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PAPER

Lipid peroxides in obese patients and effects of weight loss with orlistat on lipid peroxides levels

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OBJECTIVE: Obesity is a well-known risk factor of atherosclerosis. Recent studies showed that obesity is associated with enhanced lipid peroxidation. The aim of this study is to investigate the effect of weight reduction with orlistat treatment on lipid peroxidation levels. We assessed lipid peroxidation by measuring the concentration of plasma malondialdehyde (MDA).
DESIGN: A randomized, controlled, open-label 6-month study.
SUBJECTS: In total, 36 obese (body mass index (BMI) $>30\,kg/m^2$) and 11 healthy age-matched control subjects were enrolled in the study.
MEASUREMENTS: Fasting glucose, triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol and MDA levels were measured in both groups. Obese subjects received orlistat, 120 mg three times daily together with hypocaloric diet. After 6 months of treatment laboratory tests were repeated.
RESULTS: MDA levels were significantly higher in obese patients than the control group ($P<0.0001$). After 6 months of treatment in obese subjects, the mean weight of the patients decreased by 6.8 kg, the BMI by $3.2\,kg/m^2$. Plasma MDA levels were significantly reduced by weight loss from 2 ± 0.77 to 0.89 ± 0.41 nmol/ml ($P<0.001$). BMI correlated with MDA levels at baseline ($r=0.6$, $P<0.0001$). Changes in BMI was positively associated with plasma MDA level reduction ($r=0.36$, $P<0.05$).
CONCLUSION: These results indicate that obesity is associated with increases in endogenous lipid peroxides. Our data show that the indicator of lipid peroxidation—MDA—falls markedly in association with weight loss with orlistat. The demonstration of decreased free radical generation has important implications for oxidative mechanism underlying obesity-associated disorders.
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Introduction

The incidence of atherosclerosis is significantly increased in obesity and it has been found that a high body mass index (BMI) was positively associated with the occurrence of coronary heart disease.[1,2] The association between coronary heart disease and obesity is likely due to a series of metabolic disorders such as insulin resistance, dyslipidaemia, hypertension and abnormalities in haemostatic functions.[3,4] However, these factors do not explain the complete process of atherogenesis in obese subjects.

Several studies support hypothesis that oxidation of low-density lipoproteins (LDL) promotes atherogenesis.[5,6] Oxidized LDL exerts several biological effects that may contribute to the initiation and progression of the atherosclerotic process. It is believed that the free radical process known as lipid peroxidation is involved in the oxidative modification of LDL. One of the most frequently used biomarkers providing an indication of lipid peroxidation level is the plasma concentration of malondialdehyde (MDA), one of several by-products of lipid peroxidation processes.[7] Recent studies showed that obesity is associated with enhanced lipid peroxidation.[8,9]

Pharmacological agents are often used in the treatment of obesity. Orlistat is an inhibitor of the gastrointestinal lipase that reduces the absorbtion of dietary fat.[10] Orlistat has been shown to reduce weight in obese subjects with an increased risk of cardiovascular disease.[11,12] In this study we investigated the effect of weight reduction with orlistat treatment on MDA levels, a marker of lipid peroxidation, in obese subjects.

Methods

A total of 36 nondiabetic obese patients (seven male and 29 female) with a mean age of 49.7 ± 8 y were studied. In total,

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11 nonobese patients (three male, eight female) with a mean age 46.2±7 y were enrolled into the study as a control group. Obesity is defined as a BMI greater than 30 kg/m^2.

Exclusion criteria included diabetes mellitus, receiving hormone replacement therapy, pregnancy, lactation, psychiatric or neurological disorders, alcohol abuse, a history or the presence of malignancy, coronary heart disease and cerebrovascular disease. Continuing use of antihypertensive medication was permitted provided that the dose had been stable for at least 3 months before entry into the study. None of the subjects were taking any medication known to influence lipid behavior.

All of these obese subjects had received advice on dietary restriction and lifestyle modification, but remained obese for at least 6 months before recruitment to the study. Informed consent was obtained from all participants after having the purpose, possible risks associated with the study explained to them.

Eligible subjects underwent an assessment including documentation of medical history, physical examination, anthropometric indices and measurement of laboratory variables. After an overnight fast, blood was drawn from an antecubital vein for determination of biochemical parameters and MDA levels.

All obese subjects were given orlistat 120 mg three times daily, with appropriate instructions and warnings about the adverse effects. Subjects returned to the clinic at monthly intervals. At each visit vital signs (systolic and diastolic blood pressure, heart rate), body weight, waist and hip circumferences were measured and BMI were calculated. Adverse events and drug tolerability were recorded throughout the study. Two patients were withdrawn from the study because of lack of compliance.

Laboratory tests were performed at baseline and at 6 months. Levels of glucose, total cholesterol and triglyceride were measured by standardized laboratory methods (Technicon Instruments Corporation, Tarrytown, NY, USA). HDL-cholesterol was quantified after precipitation of apo-B containing lipoproteins with dextran-sulfate and magnesium chloride. The LDL-cholesterol level was calculated with the Friedewald formula.[13]

Plasma MDA level was measured by high-performance liquid chromatography (HPLC) method (nmol/ml plasma).[14]

Statistical analyses

All the statistical analysis were performed by using SPSS 10.0 statistical package. Results are expressed as mean±standard deviation. Unpaired *t*-tests were used for comparisons of the variables between the obese and nonobese subjects. We used paired *t*-test for comparisons of the variables between baseline and 6 months of treatment in obese subjects. Correlations were determined by Spearman correlation coefficient method. *P*-values <0.05 were considered statistically significant.

Results

Table 1 shows the biochemical values, MDA level and anthropometric indices in obese and normal control subjects. The mean weight, BMI, waist and hip circumferences, plasma total cholesterol and MDA levels were higher in obese than in nonobese subjects. The fasting glucose, triglyceride, HDL cholesterol and LDL cholesterol level were not significantly different between obese patients and the controls.

Table 2 shows the biochemical values, MDA level and anthropometric indices before and after orlistat treatment in obese subjects. After 6 months of orlistat treatment in obese subjects, the mean weight of the patients decreased by 6.8 kg, the BMI by 3.2 kg/m^2. Waist and hip circumference were also decreased significantly ($P<0.001$). Plasma levels of total cholesterol, LDL cholesterol and triglyceride decreased by 11, 14 and 38% ($P<0.001$ for all changes). Weight reduction was associated with decreasing levels of MDA. Plasma MDA levels were significantly reduced by weight loss from 2.0±0.77 to 0.89±0.41 nmol/ml ($P<0.001$).

BMI correlated with MDA level at baseline ($r=0.6$, $P<0.0001$). MDA level also correlated with total cholesterol,

Table 1 Biochemical values, MDA level and anthropometric indices of obese and nonobese patients

	Obese (n = 36)	*Nonobese (n = 11)*	P-*value*
Age (y)	49.7±8	46.2±7	NS
Sex (male/female)	7/29	3/8	NS
Weight (kg)	91.5±9.8	61±5.2	<0.001
Body mass index (kg/m^2)	36.1±3.4	22.9±1.7	<0.0001
Waist circumference (cm)	105±9.7	69±6.4	<0.001
Hip circumference (cm)	114±17	97±9	<0.001
Waist–hip ratio	0.93±0.1	0.71±0.08	<0.001
Fasting glucose (mg/dl)	90±12	87±9	NS
Total cholesterol (mg/dl)	199±36	184±15	<0.05
LDL cholesterol (mg/dl)	125±30	110±13	NS
HDL cholesterol (mg/dl)	45±6.2	45±4.8	NS
Triglyceride (mg/dl)	155±74	141±36	NS
MDA (nmol/ml)	2.0±0.77	0.63±0.14	<0.0001

MDA = malondialdehyde; NS = nonsignificant.

Table 2 Biochemical values, MDA level and anthropometric indices of obese patients before and after 6-month treatment with orlistat

	Baseline	*6 months*	P-*value*
Weight (kg)	91.5±9.8	84.7±10.1	<0.001
Body mass index (kg/m^2)	36.1±3.4	32.9±3.7	<0.001
Waist circumference (cm)	105±9.7	99±9.8	<0.001
Hip circumference (cm)	114±17	107±16	<0.001
Waist–hip ratio	0.93±0.1	0.92±0.1	NS
Glucose (mg/dl)	90±12	89±8	NS
Total cholesterol (mg/dl)	199±36	178±32	<0.001
LDL cholesterol (mg/dl)	125±30	107±25	<0.001
HDL cholesterol (mg/dl)	45±6.2	44±6.8	NS
Triglyceride (mg/dl)	155±74	134±52	<0.001
MDA (nmol/ml)	2.0±0.77	0.89±0.41	<0.001

MDA = malondialdehyde; NS = nonsignificant.

triglyceride and LDL cholesterol at baseline ($r=0.45$, $P<0.002$; $r=0.33$, $P<0.05$; $r=0.37$, $P<0.01$, respectively).

Changes in BMI was positively associated with plasma MDA level reduction ($r=0.36$, $P<0.05$).

Adverse effects were limited to the gastrointestinal tract. Gastrointestinal tract events were reported in seven patients (oily stool in three patients, increased defecation in two and oily spotting in two patients) and all of them were mild to moderate. No subject withdrew from the study because of adverse effects to the gastrointestinal tract.

Discussion

Obesity is associated with an increased risk of developing atherosclerosis. Oxidative modification of lipoproteins may play an important role in the pathogenesis of atherosclerosis. Lipid peroxidation is involved in the oxidative modifications of low-density lipoproteins and this ultimately results in the formation of atherosclerotic lesions. Malondialdehyde is one of the most frequently used indicators of lipid peroxidation. Previous studies have shown that the mean MDA levels are higher in obese individuals compared to nonobese healthy controls.[15] It is also shown that obesity is associated with increases in endogenous lipid peroxides and oxidation of low-density lipoproteins.[16] In another study it was demonstrated that lipoprotein oxidizability is enhanced in obese young women, uncomplicated by hypertension, hypercholesterolaemia, diabetes or coronary heart disease.[17] In our study we also found high MDA levels in obese subjects than in nonobese ones. Whether the increased lipoprotein oxidizability is due to enhanced oxidant challenge, or decreased antioxidant content, or changed lipoprotein composition is not fully explained. In obesity a reduced antioxidant enzyme activity of skeletal muscle, which may protect against lipid peroxidation has been observed.[18] It was also demonstrated that tumor necrosis factor-α (TNF-α) concentrations are elevated in the obese[19] and the increased concentrations of TNF-α may stimulate reactive oxygen species generation by leukocytes.[20]

A weight loss in the order of 5–10% is associated with clinically meaningful reductions with respect to all comorbidities.[21] Diet and exercise have been the cornerstone of weight management therapy. Vasankari *et al*[22] investigated the effect of weight reduction with diet and exercise on LDL oxidation in obese premenopausal women. In their study they found a 4% decrease in oxidized LDL concentration with a 13 kg decrease in body weight. However, diet and exercise have limitations, especially for weight maintenance. Pharmacological agents are often used in treatment of obesity. The weight reduction with orlistat was associated with a significant improvement in the control of cardiovascular-risk factors.[11,12] In our study we demonstrate that the reduction of weight loss achieved with orlistat was accompanied by decreasing levels of MDA in obese subjects. However, it is not certain whether orlistat has a direct effect on MDA level independent of weight reduction.

Another study failed to show decreases in isoprostanes, which is a lipid peroxidation marker, during weight reduction with orlistat.[23] They explained this result by the presence of other cardiovascular risk factors, or the concomitant pharmacological treatment that might influence isoprostane levels.

It was shown that surgical weight loss is also associated with reduction of the lipid peroxide levels.[24]

The present study has some limitations. This study was not a placebo-controlled trial. For this reason we cannot say the effect of orlistat on lipid peroxide levels was direct. It was also shown that weight loss with a dietary restriction induce a significant decrease in reactive oxygen species generation by leukocytes.[25] However the efficacy of orlistat on weight reduction has been demonstrated in obese patients in previous studies.[11,12] For many patients weight loss is difficult to achieve with a dietary restriction and lifestyle modification. Pharmacologic treatment is usually beneficial in obese patients for weight loss.

As a result, these results indicate that obesity is associated with increases in endogenous lipid peroxides. The indicator of lipid peroxidation—MDA—decreased with weight loss. Our data show that free radical generation falls markedly in association with weight loss with orlistat. The demonstration of decreased free radical generation has important implications for oxidative mechanism underlying obesity-associated disorders.

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