Orlistat Inhibits Dietary Cholesterol Absorption

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Abstract

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Objective: Orlistat decreases the absorption of dietary triglycerides by inhibiting intestinal lipases. Orlistat therapy is associated with a greater decline in plasma low-density lipoprotein-cholesterol concentrations than that expected from weight loss alone. Therefore, we evaluated the effect of orlistat treatment on dietary cholesterol absorption as a possible mechanism for the independent effect of orlistat on plasma cholesterol concentration.

Research Methods and Procedures: Cholesterol absorption from a standardized meal, containing 72 mg of cholesterol, was determined in 18 subjects with class II abdominal obesity (BMI, 35.0 to 39.9 kg/m^2) by simultaneous administration of intravenous ([$^2H_6$] cholesterol) and oral ([$^2H_5$] cholesterol) cholesterol tracers. In protocol 1 ($n = 9$), cholesterol absorption was determined on two different occasions, 10 to 20 days apart, to assess the reproducibility of the tracer method. In protocol 2 ($n = 9$), cholesterol absorption was determined with and without orlistat therapy in a prospective, randomized, crossover design to assess the effect of orlistat on cholesterol absorption.

Results: In protocol 1, cholesterol absorption from the test meal was the same on both occasions (53 $\pm$ 5% and 51 $\pm$ 5%). In protocol 2, orlistat treatment caused a 25% reduction in cholesterol absorption, from 59 $\pm$ 6% to 44 $\pm$ 5% ($p < 0.01$).

Discussion: These data demonstrate that orlistat inhibits dietary cholesterol absorption, which may have beneficial effects on lipoprotein metabolism in obese subjects that are independent of weight loss itself.

Key words: orlistat, cholesterol, stable isotope, weight loss

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Introduction

Obese persons, particularly those with abdominal obesity, are at increased risk for developing abnormalities in serum lipids, including hypertriglyceridemia, increased serum total and low-density lipoprotein (LDL) cholesterol concentrations, an increased proportion of small, dense LDL particles, and low-serum high-density lipoprotein (HDL) cholesterol (1,2). These abnormalities are clinically important because of their causal relationship with coronary heart disease (3–5). Weight loss is recommended for obese patients with dyslipidemia because it can decrease serum triglyceride, total cholesterol, and LDL cholesterol concentrations, and it increases serum HDL-cholesterol concentration (6,7). The amount of improvement in serum lipids is directly related to the amount of weight lost (7).

The key principle in achieving weight loss in obese persons is to modify lifestyle behaviors to decrease energy intake and increase physical activity. However, conventional behavior modification therapy has limited long-term success, and many obese patients who lose weight regain their lost weight over time (8). The failure to achieve permanent weight loss has led to increased interest in pharmacotherapy as an additional tool in treating obesity. All medications approved for obesity treatment, with the exception of orlistat, act as anorexiants. In contrast, orlistat decreases body weight by binding to intestinal lipases and blocking fat digestion and absorption (9,10). The results from several randomized clinical trials found that orlistat has a beneficial effect on serum cholesterol concentration that is independent of weight loss alone. Subjects given orlistat had a greater reduction in serum LDL-cholesterol concentrations than those given placebo, even after adjusting for the percentage of weight loss (11–13).

The mechanism(s) responsible for the additional cholesterol-lowering effect of orlistat is not known. A series of studies have demonstrated that cholesterol intake can affect serum cholesterol concentrations (14–20). Therefore, we hypothesized that the independent beneficial effect of orlistat on serum LDL cholesterol concentration may be mediated by inhibiting dietary cholesterol absorption. Accordingly, the aim of this study was to determine the effect of orlistat on the absorption of ingested cholesterol in obese persons. Cholesterol absorption from a test meal was evaluated by using a stable isotope tracer technique that we have

Table 1. Subject characteristics

	Protocol 1 (reproducibility) $n = 9$	Protocol 2 (orlistat) $n = 9$
Body weight (kg)	104 ± 4	109 ± 4
BMI (kg/m^2)	37 ± 1	37 ± 1
Waist circumference (cm)	107 ± 3	111 ± 3
Body fat (%)	48 ± 2	48 ± 3
Total plasma cholesterol (mg/dL)	211 ± 10	184 ± 7
LDL cholesterol (mg/dL)	135 ± 10	112 ± 4
HDL cholesterol (mg/dL)	51 ± 2	44 ± 4

Values are means ± SEM.

validated previously (21). This method has several advantages over previous methods because it does not involve radiation exposure (22,23), and it eliminates the need for cumbersome stool collections (24).

Research Methods and Procedures

Subjects

Eighteen subjects with class II (BMI, 35.0 to 39.9 kg/m^2) abdominal obesity (37 ± 3 years old; 3 men and 15 women; body mass index [BMI], 37 ± 1 kg/m^2; waist circumference, >100 cm) participated in this study. All subjects completed a comprehensive medical evaluation, which included a history and physical examination, an electrocardiogram, and standard blood and urine tests. None of the subjects had evidence of medical illnesses other than obesity, and none smoked tobacco. Fat mass and fat free mass were determined in each subject by DXA (Hologic QDR 1000/w, Waltham, MA). Fasting serum lipid and cholesterol concentrations were measured after stepwise ultracentrifugation by the Lipid Research Clinic Core Laboratory of Washington University School of Medicine (St. Louis, MO). Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine in St. Louis, MO.

Experimental Design

Two study protocols were performed. In protocol 1, the reproducibility of the cholesterol absorption test was evaluated by measuring cholesterol absorption in the same subjects on two occasions, several weeks apart. In protocol 2, the effect of orlistat on dietary cholesterol absorption was determined. One-half of the 18 subjects (eight women and one man) participated in protocol 1 and the other half (seven women and two men) participated in protocol 2 (Table 1).

Cholesterol Absorption Test Procedure

The following protocol was followed for each cholesterol absorption test. Subjects were admitted to the outpatient unit of the GCRC in the morning (between 6:30 and 7:30 AM), after they had fasted overnight. A catheter was placed in a forearm vein for blood sampling and infusion of a stable isotope-labeled tracer of cholesterol. After a baseline blood sample was obtained to determine background cholesterol enrichment, 15 mg of hexadeuterated cholesterol ([26,26,26,27,27,27-$^2H_6$] cholesterol; Medical Isotopes, Pelham, NH), dissolved in 4 mL of 10% Intralipid (Pharmacia, Inc., Clayton, NC), was given intravenously over 5 minutes. After tracer administration, the syringe containing the tracer solution was rinsed with saline, and the saline was infused through the existing intravenous line to ensure that the entire dose of tracer was given. Immediately after tracer administration, subjects ingested a test meal, which was prepared by the GCRC metabolic kitchen. This meal contained 510 calories and 72 mg of cholesterol (including the cholesterol tracer) and consisted of 240 mL of orange juice, 240 mL of whole milk, 21 g of corn flakes, and a 60-g bagel saturated with 2 g of corn oil containing 30 mg of pentadeuterated cholesterol ([2,2,4,4,6-$^2H_5$] cholesterol; Medical Isotopes). Subjects were discharged from the GCRC after consuming the test meal. Three days later, subjects returned to the GCRC in the morning (between 6:30 and 7:30 AM), after they had fasted overnight. A blood sample was obtained to measure plasma isotopic enrichment of the two cholesterol tracers.

Cholesterol tracer given orally peaks in plasma within 2 days and then slowly declines parallel to the intravenous tracer (21). Determination of the ratio of the two cholesterol tracers in plasma after 3 days of equilibration in the rapidly miscible pool of body cholesterol allows the calculation of the fractional absorption of cholesterol from the test meal. The simultaneous administration of intravenously and orally delivered tracers allows measurement of changes in plasma cholesterol absorption that are not confounded by any changes in cholesterol pool size.

Protocol 1 (Reproducibility)

Cholesterol absorption from a test meal was measured in each subject on two different occasions, 10 to 20 days apart. This amount of time is sufficient to prevent a carryover effect from the previous cholesterol absorption test. Most of the cholesterol tracer in plasma is eliminated within 10 days after oral or intravenous (IV) administration (21). Subjects were instructed to consume their normal weight-maintaining diet for the duration of the entire study.

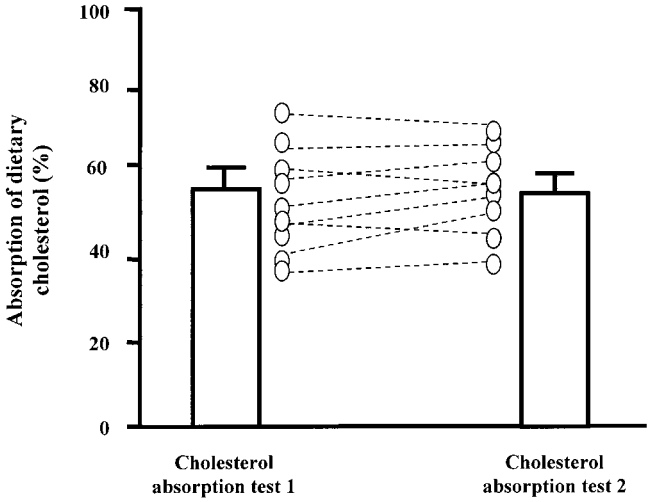

Figure 1: Reproducibility of cholesterol absorption from a test meal containing 72 mg of cholesterol (protocol 1). Nine subjects were studied twice, ~2 weeks apart.

Protocol 2 (Orlistat)

Dietary cholesterol absorption was determined on two occasions, once with and once without simultaneous ingestion of orlistat (120 mg; Hoffman LaRoche, Nutley, NJ) with the test meal. The order of tests (with or without orlistat) was randomly assigned in a prospective crossover design. Subjects were given orlistat (120 mg three times daily with meals) for 3 days before the cholesterol absorption test with orlistat was performed to allow adjustment to the drug and to simulate the effect of orlistat on cholesterol absorption that would occur during drug therapy rather than at initial drug therapy.

Preparation of Cholesterol Tracers

[26,26,26,27,27,27-$^2H_6$] Cholesterol for IV administration was dissolved in USP ethanol (20 mg of tracer/mL ethanol) and filtered through a 0.2-μm solvent-resistant filter (Millex-FG; Millipore, Bedford, MA). The limulus colorimetric assay (Bio-Whittaker, Walkersville, MD) was used to test for pyrogens and an aliquot of the solution was tested for sterility by routine culture. The tracer solution was gently mixed in warm (37 °C) 10% Intralipid. The preparation was allowed to cool to room temperature before being passed through a 1.2-μm particulate filter (IV6120; EPS, Feasterville, PA). The preparation to be infused (freezing point: −18 °C) was stored at −12 °C for up to 4 weeks in a commercial freezer that was modified with an A319 electronic temperature control unit (Johnson Controls, Milwaukee, WI) to maintain temperature to within ±1 °C. Standard refrigeration equipment was unsuitable because of excessive temperature variation and occasional freezing of the tracer with partial disruption of the intralipid particles. The infusion material was refiltered the day before each use. [2,2,4,4,6-$^2H_5$] Cholesterol for oral administration was dissolved in corn oil (15 mg of tracer per 1 g of oil) and the solution was mixed by continuous rotation for 12 hours at room temperature.

Sample Analyses

Total plasma cholesterol enrichment was determined by using gas chromatography-mass spectrometry (Hewlett Packard 5988A gas chromatography-mass spectrometer fitted with a 5-m × 0.53-mm i.d., 1-μm film thickness Xti-5 capillary column; Hewlett Packard, Palo Alto, CA) as previously described (21,25). Plasma was saponified (26) and the nonsaponifiable sterols were extracted into petroleum ether and converted to pentafluoro-benzoyl esters. Cholesterol ions were generated by negative chemical ionization, and ions at mass-to-charge ratios 581 (M + 1 of cholesterol pentafluorobenzoate), 585 (M + 5), and 586 (M + 6) were selectively monitored to determine the tracer-to-tracee ratio (TTR) of labeled and unlabeled free cholesterol in plasma.

Calculations

The percentage of cholesterol absorption from the test meal was calculated as the ratio of the two cholesterol tracers in plasma on day 4 divided by the mole ratio administered as follows.

$$\%\ \text{absorption} = \frac{\text{TTR}_{\text{oral}}}{\text{TTR}_{\text{IV}}} \times \frac{\text{IV tracer dose}}{\text{oral tracer dose}} \times 100$$

where TTR oral and TTR_{IV} represent the TTRs of the orally and intravenously administered tracers in plasma cholesterol.

Statistical Analysis

A power analysis, based on our previous data (25), suggested that six subjects would be needed to detect a 25% difference in cholesterol absorption with an α value of 0.05 and a power of 0.80.

A Student's *t* test for paired samples was used to evaluate the reproducibility of cholesterol absorption (protocol 1) and the effect of orlistat on cholesterol absorption from the test meal (protocol 2). Statistical significance was accepted at $p \leq 0.05$. All data are expressed as means ± SEM.

Results

Protocol 1 (Reproducibility)

Cholesterol absorption from the test meal on the two different occasions was 53 ± 5% and 51 ± 5%. Dietary cholesterol absorption determined during the second test in each subject was 99 ± 10% of the value measured during the first test (Figure 1).

Protocol 2

Basal cholesterol absorption from the test meal (without orlistat) was 59 ± 6%. Cholesterol absorption from the test

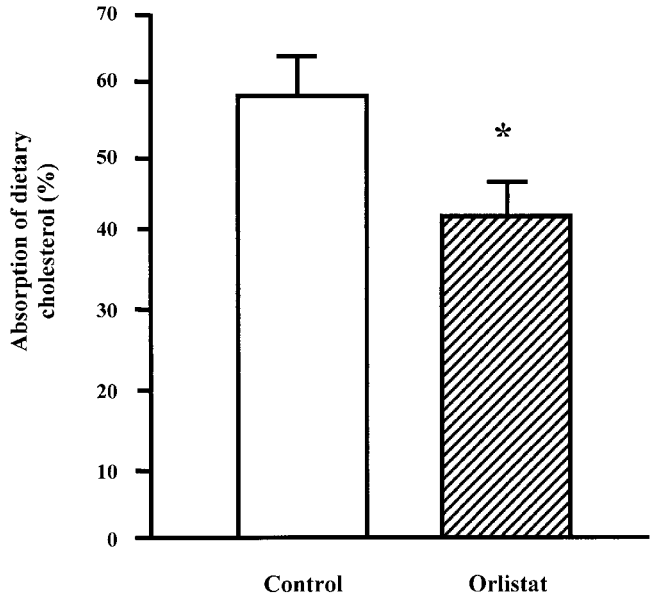

Figure 2: Effect of orlistat on cholesterol absorption from a test meal containing 72 mg of cholesterol (protocol 2). Nine subjects were studied twice (with and without orlistat), ~2 weeks apart. Values are means ± SEM. *Value different from control value, $p < 0.01$.

meal given concomitantly with orlistat was 44 ± 5% (Figure 2). Therefore, orlistat decreased the absolute amount of cholesterol absorbed from the test meal by 23 ± 5% (from 43 to 32 mg; $p < 0.01$).

Discussion

Data from several randomized, controlled trials suggest that orlistat therapy in obese subjects causes a greater decline in serum total and LDL cholesterol concentrations than that expected from weight loss alone (11–13). However, the mechanism(s) responsible for the independent effect of orlistat on plasma cholesterol is not known. In this study, we evaluated the hypothesis that orlistat inhibits dietary cholesterol absorption. Our results demonstrate that orlistat decreases the absorption of ingested cholesterol by 25% in subjects with class II abdominal obesity (BMI, 35.0 to 39.9 kg/m^2). This finding suggests that, in addition to weight loss-mediated changes in serum lipids, orlistat has beneficial effects on serum total and LDL cholesterol concentrations by reducing dietary cholesterol absorption.

Presumably, the effect of orlistat on cholesterol absorption is related to its action on intestinal lipases and impairment of fat digestion. Cholesterol is absorbed by becoming incorporated into mixed micelles, which can then pass through the thin water layer lining the small intestine to reach the luminal surface of the enterocyte. The collision of micelles with the enterocyte cell membrane results in cholesterol uptake by gut epithelia (27). Inhibition of fat digestion prevents micelle formation, and therefore, cholesterol absorption. In this study, we found that the relative reduction in cholesterol absorption induced by orlistat (~25%) is similar to the reported relative reduction in fat absorption (~30%) (10).

Data from studies that evaluated the effect of orlistat on fat absorption suggest that it is unlikely that the interpretation of our results would be affected by the amount of orlistat given, the cholesterol content of the meal, or the duration of therapy. The relationship between orlistat dose and fat absorption is curvilinear, so that increasing the dose of orlistat above 120 mg does not cause additional decreases in fat absorption (9,10). In addition, altering the amount of ingested fat, within the range of normal dietary intakes, does not change the percentage of dietary fat that is malabsorbed by orlistat treatment (9,10). It is also unlikely that the effect of orlistat on cholesterol absorption would diminish with time, because the potency of orlistat on fat absorption remains the same after 44 weeks of treatment (28).

The findings from the most carefully controlled prospective studies demonstrate that dietary cholesterol intake affects plasma cholesterol concentration, particularly in genetically susceptible persons (14–20). Up-regulation of hepatic LDL receptors is the likely mechanism responsible for the cholesterol-lowering effect of a low-cholesterol diet. Cholesterol that is absorbed by the small intestine is incorporated within chylomicrons and delivered into the systemic circulation. Adipose tissue and muscle endothelial lipoprotein lipase hydrolyze and deplete the triglyceride component of chylomicrons, leaving behind cholesterol-rich chylomicron remnants. These remnants are taken up by the liver through LDL receptor-mediated endocytosis (29,30). Hepatic cholesteryl ester content regulates LDL receptor activity, so that when hepatocyte cholesterol content is low, LDL receptor expression, and consequently, LDL receptor activity is up-regulated (29,30). Therefore, it is likely that the decrease in cholesterol absorption and delivery to the liver contribute to the orlistat-induced reduction in serum cholesterol concentration.

The effect of orlistat on cholesterol absorption could have important clinical benefits in obese patients by lowering plasma LDL cholesterol concentrations. Elevated plasma LDL cholesterol concentration is a major risk factor for coronary heart disease (3,5). It has been estimated that every 100-mg decrease in dietary cholesterol results in a 5-mg/dL decrease in plasma cholesterol (16). For example, a 25% orlistat-induced decrease in cholesterol absorption in an obese patient with a plasma cholesterol concentration of 180 mg/dL, who consumes 300 mg of cholesterol per day, could cause a 2% decline in plasma cholesterol concentration. Data from large clinical trials have shown that every 1% decrease in plasma cholesterol concentration results in a 2% decrease in the incidence of coronary heart disease (31).

Some drugs that are used for the treatment of hypercholesteremia have been shown to interfere with the absorption of dietary cholesterol. For example, clofibrate treatment reduces dietary cholesterol absorption by ~10%, whereas cholestyramine can reduce cholesterol absorption by up to 35% (32). However, orlistat is the first drug used for the treatment of obesity that decreases dietary cholesterol absorption. This effect may be particularly beneficial in obese patients because of their increased risk for hypercholesterolemia (2). The results of this study suggest that, in addition to decreasing body weight and fat absorption (16,30), the effect of orlistat on dietary cholesterol absorption contributes to its beneficial effects on plasma lipids.

In summary, the results of this study demonstrate that orlistat inhibits the absorption of dietary cholesterol. Therefore, the use of orlistat therapy in obese patients may have beneficial effects on lipoprotein metabolism that are independent of weight loss itself.

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