Genetic determinants of cholesterol and energy metabolism : implications for cardiometabolic health

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Serum CETP concentration is not associated with measures of body fat: The NEO study

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Abstract

Introduction

Adipose tissue has been postulated to contribute substantially to the serum cholesteryl ester transfer protein (CETP) pool. However, in a recent large cohort study waist circumference was not associated with plasma CETP. The aim of the present study was to further examine associations of accurate measures of body fat and body fat distribution with serum CETP concentration.

Methods

In this cross-sectional analysis of the Netherlands Epidemiology of Obesity study, we examined in 6,606 participants (aged 45-65 years) the associations of total body fat, body mass index (BMI), waist circumference, waist-to-hip ratio (WHR), abdominal subcutaneous (aSAT) and visceral adipose tissue (VAT) assessed with magnetic resonance imaging (n=2,547) and total and trunk fat mass assessed with dual-energy X-ray absorptiometry (n=909) with serum CETP concentration. Regression models were adjusted for age, ethnicity, sex, dietary intake of fat and cholesterol, physical activity, smoking and menopausal status.

Results

Mean (SD) age was 56 (6) years and BMI 26.3 (4.4) kg/m^2, 56% were women. Mean serum CETP concentration was 2.47 μg/mL. The difference in serum CETP was 0.02 μg/mL (95%CI: -0.01, 0.05) per SD total body fat (8.7%), and 0.02 μg/mL (0.00, 0.04) per SD BMI (4.4 kg/m^2). Similar associations around the null were observed for waist circumference, WHR, aSAT, VAT, total and trunk fat mass.

Conclusion

In this population-based study, there was no evidence for clinically relevant associations between several measures of body fat and serum CETP concentration. This finding implies that adipose tissue does not contribute to the CETP pool in serum.

Introduction

Cholesteryl ester transfer protein (CETP) facilitates the net flux of cholesteryl esters from high-density lipoproteins (HDL) towards (very) low-density lipoproteins ((V)LDL), coupled to a net flux of triglycerides (TG) from (V)LDL to HDL.[1] As such, CETP contributes to an atherogenic lipoprotein profile (i.e. high LDL/HDL-cholesterol (C) ratio), as has been extensively studied in both humans and in mice transgenic for human CETP.[2,3]

Because CETP contributes to an adverse lipoprotein profile and high LDL-C levels have been associated with increased cardiovascular disease risk,[4,5] a reduction of CETP activity in plasma has been proposed as a strategy to improve the lipoprotein profile and reduce cardiovascular disease.[6] This could be achieved by inhibition of plasma CETP with small molecules (i.e. CETP inhibitors), of which anacetrapib[7] and TA-8995[8] are currently being tested in clinical trials. Inhibition of CETP expression in tissues contributing to the pool of plasma CETP may be an alternative approach, but the tissues and cell types responsible for determining plasma CETP levels are still not fully elucidated.

Adipose tissue, in particular subcutaneous adipose tissue, and the liver are the most abundant sources of CETP mRNA expression1,[9–11] and hence they have both been postulated to contribute substantially to the plasma CETP pool.[1,12,13] Small studies indeed suggested that adipose tissue contributes to the plasma CETP concentration.[12,14–18] However, most of these studies compared measures of body fat content with plasma CETP activity (assessed by lipid transfer between lipoproteins)[14,16,17], instead of plasma CETP concentration by enzyme-linked immunosorbent assay (ELISA), and plasma CETP activity is highly dependent on the lipoprotein context during the measurement.[19] In addition, a wide variety of methods has been used to measure CETP activity,[19] yielding comparison of CETP activity between different studies difficult. Consequently, CETP activity does not accurately represent plasma CETP concentration and the interpretation of previous study results regarding the contribution of adipose tissue to the plasma CETP pool is therefore questionable. Few studies related plasma CETP concentration to measures of body fat. One study (n=80) found higher CETP concentrations in obese individuals (body mass index (BMI) 30-45 kg/m^2), than in lean participants (BMI 18.5-25 kg/m^2)[15], and in another study (n=44) a positive correlation was observed between total fat mass, measured with bioelectrical impedance analysis (BIA), and plasma CETP concentration.[18]

Interestingly, in a large population-based study (654 men and 780 women, 40-70 year) we recently observed that waist circumference was not associated with the plasma CETP concentration[20], which suggested that central adipose tissue does not contribute to CETP in plasma. However, waist circumference is a crude measure of adipose tissue mass

and does not discriminate between the abdominal subcutaneous and visceral fat depots. Therefore, the contribution of specific adipose tissue depots to plasma CETP remains unresolved. The aim of the present study was to examine the associations between measures of body fat and serum CETP concentration in a large, population-based study, to elucidate the contribution of adipose tissue to CETP in serum.

Materials and Methods

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study of men and women aged between 45 and 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. The present study is a cross-sectional analysis of the baseline measurements. Detailed information about the study design and data collection has been described elsewhere.[21]

NEO study participants were recruited from September 2008 until September 2012 in the greater area of Leiden, The Netherlands, to build up a population-based cohort of 6,671 participants. Men and women out of four Dutch municipalities, aged between 45 and 65 years with a BMI of 27 kg/m^2 or higher were eligible to participate. From one municipality (Leiderdorp, The Netherlands) all inhabitants aged between 45 and 65 years were invited to participate regardless of their BMI, in order to obtain a reference distribution for BMI. Participants visited the NEO study center after an overnight fast of at least 10 hours for extensive baseline measurements, including blood sampling and anthropometry. Research nurses recorded current medication use. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information.

At the study center participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices or claustrophobia). A body circumference of more than 1.70 m was an additional contraindication for undergoing MRI. Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo direct assessment of abdominal subcutaneous adipose tissue (aSAT) and visceral adipose tissue (VAT). Another random subset, approximately 15% of the total population, underwent dual-energy X-ray absorptiometry (DXA) to measure total fat mass and trunk fat mass. In the present analysis, we excluded study participants with missing data on serum CETP concentration (n=65). The present study population therefore comprises 6,606 participants.

The NEO study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Measures of body fat

Body weight and total body fat were determined with the Tanita bio-impedance balance (TBF-310, Tanita International Division, UK) without shoes and with subtraction of one kilogram (kg) to correct for the weight of clothing. BMI was calculated by dividing the weight in kilograms by the height in meters squared. During physical examination waist circumference was measured mid-way between the lower costal margin and the iliac crest and hip circumference was measured at the maximum circumference of the buttocks. Subsequently the waist-to-hip-ratio (WHR) was calculated.

aSAT and VAT were quantified by MRI. Of the total NEO study population 2,580 participants underwent MRI of the abdomen. MRI images were acquired during a breath-hold using a turbo spin echo imaging protocol (1.5 Tesla MR system, Philips Medical Systems, Best, The Netherlands). Three transverse images with a slice thickness of 10 mm were obtained at the level of the fifth lumbar vertebra. Abdominal subcutaneous and visceral fat depots were quantified by converting the number of pixels to square cm (MASS, Medis, Leiden, the Netherlands). The average of the three slices was used for analyses. In 11 participants the images were of insufficient quality for the quantification of aSAT and VAT, and therefore 2,569 participants had a successful measurement of aSAT and VAT.

DXA was performed in 915 NEO study participants. With a whole body DXA scan (Hologic Discovery A, Tromp Medical BV, Castricum, The Netherlands) total fat mass (kg) was measured. The abdominal region was defined as 20% from pelvis cut to neck cut, and trunk fat mass (kg) comprised the weight of adipose tissue in this region. 912 participants had successful measurements of total and trunk fat mass, as three DXA scans were of insufficient quality.

Serum CETP concentration

During the visit to the NEO study center venous blood samples were obtained from the antecubital vein from all participants after a 10 hour overnight fast. Aliquots of plasma and serum were stored after centrifugation at -80°C. From 11 April until 16 July 2014 CETP concentrations in serum that had undergone one previous freeze-thaw cycle were measured with enzyme-linked immune sorbent assay (ELISA) kits according to the manufacturer's instructions (DAIICHI CETP ELISA, Daiichi, Tokyo, Japan).

Covariates

Questionnaires were sent to all participants and completed at home. The general questionnaire included questions on demographic, lifestyle and clinical data. Smoking status was categorized into never smoker, former smoker and current smoker. Menopausal status

was classified as premenopausal, perimenopausal (menopausal during last year) or postmenopausal. Participants were classified as having pre-existing cardiovascular disease when a medical history of myocardial infarction, angina, congestive heart failure, stroke or peripheral vascular disease was reported via the questionnaire. Participant were classified as having diabetes when the disease was self-reported via the questionnaire, when antidiabetic medication was used, or when a fasting glucose ≥7.0 mmol/L was measured at baseline. Dietary intake of energy, fat, cholesterol and alcohol was assessed with a semi-quantitative food frequency questionnaire (FFQ)[22], and calculated from the FFQ using the 2011 version of the Dutch food composition table (NEVO-2011). Nutrient intake was expressed as energy percentage (En%) of total energy intake. Physical activity during leisure time was evaluated with the short questionnaire to assess health-enhancing physical activity (SQUASH)[23], and expressed as metabolic equivalents of task (MET)*hours/week, with MET indicating the intensity of an activity. Fasting serum total cholesterol and TG concentrations were measured with enzymatic colorimetric assays (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany) and fasting serum HDL-C concentrations with third generation homogenous HDL-C methods (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany). Fasting LDL-C concentrations were calculated using the Friedewald equation.[24]

Statistical analyses

In the NEO study, individuals with a BMI of 27 kg/m^{2}or higher were oversampled. To correctly represent baseline associations in the general population[25], adjustments for the oversampling of individuals with a BMI ≥27 kg/m^2 were made. This was done by weighting all participants towards the BMI distribution of participants from the Leiderdorp municipality[26], whose BMI distribution was similar to the BMI distribution of the general Dutch population in the age range of 45-65 years.[27] In practice, this means that participants with a lower BMI were assigned larger weights than participants with a higher BMI in the analyses. All results are based on weighted analyses, and therefore apply to a population-based study without oversampling of individuals with overweight or obesity. As a consequence, the weighted characteristics of the population are expressed in proportions instead of absolute numbers.

Descriptive statistics of the study population were summarized as mean (standard deviation, SD) or proportions and stratified by sex and total body fat. We considered total body fat assessed with BIA a more accurate measure of body fat than BMI, and categorized a total body fat percentage greater or equal to 25% as high body fat in men, and greater or equal to 35% as high body fat in women.[28–30]

Weighted linear regression analyses were performed to evaluate the associations between standardized z-scores of measures of body fat (i.e. total body fat, BMI, weight circumference, WHR, aSAT, VAT, total fat mass and trunk fat mass) and serum CETP concentration. Differences in serum CETP concentration (beta coefficients from linear regression) were expressed per SD increase in body fat measure. Standardization of body fat measures allowed for comparison of the strengths of the associations between the different body fat determinants. Crude models (model 1) were adjusted for age, ethnicity and sex (model 2). Subsequently, dietary fat intake, dietary cholesterol intake, physical activity, smoking status and menopausal status were added as confounding variables (model 3). As total body fat is strongly related to waist circumference, WHR, VAT and trunk fat mass,[31] the linear regression models of the associations between each of these variables and serum CETP concentration were additionally adjusted for total body fat percentage (model 4). Weighted mean differences and corresponding 95% confidence intervals (CI) were reported. We examined whether associations differed between men and women by including product terms between sex and measures of body fat in the regression models. As there is evidence that lipid-lowering drugs (e.g., statins, fibrates and niacin) lower the plasma CETP concentration32-37, we also examined the presence of interaction between lipid lowering drug use and measures of body fat by including product terms of lipid lowering drug use with measures of body fat into the models. When product terms were statistically significant, stratified analyses were performed.

All analyses were performed using STATA Statistical Software (Statacorp, College Station, Texas, USA), version 12.0.

Results

Population characteristics

Descriptive statistics of the 6,606 included participants are presented in Table 2.1. Serum CETP concentrations were similar for participants with high and lower body fat, both in men and women.

Lipid lowering drugs were used by 10.7% of participants (10.4% used a statin, 0.1% a fibrate, 0.1% both a statin and a fibrate, and 0.1% a bile acid sequestrant, a cholesterol absorption inhibitor or niacin). Of the lipid lowering drug users, 38% were women and mean (SD) age was 59 (5) years. For men mean (SD) total body fat was 27.8 (6.1)% and for women 40.9 (5.8)%. 5,572 participants did not use lipid-lowering drugs, 58% were women and mean (SD) age was 55 (6) years. For men mean (SD) total body fat was 24.5 (5.7)% and for women 36.6 (6.6)%.

Serum CETP concentration was higher in women (2.60 μg/mL, 95% CI 2.57, 2.63) than in men (2.31 μg/mL, 95% CI 2.28, 2.34). Figure 2.1 shows that serum CETP concentrations were lower in users of lipid lowering drugs than in non-users, both in men (1.98 μg/mL, 95% CI 1.90, 2.06 vs 2.37 μg/mL, 95% CI 2.34, 2.40) and in women (2.21 μg/mL, 95% CI 2.11, 2.32 vs 2.63 μg/mL, 95% CI 2.60, 2.66).

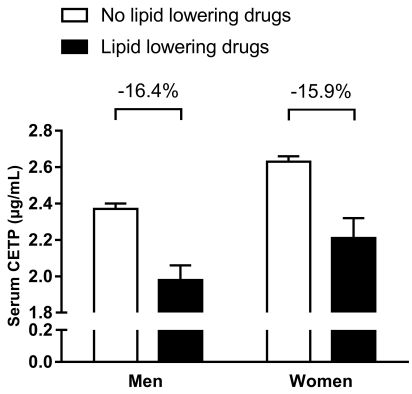

Figure 2.1: Mean serum CETP concentration in non-users and users of lipid lowering drugs, stratified by sex. Results are based on analyses weighted towards the normal BMI distribution, with n=6,606; 1,034 users of lipid lowering drugs, 5,572 non-users. Error bars represent 95% confidence intervals.

Associations between measures of body fat and serum CETP concentration

In Table 2.2 the crude and adjusted differences in serum CETP concentration per SD of the body fat measures are presented. After adjustment for all potential confounding factors total body fat, waist circumference, WHR, aSAT and trunk fat mass were not associated with serum CETP concentration. Per 4.4 kg/m^2 increase in BMI the difference in serum CETP was 0.02 μg/mL (95% CI 0.00, 0.04). Per 56.3 cm^2 increase in VAT the difference in serum CETP was -0.05 μg/mL (95% CI -0.10, -0.01). Per 7.0 kg increase in total fat mass (DXA) the difference in serum CETP was -0.06 μg/mL (95% CI -0.10, -0.02). Figure 2.2 shows the differences in serum CETP concentration per SD of the body fat measures, after adjustment for all confounding factors.

The associations between the measures of body fat and serum CETP concentration did not differ between men and women (p-values of product terms of measures of body fat with sex ranged from 0.154 to 0.988 in adjusted models). Likewise, the associations between body fat measures and serum CETP concentration did not differ between users and non-users of lipid lowering drugs (p-values of product terms of measures of body fat with lipid lowering drug use ranged from 0.053 to 0.934 in adjusted models), except for total body fat. The p-value for the product term of total body fat with lipid lowering drug use was 0.039.

Analyses stratified by use of lipid lowering drugs showed after adjustment for all potential confounding factors, that for users of lipid lowering drugs the difference in serum CETP was -0.02 μg/mL (95% CI -0.11, 0.07) per SD of total body fat (8.8%), and for non-users the difference was 0.06 μg/mL (95% CI 0.03, 0.09) per SD of total body fat (8.6%).

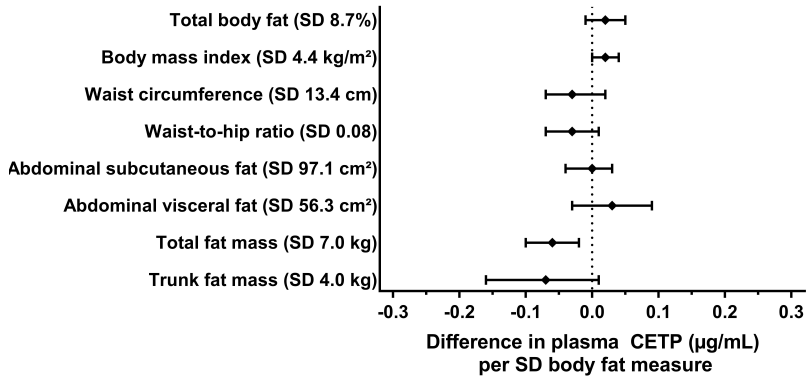

Figure 2.2: Adjusted difference[a] in serum CETP concentration per standard deviation body fat measure, for the total study population. Results are based on analyses weighted towards the normal BMI distribution, with n=6,606; magnetic resonance imaging was performed in a random subsample with n=2,547, dual-energy X-ray absorptiometry was performed in a random subsample with n=909. Error bars represent 95% confidence intervals.

[a] Beta coefficient from linear regression adjusted for age, ethnicity, sex, dietary fat intake, dietary cholesterol intake, physical activity, smoking and menopausal status. Additional adjustment for body fat percentage was restricted to the linear regression analyses of waist circumference, waist-to-hip ratio, trunk fat mass and abdominal visceral fat.

Table 2.1: Demographic and clinical characteristics of the Netherlands Epidemiology of Obesity study population (aged 45-65 years) without participants with missing data on serum CETP concentration, stratified by sex and total body fat.

Characteristics	Men		Women	
	Total body fat (%)[a]			
	<25	**≥25**	**<35**	**≥35**
Proportion of participants (%)	24	20	21	35
Ethnicity (% white)	94	97	93	95
Age (year)	56 (7)	56 (7)	55 (5)	56 (5)
Educational level (% high)[b]	55	40	55	38
Tobacco smoking (% current and former smokers)	60	72	56	60
Physical activity during leisure time (MET hours/week)	40 (37)	37 (39)	40 (26)	34 (28)
Menopausal status (% postmenopausal)	-	-	56	63
Mean daily dietary intake				
Dietary fat intake (En% derived from total fat intake)	33.9 (6.0)	34.5 (6.5)	34.2 (4.9)	34.3 (5.3)
Dietary cholesterol intake (mg/day)	219 (93)	236 (108)	178 (68)	192 (72)
Alcohol intake (g/day)	19 (19)	22 (23)	10 (9)	11 (12)
Measures of body fat				
Total body fat (%)	20.8 (3.2)	29.9 (5.1)	30.1 (3.4)	40.9 (4.0)
Body mass index (kg/m^2)	24.6 (2.2)	29.5 (3.8)	22.1 (1.7)	28.2 (4.4)
Waist circumference (cm)	92 (7)	106 (11)	76 (5)	94 (11)
Waist-to-hip ratio	0.91 (0.06)	0.98 (0.06)	0.80 (0.05)	0.87 (0.06)
Abdominal subcutaneous fat (cm^2)	167 (52)	264 (91)	180 (48)	316 (86)
Abdominal visceral fat (cm^2)	88 (43)	150 (67)	39 (18)	87 (40)
Total fat mass (kg)	20.1 (4.1)	29.6 (8.1)	19.6 (3.2)	31.9 (7.8)
Trunk fat mass (kg)	9.9 (2.6)	16.0 (4.6)	8.2 (2.0)	15.1 (4.1)
Fasting serum concentrations				
CETP (μg/mL)	2.30 (0.63)	2.32 (0.70)	2.58 (0.58)	2.61 (0.63)
Total cholesterol (mmol/L)	5.48 (1.07)	5.56 (1.21)	5.69 (0.87)	5.86 (1.01)
HDL-cholesterol (mmol/L)	1.44 (0.39)	1.24 (0.34)	1.94 (0.40)	1.64 (0.39)
LDL-cholesterol (mmol/L)	3.47 (1.00)	3.52 (1.12)	3.34 (0.81)	3.63 (0.92)
Triglycerides (mmol/L)	1.19 (0.95)	1.67 (1.15)	0.86 (0.45)	1.23 (0.71)
Comorbidity and medication				
Diabetes (%)	3	13	1	6
Cardiovascular disease (%)	6	10	3	6
Lipid lowering drugs (%)	9	22	2	10

Results are based on analyses weighted towards the normal BMI distribution, with n=6,606 (3,134 men; 3,472 women)[c]; magnetic resonance imaging was performed in a random subsample with n=2,547, dual-energy X-ray absorptiometry was performed in a random subsample with n=909.

[a] High body fat was defined as a total body fat percentage ≥25% for men and ≥35% for women. [32]

[b] High educational level: higher secondary education (according to Dutch educational system), higher vocational education, university, PhD.

[c] Missing data: n=13 for ethnicity, n=68 for educational level, n=8 for smoking status, n=3 for menopausal status, n=5 for dietary fat intake, n=4 for dietary cholesterol intake, n=4 for alcohol intake, n=120 for physical activity, n=31 for total body fat, n=7 for waist circumference, n=7 for waist-to-hip ratio, n=20 for total cholesterol, n=21 for HDL-cholesterol, n=22 for LDL-cholesterol, n=21 for triglycerides, n=28 for cardiovascular disease, n=40 for diabetes.

BMI, body mass index; CETP, cholesteryl ester transfer protein; En%, energy percent; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalents of task.

Table 2.2: Difference in serum CETP concentration per standard deviation body fat measure, for the total study population.

Body fat measure	Model	Difference in serum CETP concentration (μg/mL)[a]	95% Confidence interval
Total body fat	1	0.11	0.09, 0.13
SD: 8.7%	2	0.03	0.00, 0.06
	3	0.02	-0.01, 0.05
Body mass index	1	0.01	-0.01, 0.03
SD: 4.4 kg/m^2	2	0.03	0.01, 0.05
	3	0.02	0.00, 0.04
Waist circumference	1	-0.05	-0.07, -0.03
SD: 13.4 cm	2	0.01	-0.01, 0.03
	3	0.01	-0.02, 0.03
	4[b]	-0.03	-0.07, 0.02
Waist-to-hip ratio	1	-0.09	-0.12, -0.07
SD: 0.08	2	-0.01	-0.04, 0.02
	3	-0.01	-0.04, 0.02
	4[b]	-0.03	-0.07, 0.01
Abdominal subcutaneous adipose tissue	1	0.04	0.01, 0.07
SD: 97.1 cm^2	2	0	-0.03, 0.03
	3	0	-0.04, 0.03
Visceral adipose tissue	1	-0.1	-0.13, -0.06
SD: 56.3 cm^2	2	-0.04	-0.08, -0.01
	3	-0.04	-0.08, -0.01
	4[b]	-0.05	-0.10, -0.01
Total fat mass	1	-0.01	-0.05, 0.03
SD: 7.0 kg	2	-0.05	-0.09, -0.01
	3	-0.06	-0.10, -0.02
Trunk fat mass	1	-0.05	-0.09, -0.01
SD: 4.0 kg	2	-0.05	-0.09, -0.01
	3	-0.06	-0.10, -0.02
	4[b]	-0.07	-0.16, 0.01

Results are based on analyses weighted towards the normal BMI distribution, with n=6,606[c]; magnetic resonance imaging was performed in a random subsample with n=2,547, dual-energy X-ray absorptiometry was performed in a random subsample with n=909.
Model 1: crude.
Model 2: adjusted for age, ethnicity and sex.
Model 3: model 2 + adjusted for dietary fat intake, dietary cholesterol intake, physical activity, smoking status and menopausal status.
Model 4[b]: model 3 + adjusted for body fat percentage.

[a] Beta coefficients from linear regression; expressed per SD increase in body fat measure.

[b] Additional adjustment for body fat percentage was restricted to the linear regression analyses of waist circumference, waist-to-hip ratio, trunk fat mass and abdominal visceral fat.

[c] Missing data: n=31 for total body fat, n=7 for waist circumference, n=7 for waist-to-hip ratio, n=13 for ethnicity, n=5 for dietary fat intake, n=4 for dietary cholesterol intake, n=120 for physical activity, n=8 for smoking status, n=3 for menopausal status.

Discussion

Based on the contradiction between findings of initial small studies reporting a positive relation between body fat and plasma CETP[12,14–18], and our recent finding that waist circumference was not associated with plasma CETP concentration[20], we aimed to further examine the contribution of adipose tissue to the serum CETP concentration in a large, population-based study. In 6,606 participants of the NEO study body fat and body fat distribution were extensively characterised using anthropometric measures, BIA, MRI and DXA. There was no evidence for clinically relevant associations between measures of body fat and serum CETP concentration as assessed by ELISA. This finding implies that adipose tissue does not contribute to the CETP pool in serum.

Several previous studies showed a positive association between body fat and plasma CETP[12,14–18], but these studies were relatively small, and mostly measured plasma CETP activity. Various CETP activity assays have been developed that measure either exogenous or endogenous lipid transfer capacity of CETP. Since endogenous CETP activity measurements are dependent on the lipoprotein concentrations in plasma, such assays do not necessarily reflect CETP concentration.[19] Furthermore, several of these studies presented merely crude differences and correlations, and did not account for any potential confounding factors. In the present study total body fat, waist circumference, WHR, aSAT and trunk fat mass were not associated with serum CETP concentration after adjustment for potential confounding factors. Although BMI, VAT and total fat mass (DXA) were statistically significant associated with serum CETP, the associations were weak and in opposite directions. For example, for an individual with a length of 1.80 m, the association of BMI represents a 0.8% increase in mean serum CETP concentration (2.47 μg/mL) following a weight gain of 14.3 kg. Therefore, we consider these associations to be of no clinical relevance.

Since we and others have shown that lipid-lowering drugs, including statins, decrease the plasma CETP concentration[33–38], we tested for interaction between lipid lowering drug use and measures of body fat. Except for total body fat, there was no interaction between measures of body fat and use of lipid lowering drugs. This indicates that, although users of lipid lowering drugs have lower serum CETP concentrations, the associations between these body fat measures and serum CETP concentration do not differ between users and non-users of lipid lowering drugs. The exclusion of users of lipid lowering drugs in the model for total body fat showed a difference of 0.06 μg/mL (95% CI 0.03, 0.09) in serum CETP concentration per 8.6% increase in total body fat. We consider this association to be clinically irrelevant.

The detection of statistically significant, but clinically irrelevant associations may be explained by the large sample size of the present study, making it possible to detect even very small differences. Moreover, BMI and total body fat are less precise measures of body fat than DXA measurements [39], which were not positively associated with serum CETP concentration. We observed a weak negative association between total fat mass (DXA) and serum CETP concentration (-0.06 μg/mL per 7.0 kg higher total fat mass), arguing against the contribution of adipose tissue to CETP in serum.

It has been suggested that there are tissue specific differences in the contribution of adipose tissue to plasma CETP [12,17], as the expression of CETP mRNA in subcutaneous adipose tissue is higher than in visceral adipose tissue11. In this large study population there was no positive associations between either aSAT or VAT with serum CETP concentration (n=2,547), indicating that neither visceral nor subcutaneous adipose depots contributes to the plasma CETP concentration. Recently, we observed in 93 obese individuals that the CETP mRNA expression in the liver largely exceeds the expression in both subcutaneous and visceral adipose tissue. [20] In the liver CETP expression in confined to hepatic macrophages (Kupffer cells) and hepatic Kupffer cell content strongly correlates with plasma CETP concentration. [20]

It is of interest that weight loss was shown to decrease plasma CETP concentration [17,40–42], which at first glance may seem to be contrary to our present findings. However, it is plausible that effects other than a reduction in adipose tissue caused the observed decrease in plasma CETP concentration. Since weight reduction is accompanied by a decrease in hepatic inflammation [43,44], a reduction in Kupffer cell content could be responsible for the decrease of plasma CETP. Another mechanism that might explain a decrease in plasma CETP after weight loss is diminished activation of the liver X receptor α (LXRα). The natural ligands for LXRα are oxysterols and the expression of CETP is mainly under control of this receptor. [45,46] We speculate that a decrease in oxysterols following weight loss might lead to diminished activation of LXRα, and consequently lower CETP production. The main strength of the present study is the availability of several accurate measures of body fat and body fat distribution, in addition to information on multiple potential confounding factors. The observational design is however a limitation, as we cannot exclude the presence of residual confounding by unknown, unmeasured or not accurately measured confounding factors. Besides, the study population was predominantly white and results may therefore not apply to other ethnical groups.

In conclusion, in this large population-based study there was no evidence for clinically relevant associations of total body fat, body mass index, waist circumference, waist-to-hip ratio, subcutaneous adipose tissue (MRI), visceral adipose tissue (MRI), total fat mass

(DXA) and trunk fat mass (DXA) with serum CETP concentration. These findings imply that adipose tissue does not contribute to the CETP pool in serum. To fully understand the role of CETP in cardiovascular disease, future research is needed regarding the physiology and biological function of CETP, especially in light of the termination of the recent clinical trials with the CETP inhibitors dalcetrapib[47] and evacetrapib[48], both due to insufficient efficacy.

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