Regulation of Hepatic Lipogenesis: The Influence of Dietary Fats*

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The factors governing the rate of fatty acid synthesis by the liver have been under investigation in this laboratory. The general study procedure consisted of modifying the nutritional or endocrine state of rats and then measuring the capacity of their excised livers to convert a variety of C14-labeled substrates (glucose, fructose, pyruvate, acetate) to fatty acids. It became clear quite early that fasting for a few days drastically reduced the capacity of the liver to convert the C14 of C14-glucose (1), C14-fructose (2), and of acetate-1-C14 (3) to fatty acids, and that a single administration of glucose—but not of protein or fat—completely restored hepatic lipogenesis of the fasted rat to normal (3). The elimination of carbohydrate from the diet for only a few days before the rat was killed resulted in a loss in the ability of its liver to convert glucose (1) and acetate carbon to fatty acids (3). The effects of fasting upon the lipogenic capacity of the liver slice have been confirmed in experiments with intact rats injected with C14-acetate (4, 5). These and other experiments have given rise to the opinion that the extent of lipogenesis in the liver is linked in some manner to carbohydrate utilization (6-8).

The present report deals with the influence of dietary fats on hepatic lipogenesis. It is shown that the capacity of the liver for incorporating the C14 of C14-glucose and C14-acetate into fatty acids is related to the amount of fat ingested by the rat. Since the prolonged feeding of high fat diets may induce liver damage, the feeding of special diets described here, resembling diet (1), and acetate carbon to fatty acids (3). The effects of fasting upon the lipogenic capacity of the liver slice have been confirmed in experiments with intact rats injected with C14-acetate (4, 5). These and other experiments have given rise to the opinion that the extent of lipogenesis in the liver is linked in some manner to carbohydrate utilization (6-8).

The present report deals with the influence of dietary fats on hepatic lipogenesis. It is shown that the capacity of the liver for incorporating the C14 of C14-glucose and C14-acetate into fatty acids is related to the amount of fat ingested by the rat. Since the prolonged feeding of high fat diets may induce liver damage, the feeding of special diets described here, regardless of their fat content, was limited to 3 days. The feeding of high-fat-containing diets for this short period did not influence the glycogen or lipid content of the liver. Our findings suggest the existence of a homeostatic mechanism in the regulation of hepatic lipogenesis.

EXPERIMENTAL

Treatment of Animals—Rats of the Long-Evans strain were fed synthetic diets for 3 days before they were killed. In all cases except one (Table I) the basal diet contained 50 per cent glucose, 6 per cent salt mixture (12), 2 per cent defatted liver (VioBin), and an adequate vitamin B mixture (13). The changes in the percentages of fat in the diet were made at the expense of Cellu flour or Cellu flour together with vitamin-free casein (Nutritional Biochemical Corporation).

Preparation of Tissues and Incubation Procedure—Blood was withdrawn from the heart just before the animals were killed by a sharp blow on the head. The livers were rapidly excised and placed in cold Krebs-Henseleit bicarbonate buffer (14). Slices approximately 0.5 mm. thick were prepared with a mechanical tissue slicer (15). 500 ± 5 mg. portions of the slices were placed in the main compartment of a 50 ml. incubation flask (16) containing 5 ml. of the bicarbonate buffer (pH 7.3 to 7.4) to which either acetate-1-C14 (as the sodium salt) or glucose evenly labeled in all its carbons had been added. The C14-glucose was prepared photosynthetically and purified chromatographically by the method of Putman and Hassid (17). The tissues were incubated, with shaking, at 37.5° for 3 hours.

Analytical Procedures—The methods for determining the radioactivity in CO2 and fatty acids and for measuring as well as those plasma glucose and liver glycogen have been described elsewhere (13, 18). Plasma and liver fatty acids were determined by the method of Brody (19). The phospholipidic content of the plasma was determined by the method of King (20).

RESULTS

1. Experiments with Liver Slices

Corn Oil as Dietary Fat—In the first experiment (Table I), increasing amounts of corn oil were incorporated into the synthetic diets, all of which contained 55 per cent glucose and 22 per cent casein. No attempt was made to keep constant the caloric value per unit weight of these diets. As the percentages of corn oil in the diet were increased from 0 to 10, the conversion of the added acetate-C14 to fatty acids by liver slices was decreased. The fatty acid-C14 recoveries observed with the rats fed for 3 days, a diet containing 15 per cent corn oil were about the same as those found with the rats fed the 10 per cent corn oil diet. No change was observed in the C14O2 recoveries regardless of the level of corn oil in the diets fed.

It is worthy of note that, under the conditions of this experiment, increasing the percentage of corn oil in the diet from 0 to 15 failed to change significantly the levels of either glucose and total fatty acids of plasma or glycogen and fatty acids in liver.

In the next experiment (Table II), the caloric value per gm. of diet was kept constant, as the amount of corn oil was increased from 0 to 15 per cent, by varying the level of casein.
Regulation of Hepatic Lipogenesis

Male rats were fed the diets for 3 days. Duplicate 500 ± 5 mg. portions of liver slices prepared from each liver were incubated as described in the text. Each result is the average of two separate analyses.

**Table I**

*Influence of corn oil feeding on lipogenesis by liver slices (the caloric value per gm. of diet was not kept constant or content of corn oil varied)*

* The glucose content of the diet was 55 per cent throughout. The casein content of the diet was 22 per cent throughout.
† Mazola (Corn Products Company).

**Table II**

*Influence of corn oil feeding on lipogenesis by liver slices (caloric value per gm. of diet kept constant or content of corn oil varied)*

For experimental details see Table I.

* The glucose content of the diet was 55 per cent throughout.
† Mazola.
in the diet from 37 to 15 per cent. Even under these dietary conditions, the incorporation of acetate carbon into fatty acids was decreased as the fat in the diet was augmented.

The conversion of the C\textsuperscript{14} of glucose, the carbons of which were evenly labeled with C\textsuperscript{14}, into fatty acids and CO\textsubscript{2} by separate portions of the livers of Rats 21 to 35 (Table II) was also measured. The results were similar to those observed with acetate. Although the C\textsuperscript{14}02 recoveries in the experiments with the Cl*-fatty acid recoveries from an average value of 17.7, when the diet contained no fat, to 6.4 when the diet contained 15 per cent corn oil, it should be noted that the corresponding Cl*-fatty acid recoveries represent a 5-fold reduction.

**Changes in Dietary Protein**—Since, in the preceding experiment, we varied the casein content of the diet in order to keep the caloric value per gm. of diet constant, it became necessary to determine whether or not large differences in casein intake could modify the incorporation of acetate-1-C\textsuperscript{14} into fatty acids. In this experiment (Table III), rats were fed synthetic diets containing no fat and either 15 or 37 per cent casein. No difference in the capacity of liver slices to incorporate acetate-1-C\textsuperscript{14} into fatty acids was observed. Likewise, no difference was apparent in the oxidation of acetate-1-C\textsuperscript{14} to CO\textsubscript{2} in the two groups of animals.

It may be concluded that the depression in Cl*-fatty acid recoveries observed in Tables I and II, is neither a matter of alteration of total caloric intake nor of variation in the protein content, but rather, a response to the corn oil content of the diet.

**Experiments with Lard, Vegetable Oil, and Hydrogenated Vegetable Oil**—The results of the experiments recorded in Table IV show that the inhibitory effect on hepatic lipogenesis is not confined to corn oil. When fed for 3 days, vegetable oil, hydrogenated vegetable oil, and lard were about equally effective in decreasing the liver's capacity for lipogenesis from acetate carbon.

2. **Experiments with Intact Rats**

The experiments described so far were carried out with liver slices. Fat feeding was also shown to affect the extent of lipogenesis in the liver of the intact rat. Rats were fed isocaloric diets containing 0, 5, 10, or 15 per cent corn oil, for 3 days, and at the end of that time they were injected intraperitoneally with acetate-1-C\textsuperscript{14}. Exactly 1 hour after the injection they were killed, and their livers were rapidly excised and analyzed for C\textsuperscript{14} fatty acids (Table V). The reduction in C\textsuperscript{14} fatty acid recoveries increased with increasing amounts of dietary fat is again evident.

3. **Time and Concentration Studies with Liver Slices**

Under the conditions of our experiments, the feeding of the fat-containing diets did not increase the fatty acid content of the liver. Even so it is necessary to consider the possibility that the lower C\textsuperscript{14} fatty acid recoveries reflect a greater dilution...
of the added C\textsuperscript{14} in the liver of the fat-fed animal than in that of the rat fed the fat-free diet. As shown in Fig. 1, however, fat feeding reduced the incorporation of acetate carbon into fatty acids when 500 mg. of liver slices were incubated with 2 \mu moles of acetate as well as when 10 and 50 \mu moles of acetate were added to the incubation medium. This was true for all intervals studied, namely, 1, 2, and 3 hours. This observation, with a 25-fold variation in the amount of acetate incubated, argues

against the view that the reduced C\textsuperscript{14}-fatty acid recoveries observed with the livers of the corn oil-fed rats resulted from an enlarged two-carbon pool. It should also be noted that the C\textsuperscript{14}O\textsubscript{2} recoveries did not differ significantly in the experiments with the fat-free and fat-fed rats. This latter finding, in conjunction with the observation that the extra fat feeding did not depress the total utilization of acetate by the liver slices,\textsuperscript{1} lends support to the view that the ingestion of fat does not result in a generalized depression of the metabolic activity of the liver, but rather in a specific inhibition of lipogenesis.

4. Effect of Exogenous Insulin on Hepatic Lipogenesis of Corn Oil-Fed Rats

Since insulin is intimately involved in maintenance of hepatic lipogenesis (9, 10), we considered the possibility that the inclusion of fat in the diet had modified the release of insulin from the pancreas. The results of the experiments recorded in Table VI rule out this possibility. In normal rats fed a glucose diet, with or without added fat, the administration of insulin, twice daily for 3 days, failed to influence hepatic lipogenesis.

**TABLE V**

<table>
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<tr>
<th>Rat No.</th>
<th>Diet*</th>
<th>Average daily food intake</th>
<th>Liver</th>
<th>C\textsuperscript{14} of injected acetate-C\textsuperscript{14} recovered as fatty acids in whole liver</th>
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* The glucose content of the diet was 50 per cent throughout.

† Mazola.

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**Fig. 1. Time and concentration study with liver slices. For explanation see the text. The ordinate values are percentages of the C\textsuperscript{14} added to the incubation medium.**

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**TABLE VI**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Diet*</th>
<th>Insulin†</th>
<th>Liver</th>
<th>Added acetate-C\textsuperscript{14} recovered as:</th>
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* The glucose content of the diet was 50 per cent throughout.

† Mazola.

‡ 2 units of protamine zinc insulin (Lilly) per kilo body weight were injected subcutaneously twice daily for 3 days before the rats were sacrificed.
fatty acids. An effect of dietary fat was demonstrated in experiments with excised liver slices, as well as in the intact rat. A depressing action upon hepatic lipogenesis was observed with a variety of fats: corn oil, lard, vegetable oil, and hydrogenated vegetable oil. The sensitivity of the liver to ingested fat is shown by the fact that measurable effects on lipogenesis were noted when the fat in the diet was raised from 0 to as little as 2.5 per cent.

Because of the importance of dietary carbohydrate in the maintenance of hepatic lipogenesis (3), all diets used in this study, regardless of whether they had 0, 1, 2.5, 5, 10, or 15 per cent fat, were rich in carbohydrate and, in all experiments except one (Table 1), the diets contained exactly 50 per cent glucose. In no case did we obtain evidence that the addition of as much as 15 per cent of fat to the diet, which was fed for 3 days, lowered the glycogen content of the liver. Thus, the depressing effect of dietary fat upon lipogenesis was observed under conditions that allowed for priming of hepatic lipogenesis by carbohydrate. Our observations suggest that the lipogenic capacity of the liver is more sensitive to fat in the diet than it is to carbohydrate.

Haugard and Stadie (21) were the first to point out that, in normal rats, a positive correlation exists between the liver's capacity to incorporate acetate carbon into fatty acids and the glycogen content of the liver. Masoro et al., in their studies on cold exposure (22), found a similar correlation between lipogenesis and the total carbohydrate content of the liver. No such correlation is apparent in the data recorded in Tables I to IV.2

The effects upon hepatic lipogenesis recorded here were observed in rats fed fat-containing diets for very short periods (3 days), and the feeding of those diets did not influence the fat content of the liver. In these respects our experimental design differed from that of Brice and Okey (23), who fed diets containing 5 and 10 per cent fat, were rich in carbohydrate and, in all experiments except one (Table 1), the diets contained exactly 50 per cent glucose. In no case did we obtain evidence that the addition of as much as 15 per cent of fat to the diet, which was fed for 3 days, lowered the glycogen content of the liver. Thus, the depressing effect of dietary fat upon lipogenesis was observed under conditions that allowed for priming of hepatic lipogenesis by carbohydrate. Our observations suggest that the lipogenic capacity of the liver is more sensitive to fat in the diet than it is to carbohydrate.

The mechanism by which the feeding of fat depresses lipogenesis remains to be considered. A diet rich in fat has been shown to lower the insulin content of the pancreas (25). In view of the role of this hormone in carbohydrate utilization we were led to consider the possibility that the depressed lipogenesis produced by fat feeding may have resulted from a relative decrease in available insulin. Experiments in which insulin treatment was instituted along with fat feeding offer no support for this concept.

SUMMARY

1. Rats were fed, for 3 days, synthetic diets containing 0, 1, 2.5, 5, 10, and 15 per cent fat. The fats tested included lard, corn oil, vegetable oil, and hydrogenated vegetable oil. The glucose content of each diet, regardless of its fat content, was kept constant, namely, at 50 per cent.

2. The livers of rats fed the diet devoid of fat had the highest capacity for converting acetate carbon to fatty acids. A measurable depression in lipogenesis was observed when as little as 2.5 per cent of fat was added to the diet. When the fat content of the diet was increased to 15 per cent, the liver had lost about 90 per cent of its ability to convert acetate carbon to fatty acids. All fats tested were effective in reducing hepatic lipogenesis. Similar effects were observed when labeled glucose served as substrate.

3. These effects of ingested fat upon hepatic lipogenesis occurred in the absence of changes in (a) the glycogen and fat content of the liver and (b) the glucose and lipid content of plasma.

4. The administration of insulin failed to increase the depressed hepatic lipogenesis induced by fat feeding.

REFERENCES


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