CELLULAR MECHANISMS IN THE GENERATION OF THE LOW T_3 SYNDROME

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Ter nagedachtenis aan mijn vader

Aan Jacolien, Fraukje en Steven

CONTENTS

LIST OF ABBREVIATIONS

ATP	adenosine 5'-triphosphate
CNS	central nervous system
5'-D	5'-deiodination
Diac	3,3'-diiodothyroacetic acid
DIT	diiodotyrosine
DTT	dithiotreitol
ED	equilibrium dialysis
FT_4	free (non protein bound) thyroxine
FT_4D	free thyroxine measured by equilibrium dialysis
GSH	reduced glutathione
GSSG	oxidized glutathione
IRD	inner ring deiodination (5-deiodination)
K_i	equilibrium dissociation constant of a molecule-inhibitor complex
K_m	Michaelis constant
MCR	metabolic clearance rate
NTI	non-thyroidal illness
ORD	outer ring deiodination (5'-deiodination)
PR	production rate
PTU	propylthiouracil
REP	rapidly equilibrating pool
rT_3	reverse T_3; 3,3',5'-triiodothyronine
SEP	slowly equilibrating pool
T_4	3,3',5,5'-tetraiodothyronine; thyroxine
T_3	3,3',5-triiodothyronine
TBG	thyroxine binding globulin
TBPA	thyroxine binding pre-albumin
Tetrac	3,3',5,5'-tetraiodothyroacetic acid
TRH	TSH-releasing hormone
Triac	3,3',5-triiodothyroacetic acid
TSH	thyroid stimulating hormone
V_{max}	maximal velocity

PREFACE

The idea that iodine and thyroid function were somehow interrelated, dates from the beginning of the nineteenth century. After the discovery of iodine as a new element in 1813 (Courtois et al, 1813), and the demonstration of its presence in common sponge in 1819, Coindet, a physician in Geneva, suspected that the ancient remedy for goitre: burnt sponge, might contain iodine and he decided to start treatment of his goitrous patients with iodine (Coindet, 1820). Initially successful and taken up with enthousiasm, this therapy was gradually abandoned by the subsequent awareness of the hazards of overdosage. It was not until 1896 that Baumann could demonstrate iodine in thyroid glands (Baumann, 1896). He named his product "iodothyrin" which was soon shown to possess a similar activity compared to whole thyroid when administered to hypothyroid patients. In the years thereafter, Baumann and coworkers could demonstrate a correlation between iodine content of iodothyrin and its activity, while the iodine-free fraction of the thyroid extracts was devoid of biological activity (Baumann and Roos, 1896). Furthermore, thyroid glands of goitrous patients were shown to contain less iodine than those of non-goitrous patients (Baumann, 1896a). From that time it became generally accepted that iodine was an essential constituent and product of normally functioning thyroid glands. Further attempts to analyze and isolate the active compound in iodothyrin were unsuccessful until Kendall, in 1914, succeeded in preparing a purer crystalline substance, which he named "iodin-A" (Kendall, 1915). He later changed this name in "thyroxine" and also proposed a structure formula which best fitted his analytical data (Kendall, 1917). Although incorrect, it is interesting to note that this formula includes three iodine atoms. In 1926, Harrington definitely established the chemical structure of thyroxine and subsequently synthesized it as 3,3',5,5'-tetraiodothyronine (hence the abbreviation: T_4) (Harrington, 1926).

In the years thereafter, however, it was increasingly felt that the physiological activity of thyroid material could not be entirely accounted for by its thyroxine content and as early as 1929 Kendall suggested that "thyroxine must represent an intermediate form in the elaboration of the physiologically active compound which is probably derived directly from thyroxine" (see for a historical review Pitt- Rivers, 1978). A major breakthrough in the history of thyroid research came with the discovery of 3,3',5-triiodothyronine (T_3) by Gross and Pitt-Rivers

in 1952 (Gross and Pitt-Rivers). They could demonstrate T_3 in thyroids and plasma of ^{131}I treated rats, beef thyroids and plasma of ^{131}I-treated patients. At approximately the same time, Roche and coworkers also succeeded in synthesizing T_3 and demonstrating its presence in thyroid glands of rats given ^{131}I (Roche et al, 1952, 1952a). Synthetic L-T_3 was shown to possess a biological potency of about three times that of thyroxine (Gross and Pitt-Rivers, 1952a). Furthermore, the period between the time of administration and the peak metabolic effect was much shorter in the case of T_3 than in that of T_4. These findings and the above mentioned suggestion of Kendall, in addition to the finding of in vivo conversion of T_4 to T_3 by Pitt- Rivers et al, made them to suggest that "triiodothyronine is the peripheral hormone and that thyroxine is its precursor" (Gross and Pitt-Rivers, 1953). This statement was already made before they showed in vivo T_4 to T_3 conversion. Some in vitro studies also showed conversion of T_4 to T_3 in peripheral tissues (Albright et al, 1954), but, because other studies (Lassiter and Stanbury, 1958) did not support these observations, the subject remained dormant until 1970, when Braverman et al were able to demonstrate formation of T_3 from T_4 in athyreotic human subjects in whom substitution therapy with L- thyroxine was the only possible source of T_3. From that time the research of the peripheral metabolism of thyroid hormones has explosively expanded.

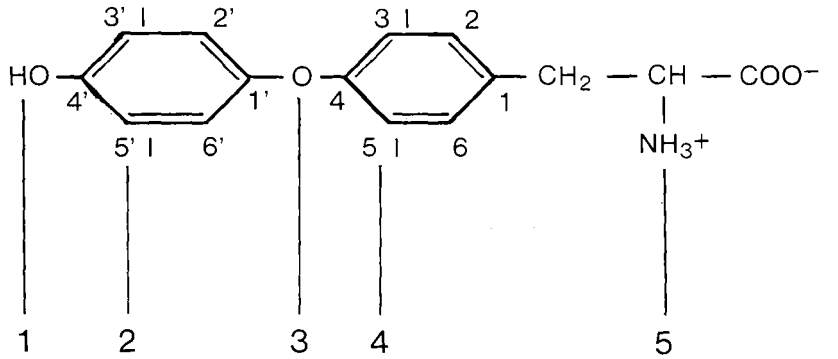
CHAPTER 1

Introduction

The principle secretory products of the thyroid gland are: thyroxine (3,3',5,5'-tetraiodothyronine) and 3,3',5-triiodothyronine, T_3. In normal conditions, with sufficient iodine intake, these hormones are produced in the thyroid in a molar ratio of about 20:1 (Nagataki et al, 1972; Chopra et al, 1973; Larsen et al, 1973), which differs considerably from their blood production rates of about 3:1 (Chopra, 1976), suggesting an extrathyroidal source for circulating T_3. In this respect, it was interesting to observe normal serum T_3 levels in L- thyroxine substituted athyreotic human subjects (Braverman et al, 1970). The only possible source of this T_3 was orally taken thyroxine, so the conclusion that T_4 was converted peripherally to T_3 was inevitable. That T_4 to T_3 conversion does occur in vivo has been repeatedly demonstrated in subsequent years (Sterling et al, 1970, Pittman et al, 1971, Surks et al, 1973). It is now widely accepted, that the major part of circulating T_3 is derived from extrathyroidal T_4 deiodination (Chopra et al, 1978). The same applies, even stronger, for 3,3',5'-triidothyronine, reverse T_3 (rT_3) (see later sections) (Chopra et al, 1978). Much work has been done to reveal the specific tissues and localization in which T_4 conversion takes place. Until recently, in vivo investigations have not yielded clear indications as to the site of thyroid hormone deiodination. The subject became even more complex when it was demonstrated that reproducible, predictable and reversible changes in T_4 deiodination, reflected by changes in serum levels of T_3 and rT_3, could be induced by several procedures. Before discussing the possible mechanisms by which these changes in serum levels of iodothyronines, which are known as the low T_3 syndrome, could take place, a summary of some aspects of peripheral thyroid hormone metabolism is given. This summary serves as a background and theoretical basis for the questions which resulted in the various investigations reported in this thesis.

Some aspects of peripheral thyroid hormone metabolism

Thyroxine, which is exclusively synthesized in the thyroid gland, is peripherally



1) Conjugation	sulphate or glucuronide
2) Outer ring deiodination	T_3 as initial product
3) Etherbond cleavage	DIT; MIT
4) Inner ring deiodination	Reverse T_3 as initial product
5) Deamination, decarboxylation	Tetrac, Triac, Diac

Fig. 1. Pathways of T_4 metabolism

metabolized via several pathways: a) deiodination, b) oxidative deamination, c) conjugation and d) etherbond cleavage (see figure 1). Deiodination is quantitatively the most important pathway, accounting for up to 80% of T_4 turnover (Chopra, 1976, Gavin et al, 1977; Engler and Burger, 1984). T_4 is also lost in bile and urine as the pure compound and T_4-conjugates, mainly in the form of glucuronyl- and sulfoconjugates. The amount of T_4 lost via these routes has been estimated at about 15% of the total T_4 production (Chopra, 1976; Chopra et al, 1978; Oddie et al, 1964). In addition, a small amount of T_4 is metabolized via oxidative deamination and decarboxylation of the alanine side chain to yield 3,3',5,5'-tetraiodothyroacetic acid (Tetrac) which can be further metabolized primarily by deiodination and to a lesser extent by conjugation (Flock et al, 1962; Green and Ingbar, 1961). Etherbond cleavage is considered to constitute a minor metabolic pathway of thyroid hormones. For a schematic representation, see fig. 2 and 3. In the following we will be predominantly concerned with the deiodinative pathway.

In assessing the thyroidal contribution to the total production of T_3, it has been assumed that the secretion of T_4 and T_3 by the thyroid, is proportional to their content in thyroglobulin (Chopra et al, 1978). Although a preferential secretion of T_3 in the rat (Haibach 1971) and rT_3 in the dog (Laurberg, 1978) was suggested, other studies (Abrams and Larsen, 1973) showed a good agreement between the T_3/T_4 ratio in thyroidal secretory products and in thyroglobulin. Because T_4 is exclusively secreted by the thyroid, the thyroidal

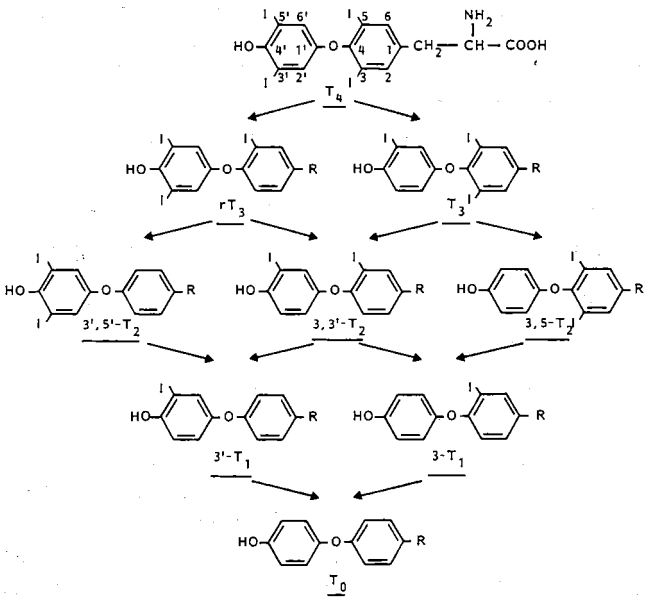


Fig. 2. Sequential 5-(↙) and 5'-(↘) deiodination of T_4

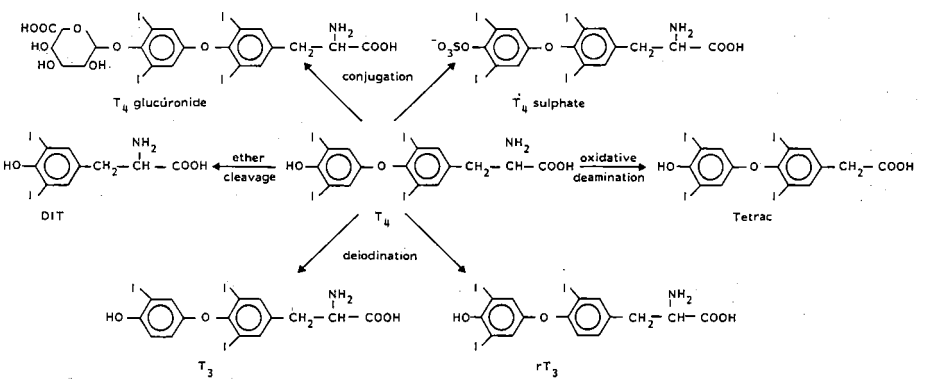


Fig. 3. Pathways of T_4 metabolism

T_3 production rate can be obtained by multiplying the T_3/T_4 ratio in thyroglobulin by the production rate of T_4. The same applies for rT_3. In this way a thyroidal contribution of 20 and 6 percent in the total daily blood production rate of T_3 and rT_3, respectively, has been accepted (table 1). It can therefore be stated, that the production of active thyroid hormone takes place mainly outside the thyroid gland (Visser, 1980). Apart from the question, where this production takes place, it was soon recognized that in serum, T_3

Table 1. Contributions of thyroidal secretion and peripheral production to total production rates of T_4, T_3 and reverse T_3

Iodothyronine	Total production	Thyroidal secretion (%) (nmol/day/70 kg)	Peripheral production
T_4	115	115 (100)	–
T_3	45	9 (20)	36 (80)
rT_3	30	2 (6)	28 (94)

Data from Chopra 1976 and Chopra et al, 1978

and rT_3 are not always present in a fixed ratio, but that reciproke changes in T_3 and rT_3 serum concentrations, with unaltered T_4 serum levels, could be detected in several conditions (vide infra). Reverse T_3 was identified in 1956 by Roche et al, both in thyroids and serum of rats. In 1974, it could be demonstrated in human serum (Chopra, 1974). In contrast to T_3, which is considered as the metabolically most active iodothyronine, rT_3 is currently regarded to be biologically inactive (Jorgensen, 1976).

Thus it was increasingly recognized that a peripherally localized regulatory system is present, by which T_4 which is also considered to possess little, if any, biological activity (see for a discussion on this subject G. Morreale de Escobar et al, 1981; M. Andreoli, 1981), is converted to the biologically active hormone: T_3 and its inactive analogue reverse T_3.

In the last decade much progress has been made in understanding the mechanisms by which these changes in serum T_3 and rT_3 take place. Firstly, changes in iodothyronines associated with caloric deprivation will be considered.

Changes in serum iodothyronines during caloric deprivation and dietary manipulations

The interest in dietary induced changes in serum iodothyronines actually began with the intriguing observation (Portnay et al, 1974) of a decreased serum T_3 level in man during starvation. After a reliable specific radioimmuno-assay for rT_3 had become available (Chopra, 1974), this observation was soon extended by the demonstration of a concomitant rise in rT_3 during caloric restriction (Vagenakis et al, 1975; Spaulding et al, 1976). Interestingly, total serum T_4 concentrations did not change, as had been shown before (Schatz et al, 1967; Verdy, 1968). Similar effects were observed in fasted non-obese L-thyroxine substituted hypothyroid patients (Croxson et al, 1977) and fasted obese euthyroid patients on a suppressive (250 ug) L-thyroxine dose (Vagenakis et al, 1975).

Since then, these observations have repeatedly been confirmed by others (Carlson et al, 1977; Azizi, 1978; Burman et al, 1979; Moreira-Andres et al, 1980), not only in starvation, but also in anorexia nervosa (Moshang et al, 1975; Miyai et al, 1975; Croxson and Ibbertson, 1977). It was, however, early recognized, that these changes in thyroid hormones were, in addition to the amount of caloric intake, also related to the dietary composition. A rise in serum T_3 was demonstrated in normal subjects during overfeeding of carbohydrate, whereas a reduction in carbohydrate was associated with a decrease in serum T_3 level (Danforth et al, 1975). This latter observation was extended (Spaulding et al, 1976) by showing that feeding obese subjects a 800 kcal diet devoid of carbohydrate, resulted in a decreased serum T_3, similar to total caloric restriction. Serum rT_3 levels, however, did not alter. If the 800 kcal diet was composed of at least 25% of carbohydrate related calories, the serum concentration of neither T_3 nor rT_3 was changed. In concert with these observations were the findings of Azizi (1978), who showed that – after fasting – refeeding with a 800 kcal mixed diet (70g carbohydrate, 50g protein and 35g fat, for 4 days) and isocaloric 100% carbohydrate diet, serum concentrations of T_3 and rT_3 returned towards normal, while a 800 kcal protein diet only restored rT_3 concentration. Burman et al (1979) could also demonstrate the efficacy of a relatively small amount (50g) of glucose (and fructose) in restoring T_3 and rT_3 serum levels after a seven day period of fasting. These findings suggested an important role of glucose metabolism in the peripheral deiodination of thyroid hormones. In particular, hepatic glucose metabolism attracted attention, after Westgren et al (1977) observed a stimulation of peripheral T_3 formation by oral but not by intravenous glucose administration in fasted subjects. In addition to this carbohydrate effect on peripheral thyroid hormone metabolism, an active role of dietary fat has been suggested (Otten et al, 1980). These authors compared changes in iodothyronines during four diets containing 1500 kcal each, composed of either 100% fat, 50% fat – 50% carbohydrate, 50% fat -50% protein or a control, mixed diet. Changes in thyroid hormones during the all-fat diet were qualitatively and quantitatively similar to those seen during fasting. In the 50% fat – 50% carbohydrate group a decrease (24%) in serum T_3 level was seen, while rT_3 concentration raised up to 34%. A similar increase in rT_3 serum levels was seen in the 50% fat – 50% protein fed subjects, but in this group the decrease in T_3 serum concentration was most pronounced. It was suggested that fat had a negative effect on peripheral thyroid hormone deiodination and this effect could (partially) be prevented by carbohydrates and proteins. With regard to inhibiting the suggested influence of fat, proteins, however, were more potent in restoring rT_3 levels than T_3 concentrations. This is supported by the above mentioned data of Azizi (1978), who observed restoring only rT_3 levels in fasted subjects refed with 800 kcal protein-diet.

Later studies, however, do not confirm all the above mentioned data. O'Brian et al (1980) observed changes in serum T_3 and rT_3 during severely restricted diets (200-600 kcal per day) similar to those in total starvation, irrespective

if calories were administered entirely as carbohydrate or protein. Also, Serog et al (1982) found that adding 110 grams of carbohydrate to a 1400 kcal/day diet, did not prevent the expected diet induced changes in T_3 and rT_3. Bogardus et al (1982) noted that adding 75g of carbohydrate to a 830 kcal/day diet containing no carbohydrate, for 6 weeks, did not prevent the decrease in serum T_3, but did prevent the increase in rT_3.

T_4, T_3 and rT_3 are tightly, but reversibly bound to serum carrier proteins, which in man include thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin. TBG has the highest affinity, but also the lowest capacity, and binds about 75% of all circulating T_4, T_3 and rT_3. TBPA has a lower affinity, but a larger capacity and binds 20% of T_4 and less than 5% of T_3. Albumin has the lowest affinity but highest capacity, binding 10% of T_4 and 20% of T_3. A small fraction of circulating thyroid hormone is non-protein-bound, the so called "free hormone". The free fractions for these hormones are: T_4: 0.02%; T_3: 0.3%; rT_3: 0.25%. The absolute free hormone concentration is obtained by multiplying total hormone levels by the free fractions. The free hormone is in equilibrium with the protein- bound fraction and in euthyroid conditions, the free hormone concentration is unchanged, despite significant changes in total hormone levels due to changes in capacity of binding proteins, for instance a rise in TBG in pregnancy. Therefore, changes in serum iodothyronines in certain conditions should also be regarded with respect to a possible change in serum binding proteins. Proteincaloric malnutrition was found to induce a decrease in TBPA and albumin, without a change in TBG (Chopra and Smith, 1975). Moreira-Andres et al (1980) showed a markedly decrease in TBPA (26%) and a small decrease in TBG (8.5%) without changes in albumin, during a two week period of caloric deprivation (500 kcal; 27g fat; 36g carbohydrate; 39g protein). However, the observed changes in T_3 and rT_3 could not in anyway be accounted for by these changes in binding proteins. Bogardus et al (1982) reached the same conclusion. The effect of caloric intake and carbohydrate on serum carrier proteins of thyroid hormones remains a matter of interest.

Welle et al (1983) found an increase in TBG and TBPA with markedly elevated serum T_3 levels during carbohydrate overfeeding. Surprisingly, free T_3 levels were also increased. The effect of carbohydrate on thyroid hormone binding proteins may also be independant of caloric intake, since TBPA is decreased in normal subjects when fed eucaloric carbohydrate restricted diets (Kelleher et al, 1983).

The possible existence of a circulating substance, inhibiting binding of thyroid hormone to its serum carrier proteins, thereby influencing the serum iodothyronine levels, will be discussed later.

In conclusion: caloric deprivation has been shown to induce changes in serum levels of thyroid hormones. These alterations are not solely dependent on the amount of caloric intake, but are also related to the composition of the diet. Carbohydrates seem to play a central role (not confirmed in all studies), but

a more active role of fat has also been suggested. The changes in serum concentrations of TBG, TBPA and albumin, observed during caloric manipulation, do not appear to account for the altered serum levels of T_3 and rT_3. As yet, the mechanism by which these dietary manoeuvres induce the above cited changes in serum iodothyronines, remains to be elucidated and will be discussed in the following sections.

Kinetics of serum iodothyronines during caloric deprivation.

An other approach to reveal the mechanism by which changes in serum iodothyronines during starvation take place, is to assess the kinetics of these hormones. Turnover kinetic studies require the intravenous administration of isotope-labeled hormone. Iodine isotopes are usually used in thyroid hormone tracer kinetic studies. Stable iodine is orally administered in order to prevent thyroidal uptake and recirculation of radioiodine, derived from the breakdown of hormone tracers. In the single compartmental model (SCM), a single (pulse) injection of radioactive iodothyronine is given intravenously. Serum tracer concentration is semilogarithmically plotted against time. The terminal slope of the plasma disappearance curve is linear, indicating that equilibrium has been reached between the plasma compartment and the rest of the body. By extrapolating this terminal part of the curve, the time-zero intercept represents the plasma tracer concentration if equilibrium existed already at that time. The volume of distribution (DV), which is actually a virtual volume and is expressed in liters plasma, can be obtained by dividing the injected dose by this zero time tracer concentration. The slope of the linear part of the curve represents the fractional catabolic rate (k), which can be calculated as $k = 0.693/T1/2$. The metabolic clearance rate (MCR) can be obtained by multiplying k and DV and refers to the volume of plasma from which a iodothyronine is cleared irreversibly per unit of time. In a steady state condition disposal of a iodothyronine equals its production rate (PR), hence PR=MCR x plasma hormone concentration. The total amount of hormone in the body (pool) can be calculated as: pool = DV x plasma hormone concentration.

If during the initial phase of distribution after the pulse injected tracer, significant degradation of a iodothyronine occurs, equilibrium between plasma and the rest of the body will be reached at a lower serum tracer concentration, with a subsequently lower extrapolated time zero tracer concentration, resulting in a higher DV and therefore overestimation of the MCR. To avoid these pitfalls, other approaches have been used like the constant infusion technique (Cavalieri et al, 1971), three compartmental model (Inada et al, 1975) and the currently more generally used non-compartmental model (NCM). This model considers a central compartment which is defined as the fast initial distribution space of iodothyronine and includes plasma pool as well as a number of other extravascular pools (compartments), reversibly exchanging the iodothyronine

with the central compartment. The term "non-compartmental" is used, because no definite compartmental localization of iodothyronine is assumed. The MCR is obtained by dividing the dose of injected labeled iodothyronine by the integrated plasma concentration determined by calculating the area under the plasma disappearance curve of the injected labeled hormone. (For mathematical background see Oppenheimer et al, 1975a, b; Gavin et al, 1977; Bianchi et al, 1978). This method also yields in addition to a MCR, a fractional turnover rate and distribution volume.

For T_3 it has been shown (Oppenheimer, 1975 a, b) that single compartmental analysis overestimates the distribution volume and MCR of T_3 by 94% and 30%, respectively. These differences are due to the relatively rapid turnover of T_3, in contrast to T_4, for which no important differences between SCM and NCM were found, most probably due to its much more slower turnover rate.

Using such methods of kinetic analysis, three studies, which can be considered as classic, have assessed the kinetic parameters of T_4, T_3 and rT_3 during caloric deprivation. In the earliest study (Vagenakis et al, 1977), nine euthyroid obese subjects were studied before and after three weeks of total caloric deprivation. The MCR and serum concentration of T_4 did not change, resulting in an unaltered PR. In contrast, T_3 PR decreased markedly, concluded from a decreased serum T_3 concentration and unchanged MCR. The decrease in T_3 PR was approximately 70%. In this study no rT_3 metabolism was investigated. Therefore, the same group (Eisenstein et al, 1978) assessed the kinetic parameters of this iodothyronine in four additional obese euthyroid patients. It should be noted that this kinetic study started on the sixth day of the fasting period, in contrast to day 21 in the former study. A 69% increase was found in serum rT_3 levels, while rT_3 MCR diminished from 96 to 68 liters/day /70 kg, resulting in a slight increase in the rT_3 PR. From these two studies it was concluded that the major cause of the lowered serum T_3 levels during fasting was a decreased production rate, while the rise in rT_3 concentration resulted from a decreased metabolic clearance rate. In a third investigation Suda et al (1978) studied the plasma peripheral kinetics of T_4, T_3 and rT_3 in seven obese fasting subjects and compared them to normal-weight persons. All volunteers were placed on a normal L-thyroxine substitution dose starting three weeks before the study. Kinetics of T_4 and rT_3 were started on day 3 of the fasting period, while labeled T_3 was injected on day 6. T_4 MCR was slightly lower in the fasted group (25%), as was the T_4 disposal rate (19%). Because all persons were on L-T_4 replacement, a decreased plasma disposal rate (=plasma production or appearance rate) is not to be expected, unless, as the authors suggested, a decrease in gastrointestinal absorption of L-T_4 is present. A possible explanation for a lowered T_4 MCR is a more intricate subject and will be discussed later. In contrast to the findings of Vagenakis (vide supra) T_3 MCR was reduced by 20%. Because serum T_3 concentration decreased much more, T_3 disposal rate was lowered by 59% compared to the fed group. Reverse T_3 MCR was

48% lower, but because of a 140% increase in plasma rT_3 concentration, the disposal rate was unchanged or slightly increased.

In conclusion: during caloric deprivation, decreased serum T_3 levels mainly result from decreased T_3 production, while increased rT_3 concentrations are due to a diminished rT_3 MCR. No major changes are observed in T_3 MCR and rT_3 PR. It should, however, be mentioned, that rT_3 tracer kinetic studies were all performed in the initial period of fasting, while it became apparent that after an initial rise in serum rT_3 (already demonstrable within 24h of starvation and which rise is maximal after one week and a half), hormone concentration subsequently gradually returns towards its prefast value (Visser et al, 1978; Carlson et al, 1977). This is in contrast to the sustained reduction of serum T_3 (Visser et al, 1978) although a concomitant rise in serum T_3 has also been reported (Suda et al, 1978; Carlson et al, 1977). As yet, no data are available as to the kinetic parameters of rT_3 after prolonged fasting. Theoretically, a gradual decrease in rT_3 serum levels could be a reflection of an increase in MCR as well as a decrease in PR. Vagenakis et al (1977) performed, as noted above, T_3 kinetic studies after 21 days of fasting and found an unchanged T_3 MCR with a decreased T_3 PR, in concert with kinetic data obtained during early fasting periods.

Enzymic activities in deiodination of iodothyronines

When it became evident that the deiodinative conversion of T_4 to T_3 and rT_3, was not a random process as had been suggested by Surks and Oppenheimer (1971), more interest arose in the biochemical background of this deiodinating process. It was only in 1975 that the enzymic nature of this reaction was established in rat liver homogenates (Hesh et al, 1975; Visser et al, 1975). In subsequent subcellular fractionation studies, most authors reported on a microsomal localization of the deiodinase activity in the liver (Visser et al, 1976; Fekkes et al, 1979; Auf dem Brinke et al, 1979; Saito et al, 1980) while others showed that the kidney enzyme is associated with the plasma membrane (Chiraseveenuprapund et al, 1978; Leonard and Rosenberg, 1978). (See for an extensive review on this subject Fekkes, 1982, thesis). Later investigations provided insight into the mechanism of enzymic deiodination and the way by which PTU inhibited this reaction (Leonard and Rosenberg, 1978 a; Visser, 1979; Visser and Van Overmeeren, 1979, 1981). In these investigations, it could be assessed that the proposed ping-pong mechanism (Visser, 1979) for thyroid hormone outer ring deiodination was correct (Visser and Van Overmeeren, 1981). It was also shown that thiol groups were essential for this enzyme activity as endogenous cofactor (Visser et al, 1976).

Originally, glutathione was advocated as the principle endogenous cofactor, because 5'-deiodinase activity changed in proportion to hepatic glutathione or non-protein sulfhydryl concentration in fasting (Visser et al, 1976; Kaplan,

1979; Balsam and Ingbar, 1979). However, a dissociation between hepatic reduced glutathione content and 5'- deiodinase activity in liver homogenates and cultured rat hepatocytes was subsequently shown (Gavin et al, 1981; Sato and Robbins, 1981). As yet, the nature of the endogenous cofactor for 5'-deiodinase activity remains a matter of investigation, but it still seems likely that hepatic reduced sulfhydryl-groups are somehow related to the observed cytosolic defect in fasting, leading to a decreased 5'-deiodinase activity. Perhaps not simply through the reduced glutathione concentration per se, but rather a change in the ratio reduced versus oxidized gluthatione. In this respect, the naturally occuring dithiol: dihydrolipoamide, has been suggested to play a role as a thiol cofactor in enzymic outer ring deiodination of T_4 and rT_3 (Goswani and Rosenberg, 1983; Sawada et al, 1985). Monodeiodinating activities have been observed in many animal tissues such as liver (vide supra), kidney (Chopra et al, 1978a), skeletal muscle (Chopra, 1977), heart (Naumann et al, 1980), pituitary (Kaplan, 1980), cerebral cortex and cerebellum (Kaplan and Yaskoski, 1980) and the thyroid itself (Erickson et al, 1981). Similar activity has been found in tissues of human origin as kidney, liver, heart, muscle, fibroblasts, polymorphonuclear leukocytes, lymphocytes, placenta and thyroid (Albright and Larson, 1959; Refetoff et al, 1972; Sterling et al, 1973; Woeber and Maddux, 1978, Kvetny, 1978; Roti et al, 1981; Ishii et al, 1982; Visser et al, 1983). It turned out that of these tissues, liver and kidney showed the greatest deiodinative activity. Initially, much of the attention was focussed on the liver and kidney, with respect to elucidate the mechanism of the alteration in iodothyronine serum levels during e.g. starvation. In addition to their high deiodinative activity in vitro, also high levels of radioactivity were noted in hepatic and renal regions during the first hours of injection of labeled thyroxine (Cavalieri et al, 1966). Furthermore, it became apparant that approximately one third of the total T_4 pool is located in the liver (Hennemann, 1981).

In 1978, a reduced 5'-deiodinase activity could be demonstrated in liver homogenates of fasted rats (Balsam and Ingbar, 1978; Kaplan and Utiger, 1978). Subsequent studies revealed that this reduction in 5'- deiodinase activity (5'-D) was due to a reduced apparent Vmax in the microsomal fraction in addition to a lowered capacity of the cytosol to support deiodination by the particulate fraction (Kaplan, 1979; Balsam et al, 1981). This lowered apparent Vmax, presumably reflecting a decreased amount of enzyme, could partially be normalized by T_4 replacement to fasting rats (which become hypothyroid during fasting) without changing the apparent K_m, provided that thiol reductants were supplemented (Balsam et al, 1981; Gavin et al, 1981). These results indicate that these changes in 5'-D in the rat result from tissue hypothyroidism, although a reduced cytosolic factor could also contribute to this effect, since T_4 and T_3 replacement of fasting rats do not fully normalize 5'D (Kaplan, 1979). Protein feeding of T_4 substituted rats seems to increase the sulfhydryl cofactor, whereas glucose feeding increases the hepatic content of active enzyme (Gavin et al, 1981).

Evidence for transmembraneous transport of iodothyronines

Based on investigations as discussed above, it was until recently generally held, that a decrease in 5'-D activity in the liver (and possibly also in other organs as well) could explain the changes in iodothyronine serum concentrations during caloric deprivation: a lowered serum T_3 level resulted from a decreased T_4 5'-deiodination, while the concomitant rise in serum rT_3 concentration was secondary to the same mechanism. However, as early as 1979, this mechanism was challenged as the sole cause for the induction of alteration in serum thyroid hormones during starvation (Jennings et al, 1979). Jennings and coworkers perfused livers of 3 days fasted rats with T_4 and observed a decreased hepatic T_4 uptake. The rate of T_4 to T_3 conversion, however, did not change during fasting. When livers were perfused similarly in the presence of PTU, no change in T_4 uptake was seen, but T_4 to T_3 conversion decreased due to inhibition of 5'D in the perfused rat liver. These results indicated that the diminished hepatic T_3 production during fasting results from decreased uptake of T_4 by the liver, rather than from changes in 5'D. A probable physiologic base for these observations was provided by the work of Krenning et al in our laboratory (Krenning et al, 1978,1979,1980,1981,1982,1983). Rao et al (1976,1981) observed a saturable and energy dependent T_3 uptake in rat liver cell suspensions. Subsequently in rat hepatocytes in primary culture, Krenning et al showed that uptake of T_4, T_3 and rT_3 was mediated by an active transport mechanism, and not, as had been suggested before (Freinkel et al, 1957; Lein and Dowben, 1961) by passive diffusion. It appeared that T_4 and rT_3 enter the cell by a common pathway that is different from that of T_3. In addition, these pathways were shown to be ATP-dependent and could be inhibited by several compounds, known to induce a low T_3 syndrome in man such as propranolol and X-ray contrast agents. Propranolol also decreased cellular ATP content, in contrast to tyropanoate, iopanoic acid and ipodate (cholecystographic agents) and amiodarone, that decreased hepatic cellular uptake of iodothyronines. It is believed that these latter agents, which show structural resemblance to thyroid hormones, inhibit hormonal uptake by competing with thyroid hormones for the uptake mechanism. Very interestingly, PTU did not influence uptake of iodothyronines in these hepatocytes, which is in concert with the observation in perfused rat liver (vide supra, Jennings et al, 1979). It also appeared that T_3 entrance into the cell was less susceptible to ATP depletion than the uptake of T_4 and rT_3. In addition to rat hepatocytes in primary culture, similar transport mechanisms for T_4, T_3 and rT_3 have been shown in cultured human fibroblasts (Docter et al, 1987). These findings were extended by showing inhibition of iodothyronine transport into rat liver cells by a monoclonal antibody (Mol et al, 1986) and the demonstration that carrier-mediated transport of thyroid hormone into rat hepatocytes is rate limiting in total cellular uptake and metabolism (Hennemann et al, 1986). That changes in transport of iodothyronines from plasma to the tissues, may, at least, significantly contribute to

the observed alterations in serum thyroid hormones, has been suggested in patients with non-thyroidal illness (N.T.I.). In these clinically euthyroid patients, serum total and free T_4 concentrations may be subnormal, normal or frankly elevated. In addition, reduced total and free T_3 and increased total and free rT_3 serum concentrations are observed. In many instances the type and magnitude of these abnormalities appear to reflect the severity of illness, as is demonstrated for instance in patients with myocardial infarction, in which an inverse relationship was shown between serum total T_4 and T_3 with infarction size (Smith et al, 1978). Furthermore, serum levels of rT_3 are higher in more severely ill patients (Alexander et al, 1982). For an excellent review on this subject, see Engler and Burger, 1984. The relationship of the severity of non-thyroidal illness and the direction and magnitude of the alterations in serum thyroid hormone levels, is complex and appears to reflect a continuum, as depicted in fig. 4. Table 2 summarizes some important features with regard to changes in thyroid hormone in these patients. In mild disease these alterations resemble those occuring in obese subjects during acute and chronic caloric deprivation (Wartofsky and Burman, 1982) and a significant role of reduced caloric intake in these ill patients is suggested by the observation that in severely

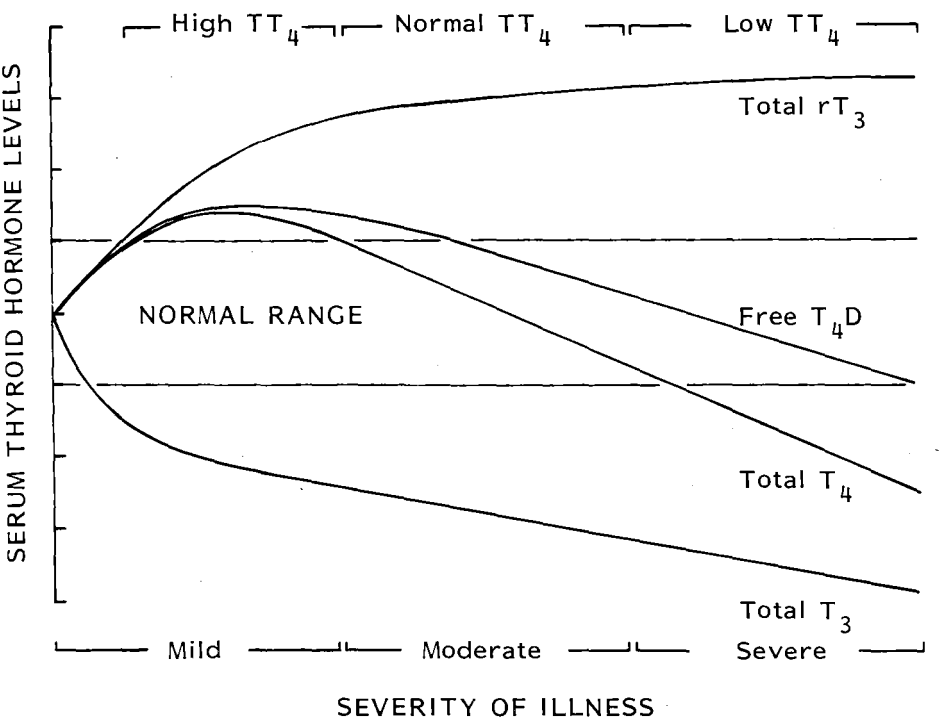


Fig. 4. Continuum of changes in serum thyroid hormone levels in non-thyroidal illness relative to the severity of the illness.

Table 2. Some important changes in thyroid function parameters and hormone kinetics in non-thyroidal illness

	Mild/moderately ill High Total T_4 State	Severely ill Low Total T_4 State
Serum total T_4	↑	↓
% free T_4 in serum (ED)	N	↑
serum free T_4 (ED)	↑	N or ↓
FT_4I	↑	↓
TBG	↑	N
Binding to carrier proteins	binding capacity ↓	hormonal binding ↓
TSH	N	N (slight ↑)
Response of TSH to iv TRH	N	N (slight ↓)
serum T_3	↓	↓
serum rT_3	↑	↑
T_4 MCR	↓	↑
T_4 PR	N	N or ↓

Data pooled from the literature, mainly from the work of Kaptein et al.

ED : equilibrium dialysis
TBG : thyroxine binding globulin
FT_4I : free thyroxine index
MCR : metabolic clearance rate
PR : production rate

ill patients serum levels of total T_3, rT_3 and T_4 returned towards normal after receiving nutritional support (Richmand et al, 1980).

As can be deducted from table 2 important changes in hormone binding occur during non-thyroidal illness. The changes in serum concentration of TBG, TBPA and albumin, however, do not appear to entirely account for the alterations in serum concentrations of T_4, T_3 and rT_3. This led Chopra et al (1979) to suggest the presence of an inhibitor in serum of patients with NTI, resulting in decreased binding of thyroid hormones to their carrier proteins. Due to its physiochemical properties, this inhibitor was thought to be an IgM-like protein or a substance complexed with IgM. Subsequently, this inhibitor was shown to be a constituent of extrathyroidal tissue during NTI and it was hypothesized that this factor leaks out of the tissues into the plasma during NTI (Chopra et al, 1985). Oppenheimer and coworkers (1982) also suggested that in NTI substances were involved in inhibiting iodothyronine binding to plasma proteins. They, however, did not reach the conclusion that this factor was an immunoglobulin. Interestingly, serum of patients with NTI showed the ability of inhibiting ^{125}I-T_4 uptake by cultured rat hepatocytes, suggesting that this inhibitor might possess biological importance. These findings were extended by Chopra and coworkers (Chopra et al 1985, 1986). They showed a very close correlation between the thyroid hormone-binding inhibitor (THBI) and an inhibitor of extrathyroidal conversion of T_4 to T_3 (IEC) in sera of patients with non-thyroidal illness. Although the exact nature of THBI and IEC is still unclear, many arguments are in favour of a lipid, more specifically

fatty acids, as being both the THBI and IEC. This is the more interesting, since serum concentration of fatty acids increase during many circumstances in which a low T_3 syndrome arises (Chopra et al, 1985). Fatty acids increase the fluidity and negative surface charges of cell membranes (Ahrens, 1981; Schmalzing, 1982) and it is feasible that these membrane effects interfere with transmembraneous transport of thyroid hormones as proposed by Krenning et al (vide supra) and substantiated by their recent demonstration that sera of patients with non-thyroidal illness and a low total serum T_4, inhibit thyroid hormone uptake by hepatocytes in primairy culture without a direct effect on deiodination (Krenning et al, 1986).

From the work of Irvine (1974) and Irvine and Simpson-Morgan (1974) it is suggested that transcapillary movement of thyroid hormones occurs as iodothyronine-protein complex in hepatic tissues, whereas for other tissues such as muscle, skin and intestine, transcapillary movement of thyroid hormone is mainly in the non-protein bound, i.e. free, form.

Because of the above mentioned changes (table 2) in serum carrier proteins of iodothyronines and the existance of a thyroid hormone binding inhibitor during non-thyroidal illness, determination of the MCR should also be regarded with respect to a changed free hormone concentration. A correction for changes in this free concentration can be made according to Oppenheimer et al (1970): free MCR= total MCR (1/FF-1) (FF: free fraction). With this correction, it appeared that free T_4 MCR was normal or slightly increased during normal or reduced free T_4 levels in the low TT_4 state of NTI and decreased in the presence of elevated free T_4 levels in the high and normal TT_4 states of NTI. It was therefore suggested that changes in the free hormone clearance rate were responsible for alterations in serum free T_4 levels. The total and free T_4 MCR may be affected by the rate of hormone transport from plasma into the tissues. The latter is determined by the quantity of T_4 in the vascular compartment and the fractional rate of hormone exit from plasma. It appeared that the fractional rate of T_4 exit from plasma was decreased to approximately 50% in the high, normal and low TT_4 states of NTI, so serum levels of total or free T_4 or the free fraction of this hormone did not determine its fractional rate of transport from plasma into the tissues. This is in contrast to the increased and reduced fractional transport rates from plasma to the tissues in the low TBG state and high TBG state, respectively, in otherwise normal subjects. These observations point to an impaired transport mechanism in the above cited situations of NTI. For more details, the reader is referred to Kaptein (Thyroid hormone metabolism, ed. G. Hennemann, 1986).

An universally decreased cellular uptake of T_4 or decreased exit of T_4 from plasma, however, cannot explain why rT_3 production remains normal. A decreased T_4 cellular availability would, in addition to a lowered T_3 production, also result in a decreased rT_3 generation. Therefore, other or additional mechanisms must be operative. Considering the model as proposed by Krenning et al (1983) in which T_4 and rT_3 enter the cell by a common pathway that

is much more sensitive to a decreased cellular ATP content than the pathway for T_3, a few assumptions have to be made in order to explain all changes in iodothyronines as seen during starvation. Firstly, it must be assumed that hormonal transport into different tissues is not inhibited to the same extent, but is most pronounced in the liver. Secondly, rT_3 production takes place mainly outside the liver in tissues not or to a lesser extent subjected to inhibition of transmembraneous transport of thyroid hormone and, thirdly, the liver is quantitatively important in the production of T_3 and the degradation of rT_3. As yet, no data are available to substantiate the first postulate. With regard to the production of T_3, rT_3 and degradation of rT_3, evidence can be presented in favour of the above cited hypothesis. This will be discussed in the next section.

Thyroid hormone deiodination is tissue-specific

Because the T_3 PR is impaired during starvation and NTI, in contrast to the unaltered rT_3 generation, it was suggested that the 5'- deiodination and 5-deiodination were accomplished by separate enzymes. The 5'-deiodination is also termed: outer ring deiodination (ORD) and 5-deiodination is referred to as inner ring deiodination (IRD). It has become increasingly evident that in rat liver IRD and ORD are mediated by the same enzyme (Fekkes et al, 1982). This conclusion can be extrapolated to the human situation. That the enzyme kinetics of rat liver deiodinase are indeed applicable to human liver, is supported by recent findings in human liver homogenates (Visser et al, 1983). Because of a low K_m and high V_{max} for IRD of rT_3, it is unlikely that the liver, although actively producing rT_3, contributes significantly to the plasma rT_3 pool (Visser et al, 1979). Reverse T_3 could not be detected in the perfusate of T_4 perfused rat livers (Köhrle et al, 1982) or incubation media of rat liver slices (Balsam and Ingbar, 1978) and cultured rat hepatocytes (Sato and Robbins, 1981) incubated with T_4. Furthermore, the net removal of rT_3 by the human liver equals about the total bodyremoval (Bauer et al, 1987). From these observations, it is now held that the liver is a major site in degradation of rT_3, and that plasma rT_3 is not derived from hepatic T_4 to rT_3 conversion. The latter is further supported by the finding that plasma production rates of rT_3 are similar in PTU and methimazole treated hyperthyroid patients (Laurberg and Weeke, 1980). Propylthiouracil (PTU) inhibits 5'-deiodination in the liver, while methimazole does not. Thus, different organs may be involved in the production and clearance of a single hormone, and these organs do not necessarily react in the same way to situations as starvation, non- thyroidal illness or changes in thyroidal hormone secretion. The 5'- deiodinating enzyme in the kidney, for instance, which is quite similar to liver 5'D (vide infra), is not reduced in activity during fasting (Kaplan et al, 1979). Moreover, it appeared that IRD and ORD activity is qualitatively and quantitatively not equally present in

Table 3. Characteristics of the pathways of iodothyronine deiodination in the rat

	Type I	Type II	Type III
Deiodination site	inner and outer ring	outer ring only	inner ring only
Substrate preference	$rT_3 >> T_4 > T_3$	$T_4 > rT_3$	$T_3 > T_4$
Kinetic pattern	ping-pong	sequential	sequential
K_m for T_4 in microsomal preparations	~ 1 μM (1-5 mM DTT)	~ 1 nM (20 mM DTT)	~ 40 nM (50 mM DTT)
Tissue localization	highest in liver, kidney and thyroid. Present in many other tissues (low activity)	anterior pituitary; CNS; brown adipose tissue; placenta (human, rat)	CNS; eye; placenta (human, rat)
Thiol reductants	Stimulatory	Stimulatory	Stimulatory
Iopanoic acid	inhibitory	inhibitory	inhibitory
PTU	inhibitory	no effect	no effect
Hypothyroidism	liver, kidney: reduced activity thyroid: increased activity	increased activity	in brain decreased activity

DTT : dithiotreitol
CNS : central nervous system
PTU : propylthiouracil

Data from Kaplan 1984

different tissues (table 3). The type of monodeiodination in the liver and kidney has been designated as type I. This type of enzymic activity is most abundant in liver and kidneys. The idea that these organs play a dominant role in the production of T_3, originates from the observation that PTU, a specific inhibitor of type I activity, induces a 50-75% decrease in serum T_3 concentration in thyroidectomized L-thyroxine substituted rats (Bernal and Escobar del Rey, 1974; Silva et al, 1982). It has, however, been shown that in rat pituitary and brain, besides low levels of type I enzyme, two other types of deiodinase activity exist, referred to as type II and III (pituitary excluded, does not contain type III activity). Type II activity concerns ORD and type III IRD. All three types are thiol- dependent, but only type I is inhibited by PTU and has a different reaction mechanism compared to type II and III. Liver and kidney do not contain type II and III activity. Very interestingly, these enzymes differ essentially in their reaction to hypothyroidism. In the rat cerebral cortex, hypothyroidism induces an increase in type II activity with a concomitant decrease in activity in type III enzyme. These changes result in an increased T_3 production and diminished T_3 degradation, thus preventing cerebral T_3 depletion. In contrast, type I activity diminishes, resulting in a lowered T_3 generation by type I containing tissues, which are believed to contribute mostly to the plasma T_3 pool. As yet, in humans no data are available with regard to changes in activity of these types of deiodinase during starvation and non-thyroidal illness.

Sulfoconjugation of iodothyronines

Regulatory mechanisms in thyroid hormone metabolism in various conditions

became even more complex when studies with cultured rat hepatocytes showed that sulfoconjugation and deiodination of T_3 and 3,3'-T_2 are strongly related processes (Otten et al, 1983, 1984; Visser et al, 1983a). Inhibition of sulfotransferase activity of cultured rat hepatocytes results in a decreased deiodination of these compounds. Sulfation of T_3 increases the V_{max} of T_3 5-deiodination by approximately 30 times, without altering the K_m (Visser et al, 1983). Subsequently, the effects of sulfation of T_4 and rT_3 were evaluated. These studies (Mol and Visser, 1985) revealed that sulfation of T_4 inhibits 5'-deiodination but 5-deiodination is greatly enchanced due to an increase in V_{max} with a concomitant decrease in K_m resulting in a 200-fold increase in the V_{max}/K_m ratio. Sulfation of rT_3 does not appreciably influence its outer ring deiodination. It remains to be established to what extent sulfation of iodothyronines could act as a possible factor in regulating thyroid hormone metabolism.

Summary

Caloric deprivation and non-thyroidal illness induce the so called low T_3 syndrome, in which reduced serum T_3 levels are found in concomitance with elevated serum rT_3 concentrations. In addition to the amount of caloric intake, manipulations in dietary composition have also been shown to influence these changes in rather specific, as yet not completely understood, ways. In caloric deprivation total serum T_4 levels are mostly unaltered, while in non-thyroidal illness total serum T_4 levels are increased, normal or decreased, roughly in parallel with the degree of illness. Kinetic analysis revealed that reduced serum T_3 levels in these situations result from a diminished production rate, while its MCR is unchanged. This is in contrast to the normal PR of rT_3 and the increase in serum levels of this iodothyronine is explained by its reduced MCR. This led to the idea that a selective decrease in ORD in the liver, as had been shown in liver homogenates from fasted rats, was responsible for the alterations in iodothyronines as cited above. However, ORD and IRD activity in rat liver could not be separated and it became evident that deiodinase activity was quantitatively and qualitatively not equally distributed over different tissues. Deiodinase activity could be attributed to three different enzymes of which some features are summarized in table 3. It also appeared that the liver must be regarded as a major site in T_3PR and degradation of rT_3. This had already been postulated by Krenning et al (1983) in order to explain the changes in serum thyroid hormones during the low T_3 syndrome alternatively i.e. by inhibition of transmembraneous transport of iodothyronines. According to this model, T_4 and rT_3 enter the hepatocyte by a common pathway that is much more sensitive to inhibitory factors than the pathway for T_3. A decreased T_4 entrance in the liver with type I enzyme activity results in a diminished T_3 production. As has been outlined above, rT_3 production in the liver does not contribute significantly to the plasma rT_3 concentration, thus no influence on

the rT_3 plasma production rate is to be expected from a decreased T_4 entrance in the liver. In contrast, a decreased entrance in the liver of plasma rT_3, which is thought to be produced by extrahepatic type III enzymic activity containing tissues, would result in an increased plasma rT_3 concentration, since the liver is considered as the major rT_3 degrading organ. In order to explain the unaltered rT_3 production rate, it must be assumed that rT_3 producing tissues are not or to a lesser extent subjected to this inhibition of transmembraneous transport of iodothyronines. Degradation of T_3 is mediated by type I and III enzymic activity. Because T_3 entrance is much less sensitive to this transport inhibition, its unaltered MCR can thus be explained. The idea that changes in transport of iodothyronines into the tissues may indeed appear to be an important factor in explaining the above mentioned alterations in serum iodothyronines, is further supported by the in vivo observations in patients with non-thyroidal illness. Taken together, the conclusion can be reached, that in explaining changes in iodothyronines during the low T_3 syndrome, several mechanisms must be taken into account, i.e. the qualitative and quantitative contribution of changes in enzymic deiodination activity, changes in cofactors and possibly sulfation of substrates for these reactions and alterations in transport of iodothyronines into the tissues. These considerations probably also apply for other conditions in which serum iodothyronines change in a predictable, reversible and reproducible way, such as during administration of propranolol, dexamethasone, amiodarone and cholecystographic agents. It is feasible that different mechanisms are operative in the diversity of conditions in which a low T_3 syndrome originates.

Scope of the thesis

The results from the work of Krenning and Docter in our laboratory, as discussed in previous sections, led to the hypothesis that inhibition of transmembraneous transport of iodothyronines, could be a major factor in inducing the hormonal changes in the low T_3 syndrome. A three-pool model of distribution and metabolism of T_4, T_3 and rT_3 was used for kinetic analysis.

Two conditions in which a low T_3 syndrome is known to originate, were studied: caloric deprivation and oral administration of propranolol.

Chapter 2 deals with alterations in kinetic parameters during caloric deprivation in 10 obese subjects. The same parameters were assessed in six normal non-obese L-T_4 replaced male students before and during D- propranolol treatment. The results obtained in this latter group are presented in chapter 3. In chapter 4 early serum disappearance and computed liver uptake of thyroxine in humans during caloric deprivation and D-propranolol administration are discussed. In addition, both parameters were assessed immediately after intravenous administration of fructose, a manoeuvre by which a prompt and significant fall in liver ATP is induced. In chapter 5 the results from the above mentioned sections are summarized.

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CHAPTER 2

EFFECTS OF CALORIC DEPRIVATION ON THYROID HORMONE TISSUE UPTAKE AND GENERATION OF LOW T_3 SYNDROME

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Abstract

Changes in thyroid hormone metabolism in the low T_3 syndrome cannot be fully explained in all conditions by a decrease in 5'-deiodinase activity. Recent observations showed that in rat hepatocytes iodothyronines are taken up by an active transport mechanism. In order to investigate if regulation i.e. inhibition of active transmembraneous transport for iodothyronines in humans may contribute to the generation of the low T_3 syndrome, tracer thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) kinetic studies were performed in ten obese subjects before and after seven days on a 240 kcal diet. Kinetic analyses were performed according to a 3-pool model of distribution and metabolism for both T_4 and T_3.

T_4 kinetics: during caloric deprivation serum total T_4 (93 $\pm$ 2.8 vs 91 $\pm$ 3.3 nmol/l) (control versus 240 kcal diet, mean $\pm$ SEM) and plasma pool (388 $\pm$ 33 vs 355 $\pm$ 19 nmol) did not change, and production rate (124 $\pm$ 11.1 vs 97 $\pm$ 6.7 nmol/d) and metabolic clearance rate (MCR) (1.32 $\pm$ 0.09 vs 1.07 $\pm$ 0.07 l/d) were significantly lower ($p < 0.005$).

Despite a significantly higher serum free T_4 (20 $\pm$ 1.2 vs 25 $\pm$ 1.9 pmol/l) ($p < 0.001$), the mass transfer rate to the rapidly equilibrating pool (REP) (195 $\pm$ 13 vs 137 $\pm$ 10.9 nmol/h) ($p < 0.005$) and the slowly equilibrating pool (SEP) (16.9 $\pm$ 3.0 vs 9.2 $\pm$ 2.3 nmol/h) ($p < 0.005$) diminished significantly, leading to smaller tissue pools (REP: 539 $\pm$ 42 vs 434 $\pm$ 36 nmol and SEP: 423 $\pm$ 52 vs 262 $\pm$ 43 nmol) ($p < 0.025$; $p < 0.005$ resp.).

T_3 kinetics: both serum total T_3 (1.7 $\pm$ 0.1 vs 0.9 $\pm$ 0.04 nmol/l) ($p < 0.001$), free T_3 (3.5 $\pm$ 0.3 vs 2.6 $\pm$ 0.2 pmol/l ($p < 0.001$), plasma pool (9.8 $\pm$ 1.0 vs 5.5 $\pm$ 0.6 nmol) ($p < 0.001$) and production rate (49 $\pm$ 4.9 vs 28 $\pm$ 2.6 nmol/d) ($p < 0.001$) diminished significantly, while MCR (27.9 $\pm$ 2.0 vs 30.5

± 2.2 l/d) remained unchanged. Mass transfer rates to the REP (19.3 ± 1.8 vs 10.1 ± 1.1 nmol/h) ($p < 0.005$) and the SEP (6.1 ± 0.5 vs 3.5 ± 0.4 nmol/h) ($p < 0.001$) were lowered by about 50%, leading to smaller tissue pools (REP: 6.8 ± 0.8 vs 4.1 ± 0.3) ($p < 0.025$) (SEP: 44.4 ± 4.5 vs 27.1 ± 2.3 nmol) ($p = 0.01$). These changes cannot be fully explained by a similar decrease of serum free T_3 (only 25%), indicating a diminished transport efficiency for T_3.

It is concluded that during caloric restriction transport of T_4 and T_3 into tissues is diminished and that this phenomenon is much more pronounced for T_4 than for T_3. It is postulated that, irrespective of any possible change in 5'-deiodinase activity, inhibition of T_4 transport per se may contribute to low T_3 production and low T_3 serum levels due to less substrate (i.e. T_4) availability in tissues.

Introduction

It was originally envisaged that iodothyronines enter the cell by simple diffusion (27). This notion was based on the lipophilicity of iodothyronines which favours the passage through the lipid bilayer of the cell membrane. In this view the translocation of iodothyronines over the cell membrane is governed by the difference in intra- and extracellular free concentration, i.e. the concentration of the non protein-bound iodothyronine. It is then to be expected that turnover of iodothyronines is more closely related to the free extracellular concentration than to the total concentration. However, normal or even decreased thyroxine (T_4) turnover has been found in several conditions for example non-thyroidal illness and the post-operative state despite the presence of increased free hormone serum levels (10). Even in euthyroid normals free T_4 concentration did not correlate with T_4 turnover (10). Moreover, in thyroxine binding globulin (TBG) deficiency with lowered serum free T_4 concentrations a normal T_4 turnover has been found. It was postulated that other factors, possibly directly related to cellular handling of T_4, may be operative (10).

Caloric deprivation in humans induces the so called low T_3 syndrome in which the total (and free) serum levels of 3,5,3'-triiodothyronine (T_3) are decreased and those of 3,3',5'-triiodothyronine (reverse T_3, rT_3) increased (3,4). Tracer kinetic studies revealed a diminution of T_3 production while rT_3 generation was unchanged (35,36). The metabolic clearance rate of rT_3 was decreased and that of T_3 unchanged (35,36). These phenomena have been explained by a decreased 5'- deiodinase enzyme activity in the liver leading to a diminished rT_3 breakdown and T_3 production. In liver homogenates of fasted rats a reduced 5'-deiodinase activity could indeed be demonstrated (9). However, a reduced 5'-deiodinase activity cannot fully explain the alterations in this syndrome since serum T_3 and rT_3 do not always change reciprocally. In contrast to a sustained reduction of serum T_3 in humans during starvation,

only a transient rise in rT_3 was observed in some studies (4). After two to three weeks rT_3 levels start to decline. Because of this and other (see discussion) considerations one should look for other factors which could participate in the induction of the low T_3 syndrome as well. Recently, we have demonstrated the presence of a specific high affinity low capacity energy dependent cellular uptake mechanism for T_4, T_3 and rT_3 in rat hepatocytes in primary culture (21-24). According to kinetic experiments, T_4 and rT_3 seem to enter the cell by a common pathway that is different from that for T_3. Both pathways have been shown to be ATP dependent (22,23). These observations led us to consider the possibility that inhibition of transmembraneous transport of iodothyronines could be a factor in the generation of the low T_3 syndrome.

To investigate this aspect we have performed tracer kinetic studies with T_4 and T_3 in obese subjects before and during a low caloric intake leading to a low T_3 syndrome. Data were analyzed according to a three compartmental model of distribution and metabolism, as developed by DiStefano et al (5, 6).

Glossary

Nomenclature used

$(i = 1, 2, 3; j = 1, 2, 3; i \neq j)$

t = time (h)
y_t = plasma activity at time t (% dose/l)
A_i = coefficient of the i^{th} exponential component (% dose)
$_i$ = exponent of the i^{th} exponential component (h^{-1})

Q_1 = size of pool 1 (plasma pool) (nmol)
Q_2 = size of pool 2 (fast or rapidly equilibrating pool (REP)) (nmol)
Q_3 = size of pool 3 (slow or slowly equilibrating pool (SEP)) (nmol)
K_{ii} = fractional turnover rate of pool i (h^{-1})
K_{ij} = fractional transport rate from pool j to pool i (h^{-1})
K_{0i} = fractional disposal rate in pool i (h^{-1})

MCR = metabolic clearance rate (l/h)
V_i = plasma equivalent distribution volume in pool i (l)

D_S = disposal rate in the slow pool
D_F = disposal rate in the fast pool
CR_S = T_4 --> T_3 conversion rate in the slow pool
CR_F = T_4 --> T_3 conversion rate in the fast pool
SR = secretion rate from the thyroid

Methods

Subjects

Ten otherwise healthy obese subjects (nine females), age 21-36 years (mean 31 years), participated in this study. All had normal thyroid function as measured by total serum T_4, T_3-resin uptake and thyrotropin response to an intravenous bolus of thyrotropin releasing hormone. The study and the protocols were approved by the Ethical Committee of the medical centre and informed consent was obtained from all participants. Medications if used were omitted for at least 1 month before the study. Body weights ranged from 75 – 155 kg (mean 113.5 kg) at the start. All subjects were at least 25% overweight, range 26 – 198 percent (mean 97%).

During the control study period patients were kept on a normal weight-maintaining diet. The study was repeated after six weeks, when the subjects were on a 240 kcal Modifast^R diet for seven days. This diet contained 33 g protein, 25.5 g carbohydrate and 0.7 g fat and adequate supplies of minerals and vitamins.

Materials and Preparations

^{125}I-T_4 (specific activity >1200 uCi/ug) and Na^{131}I for labeling were purchased from The Radiochemical Centre, Amersham, U.K. ^{131}I-T_3 was prepared by iodination of 3,5-T_2 with $^{131}I^-$ by the chloramine-T method (8). Iodination was performed by mixing 10 ul volumes of Na^{131}I, 0.5 M sodium phosphate buffer pH 7.5, 3,5-T_2 (1 ug/ul in 0.05 M sodium phosphate buffer pH 7.5) and chloramine-T (1 ug/ul in 0.05 M sodium phosphate buffer pH 7.5), in that order. After one minute the reaction was terminated by adding 100 ul sodium bisulfite (1 ug/ul in sodium phosphate buffer pH 7.5). The reaction mixture was applied on a small (10x0.5 cm) Sephadex LH-20 column and eluted with 30% ethanol in 0.05 M sodium carbonate pH 11.5 (v/v). T_3-fractions were pooled and evaporated to dryness under a stream of nitrogen.

T_3-antiserum was obtained by injecting rabbits with a T_3-bovine serum albumin conjugate in complete Freund's adjuvant. Antiserum against T_4 was purchased from Henning GmbH, Berlin. All other reagents used were of the highest purity available.

Procedures

25 uCi ^{125}I-T_4 and 30 uCi ^{131}I-T_3 in 1.5 ml of 2% human albumin in phosphate buffered saline, were administered via an intravenously inserted cannula in the arm and blood samples were drawn at regular intervals through a cannula in the contralateral arm to obtain at least six points on each of the three sections into which the plasma disappearance curve could be resolved. Patients were in supine position from one hour before and during the first four hours of the study. Later on blood samples were collected after the patients had been in supine position for at least half an hour. Samples were collected during

ten days. During the study ten drops of Lugol's solution were administered three times a day in order to prevent thyroid uptake of radioactive iodide liberated during the study. For counting ^{125}I-T_4 activity, 1 ml serum samples were precipitated with 4 volumes 10% trichloroacetic acid (TCA) and washed twice with 10% TCA. ^{131}I-T_3 was separated from serum by the method of Bianchi et al (2) with modifications. Serum samples (0.5 ml) were mixed with 0.5 ml buffer (0.08 M barbital pH 8.6, 9 mg/ml 8-anilino-naphtalene sulfonic acid (ANS)) and applied to a small (10x0.5 cm) Sephadex G-25 column equilibrated with 0.1N NaOH. Proteins, including iodoproteins and iodide were eluted with 5x0.5 ml barbital buffer (0.08 M pH 8.6, 0.6 mg/ml ANS). Thereafter ^{131}I-T_3 was eluted with 5x0.5 ml barbital buffer containing specific T_3-antibody, and fractions were pooled before counting. Rabbit T_3-antiserum (1 ml, binding capacity 2.5 nmol T_3/ml) was precipitated with 1.8 M $(NH_4)_2SO_4$ final concentration. The precipitated anti-T_3-antibody was dissolved in barbital buffer and diluted to 100 ml before use. Recovery of added labeled T_3 from serum samples was 99.0 ± 0.15% (mean ±SD, n=10). The injected dose was diluted 100 fold in the subjects' serum and processed as outlined for the serum samples, for use as standard.

Free T_4 and T_3 concentrations were determined by equilibrium dialysis according to the method of Sterling and Brenner (33).

Serum rT_3 was estimated by radioimmunoassay (38). T_4 and T_3 were measured by established radioimmunoassay procedures, using ANS to inhibit binding of the hormones to serum proteins.

Calculations

All tracer concentrations y(t), expressed as the percent injected dose per liter, were fitted to the sum of 3 exponentials as

$$y(t) = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t} + A_3e^{-\lambda_3 t}$$

where t is time, A_i is the coefficient and λ_i is the constant of the exponent of the i-th exponential. Estimation of the goodness of fit was performed by comparing the residual sum of squares with the total sum of squares of all data points. From the exponents and coefficients obtained the plasma distribution volume V_p can be calculated.

$$V_p = 100/(A_1 + A_2 + A_3)$$

and the metabolic clearance rate MCR as

$$MCR = 100/(A_1/\lambda_1 + A_2/\lambda_2 + A_3/\lambda_3)$$

Furthermore the exponents (λ_i) and coefficients (A_i) were used to calculate the turnover rates of the plasma pool (K_{11}), the rapidly equilibrating pool

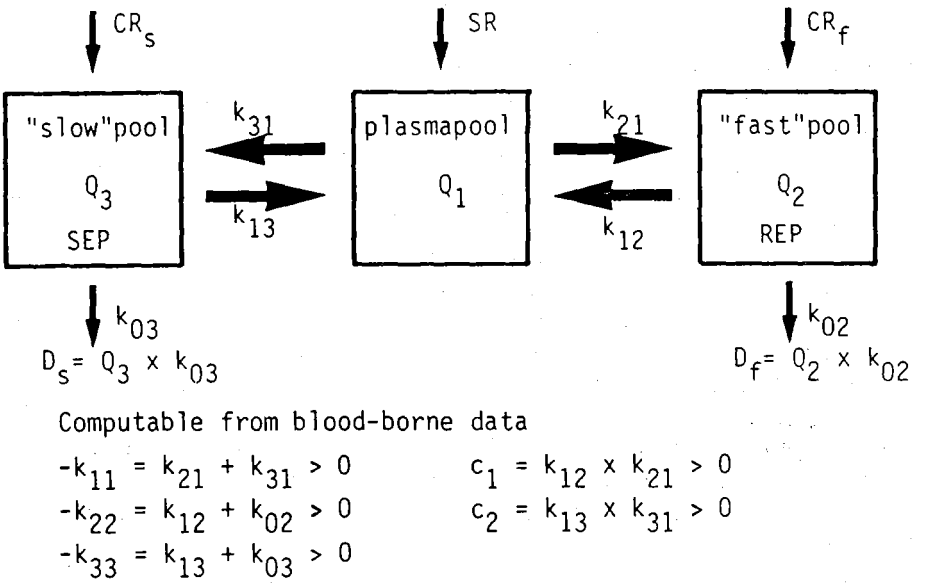

Fig. 1. Three pool model of iodothyronine distribution and metabolism. The K_{ij} values ($i \neq j$, i, j=1, 2, 3, all $K_{ij} > 0$) are fractional transport rates ($hours^{-1}$) from pool j to pool i. K_{11}, K_{22} and K_{33} are fractional turnover rates ($hours^{-1}$ of pool Q_1, pool Q_2 and pool Q_3, respectively. The relations at the bottom are the combinations of these parameters computable from data obtained by blood sampling only. For a complete list of nomenclature see Glossary. For evaluation of the T_4-model only secretion (SR) of T_4 into the plasma pool should be considered, while for evaluation of the T_3-model also T_3-production by conversion both in the slowly equilibrating pool (CR_S) and in the rapidly equilibrating pool (CR_F) should be taken into account.

(K_{22}) and the slowly equilibrating pool (K_{33}) respectively, and the constants C_1 and C_2 of the three pool model of iodothyronine distribution and metabolism depicted in fig. 1. This model and the mathematical solution was recently described by DiStefano et al. both for T_3 (5) and for T_4 (6).

Rearrangment of the terms depicted in fig. 1 gives

$$-C_1/(K_{22}+K_{02}) = K_{21} = C_2/(K_{33}+K_{03}) - K_{11}$$

which equality describes the value of K_{21}, the fractional transfer rate constant from plasma to the rapidly equilibrating pool. A minimal value for K_{21} can be found if K_{02} (the fractional disposal rate constant from REP) is arbitrarily set at zero, which means absence of disposal in this fast pool. A maximal value for K_{21} can be found if K_{03}, the fractional disposal rate constant from the SEP is put at zero, which means absence of disposal in the slow pool. It appeared that the difference between the maximal and minimal value is only small both for the T_4 and for the T_3 model (Table 1). Therefore the

Table 1. 3 pool model for T_4- and T_3-kinetics

	Upper and lower boundaries for K_{21} (fractional transfer rate from plasma to the REP). h^{-1}	
	T_4	T_3
lower bound	0.510 ± 0.037	1.929 ± 0.172
upper bound	0.523 ± 0.037	2.137 ± 0.176
difference	2.12% ± 0.20	10.6% ± 0.8

Values are means ± SE ($n = 10$).

mean of these two values was used in the further calculations. Use of this mean value inevitably divides the disposal equally between the slow and the fast pool. When K_{21} can be approximated in this way, all other parameters of the model depicted in fig. 1 can be calculated.

Further parameters used in this paper were calculated essentially as described by DiStefano et al. (5,6).

Results

In all 10 subjects a low T_3 syndrome was induced. Serum T_3 levels decreased by 46% i.e. from 1.71 ± 0.07 nmol/l (mean ± SEM) in the control period to 0.93 ± 0.04 nmol/l after 7 days of caloric deprivation. Serum rT_3 levels increased by 53% from 0.28 ± 0.04 to 0.43 ± 0.07 nmol/l respectively. Serum T_4 concentrations did not change (93 ± 2.8 versus 91 ± 3.3 nmol/l). During the first two weeks of caloric restriction a considerable body weight reduction was seen in all persons (6.4% ± 0.1).

All plasma radioactivity disappearance curves of T_4 and T_3 could be fitted to a three exponential model. A typical example for T_3 is shown in fig. 2. Residual mean square for all disappearance curves was about 0.1% of total mean squares indicating excellent fit of the data points to this model (32). The values of K_{21} and K_{31} for T_4 and T_3 may depend on the location where these iodothyronines are metabolized, i.e. the slowly and/or the rapidly equilibrating pool. Since no precise information is present in this respect it was calculated to what extent the location of the disposal of T_4 and T_3 would actually affect the K_{21} and K_{31} for these hormones. To reveal the relationship between the transfer rate constants K_{21} and K_{31} and the disposal of T_3 and T_4 in the slowly and the rapidly equilibrating pool, we have calculated all parameters of the model with values of K_{21} ranging from the minimal to the maximal value and plotted the K_{21} and K_{31} as a function of the disposal in the rapidly equilibrating pool, both for the T_4 model (fig. 3) and the T_3 model (fig. 4). A virtually absent (maximal 2.5%) change for T_4 and small change

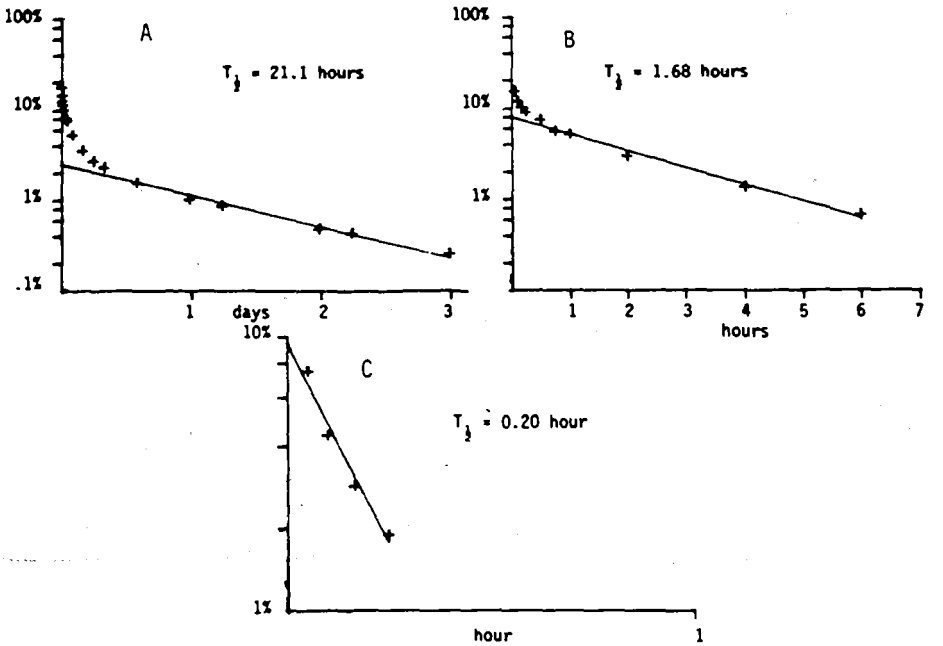

Fig. 2. Typical example of the description of a T_3-disappearance curve by a three exponent model. Panel A: plot of log y_t (in % Dose/l) against time, with the least squares regression line on the final straight part of the curve, giving estimates of coefficient A_3 and exponent λ_3. Panel B: plot of log $[y(t) - A_3e^{-\lambda_3 t}]$ against time, with the least squares regression line on the straight part of the curve, giving estimates of coefficient A_2 and exponent λ_2. Panel C: plot of log $[y(t) - A_2e^{-\lambda_2 t} - A_3e^{-\lambda_3 t}]$ against time, giving estimates of coefficients A_i and exponent λ_1. MCR=25.0 l/day; Vplasma=5.0 l; residual sum of squares=0.098, total sum of squares=64.15.

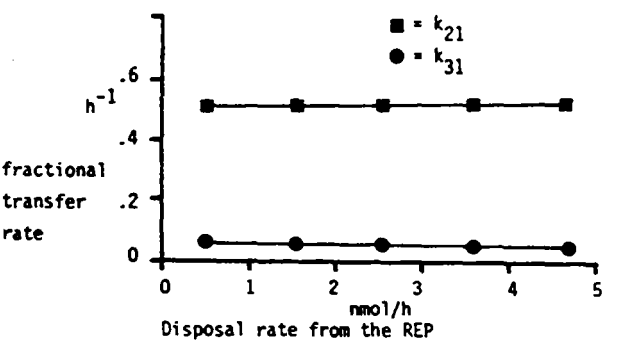

Fig. 3. 3 pool model for T_4-kinetics. Relation of the transfer rate constants to the fast pool (K_{21}) and the slow pool (K_{31}) on the disposal rate from the fast pool. Because the total disposal of T_4 (the sum of the disposals in the slow and the fast pools) is constant and can be calculated from the plasma T_4 concentration and the MCR, a similar (but inverse) relationship can be found between K_{21}, K_{31} and the disposal rate from the slow pool.

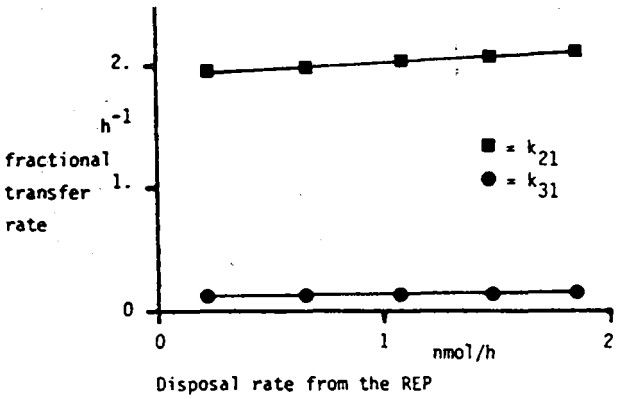

Fig. 4. 3 pool model for T_3-kinetics.
Relationship of the transfer rate constants to the fast pool (K_{21}) and the slow pool (K_{31}) on the disposal rate from the fast pool. For further details see the legend of fig. 3.

(max. 10%) for T_3 is seen for K_{21} and K_{31}, indicating that the choice of the pool in which the disposal actually takes place is not relevant for the calculations of K_{21} and K_{31}. Taking the mean of the minimal and maximal value of K_{21} will therefore give meaningfull information about the fractional transfer rates and mass transfer rates to the slow and the fast pool. Taking these considerations into account, the turnover data of T_4 and T_3 in 10 obese subjects before and during caloric deprivation can be analysed.

Thyroxine (see Table 2).

Caloric deprivation did not alter significantly plasma total T_4 levels, plasma T_4 pool and the size of the T_4 plasma compartment. Both the slowly equilibrating pool (SEP) and the total tissue pool diminished as did the mass transfer rates and fractional transfer rates to the rapidly equilibrating pool (REP) and SEP. These data indicate that transport of T_4 into the tissues is diminished during caloric deprivation, resulting in smaller tissue pools of thyroxine. Furthermore, as is illustrated in Table 2, significant increases were observed in serum levels of free T_4. It should be noted that a diminution of mass transport of thyroxine into tissues is present while free hormone levels are increased. If the mass transfer rate is divided by the free hormone concentration, a "transport efficiency ratio" is obtained, which corrects for changes in free hormone concentration. In this way a 44% and 56% transport inhibition can be calculated for REP and SEP, respectively. Without correction these values are 30% and 46% respectively. The MCR and the production rate of T_4 decreased significantly during fasting.

Table 2. T_3 and T_4 kinetics in 10 obese subjects before and during caloric deprivation

		control				caloric deprivation			
		T_4		T_3		T_4		T_3	
Production rate	nmol/d	124	(11.1)	48.6	(4.90)	97	(6.7)[a]	28.4	(2.60)[b]
MCR	l/d	1.32	(0.09)	27.9	(2.0)	1.07	(0.07)[a]	30.5	(2.2)
Plasma compartment									
free hormone concentration	pmol/l	20	(1.2)	3.5	(0.3)	25	(1.9)[b]	2.6	(0.2)[b]
hormone concentration	nmol/l	93	(2.8)	1.71	(0.07)	91	(3.3)	0.93	(0.04)[b]
size	l	4.1	(0.29)	5.6	(0.36)	3.9	(0.16)	5.9	(0.48)
pool	nmol	388	(33)	9.8	(1.0)	355	(19)	5.5	(0.57)[b]
Rapidly equilibrating compartment									
size	l	5.8	(0.45)	3.9	(0.39)	4.8	(0.42)[c]	4.5	(0.32)
pool	nmol	539	(42)	6.8	(0.82)	434	(36)[d]	4.1	(0.30)[d]
mass transfer rate from plasma	nmol/h	195	(13)	19.3	(1.75)	137	(10.9)[a]	10.1	(1.06)[a]
fractional transfer rate from plasma	h^{-1}	0.55	(0.08)	2.03	(0.17)	0.39	(0.03)[e]	1.9	(0.17)
Slowly equilibrating compartment									
size	l	4.5	(0.43)	25.8	(2.53)	2.8	(0.43)[b]	29.4	(2.24)
pool	nmol	423	(52)	44.4	(4.47)	262	(43)[a]	27.1	(2.27)[f]
mass transfer rate from plasma	nmol/h	16.9	(3.0)	6.13	(0.48)	9.2	(2.3)[a]	3.48	(0.35)[b]
fractional transfer rate from plasma	h^{-1}	0.045	(0.009)	0.64	(0.024)	0.025	(0.006)[a]	0.63	(0.022)

Values are means ± SE.MCR, metabolic clearance rate. T_4 1 nmol/l = 0.077 μg/dl; T_3 1 nmol/l = 0.065 μg/dl.
[a] $P < 0.005$; [b] $P < 0.001$; [c] $P < 0.01$; [d] $P < 0.025$; [e] $P = 0.05$ (paired t-test); [f] $P = 0.01$.

Triiodothyronine (3,5,3'-T_3) (Table 2).

All T_3 pools decreased by approximately 40% (plasma pool 44%; REP 40%; SEP 43%). Also inward mass transfer rates of the REP and the SEP decreased by a similar value (REP 48%; SEP 43%), while free T_3 decreased by approximately 25%. If a transport efficiency ratio for T_3 is calculated in the same way as outlined for T_4, a transport inhibition during caloric restriction of 30% and 23% is found for REP and SEP respectively.The fractional transfer rates did not alter.

Discussion

In general, the low T_3 syndrome is explained by diminution of 5'- deiodinase enzyme activity causing reciprocal changes in serum T_3 and rT_3. The low T_3 syndrome due to caloric deprivation or fasting in man is caused by a decrease in T_3 production and in plasma rT_3 removal (35,36). Although, until the present study, no information was present about the mechanism of these changes in man, it was hypothesized that the underlying cause is a reduced 5'-deiodinase activity in the liver. This notion was derived from studies in fasted rats where indeed a reduced liver 5'-deiodinase activity has been found (9). This decreased activity, which is caused by a decrease in enzyme protein (1,17) has been attributed to tissue hypothyroidism induced by decreased T_4 production, because enzyme activity was restored in microsomal fractions upon T_4 replacement (1,7). It was suggested, however, that 5'-deiodinase activity is only rate limiting in fasting rats, when supraphysiological concentrations of T_4 are used (15). In addition, it is not excluded, that changes in thiol availability also play a role in decreased 5'-deiodinase activity in fasted rats (37). Studies from others (15) using perfused livers of 3 days fasted rats showed a decrease in uptake of T_4, while the efficiency of T_3 production from the T_4 taken up was unimpaired. It seems, therefore, questionable to explain the low T_3 syndrome solely by diminution of 5'-deiodinase activity.

An alternative explanation for the genesis of the low T_3 syndrome may be inhibition of transport of iodothyronines across the cell membrane. From data in our laboratory (21-24) it became apparent that two different transport systems for iodothyronines can be discerned in rat hepatocytes in primary culture: one pathway shared by T_4 and rT_3 and a different one for T_3. Inhibition of these active transport systems could be achieved by a fasting-like state, i.e. incubation of hepatocytes with decreasing amounts of glucose. Based on these considerations it is attractive to speculate that transmembraneous transport of T_4 may be of rate limiting significance in peripheral T_3 production during fasting. Thus, if similar transport mechanisms for iodothyronines, as shown in rat hepatocytes, exist in man, an alternative or additional explanation for induction of the

low T_3 syndrome in these conditions can be given. Available literature (12) points to an energy dependent T_3 uptake in cultured IM-9 human lymphocytes and recently we were able to find specific energy dependent T_3 and T_4 uptake in cultured human fibroblasts (20). Therefore we have assessed the influence of a low caloric intake on mass transfer rates of T_4 and T_3 into tissue pools.

Serum disappearance curves of T_4 and T_3 could be described by the sum of three exponentials in all subjects studied. This is in concert with the results described by others (19). A three compartmental analysis as outlined in the calculations could thus be applied. From fig. 3 and 4 and table 1 it can be concluded that the fractional transfer rate constant from plasma to REP ($=K_{21}$) is not dependent on the site of the disposal. Differences between minimal and maximal values of K_{21} are virtually absent or small for T_4 and T_3 respectively. For all further calculations the mean of the maximal and minimal values of K_{21} was used, i.e. arbitrarily the disposal of both hormones was taken to occur for 50% in each pool. Because of the small differences in the transfer rate constants with respect to the assumed place of disposal, all results as shown in table 2 are valid without restrictions.

DiStefano et al. has argued both for T_3 (5), as for T_4 (6), that the REP is mainly composed of liver and kidney, both on the basis of the rapid equilibration of these tissues with plasma T_4 and T_3, as on the size of the hormone pools in these organs.

Serum free T_4 levels increased during caloric deprivation by 25%, while total T_4 levels remained unchanged. These findings correspond with earlier reports (3,18), and indicate a decrease in plasma hormone binding capacity. Others (4) found a decrease in total T_4 without a change in free T_4. During fasting a blunted TSH response to TRH is observed (3,4), which can be explained by the increased free T_4 levels, assuming that no or less inhibition of transmembreanous transport of iodothyronines arises in the pituitary from caloric deprivation. This assumption is supported by the recent finding (34) that diminished TSH secretion in the starved rat is due to enhanced thyroid hormone activity at the pituitary level. An other possibility is that inhibition of TSH secretion in caloric restriction in men is caused by a similar central mechanism reported to occur in severe non- thyroidal illness (39). In the latter group of patients the drop in TSH secretion is seen as a primary event prior to a decrease in T_4 production. A diminution in TSH secretion will give rise to a decreased production rate of T_4, as found in this study. The decrease in T_4 MCR explains the unchanged serum total T_4 level. In spite of an increased free T_4 serum level during caloric deprivation, hormone transport into REP and SEP is markedly decreased. This transport inhibition and decreased production rate can account for the observed decrease in tissue pools of T_4. In this context it is very interesting that in T_4 substituted fasting rats serum thyroxine concentration doubled within 48 hours of starvation (13). Decreased faecal excretion of T_4 was only partially responsible for this increase. In addition a reduced clearance of T_4 from plasma was found.

In this study both serum total T_3, free T_3, plasma pool and production rate (table 2) diminished significantly, while MCR remained unaltered. Mass transfer rates to SEP and REP were lowered by about 50%, leading to smaller pools (about 40%). A 50% decrease in serum total T_3 with a concomitant decrease in serum free T_3 of only 25% is likely due to a diminished plasma hormone binding capacity as seen with respect to T_4. Although a 50% decrease in T_3 transport into the tissues can be partially explained by a similar decrease in T_3 production rate, an additional factor in the decrease in inward mass transfer rates is an inhibition of transport into tissue cells because free T_3 decreased only by 25% and the free hormone concentration in the interstitial fluid equals that in plasma (14). This transport inhibition of T_4 and T_3 is particularly reflected by the decreased transport efficiency ratios, which corrects for changes in the free hormone concentration.

The much more pronounced inhibition of T_4 transport into tissues as compared to that of T_3 as found in this in vivo study, is in accordance with similar findings with ATP depleted rat hepatocytes (22). Although T_4 transfer rate into tissues, especially the REP, exceeds T_4 disposal many times, there are arguments that a decrease in T_4 influx will lead to a decrease in T_4 metabolism and hence T_3 production. Thus, we have shown that efflux of T_4 from hepatocytes is not dependent on energy and nonsaturable even far above the physiological range of free T_4 (11). Because efflux of T_4 seems to be a passive process, intracellular free T_4 is primarily dependent on the activity of the influx process. A decrease of this activity will lead to a lowering of intracellular free T_4 and thus to a diminished occupancy of enzymes involved in metabolism of T_4. This reasoning supposes the existence of a free hormone gradient over the plasma membrane. Recently the existence of such a gradient has been demonstrated (29). In this way, an attenuation of T_4 influx will result in diminished occupancy of 5'-deiodinase and consequently decreased T_3 production and also to a diminished occupancy of other binding proteins in the cell, explaining the smaller tissue pools observed. A similar mechanism was recently found in perfused rat liver (15). Diminished affinity or capacity of the intracellular binding proteins during fasting could however also (at least in part) be an explanation for these smaller pools. Unfortunately the model used in this study is not able to differentiate between these two options, and no data about changes in human tissue binding proteins during fasting are available. Taken together, it is clear from our study, that in addition to a possible inhibition of 5'-deiodinase enzyme activity, the low T_3 syndrome during fasting should at least partly be explained by inhibition of transmembraneous transport of iodothyronines.

With respect to the question if inhibition of T_4 to T_3 conversion could explain the observed findings, we have attempted to estimate the conversion ratio before and during fasting. As it has been estimated that thyroid T_3 secretion contributes about 20% to total T_3 production (26) after correction for this, a mean conversion ratio of 31% can be calculated in the control period. For calculation of the

conversion ratio during caloric deprivation the following reasoning has been pursued. As it is known that increased serum TSH predominantly stimulates thyroidal T_3 secretion over T_4 secretion it may be inferred that in the reversed situation i.e. decreased TSH activity T_3 secretion is at least as much depressed as T_4 secretion. As mean T_4 secretion in our subjects during caloric deprivation is depressed by 22%, let us assume that T_3 secretion from the thyroid is as much depressed as thyroidal T_4 secretion i.e. also by 22%. The thyroid then, will contribute with only 15% to total T_3 production. Correcting for this, a conversion ratio during caloric deprivation is calculated of 25% as compared to 31% in the control period. Admittedly these values are approximations but it seems unlikely that these differences in T_4--->T_3 conversion ratio's can fully explain a mean decrease of T_3 production during caloric deprivation of 42%. It is however quite possible that decreased 5'-deiodinase activity plays an additional role in the induction of the low T_3 syndrome during caloric deprivation.

Possibly a low T_3 syndrome can be induced by several different mechanisms which may depend on the cause, i.e. type of disease, diet, drugs etc. As outlined above, the low T_3 syndrome during fasting can be explained at least in part by 5'-deiodinase inhibition in the rat. Without having studied 5'-deiodinase activity we find evidence that inhibition of thyroxine transport into tissues may contribute to the low T_3 syndrome in humans. As to the cause of this transport inhibition only speculation is possible at this stage. Although diminished intracellular ATP is seen in perfused livers from fasted rats (31) it is not known whether caloric restriction in humans leads to a decrease of the intracellular energy charge which could result in decreased uptake of notably T_4. Another possibility arises from the notion that dietary manipulation may lead to attenuation of membrane fluidity causing a decrease in Na-K-ATPase activity of the hepatocyte (30). Since uptake of thyroid hormone is dependent on the sodium gradient over the plasma membrane (21), this mechanism may also be operative in our subjects. Finally the question is not solved to what extent the presence of a circulating inhibitor of hepatocyte iodothyronine uptake, which is found in severe non-thyroidal illness (28), plays a role in our findings.

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CHAPTER 3

THREE COMPARTMENTAL ANALYSIS OF THE EFFECTS OF d-PROPRANOLOL ON THYROID HORMONE KINETICS

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Abstract

Tracer thyroxine (T_4), 3,3',5-triiodothyronine (T_3) and 3,3',5'-triiodothyronine (rT_3) kinetic studies were performed in normal T_4 substituted subjects before and during oral d-propranolol treatment, to determine whether changes in thyroid hormone metabolism in a propranolol-induced low T_3 syndrome result from inhibition of 5'-deiodination or inhibition of transport of iodothyronines into tissues. Data were analyzed according to a three compartmental model of distribution and metabolism. T_4 plasma appearance rate decreased by 16% ($p < 0.01$), reflecting a decreased intestinal absorption of orally administered T_4 during propranolol. Serum T_4 and free T_4 levels increased significantly by 14%, while T_4 metabolic clearance rate (MCR) was lowered by 26% ($p < 0.001$). No changes were observed in size of the three T_4 compartments, or in the fractional and mass transfer rates of T_4 from plasma to the rapidly (REP) and slowly (SEP) equilibrating pools. Serum T_3, free T_3, T_3 plasma pool, T_3 mass transfer rate to REP and SEP and the T_3 pool masses were all significantly decreased during propranolol to a similar extent as the T_3 plasma production rate (PR). T_3 MCR decreased by 14% ($p < 0.05$). Serum total and free rT_3 increased, while the rT_3 MCR was substantially lowered during propranolol ($p < 0.001$). The rT_3 plasma pool, rT_3 REP and SEP, and the mass transfer rates to REP and SEP increased, while no alterations were observed in rT_3 PR and fractional transfer rates of rT_3 to REP and SEP.

It is concluded that the d-propranolol induced changes in thyroid hormone metabolism, resulting in a low T_3 syndrome, are due to inhibition of thyroid hormone deiodination. This is in contrast to the low T_3 syndrome during caloric deprivation, which results from inhibition of transport of iodothyronines into the liver.

Introduction

A variety of factors lead to a low T_3 syndrome in man, e.g. caloric deprivation (35), non-thyroidal illness (6) administration of dexamethasone (8), propyl-thiouracil (1,18), X-ray cholecystographic agents (7) and d,l-propranolol (37,39). In case of d,l-propranolol the induction of a low T_3 syndrome has been shown in normal persons, thyrotoxic and L-T_4 substituted, hypothyroid patients (14). In this syndrome serum T_3 is lowered and rT_3 increased, due to a decrease in both production rate (PR) of T_3 and metabolic clearance rate (MCR) of rT_3 (27,28). During d,l-propranolol these changes have been explained by the assumption of an inhibition of 5'-deiodinase in the liver by this compound. However, not in agreement with this seems the fact that the concentration of d,l-propranolol, which causes a 50 % inhibition of this enzyme (K_i) in vitro is about 0.5 mM (16), while therapeutic levels are much lower i.e. between 0.5 and 1 uM in serum (30). On the other hand transport of iodothyronines into rat hepatocytes in primary culture was shown to be inhibited by propranolol in a concentration as low as 1 uM, with a concomitant lowering of the intracellular ATP concentration (24). Furthermore, we could recently indicate, using tracer kinetic studies and analysis of the plasma disappearance curves with a three compartmental model of distribution and metabolism (11,12), an inhibition of mass transfer of T_4 into the rapidly equilibrating pool (REP) in obese man during caloric restriction. The REP is mainly composed of liver and kidney (11) and inhibition of T_4 transport into the REP leads to a decreased substrate availability for T_3 production as part of the underlying mechanism in the low T_3 syndrome in man during caloric deprivation (36).

Because inhibition of transport of iodothyronines apparently offers an alternative or additional mechanism in eliciting a low T_3 syndrome, tracer T_4, T_3 and rT_3 kinetic studies were performed to investigate whether the low T_3 syndrome induced by propranolol is caused by a mass transfer inhibition of T_4 and rT_3 into the REP (liver), and/or by inhibition of conversion of T_4 to T_3 and rT_3 to T_2 which reactions are both catalyzed by 5'-deiodinase. Studies were performed according to the three compartmental model of distribution and metabolism, as developed by DiStefano et al (11,12). We chose d-propranolol as experimental drug because of its efficacy in inducing a low T_3 syndrome in man (21) without having beta-blocking activity (31), which may complicate interpretation of the results.

Materials and Methods

Subjects

Six normal, healthy male students, age 22-24 years, participated in this study. All had normal thyroid function as measured by total serum T_4, T_3-resin uptake and TSH response to an intravenous bolus of TRH. The study and protocols

were approved by the Ethical Committee of the medical center and informed consent was obtained from all participants. None of these volunteers had used any medication within three months of the study. All subjects had normal weight. During the study, subjects were kept on a normal, weight maintaining diet. L-thyroxine substitution (200 ug a day) was started five weeks before the first tracer study and continued throughout the total experimental period. 5 Days after the end of the first measurement of thyroid hormone kinetics, an oral d-propranolol regimen of 80 mg t.i.d. was started and continued until the second thyroid hormone tracer kinetic experiments (which started when d-propranolol had been used for 14 days) were completed. Throughout the kinetic studies subjects were using Lugol's solution t.i.d. 10 drops, to prevent thyroidal uptake of radioactive iodide liberated after metabolism of the tracer.

Methods

^{125}I-T_4 and ^{131}I-T_3 were obtained and prepared, respectively, as described previously (36). ^{131}I-rT_3 was prepared in an essentially similar iodination procedure as described for ^{131}I-T_3, with 3,3'-T_2 as a substrate and using the chloramine-T method (20). 25 uCi ^{125}I-T_4 and 30 uCi ^{131}I-rT_3, were injected intravenously in 2 ml of 2% human serum albumin in phosphate buffered saline. Three days later 30 uCi ^{131}I-T_3 was given in a similar way. Blood samples were collected at 5, 10, 15, 30, 45 min, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14 and 16 hours for rT_3. For T_3 the same time schedule was used extended with 24, 32, 48, 56 and 72 hours. For T_4 the sampling schedule of T_3 was used during the first 3 days, followed by one sample/day during the following 6 days. Tracer T_4 was isolated from serum by TCA-precipitation, tracer T_3 by adsorption of the iodothyronines to Sephadex G-25 at high pH and subsequent elution of T_3 with specific T_3-antibodies (36). Tracer rT_3 was isolated from serum by the same procedure utilizing rabbit anti-rT_3 antiserum instead of T_3 antiserum. Recovery of added labeled rT_3 from serum samples was 96.9 ± 0.12 % (mean $\pm$ SD, $n = 6$). Further experimental conditions and procedures were essentially similar as have been described (36). Total and free hormone concentrations were measured in serum samples collected before, during and after the kinetic studies to ascertain that the kinetic studies were performed under steady state conditions. Free hormone concentrations were measured by equilibrium dialysis (36).

d-Propranolol tablets (40 mg) were obtained by a generous gift from ICI-Farma, Holland B.V. (Mrs. Dr. R. Koster).

Serum levels of propranolol were kindly determined by Mr. E. Vervloet, chemist, by high-performance liquid chromatography on a chiral phase column (38).

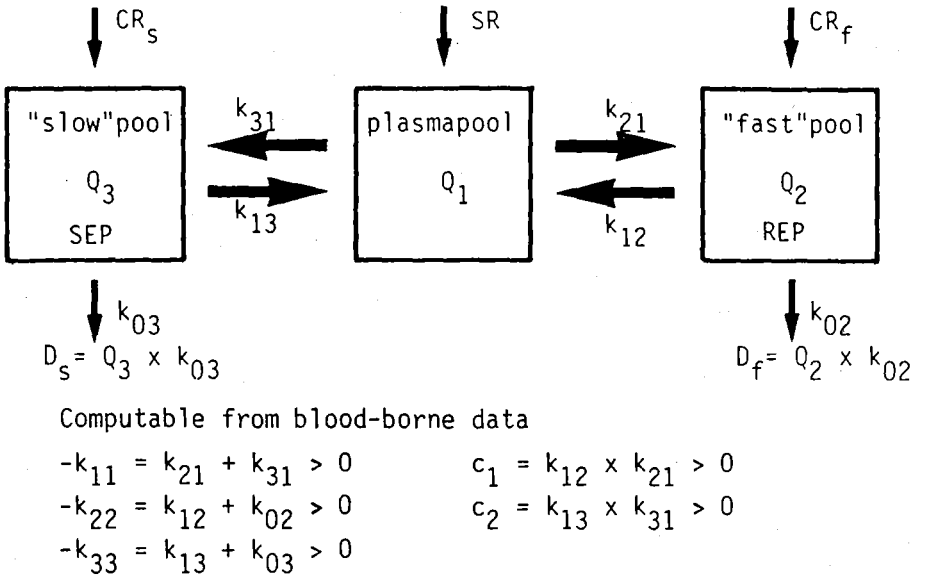

Fig. 1. Three pool model of iodothyronine distribution and metabolism. The K_{ij} values ($i \neq j$, i, j=1, 2, 3, all $K_{ij} > 0$) are fractional transport rates (hours^{-1}) from pool j to pool i. K_{11}, K_{22} and K_{33} are fractional turnover rates (hours^{-1} of pool Q_1, pool Q_2 and pool Q_3, respectively. The relations at the bottom are the combinations of these parameters computable from data obtained by blood sampling only. For a complete list of nomenclature see Glossary. For evaluation of the T_4-model only secretion (SR) of T_4 into the plasma pool should be considered, while for evaluation of the T_3-model also T_3-production by conversion both in the slowly equilibrating pool (CR_S) and in the rapidly equilibrating pool (CR_F) should be taken into account.

Calculations

Serum radioactivity of each iodothyronine was expressed as percentage dose per liter. For each iodothyronine the change of the activity as percentage dose per liter as a function of time could be described as the sum of three exponentials as

$$y(t) = \sum_{i=1}^{3} A_i e^{-\lambda_i t}$$

where t is time, A_i is the coefficient and λ_i is the constant of the exponent of the i-th exponential. A "peel-off" system of curve fitting was used, of which procedure a detailed outline can be found in the legend of figure 2. Together with the parameters estimated by linear regression on the straight parts of the curve (figure 2) the standard deviations of these parameters were calculated too. Finally it was determined whether the estimated parameters were signi-

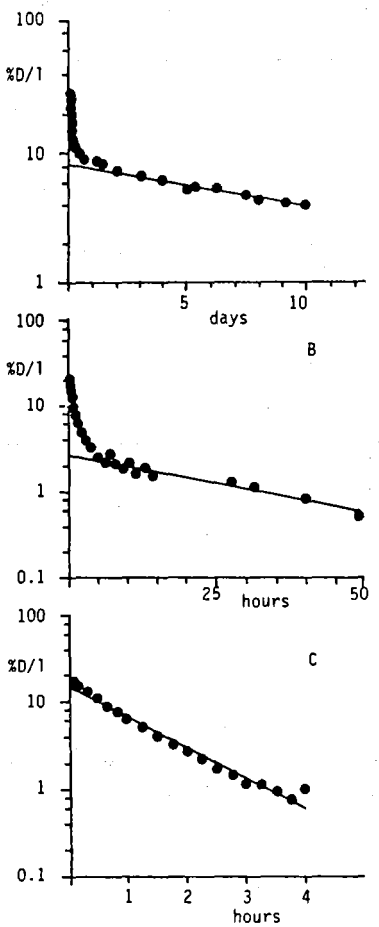

Fig. 2. Typical example of the curve fitting of a T_4-disappearance curve by a three exponent model. Panel A: plot of log y_t (in % Dose/l) against time, with the least squares regression line on the final straight part of the curve, giving estimates of coefficient A_3 7.87 (± .23 (SD)) %D/L and exponent λ_3 .0029 (± .0002) hour^{-1}. Panel B: plot of log (y(t)-$A_3e^{-\lambda_3 t}$) against time, with the least squares regression line on the straight part of the curve, giving estimates of coefficient A_2 2.68 (± .22) %D/L and exponent λ_2 0.031 (± .004) hour^{-1} Panel C: plot of log (y(t)-$A_2e^{-\lambda_2 t}$-$A_3e^{-\lambda_3 t}$) against time, giving estimates of coefficients A_1 14.88 (± 1.19) %D/L and exponent λ_1 0.80 (± 0.04) hour^{-1}. MCR=0.86 l/day; Vplasma=3.9 l; residual sum of squares = 39, total sum of squares = 7286.

ficantly different from zero. From the A_i and λ_i thus obtained, the parameters as depicted in Figure 1 of a three pool model of iodothyronine distribution and metabolism (11) could be calculated as has been outlined in detail elsewhere (36). Rearrangement of these terms gives the following formula for K_{21}

Table 1. Upper and lower values for K_{21} (fractional transfer rate from plasma to the REP).

Control period			
K_{21}	T_4 h^{-1}	T_3 h^{-1}	rT_3 h^{-1}
lowest value	0.51 (0.06)	1.34 (0.17)	0.81 (0.03)
highest value	0.52 (0.06)	1.53 (0.17)	2.59 (0.09)
difference	2.8 % (0.3)	14.0 % (1.5)	104.6 % (3.3)
During propranolol treatment			
K_{21}	T_4 h^{-1}	T_3 h^{-1}	rT_3 h^{-1}
lowest value	0.52 (0.08)	1.11 (0.26)	1.60 (0.28)
highest value	0.53 (0.09)	1.28 (0.25)	2.46 (0.30)
difference	2.0 % (0.3)	14.6 % (4.0)	43.1 % (9.4)

mean (SEM) (n=6)

$$-C_1/(K_{22}+K_{02})=K_{21}=C_2/(K_{33}+K_{03})-K_{11}$$

A minimal value of K_{21} can be found if $K_{02}=0$, i.e. no disposal in the rapidly equilibrating pool (REP). K_{21} is maximal if $K_{03}=0$, i.e. no disposal in the slowly equilibrating pool (SEP). If K_{21} is known, all other parameters can be calculated.

Results

Figure 2 shows a typical example of a three exponential curve fit of the plasma disappearance of T_4. As can be seen in the legend the fitted A_i and $_i$ (i=1,3) are highly significantly different from zero, indicating that a three exponential curve fit is valid. In this way the plasma disappearance curves of T_4, T_3 and rT_3 in all subjects before and during propranolol were best described as the sum of three exponentials, with all A_i and λ_i significantly ($p < 0.01$ or better) different from zero.

In table 1 and figure 3 the minimal and maximal values of K_{21} and K_{31} for the T_4, T_3 and rT_3 models are shown, both before and during propranolol treatment (table 1). The difference between the maximal and minimal value of K_{21} for T_4 and T_3, respectively, is small. Therefore the mean values of K_{21} were used to calculate all other parameters. This maneuver will divide the disposal of T_3 and T_4 equally between the REP and the SEP, although no

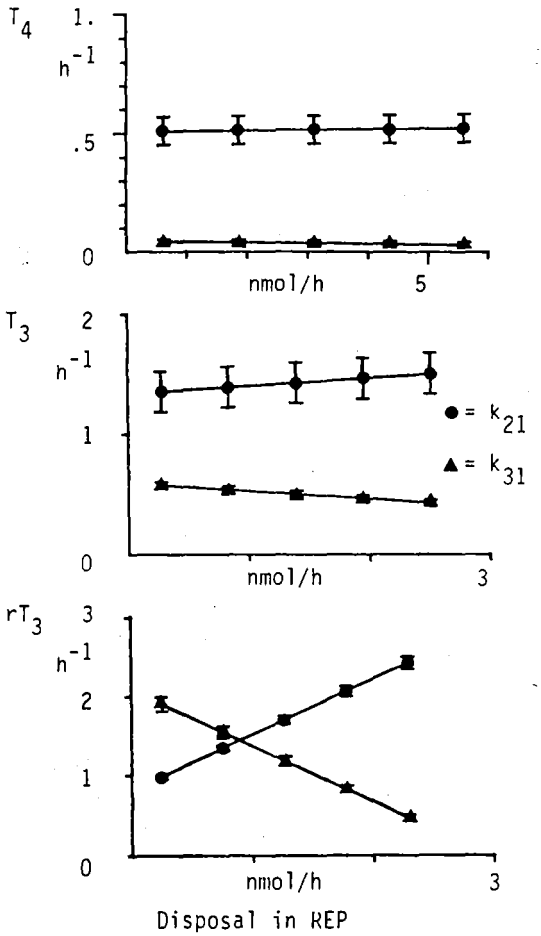

Fig. 3. Relationship of the fractional transfer rate constant from plasma to the rapidly equilibrating pool (K_{21}) and to the slowly equilibrating pool (K_{31}) on the disposal rate from the rapidly equilibrating pool, for T_4, T_3 and rT_3. As the fractional disposals from the rapidly and slowly equilibrating pools are inversely related (the sum of both being 1), the relationship between K_{21} or K_{31} and the disposal from the slowly equilibrating pool will be inverse.

data exist that this is actually the case in vivo. There is a great difference, however, between the minimal and maximal value of K_{21} for rT_3 and therefore the mean value can not be used for calculation of kinetics. Fortunately, however, more data are available for rT_3 as to the site where the disposal actually takes place. rT_3 clearance is markedly reduced in liver cirrhosis (10) and in subjects treated with PTU (32) which inhibits type I 5'-deiodinase which is mainly located in the liver (26). Recently it has been found (3) by direct blood sampling in the hepatic vein and artery in humans that the clearance of rT_3 by the liver

is equal to the total body removal of this hormone. Because of these notions and the fact that the liver is part of the REP (11), we located the disposal of rT_3 in the REP and therefore used the highest value for K_{21} to calculate all other parameters for rT_3.

A summary of the results thus obtained can be found in table 2. T_4 plasma appearance (production) rate decreased by 16% during propranolol as compared to control. The total serum T_4 concentration increased by 14%, with a similar increase of the free hormone level. T_4-MCR decreased by 26%. No changes were observed in size and pool of the three T_4 compartments, except a 23 % increase of the REP. No alterations in fractional transfer rate of T_4 to the REP or SEP could be detected.

In all subjects a low T_3 syndrome was induced by administration of d-propranolol. Total serum T_3 levels decreased by 29%, while those of total rT_3 rose by 158%. T_3 production rate and MCR decreased by 38% and 14% respectively. Serum total and free T_3, the three T_3 pools, mass transfer rates to the REP and the SEP were all decreased to a similar extent as the production rate.

Serum total and free rT_3 and plasma pool were elevated, MCR was much lower, while the production rate did not change. Propranolol did not induce alterations in sizes (in liters) and fractional transfer rates of rT_3 with regard to the REP and the SEP. Pools and mass transfer rates increased to a similar extent as the plasma rT_3 concentration.

Table 3 shows the model values which will change when the T_4 or the T_3 models are recalculated with the disposal located in the SEP. In table 4 the same values are summarized, after recalculation of the models with the disposal located in the REP. From comparison of table 3 and 4 with table 2 it can be concluded that the findings summarized in the previous paragraphs are in no way dependent on the site were the disposal actually takes place.

Propranolol levels

Serum d-propranolol levels ranged from 0.14 uM to 0.70 uM. No laevo- isomer could be detected in the serum of the participating subjects. During the control period and d-propranolol administration, blood pressure and heart rate were frequently measured. No differences were observed between these two periods. Electrocardiograms obtained in both periods were similar. No side effects were reported during the 23 days of oral administration of 3 times 80 mg daily of d-propranolol. No significant change in body weight was observed during the total study period.

Table 2. T_4, T_3 and rT_3 kinetics before and during propranolol treatment in six T_4 substituted normals.

	Mean (SEM)	CONTROL						d-PROPRANOLOL					
		T_4		T_3		rT_3		T_4		T_3		rT_3	
Production rate	nmol/d	149.5	(8.14)	66.8	(3.6)	67.0	(7.0)	126	(8.0)[c]	41.2	(3.5)[b]	69.4	(3.3)
MCR	l/d	1.53	(0.08)	37.2	(1.7)	203.6	(5.6)	1.13	(0.09)[a]	32.1	(1.6)[e]	84.0	(6.7)[a]
Plasma compartment													
free hormone conc.	pmol/l	25.2	(0.8)	5.3	(0.1)	0.29	(0.01)	28.6	(0.9)[c]	3.9	(0.06)[a]	0.77	(0.02)[a]
total hormone conc.	nmol/l	99.3	(7.7)	1.8	(0.07)	0.33	(0.03)	113	(7.1)[b]	1.28	(0.07)[a]	0.85	(0.07)[a]
size	l	4.7	(0.24)	8.2	(0.34)	4.3	(0.25)	4.5	(0.21)	8.0	(0.40)	4.1	(0.10)
pool	nmol	463	(41.6)	14.7	(0.8)	1.43	(0.18)	510	(38.3)	10.2	(0.5)[b]	3.4	(0.28)[a]
Rapidly equilibrating compartment													
size	l	6.8	(0.71)	4.7	(0.4)	12.6	(2.3)	7.3	(0.66)	4.7	(0.3)	14.6	(1.1)
pool	nmol	661	(69.3)	8.3	(0.6)	4.4	(1.11)	814	(69.7)[d]	6.0	(0.3)[d]	12.2	(1.1)[b]
mass transfer rate from plasma	nmol/h	229	(19.3)	20.7	(1.8)	3.9	(0.44)	266	(16.0)	12.2	(1.3)[a]	8.4	(0.7)[a]
fractional transfer rate from plasma	h^{-1}	0.51	(0.06)	1.43	(0.17)	2.8	(0.31)	0.53	(0.04)	1.19	(0.10)[e]	2.5	(0.1)
Slowly equilibrating compartment													
size	l	3.5	(0.16)	43.1	(5.0)	25.2	(3.2)	2.6	(0.37)	42.5	(5.0)	24.4	(1.5)
pool	nmol	341	(26.8)	76.7	(7.6)	8.4	(1.4)	302	(53.7)	53.7	(5.9)[b]	20.6	(1.8)[b]
mass transfer rate from plasma	nmol/h	15.8	(3.4)	7.5	(0.5)	0.44	(0.10)	7.5	(0.95)	5.0	(0.43)[a]	1.28	(0.19)[d]
fractional transfer rate from plasma	h^{-1}	0.037	(0.01)	0.51	(0.03)	0.35	(0.10)	0.015	(0.002)	0.49	(0.02)	0.37	(0.03)

[a]: $p<0.001$; [b]: $p<0.005$; [c]: $p<0.01$; [d]: $p<0.025$; [e]: $p<0.05$ (paired t-test; n=6)

Table 3. T_4 and T_3 kinetics before and during propranolol treatment in six T_4 substituted normals. Values were calculated with K_{21} minimal, i.e. with the disposal located in the slowly equilibrating pool.

	Mean (SEM)	CONTROL				d-PROPRANOLOL			
		T_4		T_3		T_4		T_3	
Rapidly equilibrating compartment									
size	l	6.7	(0.71)	4.4	(0.4)	7.3	(0.65)	4.4	(0.3)
pool	nmol	654	(68.6)	7.8	(0.6)	808	(69.2)[d]	5.6	(0.3)[d]
mass transfer rate from plasma	nmol/h	227	(19.2)	19.5	(1.8)	264	(15.9)	11.5	(1.3)[a]
fractional transfer rate from plasma	h^{-1}	0.51	(0.06)	1.36	(0.17)	0.53	(0.03)	1.13	(0.10)[e]
Slowly equilibrating compartment									
size	l	4.1	(0.19)	49.6	(5.7)	3.3	(0.45)	48.3	(5.3)
pool	nmol	408	(29.0)	88.2	(8.8)	387	(64.5)	61.1	(6.2)[b]
mass transfer rate from plasma	nmol/h	18.3	(3.3)	8.6	(0.5)	9.6	(1.00)	5.7	(0.46)[a]
fractional transfer rate from plasma	h^{-1}	0.042	(0.01)	0.58	(0.03)	0.019	(0.002)	0.55	(0.02)

[a]: $p<0.001$; [b]: $p<0.005$; [c]: $p<0.01$; [d]: $p<0.025$; [e]: $p<0.05$
(paired t-test; n=6)

Table 4. T_4 and T_3 kinetics before and during propranolol treatment in six T_4 substituted normals. Values were calculated with the K_{21} maximal, i.e. with the disposal located in the rapidly equilibrating pool.

	Mean (SEM)	CONTROL				d-PROPRANOLOL			
		T_4		T_3		T_4		T_3	
Rapidly equilibrating compartment									
size	l	6.8	(0.71)	4.9	(0.4)	7.4	(0.66)	5.0	(0.3)
pool	nmol	669	(70.0)	8.8	(0.6)	821	(70.2)[d]	6.3	(0.3)[d]
mass transfer rate from plasma	nmol/h	232	(19.3)	21.8	(1.8)	269	(16.1)	12.9	(1.3)[a]
fractional transfer rate from plasma	h^{-1}	0.52	(0.06)	1.51	(0.17)	0.53	(0.03)	1.26	(0.10)[e]
Slowly equilibrating compartment									
size	l	2.8	(0.24)	36.6	(4.3)	1.7	(0.31)	36.8	(4.8)
pool	nmol	276	(29.6)	65.2	(6.6)	218	(44.7)	46.4	(5.6)[b]
mass transfer rate from plasma	nmol/h	13.3	(3.4)	6.4	(0.4)	5.4	(0.92)	4.3	(0.40)[b]
fractional transfer rate from plasma	h^{-1}	0.031	(0.01)	0.43	(0.03)	0.011	(0.002)	0.42	(0.02)

[a]: $p<0.001$; [b]: $p<0.005$; [c]: $p<0.01$; [d]: $p<0.025$; [e]: $p<0.05$
(paired t-test; $n=6$)

Discussion

During propranolol treatment T_4 plasma appearance rate decreased by 16%. Lumholtz et al (27,28) already suggested that administration of d,l-propranolol, reduces intestinal absorption of L-thyroxine in substituted hypothyroid patients. However, in our subjects with intact thyroid this conclusion is less secure, since there is a possibility that propranolol could have an effect on thyroidal secretion of T_4 not suppressed by exogenous T_4. This effect cannot be mediated by beta-blocking activity because d-propranolol which was used in this study is devoid of this activity. Lumholtz et al (27,28), however, did not observe a rise in serum T_4 concentration, in contrast to the 14% rise in both total T_4 and free T_4 serum concentrations occurring in our patients. Others (39) also noticed a rise in total serum T_4 during propranolol treatment. In concert with Lumholtz et al (27), T_4 MCR was reduced during propranolol. A reduction of 26% in the T_4 MCR only partially counter-effected by a 16% decrease in plasma production could explain an increase in both total T_4 and free T_4 serum levels as observed in this study. A decrease in production rate of T_3 and metabolic clearance rate of rT_3 can (see also introduction) result from inhibition of 5'-deiodinase and/or transmembraneous transport of iodothyronines (i.e. T_4 and rT_3) into the liver. Current thinking places the liver in a dominant role in both T_3 production and rT_3 degradation. This notion results from the observation that inhibition of type I 5'-deiodinase (which is quantitatively most abundant in the liver (26)) by propylthiouracil, induces a 50-75% decrease in serum T_3 concentration in thyroidectomized L-thyroxine substituted rats (4,33). In addition, the net removal of rT_3 by the human liver equals the total body removal (3). From these studies it was also concluded that the liver probably does not contribute to plasma rT_3. This conclusion is substantiated by the fact that rT_3 could not be detected in the perfusate of T_4 perfused rat livers (23), or in incubation media of rat liver slices (2) and cultured rat hepatocytes (29) incubated with T_4. Further support is obtained by the finding of a similar production rate of rT_3 in propylthiouracil and methimazole (which does not inhibit type I deiodinase) treated hyperthyroid patients (25). The observation of an unchanged (or even increased) pool of T_4 and rT_3 in the REP (which contains liver and kidney (11,12)) indicates that limited substrate availability to 5'- deiodinase can not explain the diminished T_3 production and rT_3 MCR reported in this study. Our findings only support an inhibition of deiodination by propranolol or a metabolite thereof. Because a considerable amount of T_3 is metabolized outside the liver and, as mentioned above, the liver does not contribute to plasma rT_3, no conclusion can be drawn with regard to a possible effect of D- propranolol on liver 5-deiodination, while there seems to be no effect on 5-deiodinase in tissues outside the liver, because rT_3 production and T_3 MCR are unaltered (table 2) during propranolol treatment (and both rT_3 production and T_3 deiodination are 5-deiodination reactions).

As can be calculated from table 2, no change was found in the fraction

of T_4 disposal which was degraded via the deiodinative route (T_4 into T_3 and rT_3 before and during propranolol, 89% and 88% respectively). Therefore, a d-propranolol-induced inhibition of T_4 to T_3 conversion does not result in an increased T_4 degradation through non-deiodinative pathways as has been found during d,l-propranolol administration (9,28). In our study however, it can be calculated from table 2 that d-propranolol induced a shift in T_4 conversion away from T_3 and towards rT_3. Thus, before propranolol T_4 was equally converted i.e. 45% both to T_3 and rT_3. During propranolol the percentages were 33% and 55% respectively. By comparison of the data shown in table 3 and 4 with those presented in table 2 it can be concluded that the site where the disposal actually takes place is irrelevant. Whether the data of table 4 (disposal from the REP), table 2 (disposal equally divided between REP and SEP) or table 3 (disposal from the SEP) are used, the same conclusions have to be drawn with respect to the changes in metabolism and distribution of T_4 and T_3 induced by propranolol. Shifting the disposal from the REP to the SEP has only a minimal effect on the data concerning the REP, while the influence on the absolute value of the data of the SEP is moderate (about 30 % change), however the relative changes of the data before and during propranolol are not affected in any way.

We found a mean plasma compartment size of 4.7 l for T_4, 4.3 l for rT_3, which values are comparable, and 8.2 l for T_3, which value is much larger (table 2). On first thought one would expect similar values for the three hormones, however beside plasma this compartment also contains blood cells, and we have shown (13) that erythrocytes contain a carrier mediated uptake system for at least T_3. Therefore we have to take into account uptake of hormones by these blood cells. Consequently the measured volume of the accessible (plasma) compartment is the sum of the plasma volume and the plasma equivalent volume of the hormone bound by the blood cells. Even if there exists a comparable uptake mechanism for T_4 and rT_3 as for T_3, uptake of T_3 into for instance erythrocytes will be more pronounced than uptake of the other hormones, because the free fraction of T_3 in plasma is about ten times larger than that of T_4 or rT_3. This will lead to a relative higher proportion of T_3 bound to erythrocytes, and thus to a larger plasma volume measured.

The question remains as to how propranolol induces its effects on liver 5'-deiodinase. In vitro experiments revealed a K_i of d,l- propranolol on this enzyme of about 0.5 mM (16), which is three orders of magnitude higher than therapeutic levels in man (30). In vivo experiments show that dog liver (19) and rat liver (5,34) in contrast to some other tissues, do not accumulate propranolol relative to plasma. Actually, rat liver showed the lowest tissue concentration of propranolol after intravenous injection of the following tissues tested: adipose tissue, lung, brain, heart and muscle (5). Determination of the subcellular distribution of propranolol-binding in liver, revealed that mitochondrial and microsomal fractions contained the highest concentration of propranolol, both after administration of the drug in vivo and after incubation with homogenates.

5'-Deiodinase activity is localized in the microsomal fraction (15). No data, however, exist as to biopsy measured hepatic accumulation of propranolol in human liver. 4-OH-propranolol, a metabolite with equipotent beta blocking activity in comparison to the parent compound, has been shown to markedly inhibit 5'-deiodinase activity, at concentrations between 34-80 uM (22). However only 10 % of administered propranolol is converted to 4-OH-propranolol and therefore plasma concentrations are below 50 nM (22), which makes this compound an unlikely candidate for the inhibition of the enzyme. Furthermore, in rats hepatic accumulation (50 times) of an (undefined i.e. not being 4-OH-propranolol) metabolite of propranolol relative to plasma was demonstrated after chronic oral and subcutaneous administration of propranolol, in contrast to the parent compound (17). These data point to the possibility of a metabolite rather than propranolol itself affecting 5'-deiodinase. Of course another explanation for the disparity between the K_i of propranolol for inhibition of 5'-deiodinase and therapeutical serum levels in man may be that the estimated K_i is not the true value of the pure enzyme or the enzyme as it operates in vivo.

In summary, from our here reported kinetic data, it can be concluded that the d-propranolol-induced low T_3 syndrome results from inhibition of thyroid hormone deiodination. This is in contrast to the low T_3 syndrome caused by caloric deprivation, in which we recently (36) could indicate inhibition of transmembraneous transport of iodothyronines as the principal underlying mechanism. It seems therefore possible that a low T_3 syndrome may result from different mechanisms.

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CHAPTER 4

EARLY SERUM DISAPPEARANCE AND COMPUTED LIVER UPTAKE OF THYROXINE IN HUMANS DURING CALORIC DEPRIVATION, D-PROPRANOLOL ADMINISTRATION AND AFTER FRUCTOSE

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Abstract

Inhibition of transmembraneous transport of iodothyronines has been proposed as the underlying mechanism in inducing the low T_3 syndrome in man during caloric restriction. Propranolol, however, elicits a low T_3 syndrome in man by inhibition of thyroid hormone deiodination. Transport of thyroid hormones into cells has, in vitro, been shown to be ATP dependent. We studied T_4 serum tracer disappearance curves in a group of 4 obese subjects before and during caloric restriction and after intravenous fructose loading. Similar kinetic studies were also performed before and during an oral regimen of 3 times 80 mg D- propranolol in 6 T_4-treated normal subjects. In addition, computed T_4 hepatic uptake was determined during these conditions in both groups.

Intravenous fructose administration induced a rise in serum lactic acid and uric acid, indicating a decrease in liver ATP. This was observed in concomitance with a decreased serum tracer T_4 disappearance and computed T_4 uptake in the liver. These results suggest ATP-dependency of transport of iodothyronines into the liver in vivo. Caloric restriction also resulted in a slowing down of the T_4 serum disappearance and a decrease of liver uptake of T_4, pointing to an inhibition of transmembraneous transport of iodothyronines in this condition as well. In contrast, D-propranolol administration did not change T_4 serum disappearance and computed T_4 hepatic uptake. These latter findings are in agreement with previous observations that D- propranolol induces a low T_3 syndrome by inhibition of deiodination of iodothyronines. It is therefore concluded that the low T_3 syndrome in man can result from, at least two different mechanisms i.e. either by decreased transport of T_4 into T_3 generating (e.g. liver) tissues or by attenuated deiodination of T_4 into T_3.

Introduction

The low T_3 syndrome is characterised by a lowered serum T_3 level in concomitance with an increased serum rT_3 level, due to a decrease in T_3 production rate and rT_3 metabolic clearance rate, respectively. A variety of factors lead to a low T_3 syndrome: caloric deprivation (1,2), non-thyroidal illness (3), administration of propranolol (4), dexamethasone (5,6), propylthiouracil (PTU) (7,8) and X-ray cholecystographic agents (9,10,11). Studies in rat liver homogenates from starved rats (12) or from rats treated with PTU (13), dexamethasone (14) or propranolol (15) revealed a decreased 5'- deiodinase activity. It was therefore suggested that inhibition of this deiodinative step was responsible for the observed changes in serum iodothyronines as seen in the low T_3-syndrome. An alternative explanation, however, was given by Krenning et al (16), who showed in rat hepatocytes in primary culture that iodothyronines were taken up by an active, ATP dependent, transport mechanism, by which T_4 and rT_3 enter the cell via a common pathway that is different from that of T_3. Inhibition of notably the T_4-rT_3 transport pathway to tissues would result in an increased serum rT_3 concentration and decreased serum T_3 due to reduced intracellular T_4 availability for conversion to T_3.

The liver plays a dominant role in T_3 production and rT_3 metabolic clearance (see discussion section). To study mechanisms further that are operative in the observed changes in T_3 and rT_3 kinetics in the low T_3 syndrome, early T_4 tracer disappearance from serum (representing transport into the liver; see discussion) was examined in obese humans in three conditions: during a weight maintaining diet and during fasting and after an intravenous bolus of fructose which induces a prompt and significant fall in liver ATP (17,18). Furthermore, T_4-tracer disappearance was measured in a group of T_4 substituted normal subjects before and during administration of D-propranolol. In addition uptake in the liver was computed, using the parameters found with a three compartmental model of thyroid hormone distribution and metabolism (19,23).

Glossary

$(i = 1,2,3; j = 1,2,3; i \neq j)$

t	Time (min)
$y(t)$	Plasma activity at time t (% dose/L)
A_i	Coefficient of the i^{th} exponential component (% dose)
λ_i	Exponent of the i^{th} exponential component (min^{-1})
Q_1	Size of pool 1 (plasma pool; % dose)
Q_2	Size of pool 2 (fast or rapidly equilibrating pool; % dose)
Q_3	Size of pool 3 (slow or slowly equilibrating pool; % dose)
k_{ij}	Fractional transport rate from pool j to pool i (min^{-1})
k_{0i}	Fractional disposal rate in pool i (min^{-1})
V_p	Plasma volume (L)

Subjects and methods

Subjects

Four (three women), otherwise healthy, obese subjects, age 27-36 years (mean 30.8 years) participated in this study. All had normal thyroid function as measured by serum T_4, T_3-resin uptake and thyrotropin response to an intravenous bolus of thyrotropin releasing hormone. None of the persons used or had recently (within three months) used any medication. At the start of the study, body weight ranged from 96-117 kg(mean 108.25 kg). All subjects had at least 30% overweight according to the monograph method for assessing body weight as described by Thomas et al (20), range 32-91% (mean 61.5%). Serum T_4 tracer studies were performed three times. First during a control period, while patients were kept on a normal weight-maintaining diet. The study was then repeated after one month. The only difference with the control study was an intravenous fructose loading of 0.5 g/kg(administered in 20 minutes as 20% (w/v) solution) immediately in advance of the injection of the tracer. In the control and fasting (see below) period an equal volume of saline was similarly given. One month after the second study, the third study was done when the subjects were on a 240 kcal Modifast^R diet for seven days. This diet contains: 33 g protein, 25.5 g carbohydrate and 0.7 g fat and adequate supplies of water, minerals and vitamines.

We also analyzed early serum T_4 tracer kinetics performed in six normal males, age 22-24 years, before (control) and during the use of D-propranolol 80 mg t.i.d. . These studies were primarily intended to elucidate the underlying mechanism by which D-propranolol induces a low T_3 syndrome. The results of all other kinetic data in these subjects have been reported elsewhere (21). These six persons were euthyroid and had normal thyroid function as defined above. They were put on l-thyroxine treatment (200 ug daily) 5 weeks before the control tracer study and T_4 treatment was continued until the end of the two kinetic studies. D-propranolol was started 14 days before the second study. T_4 substitution was given to suppress thyroidal T_3 secretion rendering calculation of T_4 into T_3 production easier (see 21). The studies and protocols were approved by the Ethical Committee of the medical centre and informed consent was obtained from all participants.

Materials, preparations and calculations

^{131}I-T_4 was prepared by iodination of 3,5,3'-T_3 with ^{131}I by the chloramine-T method (22). Iodination was performed by mixing 10 ul volumes of Na ^{131}I, 0.5 M sodium phosphate buffer pH 7.5, 3,5,3'-T_3 (1 ug/ul in 0.05 M sodium phosphate buffer pH 7.5) and chloramine-T (1ug/ul in 0.05 M sodium phosphate buffer pH 7.5), in that order. The reaction was terminated after one minute by adding 100 ml sodium bisulfite (1 mg/ml in 0.05 sodium phosphate buffer

pH 7.5). The reaction mixture was applied on a small (10 x 0.5 cm) Sephadex LH-20 column and eluted with 30% ethanol in 0.05 M sodium carbonate pH 11.5 (v/v). T_4 fractions were pooled and evaporated to dryness under a stream of nitrogen. Fifty microcurie ^{131}I-L-thyroxine in 1,5 ml of 2% human albumin in phosphate buffered saline, were administered to the patients via an intravenously inserted canula in the arm. Through a catheter in the contralateral arm, blood samples were drawn at regular intervals for determination of radioactivity.

Na^{131}I for labeling of 3,5,3'-T_3 was purchased from The Radiochemical Centre, Amersham, U.K.. All other reagents used were of the highest purity available.

Percent tracer dose at the different time intervals and serum levels of T_4, free T_4 (dialysis), T_3 and rT_3 were determined as outlined previously (23). D-propranolol tablets (40 mg) were obtained by a generous gift from ICI-Farma, Holland B.V..

Uptake of tracer T_4 into the rapidly equilibrating compartment (REP) (see discussion) was calculated using a three compartmental model of thyroid hormone distribution and metabolism (19), applied by us in humans as reported in an other study (23). During the first six hours after tracer injection integrated influx of tracer T_4 into REP minus efflux from REP was computed representing net uptake in this compartment (see appendix).

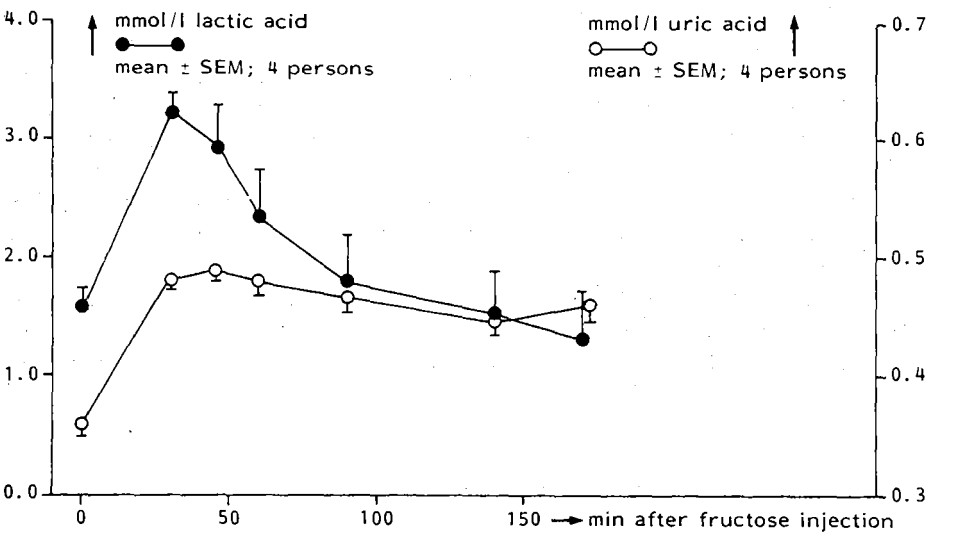

Fig. 1. Serum lactic acid and uric acid levels after i.v. fructose in 4 obese subjects.

Results

In the control and fructose periods, during which the obese subjects were kept on a weight-maintaining diet, no change in body weight occured. Serum levels of T_4, T_3 and rT_3 were normal at the time of kinetic studies in both periods. During the period of caloric restriction a mean reduction in body weight of 6.23% (± 0.61 SD) was observed. Although serum T_4 remained normal (mean ± SD) 86.0 ± 4.83 nmol/l, serum T_3 was below normal due to caloric restriction: 0.74 ± 0.05 (normal range 1.1 – 3.1 nmol/l) and rT_3 was elevated: 0.56 ± 0.045 (normal range 0.12 – 0.45 nmol/l).

Figure 1 shows mean serum levels of uric acid and lactic acid after intravenous fructose administration. A clear rise of both was achieved.

Figure 2 gives the mean decline in serum tracer T_4 expressed as percentage of the dose injected until six hours after injection. It can clearly be seen that

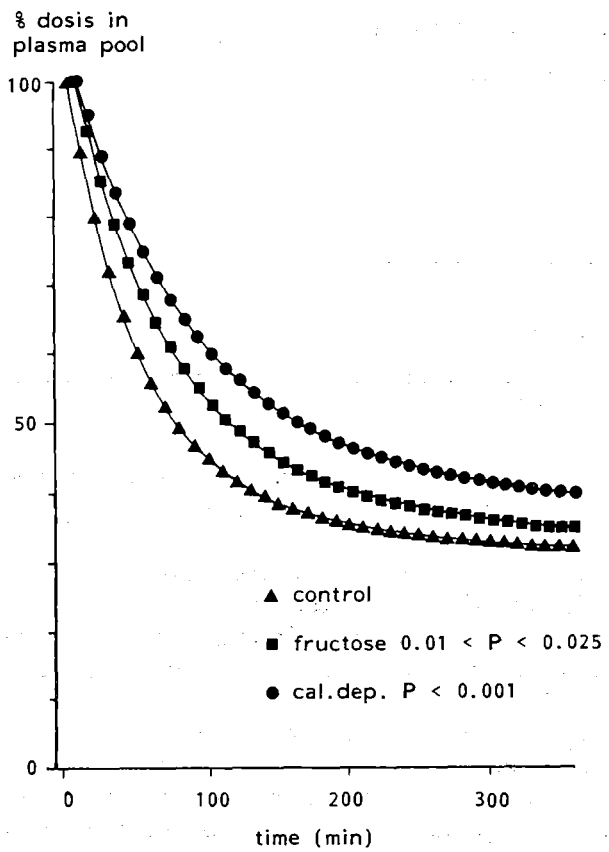

Fig. 2. Early serum tracer T_4 disappearance before and during caloric deprivation (cal. dep.) and after i.v. fructose in 4 obese subjects.

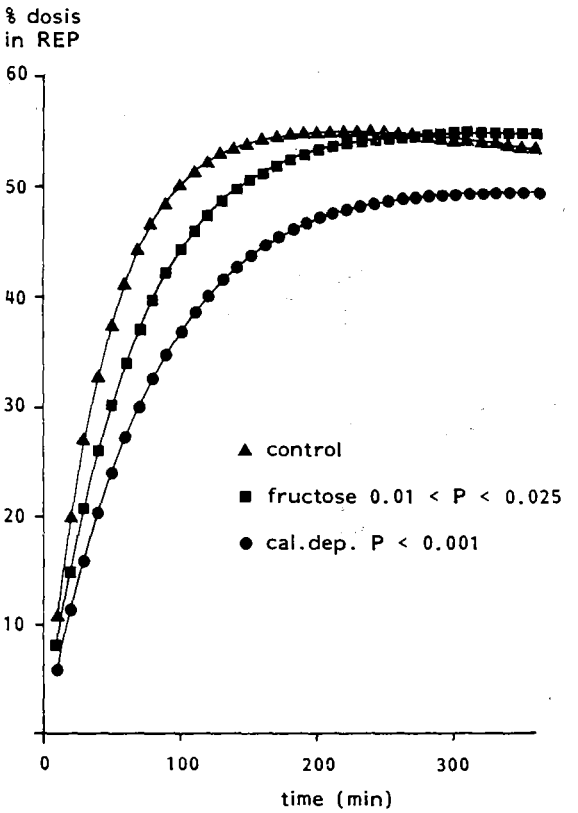

Fig. 3. Computed tracer T_4 uptake in REP before and during caloric deprivation (cal. dep.) and after i.v. fructose in 4 obese subjects.

T_4 tracer disappears more slowly from the plasma compartment during caloric deprivation and after fructose administration (paired-t-test, $p<0.001$ and $p<0.025$ respectively) as compared to control.

Figure 3 summarizes the computed uptake of T_4 in the rapid equilibrating pool, mainly composed of the liver (23), during caloric restriction and after fructose. In both conditions a significant decrease in initial hepatic uptake was found as compared to control (paired t-test $p<0.025$ for fructose and $p<0.001$ for caloric deprivation).

In figures 4 and 5, T_4 serum disappearance curves and computed T_4 hepatic uptake curves respectively in the normal T_4 treated males during the control and D-propranolol treatment are shown. A paired-t- test showed a significant increased hepatic uptake during propranolol while serum disappearance rates were not different.

All six participants developed a low T_3 syndrome during D-propranolol; serum T_3 1.28 $\pm$ 0.07 nmol/l vs control 1.8 $\pm$ 0.07 nmol/l ($p<0.001$) and

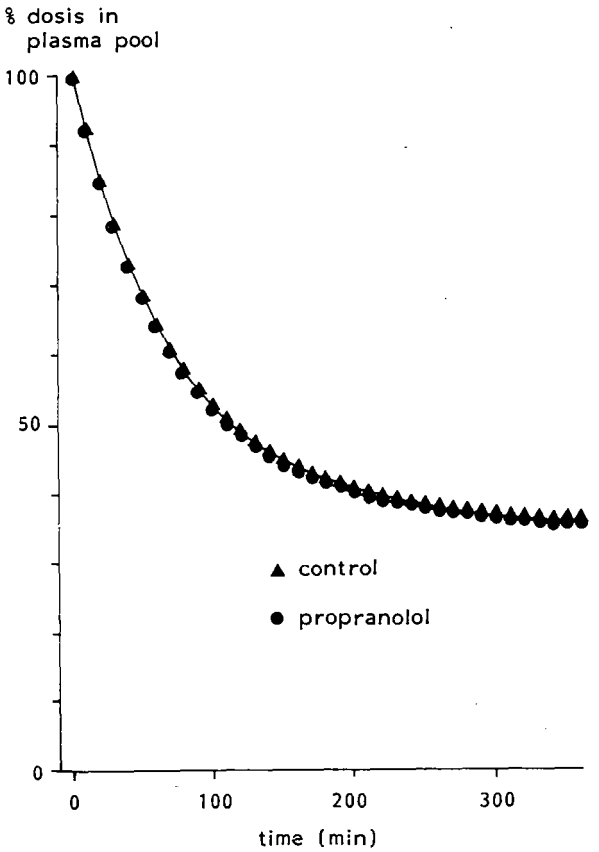

Fig. 4. Early serum tracer T_4 disappearance before and during propranolol administration in 6 normals treated with T_4.

serum rT_3 0.85 ± 0.07 nmol/l vs 0.33 ± 0.03 nmol/l) ($p<0.001$). The serum free T_4 concentration was significantly increased; 28.6 ± 0.9 pmol/l vs control 25.2 ± 0.8 pmol/l ($p<0.01$).

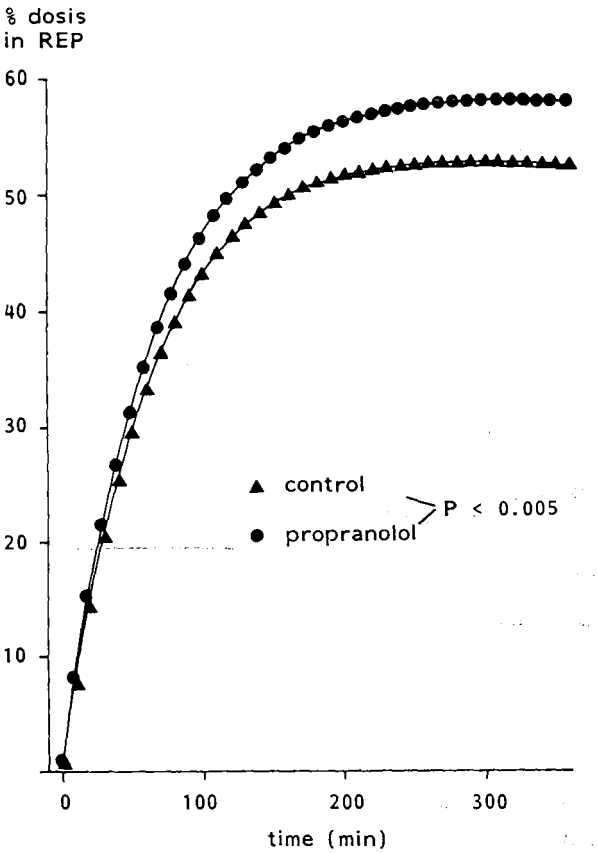

Fig. 5. Computed tracer T_4 uptake in REP before and during propranolol administration in 6 normals treated with T_4.

Discussion

T_4-tracer serum disappearance curves of each subject in each condition could be fitted to the sum of 3 exponentials, indicating a three compartmental distribution of thyroxine, i.e. a plasma pool, a rapidly equilibrating pool (REP) and a slowly equilibrating pool (SEP) (19).

Several studies (24,25,26) show time differences between various tissues for equilibration with serum radioactive thyroid hormone. Sheep, closely resembling man with respect to thyroid hormone metabolism and serum thyroid hormone binding proteins, show equilibrium of liver, kidney and lung with serum tracer thyroid hormone within 1-2 hours after injection while in intestine, heart and salivary glands equilibrium was reached after 4 hours. Brain, skin and muscle equilibrated after 32-60 hours. In man a marked difference between liver and

forearm was found (24). Several studies (27,28) showed equilibrium of radioactivity above the liver with serum T_4 tracer after 2-4 hours. Although including kidneys and lungs, the REP is predominantly constituted by the liver (23,26,29) which contains about 1/3 of the total body T_4 pool (30). Current thinking places the liver in a dominant role in both T_3 production and rT_3 degradation (23,31). These processes are greatly influenced if passage of thyroid hormones into tissues is suppressed by inhibition of transport over the cell membrane (16). Inhibition of the active transport system of T_4 and rT_3 into the liver results in changes in serum thyroid hormones as seen in the low T_3 syndrome (23). Because equilibrium of tracer T_4 between plasma and liver compartment will be reached between 2-4 hours (27,28) and since the liver is regarded as a most important organ with respect to T_3 production from T_4, we examined the T_4 serum tracer disappearance curve during the first 6 hours after injection.

From figure 1 it can be seen that intravenous fructose administration induces a prompt rise in lactic and uric acid, reflecting a marked decrease in liver ATP concentration (17,18). No changes were observed in serum total and free T_4 (not depicted). Figure 2 gives the mean serum T_4 tracer disappearance curve after fructose injection. The tracer disappears more slowly than in the control period (paired-t- test $p<0.01$), indicating an inhibition of egress from the plasma compartment. Our data suggest that this inhibition of egress from plasma results from a decreased uptake of T_4 by the liver due to ATP depletion as has been shown in rat hepatocytes in primary culture (16).

Caloric restriction resulted in an even more pronounced inhibition of T_4-tracer egress from plasma. This phenomenon was observed, despite a markedly increased free hormone level, due to a rise in the free fraction. It is, however, not known if caloric restriction induces a decrease in cellular ATP concentration in man. In 48 hour fasted rats we found a decreased liver ATP when chemically measured (van der Heyden et al, to be published), as others found when determined by ^{31}P-NMR spectroscopy (32). It has also been suggested (33) that microheterogeneity of ATP distribution can occur during fasting in cells without membrane compartmentation due to nonuniform distribution of ATP-generating and ATP consuming systems. This phenomenon may lead to diminished ATP concentration in the area of the plasma membrane, which decrease may not always be apparent when total cellular ATP is being considered. An other factor for inhibition of transmembraneous transport of iodothyronines has recently been shown by Krenning et al (34). From the work of Chopra et al (35,36) it became evident that sera of patients with non-thyroidal illness and low serum T_4 may contain an inhibitor of thyroid hormone binding to serum proteins. Krenning et al (34) showed that sera of patients with non-thyroidal illness and a low T_4 state, inhibit thyroid hormone uptake by hepatocytes in primary culture without a direct effect on deiodination. Although the exact nature of the inhibitor is not known, many arguments are in favour that it might belong to the group of fatty acids, which are known to increase during fasting. Fatty acids modulate the fluidity and negative surface charges

of cell membranes (37,38) and it is conceivable that transmembraneous transport of iodothyronines is inhibited in this way.

We could recently indicate transport inhibition of iodothyronines as a major underlying cause for inducing the changes in serum thyroid hormones as seen in the low T_3 syndrome during caloric deprivation (23). The here reported decreased disappearance of T_4-tracer from serum in caloric restriction and after fructose is compatible with the same phenomena. We also computed the uptake of the percentage of the tracer dose injected, in the REP according to the three compartmental model of thyroid hormone distribution and metabolism which we used previously (23). Figure 3 shows that during caloric deprivation and after fructose injection, the uptake of the injected T_4 tracer in the REP (mainly representing the liver) is indeed diminished. The slowest disappearance of T_4 from serum, i.e. caloric restriction, is reflected by the most pronounced inhibition of T_4 uptake in the rapidly equilibrating pool. Computed liver uptake in the control period starts to decrease soon after the maximum value has been reached so that at appr. 250 min. it is not different anymore from liver uptake in the fructose experiment.

We also analyzed non-published data from our experiments in normal T_4 treated males before and during D-propranolol in which propranolol induced a low T_3 syndrome. No changes in serum T_4 disappearance was seen (fig.4). Uptake of T_4 into the liver (rapidly equilibrating pool) was even increased probably related to the increased serum free T_4 concentration. Thus in this situation of the low T_3 syndrome no transport inhibition of T_4 into the liver was found. We could indeed attribute the low T_3 production due to propranolol to diminished T_4 into T_3 deiodination (21).

Appendix

Calculation of the uptake in the rapidly equilibrating pool (REP). The following set of equations was used:

$$\frac{dQ_1(t)}{dt} = -(k_{21} + k_{31})Q_1(t) + k_{12}Q_2(t) + k_{13}Q_3(t) \qquad \%D/t \text{ plasma} \qquad I$$

$$\frac{dQ_2(t)}{dt} = k_{21}Q_1(t) - (k_{12} + k_{02})Q_2(t) \qquad \%D/t \text{ REP} \qquad II$$

$$\frac{dQ_3(t)}{dt} = k_{31}Q_1(t) - (k_{13} + k_{03})Q_3(t) \qquad \%D/t \text{ SEP} \qquad III$$

$$Q_1(t) = y(t)V_p \qquad \%D \quad \text{plasmapool} \qquad IV$$

$$y(t) = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t} + A_3e^{-\lambda_3 t} \qquad \%D/L \text{ plasmaconc.} \qquad V$$

According to the methods as outlined in refs. 19 and 23 the constants in the equations I – V were calculated. Cumulative uptake in the REP (Q_2) was calculated by numerical solution of equation II for the time interval from 0 to 360 min., in 1 min. steps, as follows:

$$Q_2(t_n) = Q_2(t_{n-1}) + k_{21}Q_1(t_{n-1}) - (k_{12} + k_{02})Q_2(t_{n-1})$$
$$= k_{21}Q_1(t_{n-1}) + (1 - k_{12} - k_{02})Q_2(t_{n-1}) \qquad \%D \qquad \text{VI}$$

At $t_n = 0 : Q_2(t_0) = 0$ and $Q_1(t_0) = 100$ %D
therefore at 1 min (n=1)
$Q_2(t_1) = k_{21}Q_1(t_{n-1}) = k_{21}.100$ %D

Subsequently $Q_2(t_n)$ is calculated for each minute from 2 to 360 min by solving VI, using the previously found $Q_2(t_{n-1})$; and using $Q_1(t_{n-1})$ after solving of IV and V.

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CHAPTER 5

Summary

After the discovery of T_3 in 1952, it was already assumed that this iodothyronine represents the metabolically active form of thyroid hormone, while T_4, which is exclusively synthesized in the thyroid, must be regarded as a prohormone. Although conversion of T_4 to T_3 in peripheral tissues was already demonstrated in 1954, the observation (1970) that L-thyroxine substituted athyreotic persons showed normal serum T_3 levels, really focussed the attention to the peripheral activation of thyroid hormone. Apart from the question in which tissues this activating 5'-deiodination was accomplished, it was soon realized that T_3 and reverse T_3 (a biologically inactive analogue of T_3, formed by 5-deiodination of T_4) in serum were not always present in a fixed ratio, but that reciproke changes in serum levels of these hormones could be detected in several conditions. Thus, predictable, reproducible and reversible alterations in serum levels of T_3 and reverse T_3 (rT_3) could be induced by several situations of which caloric restriction, non-thyroidal illness, treatment with propranolol, dexamethasone and propylthiouracil and administration of cholecystographic agents as ipodate, are best known. In these conditions serum levels of T_3 are decreased, those of rT_3 are increased, while serum T_4 concentrations do not change greatly. These changes in serum thyroid hormone levels are known as the low T_3 syndrome.

CHAPTER 1 reviews some important aspects of peripheral thyroid hormone metabolism in relation to the generation of the low T_3 syndrome.

Kinetic studies revealed that the decrease in serum T_3 level and increase in serum rT_3 concentration result from a decreased T_3- production rate and rT_3 metabolic clearance rate, while rT_3 generation and T_3 degradation are unaltered. These findings were explained by inhibition of 5'-deiodination of T_4 and rT_3, which was supported by in vitro experiments with rat liver 5'-deiodinase.

Two important observations, however, challenged this theory as the sole explanation for the generation of the low T_3 syndrome. Firstly, it was shown that in perfused livers of fasted rats, a decreased hepatic T_4 uptake could be demonstrated while the T_4 to T_3 conversion rate was unaltered. It was also shown that inhibition of deiodination per se by propylthiouracil did not influence

T_4 uptake by the perfused liver. Subsequently, others and work from our laboratory independently showed the existence of an energy dependent uptake mechanism for T_4, T_3 and rT_3 in rat hepatocytes. It was further shown from our work that T_4 and rT_3 entered the cell by a common pathway that is different from that of T_3. The activity of these two pathways appeared to be ATP-dependent. It seemed feasable that changes in transport through these uptake systems could also (apart from any possible change in deiodination) cause the descripted changes in serum iodothyronines, as seen in the low T_3 syndrome.

The aim of this thesis was to assess to what extent changes in these two mechanisms i.e. 5'-deiodination and transmembraneous transport of iodothyronines, could be causative in the low T_3 syndrome.

Essentially, three conditions were examined: caloric deprivation, oral administration of d-propranolol and a state of (transient) ATP- depletion of the liver, induced by fructose infusion. These studies were analyzed according to a three compartmental model of distribution and metabolism of thyroid hormone.

CHAPTER 2 deals with the effects of caloric deprivation on tissue uptake of thyroid hormone. Ten obese subjects were studied before and after seven days on a 240 kcal diet. During caloric deprivation serum free T_4 levels increased by 25%, while total T_4 levels did not change. In spite of an increased free T_4 serum level, hormone transport into the tissues was markedly decreased, indicating an inhibition of transmembraneous transport. T_4 production rate was decreased, presumably due to the raised serum free T_4 concentration, resulting in a diminished TSH secretion. Because the metabolic clearance rate decreased proportionately, no change was observed in total T_4 levels. Although less pronounced in comparison with T_4, diminished T_3 transport into the tissues could also be demonstrated. This is in concert with in vitro observations in rat hepatocytes in primary culture, where transmembraneous transport of T_3 appeared to be less sensitive to inhibitory influences than T_4. Although an additional role of a decreased 5'-deiodinase activity could not be excluded, the results of the experiments, described in this chapter, point to an inhibition of transmembraneous transport of T_4 during caloric deprivation as at least an important factor contributing to low T_3 production and low T_3 serum levels, due to less T_4 availability in tissues.

In CHAPTER 3 results are described of tracer T_4, T_3 and rT_3 kinetic studies in six normal male L-thyroxine substituted subjects before and during oral d-propranolol treatment. D-propranolol induced a decrease in T_4 plasma appearance rate by 16%, presumably due to reduced intestinal absorption of L-thyroxine, although a d-propranolol effect on thyroidal secretion of T_4 not suppressed by exogenous T_4, cannot be excluded. A reduction of 26% in the T_4 metabolic clearance rate only partially countereffected by a 16% decrease in plasma appearance rate, explains an increase in both total T_4 and free T_4 serum levels. Inhibition of transport of thyroxine into the tissues could not be demonstrated. The decreased T_3 production could, thus, not be attributed

to a diminished thyroxine availability for 5'-deiodination. In addition, it could also be shown that d-propranolol did not inhibit the passage of T_3 and rT_3 through the cell-membrane. From the experiments described in chapter 3, it is concluded that the d- propranolol induced changes in thyroid hormone metabolism, resulting in a low T_3 syndrome, are due to inhibition of thyroid hormone deiodination, which is in contrast to the low T_3 syndrome during caloric deprivation (chapter 2).

CHAPTER 4 shows early serum T_4 tracer disappearance curves and computed T_4 uptake by the rapidly equilibrating pool, which is believed to be largely comprised by the liver. In concert with the observations in chapter 2, caloric deprivation results in a slower clearance of tracer T_4 from the blood in concomitance with a decrease in T_4 uptake by the rapidly equilibrating pool (liver). In chapter 3 it was shown that d-propranolol did not interfere with passage of iodothyronines through the cell-membrane. In agreement with these findings is an unalterd T_4 tracer disappearance from the blood and an unchanged computed T_4 uptake by the rapidly equilibrating pool during d-propranolol administration.

Because transmembraneous transport of iodothyronines in rat hepatocytes in primary culture was shown to be ATP-dependent, an intravenous fructose load was given to four persons. This procedure results in a prompt ATP-depletion of the liver. In accordance with this effect, a temporarily increase in serum uric acid and lactate was noted. Fructose loading resulted in a slower disappearance of thyroxine from the blood in conjunction with a decreased computed T_4- uptake by the rapidly equilibrating pool. These findings support an ATP-dependency of the transport system for iodothyronines in vivo.

In conclusion: in this thesis, experiments are described from which it can be concluded that alterations in serum thyroid hormones as observed in the low T_3 syndrome, can originate by both inhibition of 5'-deiodination and inhibition of transmembraneous transport of iodothyronines. The low T_3 syndrome during d-propranolol administration results from the former mechanism, while the latter mechanism is operating during caloric restriction. Furthermore, data are presented in support of an in vivo ATP-dependency of the transmembraneous transport system of iodothyronines.

Samenvatting

Na de ontdekking van T_3 in 1952 vermoedde men al spoedig dat de metabole activiteit van het schildklierhormoon via deze jodothyronine tot stand kwam, terwijl T_4, dat uitsluitend in de schildklier wordt gemaakt, beschouwd moest worden als een prohormoon. Hoewel al in 1954 was aangetoond, dat in perifere weefsels T_4 kon worden omgezet in T_3, richtte men pas zijn volle aandacht op deze perifere activatie van schildklierhormoon, toen bleek (1970) dat athyreote personen die met L-thyroxine werden gesubstitueerd, normale T_3 spiegels in hun bloed hadden. Naast de vraag in welke weefsels in vivo deze activerende 5'- dejodering plaats vond, bleek dat T_3 en reverse T_3 (een biologisch inactieve triidothyronine, gevormd door 5-dejodering van T_4) niet in een vaste verhouding in het bloed voorkwamen, maar dat onder verschillende omstandigheden reciproke veranderingen in de bloedspiegels van deze hormonen werden gevonden. Deze veranderingen bleken voorspelbaar, reproduceerbaar en reversibel. De bekenste situaties waarin genoemde veranderingen in bloedspiegels van T_3 en reverse T_3 (rT_3) optreden, zijn: vasten, ziekte in het algemeen bij euthyreote personen, behandeling met propranolol, dexamethason en propylthiouracil en toedienen van röntgencontrastmiddelen voor cholecystografie, zoals ipodate. Onder deze omstandigheden is de bloedspiegel van T_3 verlaagd,die van rT_3 verhoogd, terwijl de concentratie van T_4 in het bloed niet sterk verandert. Deze veranderingen in de concentraties van T_4, T_3 en rT_3 staan bekend als het laag T_3 syndroom.

HOOFDSTUK 1 geeft een overzicht van belangrijke aspecten van het perifere metabolisme van schildklierhormoon met betrekking tot het ontstaan van het laag T_3 syndroom.

Kinetische studies toonden aan dat de daling in de bloedspiegel van T_3 werd veroorzaakt door een verminderde aanmaak van dit hormoon. De stijging in de bloedspiegel van rT_3 kon verklaard worden door een verminderde afbraak. De afbraak van T_3 en de aanmaak van rT_3 bleken onveranderd. Deze bevindingen werden verklaard door aan te nemen dat de 5'-dejodering van T_4 en rT_3 was afgenomen. In vitro experimenten met 5'-dejodase uit rattelever ondersteunden deze theorie.

Twee belangrijke waarnemingen echter, zorgden er voor dat er twijfel ontstond dat remming van de 5'-dejodering de enige verklaring vormde voor het ontstaan van het laag T_3 syndroom. Allereerst bleek dat levers van gevaste ratten bij perfunderen met T_4, minder van dit hormoon opnamen dan levers van controledieren. Het percentage van het opgenomen T_4 dat in T_3 werd omgezet, was in beide groepen identiek. Tevens toonde men aan dat remming van de dejodering sec door propylthiouracil geen invloed had op de opname van T_4 tijdens de perfusie. Vervolgens werd in verschillende laboratoria, onafhankelijk van elkaar, het bestaan van een energie-afhankelijk opname systeem voor jodothyronines aangetoond. Zo konden Krenning en Docter in ons laboratorium in ratte-hepatocyten in primaire kweek, een actief energie-afhankelijk opnamesysteem

aantonen, waarbij T_4 en rT_3 de cel binnen worden gevoerd via eenzelfde pad, terwijl T_3 via een afzonderlijk opnamemechanisme de cel binnenkomt.

De activiteit van deze twee opname systemen bleek ATP-afhankelijk. Het bleek mogelijk om, met enige aannames, via veranderingen in dit transportsysteem, eveneens een verklaring te geven voor de veranderingen in schildklierhormoon, optredend tijdens het laag T_3 syndroom.

Het doel van dit proefschrift was vast te stellen in hoeverre veranderingen in deze twee mechanismen, namelijk 5'-dejodering en transport van jodothyronines door de celmembraan, bijdragen in het ontstaan van het laag T_3 syndroom.

Drie situaties werden onderzocht: vasten, orale toediening van d- propranolol en een toestand waarbij een passagere ATP-depletie van de lever optreedt ten gevolge van intraveneuze toediening van fructose. De resultaten van de experimenten werden geanalyseerd volgens een systeem waarbij de verdeling en het metabolisme van schildklierhormoon werden benaderd via een driecompartimenten model.

In HOOFDSTUK 2 wordt het effect van vasten op de opname van schildklierhormoon door de weefsels besproken. Tien adipeuze personen werden bestudeerd voor en na een periode van zeven dagen waarbij zij een dieet kregen bestaande uit 240 Kcal. Tijdens deze periode met beperkte toevoer van calorieën steeg de vrije T_4 concentratie met 25% ten opzichte van de controleperiode. De totale T_4 concentratie veranderde niet. Ondanks de stijging van de vrije T_4 concentratie nam het transport van thyroxine naar de weefsels aanzienlijk af, hetgeen wijst op een remming van transport van T_4 door de celmembraan. De aanmaak van T_4 was verminderd, waarschijnlijk ten gevolge van een verminderde TSH secretie, veroorzaakt door de stijging van de vrije T_4 concentratie. Omdat de metabole klaring van T_4 evenredig was afgenomen, werd geen verandering in de totale T_4 concentratie waargenomen. Het transport van T_3 naar de weefsels bleek eveneens verminderd, hoewel minder uitgesproken dan bij T_4. Dit is in overeenstemming met de in vitro bevindingen in rattehepatocyten waar het transport van T_3 door de celmembraan minder gevoelig bleek voor remmende invloeden. Hoewel het niet is uitgesloten dat een verminderde 5'-dejodering eveneens een bijdrage levert aan de verminderde T_3 productie, geven de resultaten uit dit hoofdstuk duidelijk aan, dat remming van transport van thyroxine door de celmembraan, op zijn minst een belangrijke factor vormt in de verminderde T_3 aanmaak tijdens vasten.

In HOOFDSTUK 3 worden de resultaten beschreven van kinetische studies met radioactief gemerkt T_4, T_3 en rT_3 bij zes normale, mannelijke personen, gesubstitueerd met L-thyroxine, voor en tijdens orale toediening van d-propranolol. Tijdens d-propranolol toediening nam de plasma appearance rate van T_4 met 16% af, waarschijnlijk ten gevolge van een verminderde resorptie van thyroxine in de darm, hoewel een effect van d-propranolol op de secretie van T_4 door de schildklier, die mogelijk niet geheel was onderdrukt door exogeen toegediend T_4, niet geheel kon worden uitgesloten. De toename van zowel de

concentratie van het vrije T_4 als het totale T_4 gehalte, kan worden verklaard, doordat de afname van de metabole klaring van T_4 met 26% slechts ten dele wordt gecompenseerd door een daling in de plasma appearance rate van 16%. Een remming van het transport van thyroxine naar de weefsels kon niet worden aangetoond. De verminderde T_3 productie kon dus niet worden toegeschreven aan een verminderde beschikbaarheid van T_4 voor 5'-dejodering. Zo bleek ook dat de passage van T_3 en rT_3 door de celmembraan niet was geremd. Uit de resultaten beschreven in hoofdstuk 3, kan worden geconcludeerd dat de veranderingen in het metabolisme van schildklierhormoon tijdens het laag T_3 syndroom veroorzaakt door d-propranolol, ontstaan door een remming van dejodering van schildklierhormoon en niet, zoals bij vasten, door remming van het transport van schildklierhormoon door de celmembraan (hoofdstuk 2).

In HOOFDSTUK 4 worden curves getoond die de verdwijning van radioactief gemerkt T_4 uit het serum weergeven in de tijd. Tevens wordt de berekende opname van T_4 door het snel equilibrerende compartiment in de tijd aangegeven in dezelfde situaties. Men neemt aan dat het snel equilibrerende compartiment voornamelijk uit de lever bestaat. In overeenstemming met de bevindingen uit hoofdstuk 2, blijkt radioactief gemerkt T_4 langzamer uit het bloed te verdwijnen tijdens vasten. Tegelijkertijd blijkt het snel equilibrerende compartiment (lever) minder T_4 op de te nemen. In hoofdstuk 3 bleek dat d- propranolol de passage van jodothyronines door de celmembraan niet belemmert. Volgens verwachting bleek de verdwijning van T_4 uit het bloed en de opname van dit hormoon door het snel equilibrerende compartiment, tijdens d-propranolol toediening onveranderd ten opzichte van de controle periode.

Omdat in rattehepatocyten was gebleken, dat het transport systeem voor jodothyronines ATP-afhankelijk was, kregen vier personen intraveneus fructose toegediend. Dit veroorzaakt een snelle daling van ATP in de lever. Deze tijdelijk fructose toediening resulteerde in een langzamere verdwijning van T_4 uit het bloed in combinatie met een verminderde opname van thyroxine door het snel equilibrerende compartiment. Deze resultaten steunen een ATP-afhankelijkheid van genoemd transport systeem voor jodothyronines.

Samenvattend kan uit de in dit proefschrift beschreven experimenten worden geconcludeerd, dat veranderingen in de bloedspiegels van schildklierhormonen als waargenomen tijdens het laag T_3 syndroom, kunnen ontstaan door zowel remming van de 5'-dejodering als door remming van transmembraneus transport van jodothyronines. Het laag T_3 syndroom tijdens d-propranolol ontstaat via remming van de 5'- dejodering, vasten veroorzaakt een remming van transport van schildklierhormoon door de celmembraan. Tevens worden gegevens gepresenteerd die een in vivo ATP-afhankelijkheid van dit transport systeem ondersteunen.

NAWOORD

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Joke Nijsse verzorgde op uitstekende wijze de uiteindelijke invoer van het manuscript in de tekstverwerker.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 8 november 1952 te Rotterdam geboren. Na het behalen van het gymnasium-beta diploma in 1971 (Sint Franciscus College, Rotterdam) studeerde hij geneeskunde aan de Medische Faculteit Rotterdam, alwaar in februari 1978 het artsexamen werd afgelegd. Aansluitend vervulde hij zijn militaire dienstplicht bij de Koninklijke Landmacht. In 1979 ving hij zijn opleiding tot internist aan op de afdeling Inwendige Geneeskunde III van het Academisch Ziekenhuis Rotterdam - Dijkzigt (Hoofd: Prof. Dr. J.C. Birkenhäger). Op deze afdeling werd dit proefschrift bewerkt onder leiding van Prof. Dr. G. Hennemann. Op 1 januari 1985 volgde inschrijving in het specialistenregister. Sedert 1 april 1985 is hij als internist verbonden aan het Sint Jozef Ziekenhuis te Gouda.