Hepatic cholesterol metabolism in obesity: activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase

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Abstract

Obesity is often associated with an elevated total body cholesterol synthesis. In order to evaluate the role of hepatic cholesterogenesis in this phenomenon, we assayed the rate-limiting step in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the microsomal fraction of liver biopsies obtained operatively from ten morbidly obese (relative body weight > 155%) subjects. Eighteen normal-weight patients (relative body weight < 120%) with cholelithiasis served as controls. Hepatic HMG-CoA reductase activity, expressed as pmol·min⁻¹·mg protein⁻¹, was 60% higher in the obese subjects compared to the gallstone patients (P < 0.05). Microsomal protein concentration was lower in the obese patients, so that enzyme activity calculated per gram liver was not significantly different between the two groups. However, mevalonate formation, expressed in terms of total organ activity, was higher in the obese than in the nonobese group. The results suggest that the liver is a major contributor to the increased cholesterol production seen in obesity. — Angelin, B., L. Backman, K. Einarsson, L. Eriksson, and S. Ewerth. Hepatic cholesterol metabolism in obesity: activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Lipid Res. 1982. 23: 770-773.

Supplementary key words cholesterol synthesis • liver biopsy

An enhanced production of cholesterol in obesity has been established by several in vivo studies (1–3). The relative contribution of different tissues to this increase in cholesterol synthesis has not been determined. Although most organs have the capability to synthesize cholesterol, the predominant sites of cholesterogenesis are the liver and the intestine (4, 5).

The present study was undertaken to test the hypothesis that hepatic cholesterol synthesis is increased in obesity. The activity of the rate-limiting enzymatic step in cholesterol biosynthesis (6), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (mevalonate:NADP oxidoreductase, E.C. 1.1.1.34), was assayed in liver microsomes from morbidly obese patients and from normal-weight controls.

MATERIALS AND METHODS

Subjects

Altogether, ten obese patients (eight females and two males) were studied. They all had relative body weights exceeding 155% and they had all been admitted for gastric operation for obesity. Basal data on these patients 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. Eighteen patients with uncomplicated gallstone disease, admitted for elective cholecystectomy, served as controls. They had relative body weights less than 120%. Clinical data on these subjects are summarized in Table 1.

None of the patients studied had been treated with drugs affecting lipid metabolism, including oral contraceptives, for at least 2 months prior to operation. No clinical or laboratory evidence of kidney or thyroid disease, diabetes mellitus, hyperlipidemia, or addiction to alcohol or narcotics was present. Five of the obese subjects had slightly elevated aminotransferase levels, whereas other liver function tests were within normal limits. Morphological evidence of fatty infiltration was seen in operative liver biopsies in eight of the obese subjects and in four of the gallstone patients, but no other histological abnormalities were observed.

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 for...
2–3 days. The amount of calories was chosen to keep body weight constant. All operations were performed between 8 and 9 AM after a 12-hr fast. Anesthesia was given in a standardized way (cf. 7, 8).

After opening the abdomen, a 2–4-g liver biopsy was obtained from the left lobe of the liver. The biopsy was immediately placed in ice-cold buffer and transported within 10 min to the laboratory, where the preparation of microsomes was started (see below).

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

Materials

\[3^{-14}C\]HMG CoA (sp act 20 µCi/mg) and [5-\(^3\)H]DL mevalonic acid (sp act 25 µCi/mg) were obtained from New England Nuclear Corp., Boston, MA. The radioactive HMG CoA was diluted with unlabeled material, obtained from P-L Biochemicals, Inc., Milwaukee, WI, to yield a final specific radioactivity of 1.45 pCi/mg.

Unlabeled DL-mevalonic acid lactone, NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase was obtained from Sigma Chemical Co., St. Louis, MO.

Preparation of liver microsomal fraction

About 1 g of the liver biopsy was used to form a 10% (w/v) homogenate. This was prepared using a Potter-Elvehjem homogenizer equipped with a loosely fitting Teflon pestle in a medium of 0.3 M sucrose, containing nicotinamide, 0.075 M, EDTA, 0.002 M, and mercaptoethanol, 0.02 M (buffer A). The homogenate was centrifuged at 20,000 g for 15 min, and the supernatant fluid was centrifuged at 100,000 g for 60 min to obtain the microsomal fraction. This was then washed with buffer A, recentrifuged at 100,000 g for 30 min, and suspended in 0.17 M phosphate buffer, pH 7.4, with mercaptoethanol, 0.034 M, to a volume corresponding to that of the 20,000 g supernatant fluid.

The protein concentration of the microsomal fraction was determined according to Lowry et al. (9). The recovery of microsomal protein was determined by assaying a microsomal marker enzyme, NADPH-cytochrome c reductase, as described by Eriksson (10).

Assay of HMG CoA reductase activity

The microsomal fraction (0.15 ml, 0.15–0.5 mg of protein) was preincubated at 37°C for 10 min in a total volume of 0.80 ml containing 100 mM phosphate buffer (pH 7.2), 3 mM NADP, 10 mM glucose-6-phosphate, 5 units of glucose-6-phosphate dehydrogenase, and 20 mM mercaptoethanol. Thereafter, 0.5 µCi (0.4 mM) of \[3^{-14}C\]HMG CoA, dissolved in 50 µl of distilled water,
rank sum test, and correlations were tested with Spearman's method (11).

RESULTS

In the controls there was no difference between males and females with regard to enzyme activity (Table 2). There was a considerable range of activities, from 14.4 to 76.7 pmol·min⁻¹·mg protein⁻¹. No relationship was seen between age, body weight, or relative body weight on the one hand and enzyme activity on the other.

The obese patients had a significantly higher enzyme activity (60.7 ± 9.9 pmol·min⁻¹·mg protein⁻¹) compared to the controls (38.7 ± 4.4 pmol·min⁻¹·mg protein⁻¹, P < 0.05), and all obese subjects except two displayed activities above the mean encountered in the control series (Table 2). There was no difference between obese subjects with and without gallstone disease. The microsomal protein concentration was about 33% lower in obese compared with nonobese subjects (P < 0.01).

The recovery of microsomal protein was the same in nonobese gallstone patients (mean 49%, range 45–57, n = 5) and obese subjects (mean 48%, range 43–53, n = 3). Therefore, when HMG CoA reductase activity was calculated as pmol of mevalonate formed per g liver tissue there was no longer any significant difference between the two groups of subjects (Table 2).

DISCUSSION

In the present work, a highly standardized procedure was used, not only with regard to the incubation conditions but also to the timing of the operation, the procedures of anesthesia, biopsy location, etc. As discussed previously (7, 8), such precautions are necessary to provide results suitable for group comparisons. The absolute figures for hepatic HMG CoA reductase activity obtained in the gallstone patients in the present study are higher than those previously reported (7, 8). This fact is due mainly to some changes of the enzymatic assay. Particularly, preincubation of the microsomal preparation with cofactors was shown to considerably increase measured activity.

The fivefold range of measured enzyme activities seen in the present series of normolipidemic subjects with gallstone disease is in agreement with our previous observations (7, 8). The lack of relationship between enzyme activity and age, sex, and absolute or relative body weight in this group of subjects also confirms previous work (7, 8, 12). This is of particular importance inasmuch as the two groups of patients presented here were not fully comparable in regard to age distribution. Furthermore, only about half of the obese patients displayed gallstone disease. Although some authors have reported higher levels of HMG CoA reductase activity in patients with cholesterol gallstones compared to patients with peptic ulcer (12, 13), we have not observed any differences in enzyme activity between patients with cholesterol gallstones and with adenomyoma of the gallbladder wall, strongly suggesting that hepatic HMG CoA reductase activity is not increased in normolipidemic cholesterol gallstone disease (8). We would therefore conclude that the patients with uncomplicated cholelithiasis reported in the present work are suitable controls for the markedly obese patients studied.

The mean hepatic microsomal HMG CoA reductase activity in the obese subjects and the controls is shown in Table 2.

<table>
<thead>
<tr>
<th>Patients</th>
<th>HMG CoA Reductase Activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>pmol·min⁻¹·mg protein⁻¹</td>
</tr>
<tr>
<td>Obese subjects</td>
<td></td>
</tr>
<tr>
<td>1. F</td>
<td>50.5</td>
</tr>
<tr>
<td>2. F</td>
<td>62.3</td>
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<tr>
<td>3. F</td>
<td>45.1</td>
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<td>4. F</td>
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<td>5. F</td>
<td>49.7</td>
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<tr>
<td>6. F</td>
<td>32.0</td>
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<tr>
<td>7. F</td>
<td>143.0</td>
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<tr>
<td>8. F</td>
<td>57.2</td>
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<tr>
<td>9. M</td>
<td>74.7</td>
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<tr>
<td>10. M</td>
<td>37.5</td>
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<tr>
<td>Total (8 females, 2 males)</td>
<td>60.7 ± 9.9b,c</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls</th>
<th>HMG CoA Reductase Activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (10)</td>
<td>38.4 ± 7.2</td>
<td>1331 ± 180</td>
</tr>
<tr>
<td>Males (8)</td>
<td>38.2 ± 5.9</td>
<td>1806 ± 248</td>
</tr>
<tr>
<td>Total (18)</td>
<td>38.7 ± 4.4</td>
<td>1542 ± 155</td>
</tr>
</tbody>
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a, F, female; M, male. b Values are means ± SEM. c Significantly different from controls, P < 0.05.
activity was about 60% higher in the obese patients than in the controls, indicative of either an increased amount of the enzyme protein or an enhanced activity of the enzyme present (6). Due to the fact that the microsomal protein content per gram of liver was reduced in the obese patients, the calculated mevalonate formation per gram tissue was not significantly increased. However, as the total liver weight is considerably increased in obesity, the finding of an increased microsomal enzyme activity does correspond to an enhanced hepatic cholesterol production in obesity. Thus, using the figures 1.5 and 3.0 kg for liver weight in normals and in the obese, respectively (14), the present results would correspond to a mevalonate formation of 2.3 \( \mu \text{mol min}^{-1} \) in the control liver and 5.4 \( \mu \text{mol min}^{-1} \) in the obese liver. Thus, an increased hepatic cholesterol synthesis would appear to explain at least a major part of the enhanced total body cholesterol production often observed in obesity (1–3). In agreement with this contention, Angel and Bray (14) recently demonstrated that hepatic cholesterologenesis, measured as \(^3\text{H}_2\text{O}\) incorporation into sterols, clearly predominated over other sources of cholesterol production in obese subjects.

The reason for this increased cholesterol biosynthesis in the liver of obese subjects cannot be decided presently. There was no relation of the HMG CoA reductase activity to the mass of fat tissue, reflected as absolute or relative body weight. Obesity is often associated with elevated levels of plasma free fatty acids, which may affect enzyme activity. Goh and Heimberg (15) have shown that the inflow of free fatty acids to the liver may influence the hepatic HMG CoA reductase activity in the rat. The possible importance of regulatory hormones, such as insulin and glucagon, in the determination of hepatic cholesterologenesis must of course be considered, too. Studies aimed at defining the role of these factors in the pathogenesis of cholesterol overproduction found in obesity are currently in progress.

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**REFERENCES**