Effect of hyperthyroidism and hypothyroidism on lipid and carbohydrate metabolism of the perfused rat liver

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(Received 10 November 1980/Accepted 6 January 1981)

1. Livers from hyper- and hypo-thyroid male fed rats were perfused with whole blood and their metabolism was compared with euthyroid controls. 2. Hyperthyroid livers produced more bile than controls and hypothyroid livers produced less. 3. Glucose output by all livers was similar; glycogen declined only during perfusion of hyperthyroid livers. Lactate uptake increased in hyperthyroid but decreased in hypothyroid livers. These results may be explained by changes in oxidation of carbohydrate rather than in gluconeogenesis. 4. Secretion of triacylglycerol was decreased in hyperthyroid and not changed significantly in hypothyroid livers. 5. Fractional extraction of infused [1-¹⁴C]oleate was unaltered. Hyperthyroid livers oxidized more oleate to CO₂ and ketone bodies, esterified less and incorporated less into lipoproteins of $d < 1.006$. Hypothyroid livers oxidized and esterified oleate to the same extent as controls; their decreased O₂ consumption was due to diminished oxidation of other (non-lipid) substrates; ¹⁴C-labelled ketone-body formation was increased, but at the expense of ¹⁴CO₂ production. 6. Lipogenesis (measured with ³H₂O) was unaltered in hyperthyroid but was decreased in hypothyroid livers. Incorporation of ³H and ¹⁴C into triacylglycerol relative to phospholipid decreased in hyperthyroid and increased in hypothyroid livers. Cholesterol synthesis was similar in all perfusions. 7. During oleate infusion, the cytosolic redox state, as indicated by the perfusate [lactate]/[pyruvate] ratio, was decreased in hyperthyroid and increased in hypothyroid livers. No change in [3-hydroxybutyrate]/[acetoacetate] was detected. 8. The importance of relating the concentration of plasma non-esterified fatty acids to the interpretation of metabolic data obtained under differing thyroid status is emphasized.

In addition to its role in controlling the metabolic rate, thyroid hormone has been implicated in the regulation of the glycolytic (Hoch, 1962), gluconeogenic (Freedland & Krebs, 1967) and pentose phosphate (Glock *et al.*, 1956) pathways. It is also involved in the regulation of lipid metabolism (Masoro, 1962; Brown *et al.*, 1966; Greenbaum *et al.*, 1967) and modifies the redox state of cell compartments (Glock & McLean, 1955; Werner & Berry, 1974). As well as acting directly, thyroid hormone potentiates the action of lipolytic hormones such as glucagon, catecholamines and corticotropin and may enhance the action of insulin (Hoch, 1962).

Thyroid dysfunction is characterized by alterations in carbohydrate (Freinkel & Metzger, 1971), lipid (Nikkilä & Kekki, 1972) and lipo-

protein metabolism (Walton *et al.*, 1965), consequently changing the concentration and composition of plasma lipoproteins (Cornwell *et al.*, 1961). In hyperthyroid patients the turnover of low-density-lipoprotein apoprotein is increased (Walton *et al.*, 1965), and the plasma cholesterol concentration is decreased. Hypothyroidism in man is associated with an increase in plasma cholesterol, particularly in low-density lipoproteins and often with elevated plasma VLD lipoprotein, and there is a positive correlation with premature atherosclerosis (Chait, 1973). Although it is known that myxoedemic patients have decreased rates of low-density-lipoprotein clearance from the circulation (Walton *et al.*, 1963), it is not known with certainty if the elevated concentration of VLD lipoprotein is due to increased secretion by the liver or to decreased clearance by the tissues.

In the present paper, we report experiments on the perfused rat liver to investigate the role of thyroid

Abbreviation used: VLD lipoprotein, very-low-density lipoprotein ($d < 1.006$).

hormone in hepatic metabolism, particularly its involvement in the process of gluconeogenesis, oxidation, synthesis and esterification of fatty acids and the secretion of lipoproteins. A preliminary account of this work has been published (Laker & Mayes, 1978).

Materials and methods

Animals

Livers from euthyroid (controls), hyperthyroid and hypothyroid male Wistar rats were perfused. The animals, obtained from A. Tuck and Sons, Rayleigh, Essex, U.K., were kept under constant conditions (25°C, lighting from 09:00–01:00h) and ate Diet 86 (E. Dixon, Ware, Herts., U.K.) *ad lib.* for a minimum of 3 weeks before experimentation. Control liver donors continued on this diet until they attained 345–360g, the weight adopted for all perfusions.

In the absence of data on spontaneous hyperthyroidism in rats, it was decided to administer that dose of thyroid hormone considered by Oppenheimer *et al.* (1978) to just saturate the hepatic nuclear receptor sites, as reflected by the activities of mitochondrial *sn*-glycerol 3-phosphate dehydrogenase (EC 1.1.99.5) and cytosolic malate dehydrogenase (decarboxylating-NADP⁺; EC 1.1.1.40). Large fluctuations in blood hormone concentration, which result from parenteral administration, were avoided by adding thyroxine to the diet. Sodium L-thyroxine (7.5mg; Sigma Chemical Co., Poole, Dorset, U.K.) was dissolved in 50 μ l of 6M-KOH, diluted to 75 ml with water and poured evenly over 500g of the pellet diet, which was allowed to dry overnight (Tyler, 1971). Rats (330–340g) were fed thyroxine-treated pellets *ad lib.* for 7 days before perfusion. This treatment (approx. 420 μ g of thyroxine/rat per 24h) produced a typically hyperthyroid animal, but did not uncouple oxidation and phosphorylation (D. D. Tyler, unpublished work) or prevent growth. A hypothyroid state was induced by adding 6-n-propyl-2-thiouracil (Sigma Chemical Co.) to the drinking water (0.05%) of 320–345g rats fed on Diet 86 for 14 days before perfusion (Debons & Schwartz, 1961).

Six rats from each of the three treatment groups intended for liver perfusion were housed singly and their food, water consumption and weight were recorded. After treatment, the animals were anaesthetized with sodium pentobarbital (60mg/kg body wt.; Abbott Laboratories, Queenborough, Kent, U.K.) and samples were taken to determine non-esterified fatty acids, triacylglycerol and cholesterol in serum, and glycogen, triacylglycerol and cholesterol in the liver.

Liver perfusions

Since a change in thyroid status *in vivo* alters the

concentrations of some blood metabolites, and may affect not only the rate of hepatic blood flow but also the O₂ binding characteristics of haemoglobin (Hoch, 1974), a standard perfusate of whole blood from normal fed rats was used at constant flow rate and at constant *p*O₂ in all perfusions. This was prepared by defibrination followed by dialysis for 24h against a bicarbonate buffer (Krebs & Henseleit, 1932) containing half the specified concentration of Ca²⁺, glucose (250mg/100ml) and the concentrations of plasma amino acids recorded by Bloxham (1971) for fed rats. Liver donor rats were anaesthetized between 09:00 and 10:00h with sodium pentobarbital. Livers were perfused *in situ* for 90min with 133ml of the dialysed blood, using the apparatus and techniques described by Mayes & Felts (1966), but with the substitution of a silicone-rubber gas exchanger. The liver temperature was maintained at 37±0.1°C, the *p*O₂ of the inflowing blood was stabilized at 12–13kPa (90–100mm Hg), and its rate of flow was 12ml/min (approx. 1.0ml/min per g of liver).

Oleic acid (141mg; Sigma Chemical Co.) labelled with 1 μ Ci of [1-¹⁴C]oleic acid (sp. radioactivity 50Ci/mol) was dissolved in 0.9ml of 1M-KOH and 1.8ml of water, and mixed at 40°C with 0.5g of crystalline bovine serum albumin (Armour Pharmaceutical Co., Eastbourne, Sussex, U.K.) dissolved in 7.3ml of 0.9% NaCl. This was introduced into the perfusate at time zero as a rapid (2.73min) priming infusion followed by a slower constant infusion to maintain a serum non-esterified fatty acid concentration of approx. 0.55 μ mol/ml. At 1.5min after the start of the fatty acid infusion, 5mCi of ³H₂O in 1.0ml of 0.9% NaCl was added to the perfusate with rapid mixing.

Analytical techniques

The *p*O₂ of the blood entering and leaving the liver was monitored continuously using O₂ electrodes (Radiometer, Copenhagen, Denmark). The O₂ consumption was calculated from the *p*O₂ and the flow rate, using the oxyhaemoglobin dissociation curve for rat blood.

Samples of blood were withdrawn at times zero and 90min and chilled in ice. The concentrations of perfusate glucose, lactate, pyruvate, acetoacetate and 3-hydroxybutyrate were estimated. Serum was separated from the blood perfusate by centrifugation and its content of non-esterified fatty acids was determined. Serum was divided into *d* < 1.006 and *d* > 1.006 fractions by ultracentrifugation. After 90min of perfusion, blood was flushed from the liver with 40ml of ice-cold 0.9% NaCl; a portion of liver was removed (0.5–1.0g) for estimation of glycogen and the remainder was used for lipid analysis. Both triacylglycerol and total cholesterol were determined in chloroform/methanol (2:1, v/v) extracts of

serum fractions and of liver samples. The constituent lipid classes were separated by t.l.c. and their radioactivity was determined by liquid-scintillation counting. Lipid extracts of serum and liver were hydrolysed, and rates of fatty acid and cholesterol synthesis were calculated from the ^3H incorporation into total fatty acids and total sterols. Radioactivity in respiratory CO_2 and in blood ketone bodies was measured, care being taken to eliminate radioactive contamination due to $^3\text{H}_2\text{O}$. Details of all materials and methods used in the above estimations have been described previously (Laker & Mayes, 1979). Results are reported as means $\pm$ S.E.M. and analysed statistically by Student's *t* test.

Results

The metabolic state of liver donors is shown in Table 1. These results summarize the effects of the treatment used to produce a hyper- or hypo-thyroid condition and also provide baseline data for the condition immediately before liver perfusion. Knowledge of growth rates during each treatment enabled liver donors to be chosen within the weight range 345–360 g after treatment.

Liver perfusions

The average liver weight in control perfusions (11.7 ± 0.6 g) did not differ significantly from that in the hyperthyroid and hypothyroid groups. The control rate of bile production was 1.0 ± 0.1 ml/h. Hyperthyroid livers produced significantly more bile, at a rate of 1.3 ± 0.1 ml/h ($P < 0.01$), an effect that was not found in hyperthyroid rats with bile-duct fistulae (Byers & Friedman, 1973) or in hyperthyroid livers perfused by Keyes & Heimberg

(1979). However, a decreased production by hypothyroid livers, which we found to be 0.6 ± 0.0 ml/h ($P < 0.01$), was observed by these investigators.

The O_2 consumption of perfused livers from control, hyperthyroid and hypothyroid animals was 4.5 ± 0.3 , 6.0 ± 0.3 and 3.3 ± 0.2 $\mu\text{mol/min}$ per g respectively. This represented an increase of 34% in hyperthyroid livers ($P < 0.01$) and a decrease of 25% in hypothyroid livers ($P < 0.02$), which established the thyroid status of the preparations, being comparable with the 30% increase or decrease of similarly treated rats recorded by Lindrös (1970).

Carbohydrate metabolism

Liver glycogen and perfusate glucose. After perfusion, the glycogen concentration of control livers was $2.0 \pm 0.4\%$ of the wet weight, and that of hyperthyroid livers was only $0.2 \pm 0.1\%$ ($P < 0.001$), whereas hypothyroid livers contained a similar concentration ($2.4 \pm 0.6\%$) to that of controls. Thus, during perfusion, glycogen concentrations did not change significantly in euthyroid or hypothyroid livers, but declined significantly ($P < 0.01$) in hyperthyroid livers (cf. Table 1). However, the perfusate glucose stabilized at a similar concentration in all three groups, being 10.1 ± 0.3 $\mu\text{mol/ml}$ in controls.

Pyruvate and lactate. The initial perfusate pyruvate concentration in euthyroid control perfusions was 0.4 ± 0.0 $\mu\text{mol/ml}$; in the hyperthyroid group it was significantly less, at 0.2 ± 0.1 $\mu\text{mol/ml}$; in the hypothyroid group it was greater, at 0.6 ± 0.0 $\mu\text{mol/ml}$. However, all perfusates showed a similar increment of 0.11 ± 0.03 μmol of pyruvate/ml during perfusion. The initial perfusate lactate concentration in perfusions of hyperthyroid livers (Fig. 1a) was half of the control value ($P < 0.001$), whereas that in

Table 1. Summary of effects of inducing either a hyperthyroid or hypothyroid condition in male rats

Eighteen rats (320–345 g) were divided into three groups. One group was maintained on the stock diet (controls), the second group was fed a thyroxine-treated pellet diet for 1 week (hyperthyroid) and the third group was given 6-n-propyl-2-thiouracil in the drinking water for 2 weeks (hypothyroid). At the end of the period of treatment, each rat was weighed, anaesthetized with pentobarbital and samples of blood and liver were analysed by methods described in the text. The results are expressed as means $\pm$ S.E.M., and were evaluated for significance against the control group, using Student's *t* test. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$. For hypothyroid animals, † refers to the final 7 days of treatment. Both non-esterified and esterified cholesterol were included in the determination of cholesterol.

	Control	Hyperthyroid	Hypothyroid
Increase in body wt. (g/7 days)†	23.8 ± 4.9	$10.0 \pm 1.8^*$	20.0 ± 4.7
Food consumption (g/7 days)†	182.5 ± 10.5	195.8 ± 8.5	$120.5 \pm 7.1^{***}$
Water consumption (ml/7 days)†	245.8 ± 24.9	187.5 ± 11.7	$119.4 \pm 11.1^{**}$
Liver glycogen (% wet wt.)	2.03 ± 0.12	$0.64 \pm 0.14^{***}$	2.47 ± 0.55
Plasma non-esterified fatty acids ($\mu\text{mol/ml}$)	0.40 ± 0.04	$0.66 \pm 0.09^*$	$0.20 \pm 0.03^{**}$
<i>d</i> < 1.006-lipoprotein triacylglycerol fatty acid ($\mu\text{mol/ml}$)	3.09 ± 0.26	3.08 ± 0.42	$1.33 \pm 0.03^{***}$
Liver triacylglycerol ($\mu\text{mol/g}$)	10.08 ± 0.97	$6.87 \pm 0.94^*$	12.12 ± 0.84
<i>d</i> < 1.006-lipoprotein cholesterol ($\mu\text{mol/ml}$)	0.36 ± 0.02	0.33 ± 0.05	$0.25 \pm 0.03^*$
<i>d</i> > 1.006-lipoprotein cholesterol ($\mu\text{mol/ml}$)	1.23 ± 0.09	1.24 ± 0.09	1.34 ± 0.14
Total serum cholesterol ($\mu\text{mol/ml}$)	1.59 ± 0.08	1.57 ± 0.14	1.59 ± 0.16
Liver cholesterol ($\mu\text{mol/g}$)	5.35 ± 0.23	5.32 ± 0.36	5.14 ± 0.32

Table 2. *Effect of thyroid status on the content and output of triacylglycerol and cholesterol of perfused livers*

Livers from fed rats were perfused for 90 min under conditions described in the text and infused continuously with non-esterified fatty acid (oleate). Control, no pretreatment; hyperthyroid, pretreated for 1 week with thyroxine in the diet; hypothyroid, pretreated for 2 weeks with 6-n-propyl-2-thiouracil in the drinking water. Results are expressed as means $\pm$ S.E.M. for seven perfusions. Significance of differences from the control group was evaluated by Student's *t* test: *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$.

		Control	Hyperthyroid	Hypothyroid
Production of triacylglycerol in VLD lipoproteins	(a) Total in 90 min (μmol of triacylglycerol fatty acids)	153.0 $\pm$ 26.7	67.5 $\pm$ 11.8*	107.3 $\pm$ 27.4
	(b) Triacylglycerol fatty acids ($\mu\text{mol/h}$ per g of liver)	8.9 $\pm$ 1.6	4.1 $\pm$ 0.7*	5.4 $\pm$ 1.3
Production of cholesterol in VLD lipoproteins	(a) Total in 90 min (μmol of cholesterol + cholesteryl ester)	9.6 $\pm$ 1.8	5.4 $\pm$ 1.6	3.6 $\pm$ 2.1
	(b) Cholesterol + cholesteryl ester ($\mu\text{mol/h}$ per g of liver)	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1*
Liver triacylglycerol (μmol of triacylglycerol fatty acids/g)		39.6 $\pm$ 6.6	26.8 $\pm$ 4.1	61.5 $\pm$ 6.1*
Liver cholesterol (μmol of cholesterol + cholesteryl ester/g)		5.3 $\pm$ 0.2	6.4 $\pm$ 0.2**	5.3 $\pm$ 0.1

Fig. 1. *Effect of thyroid status on lactate and pyruvate metabolism of perfused livers*

Initial (□) and final (▨) values after 90 min of perfusion are shown. All livers were from fed rats and were infused continuously during the experimental period with oleate complexed to serum albumin. Control, no pretreatment; hyperthyroid, pretreated for 1 week with thyroxine in the diet; hypothyroid, pretreated for 2 weeks with 6-n-propyl-2-thiouracil in the drinking water. The results are expressed as means $\pm$ S.E.M. for seven perfusions.

the hypothyroid group was greater ($P < 0.05$). Lactate concentration remained stable during perfusions of control and hyperthyroid livers, but increased substantially during perfusion of hypothyroid livers ($P < 0.001$). Consequently, the [lactate]/[pyruvate] ratio (Fig. 1b) in perfusates of hyperthyroid livers was decreased both initially ($P < 0.02$) and at the end of perfusion ($P < 0.001$) compared with controls, whereas the ratio in perfusates of hypothyroid livers was decreased initially ($P < 0.02$), but was increased after 90 min of oleate infusion ($P < 0.01$).

Lipid metabolism

Non-esterified fatty acids. The oleate infusion in control perfusions caused the concentration of serum non-esterified fatty acids to increase rapidly from $0.25 \mu\text{mol/ml}$ and stabilize at $0.54 \pm 0.03 \mu\text{mol/ml}$, this being intermediate between the mean values found in hyperthyroid and hypothyroid rats *in vivo* (Table 1). Both hyper- and hypo-thyroid livers maintained comparable serum non-esterified fatty acid concentrations throughout the perfusions, i.e. 0.51 ± 0.02 and $0.54 \pm 0.02 \mu\text{mol/ml}$ respectively, demonstrating that a change in hepatic thyroid status did not alter the fractional extraction of non-esterified fatty acid from serum.

Ketone bodies. Hyper- and hypo-thyroid livers maintained perfusate acetoacetate and 3-hydroxybutyrate concentrations within the control range, both at the start and termination of oleate infusion. Consequently, total ketone-body production did not differ significantly (control, $112.5 \pm 14.2 \mu\text{mol}$; hyperthyroid, $121.5 \pm 6.4 \mu\text{mol}$; hypothyroid, $107.5 \pm 6.2 \mu\text{mol}$). The perfusate [3-hydroxybutyrate]/[acetoacetate] ratio in each experimental group did not differ from that in the control group,

which was 2.2 ± 0.4 initially and 1.6 ± 0.2 after 90 min.

Lipoprotein secretion. The rate of secretion of triacylglycerol in VLD lipoproteins (Table 2) was decreased significantly in hyperthyroid livers ($P < 0.05$). The mean rate was less in hypothyroid livers, but the difference compared with controls was not statistically significant. Similar trends towards a decreased output of cholesterol in VLD lipoproteins in hyper- and hypo-thyroid livers were also noted (Table 2).

Liver lipid. Comparison of Tables 1 and 2 indicates that, during perfusion, triacylglycerol increased substantially in hypothyroid livers ($P < 0.05$) and cholesterol increased in hyperthyroid livers ($P < 0.01$).

Fig. 2. Effect of thyroid status on the course of oxidation of [$1\text{-}^{14}\text{C}$]oleate to $^{14}\text{CO}_2$ in perfused livers

Livers from fed rats were infused continuously for 90 min with [$1\text{-}^{14}\text{C}$]oleate complexed to bovine serum albumin to give a constant value of $0.55 \mu\text{mol}$ of non-esterified fatty acid per ml of serum. The data show the incorporation of ^{14}C into gaseous CO_2 , which was continuously absorbed into 2M-NaOH . ●, Control; ■, hyperthyroid, pretreated for 1 week with thyroxine in the diet; ▲, hypothyroid, pretreated for 2 weeks with 6-n-propyl-2-thiouracil in the drinking water. The results are expressed as means $\pm$ S.E.M. for seven perfusions.

Fate of ^{14}C -labelled non-esterified fatty acid

Oxidation of [$1\text{-}^{14}\text{C}$]oleate. In all perfusions the time course of ^{14}C incorporation into gaseous CO_2 was linear from 30 to 90 min (Fig. 2). There was a marked increase in oxidation by hyperthyroid livers ($P < 0.001$) and a decrease in perfusions of hypothyroid livers ($P < 0.05$ at 30 and 60 min; $0.05 < P < 0.1$ at 90 min). Whereas the increased oxidation in hyperthyroid livers was reflected by an increased incorporation of ^{14}C into ketone bodies (Fig. 3a), the decreased oxidation to $^{14}\text{CO}_2$ in hypothyroid livers was compensated by an increased incorporation into ketone bodies, so that total oxidation of oleate was similar to the control value.

Fig. 3. Effect of thyroid status on the conversion of [$1\text{-}^{14}\text{C}$]oleate to oxidized and esterified products by perfused livers

Livers from fed rats were infused for 90 min with [$1\text{-}^{14}\text{C}$]oleate complexed to serum albumin. Incorporation into total oxidized products (a) include: CO_2 and blood bicarbonate, □; total ketone bodies, ▨. Incorporation into total esterified products (b) include both lipids of the liver and serum lipoproteins. Control, no pretreatment; hyperthyroid, pretreated for 1 week with thyroxine in the diet; hypothyroid, pretreated for 2 weeks with 6-n-propyl-2-thiouracil in the drinking water. Mean values $\pm$ S.E.M. are shown, with the numbers of perfusions given in parentheses.

Incorporation of [$1\text{-}^{14}\text{C}$]oleate into liver lipids and serum lipoproteins. Total esterification of labelled serum non-esterified fatty acids (the sum of ^{14}C incorporated into liver lipids plus serum lipoproteins) was markedly decreased ($P < 0.001$) in perfusions of hyperthyroid livers (Fig. 3b). Although total esterification was greater in hypothyroid livers than in controls, the difference was not statistically significant. The decreased incorporation into lipids of hyperthyroid livers (Table 3) was accounted for, almost entirely, by the decreased incorporation into triacylglycerol. Incorporation into liver triacylglycerol during hypothyroid perfusions was significantly increased.

Decreased esterification by hyperthyroid livers was reflected by decreased incorporation of [$1\text{-}^{14}\text{C}$]oleate into VLD-lipoprotein total lipids (Table 3), the effect also being significant in the tri-

acylglycerol fraction, where most of the radioactivity was incorporated. Hypothyroid and control livers incorporated similar quantities of ^{14}C into VLD-lipoprotein lipids. The distribution of ^{14}C in the $d > 1.006$ serum lipoprotein lipids was similar in all perfusions, most of the radioactivity being present as non-esterified fatty acid derived from the oleate infusion.

Lipogenesis and cholesterolgenesis. Control livers synthesized $53.7\text{ }\mu\text{mol}$ of fatty acid in 90 min and the value for hyperthyroid livers was similar (Table 4). However, livers from hypothyroid rats incorporated significantly less ^3H into lipids both within the liver and in secreted VLD lipoproteins.

Triacylglycerol accounted for 60.9% of the total ^3H present in lipids of control livers, with 32.4% present in phospholipids, a distribution ratio of 1.90 ± 0.11 . The ratio was decreased to 0.81 ± 0.05

Table 3. *Effect of thyroid status on the incorporation of [$1\text{-}^{14}\text{C}$]oleate into liver and VLD-lipoprotein lipids during liver perfusion*

Livers were perfused under the conditions described in the text. [$1\text{-}^{14}\text{C}$]Oleate complexed to serum albumin was infused continuously for 90 min. Lipid extracts from liver (a) and from ultracentrifugally prepared VLD lipoproteins (b) were separated by t.l.c. and assayed for radioactivity. Control, no pretreatment; hyperthyroid, pretreated for 1 week with thyroxine in the diet; hypothyroid, pretreated for 2 weeks with 6-n-propyl-2-thiouracil in the drinking water. 'Minor lipids' include monoacylglycerol, diacylglycerol, cholesterol, cholesteryl esters and non-esterified fatty acids. Results are expressed as means $\pm$ S.E.M. for seven perfusions. Significance of differences from the control group was tested by Student's t test: *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

	Proportion of [$1\text{-}^{14}\text{C}$]oleate infused (%)		
	Control	Hyperthyroid	Hypothyroid
(a) Liver lipids			
Total	39.3 ± 1.8	$25.7 \pm 0.7^{***}$	44.6 ± 1.9
Triacylglycerol	26.5 ± 2.0	$13.0 \pm 1.0^{***}$	$33.6 \pm 2.5^*$
Phospholipid	11.5 ± 1.0	11.1 ± 0.4	9.3 ± 0.8
Minor lipids	1.3 ± 0.1	1.5 ± 0.1	1.7 ± 0.1
(b) VLD-lipoprotein lipids			
Total	20.6 ± 1.1	$14.0 \pm 1.1^{**}$	19.1 ± 1.3
Triacylglycerol	18.5 ± 1.0	$12.9 \pm 1.3^{**}$	18.2 ± 1.4
Phospholipid	0.8 ± 0.3	0.3 ± 0.3	0.1 ± 0.1
Minor lipids	1.1 ± 0.2	0.9 ± 0.1	0.8 ± 0.1

Table 4. *Effect of thyroid status on fatty acid synthesis of perfused livers*

Livers were perfused for 90 min with a constant infusion of [$1\text{-}^{14}\text{C}$]oleate and addition of $^3\text{H}_2\text{O}$. Control, no pretreatment; hyperthyroid, pretreated for 1 week with thyroxine in the diet; hypothyroid, pretreated for 2 weeks with 6-n-propyl-2-thiouracil in the drinking water. Hepatic fatty acid synthesis was calculated from the incorporation of ^3H , assuming that the average molecule of fatty acid synthesized has 31.7 carbon-bound hydrogen atoms, of which 13.3 are derived from $^3\text{H}_2\text{O}$ (Windmueller & Spaeth, 1969). The results, expressed as means $\pm$ S.E.M., were evaluated for significance against the control group, using Student's t test. The number of experiments in each group is given in parentheses. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$.

	Fatty acid synthesized (μmol)		
	Control (7)	Hyperthyroid (7)	Hypothyroid (5)
Present in liver lipid (A)	39.24 ± 7.63	44.07 ± 3.49	$20.69 \pm 2.61^*$
Present in VLD-lipoprotein lipid (B)	11.21 ± 1.86	13.18 ± 1.92	$4.01 \pm 0.49^{**}$
Present in serum $d > 1.006$ -lipoprotein lipid (C)	3.28 ± 7.63	3.85 ± 0.62	3.14 ± 0.41
Total synthesized (A + B + C)	53.74 ± 8.63	61.07 ± 4.58	$26.31 \pm 3.44^*$
Production rate ($\mu\text{mol/h}$ per g of liver)	3.06 ± 0.46	3.73 ± 0.25	$1.29 \pm 0.14^{**}$

in hyperthyroid livers ($P < 0.001$) and increased to 3.91 ± 0.50 in hypothyroid livers ($P < 0.01$). The distribution ratio of [^{14}C]oleate between hepatic triacylglycerols and phospholipids (see Table 3) showed a parallel trend, i.e. control, 2.44 ± 0.32 ; hyperthyroid, 1.20 ± 0.12 ($P < 0.01$); hypothyroid, 4.00 ± 0.81 ($P < 0.1$).

The percentage distribution of ^3H between the VLD-lipoprotein lipid classes was similar in all perfusions, most of the radioactivity being in triacylglycerol ($92.2 \pm 4.1\%$ in controls), with a small percentage in phospholipid (2.7 ± 3.5) and a negligible fraction in other lipid classes.

Control livers synthesized $5.77 \mu\text{mol}$ of cholesterol during perfusion for 90 min, a rate of $0.32 \pm 0.04 \mu\text{mol/h}$ per g of liver. The rates of cholesterol synthesis by livers both from thyroxine-treated rats (0.25 ± 0.03) and from propylthiouracil-treated rats (0.30 ± 0.07) were not significantly different.

Discussion

Thyroxine treatment did not alter hepatic glucose output since perfusate glucose concentrations were maintained at the control value. As, in addition, the decreased hepatic content of glycogen found *in vivo* declined further during perfusion, there was no indication of increased gluconeogenesis. Consequently, increased uptake of lactate by the hyperthyroid livers, indicated by the decreased perfusate concentration, must be ascribed to enhanced oxidation rather than gluconeogenesis. In contrast, the general increase in gluconeogenic enzyme activities (e.g. Bottger *et al.*, 1970) associated with thyroid hormone treatment has been assumed to result in augmented gluconeogenesis. Our use of whole-blood perfusates ensures physiological rates of O_2 consumption, allowing the channelling of lactate and pyruvate into oxidative pathways rather than gluconeogenesis.

Prior treatment with propylthiouracil did not alter hepatic glucose output since the perfusate glucose concentration was maintained at the control value, and also, comparison of glycogen concentrations showed no change during perfusion. Thus, no effect of hypothyroidism on gluconeogenesis was indicated. The decreased uptake of lactate and pyruvate probably results from less carbohydrate oxidation, due to the increased energy requirement of the hypothyroid liver. This may be mediated by a decrease in pyruvate dehydrogenase activity (Baquer *et al.*, 1976).

The altered concentration of serum non-esterified fatty acids that accompanied differences of thyroid status *in vivo* (Table 1) is likely to be due to changes in adipose tissue regulation of the output of non-esterified fatty acids (Challoner, 1970), since

their fractional extraction by the perfused liver was not altered in the hyper- or hypo-thyroid state.

Hyperthyroidism altered the partition of non-esterified fatty acids between oxidation and esterification, in favour of the former. Presumably, the increased requirement for respiratory fuel, as indicated by the increased O_2 consumption, was the primary stimulus in causing more oxidation of oleate to CO_2 and to ketone bodies and the decreased esterification followed secondarily. However, the increased plasma non-esterified fatty acid concentration, found in hyperthyroid animals, would allow more total extraction of fatty acids *in vivo*. This would not only meet the extra oxidative requirements but may be sufficient to enable the esterification rate to approach or even exceed the control value, in agreement with the fact that plasma VLD-lipoprotein triacylglycerol did not change markedly *in vivo* in our hyperthyroid rats. Increased total hepatic extraction may also explain the apparent increased production of lipoprotein triacylglycerol in thyrotoxic patients whose serum non-esterified fatty acid concentrations are double the normal value (Nikkilä & Kekki, 1972).

Total fatty acid oxidation of hypothyroid livers remained at the control value, suggesting that the decreased O_2 consumption was at the expense of carbohydrate oxidation, as also indicated by the decreased lactate uptake. However, the increased oxidation of fatty acids to ketone bodies and compensatory decreased oxidation to CO_2 may be a device to minimize ATP production per mol of fatty acid oxidized, a metabolic pattern observed previously in livers from starving rats (Mayes & Felts, 1967).

The relative decrease in incorporation of [^{14}C]oleate into liver triacylglycerol compared with its incorporation into VLD-lipoprotein triacylglycerol by hyperthyroid livers may be explained by an increased turnover of hepatic triacylglycerols due to increased rates of both esterification and lipolysis (Glenny & Brindley, 1978). Production of VLD lipoprotein was related to the balance between oxidation and esterification, the decreased release of triacylglycerol from hyperthyroid livers resulting from diversion of long-chain fatty acids into oxidative pathways. Keyes & Heimberg (1979) found an increased triacylglycerol output by perfused livers of animals treated with propylthiouracil for 7 days, but after 14 days, output was not significantly different to that of livers from normal fed controls, in agreement with our studies. Present investigations also showed that although the production of VLD-lipoprotein triacylglycerol from labelled non-esterified fatty acid was similar to controls, the contribution from lipogenesis was less. Of note was the low production of VLD-lipoprotein cholesterol by perfused livers from hypothyroid rats, which cor-

related with the decreased concentration *in vivo*. The lack of a marked increase in $d > 1.006$ -lipoprotein cholesterol in hypothyroid rats is probably because rat plasma contains little low-density lipoprotein.

An increased potential for hepatic long-chain fatty acid synthesis in hyperthyroid animals is indicated by increased activities of lipogenic enzymes (see e.g. Roncari & Murthy, 1975). Our results show no marked increase in lipogenesis, which may be due to the antilipogenic effect of the raised concentration of perfusate non-esterified fatty acids (Mayes & Topping, 1974), and do not support an extensive increase in substrate cycling involving synthesis and oxidation of fatty acids (Diamant *et al.*, 1972). Whether or not this occurs *in vivo* may depend on the prevailing non-esterified fatty acid concentration. The recovery of relatively more ^3H within phospholipids and less within triacylglycerol fractions in hyperthyroid livers may result from enhanced synthesis of unsaturated fatty acids (Faas *et al.*, 1977), which are esterified preferentially into phospholipids, the reverse trend being found in hypothyroid livers.

There was no detectable alteration in the rate of cholesterol synthesis by hyperthyroid or hypothyroid livers as measured with $^3\text{H}_2\text{O}$. This result contrasts with measurements of acetate incorporation into hepatic sterols (see e.g. Dayton *et al.*, 1960), which have implied that cholesterol synthesis is increased in the hyperthyroid and decreased in the hypothyroid condition.

Redox state

A high substrate potential, caused by a decreased oxidative requirement in the presence of fatty acid, resulted in a more reduced cytosolic redox state, as shown by the increased perfusate [lactate]/[pyruvate] ratio in hypothyroid liver perfusions. It has been suggested (Bremer *et al.*, 1974) that there is preferential access to the respiratory chain by those reducing equivalents formed in β -oxidation that are linked to flavoprotein dehydrogenases. This may result in inhibition of one or more of the shuttles transferring reducing equivalents from the cytosol into the mitochondria via NAD-linked mitochondrial dehydrogenases. An increase in the [NADH]/[NAD $^+$] ratio of the cytosol and an increased [lactate]/[pyruvate] ratio would follow, as in the present investigations. Conversely, the opposite mechanism may be envisaged in hyperthyroid livers where a low substrate potential can be invoked to explain the decreased perfusate [lactate]/[pyruvate] ratio. Alternatively, or in addition, the increased activity of hepatic mitochondrial glycerol 3-phosphate dehydrogenase (EC 1.1.99.5) that occurs after thyroxine feeding in the rat (Lee *et al.*, 1959) may increase the rate of the glycerol 3-phosphate shuttle (Werner & Berry, 1974). This

would enhance the transfer of reducing equivalents from the cytosol into the mitochondrion, decreasing the cytosolic [NADH]/[NAD $^+$] ratio. However, *in vivo*, the raised concentration of plasma non-esterified fatty acids in hyperthyroid rats may increase the substrate potential explaining the increased hepatic redox state reported by Tobin *et al.* (1979).

Numerous effects of changes in thyroid status have been described *in vivo*. Use of the perfused liver preparation has enabled us to delineate those hepatic effects, due presumably to enzyme adaptation, which are fundamental to this organ and which persist in the absence of extrahepatic influence.

We acknowledge the technical assistance of Mrs. Neena Sahni and Miss Maureen Dinsdale. This work was supported by the Medical Research Council.

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