese patients, as indicated in marked decrease in resting MDA (Table 2), despite that acute exercise has been shown to cause increase in MDA levels (Marzatico et al. 1997).

It is important to emphasize that weight loss without improvement in oxidative capacity may not provide adequate level of health benefits for obese patients who have high oxidative stress. By achieving weight loss associated with higher fat mass loss and improved oxidative status as indicated by decreased MDA, aerobic exercise training as an adjunct to orlistat therapy may provide further benefits in obesity management. Considering marked decreases in antioxidant vitamins and high oxidative stress following a short-term orlistat therapy, one should include either including additional vitamins to diet, which will support to decreased oxidative system, or an aerobic exercise training program to reduce oxidative stress. Consequently, the weight loss strategies should be aimed to ameliorate oxidative stress in addition to weight loss in obese patients.

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