Statin effects on metabolic profiles: data from the PREVEND IT trial

Statin effects on metabolic profiles

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Abstract

Background: Statins lower cholesterol by inhibiting HMG-CoA reductase, the rate-limiting enzyme of the metabolic pathway that produces cholesterol and other isoprenoids. Surprisingly little is known about their effects on metabolite and lipoprotein subclass profiles. We therefore investigated the molecular changes associated with pravastatin treatment compared to placebo administration, using a nuclear magnetic resonance (NMR)-based metabolomics platform.

Methods and Results: We performed metabolic profiling of 231 lipoprotein and metabolite measures in the PREVEND IT study, a placebo-controlled randomized clinical trial designed to test the effects of pravastatin (40 mg once daily) on cardiovascular risk. Metabolic profiles were assessed at baseline and after 3 months of treatment. Pravastatin lowered low-density lipoprotein cholesterol (LDL-C; change in SD units [95% CI]: -1.01 [-1.14, -0.88]), remnant cholesterol (change in SD units [95% CI]: -1.03 [95% CI : -1.17, -0.89]) and apolipoprotein B (apoB, change in SD units [95% CI]: -0.98 [95% CI : -1.11, -0.86]) with similar effect magnitudes. In addition, pravastatin globally lowered levels of lipoprotein subclasses, with the exception of high-density lipoprotein (HDL) subclasses, which displayed a more heterogeneous response pattern. The lipid lowering effect of pravastatin was accompanied by selective changes in lipid composition, particularly in the cholesterol content of very low-density lipoprotein (VLDL) particles. In addition, pravastatin reduced levels of several fatty acids, but had limited effects on fatty acid ratios.

Conclusions: These randomized clinical trial data demonstrate the widespread effects of pravastatin treatment on lipoprotein subclass profiles and fatty acids.

Clinical Trial Registration: NCT03073018 (https://clinicaltrials.gov)

Keywords: statin, lipoprotein subclasses, fatty acids, metabolomics, clinical trial

Introduction

Statins hamper cholesterol production in the liver through inhibition of HMG-CoA reductase, which, in turn, stimulates hepatic synthesis of low-density lipoprotein (LDL) receptors as a compensatory mechanism. These receptors bind to apoB-rich lipoproteins and facilitate their absorption by hepatocytes, leading to a further reduction in plasma cholesterol levels.[1] The cardiovascular risk reduction achieved through statins is believed to primarily result from their LDL cholesterol (LDL-C) lowering properties.[2] Lowering of LDL-C has therefore been identified as the primary treatment target of statin therapy.[3] However, statins act early in the mevalonate pathway and have the potential to extensively modify the metabolic profile in addition to their effect on cholesterol metabolism. This has led to the hypothesis that statins may provide cardioprotective benefits beyond LDL-C reduction. While there is mounting evidence underpinning the therapeutic capacities of such pleiotropic statin effects,[4-6] little is known about the underlying molecular pathways.

Nuclear magnetic resonance (NMR)-based metabolic profiling has evolved into a versatile high-throughput tool for biomarker discovery that allows simultaneous quantifications of numerous molecules, ranging from amino acids to a variety of lipoprotein subclass measures. Metabolic profiling has been widely used both in epidemiology and in drug research.[7-9] Better characterization of the metabolic footprint of statins may provide novel insights into their mechanisms of action and help guide drug discovery. A recent study of four observational population-based cohorts investigated the longitudinal effects of statins on metabolic profiles by comparing users to non-users, followed by confirmatory Mendelian randomization analysis.[9] Besides cholesterol lowering, statins influenced fatty acid levels, whereas amino acids and other metabolites were not substantially altered. While this study revealed extensive changes

in routine lipid measures, little is known about the effect of statins on lipoprotein subclass profiles, even though mounting evidence suggests distinct roles for lipoprotein subclasses in the pathophysiology of cardiovascular disease.[10-12] In addition, no study has yet comprehensively investigated the metabolic effects of statin therapy in a placebo-controlled randomized setting. Here we present the first data on pravastatin treatment derived from the Prevention of Renal and Vascular End-stage Disease Intervention Trial (PREVEND IT) study, a randomized placebo-controlled clinical trial. In addition to previously quantified parameters, including lipids, fatty acids, amino acids and glycolysis metabolites, we report results for over 160 measures of lipoprotein subclasses. An overview of lipoprotein subclasses is given in Table 1.

Materials and Methods

Subjects

Details on the PREVEND IT study have been published elsewhere.[13] Briefly, PREVEND IT is a double-blind, placebo-controlled clinical trial, in which participants were randomized to 20 mg fosinopril or matching placebo and 40 mg pravastatin or matching placebo. PREVEND IT participants were recruited from the PREVEND program, which investigated the influence of microalbuminuria on cardiovascular and renal risk. The main inclusion criteria for PREVEND IT were a urine albumin concentration of >10 mg/L in one morning spot sample and at least once a concentration of 15 to 300 mg/24 h in two successive 24-hour urine samples, a blood pressure of <160/100 mm Hg, no hypertensive treatment and a total serum cholesterol concentration < 8.0 mmol/L (or <5.0 mmol/L in case of prior myocardial infarction) and no lipid-lowering treatment. 864 subjects were randomized to receive study

medication (see above) after giving informed consent. Blood samples for metabolic profiling were limited by sample availability and could be obtained in 394 participants at baseline and after 3 months of treatment. The study was approved by the Institutional Review Board and was conducted in according to the guidelines of the declaration of Helsinki.

Laboratory Measurements

Fasting blood samples were drawn before treatment onset (baseline) and at the 3-month medical review (N=394). Metabolic profiling was performed in EDTA anticoagulated plasma samples using high-throughput 1H NMR metabolomics (Brainshake Ltd, Helsinki, Finland), as previously described[7]. This method provides accurate quantification of 231 lipoprotein and metabolite measures, including routine lipids, lipoprotein profiles with 14 lipoprotein subfractions, glycolysis related metabolites, amino acids, ketone bodies, fluid balance related metabolites and one inflammatory marker (Supplemental Table 1). Recent studies have demonstrated that NMR measurements quantified with this platform are in good agreement with routine clinical chemistry assays.[8] Representative coefficients of variation (CVs) for this platform have been reported elsewhere.[14]

Statistical analysis

Correlations between different lipoprotein and metabolite measures were calculated using Spearman's correlation coefficients. The effect of statin treatment on each NMR measure was assessed by linear regression on the change during the treatment period, as previously described.[9] The effect estimates (regression coefficients) can be interpreted as the difference between change over time in the

pravastatin group and change over time in the placebo group. To facilitate comparison between regression coefficients from different lipoprotein and metabolite measures, effect estimates were scaled to baseline SD units. The scaled effect estimates thus represent changes over time in baseline SD units attributable to pravastatin treatment. We also performed a sensitivity analysis adjusted for sex as the pravastatin group showed a higher percentage of male patients. Since many NMR measures were highly correlated (see Supplemental Table 1), we accounted for multiple testing by correcting the nominal level of significance for the number of independent tests, which was estimated by the method of Li and Ji,[15] using the matrix spectral decomposition (matSpD) tool (http://gump.qimr.edu.au/general/daleN/matSpD/). The number of independent tests was estimated to be 85, yielding a corrected significance threshold of 0.05/85=0.00059.

Results

Baseline characteristics and NMR measures

Baseline characteristics of all patients included in this study are listed in Table 2. Of 394 participants, 195 received pravastatin and 199 placebo during the 3-month treatment period. A summary of all 231 lipoprotein and metabolite measures can be found in Supplemental Table 1. NMR and available clinical chemistry measures showed strong correlations for baseline and post-treatment measurements (Supplemental Table 2), indicating consistency between different analytical methods. Heat maps of correlations between NMR measures are displayed in Supplemental Figure 1, revealing substantial correlation within lipoprotein subclasses, between amino acids and between fatty acids.

Statin effects

We compared each NMR measure separately between the pravastatin group and controls, using linear regression. To facilitate comparison between different measures, longitudinal changes associated with pravastatin treatment were scaled to baseline SD units. After the 3-month treatment period, a total of 150 NMR measures were significantly altered ($p<0.00059$) between the pravastatin group and the control group. Absolute concentration changes are given for all lipoprotein and metabolite measures in Supplemental Table 3. Additional sensitivity analysis adjusted for sex provided similar findings, suggesting that our results were not confounded by the imbalance in sex ratio between the pravastatin group and the control group (Supplemental Table 4).

As compared with placebo, pravastatin reduced levels of conventional lipid measures (Figure 1), including total serum cholesterol (change associated with pravastatin in SD units [95% CI]: -1.01 [-1.14, -0.88]; $p=7.3x10^{-41}$), LDL-C (change in SD units [95% CI]: -1.01 [-1.13, -0.88]; $p=6.7x10^{-42}$) and total serum triglycerides (change in SD units [95% CI]: -0.46 [-0.60, -0.33]; $p=1.8x10^{-11}$), whereas HDL-C levels were not affected by statin treatment (change in SD units [95% CI]: -0.01 [-0.11, 0.09]; p=0.829). However, pravastatin significantly increased cholesterol in large lipid-rich HDL2 particles (change in SD units [95% CI]: 0.18 [0.08, 0.27]; p=0.00048) and decreased cholesterol in small less dense HDL3 particles (change in SD units [95% CI]: -0.69 [-0.87, -0.51]; $p= 3.1x10^{-13}$).

Moreover, pravastatin treatment markedly lowered remnant cholesterol levels (change in SD units [95% CI]: -1.03 [-1.17, -0.89]; $p=2.0x10^{-38}$), which reflects the total cholesterol content in very large-density lipoprotein (VLDL; change in SD units [95% CI]: -0.88 [-1.02, -0.74]; $p=2.1x10^{-29}$) and intermediate-density lipoprotein (IDL;

change in SD units [95% CI]: 1.03 [-1.16, -0.89]; $p=1.3x10^{-39}$). The effect of pravastatin on apolipoprotein B (apoB; change in SD units [95% CI]: -0.98 [-1.11, -0.86]; $p=1.1x10^{-44}$) was comparable to the change in LDL-C. Pravastatin globally lowered levels of VLDL, LDL and IDL subclasses (Figure 2), whereas changes in HDL subclasses were less consistent, with significant increases across large HDL subclasses measures and a reduction in small and very large HDL-C.

Particle concentrations of all VLDL, IDL and LDL subclasses decreased in response to statin treatment. IDL was the subclass with the greatest change in particle concentration (change in SD units [95% CI]: -1.04 [95% CI: -1.17 to -0.91]; $p=7.6x10^{-45}$). In addition, we analyzed the lipid composition of different lipoprotein subclasses, expressed as the ratio of individual lipid concentrations to the total lipid concentration (Figure 3). Pravastatin treatment markedly lowered the cholesterol and cholesteryl ester to total lipids ratio in IDL and across all LDL subclasses, concomitant with an elevated relative content of free cholesterol and phospholipids in LDL. Furthermore, pravastatin selectively reduced cholesterol ratios in small and medium VLDL particles. In parallel with cholesterol and triglycerides, pravastatin lowered fatty acid concentrations (Figure 4), particularly ω-6 fatty acids (change in SD units [95% CI]: -0.85 [95% CI : -1.00, -0.71]; $p=3.5x10^{-26}$), total polyunsaturated fatty acids (PUFA, change in SD units [95% CI]: -0.84 [95% CI : -0.98, -0.69]; $p= 3.4x10^{-26}$). By contrast, pravastatin treatment only altered the saturated fatty acid to total fatty acid ratio (SFA/FA; change in SD units [95% CI]: 0.51 [95% CI : 0.29, 0.74]; $p= 9.4x10^{-6}$) and the lineolic acid to total fatty acid ratio (LA/FA; change in SD units [95% CI]: -0.35 [95% CI : 0.48, 0.21]; $p= 7.2x10^{-7}$), but produced no changes in other fatty acid ratios. Glycolysis-related metabolites, amino acids and other meatbolites remained unchanged.

We next evaluated the effect of pravastatin on correlations between different NMR measures. Results are illustrated in a correlation difference map that provides a post-treatment comparison between the pravastatin group and controls (Supplemental Figure 2). Pravastatin induced negative associations between the relative cholesterol content of medium HDL and cholesterol levels in small VLDL, IDL and LDL. We observed similar, but weaker effects for absolute cholesterol concentrations in medium HDL. Conversely, pravastatin strengthened or induced positive correlations between the phospholipid-to-total lipids ratio in medium HDL and lipid concentrations in other lipoproteins. Furthermore, correlations between absolute lipid concentrations and the relative lipid content were altered across VLDL subclasses. Finally, lactate and pyruvate showed weaker associations with lipid concentrations in VLDL following pravastatin treatment.

Discussion

This is the first placebo-controlled NMR study to assess metabolic changes associated with statin treatment, using data from the PREVEND IT trial. Our study adds to previous findings from observational NMR studies and additionally explored statin-induced changes in over 160 novel measures of lipid concentrations and lipid composition for 14 lipoprotein subclasses. Besides the well-known effects on LDL-C, statins altered a wide range of lipids and concentrations of fatty acids. These findings are supported by observational studies comparing statin users to non-users,[9,16] and fit with previous clinical trial data on fatty acids.[17] By contrast, pravastatin treatment only altered LA/FA and SFA/FA, but had no effect on other fatty acid ratios. In addition, pravastatin globally lowered levels of lipoprotein subclasses, except for HDL

concentrations, which displayed a more intricate response pattern. Detailed lipid profiling revealed that the substantial lowering of VLDL-C, IDL-C and LDL-C was paralleled by more selective changes in lipid composition of different lipoprotein particles. Finally amino acids and other metabolites were not affected by statin treatment.

Statins not only act on LDL, but also on other apoB-rich lipoproteins. In our study, pravastatin reduced apoB and LDL-C with similar effect magnitudes. ApoB has been proposed as a more robust cardiovascular risk marker than LDL-C, supporting the use of apoB as an alternative treatment target for statin therapy.[18,19]

Consistent with previous findings,[9,20] statin treatment substantially lowered cholesterol in apoB-containing, triglyceride-rich remnant particles, including IDL and VLDL. Since VLDL is the main carrier of triglycerides, remnant cholesterol is strongly associated with triglyceride levels. Although triglycerides are well-established markers of cardiovascular risk, their relationship with atherogenesis is not straightforward.[21] By contrast, remnant cholesterol is likely to play a causal role in cardiovascular disease risk.[22,23] In line with this, remnant cholesterol is associated with both ischemic heart disease and low-grade inflammation.[24] Compared with VLDL-C and IDL-C, HDL-C showed a more complex response to statin treatment, with cholesterol depletion of small HDL3 particles and slight cholesterol enrichment of larger HDL2 particles. Prospective cohort studies have consistently reported inverse associations between HDL-C levels and risk of cardiovascular disease,[25] whereas findings from recent Mendelian randomization studies[26,27] and the failure of HDL-raising drugs to improve cardiovascular outcomes[28] may argue against a causal role for HDL-C in cardiovascular disease per se. Findings from experimental studies suggest that HDL3 and HDL2 differ in their cardioprotective capacities.[29] However, the relationship between different HDL

subclasses and cardiovascular risk remains a matter of debate as results from observational studies are inconclusive.[30]

It has been suggested that small dense LDL particles are more atherogenic than larger LDL species as they are readily taken up by the arterial wall, are cleared from circulation at reduced rates due to their low affinity for LDL receptors and are more susceptible to oxidation, promoting the formation of atherosclerotic plaques.[11,13] This is supported by large cohort studies, demonstrating that concentrations of small rather than large LDL particles are associated with future cardiovascular risk after adjustment for non-lipid risk factors.[31-33] However, effect estimates for small LDL particles are not superior to total LDL concentrations and do not improve risk prediction beyond routine lipid measures.[31] Moreover, a systematic review of NMR studies found no association of LDL subclasses with cardiovascular disease after adjustment for other lipid measurements.[34] Experimental findings suggest that, similar to LDL particles, the atherogenic capacity of VLDL may depend on particle size as large VLDL subpopulations are unable to enter the arterial wall and are thus less likely to contribute to the formation of atherosclerotic plaques.[35] However, there is little evidence from clinical studies that smaller and larger VLDL particles differ in their atherogenic potential. While different lipoprotein subclasses may play distinct roles in the pathophysiology of cardiovascular disease, pravastatin treatment lowered lipoprotein particle concentrations and lipid concentrations across VLDL, IDL and LDL subclasses, which may be an indirect consequence of enhanced clearance and/or reduced synthesis of these lipoproteins.

While the cholesterol-to-total-lipids ratio was decreased in IDL and all LDL subpopulation, pravastatin selectively reduced the cholesterol content of small and medium VLDL, raising the possibility that statins specifically target potentially

atherogenic VLDL subpopulations.[35]At the same time, pravastatin lowered the triglyceride to total lipids ratio across all HDL subpopulations, but increased the triglyceride content of several VLDL and LDL subclasses as well as IDL. These changes in lipid composition may be attributable to statin effects on the reverse cholesterol transport pathway, in which cholesteryl ester transfer protein (CETP) transfers cholesteryl esters from HDL to triglyceride-rich, apoB-containing lipoproteins (LDL, IDL and VLDL) in exchange for triglycerides.[36] The statin-induced decrease in lipoprotein concentrations is associated with reduced CETP activity, resulting in cholesterol enrichment of HDL and cholesterol depletion of apoB containing lipoproteins.[37-39] Consistent with reduced CETP activity, pravastatin induced negative correlations between the cholesterol content of medium HDL and cholesterol levels in non-HDL particles. Correlation coefficients for other HDL subpopulations, however, were only moderately altered after pravastatin treatment.

The relative reduction in LDL cholesterol was associated with no or only minor changes in the triglyceride content of LDL particles. By contrast, there was triglyceride enrichment of IDL as well as medium and large VLDL particles. Statin-induced lowering CETP activity may also hamper TG transfer from VLDL and IDL to LDL,[40] which would explain the increased relative triglyceride content of IDL and VLDL. Besides lowering the cholesterol content of IDL and LDL, pravastatin treatment led to a relative increase in phospholipids and free cholesterol, which may result from reduced enzymatic cholesterol esterification due to blocked cholesterol synthesis.[41] Taken together, detailed analysis of lipoprotein subclasses revealed selective changes in lipid composition, whereas lipid concentrations were reduced across all VLDL, IDL and LDL subclasses, following pravastatin treatment.

Several studies have shown that besides lipid lowering, statins alter fatty acid levels.[9,16,17] Since the vast majority of circulating fatty acids are bound in triglycerides, cholesteryl esters and phospholipids,[17] the reduction in fatty acid levels associated may result from the statin-induced decrease in lipoproteins providing the main source of circulating lipids. Alternatively, statins may interfere with fatty acid metabolism through different molecular pathways. Simvastatin treatment increases metabolic indices indicating elevated activity of elongases and desaturases,[17] two enzymes that catalyze the formation of highly unsaturated long-chain fatty acids. Moreover, statin treatment may stimulate hepatic uptake and beta-oxidation of fatty acids by enhancing expression of peroxisome proliferator-activated receptors (PPARs).[42] We observed elevated SFA/FA and reduced LA/FA, but no effects on other fatty acid ratios, which more appropriately reflect fatty acid metabolism than fatty acid concentrations given the lipoprotein-lowering effect of statins. By contrast, a recent observational study reported stronger effects on docosahexaenoic acid (DHA)/FA, whereas SFA/FA was unchanged after statin treatment.[9] In this study, however, information on statin type and dosage was not available. Consistent with our findings, data from a clinical trial suggest that simvastatin does not enhance DHA/FA.[17] Interestingly, studies comparing different statins reported that pravastatin, in contrast to other statins, did not influence selected fatty acid ratios, indicating that changes in fatty acid metabolisms depend on the statin type.[43,44] While the decrease in LA/FA is supported by other studies,[9,17] the underlying metabolic processes remain unclear. Statins increase lecithin:cholesterol acyltransferase (LCAT) activity, which synthesizes cholesteryl esters from cholesterol and fatty acids.[45] Since LA is the preferential substrate of LCAT, elevated LCAT activity would be consistent with higher LA/FA. Collectively, changes in absolute fatty

acid levels are mainly driven by statin-induced lipid lowering, whereas statin effects on fatty acid metabolism remain uncertain and may differ between statins.

Pyruvate and lactate showed weaker correlations with VLDL-related measures after pravastatin treatment, whereas absolute concentrations of these two metabolites remained unchanged. In addition to producing lactate, pyruvate is involved in glucose and fatty acid metabolism by forming acetyl-coenzyme A, which is involved in fatty acid synthesis.[46] Fatty acids, in turn, are joined with glycerol to form triglycerides, the main component of VLDL. Pyruvate and lactate as a metabolic product of pyruvate are thus associated with enhanced hepatic VLDL synthesis and consequently should show a positive correlation with serum VLDL levels. This is in line with the correlation patterns of pyruvate and lactate in the placebo group (Supplemental Figure 2B). Statins, however, facilitate hepatic uptake of non-HDL particles, including VLDL, by increasing LDL receptor activity.[1] The resulting decrease in VLDL levels coupled with unchanged pyruvate and lactate levels is consistent with weaker correlations in the pravastatin group (Supplemental Figure 2A).

Our study was powered to detect a large number of significant changes in lipoprotein and metabolite measures after pravastatin treatment, underscoring the strengths of a placebo controlled randomized setting with pre/post treatment comparisons, which limits potential sources of confounding to a minimum. We report associations for 231 NMR measures, including over 160 novel measures of lipid concentrations and lipid composition for different lipoprotein subclasses. No other study has assessed the effect of statins on lipoprotein subclasses in such detail. However, further research is warranted to confirm our findings on lipoprotein subclasses as we did not replicate our results in an independent study. In comparison with a recent observational study that used the same NMR metabolomics platform,[9] we

observed more moderate effects of statin treatment on several lipid measures, including LDL-C, apoB and apoA1. Würtz et al. compared statin users, who commenced statin treatment, to non-users. While information on statin type and dosage was not available for this study, all statin users had an indication for statin therapy, such as hypercholesterolemia, suggesting that many of them underwent aggressive treatment. In our study, however, participants were randomly assigned to a moderate dose of a relatively week statin,[47] which may account for the lower effect estimates.

In conclusion, metabolic profiling in a randomized clinical trial revealed causal associations of statin treatment with globally reduced lipid levels across lipoprotein subclasses, accompanied by more selective changes in the lipid composition of lipoproteins. Additionally, pravastatin treatment lowered fatty acid concentrations, but had limited effects on fatty acid ratios. In line with previous findings[9], statin treatment did not alter concentrations of non-lipid measures, such as amino acids and glycolysis-related metabolites, suggesting that these metabolites do not reflect pleiotropic statin effects. Our findings demonstrate that high-throughput metabolic profiling is emerging as a powerful tool to dissect a drug's metabolic footprint, providing important information that may be used to improve current treatments.

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Disclosure

None

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Tables:

Lipoprotein	Subclass	Average particle diameter (in nm)[a]
VLDL	XXL	>75
	XL	64.0
	L	53.6
	M	44.5
	S	36.8
	XS	31.3
IDL		28.6
LDL	L	25.5
	M	23.0
	S	18.7
HDL	XL	14.3
	L	12.1
	M	10.9
	S	8.7

Table 1. Average particle size (diameter in nm) for different lipoprotein subclasses. HDL: high-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; XXL: extremely large; XL: very large; L: large; M: medium; S: small; XS: very small.

[a]Average particle diameters adapted from [8]. Cut points for size ranges can be approximated by the midpoint between the average diameters of two consecutive lipoprotein subclasses, e.g. the lower bound of XS-VLDL is approximately 30 nm.

Variable	**Placebo (n=199)**	**Pravastatin (n=195)**
Age, mean (SD)	50.6 (11.1)	51.5 (11.5)
Male, %	121 (60.8)	141 (72.3)

BMI, mean (SD)	26.5 (4.5)	26.3 (4.1)
Current smoker, %	79 (39.7)	82 (42.1)
SBP (mm Hg), mean (SD)	130.6 (17.3)	131.6 (18.3)
DBP (mm Hg), mean (SD)	75.8 (9.9)	76.6 (9.4)
Cholesterol (mmol/l), mean (SD)	5.9 (1.0)	5.9 (1.1)
HDL (mmol/l), mean (SD)	1.0 (0.3)	1.0 (0.3)
LDL (mmol/l), mean (SD)	4.1 (0.9)	4.2 (1)
Triglycerides (mmol/l), mean (SD)	1.6 (1.0)	1.6 (1)
Glucose (mmol/l), mean (SD)	5.1 (1.2)	5.0 (1)
Creatinine (µmol/l), mean (SD)	84.0 (15.1)	86.4 (13.2)
Medication use, %		
Beta-blockers	4 (2.0)	0 (0.0)
Nitrate	2 (1.0)	0 (0.0)
Diuretics	4 (2.0)	0 (0.0)
Calcium channel blockers	0 (0.0)	1 (0.5)
Digoxin	1 (0.5)	2 (1.0)

Table 2. Baseline characteristics. Lipids, glucose, and creatinine as measured by clinical chemistry. SD: standard deviation; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Figures:

Figure 1. Concentration changes in lipids and lipid-related measures associated with pravastatin treatment (n=195) compared with placebo treatment (n=199). Effect estimates indicate changes over the treatment period (3 months) associated with pravastatin treatment in baseline SD-units. Error bars represent 95% confidence intervals. The dotted line shows the effect estimate for LDL-C. Red marks indicate significant changes (p<0.00059). HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; D: diameter; C: cholesterol; TG: triglycerides; DAG: diacylglycerol; PG: phosphoglycerides; PC: phosphatidylcholine; SM: sphingomyelins; Total Chol: total cholines; apoA1: apolipoprotein A1; apoB: apolipoprotein B.

Figure 2. Changes in lipid concentrations across lipoprotein subclasses associated with pravastatin treatment (n=195) compared with placebo treatment (n=199). Effect estimates indicate changes over the treatment period (3 months) associated with pravastatin treatment in SD-units. Error bars represent 95% confidence intervals. The dotted line shows the effect estimate for LDL-C Red marks indicate significant changes (p<0.00059). XXL: extremely large; XL: very large; L: large; M: medium; S: small; XS: very small; HDL: high-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; P: particle concentration; L: total lipids; PL: phospholipids; C: cholesterol; CE: cholesteryl esters; FC: free cholesterol; TG: triglycerides.

Figure 3. Changes in lipid composition across lipoprotein subclasses associated with pravastatin treatment (n=195) compared with placebo treatment (n=199). Effect estimates indicate changes over the treatment period (3 months) associated with pravastatin treatment in baseline SD-units. Error bars represent 95% confidence intervals. The dotted line shows the effect estimate for LDL-C. Red marks indicate significant changes (p<0.00059). %: lipid concentration relative to total lipd concentration; XXL: extremely large; XL: very large; L: large; M: medium; S: small; XS: very small; HDL: high-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; P: particle concentration; L: total lipids; PL: phospholipids; C: cholesterol; CE: cholesteryl esters; FC: free cholesterol; TG: triglycerides.

Figure 4. Concentration changes in fatty acids, amino acids and other metabolites associated with pravastatin treatment (n=195) compared with placebo treatment (n=199). Effect estimates indicate changes over the treatment period (3 months) associated with pravastatin treatment in baseline SD-units. Error bars represent 95% confidence intervals. The dotted line shows the effect estimate for LDL-C. Red marks indicate significant changes (p<0.00059). FA: fatty acids; Unsat Deg: degree of unsaturation; DHA: docosahexaenoic acid; LA: lineolic acid; CLA: conjugated lineolic acid; ω-3 FA: omega-3 fatty acids; ω-6 FA: omega-6 fatty acids; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: saturated fatty acids; bOHbut: 3-hydroxybutyrate; Gp: glycoprotein acetyls.

Supplemental Tables

Supplemental Figures