THE EFFECT OF DIET ON HEPATIC CHOLESTEROL SYNTHESIS IN ALLOXAN DIABETIC RATS

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The effect of dietary composition on cholesterol formation in the liver of alloxan diabetic rats was studied. Cholesterol formation in these animals was maintained at a subnormal level when the diet had a high content of simple sugar (sucrose, glucose or fructose) without fat, but was severely impaired with diets of all other compositions tested. The possible mechanism of maintenance of hepatic cholesterol synthesis in diabetic rats on a diet with a high content of simple sugar without fat is discussed. The rate of cholesterol synthesis in the liver was not always proportional to the plasma cholesterol level.

It is known that, in the diabetic state, lipid metabolism as well as carbohydrate metabolism is abnormal. In general, alloxan diabetic rats show an increased level of serum cholesterol, similar to human cases of uncontrolled diabetes.

This hypercholesterolemia in diabetic rats is thought to be due to a decrease in the rate of either conversion of cholesterol to cholic acid (1) or a turnover of cholesterol in the liver (2).

However, it is still uncertain whether cholesterol formation in the liver of diabetic rats increases or decreases, since there are reports of reduced (3-6), increased (7-10) or normal (11-13) cholesterol formation in the liver of diabetic rats. These discrepant results are apparently due to differences in the dietary conditions used. For example, Chaikoff et al. (7-9) reported increased cholesterol formation in the liver of diabetic rats on a diet containing 60% glucose without fat, but Elwood et al. (4, 5) reported reduced synthesis in rats on a diet containing fat.

This paper reports studies on the effects of dietary composition on hepatic cholesterol synthesis in diabetic rats.

Results showed that cholesterol synthesis was maintained at a subnormal
level in animals on a diet containing a high content of simple sugar without fat, but decreased in animals on other diets.

The effect of a high content of simple sugar without fat on hepatic cholesterol formation in diabetic rats is discussed.

METHODS

Animals. Male Sprague-Dawley rats, initially weighing about 150 g, were used. Diabetes was induced by a single intramuscular injection of 120 mg of alloxan monohydrate per kg body weight (4.0% solution in 0.9% NaCl).

Control rats were injected with 0.9% NaCl, instead of alloxan solution. The rats were kept in individual cages and given food and water ad libitum. Food consumption, weight change and urinary glucose were recorded daily. Urinary glucose was estimated with Tes-Tape.

Animals with a plasma glucose level of more than 250 mg per 100 ml of plasma were regarded as diabetic.

Diets. Both control and diabetic rats were given the control diet for 5 days and then were maintained on the diet shown in Table 1 for 10 days.

<table>
<thead>
<tr>
<th>Table 1. Composition of diet (%)</th>
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<tbody>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>-----</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Corn starch</td>
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<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Oli</td>
</tr>
<tr>
<td>Salt mix</td>
</tr>
<tr>
<td>Vit. mix</td>
</tr>
<tr>
<td>Choline Cl</td>
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<tr>
<td>Cellulose</td>
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</table>

Determination of $^{14}$C-cholesterol formation from acetate-$^{14}$C. Blood was withdrawn from the inferior vena cava of animals under ether anesthesia. Then the liver was quickly excised, weighed, and placed in a cold Krebs-Ringer phosphate buffer (pH 7.4). Liver slices were prepared with a Stadie-Riggs slicer. The slices were gently blotted with filter paper and approximately 400 mg of tissue were placed in a 50 ml Erlenmeyer flask with 5 ml of Krebs-Ringer phosphate buffer (pH 7.4), containing 1 $\mu$Ci of sodium acetate-$^{14}$C (specific activity 46.1 mCi/mmol Daiichi Pure Chemical Co., Ltd., Tokyo) and 10 $\mu$moles of unlabeled sodium acetate.
The flasks were filled with 100% O₂, closed with rubber stoppers, and incubated with shaking for 2 hr at 37°C. After incubation, the slices were removed, washed 3 times with a buffer, and placed in a test tube with 5 ml of 30% KOH.

Cholesterol was extracted after saponification, by the method of Chernick et al. (14), and was determined in an aliquot of the preparation as described below. Synthesized cholesterol was precipitated by adding digitonin and allowing the mixture to stand overnight. It was washed successively with acetone and ethyl ether, and dissolved in methyl alcohol.

Radioactivity was counted in a Packard Tri Carb liquid scintillation counter.

Experiments with liver slices were carried out between 11 a.m. and 2 p.m. to avoid the influence of the circadian rhythm of cholesterol synthesis (15).

Blood analyses. The blood glucose level was determined by the Somogyi-Nelson method (16) and plasma triglyceride by a modification (17) of the Van Handel method (18). Cholesterol in the plasma was measured by the Zak-Henly method (19).

RESULTS

After alloxan treatment, the diabetic rats consumed less of their diet than the controls and their body weights decreased for several days. These rats were maintained on the control diet for 5 days and then given the experimental diets shown in Table 1 for 10 days. Average daily food intakes are expressed in mean values for the last eight days before sacrifice (Table 2). Food intake of diabetic rats on all diets increased 30 to 40% more than those of control animals. However, the control rats gained about 80 to 100 g body weight in the experimental period, while the diabetic animals gained less than 30 g, or even lost weight.

Figure 1 shows the wet weights of the livers and small intestines of control and diabetic animals on the various diets. The liver weight of diabetic animals on sucrose or fructose diets exceeded that of control animals. The wet weight of the small intestine of diabetic rats was much more than that of the controls when expressed as wet weight per 100 g body weight and, even when expressed as wet weight per rat, it was more than that of control animals. Liver weight, expressed as wet weight per 100 g body weight, was similar in diabetic and control rats, except in animals on sucrose or fructose diets.

Both normal and diabetic rats on sucrose or fructose diets showed a marked increase in the plasma level of triglyceride but, in animals on the starch diet, the level was lower than in animals on the control diet (Fig. 2).

The plasma cholesterol level of diabetic rats was higher than that of control rats, irrespective of the diet (Fig. 3). The cholesterol content of the liver tended to be slightly higher in diabetic animals than in controls (Fig. 4A). Results on the influence of dietary composition on hepatic cholesterol synthesis from acetate-
Table 2. Changes in body weight and food intake in control and alloxan diabetic rats.

<table>
<thead>
<tr>
<th>Fat</th>
<th>Control</th>
<th>Starch</th>
<th>Sucrose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats N</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>N</td>
<td>143±5</td>
<td>145±2</td>
<td>142±2</td>
<td>143±2</td>
</tr>
<tr>
<td>N</td>
<td>150±5</td>
<td>155±3</td>
<td>150±4</td>
<td>145±4</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>96±7</td>
<td>92±7</td>
<td>94±6</td>
<td>91±3</td>
</tr>
<tr>
<td>(g/2 weeks)</td>
<td>12±6</td>
<td>14±7</td>
<td>17±3</td>
<td>20±3</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>18±0.9</td>
<td>20±1.1</td>
<td>18.3±0.7</td>
<td>14.0±0.1</td>
</tr>
</tbody>
</table>

a Values represent means ± S.D.

b N: normal rat; D: alloxan diabetic rat.

c expressed as the mean for period of 8 days before sacrifice.
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Fig. 1. Values were means ± S.D. Open and solid bars represent normal and alloxan diabetic rat, respectively.

1-14C are shown in Fig. 4B. In diabetic rats on all diets containing fat, hepatic cholesterol formation was severely impaired. Similar impairment was observed in animals on a stock diet (results not shown).

However, with liver slices from diabetic rats on a diet containing a high content of simple sugar without fat, incorporation of acetate into cholesterol was maintained at nearly the normal level.

DISCUSSION

In the present work, the wet weights of the small intestine and liver of diabetic rats, expressed as wet weight per 100 g body weight, were about 100% and 10%, respectively, more than those of control rats. The difference in liver weight might be due to the difference in body weights of the two groups. However, the small intestines of diabetic rats weighed about 1.5 times more than those of normal rats, even when expressed as weight per rat. Thus, the small intestine becomes hypertrophic or hyperplastic in the diabetic state. In this respect, it is interesting that Durand et al. (20) reported that in growing rats, which show progressively increasing food intakes, the weight of the small intestine per 100 g body weight...
Fig. 2. Values were means ± S.D. Open and solid bars represent normal and alloxan diabetic rats, respectively.

Fig. 3. Values were means ± S.D. Open and solid bars represent normal and alloxan diabetic rats, respectively.
Fig. 4A
Fig. 4. Values were means ±S.D. Open and solid bars represent normal and alloxan diabetic rat, respectively. * 400 mg of tissue were incubated for 2 hr in 5 ml Krebs-Ringer phosphate buffer (pH 7.4) containing 1 μCi sodium acetate-1-14C and 10 μmoles unlabeled sodium acetate at 37°C. ** Values expressed as μmoles of incorporated acetate into the digitonin precipitable sterol per g liver for 2 hr.

increases. Diabetic rats consume more food than control rats, so the hypertrophy or hyperplasia observed in these animals might be due to hyperphagia.

It has been observed that the plasma level of lipids is greatly influenced by dietary conditions. Moreover, it has been shown that the level of plasma triglyceride is higher in animals on a diet rich in sucrose or fructose, than in those on a diet rich in starch (21, 22). The present results confirms these findings.

Hepatic cholesterol synthesis is usually measured in terms of the incorporation of acetate-14C into cholesterol. In this measurement, caution must be taken to avoid dilution of acetate-14C by endogenous acetate. In this respect, it is significant that the liver acetyl CoA level in diabetic rats has been found not to be above normal (23). Moreover, we added a rather high concentration of acetate to the incubation medium to reduce dilution by endogenous acetate.
ELWOOD et al. reported impaired sterol-$^{14}$C formation from acetate-$^{14}$C in alloxan diabetic rats (4, 5), and similar findings were reported by others (2, 3, 12). CHAIKOFF and his collaborators (7–9), however, found that liver slices from alloxan diabetic rats on a diet containing 60% glucose without fat had an increased capacity to convert acetate-$^{14}$C to sterol. In our experiments, similar results were observed under the same conditions.

Later, CLARENBURG and CHAIKOFF (9) reported that the great discrepancies between results obtained in their laboratory (7–9) and elsewhere (2–5, 11, 12) were due to differences in the diets of the animals. They considered that the discrepancies were due to the glucose content of the diets. We found that hepatic cholesterol synthesis in the diabetic state was maintained at almost the normal level not only with a diet of high glucose content, but also with diets rich in other simple sugars. In addition, we found that to maintain nearly normal hepatic cholesterol synthesis, it was essential to exclude any fat from the diet. This is clearly shown in Fig. 4B, where hepatic cholesterol synthesis is seen to be very low in diabetic rats on a diet with a high content of simple sugar and with fat.

Other workers (10) have reported that a diet containing a high content of glucose without fat is useful for maintaining hepatic cholesterol synthesis in diabetic rats, but no one has previously examined the essential composition of the diet for cholesterol synthesis.

In the diabetic state, glycolysis and fatty acid synthesis are depressed, while gluconeogenesis and fatty acid oxidation are accelerated under normal dietary conditions. However, it has been shown that hepatic glucokinase activity is induced by feeding a high glucose diet without fat both in normal and diabetic rats, although the induction is low in diabetic animals (24). Furthermore, it has been found that fructose administration restores the capacity of diabetic liver to synthesize fatty acids from acetate (25). These results strongly suggest that alterations in carbohydrate and lipid metabolisms caused by feeding a diet containing a high content of simple sugar without fat are beneficial for maintaining hepatic cholesterol synthesis in diabetic animals. Further studies are necessary on the precise mechanism of the phenomenon.

Hepatic cholesterol synthesis is greatly influenced by dietary conditions, as discussed above. However, hypercholesterolemia is observed in diabetic rats irrespective of the dietary conditions. This indicates that the rate of cholesterol synthesis in the liver is not always proportional to the level of plasma cholesterol and suggests that other factors, such as the rate of conversion of cholesterol to cholic acid and/or of secretion of cholesterol into the bile may influence the plasma cholesterol level.

REFERENCES