Effects of Triiodothyronine on Biosynthesis and Secretion of Triglyceride by Livers Perfused in Vitro with [3H]Oleate and [14C]Glycerol*

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Livers from euthyroid and hyperthyroid (triiodothyronine-treated) rats were perfused in vitro with tracer amounts of [14C]glycerol and substrate quantities of [3H]oleate. Hepatic uptake of total free fatty acid, [3H]oleate and [14C]glycerol were similar regardless of hormonal treatment. Output of triglyceride was 4.46 ± 0.48 μmol/g and 2.14 ± 0.36 by livers from euthyroid and hyperthyroid animals, respectively. Although total incorporation of [14C]glycerol into perfusate triglyceride was similar in both groups, the specific radioactivity of triglyceride was approximately 30% higher in the hyperthyroid. The glycerol-3-phosphate content of livers from triiodothyronine (T3)-treated rats was 52.3% that of livers from euthyroid rats (0.80 ± 0.15 versus 1.53 ± 0.16 μmol/g, respectively). After 4 h of perfusion, the same relationship was observed. The specific radioactivity of glycerol-3-phosphate following perfusion with [14C]glycerol in livers from T3-treated rats was twice that of the euthyroid. Synthesis of triglyceride, calculated from glycerol-3-phosphate specific radioactivity, was 4.62 ± 0.44 μmol/g/4 h by livers from euthyroid rats and 1.95 ± 0.27 after T3 treatment. The incorporation of [3H]oleate into perfusate triglyceride by livers from hyperthyroid rats was approximately 39% of the euthyroid, while the specific radioactivity was approximately equal in both groups. Triglyceride synthesized from [3H]oleate was calculated to be 2.00 ± 0.50 μmol/g of liver/4 h for livers from T3-treated animals and 4.29 ± 0.57 for euthyroid controls. The rates of synthesis of perfusate and hepatic triglyceride, and output of perfusate triglyceride, by livers from hyperthyroid rats were lower than in the euthyroid, and similar values were obtained whether calculated by chemical assay, or from radioactive incorporation of [3H]oleate or [14C]glycerol. Additionally, livers from T3-treated rats had higher rates of ketogenesis, secreted less glucose, and produced more 14CO2 from glycerol than did livers from euthyroid rats. Clearly, livers from hyperthyroid rats synthesize and secrete less triglyceride (i.e., very low density lipoprotein) than do livers from euthyroid animals. These conclusions were reached whether glycerol or free fatty acid was used as a precursor and, moreover, stress the importance of utilizing the actual in situ specific activities of precursors for calculating the rates of biosynthesis of triglyceride by the liver.

It was reported previously from our laboratory that livers from rats treated with triiodothyronine secreted less VLDL triglyceride and had higher rates of ketogenesis than did livers from euthyroid rats, even though the uptake of free fatty acid by the livers was not altered (1, 2). Thyroid hormones directed metabolism of fatty acids away from pathways of esterification (triglyceride synthesis) and into oxidative pathways (ketogenesis and CO2 formation). Observations with the perfused liver in agreement with ours were reported by Laker and Mayes (3); human studies of Abrams et al. (4) were consistent with our findings. However, studies from other laboratories (5-7) have been at variance with our conclusions. Using cell-free liver preparations (800 × g supernatant), Roncarli and Murthy (5) concluded that the synthesis of triglyceride from [14C]glycerol-phosphatidylcholine was higher in thyroxine-treated rats than in controls. Total glycerolipid synthesis, however, was not altered. Glenny and Brindley (6) measured the rate of glycerolipid synthesis in vivo 1 min after intraportal administration of [14C]palmitate and [14C]glycerol to rats; triglyceride synthesis was measured as the percentage of total glycerolipid synthesis. These authors reported that the rate of triglyceride synthesis from [14C]palmitate was the same in euthyroid and hyperthyroid states, whereas the rate of synthesis from [3H]glycerol was higher in hyperthyroid than in euthyroid states. The absolute rate of triglyceride synthesis by hyperthyroid livers was, however, not different from controls. The rate of triglyceride synthesis from [3H]glycerol was previously estimated by Nikkila and Kekki (7) in man as a function of the plasma triglyceride turnover rate. They concluded that the rate of splanchnic triglyceride production was greater in hyperthyroidism than in the euthyroid state and, moreover, was not reduced on treatment of the thyrotoxicosis.

The use of glycerol as a precursor for synthesis of triglyceride may lead to incorrect interpretation of the data, if one neglects to consider the magnitude of the glycerol-3-phosphate pool of the liver through which glycerol must pass during biosynthesis of triglyceride. Schimassek et al. (8) and Sestoft et al. (9) had reported earlier that the hepatic content of the...
Triiodothyronine and Biosynthesis of Triglyceride

Glycero-3-phosphate in the hyperthyroid state was about half that of the euthyroid. The smaller pool size of G3P in the hyperthyroid liver would dilute a tracer glycero-3-phosphate molecule less, resulting in higher specific activity of the G3P in the hyperthyroid than in the euthyroid state.

The calculated rate of triglyceride synthesis should be identical whether measured with either free fatty acid or glycero-3-phosphate as a radioactive tracer. To avoid erroneous conclusions, it is essential that the actual precursor specific activity be used for calculation of rates of synthesis of triglyceride, and not the specific activity of the added tracer glycero-3-phosphate or fatty acid. To reconcile apparently divergent data, we perfused livers from euthyroid and hyperthyroid rats simultaneously with $^{14}$C glycero-3-phosphate and $^3$Holeate, determined the specific activity of hepatic G3P and oleate taken up by the liver, and evaluated the effects of triiodothyronine on hepatic lipid metabolism.

**EXPERIMENTAL PROCEDURES**

*Treatment of Animals and Perfusate Conditions—Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 175-200 g were acclimatized for 1 week to 12-h (0600-1800 h) light-dark cycles in our animal facility before the experiment was started. Animals were made hyperthyroid by intraperitoneal administration of T$_3$ with Alzet (Model 2001) osmotic minipumps (10). The T$_3$ was administered at the nominal rate of 0.6 µg/rat/day (44.7 ± 0.07 µg/100 g body weight/day) with pumps containing 40 µg T$_3$/device. The T$_3$ was dissolved in n-butanol:propylene glycol (0.5:9.5) (v/v). Control EU rats received the pumps containing only the T$_3$ vehicle. All animals were treated for 7 days and were allowed free access to water and powdered Purina laboratory chow. Food consumption by each rat was measured daily. At the end of the treatment period, the rats were anesthetized with diethyl ether and the livers were removed surgically and perfused in a recirculating system used routinely in this laboratory (11). Immediately before cannulation of the portal vein, 4-5 ml of blood were withdrawn from the abdominal aorta for measurement of serum levels of triglyceride, FFA, and T$_3$. The composition of the perfusate was described previously (1). The initial volume of the perfusate was 70 ml and the hematocrit was 30%. The liver was equilibrated for 20 min with the initial medium; infusion was then begun of a 6% bovine serum albumin-sodium oleate complex (1) in Ca$^{2+}$ and Mg$^{2+}$-free Krebs-Henseleit buffer, pH 7.8, containing also 50 µCi of $^3$Holeate and 20 µCi of $^{14}$Cglycerol. The infusion rate of 11.7 ml/min supplied the liver with 166 µmol of oleate/H, but only tracer amounts of glycero-3-phosphate. At the start of the infusion, and 2 and 4 h later, aliquots of perfusate were removed for analysis. The experiment was terminated at the end of 6 h. A lobe of liver was tied off with an umbilical suture, excised rapidly, and frozen in liquid nitrogen. The frozen liver was ground into powder, and a weighed quantity was used to extract glycero-3-phosphate. The remaining liver was perfused with about 40 ml of ice-cold 0.9% NaCl to rinse out residual perfusate. Nonhepatic tissues were removed and the liver was blotted dry and weighed. One gram of the liver was homogenized with 4.0 ml of ice-cold 0.9% NaCl, and 1.0 ml of the homogenate was extracted and analyzed for hepatic content of lipids.

Analytical Procedures—Lipids were extracted from rat serum, perfusate, and liver samples as described previously (11, 12). The extracts were fractionated by thin layer chromatography and the free fatty acid and triglyceride were assayed as described previously (13). Aliquots of the triglyceride and free fatty acid fractions were evaporated to dryness in glass scintillation vials and the residues were dissolved in Biocount by shaking overnight. $^{14}$C and $^3$H radioactivity was determined, and the specific activity (dpm/pmol) was calculated.

To determine the specific radioactivity of G3P, the G3P extract from livers perfused with radioactive substrates was treated according to the procedure of Conyers et al. (19) to remove cations and perchloric anions. The G3P was then separated by thin layer chromatography on PEI-cellulose plates. After elution of the G3P band from the PEI-cellulose, the radioactivity of the G3P was determined, and the specific activity (dpm/pmol) was calculated.

To determine the variation of the hepatic content of G3P during the course of a perfusion, additional livers from hyperthyroid and euthyroid rats were perfused as described above. At the same time that aliquots of perfusate were sampled, a lobe of the liver was tied off, excised, and frozen in liquid nitrogen. The frozen tissue was treated as described above and used for extraction and assay for G3P. Additionally, livers from T$_3$-treated rats, euthyroid rats which received the T$_3$-vehicle only, and euthyroid rats not given any treatment were assayed for concentrations of G3P. These livers were removed from the animals and analyzed for G3P without being perfused.

Estimation of $^{14}$CO$_2$—The production of $^{14}$CO$_2$ from $^{14}$Cglycerol by the perfused livers was measured by trapping $^{14}$CO$_2$ evolved as described previously (13). The $^{14}$CO$_2$ was collected and stored in a hyamine hydroxide, and the radioactivity was determined by liquid scintillation spectrometry (3).

Materials—Bovine serum albumin, Fraction V (Miles Laboratories, Elkhart, IN) was purified before use (1). Oleic acid (99% purity) was purchased from Nu-Chek-Prep, Inc., Elsian, MN. $^{14}$Coleic acid (specific activity, 5.7 Ci/mmol) and $^3$Holeate (specific activity, 19.8 Ci/mmol) were obtained from the England Nuclear Corp. PEI-Cellulose MN and Silica Gel-G thin layer chromatography plates were obtained from Analtech, Newark, DE. Biocount scintillation fluid was purchased from Research Products International Corp., Mount Prospect, Ill.

**RESULTS**

Triiodothyronine-treated and euthyroid rats lost weight the day following intraperitoneal implantation of the osmotic minipumps, after which the body weight gradually returned toward the pretreatment level. Food consumption by the two groups was similar during the treatment period. The liver weights and ratios of liver weight/body weight were identical in both groups (Table I). Treatment of animals with T$_3$ altered the serum concentrations of the free fatty acid and triglyceride. The serum concentration of free fatty acid was higher in T$_3$-treated rats than in euthyroid animals (0.735 ± 0.12 versus 0.211 ± 0.04 µmol/ml, p < 0.001), while the serum triglyceride concentration was lower in the hyperthyroid than in the euthyroid animals (0.135 ± 0.02 versus 0.211 ± 0.02 µmol/ml, p < 0.02). Average concentrations of serum T$_3$ in normal and treated animals were 77.2 ± 6.2 and 311.0 ± 21.4 ng/dieter, respectively (p < 0.001).

In agreement with earlier observations (1, 2), the volume of bile secreted by perfused livers from T$_3$-treated rats exceeded that of the control group (Table I). Perfusion flow rate was...
identical in both groups. Uptake of infused free fatty acid measured by mass or radioactivity was not affected by treatment with T₃, and was linear with time during the experiment (Fig. 1). The specific radioactivity of the free fatty acid taken up by the liver was similar in both groups (26.4 (×10⁶) ± 5.2 for the euthyroid and 27.7 (×10⁶) ± 5.7 (dpm/μmol) in the hyperthyroid). Furthermore, as shown in Fig. 1, treatment with T₃ did not alter the uptake of [¹⁴C]glycerol by livers from T₃-treated or euthyroid rats.

The concentrations of glycerol-3-phosphate were lower in nonperfused livers from T₃-treated rats than from euthyroid rats (Table II). Glycerol-3-phosphate also was determined in livers from euthyroid and T₃-treated rats at the end of perfusion in experiments with radioactive substrates. Another group of livers from euthyroid and T₃-treated rats were perfused without tracer glycerol and with unlabeled oleate, and in these experiments, the variation of glycerol-3-phosphate concentration during perfusion was determined after 2 and 4 h of perfusion. For all perfused livers, glycerol-3-phosphate levels were lower at the end of the experiment than were the basal levels measured in the nonperfused livers (Table II). Under the conditions of these experiments, the glycerol-3-phosphate content of the perfused euthyroid livers declined to approximately 59% (p < 0.05) of euthyroid basal levels, while it declined to 64% (p < 0.2 > 0.05) of basal level for the livers from T₃-treated animals. Regardless, the concentration of G3P in euthyroid livers at the end of perfusion was higher than in the hyperthyroid livers. In the experiments in which [¹⁴C]glycerol and [³H]oleate were infused, the G3P content in livers from T₃-treated rats was 52.9 ± 9.4% of that in euthyroid livers. However, the specific activity of the [¹⁴C]glycerol-3-phosphate purified from liver extracts after 4 h of perfusion was about twice as high in livers from T₃ rats compared to the euthyroid (Table III). Although there was a progressive decrease in the hepatic content of G3P during perfusion, the relative decrease in the hyperthyroid and euthyroid livers calculated from the ratio of G3P concentration at 4 h to that at 2 was similar in both groups (Table II). The specific activity of G3P obtained at 4 h, therefore, was a reasonable estimate of the precursor pool, during the course of the perfusion, and was used for calculation of rates of synthesis of triglyceride.

### Table I

**Functional parameters for perfusion of livers from euthyroid and hyperthyroid rats**

| Parameter                              | EU          | T₃          |
|----------------------------------------|-------------|-------------|
| Body weight (g)                        | 219 ± 8.9   | 213.9 ± 5.5 |
| Food consumption (g/day)               | 13.5 ± 0.8  | 15.0 ± 0.8  |
| Liver, wet weight (g)                  | 8.54 ± 0.33 | 8.37 ± 0.33 |
| (Liver weight/body weight)             | 3.91 ± 0.11 | 3.94 ± 0.20 |
| 100                                    |             |             |
| Perfusate flow rate (ml/min/g liver)   | 2.6 ± 0.20  | 2.7 ± 0.30  |
| Bile output (μl/g/4 h)                 | 209 ± 19    | 303 ± 33"   |

"Significance of difference from control is p < 0.025.

### Table II

**Hepatic content of glycerol-3-phosphate**

The G3P content of the nonperfused liver was presumed to represent the basal level. G3P was extracted from the liver at the end of 4 h of perfusion unless indicated otherwise. The specific activity of G3P was calculated from the purified extract in which mass was assayed enzymatically and radioactivity was determined. In Experiment II, G3P was extracted and assayed from liver samples taken at the indicated times without interruption of the perfusion. Excision of a lobe of the liver for analysis of G3P at 2 h did not adversely affect the subsequent liver perfusion. Values are mean ± S.E. Numbers of livers are in parentheses. Significance of differences between basal G3P versus end of perfusion are: for T₃, p < 0.2 > 0.05, and for EU, p < 0.05. In Experiment II, significance of differences between G3P concentration at 2 h versus that at 4 h are: for T₃, p < 0.1 > 0.05, and for EU, p < 0.005.

![Fig. 1. Uptake of [¹⁴C]glycerol and free fatty acid by perfused livers from T₃-treated and euthyroid rats. Livers were perfused simultaneously with glycerol and oleic acid as outlined in the text. Free fatty acid was separated from other perfuse lipids, and both mass and radioactivity were measured. The uptake of [¹⁴C] glycerol (A), total free fatty acid (B), and [³H]oleic acid (C) were calculated as previously described (11). Per cent uptake was calculated from total amount of substrate infused. Each point represents mean ± S.E. of 6 perfusion experiments.](image)
Livers from euthyroid and T$_3$-treated rats were perfused with a medium containing [14C]glycerol and [3H]oleate. The methods for isolation and assay of mass and radioactivity of perfusate triglyceride, and calculations are described in the text. Values are mean ± S.E. (n = 6, except where indicated).

### TABLE III

|                | EU | T$_3$ |
|----------------|----|------|
| Mass output (μmol TG/g) | 4.46 ± 0.48 | 2.14 ± 0.36$^*$ |
| Total incorporation into TG (dpm/μmol) | 69,541 ± 9,161 | 53,951 ± 11,250 |
| Fractional conversion to TG (% of [14C]glycerol infused) | 0.161 ± 0.023 | 0.125 ± 0.026 |
| Specific activity of TG (dpm/μmol) | 15,984 ± 1,595 | 25,882 ± 4,849$^*$ |
| Rate of synthesis of TG (dpm/μmol/μCi [14C]glycerol infused) | 830 ± 103 | 1,352 ± 290 |
| Specific activity of G3P (dpm/μmol) | 13,788 ± 1,820 (5) | 27,566 ± 6,062 (4)$^*$ |
| Incorporation of glycerol in TG (μmol of glycerol/g/4 h) | 4.62 ± 0.44 (5) | 1.95 ± 0.27 (4)$^*$ |

$^*$ Statistical significance was estimated by Student’s t test at p < 0.005.

Biosynthesis and Secretion of Triglyceride—As predicted from previous studies (1, 2), livers from euthyroid animals secreted more triglyceride into the perfusate than did livers from T$_3$-treated animals, whether measured in terms of mass or radioactivity. Livers from euthyroid animals put out approximately twice as much triglyceride as that secreted by livers from T$_3$-treated rats (Fig. 2; the mean for all experiments with or without radioactive substrates was 2.10 ± 0.22 μmol/g of liver/4 h for T$_3$ versus 5.16 ± 0.51 μmol/g of liver/4 h for euthyroid; p < 0.001). In the livers perfused with [14C]glycerol and [3H]oleate, total incorporation of [14C] into perfusate triglyceride (dpm/g of liver) was not significantly different with livers from either euthyroid or T$_3$ animals (Table III). In contrast, incorporation of [3H]oleate into triglyceride was significantly higher in the euthyroid group than in the T$_3$-treated animals (Table IV). The fractional conversion of infused [14C]glycerol to [14C]triglyceride also was about the same in the two thyroid states. However, the specific activity (dpm/μmol) and the relative specific activity of the triglyceride synthesized from [14C]glycerol (dpm/μmol/μCi of [14C]glycerol-infused) were higher in T$_3$ than in euthyroid livers. The rate of incorporation of [14C]glycerol into triglyceride secreted by the livers was calculated as follows:

\[
\text{Rate of incorporation} = \frac{\text{dpm incorporated into triglyceride/g/4 h}}{\text{specific activity of precursor}}
\]

\[
= \frac{\text{dpm/g/4 h}}{\text{dpm/μmol}}
\]

\[
= \frac{\text{μmol precursor/g/4 h}}{1}
\]

Since 1 mol of G3P is converted to 1 mol of triglyceride, this calculation with [14C]glycerol is also equal to moles of triglyceride synthesized. Clearly, the rate of synthesis of triglyceride from [14C]glycerol was higher in livers from EU than from T$_3$-treated animals, even though the specific activity of the triglyceride was higher in the T$_3$ group. Although an identical conclusion was reached when the rate of synthesis of triglyceride from [3H]oleate was calculated, certain differences are important. As shown in Table IV, the total incorporation and fractional conversion of [3H]oleate to triglyceride was higher with livers from euthyroid rats than with those from hyperthyroid animals. The relative specific activity of the perfusate triglyceride were similar in the euthyroid and the T$_3$ group. The specific activity of the [3H]oleate taken up by the liver was identical in both groups. The incorporation of [3H]oleate into triglyceride (μmol of oleate/g/4 h) by livers from euthyroid rats was greater than that by T$_3$ livers. Estimation of μmol of triglyceride synthesized from [3H]oleate was calculated from the μmol of oleate incorporated, with the following corrections. Although 3 mol of fatty acid are contained per mol of triglyceride, all the fatty acid moieties are not oleate. It was observed in previous studies from this laboratory (11, 21) that the fatty acid composition of triglyceride secreted by livers perfused in the absence of exogenous supply of oleic acid consisted of approximately 30–35% oleic acid. When oleic acid was infused at the rate employed in the present experiments, the fatty acid composition of the secreted triglyceride was approximately 55–65% oleic acid. Clearly, the secreted triglyceride did not derive all its fatty acid from the exogenously supplied oleic acid. At best, the triglyceride-oleate concentration increased by a factor of 1.5–2.0 when oleic acid was infused under these experimental conditions. When the correction factor of 1.5 is applied in the calculation of the rate of triglyceride synthesis, the values of 4.29 ± 0.57 and 2.00 ± 0.50 (p < 0.005) are obtained for the euthyroid and hyperthyroid, respectively. These data are similar to those calculated from mass measurement or from incorporation of [14C]glycerol.

The incorporation of glycerol and oleate into triglyceride was examined further by enzymatic and cytochrome oxidase. There was no measurable 14C radioactivity in the isolated triglyceride-oleate fraction from either EU or T$_3$ livers. Therefore, under the conditions of these experiments, there was no significant lipogenesis (i.e., fatty acid synthesis) from glycerol by livers from either euthyroid or hyperthyroid rats.

At the end of the perfusion experiments, the concentration of triglyceride (μmol/g) in all livers from T$_3$-treated rats was only 46% of that in EU livers (Fig. 2). For those livers perfused with radioactive substrates, the concentration in livers from T$_3$-treated rats was about 50% of that of the EU group (Table V). Total incorporation and fractional conversion of [14C]glycerol into hepatic triglyceride were higher in livers from EU rats than in the hyperthyroid (Table V) while specific activity and relative specific activity of the triglyceride tended to be lower in the EU than in T$_3$ group. Incorporation of glycerol (μmol of glycerol/g/4 h) into triglyceride was higher in the EU than in T$_3$ livers, and the calculated rate of triglyceride synthesis (μmol of triglyceride/g/4 h) was similar to the mass of triglyceride determined by chemical assay. Similarly, as shown in Table VI, the total incorporation and fractional conversion of [3H]oleate into hepatic triglyceride were similar in the two thyroid states. The rate of synthesis of hepatic triglyceride, calculated as for perfusate triglyceride, was higher in the EU than in the T$_3$ livers and similar to that determined by chemical assay.

An interesting estimate of the utilization of each radioactive
Fig. 2. Effects of treatment with triiodothyronine on CO₂ production from [¹⁴C]glycerol, triglyceride output, hepatic triglyceride, and ketogenesis by perfused livers. For triglyceride output and hepatic triglyceride (A) and ketogenesis (B) each point represents mean ± S.E. of 8-10 experiments. For CO₂ production (C), the rate of production (inset) and cumulative output were calculated as previously described (11); values are mean ± S.E. for perfusions. For output of triglyceride and ketogenesis, statistical significance of differences between T₃ and EU were estimated by Student’s t-test: **, p < 0.005; ***, p < 0.001. For CO₂ production, statistical significance between T₃ and EU was calculated by Student’s t-test and regression analysis: α, p < 0.1 > 0.05; *, p < 0.05.

precursor by the liver for synthesis of perfusate and hepatic triglyceride was obtained by calculating the relative enrichment of each isotope in the triglyceride produced. The ratio \(^{3}H/^{14}C\) in the perfusate and hepatic triglyceride was compared with the ratio of the infused precursors (Table VII). It can be seen that the ratio \(^{3}H/^{14}C\) was greater in the synthesized triglyceride than in the infusate, and that the ratio \(^{3}H/^{14}C\) in either perfusate or hepatic triglyceride was greater in the EU group than in the hyperthyroid. These data suggest that relatively more fatty acid than glycerol was utilized for triglyceride synthesis by livers from both euthyroid and hyperthyroid rats, that livers from T₃-treated rats converted relatively less free fatty acid into triglyceride than livers from euthyroid rats, and that glycerol enters into many pathways other than lipid metabolism.

Our findings on glucose output confirmed earlier reports from this laboratory. Glucose output, which in these experiments, represents primarily glycogenolysis, at the end of 4-h perfusion by livers from T₃-treated rats was significantly less than that for EU livers (46.2 ± 4.4 µmol/g/ vs. 76.3 ± 9.4, respectively; p < 0.01).

Ketogenesis and \(^{14}CO₂\) Output—T₃-treatment altered oxidation of oleate and \([^{14}C]\)glycerol by perfused livers. Ketogenesis was significantly elevated in livers from T₃-treated rats compared to those from euthyroid rats (Fig. 2B), in agreement with previous reports from this laboratory (1, 2) and others (3, 22, 23). There was some conversion of \([^{14}C]\)glycerol into total ketones, but there was no significant differences between groups. Incorporation was 28.14 ± 5.24 dpm × 10⁵/g versus 23.28 ± 5.64, for T₃ and EU groups, respectively. The generation of \(^{14}CO₂\) from \([^{14}C]\)glycerol by livers from T₃-treated animals was higher than with livers from EU rats (86.72 ± 9.6 dpm × 10⁴/g/4 h compared to 59.66 ± 8.9 dpm × 10⁴/g/4 h; p < 0.1 > 0.05, Fig. 2C).
Triiodothyronine and Biosynthesis of Triglyceride

Use of [1-14C]oleate for measurement of synthesis of perfusate triglyceride in livers from normal and T3-treated rats

Livers from euthyroid and T3-treated rats were perfused with a medium containing [14C]oleate and [1-14C]oleate. The methods for isolation and assay of mass and radioactivity of triglyceride, and calculations, are described in the text. Calculation of mol of oleate incorporated by 1.5, as discussed in the text. Values are mean for 6 perfusions in each group.

| Measurements                                    | EU         | T3         |
|-------------------------------------------------|------------|------------|
| Mass output (µmol TG/g)                         | 4.46 ± 0.48| 2.14 ± 0.36 |
| Total incorporation into TG (dpm/µg)            | 1,665,040 ± 380,420 | 677,940 ± 154,760 |
| Frac. conversion to TG (dpm/µg/µCi)             | 9.96 ± 1.23 | 4.44 ± 0.98 |
| Relative specific activity ofTG (dpm/µmol/µCi)  | 4,816 ± 0.49 | 4,654 ± 960 |
| Rate of synthesis of TG                         | 264,110 ± 52,470 | 276,630 ± 56,960 |
| Specific activity of oleate (dpm/µmol)          | 6.44 ± 0.85 | 3.01 ± 0.75 |
| Incorporation of oleate into TG µmol oleate/g/4 h | 4.29 ± 0.57 | 2.00 ± 0.50 |
| Synthesis of TG µmol TG/g/4 h                   |            |            |

*Statistical significance of differences between EU and T3 group by Student's t test is p < 0.005.

Table V

Use of [1-14C]glycerol for measurement of synthesis of hepatic triglyceride in livers from normal and T3-treated rats

At the end of each perfusion with [14C]glycerol and [1-14C]oleate, the concentration and synthesis of triglyceride in livers from normal and T3-treated rats were determined as described in the text. Values are mean ± S.E. (n = 6, except where indicated).

| Measurements                                    | EU         | T3         |
|-------------------------------------------------|------------|------------|
| Mass content (µmol TG/g)                        | 3.07 ± 0.48| 1.82 ± 0.35 |
| Total incorporation into TG (dpm/µg)            | 1,310,720 ± 26,450 | 551,940 ± 114,330 |
| Frac. conversion to TG (dpm/µg/µCi)             | 0.756 ± 0.12 | 0.319 ± 0.06 |
| Relative specific activity of TG (dpm/µmol/µCi)  | 4,740 ± 637 | 4,172 ± 500 |
| Rate of synthesis of TG                         | 264,110 ± 52,470 | 276,630 ± 56,960 |
| Specific activity of oleate (dpm/µmol)          | 5.10 ± 0.84 | 2.07 ± 0.37 |
| Incorporation of oleate into TG µmol oleate/g/4 h | 3.40 ± 0.56 | 1.38 ± 0.25 |

*Statistical significance of differences between EU and T3 group by Student's t test is p < 0.005.

Discussion

The influence of thyroid hormones on plasma triglyceride concentration appears to be variable. It was reported that in conditions of elevated thyroid hormones, plasma triglyceride may be increased (7, 22, 24), decreased, (4, 25, 26) or normal (4, 24, 25). The variability may perhaps reflect the severity of the hyperthyroidism. Studies designed to elucidate how thyroid hormones alter degradation and/or biosynthesis of triglyceride have also yielded conflicting conclusions. Sandhofer et al. (28) observed hypotriglyceridemia in their hyperthyroid patients; they also studied splanchic triglyceride synthesis from [1-14C]palmitate and suggested that the hypotriglyceri-
demia may have been due to decreased synthesis of triglycerides, since they did not observe any change in triglyceride turnover or clearance. This suggestion was not supported by Nikkila and Kekki (7) who observed slight hyperglycemia in their hyperthyroid patients; their study led to the conclusion that the rate of triglyceride synthesis from [3H]glycerol was increased. Previous reports from our laboratory (1, 2) and the data reported here establish clearly that under controlled conditions, perfused livers from T3-treated rats directed metabolism of oleic acid away from pathways of esterification (primarily triglyceride synthesis) and secretion of the VLDL and into pathways of oxidation (Co2 production and ketogenesis). In contrast, livers from hypothyroid rats utilized fatty acids in a direction opposite of those of the hyperthyroid (1, 2). Moreover, our present data indicate that the rate of triglyceride synthesis measured by chemical assay, or by incorporation of either [1-14C]glycerol or [1-14H]oleate as precursor, gave similar results. The rate of triglyceride synthesis was calculated from the in situ specific activity of the radioactive precursor. This was particularly important with the use of [1-14C]glycerol for measurement of synthesis. Similarly, synthesis from [1-14H]oleate was calculated using the specific radioactivity of the oleate actually taken up by the liver. It is essential to use the actual specific activity of C2P to estimate the rate of triglyceride synthesis, since the hepatic concentration of glycerol-3-phosphate in T3-treated rats is reduced compared to the euthyroid, as observed in these experiments and reported previously by Schimassek et al. (8) and Sestoft et al. (9). The reduced glycerol 3-phosphate level could be attributed in part to the enhanced catalytic properties of glycerol-3-phosphate dehydrogenase by thyroid hormones (27). Clearly, administration or infusion of a tracer quantity of radioactive glycerol into euthyroid and hyperthyroid livers would mean that the infused [1-14C]glycerol would be diluted to a lesser extent in the hyperthyroid than in the euthyroid livers. Hence, the specific activity of glycerol-3-phosphate would be higher in the hyperthyroid than in the euthyroid liver. In the present experiments, where the specific activity of glycerol-3-phosphate in the livers from T3-treated rats was twice that of the euthyroid, total 14C incorporation into triglyceride by the T3-treated livers would have to be twice that of the euthyroid group to exhibit equal rates of synthesis of triglyceride. However, as stated previously, diacylglycerol acyltransferase (31) which would lead to decrease of diglyceride is below that required to saturate the a-glycerophosphate acyltransferase (31). The enzyme-substrate relationship is compatible with our observation of a decreased synthesis of triglyceride in the hyperthyroid rats. Moreover, enhanced gluconeogenesis in hyperthyroidism (34) is a frequently reported observation, and this may further decrease the pool of glycerol-3-phosphate available for triglyceride synthesis.

In addition to hepatic changes induced by thyroid hormone, plasma free fatty acid concentration is elevated by T3-treatment, as shown by others (7, 22, 25) and in this report; the increased availability of free fatty acid substrate to the liver in vivo may increase triglyceride production (29, 30). Even though the hyperthyroid state is also associated with increased oxidation of free fatty acids (1-3, 22, 32), the elevated plasma free fatty acids, nevertheless, may be sufficient to increase plasma triglyceride concentrations above normal values in the hyperthyroid animals. Similar conclusions were obtained in studies of hepatic metabolism of free fatty acids in experimental insulin deficiency (29). It has also been shown that plasma clearance of triglyceride is enhanced in hyperthyroid patients (4, 7, 26). This enhanced clearance is another factor which must be considered as contributing to the variability of plasma triglyceride concentrations in the hyperthyroid patient, the total incorporation into perfusate triglyceride is approximately equal in both euthyroid and hyperthyroid livers, while the incorporation into hepatic triglyceride was higher in the euthyroid than in the hyperthyroid animals. Therefore, for both perfusate and hepatic triglyceride, the calculated rate of triglyceride synthesis was lower in the livers from T3-treated rats than in those from euthyroid controls. The studies reported by Laker and Mayes (3) and Abrams et al. (4) showed, in agreement with our data, that the rate of triglyceride production under hyperthyroid conditions was lower than that in euthyroid. Although the data are not strictly comparable, Yokahi (28) reached similar conclusions that thyroxine treatment of rats caused inhibition of ethanol-induced elevation of a-glycerophosphate and the concomitant accumulation of serum and hepatic triglyceride.

Other investigators had calculated, contrary to our data, a higher rate of hepatic triglyceride synthesis in the hyperthyroid state than in the euthyroid (5-7, 22). In the study of Roncari and Murthy (5), triglyceride synthesis was calculated as the percentage of [1-14C]glycerol-3-phosphate incorporated into triglyceride. Gienny and Brindley (6) measured triglyceride synthesis by rats in vivo in terms of the percentage of total glycerolipid synthesized; in their report, triglyceride synthesis from [1-14C]palmitate was not altered by thyroxine treatment, whereas synthesis from [3H]glycerol was higher in the hyperthyroid than in the euthyroid condition. These two studies did not consider the endogenous pools of hepatic glycerol-3-phosphate. In comparison with the data reported here, the rates of triglyceride synthesis calculated by Roncari and Murthy (5) and Glenny and Brindley (6) would be equivalent to the calculated relative specific activity of triglyceride, shown in Table III. Calculation of triglyceride synthesis based on specific activity of the triglyceride produced from radioactive glycerol in hyperthyroidism is erroneous, because synthesis is an inverse function of the precursor specific activity. The report of Nikkila and Kekki (7) measured the rate of triglyceride synthesis in human patients in terms of turnover rate. It was not clear from their report how the calculated turnover rate translated into rate of triglyceride synthesis. In the three studies referred to above (5-7), radioactive glycerol or glycerol-3-phosphate was used as precursor and in none of the studies was the endogenous pool of glycerol-3-phosphate measured. Roncari and Murthy (5) incubated cell-free preparation with excess of glycerol-3-phosphate, an experimental condition that might also mask the T3-induced decrease of hepatic glycerol-3-phosphate concentration reported here and by others (8, 9). In the in vivo studies, liver biopsies were not done. Therefore, it is appropriate for us to suggest that, although the data reported in these studies are correct and accurate, the conclusions are erroneous.

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