Acute caloric restriction counteracts hepatic bile acid and cholesterol deficiency in morbid obesity

● S. Straniero[1], F. Rosqvist[2], D. Edholm[3], H. Ahlström[4], J. Kullberg[4], M. Sundbom[3], U. Risérus[2] & M. Rudling[1]

From the [1]Department of Medicine, Karolinska University Hospital at Huddinge, Huddinge, Stockholm; [2]Department of Public Health and Caring Sciences and Clinical Nutrition and Metabolism; [3]Department of Surgical Sciences; and [4]Department of Radiology, Uppsala University, Uppsala, Sweden

Abstract. Straniero S, Rosqvist F, Edholm D, Ahlström H, Kullberg J, Sundbom M, Risérus U, Rudling M (Karolinska University Hospital at Huddinge, Huddinge, Stockholm; Uppsala University, Uppsala, Sweden). Acute caloric restriction counteracts hepatic bile acid and cholesterol deficiency in morbid obesity. *J Intern Med* 2017; **281**: 507–517.

Background. Bile acid (BA) synthesis is regulated by BA signalling in the liver and by fibroblast growth factor 19 (FGF19), synthesized and released from the intestine. In morbid obesity, faecal excretion and hepatic synthesis of BAs and cholesterol are strongly induced and caloric restriction reduces their faecal excretion considerably. We hypothesized that the high intestinal food mass in morbidly obese subjects promotes faecal excretion of BAs and cholesterol, thereby creating a shortage of both BAs and cholesterol in the liver.

Methods. Ten morbidly obese women (BMI 42 ± 2.6 kg m^{-2}) were monitored on days 0, 3, 7, 14 and 28 after beginning a low-calorie diet (800–1100 kcal day^{-1}). Serum was collected and liver size and fat content determined. Synthesis of BAs and cholesterol was evaluated from serum markers, and the serum levels of lipoproteins, BAs, propro-tein convertase subtilisin/kexin type 9 (PCSK9), insulin, glucose and FGF19 were monitored. Fifty-four nonobese women (BMI <25 kg m^{-2}) served as controls.

Results. At baseline, synthesis of both BAs and cholesterol and serum levels of BAs and PCSK9 were elevated in the obese group compared to controls. Already after 3 days on a low-calorie diet, BA and cholesterol synthesis and serum BA and PCSK9 levels normalized, whereas LDL cholesterol increased. FGF19 and triglyceride levels were unchanged, and liver volume was reduced by 10%.

Conclusions. The results suggest that hepatic BAs and cholesterol are deficient in morbid obesity. Caloric restriction rapidly counteracts these deficiencies, normalizing BA and cholesterol synthesis and circulating PCSK9 levels, indicating that overproduction of cholesterol in enlarged peripheral tissues cannot explain this phenotype. We propose that excessive food intake promotes faecal loss of BAs and cholesterol contributing to their hepatic deficiencies.

Keywords: bile acid synthesis, cholesterol synthesis, proprotein convertase subtilisin/kexin type 9.

Introduction

The prevalence of obesity is increasing globally to reach epidemic levels. Although the causes for this increase are multifactorial, a key factor is excessive food intake [1]. Bile acids (BAs) facilitate fat absorption. Their synthesis is regulated by negative feedback [2] from BAs interacting with farnesoid X receptors (FXRs) in the liver and intestines; the latter induces fibroblast growth factor 19 (FGF19) [3] that suppresses the gene expression of *CYP7A1* encoding the rate-limiting enzyme in BA synthesis, cholesterol 7-alpha hydroxylase [2].

Intestinal food contents bind BAs, promoting their faecal loss, which in turn is compensated for through induced BA synthesis.

The synthesis of BAs and the synthesis of cholesterol both correlate with body mass index (BMI) [4–6], and studies using different techniques have established that they are both considerably increased in morbid obesity [5–11], as is the clearance of LDL from blood, despite the fact that the hepatic synthesis of the LDL precursor very low-density lipoprotein is increased [7, 12]. Importantly, it has long been established that the faecal

excretion of cholesterol and particularly of BAs is increased in morbid obesity by ~1.5-fold [6–8, 10, 13]. Also of note, studies have consistently established that long-term weight reduction (13–70 kg) from caloric restriction results in normalized levels of faecal excretion of BAs and cholesterol [6, 14]. Further, and fundamental for the present study, it has also been established that acute caloric restriction (300 kcal day^{-1}) very rapidly reduces faecal BA excretion by about 40% as measured in pooled stool collections already from the first 2 to 3 days on a low-calorie diet (LCD) [13]. The faecal excretion of cholesterol is also reduced but to a lower extent and more slowly [13, 14]. Recently, plasma BAs were reported to be elevated in morbidly obese individuals [5, 15], suggested to be due to reduced active uptake of BAs from plasma to the liver as a result of a decrease in hepatic Na$^+$-dependent taurocholate co-transporting polypeptide (NTCP) expression [5].

Together, the above observations strongly suggest that BAs and cholesterol are deficient in the liver in morbid obesity (for overview, see Fig. 1). To the best of our knowledge, the underlying mechanism for the development of this phenotype amongst morbidly obese individuals has not been addressed. However, it has been suggested that the elevated synthesis of both cholesterol and BAs in obesity is due to overproduction of cholesterol in large peripheral fat tissues, as the reduction or normalization of BMI results in reduced or normalized total synthesis of cholesterol in the body [6, 14]. In the liver, the presumably abundant
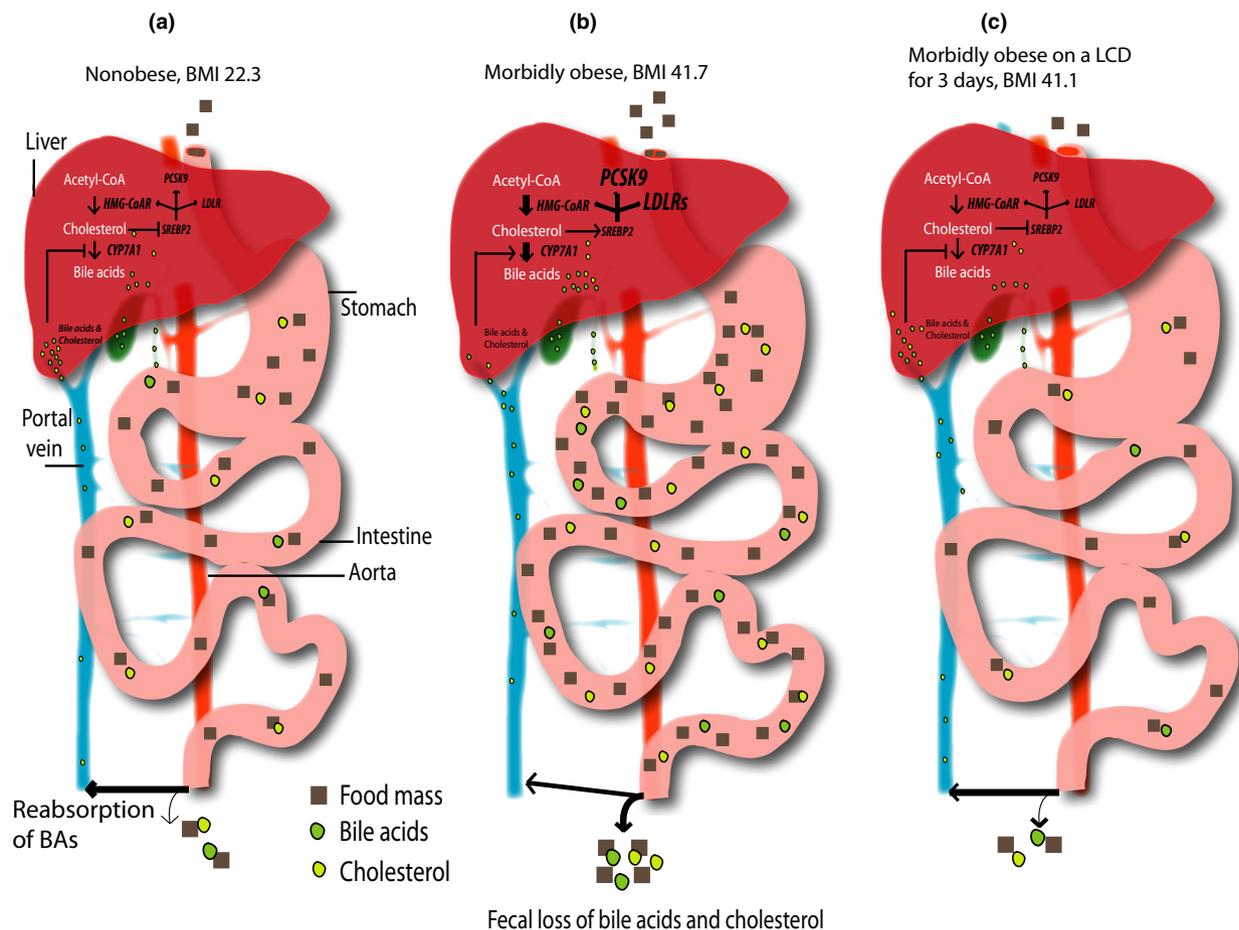

Fig. 1 *Schematic overview of the metabolic situation in nonobese control subjects (a), morbidly obese women (b) and morbidly obese women on a low-calorie diet (LCD) for 3 days (c).*

cholesterol should control the level of BA synthesis via a feed-forward mechanism [6, 7, 11]. Further, it has been suggested that the pronounced reduction in faecal excretion of BAs that presents following caloric restriction may be due to the reduced synthesis of BAs that occur in parallel [13], although why BA synthesis is indeed reduced following caloric restriction has not been addressed.

We reasoned that the described phenotype regarding cholesterol and BA metabolism may arise from a common factor amongst morbidly obese individuals that is strongly related to BMI. We hypothesized that such a factor could be the higher food intake in these individuals that will increase the intestinal food mass which in turn will promote the faecal excretion of BAs and cholesterol, thereby creating a state of BA and cholesterol deficiency in the liver. The deficiency of BAs in turn will induce the expression of CYP7A1 to produce more BAs, which further depletes cholesterol in the liver, contributing to the transcriptional induction of steroid regulatory element-binding protein-2-driven genes such as those encoding HMGCoA reductase (cholesterol synthesis) and proprotein convertase subtilisin/kexin type 9 (PCSK9), a

protein that promotes degradation of LDL receptors [16, 17].

To test this hypothesis, we analysed material from a study in which morbidly obese individuals were monitored for 4 weeks after switching to an LCD [18]. The primary aim of that study was to evaluate the time required to reduce liver volume prior to laparoscopic gastric bypass surgery. Because changing to a liquid LCD will essentially empty the intestines of formed contents within 72 h [19], we considered the study suitable to test our hypothesis. If correct, several of the established morbid obesity-related changes in BA and cholesterol metabolism should normalize within a few days on an LCD.

Materials and methods

Participants and study design

The study design has been described elsewhere [18]. Briefly, 10 morbidly obese women awaiting laparoscopic gastric bypass surgery were recruited for a prospective observational study during 4 weeks of LCD treatment. Their baseline data are shown in Table 1. The study was approved by the regional ethical review board in Uppsala, and

Table 1 *Clinical and laboratory data from 10 morbidly obese and 54 nonobese women at baseline derived from a previous cohort [4]*

	Obese women[a] (n = 10) Mean ± SEM	Nonobese women[a,b] (n = 54) Mean ± SD	P value
Age (years)	42.7 ± 2.8	38.3 ± 7.2	0.093
Body weight (kg)	114.4 ± 3.8	62.1 ± 6.3	<0.0001
BMI (kg m^{-2})	41.7 ± 0.82	22.3 ± 1.67	<0.0001
Total cholesterol (mmol L^{-1})	4.9 ± 0.27	4.9 ± 0.84	0.843
LDL cholesterol (mmol L^{-1})	3.1 ± 0.23	2.8 ± 0.79	0.289
HDL cholesterol (mmol L^{-1})	1.1 ± 0.08	1.7 ± 0.32	<0.0001
Total TG (mmol L^{-1})	2.0 ± 0.43	0.9 ± 0.35	<0.0001
C4 (ng mL^{-1})	28.5 ± 9.4	12.6 ± 9.9	0.002
C4/c (mg mol^{-1})	5.6 ± 1.63	2.6 ± 1.87	0.001
Total BAs (ng mL^{-1})	784 ± 140	457 ± 263	0.002
Insulin (mU L^{-1})	22 ± 9.4	4.2 ± 2.0	<0.0001
Lathosterol (ng mL^{-1})	1615 ± 201	1213 ± 411	0.012
Lathosterol/c (mg mol^{-1})	327 ± 39	248 ± 72	0.007
PCSK9 (ng mL^{-1})	349 ± 26	268 ± 104	0.023
FGF19 (pg mL^{-1})	101 ± 24	120 ± 51	0.320

[a]All women <50 years of age; [b]BMI <25 kg m^{-2}. TG, triglycerides; C4, 7α-hydroxy-4-cholesten-3-one; C4c, C4 corrected for total cholesterol; lathosterol/c, lathosterol corrected for total cholesterol; BA, bile acid; PCSK9, proprotein convertase subtilisin/kexin type 9; FGF19, fibroblast growth factor 19.

written informed consent was obtained from all participants. The study was registered at www. ClinicalTrials.gov (#NCT01842425). A liquid LCD was given (Modifast; Impolin AB, Stockholm, Sweden), and four or five sachets were provided daily, amounting to a total energy content of 800–1100 kcal day^{-1} (52% carbohydrates, 25% protein, 21% fat). Body composition was determined by bioimpedance analysis, and liver volume and intrahepatic fat were determined using magnetic resonance imaging in the mornings of days 0, 3, 7, 14 and 28. Fluid consumption was restricted to a total of 500 mL water during the 12 h before each examination to facilitate comparisons between measurements. Overnight fasting blood was collected at these time-points and isolated serum stored at −80 °C. Fifty-four healthy women, with a BMI <25 kg m^{-2}, from a previously described cohort [4] were included in the study as the control group.

Serum levels of proteins, lipids, lipoproteins, glucose and insulin

Levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose and insulin in obese subjects were measured using standardized routine methods at Uppsala University Hospital; all these variables were measured in the nonobese control subjects at Karolinska University Hospital using routine techniques, except serum insulin which was determined using an ultrasensitive enzyme-linked immunosorbent assay (ELISA; Cat. No. 10-1132-01; Mercodia, Uppsala, Sweden). Serum PCSK9 and FGF19 levels were determined by ELISA (Cat. No. CY-8079; CircuLex, Cyclex Co., Ltd, Nagano, Japan and Cat. No. DF1900; R&D Systems Minneapolis, MN, USA, respectively) according to the manufacturers' instructions.

Serum markers of the synthesis of BAs and total body cholesterol

Serum levels of 7α-hydroxy-4-cholesten-3-one (C4), an established marker of BA synthesis [20–23], were measured as described [22]. Unesterified lathosterol, a serum marker of total body cholesterol synthesis [24], was determined by gas chromatography–mass spectrometry [25]. C4 and lathosterol levels were corrected for total serum and total cholesterol (C4c and lathosterol/c), respectively, as described previously [24, 26]. Serum BAs were determined by liquid chromatography–tandem mass spectrometry using deuterium-labelled BA standards.

Statistical analysis

Unpaired Student's *t*-test was used to assess differences between baseline data from the 10 morbidly obese patients and from the 54 normal healthy women. A *P* value <0.05 was considered statistically significant. Responses after 3 days on the LCD were analysed by paired Student's *t*-test versus baseline values at day 0 using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA). Unless otherwise stated, values are presented as mean ± SD.

Results

Baseline measures in morbidly obese women and nonobese control subjects

Clinical and laboratory data from 10 morbidly obese women and 54 healthy nonobese women (BMI <25 kg m^{-2}) are compared in Table 1. Total and LDL cholesterol levels were similar in both groups, whereas HDL cholesterol was 35% lower in obese (*P* < 0.0001) than in nonobese women, in line with previous findings [7, 11, 27]. Serum total triglyceride levels in obese women were elevated by 122% (*P* < 0.0001) [11, 27, 28]. Next, we evaluated BA synthesis and cholesterol synthesis using the serum markers C4c [22, 26] and lathosterol/c [4, 24], respectively. As expected from previous findings [5–7, 11, 29], BA synthesis (2-fold increase; *P* = 0.001), total cholesterol synthesis (31% increase; *P* = 0.007) and total serum BAs (62% increase; *P* = 0.002) were all higher in obese women [5, 15].

Then, we analysed the circulating levels of PCSK9, a posttranscriptional regulator of LDL receptors produced and secreted by the liver, and regulated by sterol regulatory element-binding protein-2 [17]. PCSK9 expression may increase in situations in which cholesterol is deficient in the liver, such as during statin treatment [17]. In line with previous investigations of this protein and BMI [30, 31], we found that, similar to lathosterol/c, circulating PCSK9 was increased in morbidly obese women by 30% (*P* = 0.023). The finding that both lathosterol/c and PCSK9 were increased supports the view that cholesterol is deficient in the liver in morbid obesity. Serum levels of FGF19, a suppressor of BA synthesis [32], were unaltered (*P* = 0.32). Serum insulin levels were higher in obese than in nonobese women.

Thus, the baseline metabolic profiles of these 10 morbidly obese women were consistent with those

previously reported for this patient population and, in addition, circulating PCSK9 was found to be increased, also consistent with this phenotype.

Responses to caloric restriction in morbidly obese women

To test our hypothesis, we monitored the responses obtained after switching to an LCD. We anticipated that the confirmed phenotype of these morbidly obese women, except for hypertriglyceridaemia, should approximately normalize within a few days on the diet. Thus, our primary focus was to evaluate the changes observed after 3 days on the LCD, the shortest time-point in the study. At this early time-point, body weight was reduced by an average of 1.9 kg, mainly due to drainage of water-rich glycogen from liver and muscle, whereas large peripheral fat depots remained essentially intact [18]. Liver volume was reduced by 10% [18], whilst BA synthesis, doubled in the obese group compared to controls, was reduced by 46% ($P = 0.024$) and thus normalized (Fig. 2a). Total BAs in serum were reduced by 50% ($P = 0.016$), but returned towards initial elevated levels with time (Fig. 2b); in particular, glycine-conjugated forms of BAs returned towards baseline levels after the initial reduction; however, the unconjugated forms of cholic acid, chenodeoxycholic acid and ursodeoxycholic acid remained reduced throughout the dietary period (Fig. 3).

Circulating levels of the BA synthesis suppressor FGF19 were not altered on day 3 ($P = 0.68$) and remained unaltered for the entire dietary period (Fig. 2c), indicating that circulating FGF19 was not likely to be the cause of the decrease in BA synthesis.

In addition, the induced synthesis of cholesterol (lathosterol/c) in the obese women normalized at this time-point ($P = 0.0047$) (Fig. 4a), as did serum PCSK9 levels ($P = 0.0059$) (Fig. 4b). Serum LDL cholesterol increased transiently by 9% ($P = 0.0062$) after 3 days on the diet (Fig. 4c), whereas plasma total triglycerides did not change at this time-point ($P = 0.42$) (Fig. 5a). The initial fasting serum insulin levels, at the higher end of the normal range (22 mU L^{-1}), were reduced by 22% ($P = 0.028$) on day 3 (Fig. 5b), and remained reduced throughout the dietary period, whilst blood glucose was stable (Fig. 5c), as previously reported [18].

Discussion

A long series of previous studies have identified consistent metabolic changes in cholesterol and BA
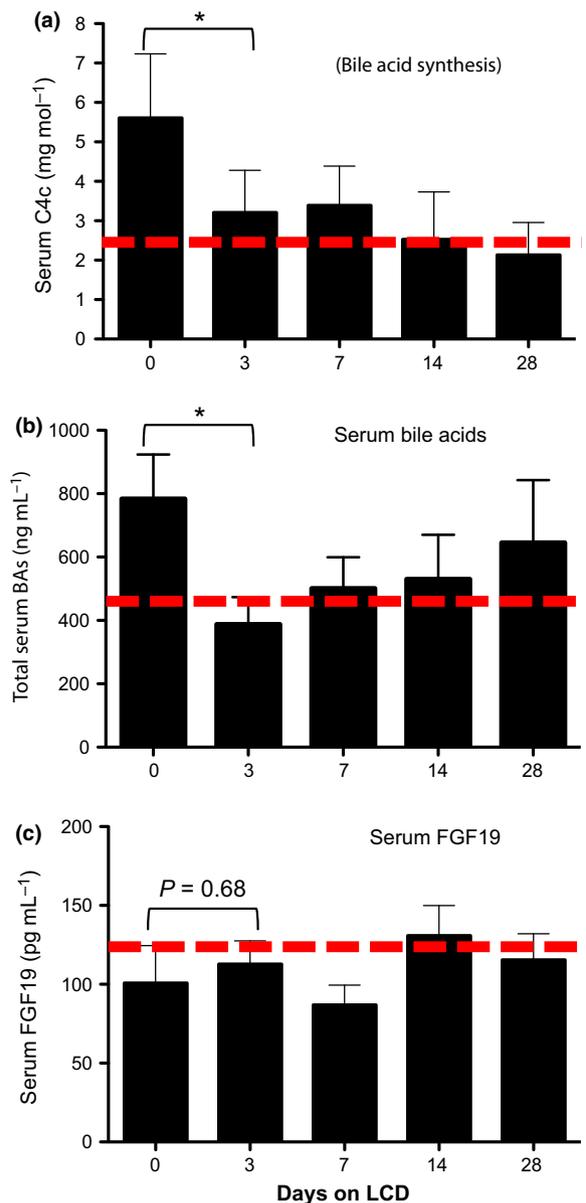

Fig. 2 *Effects of a low-calorie diet (LCD) in 10 morbidly obese women on BA synthesis (C4c) (a), serum total BAs (b) and circulating FGF19 (c). Overnight fasting blood samples were taken on day 0, and then participants were given the LCD for 28 days. Overnight fasting blood samples were drawn on the indicated days. Data show mean±SEM. *P < 0.05. In all panels, dashed horizontal lines indicate mean baseline levels in nonobese women (see Table 1).*

metabolism in obese humans. A key requisite for performing the current investigation was the fact that the synthesis of both BAs and cholesterol is
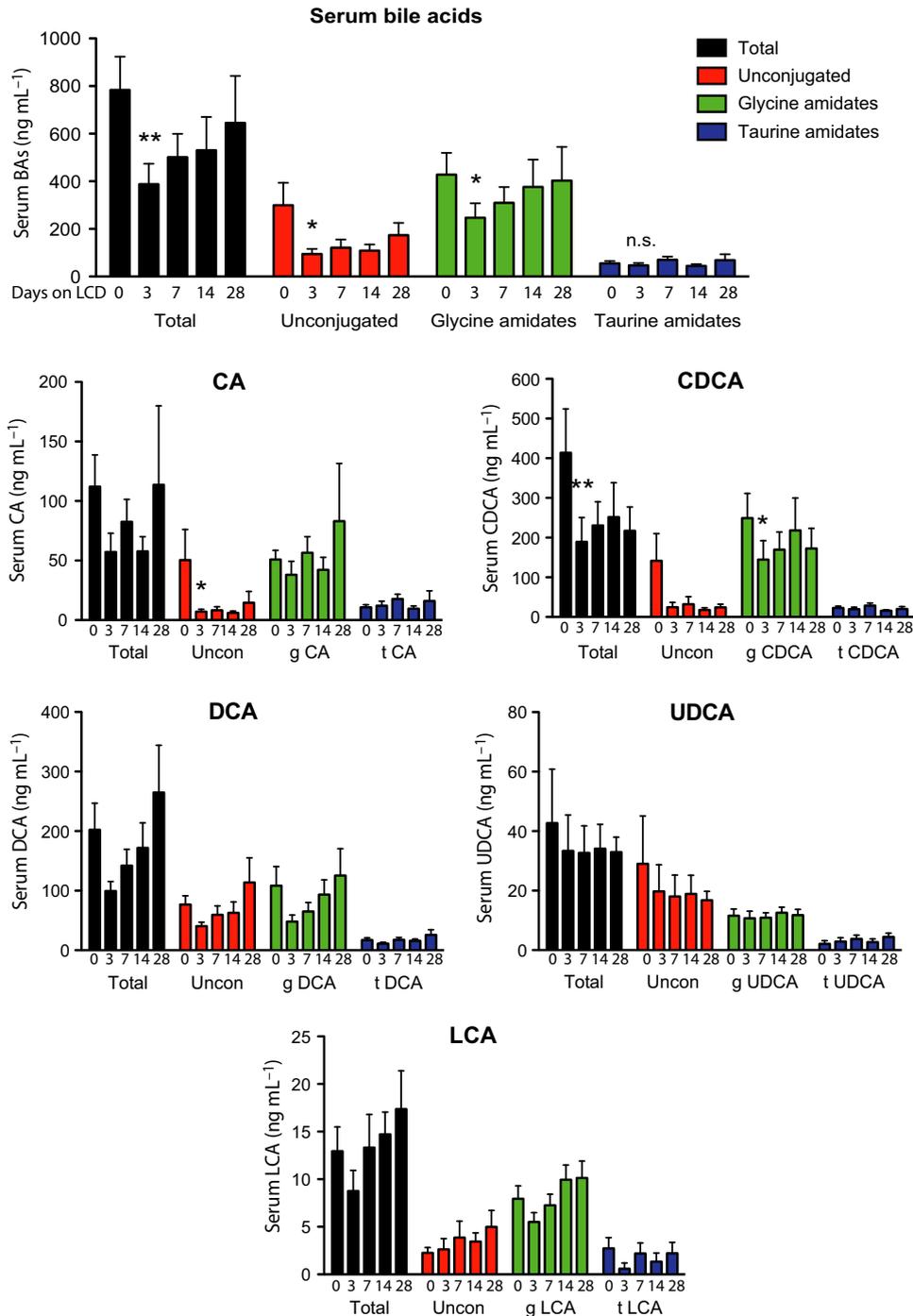
Fig. 3 *Effects of a low-calorie diet (LCD) in 10 morbidly obese women on taurine and glycine-conjugated and unconjugated serum bile acids (BAs). CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; g, glycine; t, taurine. Data show mean±SEM. *P < 0.05; **P < 0.01; n.s. not significant.*
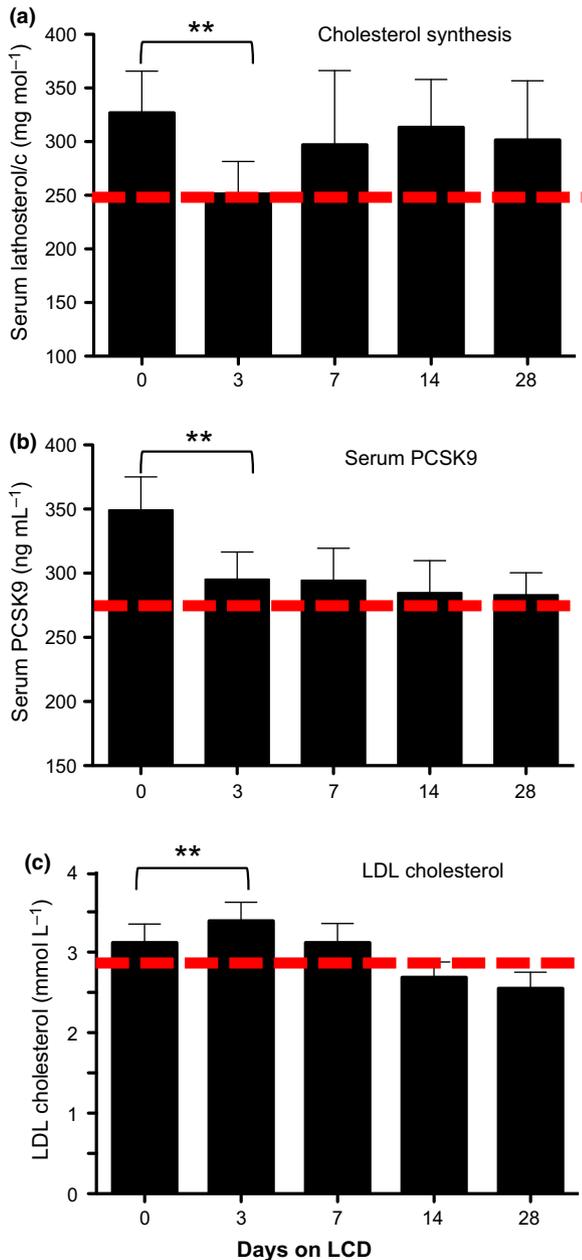
Fig. 4 *Effects of a low-calorie diet (LCD) in 10 morbidly obese women on cholesterol synthesis [lathosterol corrected for total cholesterol (lathosterol/c)] (a), circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) (b) and serum LDL cholesterol (c). Data show mean ± SEM. **P < 0.01. In all panels, dashed horizontal lines indicate mean baseline levels in healthy women (see Table 1).*

Fig. 5 *Effects of a low-calorie diet (LCD) in 10 morbidly obese women on serum total triglycerides (TGs) (a), serum insulin (b) and serum glucose (c). Data show mean ± SEM. *P < 0.05. In panels a and b, dashed horizontal lines indicate the mean baseline levels in healthy women (see Table 1).*

known to be strongly induced in morbid obesity [5–11] and that their faecal excretion is increased in proportion [6–8, 10, 13]. Another precondition was
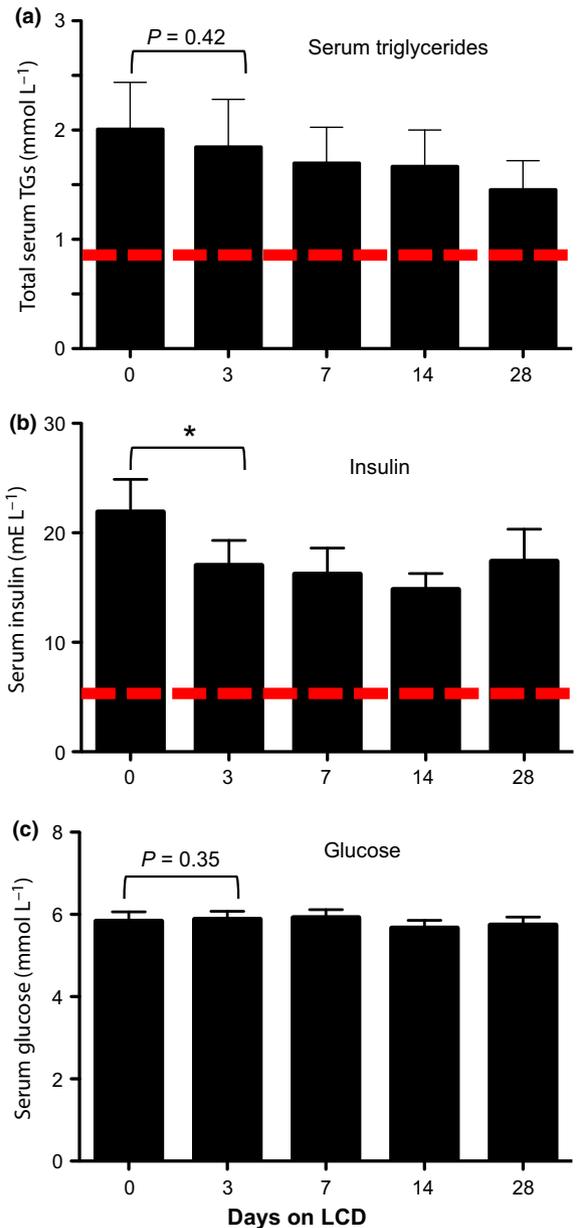
that consistent data have established that caloric restriction or fasting rapidly reduces the faecal loss of BAs in particular and also of cholesterol [13]. In humans, faecal excretion is essentially the only

way to eliminate BAs, and therefore, at steady state, the level of faecal excretion of BAs changes in proportion to BA synthesis and vice versa. Therefore, the fact that we could confirm that synthesis of both BAs and cholesterol was clearly induced at baseline in the 10 morbidly obese participants strongly suggests that their faecal excretion of BAs was induced at baseline.

We hypothesized that the excessive intake of food in morbidly obese subjects increases the intestinal food mass that in turn, due to its increased BA and cholesterol binding capacity, will promote the faecal excretion of BAs and cholesterol. If correct, a prompt and severe reduction in food intake should rapidly normalize the above-described phenotype before any major weight reduction presents (Fig. 1). Accordingly, a series of responses should quickly occur after switching to a liquid LCD: (i) faecal loss of BAs decreases, as has previously been clearly established [13, 14]; (ii) more BAs will thus return to the liver and, through hepatic FXR signalling, BA synthesis should be suppressed, which in turn will increase liver cholesterol. This is supported by the finding of unaltered FGF19 levels in serum, suggesting that mechanisms other than FGF19 pathway are involved in the regulation of BA synthesis in this situation, presumably directly through FXRs within the liver; (iii) the 10% reduction in liver volume [18] will consequently likewise increase hepatic cholesterol and BA concentrations; (iv) the increase in hepatic cholesterol will suppress transcription of LDL receptors, serving to reduce the induced plasma clearance of LDL [7, 12], thereby leading to an increase in plasma LDL cholesterol (Fig. 4c); and (v) also due to the increased liver cholesterol, circulating PCSK9, regulated by steroid regulatory element-binding protein-2, will be reduced thereby serving to increase LDL receptor half-life, which will somewhat dampen the effect of reduced production of LDL receptors. Several of these predicted responses were observed in the current study, and, importantly, they presented as anticipated after 3 days of the LCD, a time-point when body weight loss was merely 1.9 kg and essentially due to the loss of carbohydrates and water [18].

Based on both the current and previous results, we propose that the induced BA synthesis in obese individuals is a response to compensate for hepatic BA deficiency. Further, in the current study, we observed normalization of the induced synthesis of BAs and cholesterol and of the elevated PCSK9

levels in the morbidly obese group after 3 days on the LCD, when BMI and fat depots were essentially unchanged [18]. These findings do not support the view that this phenotype is caused by the overproduction of cholesterol in large peripheral fat tissue depots.

Our hypothesis could explain why this phenotype appears in morbidly obese subjects and is in line with previous studies that consistently show that fasting or caloric restriction reduces the faecal loss of BAs in balance with the reduction in BA synthesis [13, 33]. The strong correlation between cholesterol synthesis and BMI [6, 10] is interesting as BMI is an indicator of the amount of food ingested over time. In addition, synthesis of BAs correlates with BMI [4, 5, 34]. In morbid obesity, the high food intake may indeed create BA and cholesterol deficiencies leading to compensatory induction of their syntheses. These findings are in line with results from studies in which the synthesis of both BAs and cholesterol was determined using different methods [6, 11].

However, the reason why serum BAs decreased after 3 days on the LCD is likely to be related to the reason why serum BAs are increased in morbid obesity [5, 15]. A mechanism that may explain the former is an increased active hepatic uptake of BAs from the blood. It was recently suggested that NTCP-mediated hepatic uptake of conjugated BAs is induced by insulin [5]. Therefore, reduced insulin sensitivity may explain why serum BAs are increased in morbid obesity, as insulin insensitivity is common in such individuals. After 3 days on the LCD, insulin signalling improved as reflected by slightly but significantly reduced serum insulin levels. LCD-induced improvement in insulin signalling may therefore shift BAs from serum to liver to directly suppress BA synthesis. However, considering that the concentration of BAs in human plasma is about 1/60 of that in liver [35], a reduction of total plasma BAs by 400 ng mL^{-1} (from day 0 to day 3, Fig. 2b) should increase hepatic BA levels by about 5% if all these BAs are directed into the liver. We also observed that the reduction in conjugated serum BAs on day 3 was temporary, whereas unconjugated BAs (Fig. 3) as well as serum insulin levels (Fig. 5b) remained reduced throughout the dietary period.

A role of insulin in BA homeostasis was previously suggested from 4 weeks of insulin treatment in

obese Indians with uncontrolled diabetes during an adapted diet to maintain body weight. The BA pool was reduced by 35% and faecal excretion of BAs was reduced by 36%, whilst cholesterol balance revealed that total cholesterol synthesis was reduced by 19%. Interestingly, to maintain body weight constant, caloric intake had to be lowered by as much as 40%, making it impossible in this elegant study to determine whether the insulin treatment or the reduced food intake, or both, was responsible for the reduced faecal excretion of BAs [36].

The described phenotype of morbidly obese individuals may explain why serum cholesterol levels are frequently paradoxically normal in this increasing population of patients [27]. Further, they are at very high risk of developing cholesterol gallstones [37, 38] indicating that BMI may be the strongest of all risk factors for gallstone disease. A similar phenotype with consistent increases in the synthesis of BAs and cholesterol has been reported by several groups for patients with cholesterol gallstones [39–42]. It was concluded that increased faecal loss of BAs through dietary factors may serve as the driving force for this phenotype [39]. It is therefore of interest that this phenotype may actually be a consequence of the level of food intake amongst morbidly obese individuals. It is noteworthy that BMI, which correlates with BA synthesis [4, 5, 34], has been identified as a causal factor for symptomatic gallstone disease in a Mendelian randomization study in which the fat mass and obesity-associated gene (*FTO*) rs9939609 polymorphism was used as marker for BMI [43]. The rs9939609 polymorphism has been shown to be linked to 7–16% increased intake of energy from food without any changes in energy expenditure [44–46]. Considering the robust link between food intake and BMI, it remains to be determined whether the level of food intake was an important cause for why BMI was concluded to be the cause for gallstone disease.

The current study has several limitations. First, there were only 10 obese participants in the study. However, this was compensated for by the fact that individual variations could be minimized through pairwise comparisons. Secondly, it could be considered that faecal excretion of BA and cholesterol should have been measured in the present study. However, as mentioned above, there are extensive and highly consistent data on faecal excretion of BAs

in obesity and following dietary manipulations, which have already outlined certain basal metabolic conditions in obesity [6, 14, 47]. Reconfirmation of such data will not add any further information to determine the extent to which intestinal food contents specifically contribute to induce faecal excretion of BAs and cholesterol in morbid obesity.

Conclusions

In the current study, we found that elevated synthesis of BAs and cholesterol and circulating PCSK9 levels in morbidly obese women all normalize after 3 days on an LCD. These findings, together with previously established metabolic characteristics of morbid obesity, strongly suggest that higher intestinal contents in morbidly obese individuals, due to excessive food intake, are likely to promote faecal loss of BAs and cholesterol. The subsequent shortage of cholesterol and particularly of BAs therefore characterizes a metabolic phenotype amongst morbidly obese individuals that is quickly and largely normalized when the excessive food intake is rapidly reduced.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Authors' contributions

SS performed the analyses, collected and interpreted the data and wrote the manuscript; MR outlined the hypothesis, interpreted the data and wrote the manuscript; UR, FR and DE revised the manuscript; HA and JK edited the manuscript; MS was responsible for the LCD study, recruited patients and revised the manuscript.

References

1 Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 2011; **34:** 1249–57.

2 Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; **72:** 137–74.

3 Kir S, Kliewer SA, Mangelsdorf DJ. Roles of FGF19 in liver metabolism. *Cold Spring Harb Symp Quant Biol* 2011; **76:** 139–44.

4 Galman C, Angelin B, Rudling M. Pronounced variation in bile acid synthesis in humans is related to gender, hypertriglyceridaemia and circulating levels of fibroblast growth factor 19. *J Intern Med* 2011; **270:** 580–8.

5 Haeusler RA, Camastra S, Nannipieri M *et al.* Increased bile acid synthesis and impaired bile acid transport in human obesity. *J Clin Endocrinol Metab* 2016; **101:** 1335–44.

6 Miettinen TA. Cholesterol production in obesity. *Circulation* 1971; **44:** 842–50.

7 Kesaniemi YA, Grundy SM. Increased low density lipoprotein production associated with obesity. *Arteriosclerosis* 1983; **3:** 170–7.

8 Miettinen TA, Gylling H. Cholesterol absorption efficiency and sterol metabolism in obesity. *Atherosclerosis* 2000; **153:** 241–8.

9 Renner O, Harsch S, Matysik S, Lutjohann D, Schmitz G, Stange EF. Upregulation of hepatic bile acid synthesis via fibroblast growth factor 19 is defective in gallstone disease but functional in overweight individuals. *United European Gastroenterol J* 2014; **2:** 216–25.

10 Simonen PP, Gylling H, Miettinen TA. Body weight modulates cholesterol metabolism in non-insulin dependent type 2 diabetics. *Obes Res* 2002; **10:** 328–35.

11 Stahlberg D, Rudling M, Angelin B *et al.* Hepatic cholesterol metabolism in human obesity. *Hepatology* 1997; **25:** 1447–50.

12 Egusa G, Beltz WF, Grundy SM, Howard BV. Influence of obesity on the metabolism of apolipoprotein B in humans. *J Clin Invest* 1985; **76:** 596–603.

13 Kudchodkar BJ, Sodhi HS, Mason DT, Borhani NO. Effects of acute caloric restriction on cholesterol metabolism in man. *Am J Clin Nutr* 1977; **30:** 1135–46.

14 Bennion LJ, Grundy SM. Effects of obesity and caloric intake on biliary lipid metabolism in man. *J Clin Invest* 1975; **56:** 996–1011.

15 Cariou B, Chetiveaux M, Zair Y *et al.* Fasting plasma chenodeoxycholic acid and cholic acid concentrations are inversely correlated with insulin sensitivity in adults. *Nutr Metab (Lond)* 2011; **8:** 48.

16 Hua X, Yokoyama C, Wu J *et al.* SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. *Proc Natl Acad Sci U S A* 1993; **90:** 11603–7.

17 Lambert G, Sjouke B, Choque B, Kastelein JJ, Hovingh GK. The PCSK9 decade. *J Lipid Res* 2012; **53:** 2515–24.

18 Edholm D, Kullberg J, Karlsson FA, Haenni A, Ahlstrom H, Sundbom M. Changes in liver volume and body composition during 4 weeks of low calorie diet before laparoscopic gastric bypass. *Surg Obes Relat Dis* 2015; **11:** 602–6.

19 Chaddock G, Lam C, Hoad CL *et al.* Novel MRI tests of orocecal transit time and whole gut transit time: studies in normal subjects. *Neurogastroenterol Motil* 2014; **26:** 205–14.

20 Axelson M, Aly A, Sjovall J. Levels of 7 alpha-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. *FEBS Lett* 1988; **239:** 324–8.

21 Axelson M, Bjorkhem I, Reihner E, Einarsson K. The plasma level of 7 alpha-hydroxy-4-cholesten-3-one reflects the activity of hepatic cholesterol 7 alpha-hydroxylase in man. *FEBS Lett* 1991; **284:** 216–8.

22 Galman C, Arvidsson I, Angelin B, Rudling M. Monitoring hepatic cholesterol 7{alpha}-hydroxylase activity by assay of the stable bile acid intermediate 7{alpha}-hydroxy-4-cholesten-3-one in peripheral blood. *J Lipid Res* 2003; **44:** 859–66.

23 Sauter G, Berr F, Beuers U, Fischer S, Paumgartner G. Serum concentrations of 7alpha-hydroxy-4-cholesten-3-one reflect bile acid synthesis in humans. *Hepatology* 1996; **24:** 123–6.

24 Kempen HJ, Glatz JF, Gevers Leuven JA, van der Voort HA, Katan MB. Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J Lipid Res* 1988; **29:** 1149–55.

25 Lund E, Sisfontes L, Reihner E, Bjorkhem I. Determination of serum levels of unesterified lathosterol by isotope dilution-mass spectrometry. *Scand J Clin Lab Invest* 1989; **49:** 165–71.

26 Galman C, Angelin B, Rudling M. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. *Gastroenterology* 2005; **129:** 1445–53.

27 Vierhapper H, Nardi A, Grosser P. Prevalence of paradoxically normal serum cholesterol in morbidly obese women. *Metabolism* 2000; **49:** 607–10.

28 Hunt SC, Williams RR, Adams TD. Biochemical and anthropometric characterization of morbid obesity in a large Utah pedigree. *Obes Res* 1995; **3(Suppl 2):** 165S–72S.

29 Angelin B, Backman L, Einarsson K, Eriksson L, Ewerth S. Hepatic cholesterol metabolism in obesity: activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J Lipid Res* 1982; **23:** 770–3.

30 Chan DC, Pang J, McQuillan BM *et al.* Plasma proprotein convertase Subtilisin/Kexin Type 9 as a predictor of carotid atherosclerosis in asymptomatic adults. *Heart Lung Circ* 2016; **25:** 520–5.

31 Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. *J Clin Endocrinol Metab* 2009; **94:** 2537–43.

32 Potthoff MJ, Kliewer SA, Mangelsdorf DJ. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. *Genes Dev* 2012; **4:** 312–24.

33 Duane WC, Ginsberg RL, Bennion LJ. Effects of fasting on bile acid metabolism and biliary lipid composition in man. *J Lipid Res* 1976; **17:** 211–9.

34 Steiner C, Othman A, Saely CH *et al.* Bile acid metabolites in serum: intraindividual variation and associations with coronary heart disease, metabolic syndrome and diabetes mellitus. *PLoS ONE* 2011; **6:** e25006.

35 Setchell KD, Rodrigues CM, Clerici C *et al.* Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. *Gastroenterology* 1997; **112:** 226–35.

36 Bennion LJ, Grundy SM. Effects of diabetes mellitus on cholesterol metabolism in man. *N Engl J Med* 1977; **296:** 1365–71.

37 Amaral JF, Thompson WR. Gallbladder disease in the morbidly obese. *Am J Surg* 1985; **149:** 551–7.

38 Maclure KM, Hayes KC, Colditz GA, Stampfer MJ, Speizer FE, Willett WC. Weight, diet, and the risk of symptomatic gallstones in middle-aged women. *N Engl J Med* 1989; **321:** 563–9.

39 Galman C, Miquel JF, Perez RM *et al.* Bile acid synthesis is increased in Chilean Hispanics with gallstones and in gallstone high-risk Mapuche Indians. *Gastroenterology* 2004; **126:** 741–8.

40 Muhrbeck O, Wang FH, Bjorkhem I, Axelson M, Einarsson K. Circulating markers for biosynthesis of cholesterol and bile acids are not depressed in asymptomatic gallstone subjects. *J Hepatol* 1997; **27:** 150–5.

41 Sauter GH, Moussavian AC, Meyer G, Steitz HO, Parhofer KG, Jungst D. Bowel habits and bile acid malabsorption in the months after cholecystectomy. *Am J Gastroenterol* 2002; **97:** 1732–5.

42 Sauter GH, Munzing W, von Ritter C, Paumgartner G. Bile acid malabsorption as a cause of chronic diarrhea: diagnostic value of 7alpha-hydroxy-4-cholesten-3-one in serum. *Dig Dis Sci* 1999; **44:** 14–9.

43 Stender S, Nordestgaard BG, Tybjaerg-Hansen A. Elevated body mass index as a causal risk factor for symptomatic gallstone disease: a Mendelian randomization study. *Hepatology* 2013; **58:** 2133–41.

44 Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med* 2008; **359:** 2558–66.

45 Haupt A, Thamer C, Staiger H *et al.* Variation in the FTO gene influences food intake but not energy expenditure. *Exp Clin Endocrinol Diabetes* 2009; **117:** 194–7.

46 Speakman JR, Rance KA, Johnstone AM. Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. *Obesity (Silver Spring)* 2008; **16:** 1961–5.

47 Nestel PJ, Monger EA. Turnover of plasma esterified cholesterol in normocholesterolemic and hypercholesterolemic subjects and its relation to body build. *J Clin Invest* 1967; **46:** 967–74.

Correspondence: Mats Rudling, Karolinska, University Hospital at Huddinge, C2-94, S-14186, Stockholm, Sweden.
(fax: +46 8 7110710; e-mail: mats.rudling@ki.se).