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ORIGINAL ARTICLE

Lipid-lowering therapy does not affect the postprandial drop in high density lipoprotein-cholesterol (HDL-c) plasma levels in obese men with metabolic syndrome: a randomized double blind crossover trial

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Summary

Introduction The postprandial lipid metabolism in metabolic syndrome patients is disturbed and may add to the increased cardiovascular risk in these patients. It is not known whether postprandial high density lipoprotein-cholesterol (HDL-c) metabolism is also affected and whether this can be influenced by statin and/or ezetimibe treatment.

Methods Prospective, randomized, double blind, crossover trial comparing simvastatin 80 mg with simvastatin/ezetimibe 10 mg/10 mg treatment for 6 weeks on postprandial HDL-c metabolism in 15, nonsmoking, male, obese metabolic syndrome patients (Adult Treatment Panel III, ATPIII). Only study medication was allowed. HDL-c concentrations, cholesteryl ester transfer (CET), CET protein (CETP) mass and adiponectin were measured before and after oral fat loading. ClinicalTrials.gov NCT00189085.

Results Plasma HDL-c levels remained stable during continuous fasting following an overnight fast. Pre-fat load HDL-c concentrations without treatment, after simvastatin and simvastatin/ezetimibe treatment were 1·15 ± 0·04, 1·16 ± 0·05 and 1·11 ± 0·04 mmol/l. Fat load induced a 11% drop in HDL-c plasma levels; 1·02 ± 0·05 mmol/l ($P < 0·001$) which was not affected by either therapy. Triglyceride levels during fat load were similar after both treatments. Total CET increased from 9·73 ± 0·70 to 12·20 ± 0·67 nmol/ml/h ($P = 0·004$). Four hours after fat loading CETP mass was increased while adiponectin levels were decreased, irrespective of treatment.

Discussion HDL-c levels decrease as CET increases after fat loading in obese metabolic syndrome patients. This is not influenced by either simvastatin or simvastatin/ezetimibe treatment. After fat loading, CETP mass and CET increased, and adiponectin decreased pointing towards a potential role for intra-abdominal fat. Decreased postprandial HDL-c levels may contribute to the increased cardiovascular risk in metabolic syndrome patients on top of already low HDL-c levels.

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Introduction

The clustering of cardiovascular risk factors associated with abdominal obesity (elevated blood pressure, elevated triglycerides and fasting glucose plasma levels and low plasma levels of high density lipoprotein-cholesterol (HDL-c) is often referred to as metabolic syndrome.[1] The prevalence of metabolic syndrome is increasing and metabolic syndrome subjects are at increased risk for cardiovascular morbidity and mortality and for type 2 diabetes.[1] The underlying pathophysiology of metabolic syndrome is not fully understood but insulin resistance and abdominal obesity, with associated alterations in adipocyte metabolism are main characteristics.[2,3]

Low plasma HDL-c, associated with abdominal obesity and insulin resistance[4,5] is a strong and independent risk factor for future cardiovascular events and mortality.[6] Apolipoprotein A1 (ApoA1), an essential component of HDL particles, is synthesized in both liver and intestine. Interaction of ApoA1 with the ABCA1 receptor on the luminal side of the vascular endothelium results in removal of cholesterol from the vessel wall and subsequent maturation of HDL particles.[7] A constant remodelling of HDL particles occurs through the action of both phospholipids transfer protein (PLTP) and cholesteryl ester transfer protein (CETP). CETP is, like adiponectin, visfatin, tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) produced by adipocytes[8] and is the main facilitator of cholesteryl

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ester transfer (CET) in exchange of triglycerides between ApoA1 (HDL) and ApoB-containing lipoproteins (VLDL, IDL and LDL).[9] In metabolic syndrome and insulin-resistant subjects, postprandial dyslipidemia is characterized by prolonged presence of atherogenic triglycerides-rich lipoproteins particles (TGR) in the circulation due to increased production of very low density lipoprotein (VLDL-c) and reduced lipolysis as a result of diminished lipoprotein lipase (LPL) activity.[10] Little is known about postprandial HDL metabolism in insulin resistant and obese metabolic syndrome patients. In small studies in healthy subjects[11] nonoverweight patients with coronary artery disease[12] or diabetes[13] and in habitual smoking subjects[14] fat loading induced a decrease in plasma HDL-c levels.

Statins, alone or in combination with ezetimibe, are the most commonly used lipid-lowering therapies for cardiovascular risk reduction. In general, both therapies increase fasting HDL-c plasma levels between 3 and 9% and ApoA1 levels with 6%.[15] However, atorvastatin did not affect a decrease in postprandial HDL-c plasma concentrations in male hypertriglyceridemic patients.[16] The effect of statin alone or in combination with ezetimibe on postprandial HDL-c in obese metabolic syndrome patients is not known.

Aim of the present study was (i) to investigate the effects of an oral fat load on HDL-c metabolism in obese patients with metabolic syndrome and (ii) to compare the effects of high-dose simvastatin monotherapy with the combination of low-dose simvastatin with ezetimibe on post-fat load HDL-c metabolism.

Subjects and methods

Subjects

A total of 32 nonsmoking male subjects, aged 18–70 years, were recruited by advertisement in a local newspaper which called for subjects with waist circumference > 102 cm. After screening, 15 subjects consented to participate in this HDL-c study and fulfilled the diagnostic criteria for metabolic syndrome according to the Adult Treatment Panel III (ATPIII) criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.[17] Metabolic syndrome was diagnosed if ≥ 3 of the following abnormalities were present:

1 abdominal obesity (waist circumference > 102 cm)
2 high blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic)
3 hypertriglyceridemia (serum triglycerides ≥ 1·70 mmol/l (150 mg/dl))
4 low HDL-c (serum HDL-c < 1·04 mmol/l (40 mg/dl))
5 high fasting glucose (fasting serum glucose ≥ 6·1 mmol/l (110 mg/dl))

Glucose level ≥ 7·8 mmol/l after a standardized oral glucose tolerance test was also regarded as fulfilling the glucose criterion. Patients with thyroid stimulating hormone (TSH) levels > 5·0 mU/l, with clinical symptoms of hypothyroidism, serum aspartate transferase (ASAT) or serum alanine transferase (ALAT) > 2 times the upper limit of normal or serum creatinine > 1·7 times the upper limit of normal were excluded. Patients with metabolic syndrome and either very high blood pressure (≥ 180/110 mmHg) or a body mass index (BMI) > 35 kg/m^2 were not included in the present study for medical and ethical reasons. These patients were referred to their general practitioner for (immediate) medical attention. Other relevant exclusion criteria were the presence of macrovascular disease, use of lipid-lowering medication and/or use of blood pressure-lowering medication since they may affect insulin sensitivity and adipocyte function[18] HbA1c > 6·5% or plasma triglycerides > 8·0 mmol/l. The local Ethics Committee approved the study and all participants gave their written informed consent. Given the fluctuations in fasting and postprandial HDL-c plasma levels in women due to fluctuating levels of oestrogens during menstrual cycles, we decided not to include women in the present study.

Study design

In this prospective, randomized, double blind, crossover trial patients received once daily simvastatin 80 mg or the combination of simvastatin 10 mg and ezetimibe 10 mg during 2 periods of 6 weeks (Fig. 1). Between both treatment periods patients had a washout period of 4 weeks, after which crossover of therapy occurred. Before the initial therapy and after each treatment period of 6 weeks an oral fat load was performed after an overnight fast of

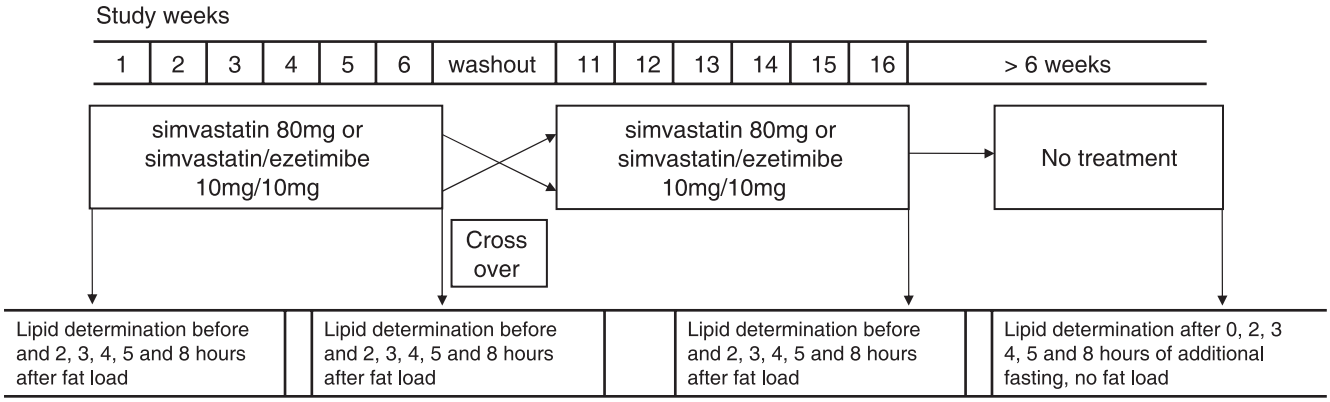

Fig. 1 Crossover study design.

at least 10 h. Randomization was performed at the pharmacy department of the institute with the use of subblocks, with four patients per subblock. Venous blood samples for plasma lipids determination were obtained in all 15 patients before and at 2, 3, 4, 5 and 8 h after the oral fat load. Further, plasma lipid levels were also determined during 8 h of additional fasting following an overnight fast of at least 10 h after a period of at least 6 weeks without lipid-lowering therapy. Plasma CETP mass was measured before and 3, 4 and 8 h after the oral fat load. The study was approved by the Ethical Review Board of the University Medical Center Utrecht (UMCU) and was carried out in a Good Clinical Practice-certified research unit. All subjects gave written informed consent.

Anthropometric measurements

Patient's weight and height were measured without heavy clothing and shoes. BMI was calculated as weight to height squared. Waist circumference was measured halfway between the lower rib and the iliac crest. Total body fat percentage was estimated by using Omron body fat monitor BF306 (Omron Matsusaka Co. Ltd., Matsusaka, Japan).

Oral fat load

For the fat load, fresh cream was used with a 40% (weight/volume) fat emulsion with a poly unsaturated : saturated fat ratio of 0·10, containing 0·001% (w/v) cholesterol and 3% (w/v) carbohydrates representing a total energy content of 3700 kCal/l. Cream was ingested at a dose of 50 g fat and 3·75 g glucose per m^2 body surface (with a maximum of 250 ml) within 5 min. Participants remained supine during the day and were only allowed to drink mineral water.

Laboratory assessments

In all 15 patients, fasting blood was sampled at each visit to determine plasma levels of glucose, creatinine, homocysteine, ALAT, ASAT, total cholesterol, LDL-c and HDL-c, triglycerides, free fatty acids and high sensitive C-reactive protein (hs-CRP). ApoE genotyping, TSH and HbA1c were measured only at the first visit. Plasma was isolated by immediate centrifugation for 15 min at 800 ***g*** at 4 °C before storage at –80 °C. Plasma cholesterol, triglycerides, HDL-c and LDL-c, ApoA1 and ApoB were measured using commercially available assays (Wako, Osaka, Japan) on a Cobas Mira auto-analyser (ABX). All other blood samples were measured in a local research facility according to standardized ISO 9001 : 2000 regulations. Total serum CET was assayed as described previously.[19] In brief, [^{3}H] cholesterol was equilibrated for 24 h with plasma-free cholesterol at 4 °C followed by incubation of the sample at 37 °C for 3 h. Subsequently, VLDL + LDL were precipitated by addition of phosphotungstate/$MgCl_2$. Lipids were extracted from the precipitate and the cholesteryl esters were isolated on silica columns and radioactivity was counted. A two-antibody sandwich immunoassay of CETP was set up according to Mezdour *et al.*[20] with major modifications as described below. The coating was performed with a combination of monoclonal antibodies TP1 (5 mg/ml in PBS) and TP2 (2·5 mg/ml) during an overnight incubation at 70 °C. To prevent nonspecific binding, plates were blocked with 1% BSA at room temperature for 2 h. Samples were tested in 20, 40 and 80-fold dilution whereas standard plasma was diluted from 10- to 160-fold with 0·1% Triton X-100 and 1% BSA in PBS. As a secondary antibody TP20, labelled with digoxigenine, was used. Antidigoxigenine Fab fragments coupled to peroxidase were added. Absorbance, after addition of TMB, was read after 30 min incubation and termination of the reaction with H_2O_2 at 450 nm. The interassay and intraassay coefficient of variance were 7·8% and 6·0%, respectively. Plasma adiponectin levels were determined by using a commercially available kit (Quantitative enzyme immunoassay technique, R & D Systems Inc, Minneapolis, MN).

Statistical analyses

Baseline characteristics are presented as mean ± SD. Fasting and post-fat load lipid levels, CET and CETP mass are expressed as mean ± SEM. The postprandial variations of lipids were integrated as area under the curve (AUC) (mean ± SEM) and were calculated by the trapezoidal rule using GraphPad Prism version 4·00 for Windows (GraphPad Software, San Diego, CA). Netto AUCs (dAUC) were calculated after correction for baseline values. Differences in AUC and dAUC between simvastatin 80 mg and combination therapy of simvastatin/ezetimibe 10 mg/10 mg were analysed by paired *t*-test; statistical significance was taken at the 5% level. Carry-over and period effects were calculated with independent samples *t*-test.[21] Calculations were performed using spss for Windows version 12·1 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics of study population

The baseline characteristics of the 15 patients of this study are shown in Table 1. Mean age was 55 ± 6 years and levels of metabolic syndrome parameters were: waist 110 ± 7 cm, systolic blood pressure 139 ± 13 mmHg, diastolic blood pressure 90 ± 6 mmHg, fasting triglycerides 1·59 ± 0·13 mmol/l, glucose 6·2 ± 0·7 mmol/l and fasting HDL-c 1·15 ± 0·04 mmol/l. The BMIs of the study patients ranged between 25·1 and 35·0 kg/m^2. None of the patients used any medication other than the study medication. Patients remained on a stable diet during the study. Weight, waist circumference and body fat percentage remained stable during the study.

HDL-c plasma concentrations during a prolonged fasting state and after oral fat loading

HDL-c plasma levels remained stable during 8 h of additional fasting after an overnight fast. At $t = 0$ HDL-c was 1·21 ± 0·06 mmol/l. The AUC was 10·2 ± 0·5 mmol h/l and after baseline correction 0·2 ± 0·1 mmol h/l (Table 2). HDL-c concentrations dropped 11·3% from 1·15 ± 0·04 to 1·02 ± 0·05 mmol/l after fat load reaching the lowest level after 4 h; $P < 0·001$ (Fig. 2a). Eight hours after the oral fat load HDL-c levels almost returned to baseline levels. The dAUC of HDL-c after oral fat load was –0·6 ± 0·1 ($P < 0·001$, compared to dAUC after additional fasting). In 8 of the 15 patients the fasting

Table 1. Baseline characteristics of the study population

	No treatment ($n = 15$)	After 6 weeks simvastatin 80 mg ($n = 15$)	After 6 weeks simvastatin/ ezetimibe 10 mg/10 mg ($n = 15$)
Age (years)	55 ± 6	–	–
Height (m)	1·83 ± 0·06	–	–
Weight (kg)	99·6 ± 12·1	100·1 ± 11·9	99·8 ± 12·0
Body mass index (kg/m^2)	29·7 ± 2·8	29·9 ± 2·7	29·8 ± 2·7
Body fat (%)	31 ± 3	31 ± 3	30 ± 3
Laboratory parameters			
ASAT (U/l)	34 ± 5	36 ± 9	36 ± 13
ALAT (U/l)	44 ± 15	48 ± 18	51 ± 19
Creatinin kinase (U/l)	102 ± 40	132 ± 66	108 ± 52
Creatinine clearance (ml/min)*	110 ± 20	120 ± 23	124 ± 21
TSH (mIE/l)	1·6 ± 0·8	–	–
HbA1c (%)	5·7 ± 0·4	–	–
Hs-CRP (mg/l)	3·1 ± 1·9	5·9 ± 3·6	5·4 ± 2·9
Total cholesterol (mmol/l)§	5·7 ± 0·2	3·8 ± 0·2‡	3·9 ± 0·2‡
LDL-cholesterol (mmol/l)§	3·8 ± 0·2	2·2 ± 0·1‡	2·2 ± 0·1‡
VLDL-cholesterol (mmol/l)§	0·70 ± 0·06	0·47 ± 0·07‡	0·53 ± 0·09
Apolipoprotein B (mg/dl)§	100 ± 4	70 ± 4‡	73 ± 5‡
Apolipoprotein A1 (mg/dl)§	114 ± 2	112 ± 4	107 ± 3
Insulin (mU/l)	18 ± 8	16 ± 9	18 ± 8
Homocysteine (µmol/l)	9·5 ± 1·6	8·9 ± 2·2	9·0 ± 2·4
HOMA-IR	5·1 ± 2·4	4·5 ± 2·4	4·9 ± 2·4
Components of the metabolic syndrome			
Glucose (mmol/l)	6·2 ± 0·5	6·2 ± 0·6	6·2 ± 0·7
Waist (cm)	110 ± 7	110 ± 5	110 ± 7
Systolic blood pressure (mmHg)	139 ± 13	139 ± 15	132 ± 9
Diastolic blood pressure (mmHg)	90 ± 6	88 ± 8	87 ± 5
Triglycerides (mmol/l)§	1·59 ± 0·13	1·12 ± 0·12†	1·28 ± 0·10‡
HDL-c (mmol/l)§	1·15 ± 0·04	1·16 ± 0·05	1·11 ± 0·04

*Cockcroft–Gault formula, †$P < 0·05$ *vs.* no treatment, ‡$P < 0·01$ *vs.* no treatment.
Mean ± SD, §Mean ± SEM.

Table 2. Area under the curve (AUC) of plasma lipids parameters after an overnight fast, with and without fat load and after oral fat loading with and without treatment

	Additional fasting, no fat load ($n = 15$)	No treatment, fat load ($n = 15$)	*P*-value*	After 6 weeks simvastatin 80 mg ($n = 15$)	After 6 weeks simvastatin/ ezetimibe 10 mg/10 mg ($n = 15$)	*P*-value†
AUC for lipid parameters						
Total cholesterol	48·2 ± 1·8	46·1 ± 1·8	0·06	30·1 ± 1·3	31·2 ± 1·6	0·47
Triglycerides	14·2 ± 1·4	19·3 ± 1·5	0·03	14·2 ± 0·9	16·3 ± 1·2	0·06
HDL-c	10·2 ± 0·5	8·6 ± 0·4	< 0·001	8·5 ± 0·3	8·4 ± 0·4	0·73
ApoA1	1047 ± 27	906 ± 19	< 0·001	880 ± 25	850 ± 24	0·35
Baseline-corrected AUC (dAUC) for lipid parameters						
Total cholesterol	0·3 ± 0·4	0·6 ± 0·5	0·48	−0·5 ± 0·6	0·4 ± 0·5	0·08
Triglycerides	0·0 ± 0·5	6·6 ± 0·8	< 0·001	5·3 ± 0·6	6·0 ± 0·7	0·40
HDL-c	0·2 ± 0·1	−0·6 ± 0·1	< 0·001	−0·7 ± 0·2	−0·4 ± 0·2	0·37
ApoA1	6·2 ± 8·1	–5·1 ± 8·1	0·36	–20·9 ± 16·3	–5·9 ± 11·0	0·69

Mean ± SEM.
**P*-value; additional fasting, no fat load *vs.* no treatment, fat load.
†*P*-value; simvastatin 80 mg *vs.* simvastatin/ezetimibe 10 mg/10 mg.

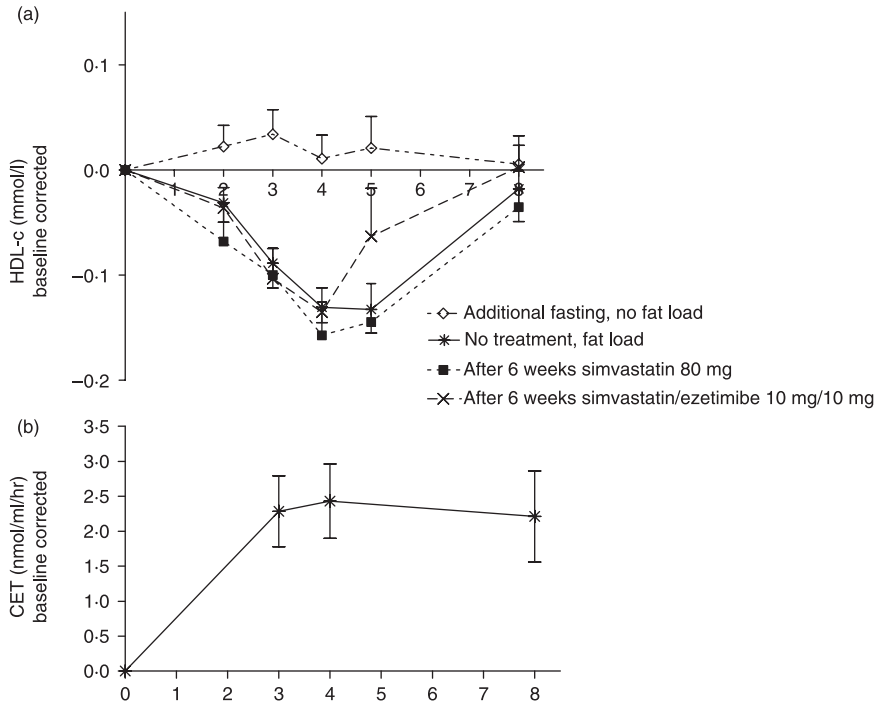

Fig. 2 (a) HDL-c baseline-corrected concentrations (mean ± SEM) after an overnight fast, with and without fat load and after oral fat loading with and without treatment. (b) Total cholesteryl ester transfer (mean ± SEM) after oral fat load without treatment.

Table 3. Fasting and post-fat load CETP and adiponectin levels

Hours after fat load	No treatment, fat load ($n = 15$)	After 6 weeks simvastatin 80 mg ($n = 15$)	After 6 weeks simvastatin/ezetimibe 10 mg/10 mg ($n = 15$)
CETP mass (μg/ml)			
0	1·65 ± 0·19	1·38 ± 0·12	1·30 ± 0·11
3	1·91 ± 0·20	1·61 ± 0·14	1·42 ± 0·13
4	1·59 ± 0·12	1·43 ± 0·12	1·32 ± 0·13
8	1·55 ± 0·16	1·48 ± 0·012	1·39 ± 0·14
Adiponectin (μg/ml)			
0	5·0 ± 0·6	4·8 ± 0·5	4·7 ± 0·5
3	5·0 ± 0·6	4·7 ± 0·5	4·3 ± 0·5
4	4·7 ± 0·6	4·5 ± 0·5	4·2 ± 0·5
8	4·5 ± 0·5	4·4 ± 0·05	4·4 ± 0·5

Mean ± SEM.

HDL-c plasma concentration was < 1·04 mmol/l, the cut-off level used in the NCEP ATPIII metabolic syndrome criteria. There was no difference in the post-fat load drop in HDL-c in patients with a fasting HDL-c concentration below or above 1·04 mmol/l. During prolonged fasting plasma ApoA1 concentration remained stable; the dAUC was 6·2 ± 8·1 mg h/dl. ApoA1 plasma levels decreased to a minimum at 5 h post-fat load (from 114·2 ± 2·0 to 111·8 ± 2·2 mg/dl), dAUC –5·1 ± 8·1 ($P = 0·36$, compared to dAUC after prolonged fasting) (Table 3). As expected, plasma triglycerides levels were significantly higher after fat load compared with prolonged fasting (dAUC 6·6 ± 0·8 compared to 0·0 ± 0·5, $P < 0·001$). The plasma concentrations of ApoB-containing lipoproteins remained also stable during 8 h of additional fasting following an overnight fast.

Effect of simvastatin 80 mg or combination of simvastatin/ezetimibe 10 mg/10 mg on fasting and post-fat load HDL-c plasma concentrations

The fasting HDL-c plasma level was 1·15 ± 0·04 mmol/l before the study and changed marginally after simvastatin (1·16 ± 0·05 mmol/l) or simvastatin/ezetimibe therapy (1·11 ± 0·04 mmol/l). Neither treatment regime did affect post-fat load plasma HDL-c or ApoA1 concentrations (Table 2). Fasting plasma LDL-c concentrations after both treatments decreased to exactly the same level (2·2 ± 0·5 mmol/l) from baseline (–43%). The increase of plasma triglyceride levels was not affected by either simvastatin 80 mg or simvastatin/ezetimibe 10 mg/10 mg (dAUC 5·3 ± 0·6 *vs.* 6·0 ± 0·7 mmol h/l, $P = 0·40$). No

carry-over or period effects between the two treatment periods were observed for fasting lipid concentrations or post-fat load lipid profiles.

CET and CETP mass after oral fat loading

Plasma CET in patients without study medication reached a maximum at 4 h post-fat load (from 9·73 ± 0·70 to 12·20 ± 0·67 nmol/ml/h, an increase of 25·4% ($P = 0·004$). Eight hours after the fat load plasma CET was 11·98 ± 0·80 nmol/ml/h (Fig. 2b).

Plasma CETP mass concentrations were comparable after treatment with simvastatin 80 mg (1·38 ± 0·12 μg/ml) and simvastatin/ezetimibe 10 mg/10 mg (1·30 ± 0·11 μg/ml) and were lower compared with fasting plasma levels without treatment (1·65 ± 0·19 μg/ml) (Table 3). Ingestion of a fat load in subjects without treatment and after simvastatin 80 mg monotherapy resulted in an increase of 16% in plasma CETP mass, 3 and 4 h postprandial, respectively. CETP mass increased 10% after fat loading, compared with fasting levels, after simvastatin/ezetimibe 10 mg/10 mg treatment.

Adiponectin plasma concentrations after an oral fat loading

No significant differences in baseline plasma adiponectin levels were observed between the control period and the two treatment regimens. However, adiponectin plasma concentrations decreased after oral fat loading. In subjects without treatment, a decrease from 5·0 ± 0·6 to 4·5 ± 0·5 μg/ml (–10%) (Table 3) was found 8 h postprandially, whereas after treatment with simvastatin 80 mg and simvastatin/ezetimibe 10 mg/10 mg, adiponectin plasma levels were also decreased 8 h after the oral fat load (–8·4% and –6·4%, respectively).

Discussion

In the present randomized, double blind, crossover trial, HDL-c plasma levels decreased after a standardized oral fat load as a model of acute hypertriglyceridemia due to increased CET in male obese metabolic syndrome patients. Treatment with high-dose simvastatin monotherapy or low-dose simvastatin in combination with ezetimibe did not prevent the acute decrease in post-fat load HDL-c concentrations.

HDL has antiatherogenic properties by facilitating cholesteryl ester transport to the liver from peripheral tissues, including the vessel wall, and further by exerting antioxidant, antithrombotic and anti-inflammatory effects.[22,23] A low fasting plasma HDL-c concentration is, besides a component of metabolic syndrome, an independent risk factor for the development of cardiovascular diseases.[5,6] The present study in nonsmoking, obese metabolic syndrome subjects, using oral fat loading as an acute model of increased plasma triglyceride levels, showed that HDL-c plasma levels decreased after an oral fat load on top of already low fasting HDL-c plasma concentrations. This decrease occurred regardless of lipid-lowering therapy and is greatly dependent upon an induction of CET in the presence of elevated levels of its substrate: triglycerides. In another study in habitual smoking subjects and in six normolipidemic patients with coronary artery disease, HDL-c plasma levels decreased after an oral fat load compared to healthy controls.[12,14] Both in type 2 diabetes patients and healthy controls, HDL-c levels declined after a mixed meal during 6 h.[13] Since humans are most part of the day in a postprandial state, it may be hypothesized that the increased risk associated with metabolic syndrome is partly caused by further decreased postprandial HDL-c plasma levels in these patients.[24] Postprandial triglycerides metabolism strongly influences HDL-c metabolism and lipoprotein composition through the action of CET. CET in plasma is modulated by CETP plasma levels and activity, plasma triglyceride concentrations, smoking and alcohol consumption.[25] Given the fact that adipose tissue is a major site of CETP production and obesity is associated with elevated CETP activity and CETP protein mass,[8,26] it is thought that elevated CETP plasma levels are a link between (visceral) obesity, insulin resistance and low HDL-c levels, all features present in metabolic syndrome. Second, CETP gene expression in large adipocytes may be even more enhanced in response to dietary cholesterol and saturated fatty acids, leading to elevated CETP mRNA levels and increased plasma activity in animal models and humans.[8,27] In accordance, in the present study, CETP mass increased directly after the fat load intake, indicating that the increased CET may in part be the result of higher CETP mass. The observed decline in adiponectin plasma levels supports the hypothesis of an altered adipocyte secretion rate after an oral fat load.

Using an acute model for plasma triglycerides increase, the decrease in post-fat load HDL-c plasma concentrations was not affected by treatment with high-dose simvastatin or the combination therapy of low-dose simvastatin/ezetimibe. Statins may decrease plasma CET indirectly by reducing hepatic VLDL synthesis, following HMG-CoA-reductase inhibition and LDL-receptor up-regulation leading to a reduced number of circulating donor particles for CET[28] which might be accompanied by an increase in fasting plasma HDL-c concentrations.[29] The post-fat load increase of plasma triglycerides was similar after both treatments, despite the fact that both treatments significantly reduced baseline plasma triglyceride levels. This opens up the question whether additional treatment with a CETP-inhibitor blocks the decrease in HDL-c levels due to enhanced CET after acute hypertriglyceridemia.

Given fluctuations in HDL-c plasma concentrations during the menstrual cycle, the results of the present study cannot automatically be extrapolated to female subjects. In the present study, only obese patients with metabolic syndrome were included. It is not known whether the results can be extrapolated to nonobese metabolic syndrome patients. Other potential limitations of our current study comprise the definition used for metabolic syndrome. Although other diagnostic criteria do exist, the ATPIII definition is most commonly used and is easily applicable in clinical practice. Crossover and carry-over effects cannot completely be ruled out but are unlikely to have affected the results, considering the half-life time of the study drugs and the 4 weeks washout period, given the 6-week treatment period leads this to a 10-week time lag between the last dose of one treatment and measuring the effects of the other treatment. Finally, it should be noted that the oral fat load consisted of a nonphysiological load of triglycerides.

In conclusion, plasma high density lipoprotein-cholesterol (HDL-c) levels decreased after an oral fat load in male obese patients with

metabolic syndrome which is likely to be the result of increased cholesteryl ester transfer, in part initiated by elevated plasma cholesteryl ester transfer protein mass and elevated plasma triglycerides. Treatment with high-dose simvastatin or with the combination of low-dose simvastatin and ezetimibe did not influence the acute increase in triglycerides or the decrease in postprandial HDL-c levels. Postprandial decrease in HDL-c plasma levels may contribute to the increased cardiovascular risk in metabolic syndrome patients already having low HDL-c plasma concentrations.

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There are no conflicts of interest for all authors.

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