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HDL cholesterol efflux capacity and cholesteryl ester transfer are associated with body mass, but are not changed by diet-induced weight loss: A randomized trial in abdominally obese men

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ABSTRACT

Background and aims: Obesity is associated with a lower HDL-mediated cholesterol efflux from macrophages and a higher CETP (cholesteryl ester transfer protein) activity, but effects of weight loss are not clear. In addition, associations with visceral and subcutaneous adipose tissue are not known. We therefore investigated effects of diet-induced weight loss on HDL-mediated cholesterol efflux and cholesterol ester (CE) transfer in abdominally obese men. Differences between normal-weight and abdominally obese men were also examined.

Methods: Twenty-five apparently healthy, normal-weight men (waist circumference: <94 cm) and 52 abdominally obese men (waist circumference: 102–110 cm) were included. Abdominally obese subjects were randomly allocated to a dietary weight-loss intervention group or a no–weight loss control group. Individuals from the intervention group followed a very-low-calorie diet for 6 weeks to obtain a waist circumference below 102 cm, followed by a 2-week weight-stable period. Cholesterol efflux was measured in BODIPY-labeled murine J774 macrophages. CE transfer was measured by quantifying the transfer of CE from radiolabeled exogenous HDL to apoB-containing lipoproteins.

Results: Cholesterol efflux capacity was 9 percentage point (pp) lower in abdominally obese than in normal-weight men ($p \leq 0.001$), while CE transfer was 5 pp higher ($p \leq 0.01$). Diet-induced weight-loss of 10.3 kg did not change cholesterol efflux and CE transfer. In addition, stepwise regression analysis did not suggest that the different fat depots are differently related to efflux capacity and CE transfer.

Conclusions: After a 2-week weight-stable period, dietary weight loss of 10 kg did not improve ABCA1-mediated cholesterol efflux and CE transfer in abdominally obese men.

1. Introduction

The role of high-density lipoproteins (HDL) in the prevention of coronary heart disease (CHD) is not clear, as raising HDL-cholesterol (HDL-C) concentrations *per se* is not causally related to a lower risk for CHD [1, 2]. However, it becomes more and more accepted that improving HDL functionality is a better target to reduce CHD risk. HDL has many anti-atherogenic properties, including anti-inflammatory, anti-oxidant, anti-apoptotic, anti-thrombotic and vasodilating effects [3]. However, its involvement in the reverse cholesterol transport (RCT) pathway, which is the ability of HDL to remove and transport the excess of cholesterol from peripheral cells such as residing macrophages in the arterial wall to the liver for excretion, represents its main anti-atherogenic effect [3, 4]. Indeed, an inverse association between cholesterol efflux with atherosclerosis and cardiovascular mortality has been reported, independent of HDL-C concentrations [5, 6].

HDL-mediated cholesterol efflux from macrophages is facilitated by several transporters, such as ABCA1, ABCG1 and SR-BI [3],

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and related to numerous factors, including HDL particles size and composition as well as differences in baseline characteristics between subjects. One of these factors is body fat. Although not all obese subjects have increased levels of inflammatory markers [7], obesity is frequently associated with chronic low-grade inflammation [8]. As pro-inflammatory cytokines reduce in vitro cholesterol efflux capacity [9, 10], abdominally obese subjects may even have a further lowered cholesterol efflux [11, 12], because visceral fat secretes more cytokines and adipokines than subcutaneous fat [8]. In addition, adipocytes may be involved in the transport of cholesterol to HDL particles, which is reduced in inflamed adipocytes [13]. Obese subjects also have an increased mass and activity of cholesteryl ester transfer protein (CETP) mediating the exchange of cholesteryl esters (CE) and triacylglycerol (TAG) between HDL and apoB100-containing lipoproteins [14]. However, not much is known about the relationship between cholesteryl ester (CE) transfer and cholesterol efflux, even though CE transfer is inversely associated with HDL particle size and composition [15, 16], which both play an important role in cholesterol efflux [17].

Although cross-sectional differences in efflux capacity between various BMI-groups have previously been reported [11, 12, 18], the effects of diet-induced weight loss on cholesterol efflux capacity are less consistent. Some studies have reported a decrease in cholesterol efflux after weight loss [19, 20], while others have reported no effects [21]. Also, relationships with the different fat depots are not known. Finally, the reported studies were lacking a no-weight loss control group. Therefore, we measured in a randomized controlled trial the effects of diet-induced weight loss on HDL-mediated cholesterol efflux and CE transfer in abdominally obese men. Cross-sectional differences at baseline between normal-weight and abdominally obese men were also investigated.

2. Materials and methods

2.1. Subjects and design

To examine the effects of weight-loss on cholesterol efflux capacity of HDL, samples from a previous study were used. In that study, effects of diet-induced weight loss on cardiometabolic risk markers and vascular function markers in abdominally obese men (waist circumference between 102 and 110 cm) were examined. Details of this study have already been published [22, 23]. Briefly, baseline measurements were completed in 54 abdominally obese men. One man dropped out before randomization and 53 men were randomly assigned to the weight-loss or no-weight loss control group. One participant discontinued the control intervention because of illness, while another man dropped out because of non-compliance with the weight-loss program. Another man from the weight-loss group stopped for personal reasons. Thus, eventually 50 abdominally obese men completed the entire weight-loss study. At the end of the study, one participant was excluded from the analyses, because of unreported use of antihypertensive medication during the study. Therefore, data from 49 abdominally obese men were used for the present analysis. For comparison, a group of 25 age-matched normal-weight men (waist circumference <94 cm) was included. All 74 subjects were apparently healthy, aged between 18 and 65 years, and had a stable body weight (weight gain or loss <3 kg within the previous 3 months). Use of medically prescribed drugs known to affect lipids, glucose and blood pressure were not allowed. The study was conducted according the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Ethics Committee of Maastricht University Medical Centre. Written informed consent was obtained from all participants before entering the study. The study was registered at clinicaltrials.gov as NCT01675401.

Subjects assigned to the weight-loss program (n = 25) had to consume daily a very-low-calorie diet (VLCD; Modifast; Nutrition et Santé Benelux) providing 2.1 MJ (500 kcal) and a maximum of 250 g vegetables or fruit (except bananas) each day for 4 weeks to reach a waist circumference below 102 cm, the cut-off value for one of the criteria to diagnose of the metabolic syndrome [24]. If the waist circumference was not below 102 cm after 4 weeks, subjects had to consume the VLCD for another week, while the calorie-restricted diet was used for 1 week. After the VLCD period, subjects were prescribed a mixed-solid, calorie-restricted diet, which provided 4.2 MJ/day for 2 weeks, as a transition period. The composition of this diet was in agreement with the Dutch dietary guidelines. In weeks 7 and 8, subjects consumed daily menus based on the new estimated energy requirements based on their newly achieved body weight in order to keep them weight stable for two weeks. Participants assigned to the no-weight loss control group (n = 27) were asked to maintain their habitual diet and use of alcohol throughout the total study period. In addition, all subjects were requested not to change their physical activity levels and to write down daily any protocol deviations in a diary. Diaries were checked at each visit and no deviations were noted that could have influenced the results. The 25 normal-weight participants were only measured once during the study and were not subjected to any intervention.

2.2. Blood sampling

Fasting blood samples were collected at the start and at the end of the intervention period. EDTA and NaF tubes were centrifuged at 1300 × g for 15 min at 4 °C to obtain plasma. Serum samples were obtained after blood samples were centrifuged at 1300 × g for 15 min at 21 °C [22]. The supernatants were transferred into 1.5 ml Eppendorf tubes and stored immediately at −80 °C until further analysis.

2.3. Measurements

Subcutaneous and visceral adipose tissue volumes were measured as described [22, 23]. The percentage of body fat was assessed by taking skinfold measurements, using the Durnin and Womersley formula [25].

Serum samples were used to measure concentrations of HDL-C (CHOD/PAP method; Roche Diagnostics System, Hofmann-La Roche Ltd., Basel, Swiss), TAG (GPO-Trinder; Sigma Diagnostics, St Louis, USA), apoA-I and apolipoprotein B100 (apoB100) (Horiba ABX, Montpellier Cedex, France), insulin, and C-peptide (RIA, Millipore, Linco Research, Missouri, USA). NaF plasma samples were used for glucose measurements (Horiba ABX, Montpellier Cedex, France). The HOMA-IR was calculated to estimate the degree of insulin resistance [26]. EDTA plasma samples were used to measure high-sensitive C-reactive protein concentrations (hsCRP) by using a multiarray detection system based on electrochemiluminescence technology (SECTOR Imager 2400; Meso Scale Discovery). For all analyses, all samples from one subject were analyzed within the same run.

The ability of HDL particles to promote cholesterol efflux from macrophages was measured as described previously [27]. Briefly, murine J774 macrophages were first cultivated and incubated overnight with 14.42 μg/ml BODIPY-cholesterol, i.e. 50 000 cells were seeded in a total incubation volume of 150 μl in a 96-well plate. Next, macrophages were equilibrated for 4 h with 1 μl/ml (final concentration: 0.005 mg/ml) of an ACAT inhibitor (Acyl CoA Acyltransferase Inhibitor (Sandoz 58-035) purchased from Sigma-Aldrich) and activated overnight with 4 μl/ml (final concentration: 0.2 mmol/L) cAMP to upregulate the expression of the

cholesterol-efflux transporter ABCA1. Next, in plasma samples from the volunteers in the study, apolipoprotein B containing lipoproteins were precipitated using tungstophosphoric acid hydrate and magnesium chloride. After spinning, the supernatant containing the HDL particles was added to the labeled macrophages and incubated for 4 h. All steps were performed in the presence of 1 μl/ml of ACAT inhibitor to block cholesterol esterification. After 4 h incubation, the 96-well plates were centrifuged for 5 min at 800 rpm at room temperature, and the supernatant of each sample was pipetted into a Fluoro Nunc plate (236105, Thermo Scientific, Waltham, Massachusetts, USA). The fluorescence intensity from each sample was quantified using the Tecan Infinite 200 plate reader (excitation 482 nm, emission 515 nm). Serum controls from two healthy subjects were included in each assay as reference. The efflux capacity values for each subject were expressed relative to the average efflux capacity value of these two serum pools, which was set to 100%. Each sample was analyzed in triplicate.

Cholesteryl ester (CE) transfer measurements were quantified by determining the transfer of CE from radiolabeled exogenous HDL to apoB-containing lipoproteins. As described [28, 29], HDL particles from plasma samples were isolated by ultracentrifugation, and radiolabeled with ^{3}H-cholesterol. Whole serum from each participant was then incubated with the radiolabeled HDL. After incubation, apoB-containing lipoproteins were precipitated with dextrane sulphate/$MgCl_2$. After low-speed centrifugation, the supernatant was transferred into a scintillation vial and the radioactivity was determined using a beta counter. Lipoprotein deficient plasma was used as negative control. CE transfer was measured as the percentage of total radiolabeled CE transferred to apoB-containing lipoproteins, normalized to the initial amount of radioactivity present in the supernatant (3000 counts per minute). Thus, the higher the percentage of radioactivity in the supernatant, the lower the percentage of CE transferred to the apoB-containing lipoproteins pellet.

2.4. Statistics

Results are presented as means ± standard deviations, unless otherwise indicated. Cross-sectional differences at baseline between normal-weight and abdominally obese men were analyzed by an independent-samples *t*-test. Differences in changes between the groups, i.e. diet-induced weight-loss and no–weight loss control treatments, were tested using the one-way ANCOVA with adjustment for baseline values. Pearson correlation coefficients were used to examine cross-sectional relations between cholesterol efflux and CE transfer with anthropometric measurements and cardiometabolic risk markers (age; BMI; waist-to-hip ratio, visceral, subcutaneous and total fat; concentrations of HDL-C, apoA-I, apoB100, TAG, CRP, glucose, C-peptide and insulin; and HOMA-IR). Because the CRP data were not normally distributed, as assessed with the Shapiro-Wilk test, Spearman's rho coefficient was used to examine correlations. Using stepwise regression analysis, the independent contributions of the cardiometabolic risk markers on plasma cholesterol efflux capacity and CE transfer was examined. Only parameters that correlated significantly were initially entered into the model. A p value ≤ 0.05 was considered significant. All statistical analyses were performed with SPSS 20.0 for Mac OS.

3. Results

3.1. Baseline characteristics of the study population

Baseline characteristics of the 25 normal-weight and 52 abdominally obese men are presented in Supplemental Table 1. Age was comparable between the normal-weight and abdominally obese men. As expected, BMI, visceral and subcutaneous fat, and total fat mass were significantly higher in the obese men. Further, fasting serum concentrations of HDL-C were 0.15 percentage point (pp) lower in the abdominally obese men ($p \leq 0.01$), while those of apoB100, TAG and CRP were higher ($p < 0.05$ for all variables). Cholesterol efflux capacity was 9 pp lower in obese men, whereas CE transfer was 5 pp higher ($p \leq 0.01$ for both variables). Finally, serum apoA1 concentrations were comparable between the normal-weight and abdominally obese men.

3.2. Effect of the weight-loss intervention

Forty-nine abdominally obese subjects completed the intervention study. In the 23 subjects in the weight loss group, body weight decreased on average by 10.3 kg, BMI by 3.1 kg/m^2, visceral fat by 0.85 L, subcutaneous fat by 0.84 L, and fat mass by 4.4 pp, compared with the control group [22, 23]. As planned, during the last two weeks of the study, body weight was stable [22]. Weight loss did not change serum HDL-C concentrations, while apoA-I concentrations were significantly decreased after weight-loss by 0.07 g/L (95% CI: −0.14, −0.01 g/L; $p = 0.03$). ApoB100 concentrations were also decreased by 0.17 g/L (95% CI: −0.24, −0.10 g/L; $p = 0.04$). However, cholesterol efflux and CE transfer did not change significantly (Table 1).

3.3. Correlations between HDL parameters

At baseline, cholesterol efflux capacity was significantly associated with BMI ($r = -0.349$; $p < 0.01$; $n = 77$), as well as with visceral, subcutaneous and total fat [($r = -0.285$; $p \leq 0.01$; $n = 76$), ($r = -0.340$; $p < 0.01$; $n = 76$), ($r = -0.302$; $p < 0.01$; $n = 77$), respectively]. Finally, CRP concentrations showed a negative correlation with cholesterol efflux capacity ($r = -0.292$; $p \leq 0.01$; $n = 77$). Stepwise multivariate linear regression including the significant variables showed that only BMI remained as a significant predictor of cholesterol efflux capacity ($\beta = -1.1$; $p \leq 0.001$).

CE transfer at baseline was associated with BMI ($r = 0.312$; $p < 0.01$; $n = 77$), the waist-to-hip ratio ($r = 0.365$; $p \leq 0.001$), and concentrations of HDL-C ($r = -0.473$; $p < 0.001$; $n = 77$), apoA-I ($r = -0.267$; $p < 0.05$; $n = 77$), apoB100 ($r = 0.580$; $p < 0.001$; $n = 77$), TAG ($r = 0.430$; $p < 0.001$; $n = 77$) and CRP ($r = 0.260$; $p < 0.05$; $n = 77$). Moreover, CE transfer showed a significant positive association with C-peptide, insulin concentrations and the HOMA-IR ($r = 0.475$; $r = 0.450$ and $r = 0.440$, respectively; $p < 0.001$ for all three variables). Stepwise linear regression analysis including these significant variables showed that HDL-C ($\beta = -11.9$; $p < 0.001$) and apoB100 ($\beta = 16.5$; $p < 0.001$) predicted CE transfer.

4. Discussion

In this study, we found that ABCA1-mediated cholesterol efflux from cholesterol- loaded macrophages to HDL is higher in normal-weight subjects than in abdominally obese men. However, diet-induced weight loss in the abdominally obese men did not improve cholesterol efflux. Comparable results were observed for CE transfer and HDL-C concentrations, while apoA-I concentrations were even decreased after weight loss.

So far, only a few other studies addressed the effect of dietary induced weight loss on HDL-mediated cholesterol efflux. In these studies, however, no-weight loss control groups were not included, while results were highly variable. Weight-loss of 25 kg induced by a VLCD within a period of 16 weeks reduced cholesterol-efflux, while HDL-C concentrations were not changed and apoA-I concentrations were increased [19]. In another study, weight loss of

Table 1
Effects of dietary weight-loss intervention on HDL related parameters in abdominally obese men

	Normal-weight men	Abdominally obese men Diet-induced weight-loss group		Abdominally obese men No-weight loss control group		Weight-loss effect
	Baseline (n = 25)	Baseline (n = 25)	Follow-up (n = 23)	Baseline (n = 27)	Follow-up (n = 26)	Change[a]
Cholesterol efflux (% pools)	104.2 ± 7.9**	96.3 ± 12.9	92.5 ± 14.1	94.8 ± 8.8	94.2 ± 12.8	−2.2 [-8.09; 3.74]
CE transfer (%)	54.7 ± 6.9*	58.5 ± 6.5	56.8 ± 6.4	60.8 ± 7.4	61.0 ± 7.7	−2.0 [-4.55; 0.6]
ApoA-I (g/L)	1.28 ± 0.18	1.24 ± 0.14	1.18 ± 0.16	1.25 ± 0.16	1.25 ± 0.17	−0.07 [-0.14; −0.01]#
HDL-C (mmol/L)	1.26 ± 0.27*	1.12 ± 0.20	1.13 ± 0.21	1.10 ± 0.24	1.11 ± 0.26	−0.02 [-0.10; 0.06]
ApoB100 (g/L)	0.85 ± 0.15**	1.06 ± 0.25	0.88 ± 0.22	1.10 ± 0.20	1.08 ± 0.18	−0.17 [-0.24; −0.10]##

Values are means ± SD.
Significantly different from abdominally obese men (independent-samples *t*-test): *$p \leq 0.01$; **$p \leq 0.001$.
[a] Changes are expressed as mean [95%CI], and were obtained with one-way ANCOVA with baseline values as covariate: #$p \leq 0.05$; ##$p \leq 0.001$.

nearly 10 kg after 4–6 weeks reduced ABCA1- and ABCG1-mediated cholesterol efflux to HDL. Like in our study, HDL-C concentrations were not changed, while apoA-I concentrations were decreased [20]. In a third study with overweight or obese women, a decrease of about 2 kg after 6 months induced by caloric restriction plus low-intensity exercise, decreased HDL-C concentrations, while apoA-I concentrations as well as ABCG1- and SR-BI-mediated efflux pathways were not changed. Surprisingly, ABCA1-mediated efflux was decreased, although the exercise regime might also have influenced the results [21]. In three other studies, effects of surgery-induced weight loss were examined. Weight loss in these studies was of course larger and were, respectively, 20 kg [30], 25 kg [31] and 56 kg [32]. Despite increases in HDL-C in all three studies, effects on cholesterol efflux - or the transporters involved - were not consistent. In one study, SR-BI- and ABCG1-mediated cholesterol efflux was increased, while the ABCA1-mediated cholesterol efflux was decreased [30]. In contrast, cholesterol efflux was increased after upregulation of ABCA1 expression in the two other studies [31, 32]. No clear reason for these discrepant findings can be provided. One possible explanation may relate to study duration. It has been suggested that HDL-C concentrations increase after weight-loss, but only during the subsequent weight-stable period, whereas they are even decreased during the active weight loss period [33]. In our study, subjects were weight stable for 2 weeks, which may have been too short for HDL metabolism to reach a new steady state. In the other diet-induced weight-loss studies [19-21], a weight stable period was however not included at all. Therefore, it would be of interest to examine effects on HDL metabolism after a longer-weight stable period. Another explanation may relate to the amount of weight loss, which was by far larger after surgery than after diet-induced weight loss. In addition, differences in metabolic disturbances may have played a role. Although our population was abdominally obese, they had no clinical manifestations of any disease. In contrast, in other studies effects of weight loss were examined in insulin-dependent type 2 diabetic patients [19], in patients with the metabolic syndrome [20], or in overweight women, of which 12% were diabetic [21]. Finally, changes in HDL subpopulations following weight loss might also play a role. Indeed, weight reduction is associated with an increase in HDL_2 particle concentrations and a decrease in HDL_3 particle concentrations [34]. In addition, the increase in HDL_2 following surgery-induced weight loss was associated with an improvement in both ABCG1- and SR-BI-efflux pathways. In contrast, ABCA1-mediated efflux was decreased, suggesting that preβ-1 HDL concentration was decreased by weight loss [30]. Possibly, weight loss has a greater effect on the ABCG1 and SR-BI-mediated efflux pathways than on the ABCA1-mediated efflux. In addition, when using apoB-depleted serum as cholesterol acceptor, not only (preβ-1) HDL, but also other factors such as albumin, may contribute to the cholesterol efflux [35] However, it should be noted that with our assay we did observe cross-sectional differences in cholesterol efflux between the abdominally obese and the normal-weight men at baseline. This suggests that ABCA1-mediated cholesterol efflux is related to body mass.

To the best of our knowledge, the relationship between fat distribution and cholesterol efflux capacity has not been investigated so far. Visceral fat is thought to secrete more pro-inflammatory cytokines than subcutaneous fat [8]. As inflammation is negatively associated with cholesterol efflux capacity [36], this could theoretically explain the lower cholesterol efflux capacity in obese subjects. In fact, our lean subjects also had lower CRP concentrations than the abdominally obese subjects (Supplemental Table 1). However, CRP levels were not changed after the weight loss intervention. It has been estimated that for each 1 kg of weight loss, CRP levels decreases with 0.13 mg/L [37]. Possibly, the amount of weight loss in our study was too small to observe any statistically significant effect [22].

In contrast to other studies [38-40], no association between cholesterol efflux and the waist-to-hip ratio was found. Efflux capacity was negatively associated with both visceral and subcutaneous fat, but after stepwise linear regression analysis, only BMI remained a significant predictor of cholesterol efflux capacity. As all the abdominally obese men a waist circumference between 102 and 110 cm, it is possible that the range in the waist-to-hip ratio was not large enough.

Reductions in CE transfer have been reported after surgery-induced weight loss [14, 30, 41]. However, not many studies have addressed the effects of diet-induced weight loss on CE transfer. In a small study with 4 women without control group, 2 months of calorie restriction was associated with a reduction in both CETP activity and mass [42]. In another study with obese men, the intake of a low-fat energy-restricted diet for 3 months did not change CE transfer, despite a 10 kg reduction in body weight [43]. A 4 weeks low caloric diet-induced weight loss decreased body weight by 4 kg and was associated with a decrease in CETP mass concentrations [44]. Finally, caloric restriction for 16 weeks was accompanied by a decrease in CETP concentration [19]. However, CE transfer was not investigated in these two latter studies [19, 44].

As in other studies [14, 42, 45, 46], BMI was positively associated with CE transfer, which might be related to the CETP synthesis in adipose tissue [47]. In fact, it has been reported that CETP mRNA levels in adipose tissue are positively associated with plasma CETP concentrations [48]. As CETP expression is higher in subcutaneous fat than in visceral fat [49], and the subcutaneous fat depot is larger, it can be expected that CETP mass is more strongly correlated with subcutaneous fat, as shown by Arai et al. [42]. However, these findings were not confirmed in a recent large cross-sectional study examining associations between fat distribution and CETP concentrations [50]. Although CE transfer does not necessarily reflect CETP concentrations [51], we also found no evidence that the

various fat depots were differently associated with CE transfer.

The relationship between CE transfer and cholesterol efflux is not clear. In one study, elevated CE transfer was positively associated with cholesterol efflux capacity in women, but not in men [52]. In addition, in patients at risk for cardiovascular disease, a high plasma concentration of CETP was associated with a high efflux capacity, and CETP mass was found to significantly predict cholesterol efflux capacity [53]. Unfortunately, we did not measure CETP mass in the present study. However, no association between CE transfer and cholesterol efflux capacity was found.

In conclusion, the present study shows that in men ABCA1-mediated macrophage cholesterol efflux and CE transfer are associated with body mass. However, these parameters were not changed after a six weeks diet-induced weight loss of 10 kg in abdominally obese men followed by a 2 weeks weight-stable period. Future studies should address the importance of a longer period of stable body weight and the involvement of other transporters, such as ABCG1 and SR-BI, on cholesterol efflux from macrophages.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

The authors' responsibilities were as follows; C.P.T.: performed the statistical analyses, interpreted the data, and wrote the manuscript; J.P.: designed the study, interpreted the data, and wrote the manuscript; P.J.J.: designed the study, performed the intervention study, and wrote the manuscript; M.K.: performed the cholesterol efflux and CETP experiments; Y.H.A.M.K.: designed the study, performed the intervention study, and wrote the manuscript; C.G.S.: designed the study, and wrote the manuscript; A.R.: performed the cholesterol efflux and CETP experiments, and wrote the manuscript; R.P.M.: designed the study, interpreted the data, had overall responsibility for the study, and wrote the manuscript. All authors read and approved the final manuscript. None of the authors declared any financial or personal conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.04.029.

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