

Adaptive Changes in Transmembrane Transport and Metabolism of Triiodothyronine in Perfused Livers of Fed and Fasted Hypothyroid and Hyperthyroid Rats

Marion de Jong, Roel Docter, Hans van der Hoek, Eric P. Krenning, and Georg Hennemann

The transport and subsequent metabolism of triiodothyronine (T_3) were studied in isolated perfused livers of euthyroid, hypothyroid, and hyperthyroid rats, both fed and 48-hour-fasted. T_3 kinetics (transport and metabolism) during perfusion were evaluated by a two-pool model, whereas the metabolism of T_3 was also investigated by determination of T_3 breakdown products by chromatography of medium and bile. For comparison of groups, metabolism was corrected for differences in transport. Transport parameters in fed hypothyroid livers were not significantly changed as compared with euthyroid livers, whereas metabolism was decreased. In fed hyperthyroid livers, fractional transfer rate constants for influx (k_{21}) and efflux (k_{12}) were decreased and metabolism, corrected for differences in intracellular mass transfer, was increased. Furthermore, for transport in hyperthyroid livers it was shown that only total mass transfer (TMT) into the metabolizing liver compartment (not into the nonmetabolizing liver compartment) was decreased. Transport and metabolic parameters in fasted hypothyroid livers were decreased as compared with euthyroid fed livers. In fasted hyperthyroid livers, transport and metabolism were not significantly different as compared with that in euthyroid fed livers, so transport was increased versus hyperthyroid fed livers. It appeared therefore that fasting normalized the effects of hyperthyroidism on both the transport and metabolic processes of T_3 in the liver. The present study demonstrates normal transport and decreased metabolism in livers of hypothyroid fed rats and decreased transport and increased metabolism in livers of hyperthyroid fed rats. In livers of hypothyroid fasted rats transport and metabolism were decreased, whereas in livers of hyperthyroid fasted rats transport and metabolism were not significantly different from that in euthyroid fed livers. These changes might favor tissue euthyroidism despite the altered thyroid and nutritional state, and can therefore be seen as adaptation mechanisms to these altered states at the tissue level.

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THE EFFECTS OF hyperthyroidism and hypothyroidism on thyroid hormone metabolism in vitro and in vivo have been extensively investigated.¹⁻⁵ However, these effects are as yet incompletely understood. In hyperthyroid humans, both decreased⁶ and increased⁷ metabolic rates for thyroid hormones are reported, whereas hypothyroid humans are reported to have an increased fractional conversion rate of thyroxine (T_4) to triiodothyronine (T_3).^{7,8} Conversion of thyroid hormone mostly takes place by deiodination and conjugation reactions.⁹ Deiodination of T_4 , T_3 , and reverse T_3 (rT_3) is catalyzed by at least three different deiodinating enzymes (type I, II, and III) located in different tissues.^{10,11} The liver quantitatively plays an important role in deiodination because of the presence of type I deiodinase.¹⁰ Hyperthyroidism increases deiodination of iodothyronines by rat liver homogenates.^{4,5} In hypothyroidism, rat tissue deiodinase activities for all iodothyronines were decreased, as was found in homogenates and tissue-slice studies.^{4,5} However, the latter data were obtained in broken-cell preparations, which have several drawbacks. Sulfation, for example, occurs only in intact cells and greatly potentiates deiodination, especially of T_3 .⁹ However, in intact isolated liver cell cultures, there is no normal relationship between transport of iodothyronines from the plasma into the cells and subsequent metabolism. Intact liver perfusion is therefore presumably the most physiological in vitro technique, since in this system the importance of other processes in thyroid hormone metabolism, such as transport over the cell membrane, can be investigated.

As for transport, we have demonstrated the presence of specific energy-dependent cellular uptake mechanisms for T_4 , T_3 , and rT_3 in rat hepatocytes.¹²⁻¹⁴ According to kinetic experiments, T_4 and rT_3 appear to enter the cell by a common pathway, which is different from that for T_3 .

Evidence has been presented for two distinct pathways for T_4 and T_3 transport in the perfused rat liver¹⁵ and in vivo in the human liver¹⁶ as well. Recently, we reported a discrimination between transport to a nonmetabolizing, presumably extracellular liver compartment and transport into an intracellular, metabolizing liver compartment in the perfused rat liver.^{17,18}

To investigate whether changes in plasma membrane transport or in subsequent metabolism of T_3 occur in hyperthyroidism and hypothyroidism, we studied these processes using a recirculating rat liver perfusion system. The effects of fasting in combination with hypothyroidism and hyperthyroidism were also investigated, since little attention has been paid until now to the influence of the nutritional state on thyroid hormone handling during hypothyroidism and hyperthyroidism.

MATERIALS AND METHODS

Materials

Male Wistar rats weighing 200 to 250 g were used in all experiments. Rats were made hyperthyroid by daily intraperitoneal injection of T_4 (10 μ g/100 g body weight) for 15 days. Hypothyroidism was induced by addition of 0.05% mercaptomethylimidazole to

From the Departments of Internal Medicine III and Nuclear Medicine, Medical Faculty, Erasmus University, Rotterdam, The Netherlands.

Submitted May 25, 1993; accepted February 14, 1994.

Supported in part by the Foundation for Medical Research MEDIGON (900-540-191).

Address reprint requests to Marion de Jong, PhD, Department of Internal Medicine III, University Hospital Dijkzigt, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

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0026-0495/94/4311-0005\$03.00/0

the drinking water for at least 3 weeks. Hyperthyroidism and hypothyroidism were confirmed by estimation of T_4 and thyrotropin (TSH) levels in plasma by radioimmunoassay (T_4) and an ultrasensitive TSH assay of which the lower limit of detection was 0.18 ng TSH/mL. In studies on the effect of fasting, food was withdrawn for 48 hours, but free access to drinking water was maintained.

$L-T_3$ was obtained from Sigma (St Louis, MO) and [$3^{-125}I$] T_3 (specific activity > 1,200 $\mu\text{Ci}/\mu\text{g}$) from Amersham International (Aylesbury, UK). All other reagents were of the highest purity commercially available.

Experimental Procedures

Livers were isolated and perfused in a recirculating system at 37°C, essentially as described by Meijer et al.¹⁹ The medium used in all experiments was 150 mL Krebs-Ringer medium with addition of 1% or 4% bovine serum albumin (BSA). The medium was gassed with carbogen (95% O_2 , 5% CO_2 , 400 mL/min). The functional state of the organ was monitored by its outer appearance and measurement of medium flow (40 mL/min), bile flow (~0.7 mL/h), pH of the medium (7.43), and enzyme release (lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase) into the perfusion medium. In short, after isolation the liver was preperfused for 0.5 hours. The experiments were started by the addition of T_3 (300 pmol to media with 1% BSA or 1,200 pmol to media with 4% BSA) and $^{125}I-T_3$ to the medium (time 0). Medium aliquots of 0.5 mL were taken at 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, and 60 minutes. Bile was collected at 10-minute intervals. Medium and bile samples were stored at -20°C until further analysis. For analysis of medium aliquots, mixtures were prepared consisting of 0.5 mL medium and 0.5 mL 1-mol/L HCl. These were applied to small Sephadex LH-20 columns (bed volume, 1 mL) equilibrated with 3 mL 0.1-mol/L HCl. Iodide was eluted with 3 \times 1 mL 0.1-mol/L HCl, and T_3 glucuronide (T_3G), T_3 sulfate (T_3S), and remaining T_3 with 8 \times 1 mL sodium acetate (0.1 mol/L, pH 4), 6 \times 1 mL H_2O , and 3 \times 1 mL NaOH (0.1 mol/L)/ethanol 50:50 (vol/vol), respectively. Fractions of 1 mL were collected and counted for radioactivity. Bile was also analyzed on Sephadex LH-20 after addition of 950 μL 0.1-mol/L HCl to a 50- μL aliquot. The same chromatographic procedure used for medium analysis was followed. Free T_3 (FT_3) concentrations in medium aliquots were determined by equilibrium dialysis according to the method of Sterling and Brenner.²⁰ The percentage of FT_3 in medium was not significantly different in all the fed and fasted groups (2.23% to 2.30%).

For analysis of T_3 kinetics, the tracer T_3 disappearance curve from the medium of each experiment was fitted to a two-exponential model, as described by Docter et al.¹⁷ Using this model consisting of a medium and a liver pool, fractional transfer rate constants for influx (k_{21}) and efflux (k_{12}), the fractional disposal rate constant (k_{02}), total mass transfer (TMT), and the total disposal were calculated. Disposal could be corrected for differences in TMT into the intracellular liver compartment, resulting in the equation metabolic capacity = disposal/intracellular mass transfer.

With the aid of experiments with 1% and 4% BSA in the medium and using the two-pool model, it is possible to discriminate between transport of T_3 to a nonmetabolizing, presumably extracellular, liver compartment and transport into the intracellular liver compartment, as described earlier.¹⁷ During the perfusion with 1% BSA,

$$TMT_{(1\%BSA)} = X + Y, \quad (1)$$

where X is the transport of T_3 to the intracellular compartment, Y

Table 1. TSH and T_4 Serum Levels in Euthyroid, Hypothyroid, and Hyperthyroid Rats

	Euthyroid (n = 7)	Hypothyroid (n = 8)	Hyperthyroid (n = 4)
TSH (ng/mL)	1.6 \pm 0.3	13.8 \pm 1.3*	0.4 \pm 0.04*
T_4 (nmol/L)	54 \pm 4	8 \pm 3*	154 \pm 14*

* $P < .001$ v euthyroid.

is the transport of T_3 to the extracellular compartment, and TMT is the total amount of T_3 transported to the liver during the perfusion. Since X is linearly related to medium FT_3 and Y is linearly related both to medium FT_3 and the medium albumin concentration,^{17,18} it follows that during the perfusion with 4% BSA in the medium

$$TMT_{(4\%BSA)} = [FT_{3(4\%BSA)}/FT_{3(1\%BSA)}]X + 4[FT_{3(4\%BSA)}/FT_{3(1\%BSA)}]Y. \quad (2)$$

In this way two equations with two unknowns (X and Y) are obtained, of which both unknowns can be solved.

Statistical evaluation was performed using one-way ANOVA followed by comparison among class means²¹ and Student's *t* test, corrected for multiple pairwise comparisons between means. Results are expressed as the mean \pm SD.

RESULTS

In hyperthyroid rats serum T_4 was significantly higher than in the euthyroid group, whereas serum TSH was significantly lower. In hypothyroid rats serum T_4 was significantly lower and serum TSH significantly higher than during euthyroidism (Table 1).

Thyroid hormone disappearance from medium was as published earlier.^{17,18} Two components of disappearance were present. The fast component (FC) is determined for a minor part by distribution through the perfusion system and the extracellular liver compartment and represents mainly uptake into the cellular compartment. The slow component (SC) represents metabolism in the liver. Two $t_{1/2}$ values were calculated from the two components of the curve of each experiment.

Fed-Liver Transport

The $t_{1/2}$ FC of medium disappearance using hypothyroid fed livers was not significantly different from that of euthyroid livers, whereas with hyperthyroid fed livers it was prolonged (Table 2), representing a decreased transport of T_3 into the liver. For hypothyroid fed livers, other transport

Table 2. $t_{1/2}$ of the FC and SC of T_3 Disappearance From the Medium Using Livers of Fed and 48-Hour-Fasted Euthyroid, Hypothyroid, and Hyperthyroid Rats (medium with 1% BSA)

	$t_{1/2}$ FC (min)	$t_{1/2}$ SC (min)
Fed		
Euthyroid (n = 7)	1.32 \pm 0.08	67.8 \pm 4.5
Hypothyroid (n = 8)	1.40 \pm 0.09	99.9 \pm 7.8*
Hyperthyroid (n = 4)	2.10 \pm 0.16*	65.5 \pm 5.9
Fasted		
Euthyroid (n = 3)	2.55 \pm 0.2*	105.6 \pm 8.7*
Hypothyroid (n = 3)	2.15 \pm 0.18*	126.5 \pm 10.2*
Hyperthyroid (n = 5)	1.41 \pm 0.10	71.3 \pm 4.9

NOTE. Results are the mean \pm SD.

* $P < .001$ v euthyroid fed.

Table 3. k_{21} and k_{12} of T₃ Transport in Livers of Fed and 48-Hour-Fasted Euthyroid, Hypothyroid, and Hyperthyroid Rats and Their Ratio (medium with 1% BSA)

	k_{21} (min ⁻¹)	k_{12} (min ⁻¹)	k_{21}/k_{12}
Fed			
Euthyroid (n = 7)	0.38 ± 0.03	0.14 ± 0.02	2.7 ± 0.3
Hypothyroid (n = 8)	0.37 ± 0.03	0.14 ± 0.03	2.6 ± 0.4
Hyperthyroid (n = 4)	0.25 ± 0.04*	0.09 ± 0.01*	2.8 ± 0.4
Fasted			
Euthyroid (n = 3)	0.20 ± 0.02*	0.17 ± 0.02	1.2 ± 0.1*
Hypothyroid (n = 3)	0.24 ± 0.02*	0.17 ± 0.01	1.4 ± 0.1*
Hyperthyroid (n = 5)	0.34 ± 0.03†	0.15 ± 0.02	2.4 ± 0.3

NOTE. Results are the mean ± SD.

* $P < .001$ v euthyroid fed.† $P < .005$ v hyperthyroid fed.

parameters such as overall k_{21} and k_{12} were also the same as for euthyroid livers. However, in hyperthyroid livers, k_{21} and k_{12} were diminished in the same direction ($P < .001$), resulting in an unchanged ratio (Table 3).

A decreased k_{21} in hyperthyroid livers represents a diminished fractional transport into the total liver. This could theoretically be caused by diminished transport to the extracellular, nonmetabolizing liver compartment, diminished transport into the intracellular liver compartment, or both, since k_{21} represents the overall influx rate constant including transport to both intracellular and extracellular hepatic compartments. Recently, we published a method to discriminate between transport to the extracellular and intracellular liver pool.^{17,18} In these studies, we showed that albumin influenced only transport to the extracellular compartment of the liver and not to the intracellular compartment. To investigate transport to the extracellular and intracellular compartments of the liver, we performed liver perfusions with 1% and 4% BSA added to the medium. The results in Table 4 show that transport to the extracellular compartment of the liver was the same in livers of euthyroid and hyperthyroid rats, whereas transport to the intracellular compartment was decreased in livers of hyperthyroid rats. Transport to intracellular and extracellular compartments was not significantly different from control values in livers of hypothyroid rats (not shown).

Table 4. Mean Total T₃ in the Medium (nmol/L), Mean FT₃ in the Medium (pmol/L), TMT (pmol/60 min), TMT/FT₃ (pmol/pmol/L free T₃), and Transport to Intracellular Liver Pool/FT₃ (=TrI) and Extracellular Liver Pool/FT₃ (=TrE) [both pmol/pmol/L free T₃] in Euthyroid and Hyperthyroid Livers Perfused With Medium With 1% and 4% BSA

	Euthyroid (n = 7)		Hyperthyroid (n = 4)	
	1% BSA	4% BSA	1% BSA	4% BSA
Mean total T ₃	0.6 ± 0.04	3.5 ± 0.2	0.6 ± 0.04	3.7 ± 0.4
Mean FT ₃	13.4 ± 0.9	24.7 ± 1.1	13.5 ± 0.9	25.8 ± 1.7
TMT	1,293 ± 96	5,995 ± 212	865 ± 87*	4,974 ± 323*
TMT/FT ₃	96.5 ± 6.8	242.7 ± 11.2	64.1 ± 5.7*	192.8 ± 13.9*
TrI/FT ₃	47.7 ± 4.3	47.7 ± 4.3	21.2 ± 2.6*	21.2 ± 2.6*
TrE/FT ₃	48.7 ± 3.3	195.0 ± 11.7	42.9 ± 4.7	171.6 ± 16.8

NOTE. Results are the mean ± SD.

* $P < .001$ v euthyroid with corresponding BSA concentration.

Fed-Liver Metabolism

The $t_{1/2}$ SC of T₃ medium disappearance was prolonged using livers of hypothyroid rats as compared with euthyroid livers, whereas that of hyperthyroid rats was not significantly different as compared with euthyroid livers (Table 2).

After uptake into the liver cells, T₃ is conjugated to T₃G or, before deiodination, to T₃S.⁹ T₃G normally appears mostly in the bile, T₃S is subsequently deiodinated, and the end-product iodide is excreted mostly to the medium.¹⁷ Results of analysis of medium and bile are depicted in Table 5. In hypothyroid livers iodide excretion was diminished as compared with that in euthyroid livers ($P < .001$); in hyperthyroid livers iodide production was not significantly different from that in euthyroid livers. Glucuronide excretion in medium and bile in euthyroid livers was the same as in hypothyroid and hyperthyroid livers. Sulfate excretion in medium and bile was diminished in hypothyroid livers ($P < .05$) and unaffected in hyperthyroid livers. In accordance with these findings, the fractional disposal rate constant (k_{02}) and the integrated T₃ disposal during 60 minutes, ie, the total amount of metabolized T₃, were decreased in hypothyroid livers ($P < .001$) and unchanged in hyperthyroid livers (Table 6). The availability of T₃ to the intracellular metabolizing enzymes is, among other things, influenced by the mass transfer of T₃ into the intracellular liver compartment during the experiment (see Discussion). Therefore, the disposal of T₃ in the three groups has to be corrected for possible differences in intracellular mass transport (=metabolic capacity). Metabolic capacity was decreased in hypothyroid livers ($P < .001$) and increased in hyperthyroid livers ($P < .001$; Table 6).

Fasted-Liver Transport

The $t_{1/2}$ FC of medium disappearance using euthyroid fasted or hypothyroid fasted livers was prolonged compared with that of euthyroid fed livers, whereas with hyperthyroid fasted livers there was no significant difference (Table 2). In accordance with these findings, as compared with that in euthyroid fed livers, k_{21} was diminished after fasting in

Table 5. Iodide, T₃G, and T₃S Excretion in Medium and Bile in Fed and 48-Hour-Fasted Euthyroid, Hypothyroid, and Hyperthyroid Livers, (medium with 1% BSA)

	% of Dose		
	Iodide	T ₃ G	T ₃ S
Fed			
Euthyroid (n = 7)	12.8 ± 1.0	13.6 ± 1.7	3.5 ± 0.2
Hypothyroid (n = 8)	5.1 ± 1.1†	13.3 ± 1.5	2.6 ± 0.9*
Hyperthyroid (n = 4)	12.3 ± 0.5	13.7 ± 2.8	3.1 ± 0.5
Fasted			
Euthyroid (n = 3)	12.1 ± 1.2	9.0 ± 1.1†	4.0 ± 0.4
Hypothyroid (n = 3)	3.4 ± 0.8†§	10.8 ± 1.2*	2.4 ± 0.8†
Hyperthyroid (n = 5)	11.2 ± 3.4	14.8 ± 1.5	4.1 ± 0.7

NOTE. Results are the mean ± SD.

* $P < .05$ v euthyroid fed.† $P < .01$ v euthyroid fed.‡ $P < .001$ v euthyroid fed.§ $P < .05$ v hypothyroid fed.

Table 6. k_{02} , Disposal, and Metabolic Capacity (metabolic capacity = disposal/intracellular mass transfer) of Livers of Fed and 48-Hour-Fasted Euthyroid, Hypothyroid, and Hyperthyroid Rats (medium with 1% BSA)

	k_{02} (min^{-1})	Disposal (pmol)	Metabolic Capacity (% of euthyroid fed)
Fed			
Euthyroid (n = 7)	0.016 \pm 0.001	155.2 \pm 20.4	100 \pm 10.8
Hypothyroid (n = 8)	0.013 \pm 0.001†	109.7 \pm 10.7†	70.6 \pm 7.9†
Hyperthyroid (n = 4)	0.016 \pm 0.001	158.0 \pm 9.4	228.1 \pm 18.6†
Fasted			
Euthyroid (n = 3)	0.012 \pm 0.001†	102.9 \pm 8.7*	76.8 \pm 3.3*
Hypothyroid (n = 3)	0.011 \pm 0.001†‡	85.0 \pm 5.6†‡	48.6 \pm 5.1†‡
Hyperthyroid (n = 5)	0.016 \pm 0.001	144.7 \pm 4.6	89.8 \pm 9.6

NOTE. Results are the mean \pm SD.

* $P < .005$ v euthyroid fed.

† $P < .001$ v euthyroid fed.

‡ $P < .025$ v hypothyroid fed.

euthyroid livers ($P < .001$) and in hypothyroid livers ($P < .001$), whereas k_{12} was unchanged. Compared with that in euthyroid fed livers, both k_{21} and k_{12} were not significantly different in fasted hyperthyroid livers, and thus increased after fasting versus fed hyperthyroid livers ($P < .005$ v hyperthyroid fed; Table 3).

Fasted-Liver Metabolism

The $t_{1/2}$ SC of medium disappearance using euthyroid fasted and hypothyroid fasted livers was prolonged compared with that of euthyroid fed livers, whereas with hyperthyroid fasted livers it was not significantly different (Table 2). As shown in Table 5, after fasting iodide production in euthyroid livers did not change. In hypothyroid livers, this production was diminished after fasting as compared with that in euthyroid fed livers ($P < .001$) and hypothyroid fed livers ($P < .05$). In hyperthyroid fasted livers the amount of iodide excreted was not significantly different from that in euthyroid fed and hyperthyroid fed livers. Glucuronide production after fasting was decreased in euthyroid livers ($P < .01$ v euthyroid fed) and in hypothyroid livers ($P < .05$ v euthyroid fed and hypothyroid fed), but not in hyperthyroid livers. In euthyroid livers sulfate production after fasting was unchanged, and in fasted hypothyroid livers it was decreased ($P < .01$ v euthyroid fed, NS v hypothyroid fed), whereas it was unaffected in hyperthyroid fasted livers (NS v hyperthyroid fed and euthyroid fed). In Table 6 it is shown that after 48 hours of fasting T_3 disposal was decreased in euthyroid ($P < .005$ v euthyroid fed) and in hypothyroid ($P < .001$ v euthyroid fed, NS v hypothyroid fed) livers, but not in hyperthyroid livers (NS v euthyroid and hyperthyroid fed). After fasting, disposal corrected for differences in intracellular mass transport was decreased in euthyroid livers ($P < .005$ v

euthyroid fed) and in hypothyroid livers ($P < .001$ v euthyroid fed, $P < .05$ v hypothyroid fed), but not in hyperthyroid livers (NS v euthyroid fed, decreased with $P < .001$ v hyperthyroid fed).

Finally, in Table 7 a summary of all changes in liver transport and metabolism of T_3 during hypothyroidism and hyperthyroidism before and after 48 hours of fasting is shown.

DISCUSSION

Adaptations of thyroid hormone kinetics to a changed thyroid hormone state are not fully understood. The metabolic rate in hyperthyroid humans has, eg, been reported to be both decreased⁶ and increased,⁷ whereas in vitro investigations in rat liver homogenates pointed to an increased T_3 5'-deiodinase activity,^{4,5} attributed to changes in 5'-deiodinase activity²²⁻²⁴ and in cytosolic cofactor.²⁴ In hypothyroid humans, the fractional conversion rate of T_4 is increased^{7,8,25}; however, studies using rat liver showed decreased hepatic 5'-deiodinase activities.²³ An increased fractional conversion rate of T_4 may be explained by increased deiodinase activity in the brain during hypothyroidism.²⁶

In most in vitro measurements, the role of active transport over the cell membrane was not considered. Studies in a human subject, rat hepatocytes, and perfused rat liver have shown transport of thyroid hormones to be rate-limiting in total cellular uptake and metabolism.^{16,27,28}

Fed Livers

In livers of hypothyroid rats transport parameters are not significantly altered, ie, k_{21} and k_{12} are not significantly different compared with those of euthyroid livers. As for metabolism, iodide production is diminished in hypothyroid livers. When only deiodination is inhibited in liver T_3 metabolism, the T_3S concentration will increase because T_3 has to be sulfated before it is deiodinated.⁹ This phenomenon was found in livers perfused with medium to which propylthiouracil (PTU) was added¹⁵; iodide production from T_3 was strongly inhibited by PTU, while T_3 conjugates accumulated.

In hypothyroid livers T_3S production was decreased as compared with that in control livers. This decrease, instead of the expected increase due to the impaired deiodination,

Table 7. Changes in Transport and Metabolism of T_3 in Livers of Euthyroid, Hypothyroid, and Hyperthyroid Rats

	Transport	Metabolism
Fed		
Euthyroid	—	—
Hypothyroid	—	↓
Hyperthyroid	↓	↑
Fasted		
Euthyroid	↓	↓
Hypothyroid	↓	↓↓
Hyperthyroid	—	—

NOTE. —, no changes v euthyroid fed; ↓, decreased v euthyroid fed; ↑, increased v euthyroid fed.

points to an inhibited sulfation as well. This is in accordance with a decreased disposal in hypothyroid livers, whereas it was not present in fed livers after PTU.

It has been reported that nuclear T₃ in euthyroid, hypothyroid, and hyperthyroid livers is derived mainly from plasma, despite present local T₄ to T₃ conversion.^{3,29} In hypothyroidism total and free plasma T₃ concentrations are decreased, thereby decreasing the supply of hormone to the liver. In case of unchanged transport but decreased metabolism, the rate of T₃ degradation in the liver will decrease (metabolic capacity is 70% of that of euthyroid fed livers) and more intracellular T₃ will be available for binding to the nuclear receptors, since the intracellular hormone concentration is determined by (1) the plasma (medium) flow rate, (2) plasma (medium) concentration of hormone and hormone-binding proteins, (3) rate constant for hormone dissociation from its binding proteins, (4) rebinding to its binding proteins, (5) influx rate constant into the cell, (6) efflux rate constant, and (7) metabolic rate constant.³⁰ Since the first four factors are constant in our liver perfusion system, only the ratio of k₂₁ to k₁₂ and the k₀₂ determine the intracellular hormone concentration. The observed effects of hypothyroidism on liver uptake and metabolism of T₃ might favor tissue euthyroidism despite the hypothyroid state.

In hyperthyroid livers, the ratio of k₂₁ to k₁₂ was unchanged. However, both k₂₁ and k₁₂ are decreased as compared with those in euthyroid livers, whereas the medium pool is the same for both livers in our liver perfusion system, leading to a decreased TMT into the intracellular liver compartment. The cause of the decreased TMT and k₂₁ is as yet not known. The synthesis of hepatic mitochondrial adenosine triphosphate (ATP) is increased during hyperthyroidism. However, *in vivo* the energy state is reduced in the hyperthyroid liver, reflecting a new balance between increased ATP synthesis and even more increased consumption.³¹ This reduced ATP availability may be the cause of the decreased transport of thyroid hormone into the hyperthyroid liver. Jennings et al,³² concerning T₄ uptake and metabolism in the perfused rat liver, also found a decrease in the hepatic uptake of T₄ in thyrotoxicosis opposite to an increase in deiodination, in agreement with our findings. With regard to increased T₃ conjugation in hyperthyroid livers (at least when corrected for transport into the metabolizing pool, which is decreased in hyperthyroid livers), at present there are to our knowledge no other studies published in which hepatic conjugation of thyroid hormones during hyperthyroidism has been investigated. However, many other hepatic enzymatic processes, including, e.g., glucuronidation of several substances, show an increased activity during hyperthyroidism.³³⁻³⁶ The attenuated transport together with the increased metabolism during hyperthyroidism protects the liver at least partially against tissue hyperthyroidism.

Fasted Livers

Fasting results in a decrease of both transport into the intracellular liver compartment and subsequent metabo-

lism of T₃ in euthyroid perfused rat livers, as previously described,¹⁷ probably due to a decrease of intracellular ATP after fasting.³⁷ This results in a decrease of the metabolic capacity to 77% of euthyroid fed livers. We did not correct for a decrease in liver weight after 48 hours' fasting, since the weight loss is mainly due to glycogen depletion and does not play an active role in thyroid hormone transport and metabolism.¹⁸ In hypothyroid fasted livers both k₂₁ and metabolism are decreased as compared with that in euthyroid and hypothyroid fed livers, whereas k₁₂ was not significantly changed. Compared with euthyroid fasted livers, because of an even further decrease of metabolism after fasting in the hypothyroid liver (metabolic capacity is 50% of that of euthyroid fed livers), the availability of liver T₃ to nuclear binding may be increased, which is favorable during hypothyroidism. A marked overlap between the changes in the rat hepatic mRNA activity profile induced by either hypothyroidism or starvation has been reviewed.³⁸ In our study, we were able to discriminate between the fasted euthyroid and fed hypothyroid state of the livers on the basis of differences in transport (Table 3) and in iodide and glucuronide production (Table 5). However, according to Mariash and Oppenheimer³⁸ hypothyroidism and fasting apparently work in the same direction with regard to the metabolic state of the liver, in accordance with the fact that after fasting in the hypothyroid liver T₃ metabolism is further decreased compared with that in the hypothyroid fed liver. Transport, which is unaffected in the hypothyroid fed liver, is also decreased after fasting. It must be noted that the results of this study apply to the rat, a species that becomes hypothyroid after fasting beyond 48 hours.^{24,39} Although our rats are fasted for only 48 hours, one has to be careful in extrapolating these results directly to other species, including humans.

In fasted hyperthyroid livers, transport is increased as compared with that in fed hyperthyroid livers. In other words, the decrease in transport effected by hyperthyroidism in fed livers is abolished by fasting. Delineating the mechanisms of this effect awaits further investigation, especially on factors regulating cellular transport activity. Metabolic capacity, which is increased in fed hyperthyroid livers, is decreased with additional fasting and comparable to T₃ metabolism in the fed euthyroid liver. Thus, alterations in transport and metabolism induced by hyperthyroidism are normalized by fasting. Our data therefore suggest that in fact the fasted "hyperthyroid" rats may have become euthyroid. These findings are in accordance with the fact that hyperthyroidism and fasting have an opposite effect on patterns of rat hepatic mRNA activity.³⁸ So, also during fasting the availability of liver T₃ to nuclear binding shows adaptations during hypothyroidism and hyperthyroidism, which are favorable during these conditions.

These changes might favor tissue euthyroidism, despite the altered thyroid and nutritional state, and can therefore be seen as adaptation mechanisms to these altered states at the tissue level.

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