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Research Article

Thyroid disease is often accompanied by changes in the concentrations of serum lipids and lipoproteins. To evaluate the hepatic contribution to the serum abnormalities in thyroid disease, we examined fatty acid metabolism in perfused livers from pair-fed rats made hypothyroid with propylthiouracil (PTU) or made hyperthyroid by treatment with triiodothyronine (T_3). The animals treated with T_3 became hyperphagic, depending on dose of drug and duration of hyperthyroidism. It was necessary, therefore, for appropriate controls, that food intake of T_3-treated rats be restricted to quantities consumed by euthyroid rats. Animals treated with PTU for 2 wk became hypophagic, and therefore, food consumption of controls was restricted to that eaten by rats receiving PTU. Dependent on dose of T_3 and duration of treatment, the output of triglyceride and glucose was diminished, whereas output of ketone bodies was increased by livers from hyperthyroid animals. In contrast, livers from PTU-treated animals secreted increased amounts of triglyceride and glucose, whereas ketogenesis was diminished. The best models for study proved to be animals treated with either 10 μg T_3/100 g body wt per d or 1 mg PTU/100 g body wt per d for 7 d. Under these conditions, all animals consumed the same quantity of food as did the euthyroid rats, but continued to display the metabolic alterations outlined above. The effects of PTU on […]

Influence of Thyroid Status on Lipid Metabolism in the Perfused Rat Liver

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ABSTRACT Thyroid disease is often accompanied by changes in the concentrations of serum lipids and lipoproteins. To evaluate the hepatic contribution to the serum abnormalities in thyroid disease, we examined fatty acid metabolism in perfused livers from pair-fed rats made hypothyroid with propylthiouracil (PTU) or made hyperthyroid by treatment with triiodothyronine (T_3). The animals treated with T_3 became hyperphagic, depending on dose of drug and duration of hyperthyroidism. It was necessary, therefore, for appropriate controls, that food intake of T_3-treated rats be restricted to quantities consumed by euthyroid rats. Animals treated with PTU for 2 wk became hypophagic, and therefore, food consumption of controls was restricted to that eaten by rats receiving PTU. Dependent on dose of T_3 and duration of treatment, the output of triglyceride and glucose was diminished, whereas output of ketone bodies was increased by livers from hyperthyroid animals. In contrast, livers from PTU-treated animals secreted increased amounts of triglyceride and glucose, whereas ketogenesis was diminished. The best models for study proved to be animals treated with either 10 μg T_3/100 g body wt per d or 1 mg PTU/100 g body wt per d for 7 d. Under these conditions, all animals consumed the same quantity of food as did the euthyroid rats, but continued to display the metabolic alterations outlined above. The effects of PTU on hepatic metabolism were readily reversible by simultaneous administration of T_3. It is clear from these data that the thyroid status of the rat regulates hepatic triglyceride formation and secretion, and ketogenesis.

Abstracts of portions of this work appeared in 1978: *Clin. Res.* **26**(5): 736A and, *Fed. Proc.* **37**(3): 258. A preliminary account of this work was presented at the Annual Meeting of the Federation of American Societies for Experimental Biology, 9–14 April 1978, Atlantic City, N. J.

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INTRODUCTION

Alterations in thyroid status appear to be associated with changes in serum triglyceride concentrations. In human hyperthyroidism, decreased (1), normal (2–4), and increased (5) serum concentrations of triglyceride have been observed. Concentrations of serum triglyceride, although usually elevated in human hypothyroidism (1–3, 5–9), have also been reported to be normal (10, 11). When hypothyroid subjects were treated with thyroid hormone preparations, the serum triglyceride concentration returned toward normal (2, 7, 9), although exceptions were reported (8). Similarly, when euthyroid hypertriglyceridemic subjects were administered thyroid hormone or thyroid hormone analogues, there was usually a reduction in serum triglyceride values (12). Because hypertriglyceridemia was not as consistent a finding in hypothyroidism as was hypercholesterolemia (10, 13), it has been postulated that the degree of hypertriglyceridemia was related to the severity or duration (6, 11) of hypothyroidism as well as to the diet (7).

The serum triglyceride concentration is regulated by entry of triglyceride into the serum from the diet in the form of chylomicrons, by hepatic synthesis and secretion of the very low density lipoprotein (VLDL),[1] and by removal of VLDL and chylomicron triglyceride from the serum by lipoprotein lipase. Serum triglyceride concentration is regulated in postabsorptive animals to a significant extent by hepatic synthesis and secretion of VLDL triglyceride from FFA substrate. The present study was designed to determine effects of hyperthyroidism and hypothyroidism on the metabolism of FFA, on output of triglyceride, and on ketogenesis by the isolated perfused rat liver.

METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) having initial weights of 150–175 g

[1] *Abbreviations used in this paper*: cAMP, cyclic AMP; PTU, propylthiouracil; T_3, 3,5,3′-triiodothyronine; VLDL, very low density lipoprotein.

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were housed in individual wire-bottom metabolic cages. Lighting was maintained between 0500 and 1700 h. The animals received daily subcutaneous injections of propylthiouracil (PTU), triiodothyronine (T_3), or PTU and T_3 between 1400 and 1600 h. When both drugs were given to the same animal, PTU and T_3 were injected at separate sites. PTU and T_3 were dissolved in 0.9% NaCl with 1 N NaOH, and the pH was adjusted to 8.5. Control (euthyroid) animals received injections of an equivalent volume of the vehicle at the same pH.

Rats treated with PTU (1.0 mg/100 g body wt per d) became hypophagic ≅8–10 d after start of treatment. To assure equicaloric consumption of food (powdered Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.) pair-feeding procedures were necessary. Therefore, food allowed the control animals was restricted to the amount consumed by the PTU-treated group. Rats treated with T_3 for 14 d became hyperphagic, but the onset of hyperphagia was dose related. Treatment with 1 μg T_3/100 g body wt or less daily for 14 d did not affect food consumption and these animals were fed ad lib. At higher doses of T_3, animals were pair-fed to the intake of the euthyroid animals.

At the end of the treatment periods, the rats were anesthetized with diethyl ether between 0900 and 1100 h. The livers were isolated surgically and perfused in vitro using procedures and apparatus described previously (14). Immediately before cannulation of the portal vein, 5 ml of blood was obtained with a heparinized syringe from the abdominal aorta of the rat. Bovine erythrocytes for the perfusate were collected by venipuncture and were washed according to the method of Exton et al. (15), except that antibiotics were omitted from the anticoagulant collection medium. The washed erythrocytes were suspended in Krebs-Henseleit bicarbonate buffer (pH 7.4), 3 g/dl bovine serum albumin and 100 mg/dl glucose. The initial volume was 70 ml and the hematocrit was 30% (vol/vol). The medium was gassed continuously with 95% O_2–5% CO_2. After a 20-min equilibration period, a complex containing 3 g bovine serum albumin and 1,419 μmol oleic acid/dl (15) was infused at a rate of 11.7 ml/h (166 μmol oleic acid/h). 5-ml samples of the perfusate were removed for analysis at hourly intervals. The experiment was terminated after 4 h of perfusion. The liver was perfused with 20 ml of ice cold 0.9% NaCl to remove residual perfusate, cleansed of adherent nonhepatic tissue, blotted, and weighed.

Lipids were extracted from the plasma- and erythrocyte-free perfusate (16). The extracts were washed, fractionated, and the lipids analyzed as reported elsewhere (17). Samples of the perfusate were hemolyzed and deproteinized with $Ba(OH)_2$-$ZnSO_4$; aliquots of the protein-free supernate were analyzed for ketone bodies (17) and glucose (glucose oxidase method, Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Output of triglyceride, ketone bodies, and glucose, and uptake of FFA were calculated as described previously (17).

All chemicals used were reagent grade and all solvents were redistilled before use. 6-*N*-Propyl-2-thiouracil and L-T_3, Na salt, were obtained from Sigma Chemical Co., St. Louis, Mo. Oleic acid (99% purity) was obtained from NuChek-Prep., Elysian, Minn. Bovine serum albumin (Fraction V powder), obtained from Pentex Biochemical, Kankakee, Ill., was purified before use (18).

Statistical analyses were performed using computerized routines (19). Perfusion parameters from the T_3 dose-response study were analyzed using multiple regression analysis. The chemical data from the T_3 dose-response experiments were analyzed using Kendall's tau (20). In both instances the doses of T_3 were transformed to log dose, to express the data in a linear fashion, before statistical analyses were performed. Hourly output data were analyzed using the Student's *t* test, except when multiple treatment groups were compared to the control. In those instances Dunnett's *t* test was employed (21).

Variations of experimental samples from control samples with a P value of <0.05 were considered statistically significant. Statistical significance is indicated in the figures by the use of asterisks: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

RESULTS

Body weights of animals in all treatment groups were similar preceding treatment with T_3 or PTU. During the 14-d treatment period, all animals gained weight. The gain in body weight, however, decreased with increasing dose of T_3 (Fig. 1), liver weight also decreased with dose of T_3. The ratio of liver weight to body weight, perfusate flow rate, and the volume of bile secreted were unchanged by treatment with T_3. Animals receiving T_3 (3 μg/100 g body wt) plus PTU were similar in all of the above parameters to animals receiving the T_3 alone, or to those treated with 0.9% NaCl only and maintained on identical food intake. The values for animal weight, liver weight, liver weight to body weight ratio, and perfusate flow rate for a separate group of pair-fed animals were not different from those recorded for rats treated with PTU for 14 d. The volume of bile secreted by the livers from PTU-treated animals was less than that seen in the controls. Other than a slight increase in perfusate flow rate ($P < 0.01$) in livers from animals treated with 10 μg T_3/100 g body wt per d for 7 d, perfusion parameters were similar to the controls, perfusate flow rate was 3.0±0.2 ml/min per g liver in livers from the control rats and 3.9±0.1 ml/min per g liver for T_3 treated animals. All perfusion parameters were similar for animals treated with either T_3 or PTU for only 3 d.

The results of treatment of rats with T_3 on FFA uptake, triglyceride output, ketone body production, and glucose output are seen in Fig. 2. These initial experiments were carried out with livers obtained from rats treated with either 0.9% NaCl or 5 μg T_3/100 g body wt per d for 14 d. The control animals were fed ad lib. and the T_3-treated animals were pair-fed to the amount of food consumed by the control animals. The uptake of FFA was unaffected by treatment with 5 μg T_3/100 g body wt. The concentration of FFA in the cell-free medium perfusing livers from T_3-treated animals (0.44 ±0.06 mM) was not different from that observed with controls (0.56±0.04 mM). However, output of triglyceride and glucose were reduced 50% and the production of ketone bodies was augmented. To determine the dependency of these changes in hepatic metabolism on dose of T_3, different groups of animals were treated for 14 d with 0.5–20.0 μg T_3/100 g body wt per d. Animals receiving >1.0 μg T_3/100 body wt per d for 14 d became hyperphagic. To insure equicaloric intake of food, these animals treated with T_3 were pair-fed

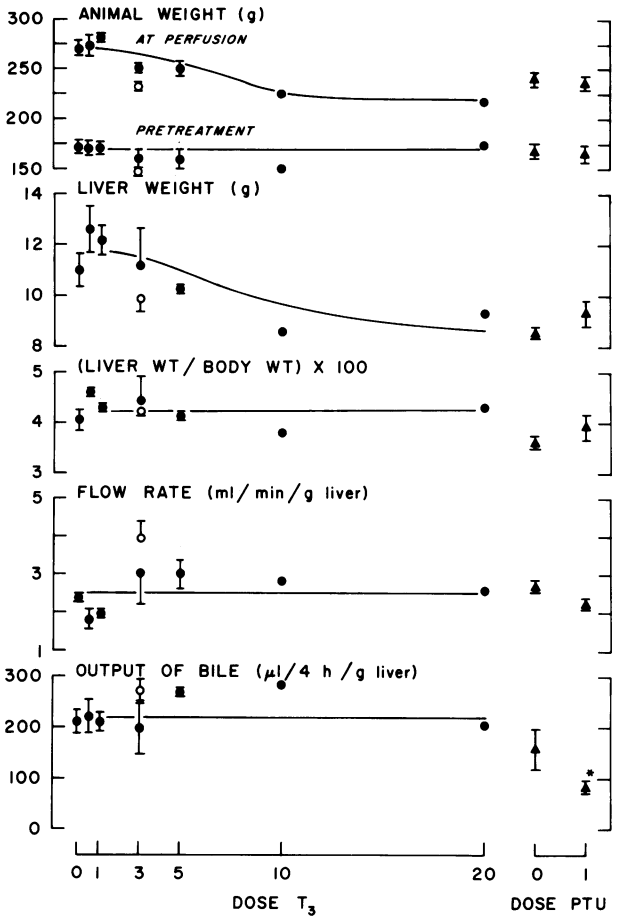

FIGURE 1 Parameters for perfusion of livers from rats treated with T_3, PTU, or with both drugs for 14 d. Separate control groups were necessary for each drug, because the drugs induced changes in food consumption. Pair-feeding was therefore required. Doses of T_3 are in μg/100 g body wt per d, whereas PTU dose was 1.0 mg/100 g body wt per d. Weights of rats before drug treatment were 150–175 g, and were similar for all groups. With increasing dose of T_3, the animal weight ($P < 0.05$) and liver weight ($P < 0.05$) at time of perfusion decreased. The doses of T_3 and number of observations in each group were: 0 μg, $n = 4$; 0.5 μg, $n = 3$; 1 μg, $n = 4$, 3 μg, $n = 4$; 5 μg; $n = 4$; 10 μg, $n = 2$; 20 μg, $n = 2$. The number of observations per dose of PTU were: 0 mg, $n = 6$, 1 mg; $n = 5$. In addition, four rats received T_3 (3 μg) and PTU, simultaneously. Values are means±SEM. ●, T_3, ▲, PTU; ○, T_3 and PTU.

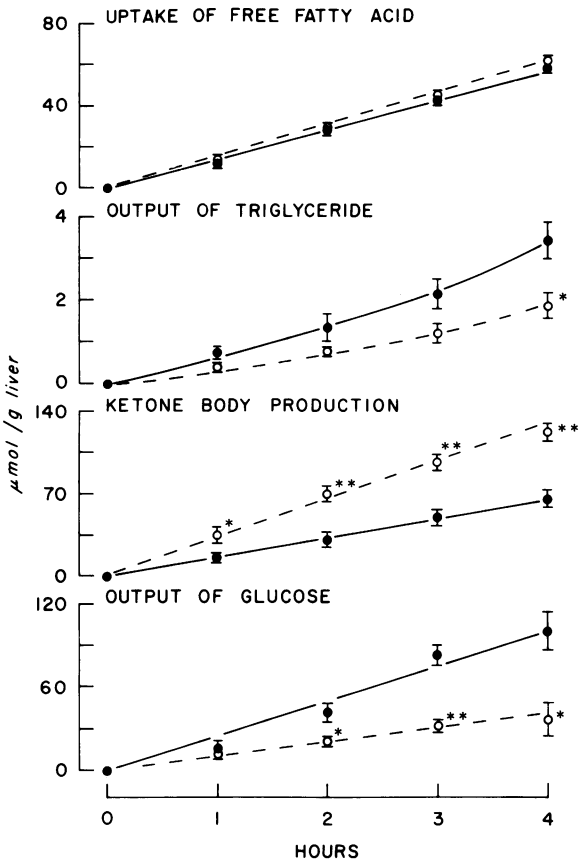

FIGURE 2 Metabolic alterations induced in perfused livers from T_3-treated rats. The dose of T_3 was 5 μg/100 g body wt per d for 14 d. Body weight at perfusion for T_3-treated animals was 250±7 g and was not different from control animals (270 ±7 g). Food consumption was restricted in the T_3-treated animals (○, $n = 4$) to the amount consumed by ad lib. fed control animals (●, $n = 4$). Values are means±SEM.

to the quantity of food consumed by the ad lib. fed controls. Regardless of dose, T_3 did not affect the uptake of FFA (Fig. 3) or the concentration of FFA in the cell-free perfusate ($\tau = 0.112$, $P > 0.05$). Concentrations of FFA in the cell-free medium were 0.56±0.04, 0.55 ±0.03, 0.49±0.02, 0.52±0.08, 0.44±0.06, 0.66, and 0.66 mM for perfused livers from animals treated with 0, 0.5, 1.0, 3.0, 5.0, 10, and 20 μg T_3, respectively. With increasing doses of T_3, output of triglyceride and glucose were depressed. Output of triglyceride was depressed significantly at the 5 μg/100 g body wt dose (t test, $P < 0.05$), and was minimal at a dose of 10–20 μg T_3/100 g body wt. Glucose output appeared to be more sensitive to T_3, being significantly decreased with 1 μg/100 g body wt (t test, $P < 0.05$), and depressed maximally at a dose of 10–20 μg T_3/100 g body wt.

The previous experiments were performed with perfused livers obtained from animals treated with T_3 for 14 d. To study the time dependency of the effects of treatment with T_3, rats were treated for 3, 7, or 14 d with 0.9% NaCl, with 10 μg T_3/100 body wt (a dose that produced maximal effects on hepatic metabolism in animals treated for 14 d) or with 3 μg T_3/100 g body wt (a dose that produced intermediate effects on hepatic metabolism in animals treated for 14 d). As in the previous experiments, T_3 did not affect the uptake of FFA or the concentration of FFA in the cell-free perfusate in any of the treatment regimens (Fig. 4). The 10-μg T_3/100 g dose produced a greater decrement at

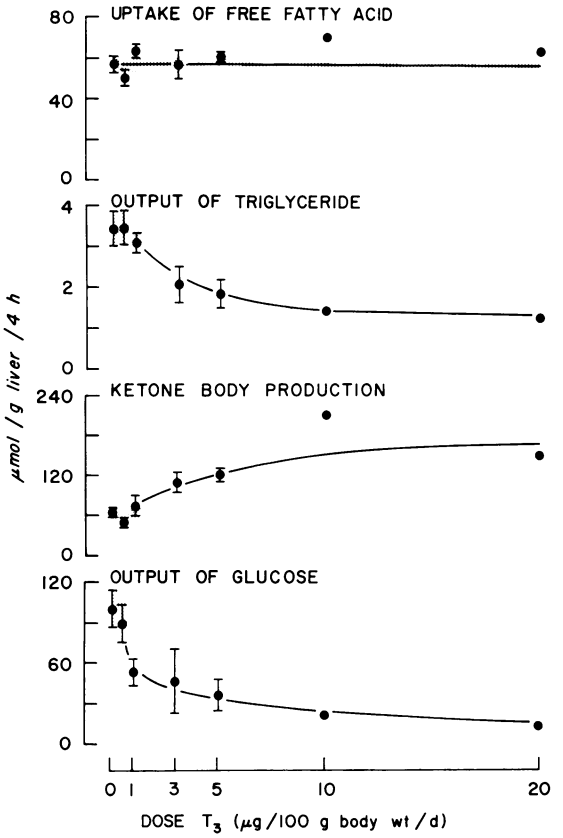

FIGURE 3 Effect of dose of T_3 on aspects of metabolism of perfused livers from rats treated for 14 d. Control animals were fed ad lib., whereas food consumption in the T_3-treated rats was restricted to the amount eaten by the controls. Numbers of observations per dose and animal weights at perfusion are those given in Fig. 1. Values represent means ±SEM. The shaded areas are means ±SEM for control animals. Data were analyzed statistically using Kendall's Tau (τ). Uptake of FFA was unaffected by dose of T_3 ($\tau = 0.26$, $P > 0.05$). However, output of triglyceride ($\tau = -0.44$, $P < 0.001$), ketone bodies ($\tau = 0.55$, $P < 0.001$) and glucose ($\tau = -0.61$, $P < 0.001$) were altered by administration of T_3.

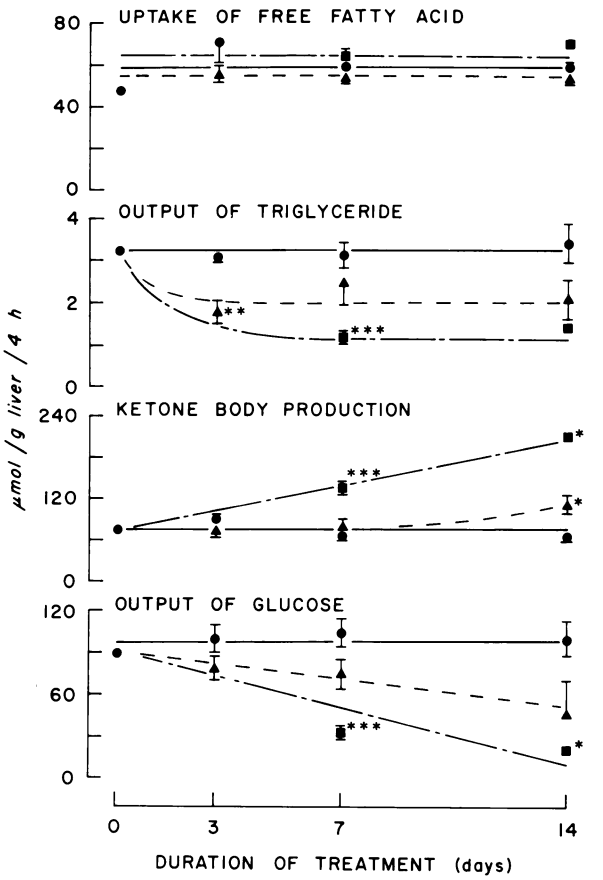

FIGURE 4 Effect of duration of treatment with T_3 on metabolism of perfused rat livers. Control animals were treated for 0 ($n = 2$), 3 ($n = 4$), 7 ($n = 8$), or 14 ($n = 4$) d with 0.9% NaCl and fed ad lib. Food consumption by rats treated with 3 ($n = 4$) or 10 ($n = 2$) μg T_3/100 g body wt per d for 14 d was restricted to amounts consumed by the controls. Animals treated with 3 μg T_3/100 g body wt per d for 3 d ($n = 4$) or 3 ($n = 4$) or 10 ($n = 4$) μg T_3/100 g body wt per d for 7 d consumed the same quantity of food as did the control group and had similar body weights at perfusion. Values are means ±SEM; 0.9% NaCl (●); 3 g T_3 (▲), 10 g T_3 (■).

14 d in triglyceride output than did the 3-μg/100 g dose; at 7 d, triglyceride output was depressed significantly with the 10-μg/100 g dose, but not with the 3-μg/100 g dose. Ketogenesis was enhanced by treatment with the 3-μg/100 g dose for 14 d, and there was an even greater effect with the 10-μg/100 g dose; at 7 d, ketogenesis was significantly stimulated by the 10-μg/100 g dose, but not by the 3-μg/100 g dose. Output of glucose was decreased significantly with the 10-μg/100 g body wt dose at 7 and 14 d.

To study the results of thyroid hormone depletion on hepatic lipid metabolism, rats were made hypothyroid by treatment with PTU. Because decreased food consumption was observed in the animals treated with PTU for 14 d, it would be incorrect to compare hepatic metabolism in these animals with metabolism in the euthyroid ad lib. fed controls that were appropriate for the T_3 dose-response curve. It was necessary therefore, that a separate group of euthyroid control animals be pair-fed to the decreased amounts of food consumed by the PTU-treated animals. As can be seen in Fig. 5, treatment of the rat with PTU for 14 d did not affect uptake of FFA by the liver or concentration of FFA in the cell-free perfusate. Output of triglyceride however, was increased ≅250% whereas ketogenesis was ≅40% of the control. Output of glucose increased ≅500% after treatment with PTU.

Under our experimental conditions, the degree of hyperthyroidism was determined by the dose-time relationship of T_3 administered. Varying degrees of hypothyroidism were produced by changing the duration of

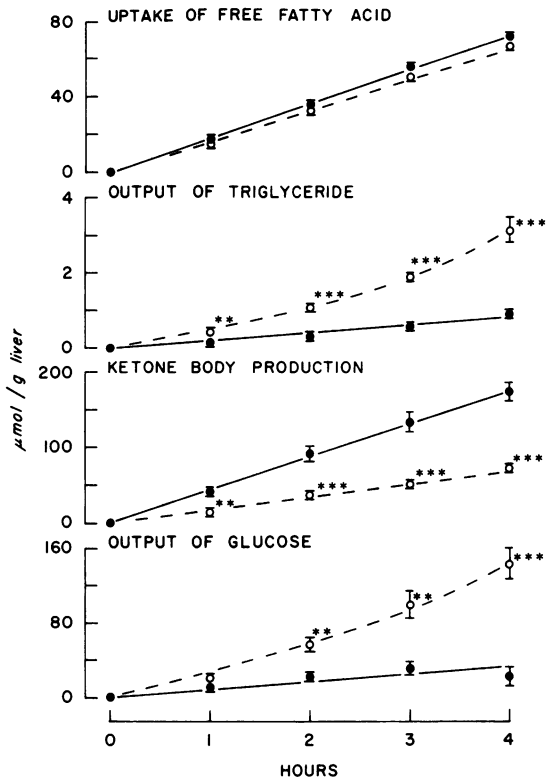

FIGURE 5 Metabolic alterations induced in perfused livers from PTU-treated animals. Dose of drug was 1 mg/100 g body wt per d for 14 d. The food allowed control animals was restricted to the amount consumed by the PTU-treated rats. Body weights at perfusion are given in Fig. 1. Values are means±SEM. 0.9% NaCl (●, n = 6); PTU (○, n = 5).

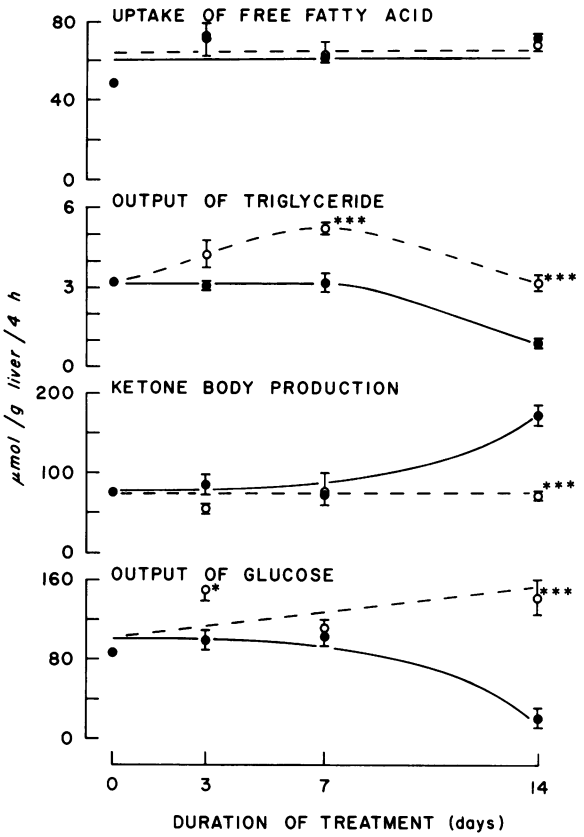

FIGURE 6 Effect of duration of treatment with PTU (1 mg/100 g body wt per d) on metabolism of perfused rat livers. PTU-treated rats were fed ad lib., and consumed amounts of food equal to those of controls until after 8–10 d of treatment, at which time they became hypophagic. Food was restricted, therefore, in 14-d control animals (n = 6) to the amounts consumed by PTU-treated rats for 14 d (n = 5). Rats treated with 0.9% NaCl for 0 (n = 2), 3 (n = 4), or 7 (n = 8) d were fed ad lib. as were PTU-treated rats for 3 (n = 4) or 7 (n = 4) d. Body weights at perfusion did not differ from controls at any time period. Values are means±SEM. Control (●); PTU (○).

treatment at constant dose of PTU, since the dose of PTU used inhibits essentially the total synthesis of thyroid hormones (22); the severity of the hypothyroidism is governed by the time required for clearance of the preexisting thyroid hormones from the body (23). For this reason, the effects on hepatic lipid metabolism of duration of treatment at constant dose of PTU were examined. Livers from pair-fed animals treated for 3, 7, or 14 d with 0.9% NaCl or PTU were studied. The decrease in food consumption in the PTU-treated animals was not observed until 8–10 d after treatment was started; before that time, control and PTU-treated animals ate similar amounts of food. Hepatic uptake of FFA and concentration of FFA in the cell-free perfusate was not altered regardless of duration of treatment with PTU (Fig. 6). Output of triglyceride was increased after the 7-d treatment whereas food consumption was still unaffected. After the 14-d treatment the effect of decreased food consumption (partial fasting in the control) is to reduce triglyceride output by livers from both control and PTU-treated animals, since they are pair-fed. Superimposed on the nutritional changes are the drug effects. A difference of ≅2 μmol/g liver per 4 h between control and PTU-treated animals remains, validating the pair-feeding procedures used for the 14-d treatment groups. Ketogenesis was depressed at 14 d relative to the control, but was unaltered at earlier treatment periods. Output of glucose was increased relative to the control at 14 d, but was not changed at 7 d. Although glucose output was increased significantly at 3 d, the physiological meaning of these particular data eludes us at the present time.

The changes in hepatic fatty acid metabolism in PTU-treated animals could conceivably have resulted from unknown factors other than reduction of thyroid hormone concentrations. Had PTU had a deleterious action on the liver similar to many hepatotoxic agents, one might have expected a decrease rather than a

stimulation of triglyceride secretion. In the following experiments, therefore, T_3 was administered concurrently with PTU to rats to determine whether hypofunction of the thyroid was indeed the cause of the metabolic changes induced by PTU. The dose of T_3 used in these experiments with PTU was 3 μg/100 g body wt, daily for 14 d. As seen in Table I, this dose of T_3 was sufficient, even with simultaneous PTU treatment, to produce the metabolic alterations of hyperthyroidism. In separate experiments, PTU was added initially to the medium perfusing livers ($n = 2$) from normal fed male animals, and was also infused with the FFA complex to give a constant concentration of 1 mM (assuming no hepatic metabolism of PTU). When compared with perfusions to which an equal volume of 0.9% NaCl was added ($n = 2$), no differences in FFA uptake, or output of triglyceride, ketone bodies, or glucose were observed. Similarly, in other experiments, T_3 was added to the perfusate and was infused with the FFA complex to maintain constant concentrations of 100 μM ($n = 2$) and 1 mM ($n = 2$) (assuming no hepatic metabolism of T_3). Again, there were no obvious differences in any of the parameters measured.

Because hepatic output of triglyceride is particularly sensitive to reduction in caloric intake, the best model for studying the metabolic effects of hypothyroidism or hyperthyroidism proved to be livers from animals treated for 7 d with either PTU or 10 μg T_3/100 g body wt. Under these conditions, food consumption was unaffected. Concentrations of FFA in the cell-free medium perfusing livers from these rats were similar; neither FFA uptake, which was linear with time, nor FFA concentration was altered by treatment with PTU or T_3 (Fig. 7). The output of triglyceride, however, was increased about 2 μmol/g liver per 4 h with livers from PTU-treated animals and decreased about the same amount with livers from T_3-treated rats. Ketogenesis was increased in the T_3-treated animal, but was not altered from control in the PTU-treated rat under these experimental conditions. Similarly, glucose output was depressed in the T_3-treated rats, but was not altered in the PTU-treated animal.

The concentration of FFA and triglyceride in plasma of the animals treated with 0.9% NaCl, PTU, or 10 μg T_3/100 g body wt for 7 d was measured in samples obtained at the time of surgery. The concentrations of triglyceride were 0.33±0.07, 0.53±0.03, and 0.53±0.06 μmol/ml plasma for T_3 (10 μg/100 g body wt), 0.9% NaCl, and PTU-treated animals, respectively. Corresponding concentrations of FFA were 0.45±0.03, 0.22 ±0.01, and 0.34±0.03 μmol/ml plasma for the T_3, control, and PTU-treated animals, respectively. The elevation of plasma FFA ($P < 0.05$) and decrease in plasma triglyceride concentration ($P < 0.05$) in T_3-treated rats differed significantly from the controls.

DISCUSSION

The thyroid status of the animal clearly is an important regulator of hepatic metabolism of FFA. When perfused with equimolar physiologic quantities of FFA (oleate), livers from PTU-treated animals secreted more triglyceride whereas livers from T_3-treated animals secreted less triglyceride than did the respective euthyroid controls, despite the fact that uptake of FFA and concentration of FFA in the cell-free perfusate were equal in all groups. Conversely, but dependent on experimental conditions, ketogenesis was stimulated in the livers from animals treated with T_3 and diminished in the livers from animals treated with PTU. In recent experiments[2] using perfused livers from rats treated with PTU (1.0 mg/100 g body wt per d) or T_3 (10 μg/100 g body wt per day) for 7 d, the proportion of [1-^{14}C]-

[2] Keyes, W. G., H. G. Wilcox, and M. Heimberg. Manuscript in preparation.

TABLE I

Effects of Treatment with T_3 and PTU Simultaneously on Hepatic Metabolism

Group	Treatment	n	Fatty acid uptake	Triglyceride output	Ketone body output	Glucose output
				μmol/g liver/h		
A	0.9% NaCl	4	57.6±3.9	3.39±0.46	67±7	100±14
B	T_3	4	57.0±7.2	2.05±0.47	109±16	47±24
C	T_3 + PTU	4	65.6±2.9	1.21±0.27	105±13	65±13
A vs. B			NS	$P < 0.10$	$P < 0.05$	NS
A vs. C			NS	$P < 0.01$	$P < 0.05$	NS
B vs. C			NS	NS	NS	NS

Animals were given drugs for 14 d. Rats treated with T_3, or T_3 plus PTU, were pair-fed to control animals. The dose of T_3 was 3 μg/100 g body wt per d. Dose of PTU was 1 mg/100 g body wt per d. Perfusion parameters are given in Fig. 1. Values are means±SEM. Data were analyzed statistically using Dunnett's *t* test.

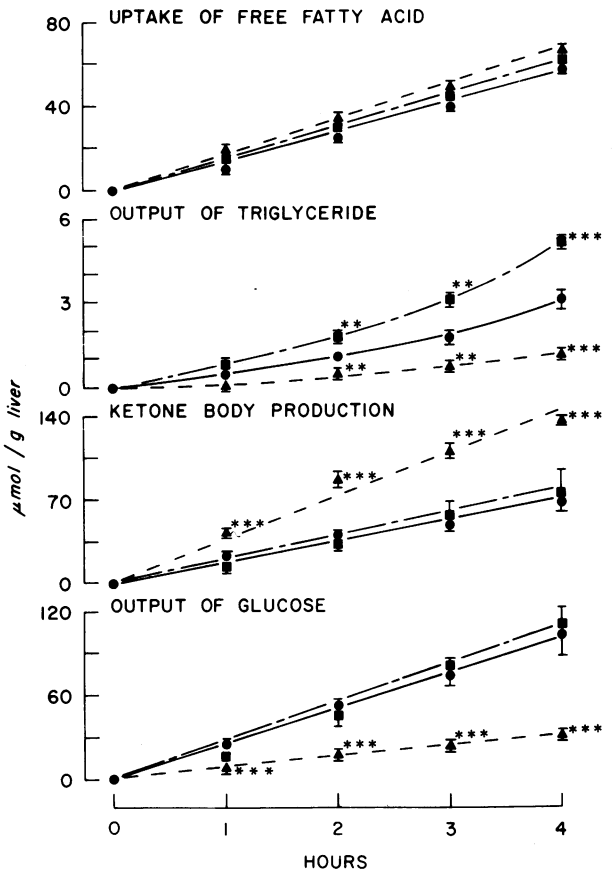

FIGURE 7 Metabolic alterations in livers from rats treated with T_3 (10 μg/100 g body wt per d), PTU (1 mg/100 g body wt per d), or 0.9% NaCl for 7 d. All rats were fed ad lib., consumed equal amounts of food, and had similar body weights at perfusion. Values are means±SEM. T_3 (▲, n = 4); PTU (■, n = 4); 0.9% NaCl (●, n = 8).

oleate esterified as total perfusate triglyceride was highly correlated with that fraction incorporated into VLDL triglyceride, in agreement with data reported in this manuscript. Moreover, hepatic output of the VLDL decreased with T_3 treatment and increased with PTU treatment, with proportional changes in triglyceride, phospholipid, and total apoprotein.

It was reported previously that hepatic oxidation of fatty acid is increased in hyperthyroidism (24, 25). Paradoxically, *de novo* synthesis of fatty acid (lipogenesis) has also been reported to be stimulated in hyperthyroidism (24, 26), although this remains controversial (27). Diamant et al. reported that oxidation of [^{14}C]palmitate was stimulated in liver slices from animals treated with T_3; simultaneously, they observed increased incorporation of [^{14}C]acetyl-CoA into fatty acids, increased activity of fatty acid synthetase, and increased activity of acetyl-CoA carboxylase by the 100,000-*g* supernate from livers of hyperthyroid rats (24). Similar observations were made by Roncari and Murthy on fatty acid synthetase and acetyl-CoA carboxylase (28); these workers moreover reported increased incorporation of ^3H_2O into fatty acids in livers of intact hyperthyroid rats. In contradiction to these data, Myant and Iliffe observed diminished incorporation of [1-^{14}C]-acetate into fatty acids by liver slices and 10,000-*g* supernatant fractions from hyperthyroid rats (27). The reasons for these discrepancies are unclear at the present time. Most of these experiments evaluating fatty acid synthesis in vitro were carried out in the absence of an exogenous supply of FFA. Under physiological conditions, however, the liver is exposed to a continuous supply of exogenous FFA. It is conceivable that the serum FFA, which may be increased in hyperthyroidism (5, 29), may depress fatty acid synthesis (30), and allow oxidation of fatty acid to be the metabolic pathway of primary importance. It is therefore important that the effects of thyroid status on hepatic fatty acid synthesis and oxidation be studied simultaneously in the same preparation in the presence of an adequate supply of exogenous FFA.

The fundamental mechanism(s) of action of thyroid hormone on the liver remain(s) to be clarified. The involvement of cyclic nucleotides is likely. It has been reported earlier from our laboratory that glucagon (31), cyclic AMP (cAMP), and dibutyryl cAMP diminish the output of triglyceride and VLDL, and stimulate ketogenesis and output of glucose by perfused livers from normal fed rats (31, 32). These effects are similar to those of T_3 reported in this paper. It is of interest that basal plasma cAMP concentrations and the peak plasma cAMP response to glucagon were elevated in human hyperthyroid subjects, in comparison to euthyroid individuals (33). Furthermore, increased activities of low K_m cyclic nucleotide phosphodiesterase (34, 35) and decreased activities of cAMP-dependent protein kinase (36) have been observed in livers from hypothyroid animals. Conversely, T_3 treatment increased hepatic cAMP concentrations (37) and hepatic protein kinase activity (38).

Glucose oxidation and gluconeogenesis from glycerol and lactate have been reported to be decreased in the hypothyroid liver, whereas increased rates of gluconeogenesis from lactate, alanine, and glycerol were observed with perfused livers from hyperthyroid rats (37, 39–42). It had been reported earlier that liver glycogen stores were depleted in hyperthyroidism (24, 27, 37). Because net glucose output under our experimental conditions resulted from glycogenolysis, diminution of output by the hyperthyroid livers would be expected. In our experiments, ketogenesis increased in livers from T_3-treated animals and decreased in livers from PTU-treated animals. The relationship of these data to ketonemia, however, remains to be determined.

The relationship of changes in thyroid status to hepatic metabolism of FFA and the concentrations of plasma

lipids and lipoproteins is of particular interest. Plasma triglyceride may be normal in many hypothyroid patients, but is usually elevated (1–3, 5–9) and may be markedly increased in myxedema (6, 10, 43). The concentration of plasma triglyceride in hyperthyroidism is more variable; concentrations generally have been reported to be decreased (1) or normal (2–4) but may be increased (5). Sandhofer et al. (1), observed that plasma triglyceride concentrations were reduced in hyperthyroid patients, and suggested that, since triglyceride clearance and turnover were unaltered in these subjects, the hypotriglyceridemia resulted from reduced triglyceride formation in the liver. Nikkila and Kekki (5), however, observed increased concentrations of plasma triglyceride in hyperthyroid subjects, associated with an augmented triglyceride production rate, a normal mean fractional removal rate, and with increased plasma concentrations of FFA and glycerol. In their studies with hypothyroid patients, increased plasma triglyceride concentrations were observed accompanied by a normal triglyceride production rate, but a marked reduction in the fractional triglyceride removal rate. The reasons for these discrepancies are unclear, but may be related to the FFA supply (44). Increased plasma FFA per se, might be expected to increase the rate of synthesis and secretion of VLDL triglyceride, in addition to the stimulatory or inhibitory actions resulting from an hormonal change. The output of triglyceride is proportional to the concentration of FFA perfusing the liver (44, 45). In the in vitro experiments reported here, uptake of FFA by livers exposed to equimolar quantities of FFA was similar in all groups; this undoubtedly is not the case with altered thyroid status in vivo. Increased plasma FFA concentrations have been observed in hyperthyroid animals and man (29). The plasma FFA concentration in rats treated for 7 d with 10 μg T_3/100 g body wt was increased over control values, and may, therefore result in increased hepatic output of triglyceride in the hyperthyroid rat in vivo. It is possible, therefore, that hepatic output of VLDL triglyceride in hyperthyroid animals in vivo might be occasionally increased above the euthyroid, primarily because of differences in plasma FFA concentration. The plasma concentration of triglyceride is, of course, also dependent on rates of peripheral utilization. Postheparin lipolytic activity is reduced in hypothyroidism and has been reported to be increased in hyperthyroid patients (2, 3, 5, 46), although there is some disagreement (3). Clearance of intravenously administered exogenous triglyceride, moreover, is depressed in hypothyroidism and accelerated in hyperthyroid patients (2, 5).

Hypercholesterolemia has been associated with hypothyroidism, and hypocholesterolemia with hyperthyroidism (8–10, 23, 47–49). The hypercholesterolemia of hypothyroidism may arise, in part, from an increased hepatic output of VLDL cholesterol, and, in part, from a decreased peripheral utilization of VLDL and low density lipoprotein cholesterol (8, 49). Although hepatic cholesterogenesis is assumed to be depressed in hypothyroidism, cholesterol catabolism is depressed even further (50). This combination of events may conceivably provide an ample pool of cholesterol for export as a moiety of the VLDL, particularly if stimulated by the supply of FFA to the liver (30). Clearly, changes in hepatic metabolism of FFA with alterations in thyroid status affect the hepatic output of triglyceride and the VLDL and therefore, may have important consequences for the concentration of plasma lipids and lipoproteins in vivo.

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