Interactions between insulin and thyroid hormone in the control of lipogenesis

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1. The effects of intragastric glucose feeding and L-tri-iodothyronine (T_3) administration on rates of hepatic and brown-fat lipogenesis *in vivo* were examined in fed and 48 h-starved rats. 2. T_3 treatment increased hepatic lipogenesis in the fed but not the starved animals. Brown-fat lipogenesis was unaffected or slightly decreased by T_3 treatment of fed or starved rats. 3. Intragastric glucose feeding increased hepatic lipogenesis in control or T_3 -treated fed rats, but did not increase hepatic lipogenesis in starved control rats. Glucose feeding increased hepatic lipogenesis if the starved rats were treated with T_3 . Glucose feeding increased rates of brown-fat lipogenesis in all experimental groups. The effects of glucose feeding on liver and brown-fat lipogenesis were mimicked by insulin injection. 4. The increase in hepatic lipogenesis in T_3 -treated 48 h-starved rats after intragastric glucose feeding was prevented by short-term insulin deficiency, but not by (–)-hydroxycitrate, an inhibitor of ATP citrate lyase. The increase in lipogenesis in brown adipose tissue in response to glucose feeding was inhibited by both short-term insulin deficiency and (–)-hydroxycitrate. 5. The results tend to preclude pyruvate kinase and acetyl-CoA carboxylase as the sites of interaction of insulin and T_3 in the regulation of hepatic lipogenesis in 48 h-starved rats. Other potential sites of interaction are discussed.

The increased rates of lipogenesis in livers of hyperthyroid rats compared with euthyroid rats may result from increased concentrations of lipogenic enzymes, e.g. acetyl-CoA carboxylase (EC 6.4.1.2) and/or increased provision of lipogenic precursors (see Sugden *et al.*, 1981b). The effect of thyroid hormone is slow on onset: in marked contrast, insulin has acute stimulatory effects on hepatic lipogenesis (reviewed by Geelen & Beynen, 1981). The stimulatory effects of insulin on hepatic lipogenesis *in vivo* in the rat can be observed after the oral administration of glucose, a treatment which produces high plasma concentrations of insulin without the hypoglycaemia associated with insulin injection (Agius & Williamson, 1980; Sugden *et al.*, 1981a, 1982a; see Stansbie *et al.*, 1976). Glucose feeding also increases lipogenesis *in vivo* (measured by incorporation of 3H from 3H_2O into fatty acid) in white and brown adipose tissue of the rat (Agius & Williamson, 1980; Sugden *et al.*, 1981a, 1982a). Again, the effects of glucose feeding on lipogenesis are mediated by insulin, since increased lipogenesis

is not observed if the rats are diabetic (Agius & Williamson, 1980; Sugden *et al.*, 1981a, 1982a).

We and others have observed that, whereas intragastric glucose feeding increases lipogenesis in brown adipose tissue of both fed and starved euthyroid rats, hepatic lipogenesis is increased only if the rats are fed (Agius & Williamson, 1981; Sugden *et al.*, 1981a, 1982a). Hepatic lipogenesis is, however, increased after glucose feeding of starved hyperthyroid rats (Sugden *et al.*, 1981b). The present work investigates the means by which hepatic lipogenesis is increased by glucose feeding in starved hyperthyroid (T_3 -treated) rats. We also describe the effects of T_3 treatment on lipogenesis in brown adipose tissue.

Experimental

Materials

Sources of materials were as described by Sugden *et al.* (1982b). (–)-Hydroxycitrate was generously given by Dr. A. C. Sullivan, Hoffman–LaRoche, Nutley, NJ, U.S.A.

Animals

Female albino Wistar rats (150–200 g) were subjected to a 12 h-light/12 h-dark cycle (light period

Abbreviation used: T_3 , L-tri-iodothyronine.

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starting at 08:00h). Rats were either fed *ad libitum* on standard rodent diet [BP Nutrition (U.K.) Ltd.] or starved for 48 h in grid-bottomed cages. Water was supplied *ad libitum*.

Animals were made hyperthyroid by subcutaneous injection of T_3 (100 μ g/100 g body wt. per day in 10 mM-NaOH/0.03% bovine serum albumin) for 2 consecutive days. Control animals were injected with an equivalent volume of 10 mM-NaOH/0.03% bovine serum albumin. Animals were killed on day 3. T_3 treatment produced moderate (6–10%) hyperphagia in the fed animals. T_3 treatment did not affect weight loss during the 2-day starvation period [mean weight loss ($\pm$ S.E.M., $n = 12$): control, 22.1 ± 0.8 g; T_3 -treated, 22.1 ± 1.1 g].

Acute diabetes was produced by the intravenous injection (under light diethyl ether anaesthesia) of streptozotocin (60 mg/kg body wt., in 0.1 M-sodium citrate buffer, pH 4.5) 1.5 h before the start of the experiment. Such treatment resulted in marked hyperglycaemia and insensitivity of brown-fat lipogenesis to glucose feeding (see Table 2), and thus presumably insulin deficiency (Schein *et al.*, 1971; see Agius & Williamson, 1980).

Measurement of lipogenesis

Experiments were started between 09:00 and 09:30 h. Glucose (2 mmol/100 g body wt., 2 M solution) or water (1.5 ml) was administered intragastrically as described by Sugden *et al.* (1981a). Insulin (2 units) or 0.15 M-NaCl was injected subcutaneously 15 min after intragastric feeding. Injection of 0.15 M-NaCl had no effects on rates of lipogenesis *in vivo* in liver or brown adipose tissue. (–)-Hydroxycitrate (0.85 mmol/kg body wt., in 0.9% NaCl, pH 7.4) or 0.15 M-NaCl was injected intraperitoneally 55 min after intragastric feeding. $^3\text{H}_2\text{O}$ (5 mCi in 0.5 ml of 0.15 M-NaCl) was injected intraperitoneally 60 min after intragastric feeding. The rats were dissected 60 min later (i.e. 120 min after intragastric feeding) as described by Agius & Williamson (1980). An arterial blood sample was withdrawn at this time for the determination of plasma $^3\text{H}_2\text{O}$ specific radioactivity and blood glucose (Slein, 1963). Tissue lipid was saponified and fatty acid extracted as described by Stansbie *et al.* (1976). The rates of lipogenesis are expressed as μ g-atoms of ^3H incorporated into tissue fatty acid/h per g wet wt. of tissue.

Enzyme measurements

Preparation of tissue extracts. Rats were under pentobarbital (60 mg/kg) anaesthesia throughout. Insulin (0.4 unit) or 0.15 M-NaCl was injected into the portal vein, as described by Taunton *et al.* (1974), 5 min after administration of pentobarbital. The livers were freeze-clamped *in situ* 15 min later (see Taunton *et al.*, 1974). The tissue was stored frozen at -70°C until preparation of tissue extracts

for enzyme assay. Samples were processed within 7 days.

(i) **Pyruvate kinase (EC 2.7.1.40).** Preparation of L pyruvate kinase (liver isoenzyme) was as described by Feliú *et al.* (1977). The activity of L pyruvate kinase was measured at phosphoenolpyruvate concentrations of 0.15 mM and 5 mM as described by Feliú *et al.* (1976). The reaction was started by the addition of extract or phosphoenolpyruvate and monitored by the decrease in A_{340} . A unit of pyruvate kinase produces 1 μ mol of pyruvate/min at 25°C .

(ii) **Acetyl-CoA carboxylase.** Frozen samples were homogenized (two periods of 5 s) in 4 vol. of ice-cold extraction medium [250 mM-sucrose, 200 mM-Tris, 20 mM-Mes (4-morpholine-ethanesulphonic acid), 2 mM-EGTA, 1 mM-dithiothreitol, pH 7.4] with a Polytron tissue homogenizer fitted with probe PT 7. After homogenization, fatty acid-free albumin was added to the extracts to give a final albumin concentration of 10 mg/ml. The extracts were then centrifuged for 30 s in an Eppendorf 5412 centrifuge. The infranant (about 1 ml) was filtered through Sephadex G-25 (10 vol.). Lactate dehydrogenase activity was measured to estimate the dilution of the extract caused by gel filtration. Portions of the filtrate were either assayed for acetyl-CoA carboxylase immediately or incubated for 20 min at 30°C with 20 mM-potassium citrate, and then assayed (as described by Halestrap & Denton, 1974). Appropriate controls were included. The initial activity (denoted as 'I') was that measured in the absence of citrate immediately after preparation of the homogenate. 'C' activity refers to the activity of the enzyme measured with 20 mM-potassium citrate in the assay medium after preincubation with citrate. The percentage of the enzyme in the active form is given by $100 \times \text{the I/C activity ratio}$.

(iii) **Carnitine acetyltransferase (EC 2.3.1.7).** This was measured at 30°C by the method of Sugden & Newsholme (1977). Liver was homogenized with a Polytron with 5 vol. of extraction medium (20 mM-potassium phosphate/2 mM-EDTA, pH 7.4), and 10 μ l of the homogenate was used for assay. The final volume of the assay was 0.1 ml. The reaction was linear with respect to time for up to 10 min.

Expression of results. Statistical significance of differences was assessed by Student's unpaired *t* test. Results are given as means $\pm$ S.E.M. for the numbers of observations given in parentheses.

Results

Effects of T_3 treatment on liver and brown-fat lipogenesis

As previously reported (Sugden *et al.*, 1981b), treatment of fed rats with T_3 for 2 days increased hepatic lipogenesis (by 194%). T_3 treatment only slightly increased hepatic lipogenesis when the rats

were starved for 48 h, and did not increase lipogenesis in livers of diabetic 48 h-starved rats (Table 1; see also Sugden *et al.*, 1981b). T_3 treatment did not increase lipogenesis in brown fat of either fed or starved rats: indeed, rates of lipogenesis in brown fat of fed rats were slightly (30%), but not significantly, decreased after T_3 treatment (Table 1). As expected, starvation decreased rates of lipogenesis *in vivo* in liver and brown fat of both control (euthyroid) and T_3 -treated rats (Table 1).

Effects of intragastric glucose feeding on lipogenesis in control and T_3 -treated rats

Glucose feeding increased hepatic lipogenesis in control and T_3 -treated fed rats by 61% and 36% respectively (Table 1). Brown-adipose-tissue lipogenesis was increased by glucose feeding by 103% in control fed rats and by 87% in T_3 -treated fed rats. Glucose-stimulated rates of brown-fat lipogenesis were less in T_3 -treated than in control fed rats (Table 1). The observed decrease in lipogenic rates in brown adipose tissue of T_3 -treated fed rats intubated with glucose (34%) is similar in magnitude to that observed with T_3 -treated fed rats intubated with water.

Glucose feeding did not increase hepatic lipogenesis in 48 h-starved control rats, although brown-fat lipogenesis was increased by 400% (Table 1). Increased rates of lipogenesis in both liver and brown fat were observed after glucose feeding of T_3 -treated 48 h-starved rats (increases of 55% and 370% respectively). Increased lipogenesis in brown fat of euthyroid and hyperthyroid starved rats, or in livers of hyperthyroid starved rats, were not observed if the rats were made diabetic by intravenous injection of streptozotocin 1.5 h before the intragastric glucose load.

Effects of insulin on liver and brown-fat lipogenesis in 48 h-starved acute-diabetic rats

The preceding results suggested that increased hepatic lipogenesis observed after glucose feeding of T_3 -treated 48 h-starved rats was the result of increased plasma [insulin]. We therefore investigated the effects of insulin treatment on lipogenesis of control and T_3 -treated 48 h-starved acute-diabetic rats (Table 2). Blood glucose concentrations were measured to test the efficacy of insulin injection. Insulin injection decreased blood [glucose] and increased brown-adipose-tissue lipogenesis (by 850%) in control (euthyroid) diabetic rats, but hepatic lipogenesis was not increased (Table 2). In addition to lowering the blood [glucose] of diabetic rats, injection of insulin into T_3 -treated diabetic rats increased lipogenesis in both brown adipose tissue and liver (Table 2), by 1666% and 153% respectively. The effects of glucose feeding on hepatic and brown-fat lipogenesis in 48 h-starved T_3 -treated animals are therefore mediated by an increase in blood [insulin].

Effects of T_3 treatment on hepatic pyruvate kinase activities in 48 h-starved rats

One consequence of glucose feeding of starved euthyroid animals is increased hepatic gluconeogenesis (see Hers, 1981). Glycogen formation from gluconeogenesis is suppressed in hyperthyroid rats (Okajima & Ui, 1979). Since gluconeogenic precursors (predominantly lactate and alanine in the 48 h-starved rat) are the substrates for the hepatic syntheses of both glycogen and fatty acids (Hems *et al.*, 1972; Salmon *et al.*, 1974; Hopkirk & Bloxham, 1977), it was considered possible that diversion of potential substrates from glycogen

Table 1. *Effect of intragastric feeding of glucose and T_3 treatment on rates of lipogenesis in vivo in liver and brown adipose tissue of fed or 48 h-starved rats*

For experimental details see the text. Rates of lipogenesis are expressed as μg -atoms of ^3H incorporated into fatty acid/h per g wet wt. of tissue. Values that are significantly different from each other are shown: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ for differences between rats not treated with T_3 or rats treated with T_3 in any experimental group; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ for differences between water-fed and glucose-fed rats.

State of rats	Liver		Brown adipose tissue	
	Water-fed rats	Glucose-fed rats	Water-fed rats	Glucose-fed rats
Fed ad libitum				
Control	10.1 $\pm$ 1.3 (5)	16.3 $\pm$ 2.1 (5)*	102.8 $\pm$ 14.6 (5)	208.7 $\pm$ 21.7**
T_3 -treated	29.7 $\pm$ 0.9 (5) $\dagger\dagger\dagger$	40.3 $\pm$ 4.2 (5) $\dagger\dagger\dagger$	71.8 $\pm$ 12.9 (5)	134.3 $\pm$ 17.6 $\dagger$
48 h-starved				
Control	3.5 $\pm$ 0.4 (7)	3.9 $\pm$ 0.3 (12)	23.5 $\pm$ 2.4 (7)	118.4 $\pm$ 12.3 (7)***
T_3 -treated	5.0 $\pm$ 0.2 (10) $\dagger\dagger$	7.6 $\pm$ 0.8 (10) $\dagger\dagger\dagger$	28.0 $\pm$ 4.3 (7)	103.8 $\pm$ 9.2 (11)***
Diabetic	5.8 $\pm$ 0.9 (8)	4.3 $\pm$ 0.1 (4)	10.0 $\pm$ 1.6 (9)	16.7 $\pm$ 1.4 (4)*
T_3 -treated diabetic	5.2 $\pm$ 0.4 (4)	4.3 $\pm$ 0.4 (5)	7.6 $\pm$ 0.2 (4)	9.6 $\pm$ 1.6 (5) $\dagger$

Table 2. *Effects of insulin treatment on rates of lipogenesis in vivo in liver and brown adipose tissue of glucose-fed 48 h-starved diabetic rats*

For experimental details see the text. Values that are significantly different from each other are shown: $\dagger\dagger P < 0.02$ for differences between control and T_3 -treated rats; $***P < 0.001$ for differences between NaCl-injected or insulin-injected rats.

	Blood glucose ($\mu\text{mol/ml}$)	Lipogenesis (μg -atoms of ^3H incorporated into fatty acid/h per g wet wt. of tissue)	
		Liver	Brown adipose tissue
Control			
NaCl-injected	17.1 ± 0.3 (4)	4.3 ± 0.1 (4)	16.7 ± 1.4 (4)
Insulin-injected	2.6 ± 0.2 (5) $***$	4.8 ± 0.7 (5)	158.9 ± 8.6 (5) $***$
T_3 -treated			
NaCl-injected	17.5 ± 1.6 (6)	4.3 ± 0.4 (5)	9.6 ± 1.6 (5) $\dagger\dagger$
Insulin-injected	3.0 ± 0.2 (5) $***$	10.9 ± 1.2 (5) $***\dagger\dagger$	169.5 ± 8.3 (5) $***$

Table 3. *Activities of pyruvate kinase L and acetyl-CoA carboxylase in livers from 48 h-starved control and T_3 -treated rats*

For experimental details see the text. Pyruvate kinase activities are expressed as units/mg of protein and acetyl-CoA carboxylase activities as nmol/min per g wet wt. The number of rats used for each determination is given in parentheses.

	Pyruvate kinase			Acetyl-CoA carboxylase		
	$v_{0.15}$	V	$100 \times v_{0.15}/V$	I	C	$100 \times I/C$
Control						
NaCl-injected	0.15 ± 0.02	0.47 ± 0.04	31.2 ± 2.3 (8)	9 ± 1	204 ± 25	4.9 ± 1.0 (8)
Insulin-injected	0.15 ± 0.03	0.44 ± 0.04	32.1 ± 3.5 (8)	13 ± 1	209 ± 22	7.0 ± 2.0 (7)
T_3 -treated						
NaCl-injected	0.12 ± 0.01	0.41 ± 0.02	29.3 ± 2.7 (8)	10 ± 2	180 ± 26	5.9 ± 1.0 (8)
Insulin-injected	0.16 ± 0.03	0.47 ± 0.05	31.7 ± 3.3 (8)	12 ± 3	164 ± 16	8.3 ± 2.0 (7)

formation towards fatty acid synthesis occurred in the livers of T_3 -treated rats (Sugden *et al.*, 1981b). For lactate and alanine to be used for hepatic glycogen synthesis rather than lipogenesis, flux through pyruvate kinase must be less than flux through phosphoenolpyruvate carboxykinase (EC 4.1.1.32) and pyruvate carboxylase (EC 6.4.1.1). In liver, pyruvate kinase is interconverted between active (dephosphorylated) and inactive (phosphorylated) forms (see Hue *et al.*, 1981). Phosphorylated pyruvate kinase is deinhibited by insulin *in vitro* (Hue *et al.*, 1981), and in meal-fed rats rates of lipogenesis correlate with total amounts and activation state of the enzyme (Hopkirk & Bloxham, 1979). For these reasons it seemed possible that pyruvate kinase was a potential site where insulin and T_3 could interact in the control of lipogenesis. We therefore investigated the effects of T_3 treatment of 48 h-starved rats on hepatic pyruvate kinase activities measured at saturating (5 mM) and subsaturating (0.15 mM) phosphoenolpyruvate concentrations (activities denoted by V and $v_{0.15}$

respectively) after injection of insulin into the portal vein. A change in activation state results in a change in $v_{0.15}$, but not in V (increased activation is associated with increased $v_{0.15}/V$). Hepatic pyruvate kinase activity was also measured at 0.15 mM-phosphoenolpyruvate with addition of fructose 1,6-bisphosphate (5 μM), which activates the phosphorylated inactive enzyme. T_3 treatment had no effect on V or $v_{0.15}$ (Table 3). The $v_{0.15}/V$ was about 0.3 in both T_3 -treated and control groups. This indicates that a substantial fraction of the enzyme was present in the inactive (fructose 1,6-bisphosphate-dependent) form. In support of this, fructose 1,6-bisphosphate addition increased $v_{0.15}$ (and thus $100 \times v_{0.15}/V$) in all experimental groups to 82.3 ± 1.9 ($n = 32$). Insulin injection was without effect on either $v_{0.15}$ or V . Similar results were obtained when the starved animals were made insulin-deficient by the intravenous injection of streptozotocin 1.5 h before tissue sampling (results not shown). In these animals the $100 \times v_{0.15}/V$ values were decreased [control (7), 22.3 ± 2.8 ; $+T_3$ (8), 24.0 ± 2.1].

Effects of (–)-hydroxycitrate

Part of the stimulatory effect of T_3 on hepatic lipogenesis *in vivo* may result from increased supply of lactate and alanine to the liver (see Sugden *et al.*, 1981b). Insulin would not be expected to increase further the precursor supply to the liver: release of lactate from muscle would be decreased. The effects of insulin on hepatic lipogenesis in T_3 -treated 48 h-starved rats are therefore presumably a result of increased precursor utilization. Lactate and alanine are utilized for fatty acid synthesis after their conversion into mitochondrial acetyl-CoA. This is condensed with oxaloacetate to form citrate, which is transferred to the cytosol and cleaved by ATP citrate lyase (EC 4.1.3.8) to oxaloacetate and acetyl-CoA. Since our data indicated that pyruvate kinase was unlikely to be a regulatory site, we investigated the effects of (–)-hydroxycitrate, an inhibitor of ATP citrate lyase (Watson *et al.*, 1969), on rates of hepatic lipogenesis in control and T_3 -treated 48 h-starved rats. The aim of the experiments was to establish whether increased flux through ATP citrate lyase was a prerequisite for stimulation by insulin of hepatic lipogenesis in T_3 -treated starved rats.

(–)-Hydroxycitrate increased rates of lipogenesis *in vivo* in water-fed control (euthyroid) rats by 40% (Table 4). A similar trend was observed with glucose-fed control rats, but the results were not statistically significant. (–)-Hydroxycitrate also increased brown-fat lipogenesis in water-fed control rats, but it prevented the increases in brown-fat lipogenesis brought about by glucose feeding (Table 4). (–)-Hydroxycitrate increased hepatic lipogenesis in T_3 -treated rats given either water (by 45%) or glucose (by 42%). Stimulatory effects of glucose feeding on hepatic lipogenesis were still observed if hyperthyroid rats were treated with (–)-hydroxycitrate, but glucose feeding did not increase brown-

fat lipogenesis in such animals (Table 4). Injection of insulin into glucose-fed (–)-hydroxycitrate-treated euthyroid rats had no effect on hepatic lipogenesis, but increased brown-fat lipogenesis (46.5%), despite the resulting hypoglycaemia (Table 4). The rates of hepatic lipogenesis in similarly treated hyperthyroid rats were 240% of those of the control rats, whereas brown-fat lipogenesis was decreased by 41%. Blood [glucose] in the control and hyperthyroid rats treated with (–)-hydroxycitrate and insulin was 2.1 ± 0.2 (4) and 2.7 ± 0.7 (4) mM respectively (not significant).

Effects of T_3 treatment on hepatic acetyl-CoA carboxylase activities in 48 h-starved rats

It is widely accepted that acetyl-CoA carboxylase is regulatory for hepatic lipogenesis, and its activity can be altered by hormones (see, e.g., Lane *et al.*, 1974). Acetyl-CoA carboxylase activity is regulated through alterations in enzyme concentration (e.g. Allmann *et al.*, 1965; Muto & Gibson, 1970), by the actions of effector molecules, e.g. citrate or long-chain acyl-CoA esters, and by phosphorylation and dephosphorylation (Geelen & Beynen, 1981). Preincubation of tissue extracts with the activator citrate is accompanied by polymerization and dephosphorylation (see Zammit & Corstorphine, 1982) and consequently activation of the enzyme. Accordingly, activity measurements made after citrate treatment (denoted by 'C') estimate total enzyme activity (i.e. enzyme concentration). The activity of the enzyme *in vivo* can be assessed by measuring initial ('I') activities in the absence of citrate activation. The fraction of enzyme in the active form is given by I/C. This is the parameter affected by hormones acting acutely by changing the phosphorylation and/or aggregation states of the enzyme, e.g. glucagon, insulin. To assess whether the stimulatory effects of insulin on hepatic lipogenesis

Table 4. *Effects of (–)-hydroxycitrate on rates of lipogenesis in vivo in liver and brown adipose tissue of 48 h-starved rats*

For experimental details see the text. Rates of lipogenesis are expressed as μg -atoms of ^3H incorporated into fatty acid/h per g wet wt. of tissue. Values that are significantly different from each other are shown: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ for differences between control and T_3 -treated rats; $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$ for differences between rats not treated with (–)-hydroxycitrate and those treated with (–)-hydroxycitrate.

State of rats	Liver		Brown adipose tissue	
	Water-fed rats	Glucose-fed rats	Water-fed rats	Glucose-fed rats
Control	3.5 ± 0.4 (7)	3.9 ± 0.3 (12)	23.5 ± 2.4 (7)	118.4 ± 12.3 (7)
T_3 -treated	4.9 ± 0.3 (4)	7.6 ± 0.8 (10) $\dagger\dagger\dagger$	28.0 ± 4.3 (7)	103.8 ± 9.2 (11)
Control + (–)-hydroxycitrate	5.0 ± 0.3 (4)*	4.5 ± 0.4 (3)	35.9 ± 2.1 (3)*	42.1 ± 2.0 (3)***
T_3 -treated + (–)-hydroxycitrate	7.1 ± 0.5 (8)** $\dagger$	10.8 ± 0.8 (10)** $\dagger\dagger\dagger$	27.1 ± 1.5 (8) $\dagger\dagger\dagger$	26.1 ± 1.2 (6)*** $\dagger\dagger\dagger$
Control + insulin + (–)-hydroxycitrate	—	3.7 ± 0.3 (4)	—	61.7 ± 5.6 (4)
T_3 -treated + insulin + (–)-hydroxycitrate	—	12.6 ± 0.9 (4) $\dagger\dagger\dagger$	—	36.5 ± 1.9 (4) $\dagger\dagger$

of starved T_3 -treated rats could be correlated with activation of liver acetyl-CoA carboxylase, we measured I and C activities in freeze-clamped livers after injection of 0.15M-NaCl or insulin into the portal vein (Table 3). Initial activities (I) were measured immediately after extraction, after filtration through Sephadex G-25 to remove pyruvate (Davies *et al.*, 1982). As indicated by Davies *et al.* (1982), this procedure eliminates the possibility that any observed stimulation by insulin could be the result of interference with the assay by pyruvate carboxylase. Initial activities were low in both control and T_3 -treated groups (Table 3). Incubation of the filtered liver extracts with potassium citrate resulted in a several-fold activation of the enzyme (Table 3). I and C activities in extracts of livers from the T_3 -treated starved rats were not significantly different from those found in extracts of livers from control (euthyroid) animals. Insulin did not increase the I/C ratio in extracts of livers from either control or T_3 -treated starved rats. I/C activity ratios ($\times 100$) for acetyl-CoA carboxylase in liver extracts from acute-diabetic 48h-starved rats were not significantly different from those in liver extracts from non-diabetic animals not treated with insulin [control (7), 5.3 ± 0.9 ; T_3 -treated (8), 4.2 ± 0.4].

Discussion

Glucose feeding of fed euthyroid rats increases liver and brown-fat lipogenesis (measured *in vivo* by using $^3\text{H}_2\text{O}$ incorporation) via an increase in blood [insulin] (Agius & Williamson, 1980; Sugden *et al.*, 1981a, 1982a). T_3 treatment of fed animals also increases hepatic lipogenesis (Roncari & Murthy, 1975; Gnoni *et al.*, 1980; Sugden *et al.*, 1981b), and the stimulatory effects of glucose feeding and T_3 treatment are additive (Sugden *et al.*, 1981b; see also Table 1). In contrast, the present results show that T_3 treatment of fed rats does not increase net lipogenesis in brown fat, and, although glucose feeding of hyperthyroid rats increases lipogenesis in this tissue, the observed rates are less than those of glucose-fed euthyroid rats. These findings are of interest, since there is a general agreement that thyroid hormones stimulate lipogenesis in white fat (e.g. Diamant *et al.*, 1972; Baquer *et al.*, 1976; Gnoni *et al.*, 1980; but see Roncari & Murthy, 1975). The metabolic fate of the fatty acids synthesized *de novo* in brown fat is not known, and the slight inhibition of net lipogenesis found in hyperthyroid-rat brown fat may reflect increased fatty acid release from the tissue; adipose-tissue lipolysis is increased in hyperthyroid animals (Goodman & Bray, 1966). Alternatively, since thyroid hormones stimulate brown-fat respiration (see Nicholls, 1979), newly synthesized fatty acids may be catabolized within the tissue.

The effects on brown-fat lipogenesis of T_3 treatment or glucose feeding of 48h-starved rats are similar to those found if the rats are fed. However, contrary to results obtained with fed rats, these treatments do not individually increase lipogenesis to any great extent in livers of 48h-starved rats (Tables 1 and 2). The stimulatory effects of glucose feeding on brown-fat lipogenesis in 48h-starved control and T_3 -treated rats, and on hepatic lipogenesis in 48h-starved T_3 -treated rats, are insulin-mediated, since they are not observed if the rats are diabetic, and are mimicked by insulin injection. It therefore appears that T_3 treatment of 48h-starved rats restores the sensitivity to insulin of hepatic lipogenesis that is lost on starvation of euthyroid rats. Two questions are posed. How does insulin increase lipogenesis in livers of starved hyperthyroid animals? How does T_3 exert its effects? Our results exclude hepatic pyruvate kinase as the control site. A key observation is that whereas the increases in lipogenesis in response to insulin in brown fat of both control and hyperthyroid 48h-starved rats are blocked by (–)-hydroxycitrate, the increases in lipogenesis in livers of starved hyperthyroid rats are not. The effects of insulin on brown-fat lipogenesis are related to increased utilization of glucose (via citrate) for rat synthesis (see Sugden *et al.*, 1982a), and thus inhibition of brown-fat lipogenesis by (–)-hydroxycitrate is not unexpected. However, since the increased rates of hepatic lipogenesis observed after insulin treatment of 48h-starved hyperthyroid rats are not inhibited by (–)-hydroxycitrate, flux through ATP citrate lyase is not rate-limiting in this tissue. One possible explanation is that in liver there is a rate-limiting step in lipogenesis distal to ATP citrate lyase, flux through which is increased by insulin. Utilization of cytoplasmic acetyl-CoA for lipogenesis depends on the activities of acetyl-CoA carboxylase and fatty acid synthase and the availability of NADPH. Thyroid hormones increase the concentration (i.e. maximal activity) of acetyl-CoA carboxylase in livers of fed rats, the enzyme is insulin-activated (see Geelen & Beynen, 1981), and in brown fat there are parallel changes in lipogenic rates and activation state of acetyl-CoA carboxylase in response to alterations of the plasma [insulin] (McCormack & Denton, 1977). However, T_3 treatment of starved rats did not increase total hepatic acetyl-CoA carboxylase activity, nor was there any significant change in the percentage of the enzyme in its activated form. Insulin treatment of control or T_3 -treated starved animals was also without effect. Others have made similar observations for livers from euthyroid animals (Stansbie *et al.*, 1976; Munday & Williamson, 1982). It appears that in starved animals the hepatic enzyme is not susceptible to short-term regulation by insulin. The low I/C ratio indicates that the enzyme is

predominantly in the inactive form (see also Zammit & Corstorphine, 1982) and, in support of this, rates of hepatic lipogenesis *in vivo* in 48h-starved rats were increased by (–)-hydroxycitrate (Table 4), which may activate acetyl-CoA carboxylase in liver. The present results do not, however, exclude the possibility that increased lipogenesis in response to insulin in livers of starved hyperthyroid (but not euthyroid) rats results from increased cytoplasmic concentrations of citrate, which can also activate the enzyme (see Geelen & Beynen, 1981). As the maximal activity of fatty acid synthase is about 10-fold higher than the initial activities of acetyl-CoA carboxylase for livers of starved rats (Zammit & Corstorphine, 1982), the enzyme is unlikely to be rate-limiting for lipogenesis unless its activity *in vivo* is severely restricted. Thyroid hormones do, however, increase the activities of fatty acid synthase and acetyl-CoA carboxylase in parallel in livers of fed and diabetic rats (e.g. Das, 1980). Similarly, although in starved-refed rats the pentose phosphate pathway can produce sufficient NADPH to satisfy the requirements of fatty acid synthesis (see Røgnstad & Katz, 1979), the maximal activities of the dehydrogenases generating NADPH for lipogenesis (glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme) are increased in livers from hyperthyroid rats (Tata *et al.*, 1963; Szepesi & Freedland, 1969; Oppenheimer *et al.*, 1977).

If the increased rates of lipogenesis observed in response to insulin in livers of starved hyperthyroid rats are not due to increases in NADPH supply or activities of fatty acid synthase and/or acetyl-CoA carboxylase, an alternative (or additional) action of insulin may be to increase the provision of cytoplasmic acetyl-CoA. The lack of inhibition by (–)-hydroxycitrate suggests that, if mitochondrial acetyl-CoA is the precursor of the cytoplasmic acetyl-CoA used for lipogenesis, it leaves the mitochondria other than as citrate. As the stimulatory effect of insulin is specific for the T₃-treated rats, it would be envisaged either that insulin selectively stimulates mitochondrial acetyl-CoA production in T₃-treated (but not euthyroid) rats, or that efflux of mitochondrial acetyl-CoA is increased in T₃-treated rats. Possible mechanisms for acetyl-CoA efflux from the mitochondria might involve the carnitine acetyl- or acyl-transferases. We found that carnitine acetyltransferase was increased by 29% in livers of T₃-treated 48h-starved rats [control (7), $1.7 \pm 0.1 \mu\text{mol}$ of acetyl-CoA utilized/min per g wet wt.; T₃-treated (8), 2.2 ± 0.1 ; $P < 0.01$], and hepatic carnitine acyltransferase is also increased in hyperthyroidism (van Tol, 1971). An alternative explanation of the lack of an inhibitory effect of (–)-hydroxycitrate is that acetyl-CoA generated in the mitochondria is not used for lipogenesis. There

have been suggestions that acetate is a major precursor for hepatic lipogenesis in starved rats (Murthy & Steiner, 1973; see Triscari & Sullivan, 1977), but the source of acetate has not been defined. The lack of a marked stimulatory effect of thyroid hormones on hepatic lipogenesis in starved rats without glucose feeding would indicate that the generation of either mitochondrial acetyl-CoA or cytoplasmic acetate is rate-limiting, and is increased by insulin.

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