Hepatic esterification rate of cholesterol and biliary lipids in human obesity

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Abstract Obesity is often associated with an increased hepatic secretion rate of cholesterol and saturated gallbladder bile. In order to evaluate the role of hepatic esterification of cholesterol in this phenomenon, we assayed the activity of acyl CoA:cholesterol acyl transferase (ACAT), which catalyzes the esterification of cholesterol, in liver microsomes obtained from 19 morbidly obese patients without gallstones undergoing vertical banded gastroplasty. Gallbladder bile was obtained and analyzed for lipid composition, cholesterol saturation, nucleation time, and occurrence of cholesterol crystals. Fourteen non-obese gallstone-free subjects undergoing cholecystectomy because of suspected polyp or adenomyoma in the gallbladder served as controls. The hepatic content of esterified cholesterol was increased by about 70% in the obese patients ($P < 0.05$). Still, the mean levels of the ACAT activity were equal in the obese and non-obese patient groups (11 ± 1 and 11 ± 2 pmol/min per mg protein, respectively). When exogenous cholesterol was added to the assay system, the activity was increased markedly in both groups. The ACAT activity was higher in obese patients with steatosis of the liver compared with those displaying normal liver morphology (12 ± 1 vs 8 ± 1 pmol/min per mg, $P < 0.05$). Obese patients did not have significantly more saturated gallbladder bile than the non-obese controls (84 ± 7 and 77 ± 8%, respectively). They had a normal nucleation time and their gallbladder bile did not contain any cholesterol crystals. We conclude that obese patients without gallstones usually have a normal esterification rate of cholesterol in the liver. Steatosis of the liver was associated with increased ACAT activity. Only few of the obese patients had saturated gallbladder bile. There was no correlation between saturation of bile and ACAT activity, indicating that the rate of esterification of cholesterol in the liver is of little regulatory importance for the cholesterol saturation of bile in humans without gallstones.—**Sahlin, S., L. Granström, U. Gustafsson, D. Ståhlberg, L. Backman, and K. Einarsson.** Hepatic esterification rate of cholesterol and biliary lipids in human obesity. *J. Lipid Res.* 1994. **35:** 484–490.

Supplementary key words ACAT • cholesterol saturation • cholesterol crystals • nucleation time

Obesity is a known risk factor for cholesterol gallstone disease. Cholesterol-saturated gallbladder bile is considered to be a prerequisite—but not the only factor of importance—for cholesterol crystal and gallstone formation in humans (1–3). Saturated bile, which contains more cholesterol than can be kept in solution by the solubilizing lipids, bile acids, and phospholipids, may be the consequence of disturbances of cholesterol, bile acid, or phospholipid metabolism or a combination of defects (1–3). According to several studies, hypersecretion of cholesterol from the liver is the most common cause of saturated bile in humans, the secretion rates of bile acids and phospholipids being normal (3, 4). Thus, hypersecretion of cholesterol is considered to be the main cause of saturated gallbladder in obese subjects (5–7).

Hypersecretion of cholesterol may be associated with and be due to different disturbances of hepatic cholesterol metabolism, e.g., increased inflow of lipoprotein cholesterol to the liver, increased hepatic synthesis of cholesterol, decreased catabolism of cholesterol to bile acids, and decreased esterification rate of cholesterol in the liver (4). An enhanced production of cholesterol in obesity has been established by several in vivo studies (8–10). In a previous in vitro study we found that the liver is a major contributor to the increased cholesterol production seen in obesity (11). Bile acid pool sizes and formation are either normal or enhanced in obesity (5–7, 10).

In the present work the hepatic acyl CoA:cholesterol acyl transferase (ACAT) activity, which catalyzes the esterification of cholesterol (12), was assayed in obese subjects and compared with that of non-obese controls. Also, the concentrations of free esterified cholesterol in liver homogenates and microsomes were determined. In addition, biliary lipid composition, nucleation time, and occurrence of cholesterol crystals in gallbladder bile were analyzed.

Abbreviations: ACAT, acyl CoA:cholesterol acyltransferase; VBG, vertical banded gastroplasty.

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MATERIALS AND METHODS

Patients

Altogether, 19 morbidly obese patients (16 females and 3 males) were studied. They all had relative body weight exceeding 150% and they all had been admitted for vertical banded gastroplasty (VBG) for obesity. Basal data on the subjects are given individually in **Table 1.** They all had constant body weight during the month preceding admittance, and none were on any specific dietary treatment. No clinical or laboratory evidence of intestinal, kidney, or thyroid disease, or addiction to alcohol or narcotics was present. Three of the obese patients displayed slight hyperlipoproteinemia and four had slightly elevated aminotransferase levels, whereas other liver function tests were within the normal limits. None of the obese patients had gallstone disease as judged by preoperative ultrasound examination and preoperative palpation of the gallbladder after it was emptied by needle aspiration. Operative liver biopsies were obtained from 17 obese patients. Fourteen normal-weight, gallstone-free subjects undergoing cholecystectomy served as controls. Cholecystectomy was performed due to preoperative suspicion of polyps or adenomyomatosis in the gallbladder and biliary pain.

Informed consent was obtained from each patient before operation. The ethical aspects of the study were approved by the Ethical Committee at Karolinska Institutet, Stockholm.

Experimental procedure

All patients were hospitalized in the surgical ward where laboratory tests and a clinical examination were performed. They were fed the regular hospital diet. The amount of calories was chosen to keep body weight constant. All operations were performed between 8 and 10 AM after an overnight fast. After opening the abdomen, the gallbladder was completely emptied of bile with a sterile needle and syringe to avoid possible stratification of bile (13). The obese patients had their gallbladders carefully examined to exclude gallstones. A biopsy specimen weighing 1–4 g was taken from the liver. A small portion of the tissue sample was sent for histological examination, and the remaining portion was immediately put into ice-cold homogenizing buffer and rapidly transported to the

TABLE 1. Clinical data and ACAT activities in obese patients and normal weight controls

Patients[a]	Age	Weight	BMI[b]	Plasma Cholesterol	Plasma Triglycerides	ACAT Activity	ACAT Activity + Exogenous Cholesterol
	yr	*kg*	*kg/m²*	*mmol/l*	*mmol/l*	*pmol/min/mg protein*	
Obese							
1.F	28	115	37	5.2	0.8		
2.F	37	96	39	5.9	2.0	4	8
3.F	43	121	41	5.9	1.1	9	18
4.F	30	114	40	4.7	1.6	7	47
5.F	32	105	40	4.2	0.7	7	59
6.F	38	108	38	5.7	2.8	9	35
7.F	37	136	51	5.0	1.3	8	58
8.F	53	114	46	6.2	1.7	21	42
9.F	45	105	42	4.5	1.5	19	40
10.F	34	106	41	5.7	0.8	8	38
11.F	27	110	42	4.2	1.1	9	21
12.F	34	108	37	6.0	1.1	11	128
13.F	47	132	49	4.4	1.5	10	26
14.F	45	111	40	5.4	2.5		
15.F	31	126	40	4.0	1.1	13	99
16.F	38	132	43	3.7	1.1	10	31
17.M	46	123	38	7.3	2.4	14	48
18.M	37	152	43	4.8	1.7	11	23
19.M	33	144	48	5.0	2.4	15	79
Total (16F, 3M)	38 ± 2	119 ± 3[d]	42 ± 1[d]	5.1 ± 0.2	1.5 ± 0.1[c]	11 ± 1	47 ± 7[f]
Non-obese (11F, 3M)	46 ± 3	67 ± 3	23 ± 1	5.8 ± 0.3	1.0 ± 0.1	11 ± 2 (4–24)	57 ± 25[e] (6–299)

Individual data and mean ± SEM (range).
[a] F, female; M, male.
[b] Body Mass Index calculated as body weight in kg/height in m².
[c] Significantly different from non-obese patients, $P < 0.05$.
[d] Significantly different from non-obese patients, $P < 0.0001$.
[e] Significantly different from ACAT activity, $P < 0.005$.
[f] Significantly different from ACAT activity, $P < 0.0005$.

laboratory where the preparation of microsomes was started. A VBG was performed in the obese patients and a regular cholecystectomy in the non-obese subjects. The suspected polyps appeared to be foldings of epithelium and the adenomyomas were confirmed.

Materials

[1-^{14}C]oleoyl coenzyme A (sp act 57.8 mCi/mmol) and [1,2,6,7-^{3}H]cholesteryl oleate (sp act 82.7 Ci/mmol) were obtained from New England Nuclear Corp., Boston MA. Deuterium-labeled cholesterol was obtained from Larodan Fine Chemicals, Malmö, Sweden. Cholesteryl oleate, cholesterol, EDTA, Triton WR-1339, and human serum albumin were purchased from Sigma Chemicals Co., St. Louis, MO. Cholesterol oxidase (Nyco-test cholesterol) and 3α-hydroxysteroid dehydrogenase (Sterognost) were purchased from Nyegaard A/S Oslo, Norway.

Determination of plasma lipids

Plasma cholesterol and triglycerides were analyzed by automated enzymatic techniques (Boehringer Mannheim Test Combination Cholesterol and Triglycerides).

Preparation of microsomes

An aliquot of the liver biopsy was minced and homogenized with a loose-fitting Teflon pestle in 9 volumes of 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose and 1 mM EDTA. The homogenate was centrifuged at 20,000 *g* for 15 min. The supernatant solution was centrifuged at 100,000 *g* for 60 min. The pellet obtained was resuspended in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA and used for assay of the ACAT activity and determination of cholesterol. The protein concentrations of the microsomes and the liver homogenates were determined by the method of Lowry et al. (14).

Assay of ACAT activity

The ACAT activity was assayed both in the absence and the presence of exogenous cholesterol (15). The assay system contained 0.1 ml of the microsomal preparation and 1 mg of fatty acid-free bovine serum albumin in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA to give a final volume of 1.0 ml. The mixture was preincubated as such for 5 min at 37°C or for 20 min at 37°C after the addition of 50 nmol unlabeled cholesterol dissolved in 600 μg of Triton. The reaction was initiated by the addition of 25 nmol (1.45 μCi) of [1-^{14}C]oleoyl coenzyme A. After 6 min the assay was stopped by the addition of 10 ml chloroform–methanol 2:1 (v/v). Tritium-labeled cholesteryl oleate (0.01 μCi) was added as an internal standard to estimate recovery followed by 1 ml of saline. The chloroform phase was collected and evaporated to dryness under N_2. The residue was resuspended in chloroform–methanol 2:1 (v/v) and subjected to thin-layer chromatography together with unlabeled cholesteryl oleate as a marker. The chromatogram was developed in hexane–ethyl acetate 95:5 (v/v). The cholesteryl oleate zone was visualized with iodine vapor and scraped off into a counting vial and analyzed for radioactivity.

Determination of liver cholesterol

The concentrations of total cholesterol in the liver homogenates and the microsomal fractions were determined by isotope dilution–mass spectrometry after addition of deuterium-labeled cholesterol as an internal standard as described previously (16, 17). Free cholesterol was determined by the same method but the hydrolysis step was omitted. The concentration of esterified cholesterol was calculated as the difference between the total and the free cholesterol in the same sample.

Analysis of biliary lipids and calculation of cholesterol saturation

Gallbladder bile was extracted with chloroform–methanol 2:1 (v/v), and the chloroform phase was analyzed for cholesterol (18) and phospholipids (19). Total bile acid concentration was determined by an enzymatic method (20) in another portion of bile. The relative concentrations of cholesterol, bile acids, and phospholipids were expressed as molar percentage of total biliary lipids. The cholesterol saturation of bile was calculated according to Carey (21) and expressed as percentage.

Analysis of cholesterol crystals and nucleation time

Bile samples were examined for typical rhomboid monohydrate cholesterol crystals by polarizing light microscopy on prewarmed slides. Nucleation time was determined by the method of Holan et al. (22) with minor modifications (23).

Statistical analysis

Data are presented as means ± SEM (standard error of the mean). Significance of differences were evaluated with Student's *t*-test and the Mann-Whitney U-test. Correlations were evaluated by linear regression and calculation of the correlation coefficient, *r*.

RESULTS

Light microscopy

All liver biopsies and the gallbladders from the gallstone-free patients were routinely examined by a pathologist not involved in the study. In the obese group, 12 of the 17 liver biopsies examined showed evidence of light to considerable fat infiltration or steatosis.

In the gallstone-free cases, some of the suspected polyps were foldings of the epithelium, others were focal cholesterolosis. No genuine neoplasm was found. In the case of adenomyomatosis, they were confirmed at operation.

ACAT activity

The ACAT activities are shown in Table 1. A wide range of enzyme activities was obtained in both the obese and non-obese groups, the mean values averaging 11 pmol/min per mg protein. When exogenous cholesterol was added to the assay system, there was a marked increase of the ACAT activity. There was no significant difference in ACAT activity between obese and non-obese subjects.

Obese patients with steatosis of the liver (n = 12) had significantly higher ACAT activity compared with those displaying normal liver morphology (n = 5), 12 ± 1 versus 8 ± 1 pmol/min per mg protein, $P < 0.05$. After addition of exogenous cholesterol to the microsomes, there was no significant difference between obese subjects with and without steatosis (54 ± 10 vs. 32 ± 9 pmol/min per mg protein).

Hepatic cholesterol content

The hepatic content of free cholesterol was similar in obese and non-obese subjects (**Table 2**). The obese group had 65–70% higher content of esterified cholesterol compared with the non-obese ($P < 0.05$). There was no significant difference in microsomal content of free and esterified cholesterol between obese and non-obese subjects.

Obese patients with steatosis had a significantly higher proportion of esterified cholesterol in the liver compared with those without steatosis (32 ± 3% vs. 16 ± 5%, $P < 0.01$).

There were no significant correlations between ACAT activity and microsomal free cholesterol in either the obese patients ($r = +0.37$) or in the non-obese controls ($r = +0.01$).

Lipid composition, cholesterol saturation, nucleation time, and occurrence of cholesterol crystals in gallbladder bile

In obese patients, the gallbladder bile contained less total lipids than in non-obese patients (**Table 3**). There was a significantly higher molar percentage of phospholipids and a correspondingly lower percentage of bile acids in the obese subjects as compared to the non-obese subjects. The cholesterol saturation of gallbladder bile tended to be higher in the obese group compared with the non-obese group but the difference did not reach statistical significance. In fact, cholesterol saturation exceeded 100% in only 5 out of 19 obese patients investigated.

Obese patients with saturated bile had ACAT activities similar to those of patients with unsaturated bile (**Table 4**). There was no significant correlations between cholesterol saturation and ACAT activity assayed with and without exogenous cholesterol in either the obese patients ($r = -0.04$ and $r = 0.31$) or in the non-obese controls ($r = -0.09$ and $r = -0.15$).

Hepatic content of free and esterified cholesterol did not differ between obese patients with saturated and unsaturated bile (Table 4). There were no significant correlations between cholesterol saturation and microsomal free cholesterol in the obese patients ($r = -0.14$) or in the non-obese controls ($r = -0.40$).

The nucleation time as determined in 8 obese patients was not significantly different from that of non-obese subjects (Table 3). None of the obese patients displayed cholesterol crystals in their gallbladder bile.

DISCUSSION

This is the first report on hepatic esterification of cholesterol in human obesity. Morbidly obese patients undergoing VBG were compared with normal weight subjects undergoing cholecystectomy. Both groups of patients were gallstone-free. The activity of ACAT—catalyzing the esterification of cholesterol—was assayed both in the absence and presence of exogenous cholesterol. The mean levels of ACAT activity were about the same in the obese and non-obese groups of patients. Addition of exogenous cholesterol increased the ACAT activity markedly in both groups.

TABLE 2. Cholesterol content of liver homogenates and liver microsomes obtained from obese patients and non-obese controls

	Liver Homogenate		Liver Microsomes	
Patients	Free Cholesterol	Esterified Cholesterol	Free Cholesterol	Esterified Cholesterol
	nmol/mg protein		*nmol/mg protein*	
Obese patients (n = 19)	41.7 ± 3.5	16.5 ± 2.2[a]	58.9 ± 3.4	14.8 ± 3.6
Non-obese subjects (n = 14)	42.4 ± 3.4	9.9 ± 2.1	54.7 ± 2.8	8.6 ± 1.1

Values given as mean ± SEM.
[a]Significantly different from non-obese patients, $P < 0.05$.

TABLE 3. Bile lipid composition, cholesterol saturation, and nucleation time in gallbladder bile of obese patients and non-obese controls

Patients	Cholesterol	Bile Acids	Phospholipids	Total Lipids	Cholesterol Saturation	Nucleation Time
		molar %		*g/dl*	*%*	*days*
1.F	6.5	70.7	22.8	9.3	90	7
2.F	5.6	85.3	9.1	9.4	143	11
3.F	7.7	64.4	27.8	13.4	92	35
4.F	7.6	60.8	31.7	15.5	85	-
5.F	9.1	61.7	29.1	12.7	108	10
6.F	5.8	63.1	31.1	4.7	80	5
7.F	11.0	60.4	28.6	10.3	134	12
8.F	8.9	52.0	39.1	9.0	111	-
9.F	4.3	67.6	28.2	6.2	58	-
10.F	5.8	58.5	35.7	12.8	67	-
11.F	4.3	67.7	28.0	13.8	52	-
12.F	4.7	69.9	25.4	6.6	66	-
13.F	5.6	62.3	32.1	5.1	76	-
14.F	9.8	57.5	32.8	5.6	130	-
15.F	6.7	64.3	29.1	7.1	87	-
16.F	3.9	67.7	28.4	14.2	46	6
17.M	3.3	67.9	28.8	15.1	38	-
18.M	3.4	67.9	28.8	3.8	50	-
19.M	5.1	71.9	22.9	6.5	76	17
Total Obese patients (n = 19)	6.3 ± 0.5	65.3 ± 1.6[a]	28.4 ± 1.4[a]	9.5 ± 0.9[a]	84 ± 7	13 ± 3
Non-obese subjects (n = 14)	5.8 ± 0.6	70.2 ± 1.2	24.0 ± 1.2	13.3 ± 1.2	77 ± 8	19 ± 3

Individual data and mean ± SEM.
[a]Significantly different from non-obese patients, $P < 0.05$.

In spite of normal ACAT activity, the hepatic content of esterified cholesterol was about 70% higher in the obese patients compared with the non-obese. The reason for that is not quite apparent. According to current concepts, the proportion of hepatic cholesterol in free or ester forms is governed not only by esterification by the ACAT enzyme but also in part by cholesteryl ester hydrolysis by neutral cholesteryl ester hydrolase (24). To our knowledge, no data on human hepatic cholesterol hydrolase are available. However, hypothetically, the increased cholesteryl ester content in obesity might be explained by a reduced activity of the hepatic cholesterol hydrolase.

An interesting finding was that obese patients with steatosis of the liver had about 50% higher ACAT activity compared with those displaying normal liver morphology. A similar observation has previously been reported by Erickson and Cooper (25). These authors assayed ACAT activity in liver biopsies obtained from five patients with Hodgkin's disease. Liver samples from two of the patients contained histologically appreciable amounts of fat. Interestingly, the ACAT activities of those two livers were considerably higher than those displaying normal histology.

Unexpectedly, we did not find any significant difference in cholesterol saturation of gallbladder bile between obese

TABLE 4. ACAT activities and cholesterol content of liver homogenates and liver microsomes obtained from obese patients with saturated and unsaturated bile

Patients	ACAT Activity	ACAT Activity + Exogenous Cholesterol	Liver Homogenate		Liver Microsomes	
			Free Cholesterol	Esterified Cholesterol	Free Cholesterol	Esterified Cholesterol
	pmol/min/mg protein		*nmol/mg protein*		*nmol/mg protein*	
Patients with saturated bile (n = 5)	9.8 ± 3.7	41.9 ± 11.2	45.6 ± 5.0	13.7 ± 4.2	56.7 ± 6.0	23.7 ± 13.7
Patients with unsaturated bile (n = 14)	11.2 ± 0.9	48.7 ± 9.2	40.3 ± 4.4	17.5 ± 2.6	59.7 ± 4.2	11.6 ± 1.0

Values given as mean ± SEM.

and non-obese patients. In fact only 30% of the obese subjects had saturated bile. None displayed cholesterol crystals in the gallbladder bile and the nucleation time was normal in all patients investigated, even in those with saturated bile. According to several previous studies, obese subjects usually have saturated gallbladder bile and are at a high risk of gallstone formation (26). One factor of importance to explain our results might be that only gallstone-free subjects were selected. Another contributing factor could be that the obese patients in the present study were comparatively young. Also, Whiting and Watts (27) have previously reported on unsaturated bile in young gallstone-free, obese subjects (mean 30 years). Thus, morbid obesity is not always associated with saturated gallbladder bile. Furthermore, in obese subjects with saturated bile, but without gallstones, the nucleation time was normal and the bile was free of cholesterol crystals and stones. This finding also underlines that factors in bile other than cholesterol saturation are necessary for cholesterol precipitation (1–3).

In this study, no correlation between cholesterol saturation of gallbladder bile and ACAT activity in non-obese or obese subjects was found. In the rat, biliary cholesterol output is correlated to hepatic ACAT activity in a reciprocal manner (28). Smith et al. (29) have recently reported on decreased hepatic ACAT activity in patients with cholesterol gallstones compared with gallstone-free controls. However, in a recent study we did not find any difference in hepatic activity between patients with cholesterol gallstones and gallstone-free controls, although the cholesterol saturation of bile was significantly higher in the gallstone patients compared with the gallstone-free (30). Neither was the hepatic ACAT activity affected in gallstone patients treated with chenodeoxycholic acid or ursodeoxycholic acid, making bile unsaturated with cholesterol (31). Thus our previous and present data do not give evidence of any major role for ACAT in the cholesterol saturation of bile in humans.

In conclusion, this study has shown that gallstone-free subjects with morbid obesity have normal hepatic ACAT activity. Steatosis of the liver is associated with an increased level of ACAT activity. Obese subjects may have unsaturated gallbladder bile and normal nucleation time. Cholesterol saturation in the bile of subjects without gallstones is not influenced by hepatic ACAT activity to any major degree.

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