



Metabolic liver inflammation in obesity does not robustly decrease hepatic and circulating CETP



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ABSTRACT

Background and aims: We recently showed that plasma cholesteryl ester transfer protein (CETP) is mainly derived from VSIG4-positive Kupffer cells. Activation of these cells by the bacterial endotoxin lipopolysaccharide (LPS) strongly decreases *CETP* expression. As Kupffer cell activation plays a detrimental role in the progression of non-alcoholic fatty liver disease (NAFLD), we aimed to study if metabolic liver inflammation is also associated with a decrease in hepatic and circulating CETP.

Methods: We collected plasma and liver biopsy samples at various stages of NAFLD from 93 obese individuals who underwent bariatric surgery. Liver lobular inflammation was histologically determined, and liver *CETP* expression, CETP positive cells, circulating CETP concentrations, and liver *VSIG4* expression were quantified.

Results: Mean (SD) plasma CETP concentration was 2.68 (0.89) $\mu\text{g/mL}$. In the presence of liver inflammation, compared to the absence of pathology, the difference in hepatic *CETP* expression was -0.03 arbitrary units (95% CI $-0.26, 0.20$), the difference in number of hepatic CETP positive cells (range 11–140 per mm^2) was -20.0 per mm^2 (95% CI $-41.6, 1.9$), and the difference in plasma CETP was -0.35 $\mu\text{g/mL}$ (95% CI $-0.80, 0.10$). Hepatic *VSIG4* expression was not associated with liver inflammation (0.00; 95% CI $-0.15, 0.15$).

Conclusions: We found no strong evidence for a strong negative association between metabolic liver inflammation and CETP-related outcomes in obese individuals, although we observed consistent trends. These data indicate that metabolic liver inflammation does not mimic the strong effects of LPS on the hepatic expression and production of CETP by Kupffer cells.

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1. Introduction

Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl esters from high-density lipoproteins (HDL) towards (very) low-density lipoproteins ((V)LDL), coupled to a net flux of triglycerides from (V)LDL to HDL, thereby contributing to an atherogenic lipoprotein profile [1]. Recently, we showed in human studies that circulating levels of CETP are mainly determined by

resident hepatic macrophages (Kupffer cells) [2], without a contribution of adipose tissue [3]. In fact, hepatic Kupffer cell content strongly correlated with both hepatic *CETP* expression and plasma CETP concentration [2].

Besides its established role in lipid metabolism, accumulating evidence suggests that CETP is involved in immunity and inflammatory processes [4]. This is in line with the primary expression of *CETP* by Kupffer cells, which play a pivotal role in inflammation and host defence against e.g. Gram-negative bacterial infections. Kupffer cells can detect lipopolysaccharide (LPS), a potent endotoxin released from Gram-negative bacteria, and induce an anti-bacterial response via the release of pro-inflammatory cytokines

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such as tumor necrosis factor α (TNF- α) and interleukin-1 β (IL-1 β) [5]. Interestingly, LPS reduces liver *CETP* expression and circulating CETP concentrations in CETP-transgenic mice [6]. Similarly, LPS, TNF- α and IL-1 β decrease *CETP* expression and CETP concentration in hamsters [7]. LPS administration also decreased plasma CETP concentrations in humans [8]. Collectively, inflammatory stimuli seem to downregulate *CETP* expression by Kupffer cells.

Recently, we showed in *CETP*-expressing mice that hepatic expression of *CETP* is confined to a specific subset of Kupffer cells that also express *Vsig4*, which is a marker of resting Kupffer cells [8]. Also in humans, hepatic *VSIG4* expression correlated with both liver *CETP* expression and plasma CETP concentration [8]. In this same study, we showed that LPS inoculation of mice markedly reduced liver *CETP* expression and production, accompanied by a similar loss of the Kupffer cell marker *Vsig4*. Combined, these data indicate that liver *CETP* expression is exclusively confined to a resting Kupffer cell subset, which loses *CETP* expression when activated by LPS.

Although the mechanisms by which inflammatory stimuli reduce *CETP* expression are not yet fully understood, they may counteract liver X receptor (LXR)-induced *CETP* expression. LXRs regulate a variety of genes to control cholesterol and lipid homeostasis and protect cells from an overload of toxic sterol [9]. *CETP* gene transcription is induced via an LXR α response element in the *CETP* promoter region [10,11]. Natural ligands for LXR α are oxidized derivatives of cholesterol (i.e. oxysterols) [12,13] and the cholesterol precursor desmosterol [14]. Interestingly, *in vitro* exposure of murine macrophages to LPS, TNF- α or interferon γ (IFN- γ) suppresses an LXR α -induced increase in *CETP* expression [15]. In addition, we recently showed that LPS also reduces *CETP* expression as induced by an LXR agonist in human macrophages [8]. Taken together, LXR α -induced expression of *CETP* by Kupffer cells may be counteracted by inflammatory stimuli.

Apart from their beneficial role in host defence [5], Kupffer cells play a detrimental role in the progression of non-alcoholic fatty liver disease (NAFLD) from simple steatosis to non-alcoholic steatohepatitis (NASH), which is characterized by liver inflammation [16–21]. With the growing prevalence of obesity worldwide, an increasing number of individuals suffer from NAFLD [22]. The central role of Kupffer cells in NAFLD is partly explained by excess free cholesterol that cannot be detoxified by esterification, leading to cholesterol crystallization not only within hepatocytes but also within Kupffer cells, which consequently activates inflammatory pathways [16,23,24]. The aim of the present study is to determine whether metabolic liver inflammation, as a component of NAFLD, is associated with a decrease in liver *CETP* expression and CETP production similar to LPS. To this end, we collected liver biopsy samples at various stages of NAFLD from a bariatric surgery cohort to histologically determine liver inflammation, and to quantify liver *CETP* and *VSIG4* expression, liver lipids, liver CETP positive cells and circulating CETP concentrations.

2. Materials and methods

2.1. Study design and study population

The study population consisted of 93 severely obese men and women who underwent elective bariatric surgery between 2006 and 2009 at the Department of General Surgery, Maastricht University Medical Center (Maastricht, The Netherlands) [25]. Subjects using anti-inflammatory drugs or with acute or chronic inflammatory diseases, degenerative diseases, and subjects reporting alcoholic intake >10 g/day were not included in this study. There were no specific dietary protocols that participants had to follow before surgery. Venous blood samples were drawn on the morning

of surgery after 8 h of overnight fasting. During surgery, wedge biopsies of the liver were taken. The study was approved by the medical ethics board of Maastricht University Medical Center, in line with the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from each participant.

2.2. Data collection

Because of the limited samples available, random samples from this study population were selected for data collection. Liver *CETP*, *VSIG4* mRNA expression were determined from microarrays of 82 samples, as described previously [2,25]. Similarly, mRNA expression of the inflammatory markers tumor necrosis factor α (TNF α), interleukin 1 β (IL1 β), interleukin 6 (IL6) and Toll-like receptor 4 (TLR4) was determined to assess the association with different components of NAFLD. Biopsy specimens were formalin-fixed and paraffin-embedded, and subsequently immunohistochemistry was performed. The number of CETP positive cells per mm [2] was obtained (n = 44) [2]. Plasma CETP concentrations were measured for 73 participants from whom plasma was available with ELISA kits (DAIICHI CETP ELISA, Alpco, Salem, USA), according to the manufacturer's instructions. We measured CETP concentration instead of exogenous CETP activity, both of which are however highly correlated [26–28].

2.3. Histology

For histological scoring of the liver biopsies, Hematoxylin-eosin and Klatskin (Masson) trichrome stains were used to assess histopathology. Samples were scored for steatosis (n = 93), lobular inflammation (n = 89), and hepatocellular ballooning (n = 89), by an experienced liver pathologist who was blinded for clinical and biochemical parameters, according to the criteria of the NAFLD activity score described by Kleiner et al. [29] (see [Supplementary Data](#)).

2.4. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy was used to measure total cholesterol, free cholesterol, cholesteryl ester and triglyceride contents in 68 liver samples. A detailed description of the method can be found in the [Supplementary Data](#).

2.5. Biochemical analyses

Plasma concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, alanine transaminase (ALT), and aspartate transaminase (AST) were measured, as described before [25].

2.6. Statistical analyses

We assessed the associations of the following determinants with CETP-related outcomes: steatosis score, lobular inflammation score, hepatocellular ballooning score, presence of NASH, plasma ALT concentration, plasma AST concentration, liver total cholesterol content, liver free cholesterol content, liver cholesteryl ester content and liver triglyceride content. For steatosis, lobular inflammation, hepatocellular ballooning, and NASH separately, the presence of disease (score ≥ 1) was compared with absence of pathology (score 0), as defined by the criteria of the NAFLD activity score [29]. As CETP-related outcome variables we used liver *CETP* expression, CETP protein positive cells, and plasma CETP concentration. Linear regression analyses were used to determine all associations. Crude models (Model 1) were adjusted for age and sex

Table 1
Characteristics of the elective bariatric surgery cohort aged 17–67 years (n = 93^a), stratified by sex.

Characteristic	Men	Women
Number of participants (% of total)	26 (28%)	67 (72%)
Age (year)	46 (11)	43 (9)
BMI (kg/m ²)	50 (10)	45 (9)
Fasting plasma concentrations		
CETP (µg/mL)	2.56 (0.92)	2.72 (0.88)
Total cholesterol (mmol/L)	4.66 (0.89)	5.26 (1.15)
HDL-cholesterol (mmol/L)	0.88 (0.27)	1.02 (0.40)
LDL-cholesterol (mmol/L)	2.90 (0.86)	3.34 (1.03)
Triglycerides (mmol/L)	1.95 (0.94)	2.33 (2.27)
ALT (IU/L)	26.6 (11.8)	26.5 (17.6)
AST (IU/L)	27.1 (10.4)	23.5 (13.1)
Liver biopsies		
Total cholesterol content (nmol/mg protein) ^b	12 (9)	37 (68)
Free cholesterol content (nmol/mg protein) ^b	8 (6)	25 (44)
Cholesteryl ester content (nmol/mg protein) ^b	4 (3)	11 (26)
Triglyceride content (nmol/mg protein) ^b	90.4 (71.1)	268 (618)
Number of patients with NAFLD activity score ≥5 (% of total men or women) ^c	4 (15%)	12 (18%)

Results are presented as mean (SD) or percentage.

^a Missing data: n = 1 for BMI, n = 20 for plasma CETP concentration, n = 6 for total cholesterol concentration, n = 7 for HDL-cholesterol, LDL-cholesterol and triglyceride concentration, n = 6 for ALT and AST concentration, n = 4 for NAFLD activity score, n = 25 for liver content of total cholesterol, free cholesterol, cholesteryl esters and triglycerides.

^b Measured with NMR.

^c A NAFLD activity score ≥5 is indicative of NASH [29].

(Model 2). Differences were expressed as beta coefficients with corresponding 95% CIs. Liver sterol and triglyceride contents and liver transaminases were non-normally distributed, and therefore we used their natural logarithms in the analyses. For interpretation purposes, beta coefficients were multiplied by ln(1.1), and differences with corresponding 95% CIs were expressed per 10% relative increase in the determinant. In addition, we assessed the correlations between NMR-determined liver sterol and triglyceride data and reported Spearman's correlation coefficient. All analyses were performed using STATA Statistical Software (Statacorp, College Station, Texas, USA), version 12.0.

3. Results

3.1. Population characteristics

Table 1 shows the characteristics of the elective bariatric surgery cohort. Body mass index (BMI) ranged from 30.6 to 73.6 kg/m². Mean age was 44 years, and most participants were women. Mean (SD) CETP concentration was 2.68 (0.89) µg/mL in the total population, 2.56 (0.92) µg/mL for men and 2.72 (0.88) µg/mL for women. 15% of men and 18% of women had a NAFLD activity score ≥5, which is indicative of NASH [29]. Plasma ALT concentration was comparable between men and women, and plasma AST concentration was somewhat lower in women. Liver sterol and triglyceride contents were higher in women than in men.

3.2. Lobular inflammation and hepatocellular ballooning tend to associate with less CETP positive cells in the liver and a lower plasma CETP concentration

Associations of the separate NAFLD activity score components, i.e. steatosis, lobular inflammation and ballooning score, with the CETP-related outcomes are presented in Fig. 1 and Supplementary table 2. The age and sex-adjusted difference in CETP mRNA expression in the liver for an inflammation score ≥1 compared with a score of 0 was −0.03 arbitrary units (95% CI −0.26, 0.20). Comparable small, null associations were observed for steatosis and ballooning scores. The number of CETP positive cells per mm² liver ranged from 11 to 140. The presence of liver lobular inflammation

was associated with a lower number of CETP positive cells of −20.0 per mm² (95% CI −41.6, 1.9). Steatosis and ballooning scores were also negatively associated with CETP positive cell number, although confidence intervals overlapped with zero. Plasma CETP concentration was within the range of 1.45–5.56 µg/mL. The presence of lobular inflammation was associated with a lower plasma CETP of −0.35 µg/mL (95% CI −0.80, 0.10), although confidence intervals overlapped with zero. Hepatocellular ballooning was associated with a −0.42 µg/mL (95% CI −0.84, −0.00) µg/mL lower plasma CETP concentration, which represents approx. 15% of the mean CETP concentration. Steatosis score also showed a negative association with CETP concentration, although the confidence intervals overlapped with zero. Steatosis, inflammation and ballooning scores were not negatively associated with *VSIG4* expression (Fig. 1, Supplementary table 2). Associations of the separate NAFLD activity score components with mRNA expressions of *TNFα*, *IL1β*, *IL6* and *TLR4* can be found in Supplementary figure 1 and Supplementary table 3. In the presence of liver inflammation *TNFα* expression was increased (0.17 arbitrary units; 95% CI 0.03, 0.31) and *IL1β* (0.35 arbitrary units; 95% CI −0.10, 0.80) and *IL6* (0.26 arbitrary units; 95% CI −0.18, 0.70) expression tended to be increased, compared to absence of liver inflammation. For the other two stages of NAFLD, i.e. steatosis and ballooning, no clear associations were observed with the mRNA expression of these genes. Also, *TLR4* mRNA expression was increased in the presence of liver inflammation (0.14 arbitrary units; 95% CI 0.02, 0.26).

3.3. CETP-related outcomes are not different in patients with a NAFLD activity score ≥5

A NAFLD activity score ≥5 was associated with a higher ALT concentration of 18.2 IU/L (95% CI 10.1, 26.3) and a higher AST concentration of 13.5 IU/L (95% CI 7.2, 19.8). However, no difference in CETP-related outcomes was observed between participants with a NAFLD activity score ≥5 compared with participants with a NAFLD activity score <5 (Supplementary figure 2, Supplementary table 4). Plasma ALT and AST concentrations were negatively associated with liver CETP expression, although associations were weak (Supplementary figure 3, Supplementary table 5). Age- and sex-adjusted differences in CETP expression were −0.020 arbitrary

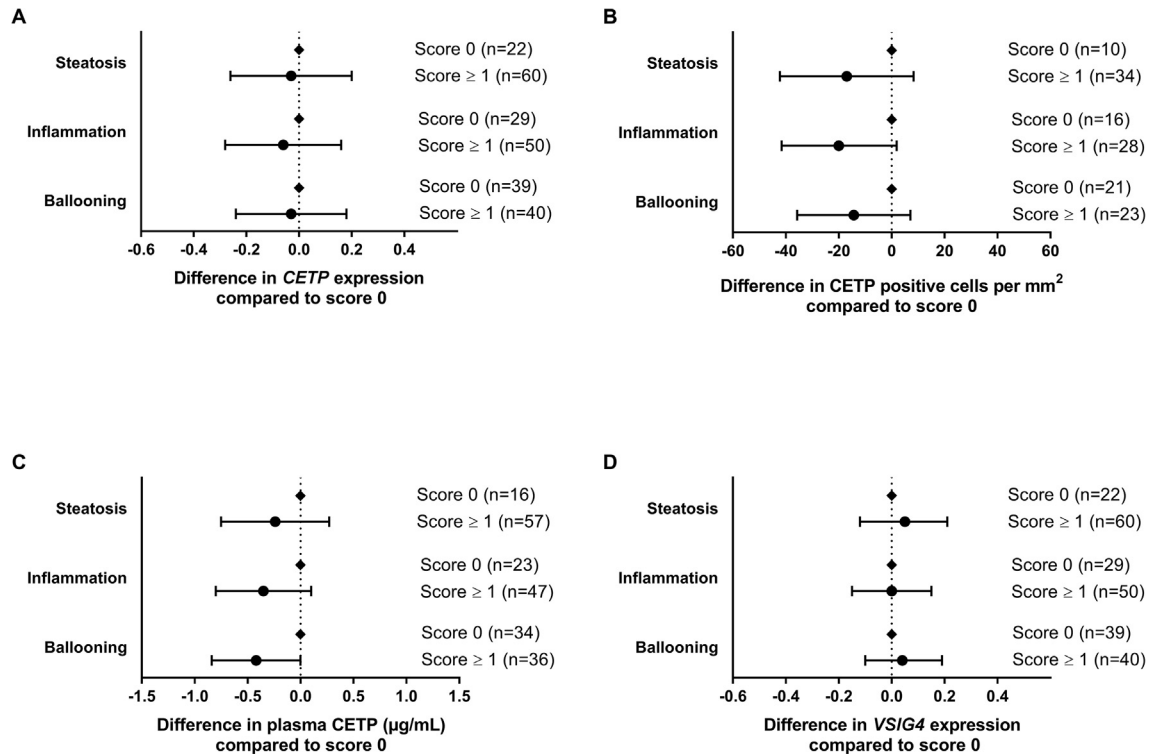


Fig. 1. CETP-related outcomes, but not liver VSIG4 expression, show a consistent trend for negative association with metabolic liver steatosis, inflammation and ballooning. Age and sex-adjusted differences (i.e. beta coefficients from linear regression) in (A) liver *CETP* mRNA expression, (B) liver CETP positive cells, (C) plasma CETP concentration, and (D) liver VSIG4 mRNA expression between absence (score 0, reference category) and presence (score ≥ 1) of liver steatosis, lobular inflammation and ballooning. Error bars represent 95% confidence interval.

units (95% CI -0.040, 0.000) and -0.024 arbitrary units (95% CI -0.047, -0.001) per 10% relative increase in plasma ALT and AST concentration, respectively. Associations of both liver transaminases with CETP positive cells and CETP concentration were also negative, although effect sizes were small and confidence intervals overlapped with zero (Supplementary figure 3, Supplementary table 5).

3.4. Associations of liver sterol and triglyceride contents with CETP-related outcomes were around the null

NMR-determined liver sterol and triglyceride data clearly show that hepatic contents of total cholesterol and free cholesterol were highly correlated ($r^2 = 0.97$, $P < 0.0001$). In addition, the correlations between the hepatic content of total cholesterol and cholesteryl esters ($r^2 = 0.77$, $P < 0.001$), and between total cholesterol and triglycerides ($r^2 = 0.71$, $P < 0.001$) were high. Strikingly, all of the associations of liver triglyceride and sterol contents with CETP-related outcomes were around the null (Fig. 2, Supplementary table 6).

4. Discussion

VSIG4-positive Kupffer cells have recently been identified as the main determinants of circulating CETP levels [2,8], but the influence of the hepatic environment on *CETP* expression and CETP production is far from elucidated. Based on previous studies, showing that inflammatory stimuli downregulate *CETP* expression in mice and *in vitro* [6,7,15,30,31], we hypothesized that hepatic *CETP* expression and circulating CETP concentrations are decreased in humans with metabolic liver inflammation. Therefore, we aimed to study the associations of histologically-determined liver

inflammation, as a component of NAFLD, with liver *CETP* expression, CETP positive cells and circulating CETP concentrations, using liver biopsy data from an elective bariatric surgery cohort. We found no strong evidence for a negative association between liver lobular inflammation and CETP-related outcomes, as 95% confidence intervals overlapped with zero. However, we did observe consistent trends towards less CETP expression, a lower number of CETP positive cells, and a lower circulating CETP concentration when liver inflammation was present. In line, we also observed trends for a negative association of ALT and AST concentrations, both markers for liver damage, with CETP-related outcomes.

It is interesting to speculate why only limited associations were observed between metabolic inflammation and CETP-related outcomes. Previous animal and *in vitro* studies showed a decrease in *CETP* expression specifically in response to infection-related inflammatory stimuli, such as LPS, IL-1 β and IFN- γ [6,7,15]. In addition, we recently showed that LPS also reduces plasma CETP concentration in humans [8]. In the present study, all participants were morbidly obese, and individuals who used anti-inflammatory drugs or had acute or chronic inflammatory diseases were not able to participate. Thus, liver inflammation in the present study was assumed to be metabolically-induced. Interestingly, metabolic liver inflammation, which is a component of NAFLD, has been shown to differ from infection-induced liver inflammation [32]. In mice, LPS and IL-1 β cause intrahepatic inflammation that is exclusively mediated by macrophages, while metabolic triggers (i.e. carbohydrate and cholesterol) of liver inflammation induce a NASH phenotype with mixed intrahepatic infiltrates including both macrophages and neutrophils [32]. Since CETP is primarily produced by hepatic macrophages, infection-related inflammatory stimuli may affect hepatic expression and production of CETP to a different extent than metabolic triggers.

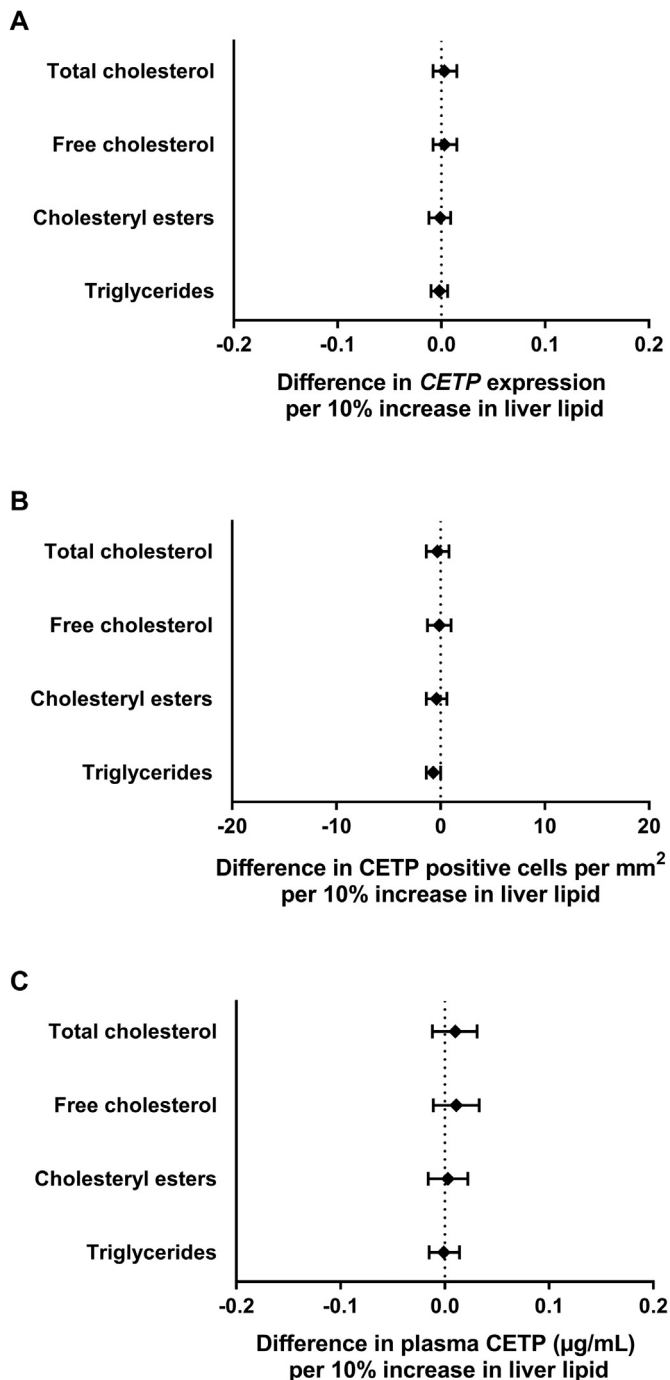


Fig. 2. Associations of liver sterol and triglyceride contents with *CETP*-related outcomes are all around the null.

Age and sex-adjusted differences^a in (A) liver *CETP* mRNA expression, (B) liver *CETP* positive cells and (C) plasma *CETP* concentration, per 10% relative increase in the plasma concentrations of ALT and AST. Error bars represent 95% confidence interval. ^a (Beta coefficients from linear regression)*ln(1.1), i.e. difference per 10% relative increase in liver sterol and triglyceride content.

Importantly, it should be noted that obesity has been associated with adverse changes in the gut microbiota [33], which may lead to elevated penetration of LPS from the gut into the circulation, a condition described as metabolic endotoxemia [34,35]. Indeed, in our study we showed that hepatic *TLR4* mRNA expression was higher in the presence of liver inflammation. The *TLR4* signalling pathway can be activated by LPS to release pro-inflammatory

cytokines, such as $\text{TNF}\alpha$ [36,37], which was also higher in our study in the presence of liver inflammation. It has been shown that low-dose LPS administration leads to a low-grade chronic inflammatory state [34], which possibly downregulates *CETP* production to a certain extent.

Notably, severe sepsis was previously shown in humans to largely decrease *CETP* concentration, i.e. by -25% after 3 days of sepsis [38], while we only observed a trend towards a negative association between metabolic liver inflammation and *CETP*-related outcomes. This is in line with a previous study in *CETP*-transgenic mice, in which we observed that long term high-fat diet-induced obesity did not affect plasma *CETP* concentration [39]. The difference between metabolic *versus* infection-induced liver inflammation is further highlighted by our observation that *VSIG4* expression, which is a marker of resting Kupffer cells, was not lower in the presence of metabolic liver inflammation, while we previously showed that LPS injection largely reduced both *Vsig4* and *CETP* expression, at least in mice [8]. Taken together, we propose that acute and/or whole-body inflammatory responses to invading pathogens are required to induce a robust reduction in *CETP* production by Kupffer cells.

In the context of infection-induced *versus* metabolic liver inflammation, it is important to consider the biological relevance underlying the relationship between *CETP* and inflammation. *CETP* mediates the bidirectional exchange of triglycerides and cholesteryl esters between (V)LDL and HDL, thereby raising LDL-cholesterol and decreasing HDL-cholesterol, which is generally regarded as a biologically unwanted proatherogenic property of *CETP*. Since HDL is known for its inflammation-modulatory properties and beneficial role in host-defence [40–43], we reason that the biological function of *CETP* in humans is modulation of the immune response via HDL. In fact, the reduction in *CETP* expression by Kupffer cells in *CETP*-transgenic mice as induced by LPS is accompanied by an increase in HDL [8]. We thus postulate that inflammatory stimuli activate resting Kupffer cells to decrease *CETP* expression and thereby raise HDL to combat invading microorganisms. In line with this hypothesis, it seems plausible that metabolic liver inflammation will not largely reduce the expression and production of *CETP*.

Comparable with liver inflammation, liver steatosis score also tended to be negatively associated with the number of *CETP* positive cells. This negative effect direction can possibly be explained by a decrease in macrophage cell number per area rather than by an absolute decrease in Kupffer cell number, as the presence of steatosis results in occupation of a larger area of the microscopic field by lipid-filled hepatocytes. This is a plausible explanation, as associations between NMR-determined triglyceride content and *CETP*-related outcomes were all around zero. NMR triglyceride measurements were expressed per mg protein, and can therefore be interpreted as mean triglyceride content per hepatocyte. Therefore, in the light of the NMR findings, the negative association between histologically-determined steatosis and *CETP*-related outcomes is most likely explained by the measurement technique.

This study is one of the first studies to include NMR spectroscopy for the quantitative analysis of several lipid and sterol classes in human liver biopsies. By using NMR spectroscopy, we revealed a strong correlation between hepatic contents of total cholesterol and free cholesterol ($R^2 = 0.97$) as well as those of total cholesterol and triglycerides ($R^2 = 0.71$). The finding that none of the measured liver sterol components (i.e. total cholesterol, free cholesterol, nor cholesteryl esters) was associated with *CETP* expression in the present study, may seem counterintuitive given that *CETP* expression is under the control of *LXR* α [10,11]. Cholesterol derivatives activate *LXR* α [12,13], which subsequently binds to an *LXR*-response element in the *CETP* promotor region to increase gene transcription [10,11]. It should be realized that we measured

cholesterol rather than the actual LXR agonists, e.g. oxysterols and desmosterol. For future studies, mass spectrometric platforms might complement NMR analysis to measure oxysterols and desmosterol, as the commonly observed low concentrations are likely out of the measurement range of NMR spectroscopy.

Notably, compared with participants with a NAFLD activity score <5, CETP-related outcomes were not different in participants with a NAFLD activity score ≥ 5 , which is indicative of NASH [29]. This score is composed of three summed, separate components (i.e. steatosis, lobular inflammation and ballooning scores) [29]. We argue that this score may not be sufficiently specific to detect an association of specific aspects of NAFLD with CETP-related outcomes, which is the main reason that we studied the associations between the different components of the NAFLD activity score and CETP-related outcomes separately. The same reasoning may also hold for plasma ALT and AST concentrations, which are relatively non-specific markers for liver function [44]. Associations of both liver transaminases with CETP-related outcomes were around the null.

The main strength of the present study is the availability of liver biopsies from a population of obese men and women, with a high prevalence of metabolic liver inflammation. Liver samples were extensively characterized with regard to CETP, sterol and triglyceride content, steatosis, inflammation and cell damage. Also, several limitations of the study design should be considered. Firstly, inherent to the observational cross-sectional design we cannot exclude residual confounding, or draw conclusions on causality. Secondly, this study possibly lacked power to identify statistically significant differences in CETP-related outcomes due to the relatively small study population size. Lastly, results may not be generalizable to other populations, as participants were selected from an obese cohort that underwent bariatric surgery. Remarkably, although NAFLD is more common in men than in women [45], in the present study liver sterol and triglyceride contents were higher in women than in men. This may suggest that bigger hepatic lipid droplets are present in women. However, sex differences in disease phenotype are far from understood. Severity of steatosis has been associated with clustering of risk factors for the metabolic syndrome. Possibly differences in these risk factors between men and women may partly explain different liver steatosis phenotypes. Moreover, hormonal factors have also been proposed to explain part of the differences between men and women [45,46]. Clearly, further research in this area is warranted.

In conclusion, we found no strong evidence for a negative association between metabolic liver inflammation and CETP-related outcomes, although we observed consistent trends. Given that infection-related inflammatory stimuli do significantly decrease *CETP* expression by Kupffer cells, our results suggest that metabolic liver inflammation affects the expression and production of CETP by Kupffer cells to a more modest extent than infection-induced liver inflammation. It is tempting to speculate that acute and/or whole-body inflammatory responses to invading pathogens are required to induce a robust reduction in CETP production by Kupffer cells. Further research into the effects of liver inflammation and host defence on hepatic CETP production is, therefore, eagerly awaited.

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

Acquisition of data: ZL, SSR, JWMG, MG, AV, RJD. Study concept and design: LLB, YW, PCNR. Analysis and interpretation of data: LLB, YW, PCNR. Statistical analysis: LLB. Drafting of the manuscript: LLB. Critical revision of the manuscript: ZL, SSR, JWMG, MG, YW, PCNR. Study supervision: PCNR.

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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.2018.06.004>.

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