Regulation of hepatic lipogenesis in starved and diabetic animals by thyroid hormone

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The effects of intragastric feeding with glucose and of the administration of L-triiodothyronine (T3) on in vivo rates of hepatic lipogenesis were investigated in control (fed ad libitum on normal diet), diabetic (fed ad libitum on normal diet), fat-fed (fed ad libitum on high-fat diet), and starved (food removed for 48 h) rats. Two days of T3 treatment increased hepatic lipogenesis in control and fat-fed animals but not in the diabetic or starved animals, although increases in lipogenesis in diabetic animals were observed after 4 days of T3 treatment.

Intragastric glucose feeding increased hepatic lipogenesis in the livers of control animals and T3-treated control animals. Such increases are mediated by an increase in the circulating insulin concentration, as increases are not observed in diabetic rats or T3-treated diabetic rats. Glucose feeding failed to increase hepatic lipogenesis in fat-fed rats or starved rats. Insulin injection together with glucose feeding increased lipogenesis in the fat-fed group but not the starved group; i.e., impaired insulin secretion following an oral glucose load may in part explain the lack of response in the fat-fed but not the starved animals. Marked increases in hepatic lipogenesis after glucose feeding were, however, observed if either the starved or the fat-fed animals were treated with T3. The physiological implications of these observations are discussed.

Rates of hepatic lipogenesis depend on a variety of nutritional and hormonal factors (for reviews see Bloch & Vance, 1977; Geelen et al., 1980). Rates of hepatic fatty acid synthesis are decreased in starved (Allmann et al., 1965), diabetic (Nakanishi & Numa, 1970), and hypothyroid (Volpe & Marasa, 1975) animals and in animals fed a high-fat diet (Carrozza et al., 1979). Conversely, rates of hepatic
Lipogenesis are increased by administration of thyroid hormones (Roncari & Murthy, 1975) or feeding a diet high in carbohydrate (see Oppenheimer et al., 1981). The decreased lipogenic rates observed in diabetes and on starvation are partly caused by decreased concentrations of acetyl-CoA carboxylase (EC 6.4.1.2) and fatty acid synthetase. It has been found that administration of L-triiodothyronine (T3) to diabetic rats restores the concentration of these enzymes to the levels found in non-diabetic animals (Das, 1980). We therefore studied the effects of such treatment on the rates of hepatic lipogenesis observed in vivo. For comparison, the effects of T3 treatment on the rates of hepatic lipogenesis of starved and fat-fed animals were investigated.

Experimental

Female albino Wistar rats (150-200 g), subjected to a 12-h-light:12-h-dark cycle (light period starting at 0800 h), were fed ad libitum on standard rodent diet (Rat/Mouse No. 3) or a high-fat diet (30% butter and 70% Rat/Mouse No. 3) purchased from BP Nutrition (U.K.) Ltd., Streffield, Witham, Essex, U.K. Although the crude-protein content of the high-fat diet was 30% less than that of the control diet, the growth rate was unaffected, and weight increases of 5.9 ± 0.2% (3 animals) and 5.6 ± 0.6% (3 animals) of the initial body weights were observed in, respectively, control and fat-fed animals over 7 days. In some experiments, food was withdrawn for 48 h; the rats were transferred to clean cages at the onset of food withdrawal to minimize coprophagia, and the dietary status of the starved animals was assessed by measurements of blood ketone body levels (see text) and hepatic glycogen content [6.2 ± 2.8 (5 animals) μmol glucose/g wet wt.]. Diabetes was induced by the intravenous administration of streptozotocin (60 mg/kg body wt.; in 0.1 M sodium citrate buffer, pH 4.5) and confirmed by whole-blood glucose concentrations greater than 15 mM, and experiments were initiated on the third day after streptozotocin injection. Animals were made hyperthyroid by subcutaneous injection of T3 [100 μg/100 g body wt./day; in 10 mM NaOH/0.03% bovine serum albumin (BSA)]. This dose of T3 produced moderate (6-10%) hyperphagia in all groups and slight (1-3%) weight loss in the starved and diabetic groups. Treatment was for 2 or 4 days (animals killed on day 3 or 5). Some animals were treated with T3 for 10 days. Such treatment was not routinely carried out as the rates of hepatic lipogenesis were not significantly different from those observed in rats treated for 4 days (results not shown). T3 treatment of the starved rats was initiated at the time of food withdrawal. Control (euthyroid) rats were injected with an equivalent volume of 10 mM NaOH/0.03% BSA. All experiments were started between 0900 h and 0930 h. Glucose (2 mmol/100 g body wt.; 2 M solution) or H2O (1 ml/100 g body wt.) were administered intragastrically as described by Sugden et al. (1981). Insulin (2 units; Isophane insulin injection, Nordisk Insulin Laboratorium, Copenhagen, Denmark) was injected subcutaneously at this time except for the starved rats, where insulin was injected 15 min after glucose loading to avoid lethal
hypoglycaemia. The rats were injected intraperitoneally with $^{3}$H$_{2}$O (5 mCi; 0.5 ml in 0.9% NaCl) 1 h after the intragastric load, anaesthetized 50 min later (with sodium pentobarbital; 60 mg/kg body wt.) and dissected at 60 min as described by Agius and Williamson (1980). The incorporation of $^{3}$H into lipid was measured as described by Stansbie et al. (1976). An arterial blood sample was withdrawn for determination of plasma $^{3}$H$_{2}$O specific radioactivity and results were expressed as µg-atoms of $^{3}$H incorporated into lipid/h per g wet wt. of tissue. Arterial blood samples were treated with HCIO$_{4}$ (2.0 ml/0.5 ml whole blood), and D-glucose (Slein, 1963), 3-hydroxybutyrate, and acetoacetate (Williamson et al., 1962) were determined in KOH-neutralized HCIO$_{4}$-extracts. Samples of liver were freeze-clamped in tongs cooled in liquid N$_{2}$ at the time of death, and frozen liver powder (1 g) was extracted with 3.0 ml of 6% (w/v) HCIO$_{4}$ for determination of the glycogen content (Keppler & Decker, 1974). The statistical significance of differences was assessed using Student's unpaired t-test. Results are given as mean ± SEM with the number of observations given in parentheses.

Results

Effects of T$_{3}$ treatment

Lipogenesis was decreased by 57% in the diabetic rats, 46% in the 48-h-starved rats, and 50-60% in the fat-fed rats (Table 1), in agreement with the results of other workers. The treatment of the control rats (fed ad libitum on normal diet) with T$_{3}$ for 2 days increased hepatic lipogenesis by 149%, but in marked contrast, the treatment of the starved or the diabetic rats with T$_{3}$ for 2 days had no significant effect on in vivo rates of lipogenesis. This result was unexpected as, at least in the case of the diabetic rats, such treatment would restore enzyme levels to at least 80% of the control levels (Das, 1980). The difference in the responses to T$_{3}$ of the control and the diabetic and/or the starved groups is not likely to be due to the elevated levels of plasma non-esterified fatty acids which would be found in diabetes and starvation; feeding a high-fat diet inhibited hepatic lipogenesis to the same extent as did starvation for 48 h or diabetes, but in this case a response to treatment with T$_{3}$ was observed, although the response was slightly impaired (2 days of treatment of fat-fed rats with T$_{3}$ increased lipogenesis by 97%; Table 1).

The maximum rates of hepatic lipogenesis were observed in rats which had been treated with T$_{3}$ for 4 days. Such treatment of control or fat-fed rats increased hepatic lipogenesis by 296% and 215% respectively. A significant (118%) increase in hepatic lipogenesis was also observed after 4 days' treatment of diabetic animals with T$_{3}$, and indeed after this period of treatment, the in vivo rates of hepatic lipogenesis in the diabetic animals were similar to those found in the control rats which were not injected with T$_{3}$.
Table 1. Rates of hepatic lipogenesis in vivo after intragastric feeding or treatment with T₃

For experimental details see the text. Rates of lipogenesis are expressed as µg-atoms of ³H incorporated into lipid/h per g wet wt. of tissue. The values are means ± S.E.M., with the numbers of rats shown in parentheses.

<table>
<thead>
<tr>
<th>State of rats</th>
<th>H₂O-fed rats</th>
<th>Glucose-fed rats</th>
<th>H₂O-fed rats</th>
<th>Glucose-fed rats</th>
<th>H₂O-fed rats</th>
<th>Glucose-fed rats</th>
</tr>
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<tr>
<td></td>
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<td>2</td>
<td>4</td>
<td></td>
<td>2</td>
<td>4</td>
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<tr>
<td>Control</td>
<td>12.3 ± 0.9 (12)</td>
<td>18.8 ± 3.9 (6)+</td>
<td>30.6 ± 1.2 (6)**</td>
<td>37.5 ± 4.1 (6)</td>
<td>48.8 ± 8.4 (3)**</td>
<td>75.5 ± 3.1 (3)+</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5.8 ± 0.9 (8)</td>
<td>4.5 ± 1.2 (3)</td>
<td>7.1 ± 1.1 (6)</td>
<td>10.5 ± 1.2 (3)</td>
<td>12.7 ± 1.4 (5)**</td>
<td>15.8 ± 3.9 (4)</td>
</tr>
<tr>
<td>48-h-starved</td>
<td>6.6 ± 1.8 (6)</td>
<td>8.2 ± 1.7 (5)</td>
<td>7.2 ± 1.0 (6)</td>
<td>18.1 ± 1.6 (6)+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat-fed (5 days)</td>
<td>6.2 ± 1.7 (3)</td>
<td>4.7 ± 0.6 (3)</td>
<td>12.2 ± 1.6 (3)*</td>
<td>19.4 ± 2.1 (3)+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat-fed (7 days)</td>
<td>4.7 ± 0.8 (3)</td>
<td>5.0 ± 0.5 (3)</td>
<td>-</td>
<td>-</td>
<td>15.0 ± 2.1 (3)**</td>
<td>25.5 ± 2.4 (3)+</td>
</tr>
</tbody>
</table>

Values that are significantly different from each other are shown: **P ≤ 0.001, ***P ≤ 0.01, *P ≤ 0.05 for differences between rats not treated with T₃ or rats treated with T₃ in any one experimental group; ††P ≤ 0.001, †P ≤ 0.05 for differences between H₂O-fed and glucose-fed rats.
Effects of intragastric feeding and insulin treatment

Lipogenesis in the livers of control rats increased after intragastric feeding with glucose (52%; \( P < 0.05 \)). Similar observations have been made by others (Stansbie et al., 1976; Agius & Williamson, 1980). Increases in hepatic lipogenesis following glucose feeding also occurred if the animals had been treated with \( T_3 \) (Table 1). The increases were such that glucose feeding of animals treated for 4 days with \( T_3 \) resulted in rates of hepatic lipogenesis which were 6-fold greater than those found in euthyroid, \( \text{H}_2\text{O} \)-fed animals (Table 1). As glucose feeding did not increase hepatic lipogenesis in diabetic animals (Table 1) or in diabetic animals which had been treated with \( T_3 \) (Table 1), it is likely that the increased lipogenesis elicited by glucose feeding in both the control and the \( T_3 \)-treated animals was secondary to an increase in insulin secretion.

Glucose feeding failed to increase hepatic lipogenesis in 48-h-starved rats or fat-fed rats which had not been treated with \( T_3 \) (Table 1). However, if the rats had been treated with \( T_3 \), marked increases in response to glucose feeding were observed (Table 1). The increase was 60-70% in the fat-fed groups (70%; \( P < 0.05 \) in the 4-day \( T_3 \)-treated rats) and 151% (\( P < 0.01 \)) in the group starved for 48 h. Starvation is associated with diminished insulin secretion (Hedeshkov & Capito, 1974; Malaisse et al., 1976), and starved hyperthyroid rats have slight hyperinsulinaemia (Okajima & Ui, 1978). It follows that our results with starved rats might be explained if insulin secretion in response to the glucose load did not occur or was diminished unless the rats were treated with \( T_3 \). This explanation is unlikely to be correct, as, firstly, the elevated blood ketone body concentrations observed in the rats starved for 48 h were decreased by glucose intubation [minus glucose, 1.47 ± 0.19 (7) mM; plus glucose, 0.22 ± 0.05 (9) mM; \( P < 0.001 \)], and secondly, the injection of insulin 15 min following glucose administration did not increase hepatic lipogenesis in the rats starved for 48 h (Table 2). Taken together these observations imply that insulin secretion in response to a glucose load occurs in animals starved for 48 h but the elevated concentration of circulating insulin fails to increase hepatic lipogenesis. In contrast to the results obtained with the starved rats, the injection of insulin

<table>
<thead>
<tr>
<th>State of rats</th>
<th>Lipogenesis (( \mu \text{g-atoms of } ^{14} \text{C} \text{H incorporated into the lipid/g wet wt. of tissue} ))</th>
<th>Blood glucose (( \mu \text{mol/l} ))</th>
<th>Lipogenesis (( \mu \text{mol/l} ))</th>
<th>Blood glucose (( \mu \text{mol/l} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>48-h-starved</td>
<td>4.2 ± 0.6 (9)</td>
<td>6.9 ± 0.4 (9)</td>
<td>3.7 ± 0.3 (5)</td>
<td>1.6 ± 0.2 (5)***</td>
</tr>
<tr>
<td>Fat-fed</td>
<td>4.7 ± 0.3 (3)</td>
<td>7.1 ± 0.3 (3)</td>
<td>13.0 ± 1.7 (3)††</td>
<td>3.4 ± 0.1 (3)***</td>
</tr>
<tr>
<td>Fat-fed + ( T_3 )</td>
<td>19.4 ± 3.1 (3)</td>
<td>9.1 ± 0.5 (3)</td>
<td>29.6 ± 1.6 (3)††</td>
<td>2.2 ± 0.1 (3)***</td>
</tr>
</tbody>
</table>

Values that are significantly different from each other are shown: ***\( P <0.001 \) for differences in blood glucose concentration between control and insulin-injected rats; ††\( P <0.02 \), †\( P <0.05 \) for differences in lipogenesis between control and insulin-injected rats.
together with the oral administration of glucose increased hepatic lipogenesis by 176% in the fat-fed rats (Table 2); i.e., insulin secretion in response to glucose is sub-optimal for effects on hepatic lipogenesis in the fat-fed animals. Although it is possible that T3 treatment increases glucose-stimulated insulin secretion in fat-fed rats, it is suggested that the effects of T3 are not mediated solely via the restoration of a normal insulin-secretory response to glucose. Thus insulin treatment also increased hepatic lipogenesis (although to a lesser extent) if fat-fed rats were treated with T3 (Table 2). Measurement of the serum insulin levels of the fat-fed animals in each experimental group would clarify this point.

The lack of effect of insulin on hepatic lipogenesis in the starved animals is not related to the observed hypoglycaemia: blood glucose levels were decreased after insulin treatment in both the starved and fat-fed groups (Table 2). One possible explanation is that in the starved, but not the fat-fed, animals, the newly formed glucose phosphates are directed towards the formation of glycogen in muscle and possibly (but see Hems et al., 1972) liver rather than towards the formation of fatty acids. If this were the case, the effects of T3 treatment on the response to oral glucose in the starved animals could be attributed to inhibition of glycogen synthesis by the hormone. Although we did not measure the muscle glycogen content in this study, we found that in control and fat-fed rats treated with T3, hepatic glycogen was less than 5% of the control values (results not shown). Others have made similar observations (e.g. Okajima & Ui, 1979a; Laker & Mayes, 1981), and in support of our suggestion of inhibition of glycogenesis by T3, it has been found that the incorporation of [14C]glucose or [3H]glucose into liver glycogen in starved rats is inhibited by 95% on the induction of hyperthyroidism (Okajima & Ui, 1979a).

Discussion

Our experiments have shown that the administration of T3 to normal rats increases the rates of hepatic lipogenesis measured in vivo by the incorporation of 3H2O into lipid (which estimates total fatty acid synthesis from all precursors). In contrast, lipogenesis (measured using 3H2O) is not increased in perfused livers from hyperthyroid animals (Laker & Mayes, 1981). The discrepancy between the results obtained in vivo and in vitro may be the result of differences in the supply of substrate precursor for lipogenesis. Whereas hepatic glycogen can provide a major source of carbon for in vitro fatty acid synthesis in livers from fed, euthyroid rats (Salmon et al., 1974), glycogen is virtually absent from livers from fed hyperthyroid animals (see above, and also Okajima & Ui, 1979a; Laker & Mayes, 1981) and it might therefore be expected that the precursor supply would limit the expression of lipogenic potential. Experimental support for this hypothesis is the observation that hepatocytes prepared from fed, hyperthyroid rats show higher rates of lipogenesis than hepatocytes prepared from fed, euthyroid rats when lipogenesis is measured by incorporation of 14C from exogenous [2-14C]-acetate into fatty acids rather than by using 3H2O (Gnoni et al., 1980a,b).
The precursor(s) for hepatic fatty acid synthesis in vivo in hyperthyroid rats is not known. Possible substrates include lactate (see Hopkirk & Bloxham, 1977) and alanine. Although the increased rates of hepatic lipogenesis observed in hyperthyroid animals probably reflect changes in the maximum capacity of the lipogenic pathway as indicated by increased activities of lipogenic enzymes (e.g. Roncari & Murthy, 1975; Gnoni et al., 1980a,b), part of the effect may be due to inhibition of glycogenogenesis and/or an increased substrate supply to the liver. The hepatic supply of both lactate and alanine is increased in hyperthyroidism (Okajima & Ui, 1979a). An increased lactate supply may be the result of stimulation of extrahepatic glucose utilization by thyroid hormone (Okajima & Ui, 1979b). Moreover, lactate uptake is increased in perfused livers from hyperthyroid rats (Bartels & Sestoft, 1980; Laker & Mayes, 1981). An increased supply of alanine to the liver may be the result of increased release of alanine from skeletal muscle (Okajima & Ui, 1979a; Carter et al., 1981). In addition the supply of both alanine and lactate to the liver may be increased as a consequence of increased hepatic blood flow (Hagenfeldt et al., 1981). Administration of T₃ to diabetic rats for 2 days did not significantly increase rates of hepatic lipogenesis, although such treatment caused marked increases in hepatic lipogenesis in non-diabetic rats. A 2-fold increase in hepatic lipogenesis was, however, observed after 4 days' treatment with T₃. Again the response to T₃ treatment was less in the diabetic than in the non-diabetic animals (increases of 118% and 296%, respectively). The reason for the difference in response to T₃ between the experimental groups is not known, but one explanation might be that insulin insufficiency decreases the utilization of blood glucose by peripheral tissues, and that consequently the supply of lactate to the liver is restricted.

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References

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