Treatment with the natural FXR agonist chenodeoxycholic acid reduces clearance of plasma LDL whilst decreasing circulating PCSK9, lipoprotein(a) and apolipoprotein C-III

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Abstract. Ghosh Laskar M, Eriksson M, Rudling M, Angelin B (Karolinska University Hospital, Huddinge, Stockholm, Sweden). Treatment with the natural FXR agonist chenodeoxycholic acid reduces clearance of plasma LDL whilst decreasing circulating PCSK9, lipoprotein(a) and apolipoprotein C-III. J Intern Med 2017; 281: 575–585.

Background. The natural farnesoid X receptor (FXR) agonist chenodeoxycholic acid (CDCA) suppresses hepatic cholesterol and bile acid synthesis and reduces biliary cholesterol secretion and triglyceride production. Animal studies have shown that bile acids downregulate hepatic LDL receptors (LDLRs); however, information on LDL metabolism in humans is limited.

Methods. Kinetics of autologous 125I-LDL were determined in 12 male subjects at baseline and during treatment with CDCA (15 mg kg⁻¹ day⁻¹). In seven patients with gallstones treated with CDCA for 3 weeks before cholecystectomy, liver biopsies were collected and analysed for enzyme activities and for specific LDLR binding. Serum samples obtained before treatment and at surgery were analysed for markers of lipid metabolism, lipoproteins and the LDLR modulator proprotein convertase subtilisin/kexin type 9 (PCSK9).

Results. Chenodeoxycholic acid treatment increased plasma LDL cholesterol by ~10% as a result of reduced clearance of plasma LDL-apolipoprotein (apo)B; LDL production was somewhat reduced. The reduction in LDL clearance occurred within 1 day after initiation of treatment. In CDCA-treated patients with gallstones, hepatic microsomal cholesterol 7α-hydroxylase and HMG-CoA reductase activities were reduced by 83% and 54%, respectively, and specific LDLR binding was reduced by 20%. During treatment, serum levels of fibroblast growth factor 19 and total and LDL cholesterol increased, whereas levels of 7α-hydroxy-4-cholesten-3-one, lathosterol, PCSK9, apoA-I, apoC-III, lipoprotein(a), triglycerides and insulin were reduced.

Conclusions. Chenodeoxycholic acid has a broad influence on lipid metabolism, including reducing plasma clearance of LDL. The reduction in circulating PCSK9 may dampen its effect on hepatic LDLRs and plasma LDL cholesterol. Further studies of the effects of other FXR agonists on cholesterol metabolism in humans seem warranted, considering the renewed interest for such therapy in liver disease and diabetes.

Keywords: apolipoprotein, chenodeoxycholic acid, LDL cholesterol, LDL receptors, lipoprotein(a), PCSK9.

Introduction

Treatment of humans with the primary bile acid chenodeoxycholic acid (CDCA), a natural farnesoid X receptor (FXR) agonist [1, 2], lowers the biliary secretion of cholesterol and reduces the cholesterol saturation of bile [3, 4]. Concomitantly, the syntheses of bile acids and cholesterol are reduced [4, 5]. CDCA has been used to dissolve cholesterol gallstones [6, 7]. Further, CDCA decreases plasma triglyceride levels in humans by reducing hepatic very low-density lipoprotein (VLDL) production [8, 9], whereas plasma LDL cholesterol (LDL-C) often increases [10, 11]. The level of plasma LDL-C is determined by the balance between its synthesis and elimination. The clearance of LDL particles from blood plasma is predominantly mediated by LDL receptors (LDLRs) in the liver [12]. Animal
studies have shown that treatment with bile acids rapidly reduces the number of hepatic LDLRs [13, 14]. In human liver cells, mRNA expression levels of LDLR, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (the rate-limiting enzyme of cholesterol synthesis) and proprotein convertase subtilisin/kexin type 9 (PCSK9), which promotes the degradation of LDLRs [15], have all been reported to be suppressed during CDCA treatment [16, 17].

In this study, we sought to explain why LDL-C increases during CDCA treatment, and whether this is influenced by changes in circulating PCSK9. For this purpose, we studied the kinetics of radiolabelled autologous LDL before, at initiation and after 3 weeks of CDCA treatment. We also analysed the levels of circulating PCSK9 before and during CDCA treatment and measured specific LDLR-binding properties, LDL particles were isolated from four of the subjects on long-term CDCA treatment and from four matched male controls. The LDL turnover study was then repeated using ultracentrifugation as described by Eriksson et al. [23]. 125I-LDL (30–60 µCi) was injected within 5 days of 125I-labelling, and blood samples were collected at 10 min and 2, 4, 6, 8, 12, 24 and 26 h after the injection, thereafter daily at 8 am for 2–3 weeks [23]. In the patients where CDCA treatment was initiated during the turnover study, samples were drawn at 8 am and 8 pm 2–3 days before and after the first dosing. CDCA was administered as 125-mg capsules (Chendal, Draco AB, Sweden) at a dose of 15 mg kg\(^{-1}\) day\(^{-1}\). Cholesterol and triglycerides in lipoprotein fractions (VLDL, LDL and HDL) were quantified from both the plasma radioactivity decay curve and the daily urine/plasma (U/P) radioactivity ratio as described [23]. Cholesterol and triglycerides in lipoprotein fractions (VLDL, LDL and HDL) prepared by ultracentrifugation were quantified repeatedly also as previously described [23, 24]. To evaluate whether CDCA treatment could influence LDLR-binding properties, LDL particles were isolated from four of the subjects on long-term CDCA therapy and from four matched male controls. The ability of these isolated LDL particles to compete with 125I-LDL from healthy controls for the degradation by normal human fibroblasts was compared as previously described [23].

The fractional catabolic rate (FCR) of 125I-LDL was calculated from both the plasma radioactivity decay curve and the daily urine/plasma (U/P) radioactivity ratio as described [23]. Studies in patients with cholesterol gallstones

In total, 22 otherwise healthy patients (four male and 18 female) with uncomplicated cholesterol gallstone disease were included in the second set
of studies (Table S2). Of these, seven were treated with CDCA at a mean dose of 15 mg kg\(^{-1}\) day\(^{-1}\) for 3 weeks prior to cholecystectomy; the remaining 15 served as untreated controls. The operations were performed between 08.00 and 09.00 after overnight fasting; standard anaesthesia was administered as previously described [25]. After opening the abdomen, a liver biopsy sample was collected and preparation of microsomes was initiated within 10 min. Cholesterol 7\(a\)-hydroxylase (CYP7A1) and HMG-CoA reductase activities were analysed as outlined previously [25, 26]. Specific heparin-sensitive LDLR binding was determined in liver homogenates also as reported [27, 28]. Fasting plasma samples from the CDCA-treated patients were obtained at baseline and in the morning before surgery.

All participants in both studies gave their written informed consent, and the studies were approved by the Ethics Committee of Karolinska Institutet.

Serum analyses

In the LDL turnover studies, plasma lipoproteins were analysed repeatedly using combined ultracentrifugation and precipitation as described by Carlson [24]. In the patients with gallstones, levels of total and HDL cholesterol, triglycerides and glucose were determined using routine clinical chemistry techniques. LDL-C was calculated according to Friedewald \[\text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \frac{\text{Triglycerides}}{5}\] [29]. Serum levels of \(7\alpha\)-hydroxy-4-cholesten-3-one (C4), a marker of bile acid production, and unesterified lathosterol, a marker of cholesterol synthesis, were determined [30, 31]; C4 and lathosterol were corrected for total cholesterol (C4/c and lathosterol/c, respectively) [31].

Serum apoA-I (reference no. KAI-002), apoA-II (KAI-003), apoB (KAI-004), apoC.II (KAI-005), apoC-III (KAI-006) and apoE (KAI-007) were analysed using immunoturbidimetric assay kits, according to the manufacturer’s instructions (Kamiya Biomedical Company, Seattle, WA, USA). A TruLab kit (reference no. 598309910046; Diasys, Holzheim, Germany) was used to measure serum Lp(a) concentration. The Respons\(\text{R}\) 910 analyser from Diasys was used for all assays. Concentrations of apolipoproteins are presented in mg dL\(^{-1}\).

Serum PCSK9 was measured using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (CY-8079; CycLex Co. Ltd., Nagano, Japan), according to the manufacturer’s instructions. Concentrations of PCSK9 are given in ng mL\(^{-1}\).

FGF19 was measured by a solid-phase two-site enzyme immunoassay from R&D system, Sweden (Human FGF19 Quantikine ELISA kits; DF1900), according to the manufacturer’s protocol. Concentrations of FGF19 are expressed in pg mL\(^{-1}\).

Insulin was determined by a solid-phase two-site ELISA (ultra-sensitive human insulin kit; 10-1132-01; Merodia, Uppsala, Sweden), according to the manufacturer’s protocol. Concentrations of insulin are expressed in mU L\(^{-1}\) (1 mU L\(^{-1}\) = 6.0 pmol L\(^{-1}\)).

Statistical analyses

Data are presented as means ± SEM. The significance of differences was tested by Student’s paired or unpaired t-test, as appropriate.

Results

Plasma LDL kinetics

The administration of 15 mg kg\(^{-1}\) day\(^{-1}\) CDCA to seven healthy male volunteers for 3 weeks was not associated with any clinical or laboratory side effects, except for an occasional tendency towards loose stools. Treatment resulted in significant increases in total cholesterol (7%) and LDL-C (10%), with trends towards decreased VLDL and HDL cholesterol (Table S3). Findings were similar in the five male patients with various forms of dyslipidaemia.

In the normal subjects, the baseline FCR of plasma LDL ranged from 0.287 to 0.435 pools per day when calculated from the plasma radioactivity curve (Table 1). The FCR independently calculated from daily measurements of the U/P radioactivity ratio was strongly correlated with that obtained from plasma data, before \((r = 0.72)\) as well as during \((r = 0.80)\) CDCA treatment, and remained constant during the study periods, indicating that steady-state conditions had been obtained. As expected, LDL clearance was reduced in the patient with heterozygous familial hypercholesterolaemia (FH) (\#8). A high basal FCR of LDL was observed in one of the hyperlipidaemic patients (\#10) and, as expected [23], in the oestrogen-treated patient (\#12).

After 3 weeks of treatment with CDCA, the repeated LDL turnover studies showed a robust
reduction in FCR of LDL (Table 1). Amongst the seven control subjects, the FCR decreased by a mean of \( -13\% \) (\( P < 0.02 \)) calculated from the plasma curves and \( -17\% \) from the U/P ratio (\( P < 0.01 \)). The results were similar in the total cohort of subjects; the mean changes in FCR from plasma curve analysis and from U/P ratio were \( -13\% \) and \( -20\% \), respectively. Interestingly, the decrease in FCR was most marked in the hyperlipidaemic patient with a very high initial clearance (\#10) and was not observed in the patient with heterozygous FH (\#8). In the control subjects, the plasma LDL-C levels were inversely correlated with the FCR before (\( r = -0.73 \)) as well as during CDCA treatment (\( r = -0.97 \)).

In the steady state, the catabolic rate of LDL equals its production rate. Comparison of estimated synthesis rates of LDL before and during CDCA treatment showed that LDL production was reduced during treatment (Table 1).

To evaluate the speed of onset of the effect of CDCA treatment on LDL clearance, we monitored closely the elimination of radioactivity from plasma around the time of initiation of bile acid feeding in the five patients. Within 1 day of CDCA administration, there were clear shifts in the plasma radioactivity decay curves, as shown for patient \#11 in Fig. 1, suggesting an acute reduction of LDL clearance. This could be convincingly demonstrated by the marked (\( -35\% \)) reduction in the daily FCR calculated from the U/P ratio (Table 2). This acute change was associated with increased mean plasma LDL-C levels (\( 6\% \)) in all subjects. Again, the largest change was observed in the two subjects with the highest basal LDL clearance rates.

When performing LDL turnover studies, it is important to consider the properties of the tracer compound. In the study of acute effects, the LDL used for radiolabelling had been harvested before treatment, and possible changes in the composition and/or conformation of the LDL particles induced by CDCA should not be relevant. However, such an effect could be present in the long-term treatment study [23]. To evaluate this possibility, we determined the ability of LDL particles isolated from four of the subjects on long-term CDCA treatment to compete with labelled LDL isolated from healthy controls for binding to fibroblast LDLR, and found it to be similar to that of LDL isolated from four matched untreated controls.

### Table 1

| Subjects | LDL-apoB (mg dL\(^{-1}\)) Before | During | FCR (day\(^{-1}\)) Before | During | Absolute catabolic rate (mg kg\(^{-1}\) day\(^{-1}\)) Before | During |
|----------|-------------------------------|--------|---------------------------|--------|----------------------------------------------------------|--------|
| Controls |                               |        |                           |        |                                                          |        |
| 1        | 100                           | 92     | 0.347                     | 0.310  | 15.62                                                    | 12.83  |
| 2        | 104                           | 87     | 0.365                     | 0.314  | 17.10                                                    | 12.89  |
| 3        | 83                            | 83     | 0.435                     | 0.327  | 16.28                                                    | 12.21  |
| 4        | 95                            | 99     | 0.315                     | 0.299  | 13.47                                                    | 13.34  |
| 5        | 89                            | 87     | 0.344                     | 0.329  | 13.80                                                    | 12.88  |
| 6        | 139                           | 137    | 0.287                     | 0.260  | 18.80                                                    | 16.03  |
| 7        | 87                            | 98     | 0.376                     | 0.307  | 14.71                                                    | 13.56  |
| Mean ± SEM | 100 ± 7                       | 96 ± 7 | 0.353 ± 0.02              | 0.307 ± 0.01* | 15.68 ± 0.71 | 13.3 ± 0.49** |
| Patients |                               |        |                           |        |                                                          |        |
| 8        | 191                           | 174    | 0.179                     | 0.203  | 15.38                                                    | 15.89  |
| 9        | 104                           | 135    | 0.284                     | 0.269  | 13.31                                                    | 16.37  |
| 10       | 73                            | 72     | 0.681                     | 0.529  | 19.34                                                    | 14.72  |
| (Mean ± SEM of all subjects) | 106 ± 11                       | 106 ± 10 | 0.361 ± 0.04              | 0.315 ± 0.03* | 15.78 ± 0.67 | 14.02 ± 0.51 |

Significantly different from before treatment: *\( P < 0.05 \); **\( P < 0.01 \).

CDCA, chenodeoxycholic acid; FCR, fractional catabolic rate.
This indicates that the reduced clearance of LDL observed is not due to major changes in the binding properties of LDL induced by CDCA treatment.

Studies in patients with gallstone disease

The seven patients with cholesterol gallstone disease who received CDCA (Table S2) also tolerated treatment well, with no laboratory abnormalities and only occasional loose stools. Treatment for 3 weeks with 15 mg kg\(^{-1}\) day\(^{-1}\) CDCA resulted in a profound suppression (~87%) of the activity of CYP7A1, the rate-determining enzyme in bile acid synthesis, compared to 15 untreated patients undergoing cholecystectomy under the same standardized conditions (Table 3). In response to the decreased hepatic demand for cholesterol during suppression of bile acid formation, the activity of the rate-limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase, was simultaneously reduced by 54%. However, there was no difference between the two groups with regard to microsomal cholesterol concentrations (Table 3). To determine whether the hepatic LDLR number is also reduced in response to CDCA treatment, we determined the heparin-sensitive binding of \(^{125}\)I-LDL (50 \mu g mL\(^{-1}\)) in liver homogenates and found a clear tendency towards suppression (~20%) in the CDCA-treated patients (Table 3).

The marked changes observed in hepatic cholesterol metabolism in this small number of patients offered an opportunity for exploratory studies of potential mechanisms related to the effects of CDCA. Frozen serum samples from five of the CDCA-treated patients (one male and four female) were available for complete analysis (Figs 2 and 3, and Fig. S1). In accordance with our findings regarding hepatic microsomal CYP7A1 and HMG-CoA reductase activities, the serum levels of C4/c, a marker of bile acid synthesis [31], and lathosterol/c, a marker of cholesterol synthesis [32], were strongly reduced by CDCA treatment (Fig. 2d,e). The concentration of circulating FGF19, a possible regulator of bile acid synthesis which is strongly related to activation of FXR by bile acids in the distal small intestine [33], was clearly enhanced during CDCA treatment (Fig. 2f).

### Table 2

| Patients | FCR calculated from U/P ratio (day\(^{-1}\)) | Serum LDL-C (mmol L\(^{-1}\)) |
|----------|-------------------------------------------|-------------------------------|
|          | Before     | During     | Before     | During     |
| 8        | 0.277      | 0.205      | 8.53       | 9.41       |
| 9        | 0.276      | 0.231      | 3.91       | 4.31       |
| 10       | 0.433      | 0.264      | 3.09       | 4.16       |
| 11       | 0.283      | 0.236      | 4.16       | 4.28       |
| 12       | 0.435      | 0.194      | 2.54       | 3.04       |
| Mean ± SEM | 0.341 ± 0.040 | 0.221 ± 0.010* | 4.54 ± 1.04 | 4.83 ± 1.18 |

Mean urine/plasma ratios 2 days immediately before and following the initiation of treatment are compared. Significantly different from before treatment: *P < 0.05.

CDCA, chenodeoxycholic acid; FCR, fractional catabolic rate.
Interesting observations were made regarding plasma lipoprotein metabolism. In agreement with the findings from the turnover studies, serum cholesterol was also significantly increased after 3 weeks of treatment with CDCA in the patients with cholesterol gallstone disease (Fig. 2a), and a clear reduction in serum triglyceride levels was seen in these subjects (Fig. 2b). The mean circulating level of PCSK9, a modulator of hepatic LDLR number [15], was reduced in all patients (by 37%; Fig. 2c). Treatment also significantly lowered the circulating levels of Lp(a), apoC-III and apoA-I (Fig. 3c–e), whereas no changes were observed for serum apoA-II, apoB, apoC-II or apoE (Fig. S1a–d).

Finally, in agreement with previous reports of increased insulin sensitivity following treatment with synthetic FXR agonists in animals [18, 19], treatment with the natural FXR agonist CDCA in humans significantly reduced serum insulin without altering glucose levels (Fig. 3a,b).

**Discussion**

In the present study, we have highlighted possible causes of the increase in circulating LDL-C during CDCA treatment in humans, including the potential involvement of PCSK9 in this process. In the first set of experiments, LDL kinetic parameters were determined before and during therapy with CDCA, both in healthy subjects and in patients in whom either an initial low or high plasma clearance of LDL would be expected. The results clearly demonstrate an effect of CDCA treatment on the metabolism of LDL. In agreement with previous studies [10, 11], the concentration of plasma LDL-C increased moderately during therapy, but the changes were more pronounced when analysing the kinetic data. Thus, there was a robust reduction of the FCR of LDL after 3 weeks of treatment in every individual except the patient with heterozygous FH. The estimated production rate in the new steady state was simultaneously reduced, resulting in a conserved pool size of LDL-apoB, and a more modest increase in LDL-C. It has previously been shown that CDCA treatment reduces the synthesis and secretion of VLDL, the precursor of LDL [8, 34], which would support the interpretation that decreased catabolism via LDLRs is a major cause of the effects of CDCA on LDL-C. Reduced production of VLDL would also explain the simultaneous reduction in LDL-apoB synthesis.

Chenodeoxycholic acid treatment did not result in altered affinity of the LDL particles for binding to the LDLR. The proportions of cholesterol and triglycerides in the particles were also essentially unchanged. It was evident that the change induced by CDCA treatment was more pronounced in individuals with a high basal FCR of LDL, and less so in the patient with heterozygous FH. Accordingly, a marked reduction in LDL clearance has been reported following CDCA treatment of two patients with cerebrotendinous xanthomatosis [35, 36]. This is a very rare disease, in which...
deficient inhibition of bile acid synthesis results in a very high basal activity of CYP7A1 and rapid LDL clearance, both of which are reduced by CDCA treatment.

In the second set of experiments, we examined the effects of CDCA on hepatic cholesterol metabolism. In agreement with previous studies [37], CDCA treatment resulted in a pronounced feedback inhibition of bile acid synthesis by a clear reduction in the activity of CYP7A1. The liver can meet its requirement for cholesterol by regulating both de novo cholesterol synthesis and LDLR-mediated LDL uptake from blood [12, 16]. In response to CDCA treatment, cholesterol synthesis measured as HMG-CoA reductase activity was markedly reduced and, in agreement with the results from the LDL kinetic studies, there was also a trend towards a reduction in LDLR-binding activity (by 20%).

Analysis of the changes in U/P radioactivity ratio and plasma decay curve during initiation of CDCA treatment indicated that the suppression of hepatic LDLRs occurred very rapidly. This would support the findings of previous in vivo studies of hepatic LDLR expression and LDL clearance following the infusion of bile acids in dogs [13]. This rapid response was of greater magnitude than that observed during long-term treatment, and was also seen in the heterozygous FH patient. These results suggest that there are counter-regulatory mechanisms in action during prolonged treatment, so that the responses following acute and longer-term

![Fig. 2 Effects of chenodeoxycholic acid (CDCA) treatment in patients with gallstones on serum levels of cholesterol (a), triglycerides (b), proprotein convertase subtilisin/kexin type 9 (PCSK9; c), 7alpha-hydroxy-4-cholesten-3-one corrected for total cholesterol (C4c; d), lathosterol corrected for total cholesterol (lathosterol/c; e) and fibroblast growth factor 19 (FGF19; f).](image-url)
perturbations of bile acid metabolism may differ to some extent.

The gene expression of the LDLR-modulating protein PCSK9 is co-regulated with the expressions of HMG-CoA reductase and LDLRs by the transcription factor SREBP2 [38]. In agreement with this, we found decreased levels of circulating PCSK9 in response to 3 weeks of CDCA treatment in the patients with gallstones. This observation implies that the long-term suppression of LDLRs induced by CDCA treatment is partly counterbalanced by a reduced level of circulating PCSK9, which should increase the number of hepatic LDLRs. Together with the reduced production of LDL particles, which is presumably the consequence of diminished VLDL secretion, this could explain why LDL-C does not reach excessive levels despite drastic reductions in cholesterol elimination as bile acids and cholesterol in the bile. Whether other mechanisms of posttranscriptional modification of LDLRs, such as increased mRNA stability [39] or changes in miRNAs [40], are relevant for the effects of CDCA in humans can only be speculated upon at present.

Activation of the nuclear bile acid receptor FXR can alter metabolism in multiple ways. The secretion of FGF19 from the terminal ileum is largely related to the transintestinal flux of bile acids [33], as also evident in the present work. Whether these increased levels of FGF19 contribute to the marked suppression of bile acid synthesis, or to any of the other effects observed following CDCA treatment, cannot be determined from the present data.

Farnesoid X receptor agonists including CDCA have been reported to influence apolipoprotein secretion from cultured human cells [18–22, 41]. In accordance with the findings of previous in vitro studies [21], the concentration of previous in vitro data [22], there was a reduction in serum apoA-I, but this was not

Fig. 3 Effects of chenodeoxycholic acid (CDCA) treatment in patients with gallstones on serum levels of glucose (a), insulin (b), lipoprotein(a) (Lp(a)) (c), apolipoprotein C-III (apoC-III) (d) and apolipoprotein A-I (apoA-I) (e).
linked to any change in HDL-C levels. In the present work, we did not observe any change in apoC-II levels, in contrast to reports from in vitro studies [41]. The marked reduction in apoC-III following CDCA treatment is interesting, because lowering of apoC-III has recently been shown to have positive effects on lipid and carbohydrate metabolism [42].

Another interesting observation in the present work is the fact that the levels of Lp(a) were robustly reduced following treatment with CDCA. Previous studies have indicated that the hepatic production of Lp(a) may be reduced in patients with biliary obstruction, in whom FXR signalling is activated [20]. The possibility that the serum level of this atherogenic lipoprotein could be influenced by CDCA treatment is of particular interest for at least two reasons: Firstly, only a few of the currently available treatment options for dyslipidaemia are able to reduce Lp(a), and, secondly, treatment with CDCA may provide a useful tool in future studies to explore the mechanisms of assembly of this lipoprotein [43]. As treatment with PCSK9 inhibitors has recently been shown to reduce Lp(a) levels [44], it is tempting to speculate that the reduction in circulating PCSK9 observed during CDCA treatment may play a role in this finding.

In conclusion, our detailed studies in a small number of well-characterized human subjects have shown that treatment with CDCA suppresses hepatic bile acid and cholesterol syntheses and LDLR numbers reduces the plasma clearance of LDL particles and thus increases levels of LDL-C. LDL production is simultaneously reduced, presumably due to suppressed VLDL production. CDCA treatment also decreases circulating levels of the LDLR-degrading protein PCSK9, which is likely to moderate the degree of reduction of hepatic LDLRs, and influences the levels of several apolipoproteins including apoC-III and Lp(a). During recent years, considerable interest has developed in the potential use of FXR agonists for the treatment of liver-related diseases, including nonalcoholic fatty liver, insulin resistance and primary biliary cirrhosis [45, 46]. A concern in many early trials has been the relatively marked increases in LDL-C observed in response to some synthetic FXR agonists [46, 47]. There is so far relatively limited information available from more detailed studies of lipoprotein metabolism during such therapy [48], and it will be important to explore the extent to which treatment with these compounds shares the modulating effects on PCSK9, apoC-III and Lp(a) observed in response to CDCA treatment.

Conflict of interest statement

The authors have nothing to disclose in relation to this manuscript. The manuscript has been handled by an external editor, Professor Olov Wiklund, Department of Molecular and Clinical Medicine at Institute of Medicine, Sahlgrenska University Hospital Gothenburg, Sweden.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Baseline characteristics of subjects participating in kinetic studies using autologous ¹²⁵I-LDL.

**Table S2.** Basal characteristics of patients with cholesterol gallstone disease.

**Table S3.** Cholesterol and triglycerides in lipoprotein fractions before and following 3 weeks of treatment with CDCA; analyzed by ultracentrifugation/precipitation.

**Figure S1.** Serum levels of a) apoA-II; b) apoB; c) apoC-II; d) apoE before and at 3 weeks of CDCA treatment in gallstone patients.