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THYROID HORMONE-INDUCED CHANGES IN GLUCONEOGENESIS AND KETOGENESIS IN PERFUSED RAT LIVER

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Summary

The regulation of ketogenesis and gluconeogenesis was studied in isolated, perfused livers from hyper- and euthyroid rats. Experimental conditions were varied with respect to lactate and fatty acid concentration in the perfusion medium and with respect to the nutritional state of the rats.

1. The rate of uptake of oleate in perfused livers was independent of the thyroid and nutritional states of the animals.

2. In livers from 48-h-fasted rats no difference was found in rates of ketogenesis between the euthyroid and hyperthyroid livers except when 10 mM lactate was present in the perfusate. In recently fed rats the rates of ketogenesis from oleate (1 mM) and endogenous substrates were low in euthyroid livers (0.45 and 0.05 μmol/min per g liver, respectively), while these rates in hyperthyroid livers were (1.333 and 0.136 μmol/min per g liver, respectively). With octanoate as substrate, high rates of ketogenesis were found in recently fed livers from both euthyroid and hyperthyroid rats (1.573 and 1.717 μmol/min per g liver, respectively).

3. Without oleate in the perfusion medium the rate of gluconeogenesis from low (1 mM) lactate concentrations in livers from 48 h-fasted-rats was slightly increased in the hyperthyroid state (0.548 μmol/min per g liver) compared to the euthyroid state (0.408 μmol/min per g liver). When lactate concentration in the perfusion medium was raised to 10 mM the rate of gluconeogenesis was increased 4-fold in the hyperthyroid livers (1.800 μmol/min per g liver) but only 20% in the euthyroid livers (0.490 μmol/min per g liver).

The presence of oleate (1 mM) had no effect on the rate of gluconeogenesis

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from low lactate concentrations in livers form 48-h-fasted animals of either thyroid state. At 10 mM lactate the inclusion of oleate caused a pronounced stimulation of gluconeogenesis in euthyroid livers (from 0.490 to 1.766 μmol/min per g liver) but not in hyperthyroid livers (from 1.800 to 1.973 μmol/min per g liver) so that the difference in the rate of gluconeogenesis between the two thyroid states disappeared.

4. The content of endogenous substrates was measured in liver biopsies taken before perfusion. The glycogen concentration was independent of the thyroid state in 48-h-fasted animals. The triglyceride content was independent of the thyroid state in recently fed animals. In recently fed animals the glycogen content was reduced by 90% in hyperthyroid animals, and in 48-h-fasted animals the triglyceride content was reduced by 50% in hyperthyroid animals.

5. The energy cost of gluconeogenesis from lactate appeared to be independent of the thyroid state.

Introduction

The hyperthyroid state is associated with a tendency to ketosis both in man [1] and experimental animals [2]. This is due at least partially to an increased rate of fatty acid mobilisation from adipose tissue [3] which causes an increased supply of free fatty acids to the liver [4,5]. However, in severely hyperthyroid patients, we recently found that the plasma concentrations of ketone bodies were elevated disproportionally to the free fatty acid concentration during the earliest period of fasting [6]; indicating that an alteration of hepatic free fatty acid metabolism might contribute to the elevation of the ketone body concentration. Such an alteration has actually been demonstrated in perfused liver from triiodothyronine-treated, fed rats, where ketone body production from oleate was found to be increased despite an unchanged fatty acid uptake [7].

The increased rates of gluconeogenesis from lactate [8] and alanine [9] in perfused liver from fasted, hyperthyroid rats has been attributed to an increased pyruvate carboxylase activity [10]. In view of the stimulation of gluconeogenesis by fatty acid oxidation in normal livers [11], the high rate of gluconeogenesis in the hyperthyroid liver may be related to an increased rate of fatty acid oxidation.

In order to investigate this possibility we have performed simultaneous measurements of ketogenesis and gluconeogenesis from lactate in perfused livers from 48-h-fasted euthyroid and hyperthyroid rats with varying gluconeogenic and ketogenic substrate loads. As preliminary experiments surprisingly failed to demonstrate any increase in ketone body production in hyperthyroid livers, the investigation was extended to comprise livers from recently fed animals. On the basis of these experiments we conclude that the principal effect of thyroid hormone excess on liver fatty acid and lactate metabolism is a deficient adaptation to feeding in hyperthyroid livers, which can be considered to be in a constantly fasted state.

Material and Methods

Chemicals. Analytical grade reagent were used when available. Enzymes and cofactors were from Boehringer, Mannheim. L-3,5,3′-Triiodothyronine (Tertroxin®) was from Glaxo Laboratories, Greenford, U.K. Bovine serum albumin (Fraction V) from Sigma, U.S.A., was defatted according to Chen [12] and extensively dialysed against 0.9% NaCl and distilled H_2O in order to remove low molecular weight impurities [13] before use.

Animals. Animals were female Wistar rats weighing about 170 g. In order to induce hyperthyroidism, the rats were given triodothyronine intraperitoneally (50 μg/100 g body weight) on alternate days for a total of three doses, the last one being given 24 h before the perfusion. The rats were kept in a light-dark cycle of 12 h beginning at 12 a.m. with access to food only during the last 6 h of the dark period in order to provide livers for perfusion from animals with food in the stomach, i.e. 'recently fed'. The rats were either used recently fed or after 48 h fasting.

Perfusion. The non-recirculating perfusion system has been described previously [14]. The perfusion medium consisted of washed bovine erythrocytes suspended in a Krebs-Ringer bicarbonate buffer modified by an increase of the inorganic phosphate concentration to 2.3 mM [15] and with an albumin concentration of 2% (w/v). Oleate or octanoate (1 mM) was added in suspension, complexed to albumin according to Ross et al. [16]. The hematocrit was 30% and the hemoglobin concentration was 5.6 mmol/l. The perfusate flow was adjusted to 1.5 ml/min per g liver. The experiments with 48 h fasted rats were initiated by 40 min perfusion with a medium containing about 1 mM lactate and 0.1 mM pyruvate as substrates (free fatty acid concentration in this medium was measured to less than 0.025 mM). Then oleate or lactate (10 mM) was added, and the perfusion was continued until a new steady state was attained with regard to oxygen uptake and glucose and ketone-body production (between 15 and 25 min). Finally lactate or oleate was added, and the perfusion was continued for 20 min with a medium containing oleate (1 mM) as well as lactate (10 mM). In the experiments with livers from fed animals, oleate (1 mM) or octanoate (1 mM) was added after 30 min of perfusion with the medium containing 1 mM lactate and 0.1 mM pyruvate as substrates, and the perfusion was then continued for 20 min. Steady state values for oxygen and metabolite concentrations in perfusate were determined as the mean of at least three individual measurements.

Assays. Enzymatic assays were employed for determination of glucose, lactate, 3-hydroxybutyrate, pyruvate and acetoacetate as reported earlier [17]. Free fatty acids were assayed colorimetrically [18]. Hemoglobin concentration and oxygen saturation was measured with automatic equipment (hemoximeter OSM 2, Radiometer, Denmark).

Triglyceride was extracted from rapidly frozen liver biopsies [19], hydrolysed and calculated after enzymatic determination of glycerol [17]. Glycogen content in biopsies was determined as glucose after hydrolysis [20]. Results are given as means ±S.E.

TABLE I

RATES OF KETOGENESIS IN LIVERS FROM 48 H FASTED HYPER- AND EUTHYROID RATS

Livers from 48-h fasted 3,5,3′-triiodothyronine treated and normal rats were perfused with media containing lactate (1 mM); lactate (1 mM) + oleate (1 mM); lactate (10 mM); lactate (10 mM) + oleate (1 mM). Measurements of the rate of acetoacetate and 3-hydroxybutyrate production were performed under steady-state conditions. Values given are mean ± S.E., or range of individual values.

	Acetoacetate production (μmol/min per g liver)		3-Hydroxybutyrate production (μmol/min per g liver)		Total ketone body production (μmol/min per g liver)	
	Triiodothyronine treated	Controls	Triiodothyronine treated	Controls	Triiodothyronine treated	Controls
Lactate, 1 mM	0.390 ± 0.135 (n = 4)	0.356 ± 0.105 (n = 4)	0.041 ± 0.015 (n = 4)	0.042 ± 0.008 (n = 4)	0.431 ± 0.155 (n = 4)	0.397 ± 0.120 (n = 4)
Lactate, 1 mM + oleate, 1 mM	0.821 ± 0.089 (n = 3)	0.554 ± 0.075 (n = 3)	0.785 ± 0.115 (n = 3)	1.081 ± 0.025 (n = 3)	1.606 ± 0.090 (n = 3)	1.635 ± 0.089 (n = 3)
Lactate, 10 mM	0.000—0.000 (n = 2)	0.038 ± 0.016 (n = 4)	0.005—0.018 (n = 2)	0.021 ± 0.008 (n = 4)	0.005—0.018 (n = 2)	0.059 ± 0.013 (n = 4)
Lactate, 10 mM + oleate, 1 mM	0.160—0.210 (n = 2)	0.272—0.426 (n = 2)	0.272—0.450 (n = 2)	0.329—0.777 (n = 2)	0.382—0.660 (n = 2)	0.601—1.203 (n = 2)

TABLE II

RATES OF KETOGENESIS IN LIVERS FROM RECENTLY FED HYPERTHYROID AND NORMAL RATS

Livers from recently fed 3,5,3′-triiodothyronine treated and normal rats were perfused with media containing lactate (1 mM); lactate (1 mM) + oleate (1 mM) and lactate (1 mM) + octanoate (1 mM). Measurements of acetoacetate and 3-hydroxybutyrate production were performed under steady state conditions. Values given are mean ± S.E. or range of individual values.

	Acetoacetate production (μmol/min per g liver)		3-hydroxybutyrate production (μmol/min per g liver)		Total ketone body production (μmol/min per g liver)	
	Triiodothyronine treated	Controls	Triiodothyronine treated	Controls	Triiodothyronine treated	Controls
Lactate, 1 mM	0.091 ± 0.021 (n = 4)	0.031 ± 0.024 (n = 4)	0.046 ± 0.027 (n = 4)	0.014 ± 0.011 (n = 4)	0.136 ± 0.026 (n = 4)	0.046 ± 0.016 (n = 4)
Lactate, 1 mM + oleate, 1 mM	0.360—0.731 (n = 2)	0.056—0.234 (n = 2)	0.745—0.829 (n = 2)	0.222—0.379 (n = 2)	1.189—1.476 (n = 2)	0.278—0.613 (n = 2)
Lactate, 1 mM + octanoate, 1 mM	0.653—0.780 (n = 2)	0.451—0.466 (n = 2)	1.009—1.078 (n = 2)	1.078—1.094 (n = 2)	1.662—1.858 (n = 2)	1.544—1.545 (n = 2)

Results

Ketogenesis. The rate of ketogenesis in the perfused liver from 48 h fasted rats is shown in Table I. The thyroid state did not influence the ketone-body formation from 1 mM lactate plus endogenous substrates. Addition of 1 mM oleate to the perfusate increased the rate of total ketone-body formation 4-fold im both hyper- and euthyroid livers. The ratio between 3-hydroxybutyrate and acetoacetate production in controls was twice that for hyperthyroid livers.

When the lactate concentration in the perfusate was raised from 1 to 10 mM a decrease in the rate of ketogenesis was observed both in the hyper- and euthyroid livers (Table I). The antiketogenic action of lactate was however, greater in the hyperthyroid livers than in control livers.

A completely different pattern was obtained with livers from recently fed animals (Table II). Under this condition the rate of total ketone-body formation from 1 mM lactate plus endogenous fatty acids was threefold higher in the fed, hyperthyroid livers as compared to the euthyroid livers. However, the rate of ketogenesis was less than 30% of that found after 48 h of fasting. In hyperthyroid livers addition of 1 mM oleate increased the rate of ketogenesis to 80% of that seen after 48 h fasting. In euthyroid livers the rate of ketogenesis after stimulation with oleate (1 mM) attained 30% of the value found after 48 h fasting. In order to circumvent the carnitine acyl transferase step, octanoate was used as the substrate for ketogenesis. The octanoate-induced rise in the rate of ketogenesis in euthyroid livers from fed animals was close to that found in the hyperthyroid livers (Table II).

The oleate uptake in the hyperthyroid livers was 0.734 ± 0.034 (n = 3) μmol/min per g liver in the fasted state and 0.750 (n = 2) μmol/min per g liver in the fed state with corresponding control values of 0.717 ± 0.031 (n = 3) μmol/min per g liver and 0.708 (n = 2) μmol/min per g liver, respectively. Thus the fatty acid uptake appears to be independent of the nutritional as well as the thyroid state.

Table III shows the oxygen uptake before and after addition of fatty acid to

TABLE III

FATTY ACID INDUCED RATES OF OXYGEN UPTAKE IN LIVERS FROM 48-H-FASTED HYPERTHYROID AND NORMAL RATS

Livers were perfused with a medium containing lactate (1 mM). After 40 min of perfusion oleate (1 mM) was added to the medium, and the perfusion continued for 20 min. Measurements of O_2 uptake were performed immediately before addition of oleate and after establishment of a new steady state. Values given are mean ± S.E., or range of individual values.

	Controls		Triiodothyronine	
	48-h-fasted	Recently-fed	48-h-fasted	Recently-fed
O_2 Uptake before oleate, (μmol/min per g liver)	3.152 ± 0.191 (n = 5)	2.126 ± 0.258 (n = 4)	4.955 ± 0.443 (n = 4)	3.762 ± 0.045 (n = 4)
O_2 Uptake after oleate, (μmol/min per g liver)	4.204 ± 0.117 (n = 3)	2.987—3.549 (n = 2)	6.173 ± 0.279 (n = 3)	4.688—4.812 (n = 2)
Oleate induced O_2 uptake, (μmol/min per g liver)	1.052	1.142	1.142	0.988

the perfusion medium. Neither triiodothyronine treatment nor prolonged fasting affects the magnitude of the fatty acid induced increase in the rate of oxygen uptake.

Gluconeogenesis. In the fasted state the rate of gluconeogenesis was higher in the hyperthyroid livers than in the euthyroid livers (Table IV). However, the concentration of lactate and the presence of fatty acids in the perfusate markedly influenced the magnitude of the difference.

At low lactate concentrations (1 mM) the glucose production in the euthyroid livers was fully accounted for by lactate plus pyruvate uptake with a ratio of glucose production to lactate plus pyruvate uptake of 0.98 ± 0.08 (n = 5) whereas the glucose production in the hyperthyroid state exceeded the lactate plus pyruvate uptake (ratio of glucose production/lactate plus pyruvate uptake 1.17 ± 0.12) (n = 4), thus indicating a gluconeogenesis from endogenous sources, probably amino acids and glycerol. The difference in glucose production of 0.150 μmol/min per g liver between the eu- and hyperthyroid state is partially accounted for by a 10% increase in the lactate plus pyruvate uptake of the hyperthyroid livers. Addition of 1 mM free fatty acid did not have any significant effect on the rate of gluconeogenesis at low lactate concentrations in either eu- or hyperthyroid livers (Table IV).

When the lactate concentration in the perfusate was raised from 1 to 10 mM in the absence of free fatty acid the rate of lactate uptake in euthyroid livers increased 2-fold to 1.850 μmol/min per g liver with a concomitant increase in the rate of pyruvate production to 0.568 μmol/min per g liver accounting for half of the extra lactate uptake (Table IV). However, the rate of gluconeogenesis showed only a minimal increase, and the ratio of glucose production to lactate plus pyruvate uptake decreased from 1.0 (see above) to 0.7. At the same time the lactate to pyruvate concentration ratio rose from 11.6 ± 0.6 (n = 4) to 26.3 ± 3.0 (n = 3). In hyperthyroid livers a markedly different response was obtained upon raising the lactate load. The rate of lactate uptake increased 5-fold to 4.815 μmol/min per g liver, and the major part of this was accounted for by a 4-fold increase in the rate of glucose production (Table IV). The rate of pyruvate release increased to 0.798 μmol/min per g liver. The lactate/pyruvate ratio was unaffected by the increased lactate concentration being 13.7 ± 1.1 (n = 4) at 1 mM lactate and 11.6 (n = 2) at 10 mM lactate).

Addition of 1 mM oleate to the perfusate with 10 mM lactate caused a 4-fold stimulation of the rate of glucose production and a 3-fold stimulation of the rate of net lactate plus pyruvate uptake in the euthyroid livers, while in the hyperthyroid livers only modest increases were seen. Thus, under conditions with ample substrates for β-oxidation, differences in the rates of gluconeogenesis from lactate between eu- and hyperthyroid livers disappeared (Table IV).

In livers from recently fed euthyroid animals there was a concomitant glucose and lactate production (Table V). In contrast, hyperthyroid livers took up lactate and produced glucose (Table V).

In Table VI are shown the increases in oxygen uptake and glucose production in the fasted state following an elevation of the lactate concentration from 1—10 mM in the perfusate. The energy cost of gluconeogenesis as estimated by the ratio between the changes in glucose production and oxygen uptake is not

TABLE IV

RATES OF GLUCONEOGENESIS IN LIVERS FROM 48-H-FASTED HYPERTHYROID AND NORMAL RATS

Livers from 48-h-fasted 3,5,3′-triiodothyronine treated and normal rats were perfused with media containing lactate (1 mM); lactate (1 mM) + oleate (1 mM); lactate (10 mM); and lactate (10 mM) + oleate (1 mM). Measurements of glucose production and lactate plus pyruvate uptake were obtained under steady state conditions. Net C-3 uptake denotes the sum of lactate and pyruvate uptake or in the experiments with 10 mM lactate in perfusion media with the difference between lactate uptake and pyruvate release. Values given are mean ± S.E., or range of individual values.

	Euthyroid, 48-h-fasting		Hyperthyroid, 48-h-fasting	
	Glucose production (μmol/min per g liver)	Net C-3 uptake (μmol/min per g liver)	Glucose production (μmol/min per g liver)	Net C-3 uptake (μmol/min per g liver)
Lactate, 1 mM	0.408 ± 0.016 (n = 5)	0.846 ± 0.055 (n = 5)	0.548 ± 0.058 (n = 4)	0.943 ± 0.077 (n = 4)
Lactate, 1 mM + oleate, 1 mM	0.384 ± 0.008 (n = 3)	0.751 ± 0.022 (n = 3)	0.576 ± 0.023 (n = 3)	0.980 ± 0.094 (n = 3)
Lactate, 10 mM	0.490 ± 0.032 (n = 4)	1.375 ± 0.122 (n = 4)	1.699—1.801 (n = 2)	3.960—4.075 (n = 2)
Lactate, 10 mM + oleate, 1 mM	1.766 ± 0.101 (n = 4)	4.025 ± 0.130 (n = 4)	1.973 ± 0.068 (n = 4)	4.967 ± 0.143 (n = 4)

TABLE V

GLUCONEOGENESIS IN LIVERS FROM RECENTLY FED HYPERTHYROID AND NORMAL RATS

Livers from 3,5,3′-triiodothyronine treated and normal rats were perfused with media containing lactate (1 mM) or lactate (1 mM) and oleate (1 mM). Values of glucose production and lactate plus pyruvate uptake were obtained after at least 25 min of perfusion. Net C-3 uptake denotes the sum of lactate and pyruvate uptake. Negative values denote net release of the metabolites. Values given are mean ± S.E.

	Euthyroid, recently fed		Hyperthyroid, recently fed	
	Glucose production (μmol/min per g liver)	Net C-3 uptake (μmol/min per g liver)	Glucose production (μmol/min per g liver)	Net C-3 uptake (μmol/min per g liver)
Lactate, 1 mM	1.371 ± 0.187 (n = 4)	—0.822 ± 0.186 (n = 4)	0.762 ± 0.076 (n = 4)	0.498 ± 0.130 (n = 4)
Lactate, 1 mM + oleate, 1 mM	1.378—1.632 (n = 2)	0.059—0.147 (n = 2)	0.729—0.780 (n = 2)	0.648—0.977 (n = 2)

TABLE VI

ENERGY COST OF GLUCONEOGENESIS IN LIVERS FROM 48-H-FASTED HYPERTHYROID AND NORMAL RATS

Livers from 3,5,3′-triiodothyronine treated rats and from normal rats were perfused with media containing lactate (1 mM) or lactate (1 mM) + oleate (1 mM). After 40 min of perfusion lactate concentrations were raised to 10 mM and perfusion continued for 20 min. Oxygen uptake and glucose production were measured immediately before the increase of perfusate lactate concentration and after establishment of a new steady state. The difference between these values (mean of two perfusions) is given in the table. Values in parenthesis denote oxygen uptake and glucose production before the increase in perfusate lactate concentration.

	Euthyroid, 48-h-fasted		Hyperthyroid, 48-h-fasted	
	+Oleate (μmol/min per g liver)	—Oleate (μmol/min per g liver)	+Oleate (μmol/min per g liver)	—Oleate (μmol/min per g liver)
I Increase in O_2 uptake induced by 10 mM lactate	1.512 (4.297)	0.057 (3.091)	1.352 (6.259)	1.274 (4.792)
II Increase in glucose production, induced by 10 mM lactate	1.435 (0.382)	0.079 (0.394)	1.323 (0.557)	1.161 (0.539)
Ratio II/I	0.95	1.39	0.98	0.91

TABLE VII

LIVER CONTENT OF TRIGLYCERIDE AND GLYCOGEN

Hepatic glycogen and triglyceride content in eu- and hyperthyroid rats, fasted for 48 h or recently fed. Values are mean ± S.E. of livers from 5 rats.

	48-h-fasted		Recently fed	
	Triiodothyronine treated	Controls	Triiodothyronine treated	Controls
Triglyceride content (μmol/g liver)	2.86 ± 0.23	6.49 ± 0.35	4.67 ± 0.33	4.90 ± 0.32
Glycogen content (mg/g liver)	1.3 ± 0.5	1.5 ± 0.2	6.6 ± 1.1	48.1 ± 1.1

increased in the hyperthyroid state. To delineate whether differences in supply of endogenous substrates might account for the differences in gluconeogenesis and ketogenesis between eu- and hyperthyroid livers a determination was made of pre-perfusion values of liver glycogen and triglyceride content (Table VII).

In livers from 48-h-fasted rats no difference was found between eu- and hyperthyroid values for glycogen content, which invariably was below 2.5 mg/g liver. The triglyceride content in the fasted-hyperthyroid livers was less than half that of corresponding controls.

In the recently fed state the glycogen content in euthyroid livers was increased by a factor of 30 compared to fasting values, while only a 4-fold increase was found in the hyperthyroid livers. No difference in triglyceride content was found between eu- and hyperthyroid livers from fed rats.

Discussion

Our results and those of Keyes and Heimberg [7], showed a several-fold increase in the rate of ketogenesis from long chain fatty acids in livers from recently fed hyperthyroid animals. In contrast we found equal rates of total ketone-body formation in livers from 48-h-fasted hyper- and euthyroid animals. The observation that the rate of ketone-body formation is increased disproportionately to the free fatty acid concentration in the earliest phase of adaptation to fasting in hyperthyroid humans is thus corroborated [6]. The principal alteration of the long chain fatty acid metabolism in the hyperthyroid liver therefore seems to be the absence of the normal suppression of ketogenesis in the fed state. The absolute values found in this study for the rate of ketogenesis and oleate uptake in livers from 48-h-fasted rats are similar to those found by Chernick et al. [21] in livers from starved-hypophysectomized rats. It appears thus that the adaptation of the liver fatty acid metabolism to fasting is largely independent of thyroid, pituitary and possibly also adrenal cortex function.

The mechanism of the thyroid hormone-induced increase in the rate of ketogenesis in the fed state cannot be fully explained from the data presented here. Because the rate of oleate uptake was independent of the thyroid state and the rate of ketogenesis was increased in hyperthyroid livers, the rate of triglyceride synthesis must be decreased in hyperthyroid livers. Such an effect has been

experimentally shown in hyperthyroid livers [7,22]. This alteration in the partition of fatty acids between esterification and oxidation might well be due to the high activity of (L-)carnitine acyltransferase (EC 2.3.1.7) in livers from fed hyperthyroid rats [23] as the experiments with octanoate in the perfusate showed a marked reduction of the difference in ketogenesis between eu- and hyperthyroid livers.

Previous investigations of the rate of gluconeogenesis in the fasted hyperthyroid liver have shown an increase of glucose production of more than 50%, when lactate in concentrations greater than 5 mM was employed as a substrate [8,24]. Recently we found that this difference between eu- and hyperthyroid livers largely disappears at physiological lactate concentrations [17]. The present data confirm that a significant thyroid hormone-induced increase in the rate of gluconeogenesis was found only at high lactate concentrations. However, addition of oleate (1 mM) abolished this difference. Thus the principal effect of hyperthyroidism on gluconeogenesis from lactate appears to be an absence of any requirement for simultaneous oxidation of exogenous fatty acid, which, on the other hand, plays a major role in the euthyroid state.

In the fed euthyroid state here was a simultaneous glucose and lactate release from the perfused livers in agreement with the findings that glucose production is largely derived from glycogenolysis and that glycolysis occurs in perfused livers in the absence of other oxidizable substrates [16]. The relatively low rate of glucose release in the fed hyperthyroid liver and the fact that lactate is taken up in such livers are in accordance with the reduced glycogen content in hyperthyroid livers (Table VII). Thus, the livers from fed hyperthyroid rats appear to be in a partially fasted state both with respect to fatty acid and glucose metabolism.

It may be concluded that the physiological consequences of the thyroid hormone-induced changes in liver metabolism of fatty acids, glucose and lactate are absolutely dependent on the nutritional state of the organism. After prolonged fasting where the plasma-free fatty acid concentration is high, the differences in gluconeogenic and ketogenic rates between intact eu- and hyperthyroid animals will be mainly due to differences in the rate of substrate delivery to the liver, while intrahepatic alterations can only be expected to play a minor role. In the fed state, however, the alterations in fatty acid and lactate metabolism seen in the isolated liver can be expected to manifest themselves under physiological conditions.

The energy cost of gluconeogenesis at high lactate concentration, as estimated from the ratio between the rates of glucose release and oxygen uptake was found to be equal in 48-h-fasted hyper- and euthyroid livers, with a value near the ideal (1.0) assuming an ADP/O ratio of 3 (Table VI). This finding is in agreement with our earlier investigation of gluconeogenesis from glycerol in perfused hyperthyroid liver [17] and consistent with the data of Tata et al. [25] and Nishiki et al. [26] which show, that the coupling of the oxidative phosphorylation is unchanged in isolated mitochondria and in intact tissue from hyperthyroid animals. However, our data are at variance with the suggestion that futile cycles in gluconeogenesis are partly responsible for thyroid hormone induced calorigenesis in isolated hepatocytes [24]. The cause of this discrepancy is not clear, but it is remarkable that relatively higher substrate-

induced rates of oxygen uptake have been reported in isolated hepatocytes, than in perfused liver preparations, [27] suggesting the existence of artifactually elevated rates of futile cycling in isolated hepatocytes.

The increase in oxygen uptake induced by addition of 1 mM oleate is independent of the thyroid or nutritional state (see Results). Therefore, an extra futile cycle between fatty acid oxidation and synthesis cannot be responsible for thyroid hormone calorigenesis. This together with the apparent normal energy cost of gluconeogenesis from lactate and glycerol makes it unlikely that alterations in carbohydrate or lipid metabolism can account for the 30% increase in oxygen uptake found in the hyperthyroid livers in this study.

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