

## **The effect of colesvelam treatment on bile acid and lipid metabolism and glycemic control in healthy men**

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## Summary

The treatment of hypercholesterolemia with bile acid (BA) sequestrants results in upregulation of BA synthesis through the classical pathway initiated by cholesterol 7 $\alpha$ -hydroxylase (CYP7A1). To characterize the detailed dynamics of serum lipid and BA concentrations and the BA synthesis rate in response to treatment with BA sequestrants and to determine whether the -203A/C promoter polymorphism of the CYP7A1 encoding gene (*CYP7A1*) affects such a response, this pilot study was carried out in healthy men (8 homozygous for the -203A allele and 8 homozygous for the -203C allele of *CYP7A1*). The subjects were treated for 28 days with colesevelam and blood was drawn for analysis before and on days 1, 3, 7, 14 and 28 of treatment.

The response of lipids, BA, fibroblast growth factor-19 (FGF19) and 7 $\alpha$ -hydroxy-4-cholesten-3-one (C4) to colesevelam did not differ between carriers of -203A and -203C alleles; their data were then aggregated for further analysis. Colesevelam treatment caused immediate suppression of FGF19 concentration and a fivefold increase in CYP7A1 activity, as assessed from C4 concentration, followed by a 17 % decrease in LDL-cholesterol. Although total plasma BA concentrations were not affected, the ratio of cholic acid/total BA rose from  $0.25\pm 0.10$  to  $0.44\pm 0.16$  during treatment at the expense of decreases in chenodeoxycholic and deoxycholic acid.

## Key words

bile acid, bile acid sequestrant, cholesterol 7 $\alpha$ -hydroxylase, LDL-cholesterol, genetics

## Introduction

Bile acid (BA) sequestrants were originally recognized as the first drugs to efficiently decrease cholesterolemia and diminish the risk of cardiovascular disease (1984a, 1984b). However, due to their low efficiency and limited patient compliance, they were replaced by more potent statins. Interest in their use was revived after the arrival of new sequestrants (colestipol, colesevelam) that were better tolerated by patients and, more importantly, after they were demonstrated to have a positive effect on glycemic control (Zieve *et al.* 2007).

The direct effect of sequestrants on BA uptake diminishes their enterohepatic recirculation, resulting in upregulation of BA *de novo* synthesis from cholesterol in the liver. The consumption of intracellular cholesterol induces an increase in LDL receptor activity and, subsequently, a drop in LDL-cholesterol concentration (Insull 2006, Couture and Lamarche 2013).

Cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), the first and key regulatory enzyme of the classical pathway of BA synthesis, plays a critical role in this process. Its expression is controlled by two main regulatory feedback mechanisms (Chiang 2009). First, BA returning to the liver binds to the hepatic farnesoid X receptor (FXR) and induces expression of the small heterodimer partner (SHP). SHP then inhibits expression of the cholesterol 7 $\alpha$ -hydroxylase gene (CYP7A1). Second, BA, after binding to FXR in enterocytes, induce the intestinal expression of fibroblast growth factor 19 (FGF19), which is then secreted into systemic circulation. FGF19 interacts with FGF receptor 4 (FGFR4) on hepatocytes and inhibits expression of CYP7A1 via the MAPK/ERK1/2 signaling pathway.

Interestingly, several functional polymorphisms have been identified in the promoter region of the CYP7A1 gene. One of them, the -203A/C polymorphism (rs 3808607), has been shown to affect cholesterolemia (Wang *et al.* 1998, Couture *et al.* 1999) and/or cholesterolemia responsiveness to diet (Hubacek *et al.* 2003, Hofman *et al.* 2004, Kovar *et al.* 2004) and also

modulate the effectiveness of statin therapy (Kajinami *et al.* 2004, 2005). It is not clear yet whether this polymorphism may also affect the response to BA sequestrant therapy in healthy men.

Increased synthesis of BA through the classical pathway initiated by CYP7A1 can also be of great importance with respect to the recently recognized role of BA as important signaling molecules involved in the regulation of lipid, glucose and energy homeostasis (Chiang 2013, Vitek and Haluzík, submitted). It has been shown that BA sequestrant treatment affects the concentrations of individual BA in circulation (Brufau *et al.* 2010), which might have an impact on their signaling role, in particular due to marked differences in the biological activities of individual BA (Liu *et al.* 2014).

Therefore, in this pilot study of healthy men, we characterized in detail the dynamics of the responses of lipids, BA, FGF19 and also 7 $\alpha$ -hydroxy-4-cholesten-3-one (C4) (a plasma marker of CYP7A1 activity (Axelson *et al.* 1991, Sauter *et al.* 1996)) to treatment with colesevelam, a novel bile acid sequestrant. To determine whether such a response is affected by the promoter polymorphism of the CYP7A1 gene, the study was carried out in subjects homozygous either for the -203A or -203C allele of CYP7A1. We also analyzed the effect of treatment on parameters related to glycemic control and thyroid hormone metabolism, which can also be affected by bile acid sequestrants (Zieve *et al.* 2007, Ockenga *et al.* 2012).

## **Methods**

### ***Subjects and study design***

Sixteen healthy men (eight homozygous for the A allele and eight homozygous for the C allele) were selected from the employees of both participating institutions and from a number of healthy subjects who participated in a preventive medical program (Vitek *et al.* 2013). The exclusion criteria included triglyceride (TG) concentration higher than 2.0 mmol/l and

abnormal liver function tests (AST > 0.58  $\mu$ kat/l, ALT > 0.75  $\mu$ kat/l). Both groups did not significantly differ in age, BMI or lipid concentration upon entering the study (Table 1).

The subjects were administered colesevelam (Cholestagel®, Genzyme Europe B.V., the Netherlands) for 4 weeks. Colesevelam (3.75 g/day) was given in one daily dose taken with a meal except of one subject who preferred to take it in two doses due to discomfort when taking six large tablets at once. Blood for determination of biochemical parameters was drawn on the first day of treatment (day 0) before application of the first dose of the drug and on days 1, 3, 7, 14 and 28 of treatment. Blood sampling was carried out in the morning in a fasting state at exactly the same time of a day in each of the subjects to diminish the effect of diurnal variation. The blood was collected in tubes with EDTA and immediately transferred to ice. A dipeptidyl peptidase-4 (DPP4) inhibitor was added immediately after blood samples were drawn for glucagon-like peptide 1 (GLP-1) analysis. Plasma samples were stored at -80°C until the analyses were performed.

The protocol of the study adhered to the Principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital in Prague, Czech Republic. All participants gave their informed consent.

### ***Analytical methods***

The concentrations of cholesterol, HDL-cholesterol and triglyceride in plasma were measured using enzymatic kits from Roche Diagnostics (Mannheim, Germany), LDL-cholesterol was calculated based on the Friedewald formula, non-esterified fatty acids (NEFA) were determined using a kit from Wako Chemicals GmbH (Germany), and glucose using a kit from Erba Lachema (Brno, Czech Republic). Insulin concentrations were determined using a radioimmunometric kit from Beckman Coulter (Prague, Czech Republic). Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR)

(Matthews *et al.* 1985). Thyroxine (T4), free thyroxine (fT4), triiodothyronine (T3), free triiodothyronine (fT3) and thyroid-stimulating hormone (TSH) were determined using radioimmunoassay kits (Beckman Coulter, Prague, Czech Republic). Fibroblast growth factor-19 (FGF-19) was measured using ELISA kit (R&D Systems, Abingdon, UK) and total GLP-1 and active GLP-1 (i.e. intact functional GLP-1 not subjected to the action of an endogenous DPP-4 inhibitor) also with ELISA kits (EMD Millipore, St Charles, USA). Serum levels of bile acids were determined as described previously (Setchell and Lawson 1989, Haluzikova *et al.* 2013). The levels of 7 $\alpha$ -hydroxy-4-cholesten-3-one (C4) were determined by HPLC and mass spectroscopy. Briefly, 100  $\mu$ l of serum and 2 ng of the internal standard (7 $\alpha$ -hydroxy-4-cholesten-3-one d7, Santa Cruz Biotechnology, USA) in 40  $\mu$ l of methanol were mixed and extracted as previously reported (Lenicek *et al.* 2008a). The purified sample was dissolved in 50  $\mu$ l of 75 % methanol, 15  $\mu$ l were injected on HPLC system (Dionex Ultimate 3000, Dionex Softron GmbH, Germany) equipped with a Hypersil GOLD column (150x2.1 mm, 3  $\mu$ m, Thermo Scientific, USA) and SecurityGuard column (Phenomenex, USA). The sample was separated by methanol (LC-MS grade, Biosolve BV, the Netherlands), water and ammonium acetate (LC-MS grade, Sigma-Aldrich, USA) at a flow rate of 0.3 mL/min (40 °C). While the ammonium acetate concentration was kept at 0.1 % (w/v) at all times, methanol concentrations (v/v) were as follows: 1-8 min 82 %-90 %; 8-10 min 90 %; 10-12 min 99 %; 12-17 min 82 %.

A triple quadrupole mass spectrometer (TSQ Quantum Access Max with an H-ESI II probe, Thermo Fisher Scientific, Inc., USA) operating in SIM mode was used as the detector. Transitions used for monitoring C4 and the internal standard were: m/z 401.4  $\rightarrow$  177.3, 401.4  $\rightarrow$  383.6 and 408.4  $\rightarrow$  184.3, 408.4  $\rightarrow$  390.6, respectively.

## ***Genotyping***

Determination of the -203A/C genotype of CYP7A1 was carried out as described earlier (Lenicek *et al.* 2008b).

## ***Statistics***

The effect of the CYP7A1 genotype on changes to all parameters during the study was evaluated using ANOVA for repeated measures with one grouping factor (genotype). Data for analysis were logarithmically transformed where necessary. ANOVA for repeated measures or its non-parametric variant (the Friedman test) were then used for analysis of the pooled data from all subjects. Corresponding post hoc tests were carried out where significant differences were detected using ANOVA or the Friedman test (GraphPad InStat 3.00, GraphPad Software, San Diego, CA, USA).

## **Results**

The treatment with colesevelam was well tolerated by all the subjects in the study and resulted in expected decrease of cholesterol and LDL-C in all of them (Table 2).

### ***The effect of the -203A/C polymorphism on CYP7A1 activity and the response of cholesterol to treatment with colesevelam***

There were no significant differences between homozygous carriers of -203A and -203C alleles for all parameters measured in the study at baseline (day 0) (Table 1). Importantly, when the ANOVA for repeated measures with one grouping factor (genotype) was used to analyze the response of lipids, C4, FGF-19, BA and parameters related to glucose control and thyroidal function to colesevelam therapy, no significant differences between carriers of -203A and -203C alleles were detected (data not shown). Interestingly, the concentration of C4 was more than twice as high, albeit not significantly, in homozygous A allele carriers as in C allele carriers on day 0 of the study ( $29.4 \pm 21.9$  vs.  $12.6 \pm 7.8$   $\mu\text{g/l}$ ,  $p = 0.115$ ). However,

the response of C4 concentration to the therapy did not differ between -203A and -203C carriers (data not shown). Therefore, all other analyses of the effect of colestevlam treatment were carried out for all sixteen subjects altogether, regardless of genotype.

### ***The effect of colestevlam treatment on lipoproteins***

Cholesterolemia decreased by 8 % after a week of treatment and remained stable till the end of the study (Table 2). This decrease was caused by an approximate 17 % decrease in LDL-cholesterol, which had already become significant after 3 days on colestevlam (Table 2, Fig. 1A). Neither HDL-C nor TG concentration was affected by the treatment. Although the mean TG concentration rose only non-significantly during colestevlam therapy, transient elevations in TG concentration were observed in 5 subjects on six occasions (triglyceridemia was more than twice as high when compared to day 0).

### ***The effect of colestevlam treatment on regulation of BA synthesis and concentration***

Concentration of FGF19 dropped to approximately one third of the baseline value as early as on the first day of therapy and remained at this level until the end of the study (Fig. 1C). Simultaneously, the concentration of C4 – a plasma marker of CYP7A1 activity – increased more than twofold on day 1 and progressed to fivefold higher levels on day 3, but did not then change further (Fig. 1D).

Treatment with colestevlam had no significant effect either on total concentrations of plasma BA (Table 2, Fig. 1B) or individual BA, except for concentration of DCA, which was markedly suppressed after 3 and 7 days of therapy (Table 2). However, the ratio of CA/total BA rose from  $0.25 \pm 0.10$  at the beginning of the study to  $0.44 \pm 0.16$  at the end and the ratio of CDCA/total BA dropped from  $0.40 \pm 0.13$  to  $0.32 \pm 0.08$  (Fig. 1E). Moreover, the share of the sum of both primary BA, CA and CDCA from the total BA concentration rose from  $0.65 \pm 0.17$  to  $0.76 \pm 0.14$  (Fig. 1F).



### ***The effect of treatment on parameters related to glycemic control and thyroidal function***

A transient 5 % decrease in glycemia was observed on day 3 of the study (Table 3).

However, treatment with colesevelam had no significant effect on insulin or on total and active GLP-1 concentrations in our group of healthy men. Also, HOMA-IR was not affected by the treatment.

Treatment with colesevelam resulted in a transient increase in TSH concentration, peaking on day 7 (Table 3). Neither total nor free thyroxin (T4) concentrations were affected by the coleveselam treatment, although both total and free triiodthyronine (T3) concentrations tended to be somewhat higher at the end of the study. The ratio of T3/T4 was approximately 10 % higher at the end of the study.

### **Discussion**

In our pilot study of healthy men, we document that treatment with colesevelam, a new generation BA sequestrant, resulted in an immediate downregulation of FGF-19 concentration followed by a severalfold increase in BA synthesis within three days and a decrease in LDL-cholesterol concentration within a week. Although plasma concentrations of total BA were not affected (presumably due to low baseline levels in the healthy individuals of the study), changes to the spectrum of individual BA, characterized by an increase in CA and a decrease in CDCA, were observed. These changes were not affected by the promoter polymorphism of *CYP7A1*.

The -203A/C polymorphism, which has been previously shown to affect cholesterolemia and cholesterol responsiveness to diet, did not seem to have any effect on the response of BA synthesis and plasma cholesterol to treatment with colesevelam in healthy volunteers. It can be assumed that due to the high loss of BA as a result of treatment with the BA sequestrant, *CYP7A1* activity must have been markedly upregulated to compensate for depletion of the

BA pool. The extent of such a depletion should not be affected by *CYP7A1* promoter polymorphism and may be the driving force in the upregulation of *CYP7A1* activity. Therefore, under these conditions, the rate of BA synthesis may not have been affected by the *CYP7A1* polymorphism.

Because there were no differences between both -203A and -203C allele homozygous carriers at all, we aggregated all subjects for all other analyses.

It is worth noting that the response of FGF-19 to the treatment was immediate – it dropped to 30 % of the baseline values as early as on day 1 and did not change further (Fig 1A). The activity of *CYP7A1*, as assessed from C4 concentration, also responded very quickly – it reached its maximum on day 3 and then did not change significantly (Fig 1A). The increased consumption of hepatic cholesterol for BA synthesis resulted in a 13 % reduction of LDL-cholesterol (Fig. 1A), which fully explains the decrease of total cholesterol concentration because the HDL-C concentration was unaffected. The final reduction of 17 % and 8 % for LDL-C and total cholesterol, respectively, seems to be in the line with other studies (Insull 2006). Importantly, although mean triglyceridemia rose during the study, the increase was not significant. When the data were thoroughly examined, we noticed transient elevations of triglyceridemia in some subjects. It has been suggested that BA downregulate triglyceride synthesis and VLDL secretion *via* a pathway involving FXR, SHP and SREBP-1c (Watanabe *et al.* 2004). It cannot be excluded that this signaling pathway is indeed attenuated in certain subjects during colesevelam treatment because hydrophilic CA, whose proportion is lower during the treatment, is a weak agonist of FXR (Wang *et al.* 1999).

Importantly, colesevelam treatment did not affect total fasting BA concentrations, probably due to their overall low plasma concentrations in our healthy subjects. However, there were marked changes in the spectrum of individual BA. Due to the high variability of individual BA concentrations, the changes to individual BA concentrations did not reach statistical

significance during the study, except for a transient decrease in DCA concentration. However, the ratio of CA/total BA increased 1.8 times within three days and then remained stable during the treatment. This increase took place at the expense of CDCA and DCA concentrations. Moreover, the ratio of (CA + CDCA)/BA also increased after the treatment, indicative of the higher contribution of primary BA to the total BA pool. These findings are in relatively close agreement with those of Brufau *et al.* (Brufau *et al.* 2010) who demonstrated that the BA pool becomes more hydrophilic after colestevlam treatment in both healthy and diabetic subjects. Based on our data, we can only speculate on the mechanisms behind these changes. First, the increase in the proportion of CA may be due to the higher affinity of colestevlam for more hydrophobic BA. But this seems unlikely because colestevlam was designed to bind to CA with higher efficiency (Insull 2006). Second, the higher proportion of CA may result from the increased activity of sterol-12 $\alpha$ -hydroxylase (CYP8B1). This enzyme is responsible for CA synthesis and its expression is regulated through the action of FXR and SHP in exactly the same way as expression of the *CYP7A1* gene (Goodwin *et al.* 2000). Third, the proportion of CA may increase because of the lower conversion to DCA in the intestine. This could provide an explanation for the transient decrease of DCA after 3 and 7 days of treatment and also for the increased proportion of primary BA in the total plasma BA pool. It cannot be excluded that BA (when bound to colestevlam) are better protected against the metabolism by intestinal microbiota or that colestevlam *per se* affects the composition and metabolism of intestinal microbiota resulting in the suppression of CA transformation.

Changes to the BA profile in plasma may have a pronounced impact on BA signaling in the systemic circulation. Some of the positive effects of colestevlam are assumed to be caused by the interaction of BA with the FXR and TGR5 receptors. However, hydrophilic CA is a weaker ligand for these receptors (Wang *et al.* 1999, Sato *et al.* 2008), so it may be then

expected that the more hydrophilic BA profile may diminish the activation of these receptors in circulation.

Despite the comprehensively described effect of colestevam treatment on glycemic control, we did not observe any significant effect of colestevam therapy on insulin, total and active GLP-1 concentrations or HOMA-IR in our healthy men. In our study, we noted only a transient decrease in glycemia. These findings are in relatively close agreement with observations that HOMA-IR improves after treatment with colestevam in diabetics only, and not in healthy controls (Brufau *et al.* 2010). Therefore, it cannot be excluded that the positive effect of BA sequestrant therapy on glucoregulation may be seen only in subjects who are insulin-resistant, not in healthy subjects with normal insulin sensitivity.

Similar to the effect of the treatment on glycemic control, only transient and modest changes were observed in levels of TSH and T3 concentrations. It has been shown that BA activate a cyclic-AMP-dependent thyroid hormone, which in turn activates type 2 iodothyronine deiodinase – an enzyme responsible for the conversion of T4 to T3 – through activation of the TGR5-signaling pathway (Watanabe *et al.* 2006). However, such an effect is unlikely after colestevam treatment, since hydrophilic CA binds to TGR5 with a lower affinity than CDCA and DCA, which are more hydrophobic (Sato *et al.* 2008).

We are aware that there are some limitations to our study – the sample size was small and the method used to evaluate BA concentrations did not allow us to discriminate between conjugated and unconjugated BA. Moreover, the concentrations of BA in fasting plasma may not have corresponded to the changes in BA concentrations in portal circulation. In addition, the study was carried out in healthy subjects and it cannot be excluded that the results in patients that are insulin-resistant may be different.

Based on our pilot study, it can be concluded that the -203A/C polymorphism of the *CYP7A1* gene does not affect the response of cholesterolemia and/or BA synthesis to

colesevelam treatment, at least in healthy subjects. Importantly, we document that CYP7A1 activity is downregulated immediately after initiation of therapy. Although the treatment does not affect plasma BA concentration, it results in pronounced changes in their spectrum, characterized by an increase in hydrophilic CA. On the other hand, it does not appear that BA sequestrant treatment has any significant effects on parameters related to glycemic control and thyroidal function in healthy, insulin-sensitive subjects.

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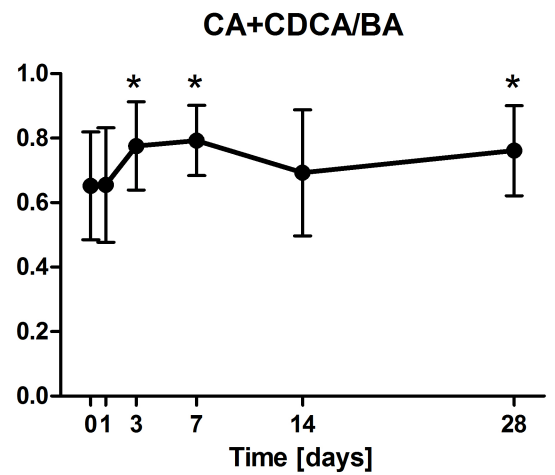
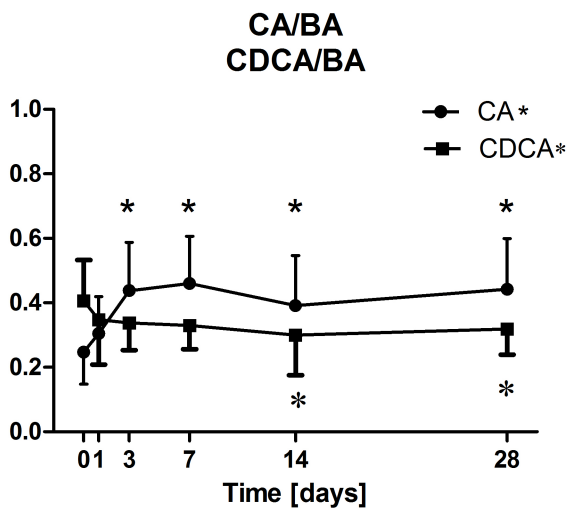
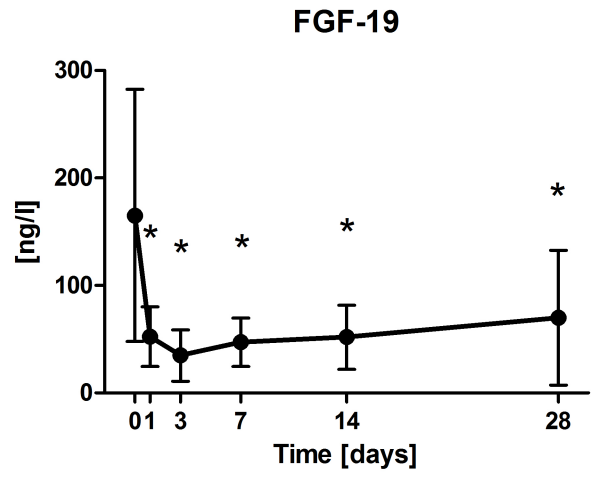
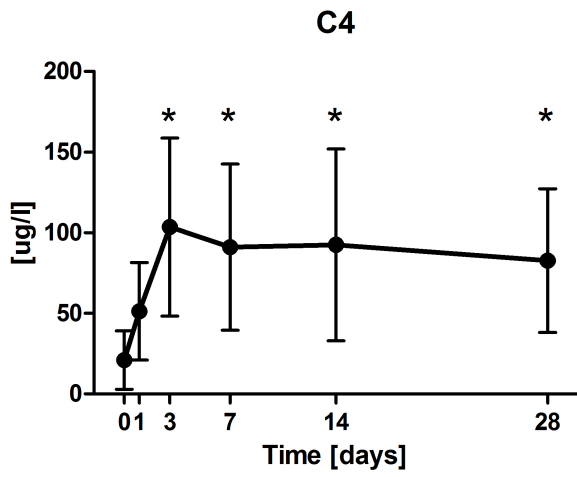
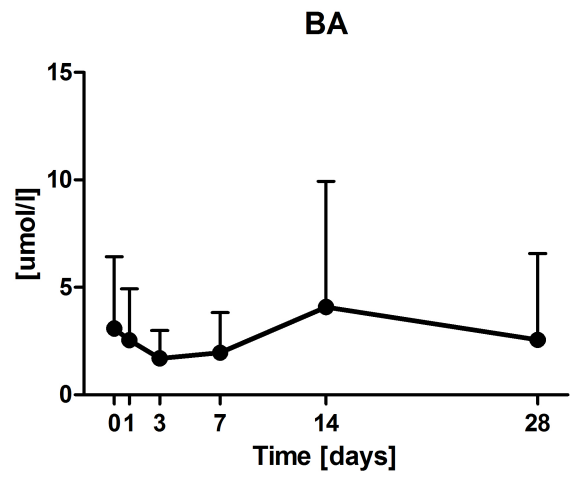
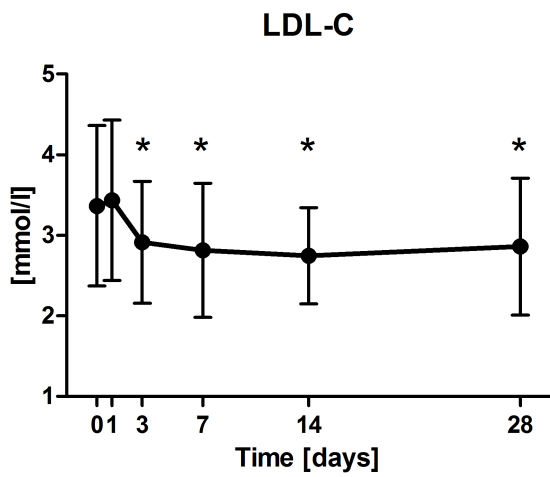
## Figure 1

**The response of selected parameters of lipid and BA metabolism to treatment with colesevelam**

Data are mean  $\pm$  SD.

LDL-C ... low density lipoprotein-cholesterol, BA ... bile acids, C4 ... 7 $\alpha$ -hydroxy-4-cholesten-3-one, FGF19 ... fibroblast growth factor-19, CA ... cholic acid, CDCA ... chenodeoxycholic acid, DCA ... deoxycholic acid.

\* ...  $p < 0.05$  vs baseline



**Table 1****Baseline characteristics of subjects separated according to the *CYP7A1* genotype**

|                          | AA                  | CC                  |         |
|--------------------------|---------------------|---------------------|---------|
| n                        | 8                   | 8                   | p value |
| age [years]              | 40.7 ± 9.0          | 43.2 ± 13.8         | 0.677   |
| BMI [kg/m <sup>2</sup> ] | 25.4 ± 2.1          | 24.0 ± 2.7          | 0.258   |
| cholesterol [mmol/l]     | 5.64 ± 0.80         | 4.94 ± 1.46         | 0.259   |
| LDL-C [mmol/l]           | 3.77 ± 0.63         | 2.97 ± 1.17         | 0.118   |
| HDL-C [mmol/l]           | 1.32 ± 0.31         | 1.41 ± 0.20         | 0.490   |
| TG [mmol/l]              | 1.23 ± 0.30         | 1.24 ± 0.67         | 0.955   |
| glucose [mmol/l]         | 5.50 ± 0.43         | 5.38 ± 0.37         | 0.547   |
| C4 [µg/l]                | 29.4 ± 21.9         | 12.6 ± 7.8          | 0.115   |
| FGF-19 [ng/l]            | 144 ± 90            | 187 ± 142           | 0.573   |
| BA [µmol/l]              | 1.60<br>(0.92-3.25) | 2.38<br>(1.07-3.43) | 0.721   |
| CA [µmol/l]              | 0.27<br>(0.14-0.78) | 0.53<br>(0.32-1.29) | 0.279   |
| CDCA [µmol/l]            | 0.53<br>(0.42-1.13) | 0.78<br>(0.51-1.52) | 0.599   |
| DCA [µmol/l]             | 0.85<br>(0.36-1.20) | 0.30<br>(0.20-0.66) | 0.270   |

Data are mean ± SD or median (IQ range), depending on the data normality. There were no significant differences between groups. Differences between groups were evaluated using the unpaired t-test or the Mann-Whitney rank-sum test, where appropriate.

AA, CC ... homozygous carriers of the -203A and -203C alleles of the *CYP7A1* gene, BMI ... body mass index, LDL-C ... low-density lipoprotein cholesterol, HDL-C ... high-density lipoprotein cholesterol, TG ... triglyceride, C4 ... 7 $\alpha$ -hydroxy-4-cholesten-3-one, FGF19 ... fibroblast growth factor-19, BA ... bile acids, CA ... cholic acid, CDCA ... chenodeoxycholic acid, DCA ... deoxycholic acid, LCA ... lithocholic acid, UDCA ... ursodeoxycholic acid. Lithocholic and ursodeoxycholic acid concentrations are not shown because they were under the limit of detection in most subjects.

**Table 2****The response of lipids, lipoproteins and bile acids to treatment with colesevelam**

|             | Day         |             |             |             |             |             | ANOVA   |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
|             | 0           | 1           | 3           | 7           | 14          | 28          |         |
| cholesterol | 5.29        | 5.33        | 5.00        | 4.88**      | 4.83**      | 4.85**      | <0.001  |
| [mmol/l]    | ± 1.19      | ± 1.13      | ± 1.02      | ± 1.0       | ± 0.86      | ± 0.91      |         |
| LDL-C       | 3.37        | 3.43        | 2.92**      | 2.81**      | 2.74**      | 2.80**      | <0.001  |
| [mmol/l]    | ± 1.00      | ± 1.00      | ± 0.76      | ± 0.83      | ± 0.60      | ± 0.85      |         |
| HDL-C       | 1.36        | 1.34        | 1.30        | 1.38        | 1.39        | 1.43        | 0.098   |
| [mmol/l]    | ± 0.25      | ± 0.26      | ± 0.29      | ± 0.27      | ± 0.30      | ± 0.30      |         |
| TG          | 1.23        | 1.22        | 1.72        | 1.51        | 1.53        | 1.36        | 0.190   |
| [mmol/l]    | ± 0.50      | ± 0.46      | ± 1.22      | ± 0.80      | ± 1.11      | ± 0.81      |         |
| BA          | 1.91        | 1.56        | 1.25        | 1.49        | 1.84        | 1.40        | 0.771 § |
| [µmol/l]    | (0.97-4.08) | (0.80-4.27) | (0.81-2.17) | (0.95-2.06) | (0.98-5.45) | (1.05-1.82) |         |
| CA          | 0.38        | 0.45        | 0.42        | 0.54        | 0.67        | 0.45        | 0.155 § |
| [µmol/l]    | (0.18-1.33) | (0.20-1.54) | (0.25-1.15) | (0.32-1.06) | (0.40-1.37) | (0.42-0.93) |         |
| CDCA        | 0.65        | 0.49        | 0.43        | 0.42        | 0.58        | 0.46        | 0.654 § |
| [µmol/l]    | (0.39-1.25) | (0.32-0.98) | (0.23-0.88) | (0.38-0.66) | (0.32-0.71) | (0.36-0.72) |         |
| DCA         | 0.44        | 0.34        | 0.17**      | 0.24**      | 0.27        | 0.26        | 0.003 § |
| [µmol/l]    | (0.24-1.15) | (0.16-0.83) | (0.12-0.33) | (0.18-0.37) | (0.16-0.55) | (0.17-0.41) |         |

Data are mean ± SD or median (IQ range). ANOVA ... significance calculated by ANOVA

for repeated measures or using the Friedman test. \*\* ... p < 0.01 vs day 0, <sup>a</sup> ... p < 0.05 vs day

1, <sup>b</sup> ... p < 0.01 vs day 3, <sup>c</sup> ... p < 0.05 vs day 7.

LDL-C ... low-density lipoprotein cholesterol, HDL-C ... high-density lipoprotein cholesterol,

TG ... triglyceride, BA ... bile acids, CA ... cholic acid, CDCA ... chenodeoxycholic acid,

DCA ... deoxycholic acid. Lithocholic and ursodeoxycholic acid concentrations are not shown

because they were under the limit of detection in most subjects.

**Table 3**

**The response of parameters of glycemic control and thyroidal function to treatment with colesevelam**

|                 | Day     |         |         |                   |                   |                     | ANOVA   |
|-----------------|---------|---------|---------|-------------------|-------------------|---------------------|---------|
|                 | 0       | 1       | 3       | 7                 | 14                | 28                  |         |
| glucose         | 5.44    | 5.33    | 5.16*   | 5.23              | 5.22              | 5.46                | 0.013   |
| [mmol/l]        | ± 0.40  | ± 0.33  | ± 0.32  | ± 0.33            | ± 0.39            | ± 0.43              |         |
| insulin         | 6.5     | 6.2     | 5.9     | 5.7               | 5.0               | 5.4                 | 0.241   |
| [mIU/l]         | ± 3.6   | ± 2.2   | ± 2.7   | ± 3.0             | ± 2.4             | ± 2.7               |         |
| HOMA-IR         | 1.61    | 1.48    | 1.36    | 1.34              | 1.16              | 1.32                | 0.171   |
|                 | ± 0.97  | ± 0.57  | ± 0.62  | ± 0.75            | ± 0.57            | ± 0.70              |         |
| active GLP-1    | 2.87    | 2.96    | 3.10    | 3.21              | 2.93              | 3.00                | 0.981   |
| [pmol/l]        | ± 0.86  | ± 0.93  | ± 1.10  | ± 1.38            | ± 0.68            | ± 0.91              |         |
| total GLP-1     | 9.00    | 8.53    | 11.08   | 10.72             | 10.24             | 10.48               | 0.150   |
| [pmol/l]        | ± 3.42  | ± 4.20  | ± 7.20  | ± 4.83            | ± 6.20            | ± 7.97              |         |
| TSH             | 3.22    | 3.00    | 2.95    | 3.81 <sup>a</sup> | 3.34              | 2.83                | 0.001   |
| [µmol/l]        | ± 1.72  | ± 1.36  | ± 1.30  | ± 1.87            | ± 1.49            | ± 1.25              |         |
| T4              | 77.2    | 77.6    | 77.4    | 77.4              | 76.8              | 75.7                | 0.943   |
| [nmol/l]        | ± 10.7  | ± 14.7  | ± 11.2  | ± 9.7             | ± 10.4            | ± 10.1              |         |
| free T4         | 15.3    | 15.4    | 15.1    | 14.9              | 15.3              | 15.1                | 0.713   |
| [pmol/l]        | ± 2.2   | ± 2.0   | ± 2.1   | ± 1.9             | ± 2.0             | ± 2.0               |         |
| T3              | 1.61    | 1.62    | 1.52    | 1.57              | 1.66              | 1.69 <sup>b</sup>   | 0.007   |
| [nmol/l]        | ± 0.20  | ± 0.22  | ± 0.15  | ± 0.23            | ± 0.31            | ± 0.25              |         |
| free T3         | 5.00    | 5.00    | 4.80    | 4.92              | 5.25 <sup>b</sup> | 5.09                | 0.011   |
| [pmol/l]        | ± 0.84  | ± 0.72  | ± 0.82  | ± 0.61            | ± 0.71            | ± 0.64              |         |
| 1000*T3/T4      | 20.2    | 20.6    | 19.8    | 20.3              | 22.0              | 22.4 <sup>b,c</sup> | 0.004 § |
|                 | ± 2.4   | ± 1.8   | ± 2.2   | ± 2.5             | ± 3.0             | ± 2.4               |         |
| free T3/free T4 | 0.331   | 0.329   | 0.319   | 0.334             | 0.349             | 0.342               | 0.385   |
|                 | ± 0.057 | ± 0.054 | ± 0.053 | ± 0.047           | ± 0.065           | ± 0.054             |         |

Data are mean  $\pm$  SD. ANOVA ... significance calculated from ANOVA for repeated measures and/or from § the Friedman test. <sup>a</sup> ...  $p < 0.05$  vs day 1, <sup>b</sup> ...  $p < 0.01$  vs day 3, <sup>c</sup> ...  $p < 0.05$  vs day 7.

HOMA-IR ... insulin resistance from the homeostasis model assessment, GLP-1 ... glucagon-like peptide 1, TSH ... thyroid-stimulating hormone, T4 ... thyroxine, T3 ... triiodothyronine.