THYROID HORMONES AND IODIDE

IN THE NEAR-TERM PREGNANT RAT

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Proefschrift

ter verkrijging van de graad van doctor

op gezag van de rector magnificus,

van de Landbouwuniversiteit Wageningen,

Dr. C.M. Karssen,

in het openbaar te verdedigen

op woensdag 1 april 1998

des namiddags te vier uur in de Aula

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Stellingen

1. De uitwisseling van T_4 uit het maternale plasma met het foeto-placentaire compartiment is een zeer snel proces.
   (dit proefschrift)

2. Tijdens marginale jodiumdeficiëntie zijn zowel de beschikbaarheid van T4 afkomstig van de moeder voor de foetussen, als de jodide opname door de foetale schildklier, en dus de foetale productie van T_4, verminderd.
   (dit proefschrift)

3. Het transport van T_4 naar de foeto-placentaire eenheid resulteert indirect in verlaging van de hoeveelheid T_3 in de organen van de moeder.
   (dit proefschrift)

4. Tijdens ondervoeding is de productie van T_4 door de schildklier verlaagd. Dit is toe te schrijven aan een intracellulair energie tekort, resulterend in een verlaagde opname van jodide.
   (Schröder-van der Elst et al. (1992) Diabetes 41:147-152)

5. De lading op de oligosaccharideketens van glycoproteinen is bepalend voor de biologische activiteit.

6. Daar in Nederland brood de voornaamste bron van jodium is, is het voor de ontwikkeling van zuigelingen van groot belang dat hun lacterende moeders voldoende brood eten.

7. Borstvoeding is wereldwijd de beste start voor een kind.
   een deskundige

8. Immunologische bepalingen hebben in hoge mate last van storende factoren.

9. De verzorging van proefdieren door de onderzoeker zelf draagt bij aan betere dierproeven.

10. Een creatieve werksfeer wordt in belangrijke mate bepaald door je directe collega's en de wetenschappelijke groep daaromheen.
Prof. Dr. T. de Lange, 28 februari 1998, de Volkskrant

11. In veel gevallen is de balans tussen ontvangen en gegeven onderwijs van AIO's verstoord.

12. Het structureel creëren van deeltijdaanstellingen is, naast een goed geregelde kinderopvang een van de voorwaarden voor het bevorderen van gelijke kansen op de arbeidsmarkt voor mannen en vrouwen.

13. Het voortdurend "updaten" van de gebruikte software heeft niet altijd een verhoging van het rendement tot gevolg.

14. In de meeste gevallen zijn slaap- en eetproblemen bij kleine kinderen niet wetenschappelijk aan te pakken.

15. Het schrijven van een proefschrift is minstens even zwaar als de zwangerschap en bevalling van een kind.

16. Promoveren op 1 april is niet per definitie een grap.

Stellingen behorend bij het proefschrift:
"Thyroid hormones and iodide in the near-term pregnant rat"

Petra Versloot
Wageningen, 1 april 1998

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1. THYROID HORMONE METABOLISM

Thyroid hormone action

Thyroid hormone is active throughout the whole body. Numerous biological effects have been described. During fetal life and childhood thyroid hormone plays an important role in development and growth. Throughout life thyroid hormones are responsible for regulation of the metabolic state.

The thyroid produces mainly thyroxine (T_4), and to a much lesser extent 3,5,3'-triiodothyronine (T_3). The greater part of T_3 is formed in the different organs by monodeiodination of T_4. T_3 has been shown to be the biologically active form of thyroid hormone. The working mechanism of T_3 is similar in all organs; T_3 exerts it action by nuclear T_3 receptors. After binding of T_3 the T_3-receptor complex interacts with the promotor of various genes by binding to a thyroid hormone response element, resulting in regulation of transcription of the target genes and influencing the specific mRNA synthesis of many target genes (47).

Peripheral thyroid hormone metabolism

In several tissues T_4 and T_3 are metabolized via different pathways. The most important is deiodination, especially because biologically inactive T_4 is metabolized into biologically active T_3 via this route. In the rat about 40 % of T_3 is secreted by the thyroid, wheras about 60 % is derived from extrathyroidal conversion of T_4 (50, 57, 58, 60).

T_4 can be deiodinated in the outer ring (in the 3' or 5' position, outer ring deiodination; ORD) or the inner ring (in the 3 or 5 position, inner ring deiodination; IRD). The ORD of T_4 results in T_3 and is regarded as the activating step. The IRD of T_4 results in reverseT_3 (rT_3), which is the so-called inactivation step. The different deiodination reactions are shown in Fig. 1. Three different deiodinase enzymes are known (29, 33). Type I iodothyronine deiodinase (ID-I) is a selenocysteine containing, nonselective enzyme, which is competitively inhibited by propylthiouracil (PTU). Both the inner and the outer ring can be deiodinated by this enzyme. It is responsible for both the production of T_3 as well as the clearance of mainly rT_3 in the liver. ID-I has been found in liver, kidney and thyroid. rT_3 is the preferred substrate, the conversion of rT_3 to 3,3'-T_2 is roughly 10^3 times more efficient than the deiodination of T_4 and T_3 (28). Type II iodothyronine deiodinase (ID-II) acts only on the outer ring and catalyzes the conversions of T_4 to T_3 and rT_3 to 3,3'-T_2. The enzyme is not inhibited by PTU. It is

present in the pituitary, central nervous system and brown adipose tissue. In these tissues, ID-II is responsible for the local supply of T_3 (26).

Type III enzyme (ID-III) catalyzes the formation of rT_3 from T_4 and the formation of 3,3'-T2 from T_3. The highest levels of this enzyme are found in the central nervous system, placenta and skin (63).

Other pathways for the metabolism of thyroid hormones are conjugation with either glucuronic acid or sulfate. Conjugation of thyroid hormones increases the water-solubility and thus facilitates their excretion via bile (62). Sulfation enhances type I deiodination of T_3 (48). A relatively minor role in thyroid hormone metabolism is played by side chain deamination and decarboxylation as well as ether-link cleavage (32).
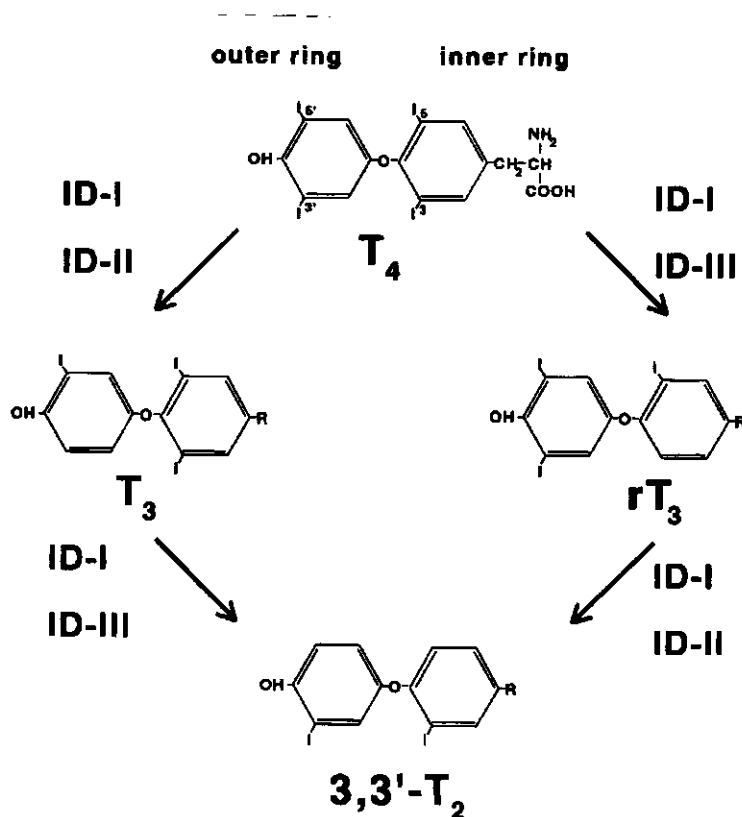

Figure 1: Deiodination pathways of thyroid hormones. ID-I, type I deiodinase; ID-II, type II deiodinase; ID-III, type III deiodinase.

Thyroid hormone synthesis in the thyroid

Thyroid hormone is synthesized in the thyroid gland, an endocrine organ located in the lower part of the throat around the trachea. The functional unit of the thyroid gland is the follicle, a single layer of follicular cells surrounding thyroglobulin (Tg)-containing colloid. T_4 and T_3 can be formed by coupling iodinated tyrosylresidues (monoiodotyrosine and diiodotyrosine) in the Tg molecule. The iodination reaction of the tyrosyl-residues takes place on the apical membrane at the colloid site and is catalyzed by thyroid peroxidase. The iodinated Tg, containing T_4 and T_3, is stored in the colloid lumen. When thyroid hormone is required the Tg molecule reenters the follicular cell. Thyroid hormones, released by hydrolysis of the Tg molecule in phagolysosomes, enter the circulation (61).

Role of iodide in thyroid hormone synthesis

Iodide is an essential element for the synthesis of thyroid hormone. Inorganic iodide is transported from the blood into the thyroid follicular cell by means of Na^+/I^- symport (34). The most important iodide-concentrating tissues in vertebrates are thyroid, placenta, salivary glands and gastric mucosa. The thyroid is the only organ which is able to organify accumulated iodide (67). The transport of iodide into the thyroid gland is an active process (61). This process is regulated by thyroid-stimulating hormone or thyrotropin (TSH) (61) and the thyroidal blood flow (2).

Regulation of thyroid hormone production

Thyroid hormone production by the thyroid gland is stimulated by TSH, which is secreted by the anterior pituitary gland. The synthesis and secretion of TSH are stimulated by thyrotropin-releasing hormone (TRH) from the hypothalamus. Both T_4 and T_3 exert a negative feedback on TSH synthesis and release, directly at the pituitary level and indirectly at the hypothalamic level by reducing the secretion of TRH (68). TSH binds to a TSH-receptor on the basal membrane of the thyroid cell and stimulates thyroid hormone synthesis at many different levels. Thyroidal iodide uptake as well as the synthesis and release of thyroid hormones is under the control of TSH (69). Since thyroid function is not abolished completely after hypophysectomy, there must be an internal autoregulatory system within the thyroid gland (49). The local production of T_3 from T_4 in several organs is regulated by the activity of the deiodinase enzymes and the availability of the precursor, T_4. During hypothyroidism the ID-I activity in liver and kidney is decreased, while the ID-II activity in brain

increases. The effects of hyperthyroidism are just in the opposite direction. Iodine deficiency does not affect ID-I activity in liver and kidney, while ID-II activity in the brain is increased (5, 25, 31). In the brain the aim of regulation is to reach homeostasis of the intracellular T_3 level.

Transport of thyroid hormones

Thyroid hormones secreted into the blood are almost entirely bound by specific binding proteins in the plasma. In humans thyroxine-binding globulin (TBG) is the most important binding protein, while in rat albumin is the most abundant binding protein for thyroid hormones. The free hormone fraction is metabolically active at the tissue level. In normal human serum, approximately 0.02 % of total T_4 and 0.2 % of total T_3 is free (53). Thyroid hormones enter the cell by means of specific transport proteins in order to exert their hormonal effects (12).

1.2. MATERNAL THYROID HORMONE METABOLISM DURING PREGNANCY

The maternal thyroid

During pregnancy thyroidal activity is stimulated by TSH and human chorionic gonadotropin (hCG) (27). In humans hCG levels are high mainly in the first half of gestation (4), whereas slightly increased TSH levels occur during the second half of gestation (22).

Enlargement of the maternal thyroid is common during normal human pregnancy (22). The uptake of radioiodide, expressed as percentage dose, is increased (1, 18). However, the availability of iodide for the maternal thyroid is decreased, due to increased renal clearance (1) and transport to the feto-placental complex during the late phase of gestation (18). Iodide is transported actively through the placenta (31). In the rat a decrease in uptake of ^{131}I by the maternal thyroid at the end of gestation has been described (16, 21, 24).

Maternal thyroid hormone concentrations and metabolism

Human pregnancy is accompanied by a rise in TBG and total T_4 and T_3 (18, 22). However, the level of free T_4 is only decreased slightly at the end of gestation (4, 7, 28, 65). Plasma TSH is increased slightly in more than 80 % of the near-term pregnant women, even though levels remain in the normal range (22).

In rat plasma T_4 and T_3 concentrations are decreased markedly during gestation (9, 41). The free fraction of T_4 is increased at the end of gestation (21), resulting in free thyroid hormone concentrations that stay within the normal range, just as in humans. Despite the considerable decrease in plasma T_4 and T_3 values, plasma TSH in the rat remains constant or is only elevated slightly at the end of gestation (9, 20, 30). It has been reported that the clearance of T_4 from the plasma is enhanced in pregnant rats (21). Both the extra-thyroidal T_4-pool and the turnover of T_4 are increased near-term. The secretion rate for T_4 is increased markedly just prior to term but equal to control values until 1 day before parturition (35). This has also been shown in mice. The fractional turnover of plasma T_4, the volume of distribution of T_4 and the T_4-secretion rate were all elevated in pregnant mice compared to virgin mice (66). Rat studies by the Madrid group have shown that in all tissues, except T_3 in the brain, T_4 and T_3 concentrations are decreased at the end of gestation. T_3 in the brain stayed within the normal range because of an increase in the local production of T_3 from T_4 as a result of an increase in ID-II activity (9, 38, 40, 43). No significant alteration in monodeiodination in liver homogenates from pregnant rats at the end of gestation was found by Yoshida et al. (70). However, a slight increase in hepatic ID-I activity in the near-term pregnant rat was described by Calvo et al. (9).

Maternal thyroid hormones and fetal development

It is well established that thyroid hormones play an important role during development, especially of the central nervous system. Until two decades ago it was generally accepted that there is no transport of maternal T_4 and T_3 to the fetus (19). However, convincing evidence for humans as well as rats has been presented that there has to be a transport of maternal thyroid hormones to the fetus. Even before the onset of fetal thyroid function T_4 and T_3 can be detected in rat (43, 44) as well as human embryos (6, 17). The concentrations of T_4 and T_3 in embryonic tissues from thyroidectomized dams were undetectable before the onset of fetal thyroid function and were still reduced in some tissues near-term, despite the onset of fetal thyroid function (43, 55). Even during normal pregnancy the maternal to fetal transfer of T_4 continues until term (37, 39).

In the human fetus with an impaired thyroid neurological damage can be prevented by initiating thyroid hormone treatment within a few days of birth. This implies that during fetal development the supply of maternal thyroid hormones was sufficient to achieve a normal development of the central nervous system (64).

1.3. IODINE DEFICIENCY

Since iodine is an essential element for thyroid hormone synthesis iodine deficiency affects the production of thyroid hormones, which results in alterations in thyroid hormone metabolism. Iodine intake is insufficient in large areas of the world. In case of severe iodine deficiency this results in a broad spectrum of iodine deficiency disorders, including miscarriage, stillbirth, congenital anomalies as well as the more familiar goiter, cretinism, impaired brain function and hypothyroidism in children and infants (23). Many effects of iodine deficiency are the result of impaired maternal thyroid functioning during pregnancy. In fact, even the effects of marginal iodine deficiency on maternal thyroid hormone metabolism and fetal development are unknown.

Effects of iodine deficiency on thyroid hormone metabolism

In nonpregnant rats it has been shown that during severe iodine deficiency the weight of the thyroid increases markedly, while the iodine content of the thyroid decreases rapidly. The T_3-to-T_4 ratio in the thyroid is increased (52, 56). Studies of 10-day-old rats have shown that the thyroidal uptake of iodide, as percentage dose, was considerably higher in iodine-deficient than in control rats (71). The plasma T_4 level is decreased and plasma TSH is increased, while circulating T_3 levels remain normal. The activities of the thyroid hormone-dependent enzymes alpha-glycerophosphatedehydrogenase and malic enzyme in liver are significantly decreased (56), implying that the hepatic T_3 levels are decreased during iodine deficiency. Despite an increase in ID-II activity tissue T_3 levels in the brain are lower during iodine deficiency which results in cerebral hypothyroidism. This is caused by the decreased availability of T_4 for the local production of T_3 (46).

Iodine deficiency during pregnancy

In areas of marginal iodine intake pregnancy constitute a goitrogenic stimulus (22). In the pregnant rat iodine deficiency results, just as in the nonpregnant rat, in an increase in the weight of the thyroid and a decrease in plasma T_4. This decrease in plasma T_4 is in addition to the decrease caused by pregnancy. (15, 45).

When iodine deficiency is severe enough to cause very low maternal plasma T_4 values, embryonic and fetal tissues become deficient in T_4 and T_3 both before and after the onset of fetal thyroid function (15, 45). Despite an increase in ID-II activity

in the fetal brain normal T_3 values cannot be achieved (45).

1.4. MATERNAL HYPOTHYROIDISM

Maternal hypothyroidism can be considered a rather common problem, especially since it is clear that maternal thyroid hormones are of great importance for a normal development of the fetal central nervous system. Untreated or inadequately treated hypothyroid women are less fertile and exhibit an increased risk of spontaneous abortion (3). For the children of mothers who were hypothyroid during pregnancy the incidence of behavioral and neurological disorders is high (36).

The thyroidectomized rat is an appropiate model for maternal hypothyroidism in rats. Thyroidectomy results in very low T_4 and T_3 levels in maternal plasma and tissues (41, 42, 51, 55). Before the onset of fetal thyroid function T_4 and T_3 are not detectable in the embryo (43). At the end of gestation fetal plasma T_4 and T_3 values have normalized (41, 51, 55). Most fetal tissues still exhibit decreased T_4 and T_3 levels on day 20 of gestation, but the difference with respect to the control fetuses is already much smaller than on day 18 (55).

In the thyroidectomized mother treated with methimazole, maternal as well as fetal hypothyroidism will occur. In this severe situation it has been shown that only T_4, and not T_3, is able to mitigate T_3 deficiency in the fetal brain (8, 41). This means that maternal T_4 is especially important for the normal development of the fetal brain.

1.5. OUTLINE OF THIS THESIS

Thyroid hormones, especially T_4, transported from mother to fetus, are of great importance for the development of the central nervous system. In most studies the point of interest is the fetal thyroid hormone status during pregnancy, especially in the event of impaired maternal and/or fetal thyroid function or iodine deficiency. However, much less is known about the effects of pregnancy on thyroid hormone and iodide metabolism in the mother during a normal, hypothyroid or iodine-deficient situation. This information is very important, because these conditions might restrict the availability of maternal thyroid hormone for the fetuses.

The aim of this study was to provide insight into maternal thyroid hormone metabo-

lism, iodide metabolism and peripheral T_3 production at the end of pregnancy. For this purpose pregnant rats were studied on day 20 of gestation.

Thyroid hormone production, metabolism and distribution were evaluated in the normal pregnant near-term rat (chapter 2). To study T_4 and T_3 kinetics nonpregnant and near-term pregnant rats were given a bolus injection of $[^{125}I]T_4$ and $[^{131}I]T_3$. The physiological parameters were estimated by means of the three-compartment model of JJ. DiStefano (10, 11).

In chapter 3, the effects of pregnancy on thyroid hormone concentrations and local conversion of T_4 to T_3 in many maternal organs are described. For this purpose a steady-state experiment was performed (13, 14, 57-59).

In chapter 4 the uptake of iodide by the thyroid gland in nonpregnant and near-term pregnant rats is compared. The uptake of iodide by the fetal thyroid gland was also estimated. Furthermore, the effects of marginal iodine deficiency on thyroidal iodide uptake were studied; what are the consequences for the availability of iodide for the fetal thyroid ? We chose to induce an iodine deficiency which is only marginal, because this situation closely reflects the iodine status in large populations in the world.

The effects of marginal iodine deficiency (chapter 5), and different levels of maternal hypothyroidism (chapter 6) on maternal thyroid hormone production, metabolism and distribution were evaluated by means of a kinetic study. Special point of interest in these studies was the availability of maternal thyroid hormones for the fetuses.

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CHAPTER 2

THYROXINE AND 3,5,3'-TRIIODOTHYRONINE PRODUCTION, META-

BOLISM, AND DISTRIBUTION IN PREGNANT RAT NEAR TERM.

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ABSTRACT

In the pregnant rat near term thyroxine (T_4) and 3,5,3'-tri-iodothyronine (T_3) concentrations are lower in plasma and extrathyroidal tissues, except T_3 in the brain. To study the changes in T_4 and T_3 kinetics a bolus injection of [^{125}I]T_4 and [^{131}I]T_3 was administered to nonpregnant controls and rats 14 and 19 days pregnant. Physiological parameters of the production, interpool transport, distribution, and metabolism of T_4 and T_3 were estimated by means of a three-compartment model. The production and partition of T_4 remained unchanged during pregnancy. The total distribution volume of T_4 was enlarged. On day 19 the plasma clearance rate was doubled, and transport to the fast pool was more than tripled. The rate of production of T_3 was slightly diminished. The plasma clearance rate was increased, but no changes were found in the interpool transport rates. These results suggest that in the pregnant rat near term the increased transport of T_4 is responsible for the distribution of the available T_4 between the maternal and the fetal compartment.

Keywords: thyroid hormone, kinetics, deiodinases, pregnancy.

INTRODUCTION

Thyroid hormones are of great importance for fetal and postnatal development, especially of the central nervous system. A deficiency of thyroid hormone during fetal development caused by maternal hypothyroidism in early gestation results in damage to the central nervous system (19, 23).

Convincing evidence has been presented that in humans substantial amounts of thyroxine (T_4) are transferred from mother to fetus. This results in normal fetal development, even in the case of congenital hypothyroidism. In such a case severe neurological damage can be prevented by initiating thyroid hormone treatment within a few days of birth (19, 29).

In humans normal pregnancy is accompanied by a rise in the serum levels of total T_4 and 3,5,3'-triiodothyronine (T_3). However, by a rise in the T_4-binding globulin the free T_4 and T_3 levels decrease during pregnancy (11). In contrast to the findings for humans, plasma T_4 and T_3 concentrations in the rat are decreased. This leads to reduced concentrations of T_4 and T_3 in the tissues, except T_3 in the brain. Mainten-

ance of the T_3 level in the brain can be attributed to increased local production of T_3 from T_4 via increased type II deiodinase activity in the brain (2, 21). Despite the considerable decrease in T_4 and T_3, plasma thyroid-stimulating hormone (TSH) remains unchanged or is only slightly increased during pregnancy (2, 8, 13).

Thyroid hormones are already present in the rat fetus before its own thyroid starts to function between *days 17* and *18* of gestation (21). Maternal T_4 and T_3 are transferred to the fetus, as shown in experiments in which the fetal thyroid is impaired. However, only T_4 can mitigate T_3 deficiency in the fetal brain (20). In normal pregnant rats T_4, but not T_3, is transported to the fetuses at the end of gestation (6, 7).

Information about secretion, distribution, and metabolism of T_3 and T_4 during pregnancy is lacking; nor is it known what the consequences of the lowering plasma thyroid hormone levels during pregnancy are for the partition of T_4 and T_3 over the tissues. This information may be obtained from kinetic studies based on the three-compartment model of distribution and metabolism developed by DiStefano (3, 4). In this model three compartments can be distinguished: (1) the plasma; (2) tissues that exchange T_4 and T_3 with plasma at a fast rate, the fast pool; and (3) the slow exchanging pool, the slow pool. Liver and kidney are considered as the main components of the fast pool, while skin, muscles and brain belong to the slow pool (3, 4). With this model it should be possible to distinguish and measure the transport of thyroid hormones to the fetal pool.

To investigate whether the decreased T_4 and T_3 levels in the pregnant rat lead to alterations in the biological end-points of thyroid hormone activity we determined the activity of α-glycerophosphate dehydrogenase (α-GPD; EC 1.1.2.1) in the liver. We have also measured hepatic type I deiodinase (ID-I) and cerebral type II and III deiodinases (ID-II and ID-III).

MATERIAL AND METHODS

Animals

Three-month-old female Wistar rats (CPB/WU, IFFA CREDO, Brussels) were used. At the start of the experiment the rats weighed 213 ± 9 g. The rats were individually housed at 22 °C, with alternating 14-h light and 10-h dark periods. The animals were fed a semisynthetic American Institute of Nutrition diet (1). The dry food was mixed with distilled water (60 % dry weight, 40 % water) containing 10 mg/l potassium iod-

ide to prevent utilization of labeled iodide by the thyroid (5). This dose of potassium iodide does not influence the T_4 and T_3 production rates (25, 26).

After two regular estrus cycles the rats were mated. The day that sperm appeared in the vaginal smear was taken as *day 0* of gestation (21). The gestational period of the rat is 22 days.

Design of the study

Two kinetic experiments were performed with three groups of rats; nonpregnant controls, pregnant rats on *day 14* and pregnant rats on *day 19*. In the first experiment the rats received only [^{125}I]T_4 ($n=6$, $n=4$, $n=5$ respectively), while in an second experiment the rats received [^{125}I]T_4 and [^{131}I]T_3 ($n=6$, $n=6$, $n=5$ respectively).

In a separate experiment we determined the activity of α-GPD and the deiodinases ID-I, ID-II, and ID-III in liver and brain of nonpregnant controls ($n=7$), 14-day pregnant rats ($n=6$), and 19-day pregnant rats ($n=6$).

Kinetic and analytical protocols

The rats received, via a cannula inserted into the right jugular vein (24), a 400 µl bolus injection of a solution containing 10 µCi [^{125}I]T_4 and 10 µCi [^{131}I]T_3 in saline containing heparin (0.3 U/ml; Organon, Tilburg, The Netherlands), ticarcillin (0.4 mg/ml; Ticarpen, Beecham S.A., Heppignies, Belgium) and 5% normal rat serum. Ticarcillin is used in the solutions containing labelled iodothyronines to prevent eventual bacterial infection and diminish artifactual deiodination. It has been used for that reason since described by Van Doorn (5).

Blood samples of 0.2 ml were taken at 1, 4.5, 10, 23, 44, 115, 202 and 315 min; 0.4 ml were drawn at 465, 600, 900, 1200 and 1440 min, as given by the optimal time schedule according to DiStefano et al. (3,4).

Plasma (50-100 µl in duplicate) was used for counting the total ^{131}I and ^{125}I activities. The plasma samples were extracted with ethanol/ammonia (25 %)(197:3 v/v) with 0.1 mM propylthiouracil (PTU). The dried extracts were dissolved in 0.1 ml 0.2 M ammonia containing carrier T_4, T_3 and potassium iodide (1 mg/10 ml) and subjected to high-performance liquid chromatography (HPLC) to separate the iodothyronines, according to the method described by Schröder-van der Elst and van der Heide (25). Plasma volume in controls and pregnant rats was determined in another experiment. Rat serum albumin (Nordic Immunological Laboratories, Tilburg, The Netherlands) was labelled with ^{125}I by the lactoperoxidase method and biologically screened (17).

A small amount of the ^{125}I-labelled albumin was injected via the cannula. At 1, 3, 5, 10, 15, 20 and 30 minutes blood samples were taken. The radioactivity in the plasma was counted, and the percentage dose per milliliter was calculated. The data were fitted to a single exponential function. The extrapolated activity at t(0) was used to determine the plasma volume [100/t(0)] (3).

The endogenous concentrations of T_4 and T_3 in plasma were measured by rat radioimmunoassay (RIA) in samples taken before the kinetic ex-periment was performed. Plasma TSH was measured by the specific RIA developed for the rat by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health (USA). Reference preparation RP-2 was used as a standard.

Calculations

The percentage doses of $[^{131}I]T_3$ and $[^{125}I]T_4$ per milliliter plasma were calculated from the total radioactivities and the $[^{131}I]T_3$ and $[^{125}I]T_4$ distributions on the HPLC chromatogram.

These percentage doses per milliliter, together with the plasma volume at $t(0)$, were individually fitted to sums of n=1 to 3 exponentials

$$Y(t)= A_1 \exp(\lambda_1 {}^* t) + A_2 \exp(\lambda_2 {}^* t) + A_3 \exp(\lambda_3 {}^* t)$$

using the program DIMSUM, in which coefficients A_i are expressed in percentage dose per ml and exponents λ_i are expressed per min (16).

To calculate 24 parameters of production, distribution and metabolism the program MAMPOOL (15) was used. The sum of the three exponential functions was fitted (by weighted least-squares regression) to the data collected for each rat individually and, together with plasma T_4 and T_3 levels, substituted in the three-compartment model, according to the kinetic T_4 and T_3 studies of DiStefano et al. (3, 4).

The calculated parameters are summarized in table 1.

All data are expressed as mean ± SE. Data were analyzed using the Statistical Package for Social Sciences (SPSS)(28). All data were subjected to one-way analysis of variance, and statistical differences among the groups were determined using the modified least significant difference method.

Table 1: Nomenclature for kinetic parameters of T_4 and T_3.

T_4-PR	Rate of thyroidal production of T_4; pmol/h
T_3-PR	Rate of total body production of T_3; pmol/h
T_3-PAR	Rate of plasma appearance of T_3; pmol/h
PCR	Plasma clearance rate; ml/h
TR_{PF}, TR_{FP}, TR_{PS}, TR_{SP}	Interpool transport rates between plasma and the fast and the slow pool, respectively in each direction; pmol/h
DR_{FO}, DR_{SO}	Irreversible rate of disappearance from the fast and slow tissue pools, respectively; pmol/h
Q_1, Q_2 and Q_3	Sizes of the plasma, fast and slow pools, respectively; pmol
V_p, V_2, V_3 and V_D	Plasma equivalent distribution volume of he plasma, fast, slow and total body pools, respectively; ml
% PF and % PS	Fraction of T_4 or T_3 in plasma transported unidirectionally to the fast and slow tissue pools, respectively
Transit time	Single mean transit time for T_4 and T_3 molecules traversing plasma and the fast and slow pools; min
Total residence time	All-pass mean residence time in the entiresystem; min

Enzyme and protein determinations

Liver mitochondrial α-GPD was measured by means of the method described by Garrib and McMurray (10).

The determinations of ID-I, ID-II, and ID-III activities were performed by the method as described by Janssen et al. (12). In short, ID-I activity was determined in liver homogenates. The final assay conditions were: 1 μM reverseT_3 (rT_3) and [^{125}I]rT_3, 0.1 M phosphate (pH 7.2), 2 mM EDTA and 5 mM dithiothrietol (DTT). Incubation time was 30 min at 37 °C. ID-II activity was determined by incubation of 0.5 nM [^{125}I]T_4 for 1 h at 37 °C with brain homogenate in the presence of 1 μM T_3, 1 mM PTU, 25 mM DTT, 0.1 M phosphate (pH 7.2) and 2 mM EDTA. ID-III activity in brain homogenates was determined by incubating 3,5-[^{125}I]T_3 for 1 h at 37 °C in the presence of 1 μM rT_3, 0.1 mM PTU, 50 mM DTT, 0.1 M phosphate (pH 7.2) and 2 mM EDTA.

All deiodinase reactions were stopped by adding 100 μl pooled human serum and 500 μl ice-cold trichloroacetic acid. Released ^{125}I⁻ was separated from protein-bound iodothyronines by centrifugation and counted.

Protein was determined by the bicinchoninic acid method (Pierce) using bovine serum albumin as standard.

RESULTS

During pregnancy we found a significant decrease in the plasma concentrations of T_4 and T_3. The TSH concentration did not change significantly (Table 2).

Table 2: Body weight and plasma thyroid hormone concentrations in nonpregnant controls and 14 and 19-day pregnant rats.

	Controls	Pregnant day 14	Pregnant day 19
BW, g	211 ± 3	274 ± 5*	315 ± 6#@
T_4, nM	33.8 ± 2.2	27.1 ± 1.6	23.3 ± 1.8@
T_3, nM	1.05 ± 0.07	0.61 ± 0.07*	0.57 ± 0.09@
TSH, ng/ml	0.29 ± 0.05	0.33 ± 0.06	0.57 ± 0.05

Values are means ± SE. Groups are, respectively, n=12; n=10 and n=10, except for T_3 (n=6; n=6; n=5). BW, body weight; TSH, thyroid-stimulating hormone. Statistical significance, $P < 0.05$: * day 14 vs. controls; @ day 19 vs. controls; # day 19 vs. day 14.

T_4 kinetics

For the T_4 kinetics animals from the two experiments were taken together. No significant differences in T_4 kinetics was found between the animals which received only [^{125}I]T_4, and those receiving [^{125}I]T_4 and [^{131}I]T_3.

Table 3 shows the mean values of the A and λ values and the fractional turnover and transport rates (k_{11}, k_{22}, k_{33}, $k_{12}k_{21}$, $k_{13}k_{31}$). The disappearance curves for T_4 from the plasma, based on the mean A and λ values for the three groups, are shown in Fig. 1. The absolute magnitude of the slopes of the regression lines describing the disappearance of T_4 from each of the three pools were significantly increased. This could imply that T_4 disappeared more quickly from all three pools in the pregnant rat. The mean results of distribution volumes, pool sizes and transport rates of T_4 are summarized in Table 4. The data are expressed per 100 g body wt and per total animal (maternal + fetal compartment). Table 5 shows the transit times of T_4 in the three pools and the mean residence time in the body.

Table 3: T$_4$ kinetic model parameters: controls and pregnant rats.

	Controls	Pregnant Day 14	Pregnant Day 19
	(n=12)	(n=10)	(n=10)
Coefficients and exponents			
A$_1$ (% dose/ml)	4.87 ± 0.15	3.59 ± 0.15*	2.81 ± 0.15 @#
λ$_1$ (1/min)	-0.22 ± 0.03	-0.47 ± 0.12	-0.83 ± 0.10@#
A$_2$ (%dose/ml)	2.39 ± 0.18	1.84 ± 0.18	1.94 ± 0.09
λ$_2$ (1/min)	-0.017 ± 0.002	-0.022 ± 0.005	-0.030 ± 0.004
A$_3$ (%dose/ml)	2.46 ± 0.07	1.92 ± 0.13*	1.71 ± 0.10@
λ$_3$ (1/min)	-0.0013 ± 0.0001	-0.0012 ± 0.0001	-0.0016 ± 0.0001@#
Fractional turnover and transport rates			
k$_{11}$ (1/min)	-0.117 ± 0.0012	-0.232 ± 0.054	-0.368 ± 0.042 @
k$_{22}$ (1/min)	-0.118 ± 0.013	-0.251 ± 0.061	-0.482 ± 0.056 @
k$_{33}$ (1/min)	-0.009 ± 0.001	-0.012 ± 0.003	-0.015 ± 0.002
k$_{12}$k$_{21}$ (1/min^2)	0.013 ± 0.003	0.083 ± 0.039	0.183 ± 0.035 @
k$_{13}$k$_{31}$ (1/min^2)	0.00013 ± 0.0000	0.00037 ± 0.0002	0.00041 ± 0.0001

Values are means ± SE. Statistical significance, P< 0.05: * day 14 vs. controls; @ day 19 vs. controls; # day 19 vs. day 14.

Table 5: Transit times and total residence time for T$_4$.

	Controls	Pregnant Day 14	Pregnant Day 19
	(n=12)	(n=10)	(n=10)
Transit time, *min*			
Plasma	9.5 ± 0.8	6.2 ± 1.0*	3.4 ± 0.8@
Fast pool	9.7 ± 1.1	6.4 ± 1.3	2.8 ± 0.8@
Slow pool	127 ± 15	124 ± 25	96 ± 32
Total residence time, min	790 ± 30	796 ± 64	631 ± 22@#

Values are means ± SE. Statistical significance, P<0.05: * day 14 vs. controls; @ day 19 vs. controls; # day 19 vs. day 14.

Table 4: Parameters of distribution volumes, pool sizes and transport rates of T_4, expressed per 100 g body wt and per total animal (maternal plus fetal compartment)

	Per 100 g Body Wt			Per total animal		
	Controls (n=12)	Pregnant Day 14 (n=10)	Pregnant Day 19 (n=10)	Controls (n=12)	Pregnant Day 14 (n=10)	Pregnant Day 19 (n=10)
PCR, ml/h	1.38 ± 0.05	1.32 ± 0.04	1.62 ± 0.04@#	2.91 ± 0.1	3.62 ± 0.1*	5.10 ± 0.1@#
T_4 PR, pmol/h	47.2 ± 3.8	35.6 ± 1.9	37.6 ± 2.5	99.6 ± 8.8	98.0 ± 6.6	118.1 ± 7.8
V_a, ml	18.0 ± 0.7	18.2 ± 0.8	17.5 ± 0.7	37.8 ± 1.3	49.9 ± 2.7*	55.2 ± 2.6@
V_p, ml	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	10.3 ± 0.2	13.7 ± 0.4*	15.6 ± 0.5@
V_2, ml	4.3 ± 0.3	4.6 ± 0.4	3.7 ± 0.3	9.1 ± 0.6	12.6 ± 1.3	11.5 ± 1.0
V_3, ml	8.8 ± 0.6	8.6 ± 0.5	8.9 ± 0.4	18.5 ± 1.2	23.6 ± 1.6	28.1 ± 1.6@
Q_{tot}, pmol	617 ± 50	492 ± 33	427 ± 33@	1298 ± 108	1356 ± 109	1343 ± 108
Q_1, pmol	166 ± 10	135 ± 7	115 ± 8@	350 ± 25	372 ± 24	360 ± 25
Q_2, pmol	145 ± 12	121 ± 10	86 ± 10@	305 ± 26	335 ± 32	270 ± 31
Q_3, pmol	305 ± 33	236 ± 23	205 ± 15@	640 ± 70	650 ± 71	644 ± 49
TR_{pfl}, pmol/h	973 ± 108	1743 ± 428	2426 ± 341@	2059 ± 240	4884 ± 1356	7528 ± 1040@
TR_{fpr}, pmol/h	950 ± 107	1726 ± 428	2407 ± 341@	2009 ± 242	4834 ± 1353	7469 ± 1037@
DR_{fo}, pmol/h	23.6 ± 1.9	17.8 ± 1.0	18.8 ± 1.2	49.8 ± 4.4	48.9 ± 3.3	59.1 ± 3.9
TR_{ps}, pmol/h	161 ± 21	162 ± 33	185 ± 28	341 ± 48	447 ± 102	576 ± 82
TR_{sp}, pmol/h	137 ± 19	144 ± 32	166 ± 27	291 ± 45	398 ± 99	517 ± 80
DR_{sp}, pmol/h	23.6 ± 1.9	17.8 ± 1.0	18.8 ± 1.2	49.8 ± 4.4	48.9 ± 3.3	59.1 ± 3.9

Values are means ± SE. See Table 1 for nomenclature. Statistical significance, $P < 0.05$: * day 14 vs. controls; @ day 19 vs. controls; # day 19 vs. day 14.
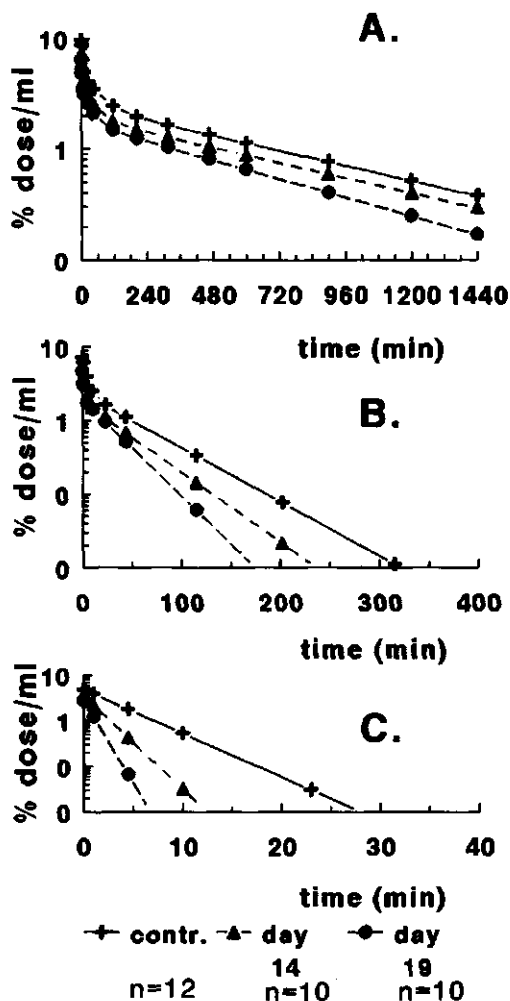
A.

B.

C.

Figure 1: T$_4$-disappearance curves for controls and 14 and 19-day pregnant rats obtained with a three exponential model.
Panel A: plot of log y(t) (in % dose/ml) 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$_3$.
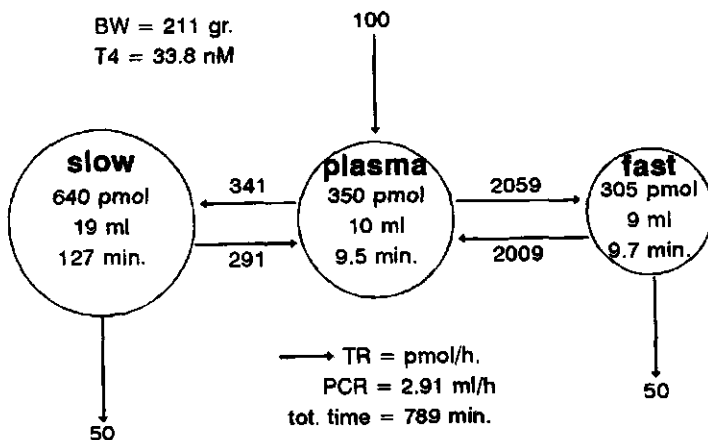*Panel B:* plot of log [y(t) - A$_3$ exp(λ$_3$*t)] against time, with the least squares regression line on the curve, giving estimates of coefficient A$_2$ and exponent λ$_2$.
Panel C: plot of log [y(t) - A$_3$ exp(λ$_3$*t) - A$_2$ exp(λ$_2$*t)] against time, giving estimates of coefficient A$_1$ and exponent λ$_1$.

The T$_4$ models for controls and 19-day pregnant rats are given in Fig. 2. For almost all parameters the value on *day 14* of gestation was somewhere between the value for the controls and that for 19-day pregnant rats.

The serum T$_4$ concentration decreased from 33.8 nM to 23.3 nM on *day 19* of gestation. The production of T$_4$ by the thyroid did not change significantly during pregnancy. The total amount of T$_4$ remained unchanged, as did the absolute mass of T$_4$ in the three pools. During pregnancy, the distribution volume of T$_4$ increased. The sizes of the plasma and slow pools increased by 50%, whereas the fast pool was only 25% larger.
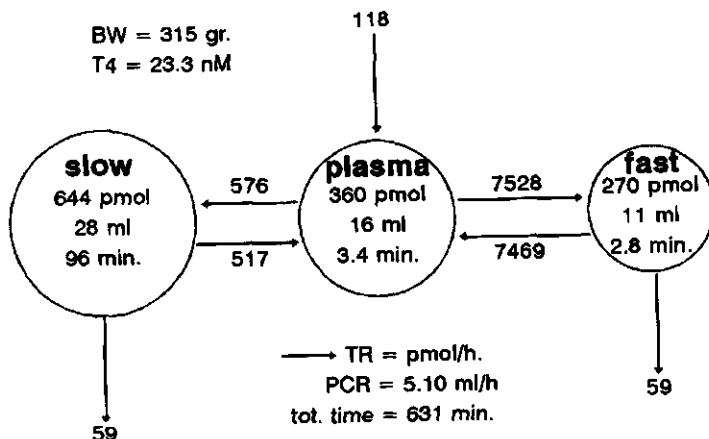
A.

BW = 211 gr.   100
T4 = 33.8 nM

slow   plasma   fast
640 pmol   341   350 pmol   2059   305 pmol
19 ml   10 ml   9 ml
127 min.   291   9.5 min.   2009   9.7 min.

⟶ TR = pmol/h.
PCR = 2.91 ml/h
tot. time = 789 min.

50   50

B.

BW = 315 gr.   118
T4 = 23.3 nM

slow   plasma   fast
644 pmol   576   360 pmol   7528   270 pmol
28 ml   16 ml   11 ml
96 min.   517   3.4 min.   7469   2.8 min.

⟶ TR = pmol/h.
PCR = 5.10 ml/h
tot. time = 631 min.

59   59

Figure 2: Mean 3-pool model of T_4 kinetics in nonpregnant controls (A) and 19-day pregnant rats (B). See table 1 for nomenclature.

Many alterations in the transport of T_4 occurred. On *day 19* of gestation the plasma clearance rate (PCR) for T_4 had increased nearly twofold. The transport to the fast pool and vice versa was more than tripled, and transport to the slow pool and back was almost doubled. The total residence time of T_4 decreased from 789 to 631 min. We found a reduction in the transit time in plasma and the fast pool to 30% of the control value. The transit time in the slow pool was 50% lower.

T$_3$ kinetics

A complete overview of the kinetic data and statistics of T$_3$ is given in Tables 6-8. The T$_3$ models for controls and 19-day pregnant rats are given in Fig. 3.

The plasma T$_3$ concentration markedly decreased during pregnancy. Although all A and λ values on *day 19* of gestation were significantly different from controls, only a few changes in the physiological parameters were found.

Table 6: T$_3$ kinetic model parameters: controls and pregnant rats.

	Controls	Pregnant Day 14	Pregnant Day 19
	(*n*=6)	(*n*=6)	(*n*=5)
	Coefficients and exponents		
A$_1$ (% dose/ml)	8.89 ± 0.31	6.63 ± 0.39*	5.88 ± 0.13@
λ$_1$ (1/min)	-1.66 ± 0.03	-1.71 ± 0.15	-1.89 ± 0.09
A$_2$ (%dose/ml)	0.79 ± 0.06	0.68 ± 0.03	0.62 ± 0.08
λ$_2$ (1/min)	-0.048 ± 0.004	-0.052 ± 0.005	-0.041 ± 0.007
A$_3$ (%dose/ml)	0.212 ± 0.018	0.195 ± 0.14	0.173 ± 0.014
λ$_3$ (1/min)	-0.0020 ± 0.0003	-0.0022 ± 0.0002	-0.0023 ± 0.0003
	Fractional turnover and transport rates		
k$_{11}$ (1/min)	-1.431 ± 0.07	-1.514 ± 0.14	-1.620 ± 0.13
k$_{22}$ (1/min)	-0.206 ± 0.016	-0.237 ± 0.020	-0.247 ± 0.030
k$_{33}$ (1/min)	-0.0093 ± 0.002	-0.0116 ± 0.001	-0.0096 ± 0.001
k$_{12}$k$_{21}$ (1/min^2)	0.239 ± 0.020	0.293 ± 0.005	0.344 ± 0.057
k$_{13}$k$_{31}$ (1/min^2)	0.0026 ± 0.0005	0.0030 ± 0.0004	0.0017 ± 0.0004

Values are means ± SE. Statistical significance, *P*< 0.05: *day 14* vs. controls; @ *day 19* vs. controls.

The plasma appearance rate and the production rate for T$_3$ were diminished when expressed per 100 g body wt, but no significant change, due to a large variation, was found per total body weight. During pregnancy the distribution volume of the plasma and the two tissue pools was enlarged. The amount of T$_3$ in the plasma and the fast pool had not changed, whereas the T$_3$ in the slow pool exhibited a sharp decrease. PCR increased. No significant differences were found in the transport rates, transit times, and total residence time for T$_3$.

Table 7: Parameters of distribution volumes, pool sizes and transport rates of T_3, expressed per 100 g body wt and per total animal (maternal plus fetal compartment)

	Per 100 g Body Wt			Per total animal		
	Controls (n=6)	Pregnant Day 14 (n=6)	Pregnant Day 19 (n=5)	Controls (n=6)	Pregnant Day 14 (n=6)	Pregnant Day 19 (n=5)
PCR, ml/h	21.7 ± 2.2	21.6 ± 0.9	21.0 ± 1.9	45.4 ± 3.9	57.0 ± 2.7	64.4 ± 4.5@
T_3 PAR, pmol/h	22.7 ± 2.8	13.4 ± 1.6	11.4 ± 1.2@	47.8 ± 5.2	35.5 ± 5.4	35.4 ± 3.7
T_3 PR, pmol/h	24.9 ± 3.1	14.7 ± 1.7*	12.9 ± 1.2@	52.3 ± 5.8	38.8 ± 5.9	40.0 ± 3.9
V_d, ml	209 ± 21	181 ± 12	169 ± 11	439 ± 36	475 ± 38	522 ± 31
V_p, ml	4.8 ± 0	5.1 ± 0.3	4.9 ± 0.1	10.1 ± 0.3	13.5 ± 0.9*	15.0 ± 0.3@
V_2, ml	28.6 ± 2.3	26.6 ± 1.3	29.1 ± 4.3	60.0 ± 3.6	70.1 ± 4.0	89.5 ± 11.2@
V_3, ml	176 ± 21	149 ± 11	135 ± 14	369 ± 37	392 ± 36	418 ± 39
Q_{tot}, pmol	218 ± 20	108 ± 8*	93 ± 13@	458 ± 36	284 ± 28*	290 ± 40@
Q_1, pmol	5.1 ± 0.3	3.1 ± 0.2*	2.8 ± 0.5@	10.7 ± 0.8	8.1 ± 0.8	8.6 ± 1.5
Q_2, pmol	29.7 ± 1.9	15.7 ± 1.2*	16.3 ± 3.5@	62.4 ± 3.1	41.6 ± 4.4	50.5 ± 9.9
Q_3, pmol	183 ± 19	89 ± 7*	74 ± 12@	385 ± 37	234 ± 24*	231 ± 31@
TR_{pf}, pmol/h	362 ± 27	230 ± 28	239 ± 56	768 ± 62	612 ± 101	742 ± 161
TR_{fp}, pmol/h	351 ± 27	223 ± 27	233 ± 55	744 ± 62	594 ± 98	725 ± 160
DR_{fo}, pmol/h	11.4 ± 1.4	6.7 ± 0.8*	5.7 ± 0.6@	23.9 ± 2.6	17.8 ± 2.7	17.7 ± 1.9
TR_{ps}, pmol/h	91 ± 13	52 ± 6*	36 ± 11@	191 ± 23	138 ± 22	113 ± 32
TR_{sp}, pmol/h	94 ± 13	54 ± 6*	37 ± 11@	199 ± 23	143 ± 23	116 ± 32
DR_{so}, pmol/h	13.5 ± 1.7	8.0 ± 1.0*	7.2 ± 0.7@	28.4 ± 3.2	21.1 ± 3.2	22.3 ± 2.2

Values are means ± SE. See Table 1 for nomenclature. Statistical significance, $P < 0.05$: day 14 vs. controls; @ day 19 vs. controls;
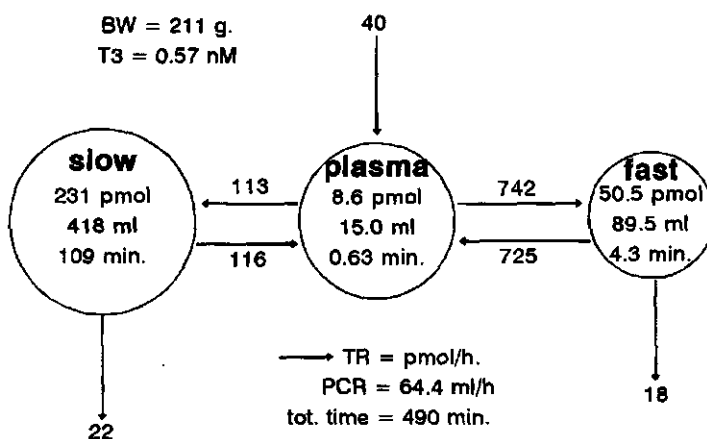
\# day 19 vs. day 14.
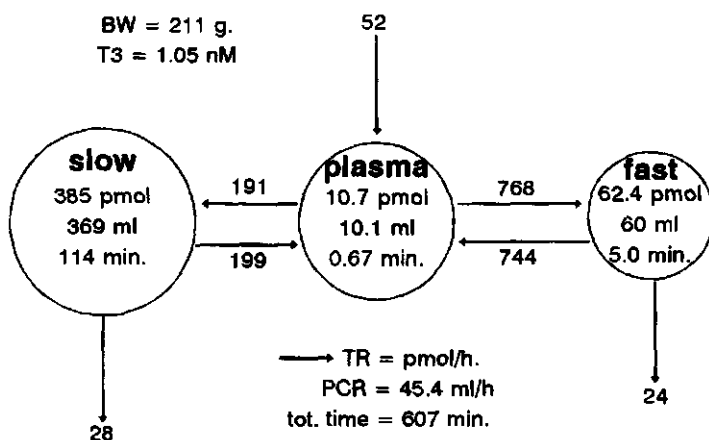
Figure 3: Mean 3-pool model of T$_3$ kinetics in nonpregnant controls (A) and 19-day pregnant rats (B). See table 1 for nomenclature.

Enzyme activities

The changes in the α-GPD activity in the liver are shown in Fig. 4. When expressed per milligram of protein there is a significant decrease in the α-GPD activity, but no change in activity is found when calculated per total liver.

Table 8: Transit times and total residence time for T_3.

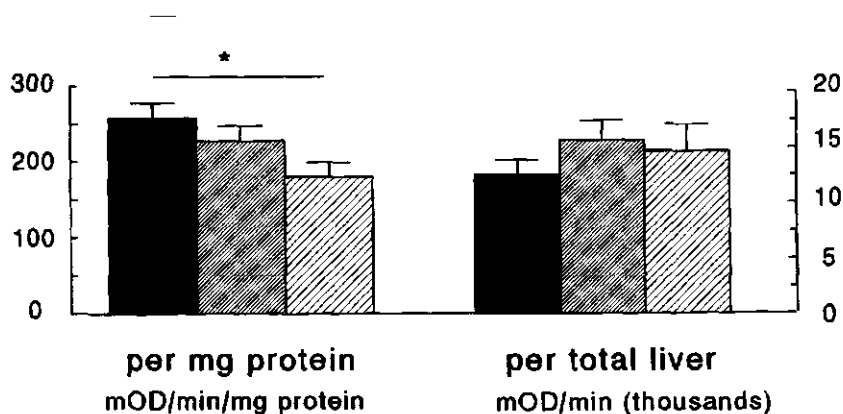|	Controls (n=6)	Pregnant Day 14 (n=6)	Pregnant Day 19 (n=5)
Transit time, min			
Plasma	0.67 ± 0.02	0.69 ± 0.07	0.63 ± 0.05
Fast pool	4.99 ± 0.36	4.38 ± 0.39	4.30 ± 0.56
Slow pool	114 ± 15	94 ± 14	109 ± 9
Total residence time, min	607 ± 76	507 ± 48	490 ± 33

Values are means ± SE.

Figure 4: α-glycerophosphate dehydrogenase activity (per mg protein and per total liver) in liver homogenates from controls (solid bars; n=7) and 14- (gray bars; n=6) and 19-day (hatched bars; n=6) pregnant rats. Values are means ± SE. $^*P < 0.05$.

Figure 5 shows the changes in deiodinase activities during pregnancy. Hepatic ID-I increased from 213 pmol/min/mg protein for controls to 270 pmol/min/mg protein on *day 19* of gestation. In the pregnant rat near term the weight of the liver increased by > 50%. The protein concentrations in the subcellular fractions were unchanged, reuslting in a relatively larger increase in the ID-I activity when calculated for the total liver. Brain ID-II had not changed on *day 14* but was markedly increased on *day 19* of gestation. The activity of ID-III in the brain remained unchanged during pregnancy. The brain weight was not significantly different in the pregnant rats.
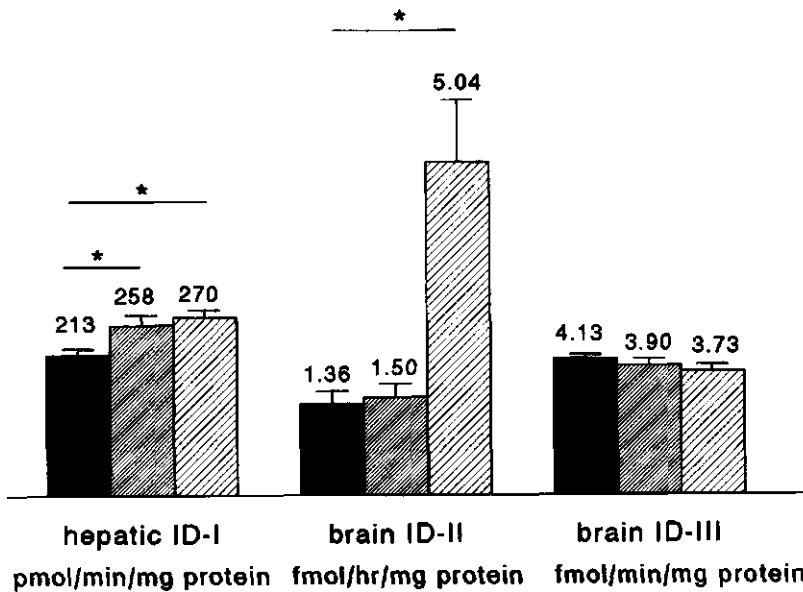
Figure 5: Deiodinase activities in total homogenates from liver (ID-I) and brain (ID-II and ID-III) from controls (solid bars; $n=7$) and 14- (gray bars; $n=6$) and 19-day (hatched bars; $n=6$) pregnant rats. Values are means ± SE. * $P < 0.05$.

DISCUSSION

In this study kinetic experiments were performed to investigate the mechanisms of the changes in thyroid hormone concentrations in the pregnant rat near term.

The kinetic parameters of thyroid hormone production, distribution, and metabolism are expressed per total animal (maternal + fetal compartment) and per 100 g body wt, because of the enormous gain in body weight due to the fetal compartment. After delivery bodyweight of the dams is nearly the same as that of age-matched nonpregnant controls; no essential alterations in bodyweight of the dams takes place during pregnancy. The volume of distribution of each pool increases proportionately to the increase in body weight. This would mean that the partition of T_4 in plasma and fast and slow pool is the same in pregnant and nonpregnant rats. When no changes are found in the proportion of T_4 in the three pools of the maternal compartment, this indicates that the developing placental and fetal compartments are made up of plasma and fast and slow pools similar to those of the mother.

Production of T_4 and T_3

Under normal conditions a lower plasma T_4 concentration will lead, by means of stimulation of TSH release, to an increase in the T_4 production rate. However, the free fraction of T_4 is increased in the pregnant rat, resulting in unchanged free T_4 plasma concentrations (9). This could explain the unchanged TSH levels.

Our experiment shows that there is no increase in thyroidal T_4 production. Others found an increased production of T_4 (7), probably because they used a noncompartmentalized method. The total amount of T_4 in the three pools remains unaltered; the reduced T_4 concentration in plasma and tissues (2) in the pregnant rat could be due to the enhanced distribution volume.

The total body production rate of T_3 was unchanged. However, the T_3 production rate has not increased proportionately to the increase in body weight, leading to a decreased T_3 production rate per 100 g body wt. This can be explained by a reduced thyroidal T_3 production and/or a decreased peripheral production from T_4. However, thyroidal T_4 production does not change essentially, and one would not expect the thyroidal production of T_3 to be specifically lowered. On the basis of the decreased plasma T_4 and T_3 levels, one would expect that the pregnant rat near term is hypothyroid. Just as in hypothyroid rats (14, 27) we found an increase in cerebral ID-II activity leading to normal tissue T_3 levels (2). However, instead of the decrease found in hypothyroid rats (14, 26), ID-I is also increased in livers of pregnant rats. This finding is supported by Calvo et al.(2). In contrast, no significant differences in hepatic and renal T_3 generation from T_4 have been observed by others (9, 30). From the increased in vitro ID-I and ID-II activities it could be expected that the production of T_3 from T_4 was increased. However, from the kinetic data this appears not to be the case. A possible explanation for this can be found in the rT_3.

The rT_3 level in plasma of pregnant rats is lower than in nonpregnant rats (2), despite the increased placental ID-III activity. However, the molar ratio of rT_3 to T_4 is doubled in the pregnant rat (2). This can result in a decreased in vivo T_3 production, since rT_3 is the preferred substrate for ID-I, whereas the in vitro activity of ID-I is increased.

Transport of T_4 and T_3

The T_4 PCR is markedly increased. Others (9) have suggested that the increased PCR is due not only to enhanced deiodination but also to the marked increase in the clearance of T_4 from plasma via the gastrointestinal pathway in the pregnant rat.

However, we did not find any significant changes in the disposal of T$_4$ from the body. We think that the major cause of the elevated PCR for T$_4$ is the result of the enlarged volumes of plasma and the slow pool.

This is also the case for the PCR for T$_3$; both the PCR and the plasma volume are increased by 50%. From our study it is clear that gestation is associated with significantly higher fractional T$_4$ turnover rates for different tissue pools. This results in enhanced transport rates for T$_4$ during pregnancy, especially for transport to the fast pool. It is unlikely that the increase in transport rate can be attributed only to the liver and kidney, which are considered to be the main organs of the fast pool.

Calvo et al. found that total liver and kidney from 21 day pregnant rats contain 35% less T$_4$ than those from the nonpregnant rat (2). In our model the total amount of T$_4$ in the fast pool remained unchanged. We suggest that an explanation for this discrepancy could be that in the pregnant rat the fast pool consists not only of the liver and kidney but also of a new, rapidly developed compartment (placentas plus fe- tuses). It is possible that this placentofetal compartment represents even "faster" thyroid hormone metabolism, but we were not able to distinguish a fourth exponent, located mathematically between plasma and the fast pool, on our disappearance curve.

In the three-compartment model the sum of the disposal rates equals the production rate in all cases. The individual values for the irreversible disposal rates from the slow and the fast pool (DR$_{so}$ and DR$_{fo}$, respectively) are rough estimates, which can vary in theory between zero and the production rate. DiStefano et al. (3) have made the assumption that the DR$_{so}$ and DR$_{fo}$ are both half the production rate. However, we think that the disappearance rate from the fast pool is larger than from the slow pool. We based this on the threefold increased transport rate from plasma to fast pool and the unaltered transport to the slow pool. This could mean that the disposal from the slow pool is the same in the nonpregnant and the near term pregnant rat. For that reason we assume that the disposal from the fast pool is increased with at least 18 pmol/h (DR$_{so}$=50; DR$_{fo}$=68 on *day 19*). We speculate that when the disposal from liver and kidney remained unaltered in the pregnant rat, this 18 pmol/h disappears from the placental and fetal compartments.

The parameters of T$_3$ transport in pregnant rats were not significantly different from those found for nonpregnant rats. This is in accordance with the results of Dubois et al. (6), who showed that T$_3$ is not transported across the placenta.

Thyroid hormone actions

Hepatic α-GPD activity per milligram protein is decreased in the pregnant rat (2, 19). This is in agreement with the lowered T_4 and T_3 concentrations in the liver. However, only when expressed per total liver can the actual enzyme activities of controls and pregnant rats be compared. This total α-GPD activity remained unchanged.

In summary, in the pregnant rat near term available T_4 is distributed between the maternal and the fetal compartment by very fast transport, presumably to the placenta and fetuses. The unchanged T_3 kinetics are in agreement with the absence of placental transfer of T_3 in the normal pregnant rat at the end of gestation. The pregnant rat does not produce more thyroid hormones to compensate for loss to the fetuses.

ACKNOWLEDGEMENTS

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CHAPTER 3

CONTRIBUTION OF T_3 PRODUCED LOCALLY FROM T_4 IN SEVERAL MATERNAL TISSUES OF THE NEAR-TERM PREGNANT RAT.

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ABSTRACT

3,5,3'-Triodothyronine (T_3) is produced by the thyroid and locally, by monodeiodina-
tion of thyroxine (T_4), in the peripheral tissues. During pregnancy the thyroid hor-
mone status in rats is altered: plasma and tissue levels of T_4 and T_3 are decreased.
We investigated the effects of pregnancy on the contribution of T_3 produced locally
in the maternal tissues by administering a continuous infusion of $[^{125}I]T_4$ and $[^{131}I]T_3$.
The transport of T_4 to almost all maternal organs diminished. Less T_3 was trans-
ported from the plasma to brown adipose tissue (BAT), liver, kidney and pituitary. In
BAT and brain the amount of locally produced T_3 decreased, despite the increase in
deiodinase type II activity in the brain. In liver the contribution of locally produced T_3
remained constant, despite an increase in deiodinase type I activity during preg-
nancy. This discrepancy between deiodinase activities and locally produced T_3 can
be explained by an insufficient availability of T_4. The decreased maternal T_4 concen-
tration, together with the transport of T_4 to the feto-placental compartment, results
indirectly in a diminished availability of T_3 in the maternal organs.

INTRODUCTION

During pregnancy maternal thyroid hormones are exceedingly important for normal
development of the fetal central nervous system (7). The biologically active form of
thyroid hormone is 3,5,3'-triiodothyronine (T_3) (13). T_3 is produced not only by the
thyroid but also locally in peripheral tissues by monodeiodination of thyroxine (T_4). In
liver, kidney and the thyroid monodeiodination of T_4 is catalyzed by the deiodinase
type I enzyme (ID-I), while in the pituitary, central nervous system and brown adip-
ose tissue (BAT) deiodinase type II enzyme (ID-II) is responsible for the conversion
of T_4 to T_3 (10).
Some tissues can adapt to alterations in plasma thyroid hormone status, and regu-
late intracellular T_3 by changing the deiodinase enzyme activities, and thus the
amount of locally derived T_3. During hypothyroidism a decrease in hepatic ID-I
activity, and an increase in brain ID-II activity have been described (9, 18). This is in
agreement with the contribution of the local conversion found in those organs; in
liver and kidney local conversion is decreased, whereas in brain an increase has
been found (4).

Especially in the central nervous system local conversion of T_4 is the main source of intracellular T_3. Therefore, when the amount of T_3 available from plasma is decreased, this can be compensated by an increase in the local conversion of T_4 to some extent (4).

In the near-term pregnant rat this condition is associated with decreased T_4 and T_3 levels in plasma and tissues (2, 12, 20). From earlier studies of T_4 and T_3 kinetics it has been suggested that in the near-term pregnant rat T_4 is transported from the plasma to the feto-placental compartment at a very fast rate (20). This leads to a decrease in the availability of T_4 for the maternal tissues. No changes were found in the transport of T_3.

Studies by the Madrid group have shown that T_4 and T_3 concentrations in maternal tissues are decreased at the end of gestation (2, 12). However, it is not known how pregnancy influences the amount of T_3 produced locally in several tissues. An indication about T_3 produced locally in several tissues is only based on measurements of deiodinase activities. It has been found that in the near-term pregnant rat hepatic ID-I as well as brain ID-II activity is increased (2, 20). The aim of this study was to compare the contribution of T_3 produced locally in maternal tissues of the non-pregnant and near-term pregnant rat. By administering a continuous simultaneous infusion of $[^{125}I]T_4$ and $[^{131}I]T_3$ (4, 5, 16, 17), T_4 and T_3 concentrations in maternal tissues, the relative contribution of plasma-derived vs. locally produced T_3, thyroidal T_4 and T_3 secretion, and the plasma-to-tissue ratios of T_4 and T_3 were determined.

MATERIAL AND METHODS

Animals
Three-month-old female Wistar rats (CPB/WU, Iffa Credo, Brussels) were used. The rats were individually housed in metabolic cages at 22 °C, with alternating 14-h light and 10-h dark periods. The animals were fed a semisynthetic American Institute of Nutrition diet (1). The dry food was mixed with distilled water (60% dry weight, 40% water) containing 10 mg/l potassium iodide.

Design of the study

Two groups of rats were used in this study, i.e. nonpregnant and pregnant rats. After two regular estrus cycles the rats were mated . The day that sperm appeared in the vaginal smear was taken as *day 0* of gestation. At the start of the infusion the rats weighed 211±4 g (controls) and 237±3 g (pregnant, *day 8*).

The continuous intravenous infusion of [^{125}I]T$_4$ (30 µCi/rat/day) was started on *day 8* of pregnancy; 4 days later [^{131}I]T$_3$ was added to the infusion fluid. The labeled iodo-thyronines were administrated at a constant rate (10 ml/day), via a cannula which was inserted into the right jugular vein, and extended to the right atrium (14) at least 4 days before the continuous infusion was started. The rats were unrestrained, and could eat and drink normally.

Urine and feces were collected from the start of infusion. The ^{125}I and ^{131}I contents were counted and expressed as a percentage of the daily infused radioactivity. The animals were in isotopic equilibrium when the sum of radioactivity in urine and feces was equal to the daily administered dose.

Labelled iodothyronines

High specific activity [^{125}I]T$_4$ and [^{131}I]T$_3$ (specific activity ~ 2200 and ~ 3500 µCi/µg, respectively) were prepared in our laboratory (8, 21). Na^{125}I and Na^{131}I were pur-chased from Amersham (Aylesbury, UK); L-T$_3$ and 3,5-L-diiodothyronine, the re-spective substrates for labelling, were obtained from Sigma (St. Louis, MO). Purity of the tracers was assessed by means of high performance liquid chromatography (HPLC). All infusions consisted of a sterile 0.9 % NaCl solution containing 0.2 mg/ml ticarcillin (Ticarpen; Beecham, Heppignies, Belgium) and 0.3 U/ml heparin (Orga-non, 0Tilburg, The Netherlands). The stock infusion solutions were stored at 4 °C in the dark. The infusion flasks were protected from light to minimize artifactual de-iodination of the tracers.

Analytical procedures

At the end of the infusion period, i.e. *day 20* of gestation for the pregnant rats, the rats were bled under light ether anesthesia. Blood was collected in heparinized tubes. To prevent artifactual deiodination propylthiouracil (PTU) was added to a final concentration of 0.1 mM (11). To free the tissues of trapped blood, the rats were perfused with 40-50 ml of a 0.9 % NaCl solution containing 3 U heparin/ml and 0.1 mM PTU; outflow was obtained by puncturing the inferior vena cava. Maternal

tissues were then immediately excised and kept on ice. Either whole small organs or weighed portions of the bigger organs were minced and homogenized in a Potter homogenizer (B. Braun, Melsungen, Germany) at 0 °C in methanol containing 0.1 mM PTU, carrier T_4 and T_3 and potassium iodide (1mg/10 ml). The pituitaries were homogenized in 1.5 ml saline containing carrier and PTU.

To determine the iodothyronine concentration a measured aliquot was taken of each tissue homogenate and the plasma; the ^{125}I and ^{131}I contents were counted. The samples were extracted with methanol-ammonia (25 %, 197:3 vol/vol) with 0.1 mM PTU. The dried extracts were dissolved in 0.1 ml 0.2 M ammonia containing carrier T_4, T_3 and potassium iodide (1 mg/10 ml) and subjected to HPLC to separate the iodothyronines. The analyses with HPLC were performed according to the method described earlier (16).

After decay (7-8 half lives) of the ^{131}I initially present in the samples the concentrations of stable T_4 and T_3 in plasma were assessed by a specific radioimmunoassay (RIA) for rats using $[^{131}$I$]T_4$ and $[^{131}$I$]T_3$ as tracers (6). Plasma TSH was measured by the specific RIA developed for the rat by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health. Reference preparation RP-2 was used as a standard.

Calculations

The levels of T_4, T_3, tissue T_3 derived from T_4 ($Lc(T_3(T_4))$) and plasma-derived T_3 ($p(T_3)T_3$) were calculated (4, 5, 16, 17).

In short:

Tissue T_4 (pmol/g wet wt)

$$\approx \frac{\text{tissue } [^{125}\text{I}]T_4 \text{ (\% dose/g)}}{\text{plasma } [^{125}\text{I}]T_4 \text{ (\% dose/ml)}} \times \text{plasma } T_4 \text{ (pmol/ml; RIA)}$$

The concentration of $[^{125}$I$]T_4$ in the tissue was corrected for trapped plasma (5).

Lc $T_3(T_4)$ was calculated as follows

$$\approx \frac{\text{tissue } [^{131}\text{I}]T_3 \text{ (\% dose/g)}}{\text{plasma } [^{131}\text{I}]T_3 \text{ (\% dose/ml)}} \times \text{plasma } [^{125}\text{I}]T_3 \text{ (\% dose/ml)}$$

$$\approx \text{tissue } [^{125}\text{I}]T_3 \text{ derived from plasma (\% dose/g)}$$

Then

tissue Lc $[^{125}I]T_3$ (% dose/g)

$\qquad = \quad$ total tissue $[^{125}I]T_3$ (% dose/g)

$\qquad\qquad\qquad$ - tissue $[^{125}I]T_3$ derived from plasma (% dose/g)

Thus

tissue Lc $T_3(T_4)$ (pmol/g)

$$= \frac{\text{tissue Lc } [^{125}I]T_3 \text{ (\% dose/g)}}{\text{plasma } [^{125}I]T_4 \text{ (\% dose/ml)}} \quad \text{X plasma } T_4 \text{ (pmol/ml; RIA)}$$

whereby $[^{125}I]T_4$ was multiplied by 2 to correct for the loss of ^{125}I from the distal ring of T_4.

The concentration of T_3 derived from plasma in the various tissues was obtained as follows

tissue T_3 derived from plasma (pmol/g)

$$= \frac{\text{tissue } [^{131}I]T_3 \text{ (\% dose/g)}}{\text{plasma } [^{131}I]T_3 \text{ (\% dose/ml)}} \quad \text{X plasma } T_3 \text{ (pmol/ml; RIA)}$$

The total level of T_3 in a tissue is the sum of the values calculated for tissue Lc $T_3(T_4)$ and $pT_3(T_3)$.

The rate of infusion of $[^{125}I]T_4$ and $[^{131}I]T_3$ and their respective blood levels were used to calculate the plasma clearance rates (PCR) for T_4 and T_3. If the plasma concentration is expressed as a percentage of the infused dose (in 1h/100 g body wt), then

PCR (ml $\cdot$ h^{-1} $\cdot$ 100 g body wt^{-1})

$$= \frac{100}{\text{\%dose (h} \cdot \text{100 g body wt}^{-1} \cdot \text{ml}^{-1})}$$

The production rate (PR) for T_4, or the plasma appearance rate (PAR) for T_3, is the PCR for T_4 or T_3 multiplied by their respective plasma concentrations.

The production of T_3 by the thyroid (ThPRT$_3$) can be calculated as follows:

since the amount of circulating T_3 derived from T_4 ($T_3(T_4)$) is given by

$$T_3(T_4) = \frac{\text{\% dose/ml } [^{125}I]T_3}{\text{\% dose/ml } [^{125}I]T_4} \text{X plasma } T_4 \text{ (pmol/ml; RIA)}$$

and $T_3(T_4($ (pmol/ml)

$$\frac{T_3(T_4(\text{ (pmol/ml)}}{\text{plasma } T_3 \text{ (pmol/ml; RIA)}} \times 100\ \% = \%\ T_3(T_4)$$

then $\{100\text{-}[\%T_3(T_4)]\} \times$ PAR T_3 = ThPRT$_3$ in picomoles per hour per 100 grams bodyweight.

All results are expressed as means ± SE. Data were analyzed using the Statistical Package for Social Sciences (19). Statistical analysis was performed by means of the Student's t test.

RESULTS

Each rat received the continuous infusion until the individual ^{125}I- and ^{131}I-radioactivities in urine and feces equaled the daily input for at least two days. This occurred in both groups after 10 days of [^{125}I]T$_4$ infusion and 7 days of [^{131}I]T$_3$ infusion. At this time the rats were assumed to be in isotopic equilibrium as far as the major pools of T$_4$, T$_3$ and their metabolites were concerned.

Table 1: Bodyweight and plasma thyroid hormone concentrations in nonpregnant controls and *20-day* pregnant rats.

	Controls (*n*=10)	Pregnant (*n*=9)
Bodyweight, g	229 ± 2.7	305 ± 4.8[*]
# fetuses		12 ± 1
plasma T$_4$, nM	30.7 ± 3.2	18.9 ± 4.8[*]
plasma T$_3$, nM	0.62 ± 0.04	0.52 ± 0.03
plasma TSH, ng/ml	0.70 ± 0.04	0.80 ± 0.11

Data are expressed as means ± SE. T$_4$, thyroxine; T$_3$, 3,5,3'-triiodothyronine; TSH, thyrotropin. $P < 0.01$: [*] pregnant vs. controls.

Table 1 shows that plasma T$_4$ decreased in pregnant rats. Plasma T$_3$ and plasma TSH did not change significantly. Plasma clearance rates (PCR) and production rates (PR) for T$_4$ and T$_3$ are expressed per total animal and per 100 g body wt (Table 2). No alterations were found in the PCR for T$_4$ and T$_3$ when expressed per 100 g

body wt, however the PCR for T$_4$ increased significantly per total animal. The production of T$_4$ remained constant for the total animal. For T$_3$ the PAR decreased per 100 g body wt but remained constant per total animal. Per total animal the contribution of T$_3$ produced by the thyroid increased, whereas extrathyroidal T$_3$ production showed a decrease.

Tabel 2: Plasma clearance rates for T$_4$ and T$_3$, production rate for T$_4$, plasma appearance rate for T$_3$ and T$_3$ production by the thyroid and local conversion from T$_4$ in nonpregnant controls and 20-day pregnant rats. All values are expressed per total animal and per 100 g body wt.

	Per total animal		Per 100 g body wt	
	Controls (n=10)	Pregnant (n=9)	Controls (n=10)	Pregnant (n=9)
PCR T$_4$, ml/hr	2.16 ± 0.21	3.47 ± 0.23*	0.95 ± 0.10	1.14 ± 0.07
PCR T$_3$, ml/hr	46.7 ± 3.6	51.7 ± 2.2	20.4 ± 1.6	16.9 ± 0.7
PR T$_4$, pmol/hr	63.3 ± 6.6	64.1 ± 5.8	27.7 ± 2.9	21.0 ± 1.8
PAR T$_3$, pmol/hr	28.5 ± 2.4	26.5 ± 1.3	12.5 ± 1.2	8.7 ± 0.4*
ThPR T$_3$, pmol/hr	7.5 ± 2.3	13.3 ± 1.6*	3.3 ± 1.0	4.3 ± 0.5
T$_3$ (T$_4$), pmol/hr	21.1 ± 2.7	13.2 ± 0.9*	9.2 ± 1.2	4.4 ± 0.3*

Data are expressed as means ± SE. PCR, plasma clearance rate; PR, production rate; PAR, plasma appearance rate; ThPR, production rate of T$_3$ by the thyroid; T$_3$ (T$_4$), production rate of T$_3$ from local conversion of T$_4$. $P < 0.05$: * pregnant vs. controls.

The total tissue concentrations of T$_4$ and T$_3$ are shown in Fig. 1. In all tissues the T$_4$ concentration diminished significantly. The total T$_3$ concentration decreased in all organs, except the ovary which exhibited an increase.

Figure 2 shows the contributions of plasma-derived and locally produced T$_3$ in the organs. The amount of T$_3$ derived from plasma decreased in brown adipose tissue (BAT), heart, kidney, liver, muscle and pituitary. The amount of T$_3$ produced locally decreased in BAT and brain. In the ovary an increase was found for T$_3$ derived from both plasma and local production. The percentage local conversion (that is the %T$_3$ locally produced from T$_4$ in relation to the total T$_3$) was lower in BAT, whereas an increase was found for the ovary. For all other organs the percentage local conversion remained constant.
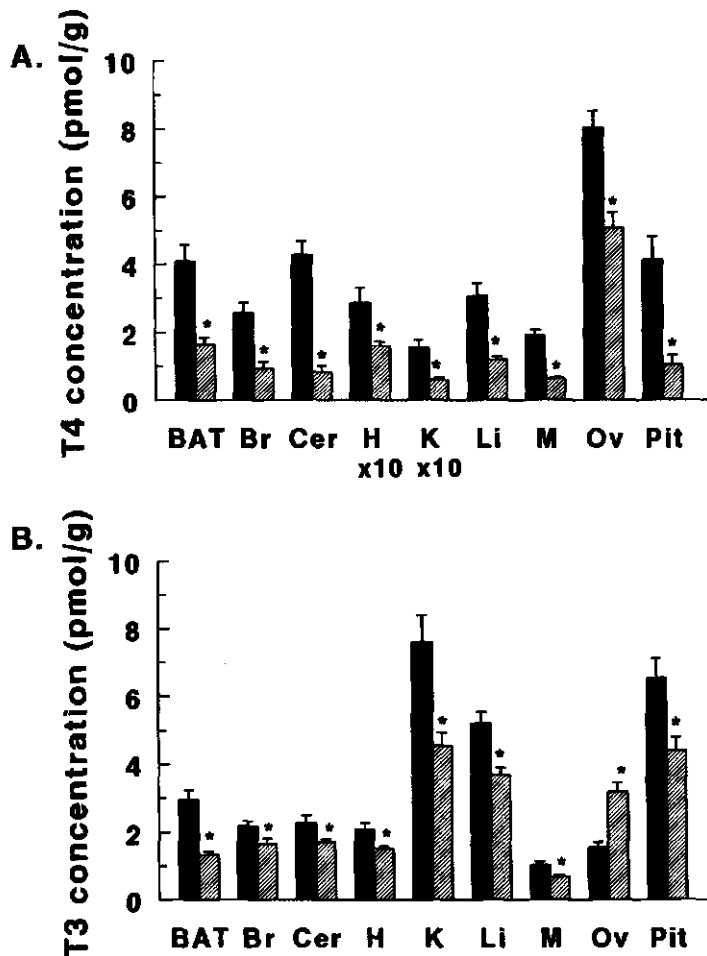
Figure 1: Concentrations of T_4 (A) and T_3 (B) in brown adipose tissue (BAT), brain (Br), cerebellum (Cer), heart (H), kidney (K), liver (Li), muscle (M), ovary (Ov) and pituitary (Pit) from nonpregnant (solid bars) and 20-day pregnant (hatched bars) rats. Values are mean ± SE. * P < 0.05.

The tissue-to-plasma ratio for T_4 decreased in all tissues, except the heart and ovary (Table 3). For $[^{131}I]T_3$ the tissue-to-plasma ratio decreased in BAT, kidney, liver and pituitary, whereas it increased in the ovary (Table 4). Table 5 shows the ratio of $[^{125}I]T_3$-to-$[^{125}I]T_4$. This ratio increased for the brain, cerebellum, liver, muscle, ovary and pituitary.

T3 (pmol/g)

plasma derived

locally produced

% Lc

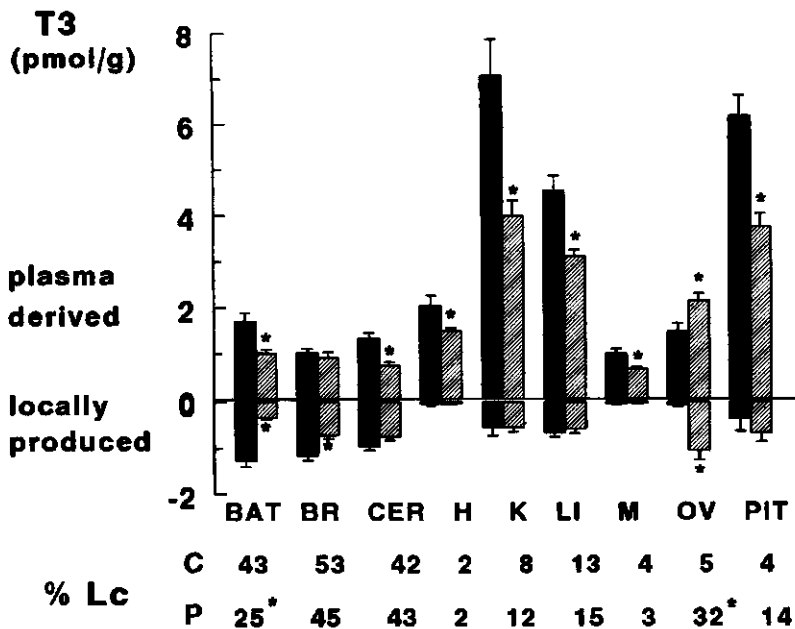|	BAT	BR	CER	H	K	LI	M	OV	PIT
C	43	53	42	2	8	13	4	5	4
P	25*	45	43	2	12	15	3	32*	14

Figure 2: Concentrations of T_3 derived from plasma and produced locally in several organs (see figure 1) from nonpregnant (solid bars) and 20-day pregnant (hatched bars) rats. Values are mean ± SE. * P < 0.05.

% Lc is the percentage local conversion (i.e. the percentage T_3 locally produced from T_4 in relation to the total T_3) in nonpregnant (C) and 20-day pregnant (P) rats.

Table 3: Tissue-to-plasma ratios for T_4 in control and 20-day pregnant rats.

	Controls	Pregnant	p value
BAT	0.144 ± 0.019	0.089 ± 0.011	< 0.05
brain	0.091 ± 0.013	0.049 ± 0.008	< 0.01
cerebellum	0.160 ± 0.027	0.041 ± 0.007	< 0.001
heart	0.091 ± 0.009	0.080 ± 0.006	ns
kidney	0.516 ± 0.062	0.319 ± 0.025	< 0.05
liver	1.017 ± 0.072	0.640 ± 0.035	< 0.001
muscle	0.066 ± 0.007	0.034 ± 0.002	< 0.001
ovary	0.283 ± 0.029	0.253 ± 0.012	ns
pituitary	0.145 ± 0.023	0.052 ± 0.012	< 0.005

Data are expressed as means ± SE.

Tabel 4: Tissue-to-plasma ratios for $[^{131}I]T_3$ in control and *20-day* pregnant rats.

	Controls	Pregnant	p value
BAT	2.76 ± 0.30	1.92 ± 0.12	< 0.05
brain	1.67 ± 0.11	1.80 ± 0.22	ns
cerebellum	2.17 ± 0.19	1.87 ± 0.11	ns
heart	3.31 ± 0.32	2.90 ± 0.13	ns
kidney	11.42 ± 1.13	7.75 ± 0.53	< 0.05
liver	7.35 ± 0.43	6.07 ± 0.29	< 0.05
muscle	1.60 ± 0.15	1.29 ± 0.08	ns
ovary	2.41 ± 0.30	4.13 ± 0.23	< 0.001
pituitary	10.14 ± 0.81	7.31 ± 0.63	< 0.01

Data are expressed as means ± SE.

Table 5: Tissue $[^{125}I]T_3$-to-$[^{125}I]T_4$ ratios in control and *20-day* pregnant rats.

	Controls	Pregnant	p value
BAT	0.728 ± 0.171	0.567 ± 0.075	ns
brain	0.796 ± 0.072	1.532 ± 0.201	< 0.01
cerebellum	0.473 ± 0.056	1.916 ± 0.315	< 0.001
heart	0.594 ± 0.062	0.511 ± 0.041	ns
kidney	0.390 ± 0.025	0.444 ± 0.042	ns
liver	0.137 ± 0.010	0.186 ± 0.012	< 0.01
muscle	0.407 ± 0.047	0.574 ± 0.060	< 0.05
ovary	0.142 ± 0.017	0.422 ± 0.041	<0.001
pituitary	1.341 ± 0.180	3.621 ± 0.837	< 0.05

Data are expressed as means ± SE.

DISCUSSION

In this study we were able to determine the contribution of local conversion of T_4 to T_3 in several tissues of the near-term pregnant rat. The method, the surgical proce-dure to insert the cannula and the subsequent continuous infusion of $[^{125}I]T_4$ and $[^{131}I]T_3$ did not have a negative effect on pregnancy. This is demonstrated by the

normal bodyweight and number and weight of fetuses in the near-term pregnant rats. In accordance with previous reports pregnancy resulted in a decrease in plasma T_4. In contrast, plasma T_3 did not decrease significantly in this study (2, 12, 20).

The plasma clearance rates for T_4 and T_3 were not altered significantly. However, when expressed per total animal instead of per 100 gram body wt an increase in the PCR for T_4 was found. This is in agreement with the results of kinetic experiments (20).

By expressing the production rates per total animal a more realistic comparison can be made between nonpregnant and near-term pregnant rats. In the pregnant rat the weight of maternal tissues is not increased in proportion to the bodyweight; the enormous gain in bodyweight is mainly due to the feto-placental compartment. However, this compartment does not contribute essentially to the production of maternal T_4 and T_3.

Despite normal plasma TSH values the production of T_3 by the thyroid is enhanced, in contrast to the thyroidal production of T_4 which remained constant. Therefore, the T_3-to-T_4 ratio for thyroid hormones produced by the thyroid of the pregnant rat is increased. This pattern is also found during iodine deficiency (15), diabetes mellitus and modified fasting (17). On the other hand, the contribution of peripherally produced T_3 entering the plasma decreased. This implies that less T_3 was produced, or reached the plasma, in those organs which contribute to circulating T_3, i.e. liver and kidney (4). The increase in thyroidal T_3 production, and the decrease in peripherally produced T_3 in the plasma together resulted in an unchanged plasma appearance rate.

The amount of T_4 decreased drastically in all organs. For some organs this had already been reported by Calvo et al. (2). The tissue-to-plasma ratio for T_4 decreased in all organs except the ovary and heart. Therefore, the transport of plasma T_4 to the maternal organs diminished, resulting in a tissue T_4 concentration which was decreased even more than the plasma T_4 level. In contrast, a marked increase in the transport from plasma to the fast pool has been found in kinetic experiments (20). However, in the normal situation the fast pool consists mainly of liver and kidney (3). We have already suggested that in the near-term pregnant rat the feto-placental compartment also belongs to the fast pool (20). This implies that less T_4 is available for the maternal organs, an hypothesis which is confirmed by the results of this study: the transport of T_4 to the maternal organs decreased.

For T_3 the total tissue concentration diminished in all organs except the ovary, which

exhibited a pronounced increase in the T_3 concentration. The ovary is the only organ in which the percentage T_3 produced locally from T_4 had increased in relation to the total T_3. This could mean that the ovary is metabolically very active at the end of gestation. It is not clear why this occurs.

The tissue-to-plasma ratio for plasma-derived T_3 ($[^{131}I]T_3$) decreased in BAT, liver, kidney and pituitary, which means that less T_3 was transported from plasma to these organs. This is also demonstrated by the decreased amount of plasma-derived T_3 in these organs.

The ratio of $[^{125}I]T_3$-to-$[^{125}I]T_4$ increased in the brain and cerebellum. Since the $[^{125}I]T_3$-to-$[^{125}I]T_4$ ratio is a marker for the activity of deiodinase enzymes, the activity of ID-II must have increased. This was confirmed by measurements of ID-II activity in the brain of near-term pregnant rats (2, 20). However, despite this increase in ID-II in the brain there was not an increase but a decrease in locally produced T_3. This has to be explained by the decrease in T_4 in the brain. In BAT the ratio of $[^{125}I]T_3$-to-$[^{125}I]T_4$ remained unchanged, indicating that no alterations in ID-II activity occur in BAT during pregnancy. Therefore the decrease in the local production of T_3 in BAT has to be the result of a diminished availability of precursor T_4.

The ratio of $[^{125}I]T_3$-to-$[^{125}I]T_4$ also increased in the liver, but not in the kidney which has to be the result of an increase in the activity of ID-I. It is known that the activity of deiodinase type I is increased in the liver of near-term pregnant rats (2, 20), indicating that an increased fraction of T_4 is monodeiodinated to T_3. In this way, despite the decrease in T_4, a normal level of locally produced T_3 could be reached in the liver.

In conclusion: in the near-term pregnant rat normal tissue T_3 values cannot be maintained. The tissue T_4 concentrations are decreased so much that normal T_3 values cannot always be reached, not even in those organs in which deiodinase activity was elevated. Further regulation is not possible because there is not sufficient T_4 available for the local production of T_3. Therefore, the transport of T_4 to the feto-placental compartment results indirectly in a T_3 deficiency in the maternal organs.

ACKNOWLEDGEMENTS

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CHAPTER 4

EFFECTS OF MARGINAL IODINE DEFICIENCY DURING PREGNANCY:

IODIDE UPTAKE BY THE MATERNAL AND FETAL THYROID.

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ABSTRACT

Iodide uptake by the thyroid is an active process. Iodine deficiency and pregnancy are known to influence thyroid hormone metabolism. The aim of this study was to clarify the effects of iodine deficiency and pregnancy on iodide uptake by the thyroid. Radioiodide was injected intravenously into nonpregnant and 19-day pregnant rats receiving a normal or marginally iodine-deficient diet. The uptake of radioiodide by the thyroid was measured continuously for 4 h. The absolute iodide uptake by the maternal and fetal thyroids at 24 h was calculated by means of the urinary specific activity. Pregnancy resulted in a decrease in the absolute thyroidal iodide uptake. Marginal iodine deficiency had no effect on the absolute iodide uptake by the maternal thyroid. The decreased plasma inorganic iodide was compensated by an increase in thyroidal clearance. A similar compensation was not found for the fetus; the uptake of iodide by the fetal thyroid decreased by 50% during marginal iodine deficiency. This can lead to diminished thyroid hormone production, which will have a negative effect on fetal development, especially of the brain.

thyroxine; 3,5,3'-triiodothyronine; plasma inorganic iodide; iodide kinetics.

INTRODUCTION

It is known that thyroid function is affected by various physiological conditions, for instance, food deprivation (24), pregnancy (10, 11, 12), and iodine deficiency (19, 23). Iodide is an essential element for the production of thyroid hormones. The uptake of iodide by the thyroid gland is an active process that is regulated by thyrotropin (TSH) (26) and the thyroidal blood flow (3). Alterations in the thyroidal uptake of iodide can cause changes in the production of thyroid hormones.

Iodine deficiency affects the physical and mental development of humans in large areas of the world (13). In rats it has been shown that during iodine deficiency the plasma thyroxine (T_4) level decreased, while the 3,5,3'-triiodothyronine (T_3) level remained unchanged (23). The weight of the thyroid and the T_3-to-T_4 ratio in the thyroid of rats on a low-iodine diet increased (19, 23). Studies of 10-day-old rats have shown that the thyroidal iodide uptake was considerably higher in iodine-deficient rats than in controls (31).

Physiological changes in thyroid function also occur during pregnancy. Normal pregnancy in humans is accompanied by a rise in T_4-binding globulin and total T_4 and T_3 (12). However, the free T_4 level is decreased at the end of gestation (29). The availability of iodide for the maternal thyroid is decreased because of increased renal clearance (1) and transport to the feto-placental complex during the late phase of gestation (10). The maternal thyroid is enlarged and the radioiodide uptake, expressed as a percentage of the dose, is increased during pregnancy (1, 10, 13).

In the rat normal pregnancy results in a decrease in plasma total T_4 and T_3 concentrations (4). This is comparable to the decrease in free T_4 in the third trimester of human pregnancy (29). The thyroidal uptake of ^{131}I is decreased in the pregnant rat (9, 11, 15). No changes were found in the urinary excretion of iodide during the last days of gestation (9). Iodine deficiency induces a further decrease in plasma T_4 in the near-term pregnant rat (8). Also the weight of the thyroid is increased by iodine deficiency in the pregnant rat (7).

Most studies of the iodide uptake in rats are performed by giving a tracer injection of radioactive isotope of iodide (^{131}I, ^{125}I or ^{123}I). After a certain period the thyroid is removed and the percentage of the injected radioactivity is calculated (9, 11, 15, 31). In our laboratory we developed a method for measuring continuously the in vivo uptake of ^{125}I by the thyroid. By means of this method it is possible to study not only the amount of iodide taken up by the thyroid, but also the kinetics of the thyroidal uptake of iodide. We were also able to calculate the absolute iodide uptake by the thyroid.

The aim of this study was to clarify the effects of pregnancy and iodine deficiency on iodide uptake by the thyroid. We used four groups of rats: nonpregnant and near-term (day 19) pregnant rats receiving a normal iodine diet or a marginally iodine-deficient diet. This marginally iodine-deficient situation closely reflects the iodine status in large populations of humans in the world. As previously described, pregnancy as well as iodine deficiency affects plasma thyroid hormone levels. However, the effects on the absolute iodide uptake by the thyroid are unknown. In particular iodine-deficient, near-term pregnant rats represent an important group. What are the effects of iodine deficiency on iodide metabolism in the near-term pregnant rat? Are the low amounts of iodide available taken up totally by the maternal thyroid or is there still iodide available for the fetuses?

MATERIAL AND METHODS

Animals

Three-month-old female Wistar rats (CPB/WU, IFFA CREDO, Brussels) were used. The rats were individually housed in metabolic cages at 22 °C, with alternating 14-h light and 10-h dark periods. The animals were fed a semisynthetic American Institute of Nutrition (AIN) diet (2) mixed with distilled water (60 % dry weight - 40 % water) and potassium iodide: 55 ng [normal iodine dose (NID)] or 2.9 ng [marginal iodine dose (MID)] per gram of feed. The marginally iodine-deficient groups received 1% $KClO_4$ in the drinking water during the first two days of the MID, at least four weeks before measurement was started. $KClO_4$ was given to accelerate thyroidal depletion of total iodine stores.

After two regular estrus cycles the rats were mated. Mating was confirmed by the presence of sperm in a vaginal lavage the following morning, called *day 0* of pregnancy.

Design of the study

In this study four groups of animals were studied:
1. nonpregnant rats on a normal diet (NID, C)
2. pregnant rats on a normal diet (NID, P)
3. nonpregnant rats on a marginally iodine-deficient diet (MID, C)
4. pregnant rats on a marginally iodine-deficient diet (MID, P)

The pregnant rats were assessed at the end of gestation, i.e. *day 19*. Fetuses are delivered on *day 21*. The more frequently used *day 20* was not suitable in this experiment, because the animals were killed and bled twenty-four hours after the injection of radioiodide.

Daily feed intake and urinary iodide excretion were determined for all rats. The mean feed intake was 30 g, resulting in a daily iodide intake of 1.3 µg for the NID groups and 66 ng for the MID groups.

Urinary iodide was determined as described by Sandell and Kolthoff (22).

The experiments were approved by the University Committee on Animal Care and Use of the Agricultural University of Wageningen.

Thyroidal iodide uptake

The rat was anesthetized with xylazine (25 µl of 2 % rompun^R/100 g body wt; sc), atropine (5 µg/100 g body wt; sc) and ketamine (50 µl of 10 % ketamine/100 g body wt; ip). The anesthesized rat was fixed in a bed of plaster. The body temperature was controlled in the rectum and maintained at 38 °C by a warming jacket.

The scintillation probe [type 42A. Nal crystal 23 mm, 1 mm thick, connected to mini-Assay type 6-20 (Mini Instruments Ltd, Essex, UK)] was placed as close as possible to the thyroid region.

To measure the thyroidal iodide uptake in the rat, a cannula inserted into the right vena jugularis (20) and a 400 µl bolus of saline containing 10 µCi carrier free Na^{125}I (Amersham, Aylesbury, UK) was injected. The radioactivity taken up by the thyroid was measured automatically every thirty seconds for 4 h and calculated as a percentage of the injected dose. The percentage dose of iodide taken up by the thyroid was fitted to the equation % dose = A [1-exp(-t/γ)], by non-linear regression analysis using the program NLFIT/FIT4EXP, which is based on the Levenberger-Marquart method. 'A' represents the maximum percentage of dose taken up by the thyroid and γ is the time constant (min).

Perchlorate discharge test

To investigate the effects of perchlorate on radioactivity in the thyroid three animals from each group underwent a perchlorate-discharge test (17). Four hours after the administration of Na-^{125}I 10 mg/kg BW potassium perchlorate was administrated i.p.. Thyroidal radioactivity was measured for another 30 minutes.

Iodide kinetics in plasma

To study the disappearance of the injected ^{125}I, plasma samples (50-100 µl blood) were taken via the cannula in the vena jugularis 1, 3, 5, 10, 16, 25, 40, 80, 150 and 240 min. after injection of the ^{125}I. Radioactivity in the plasma samples was expressed as percentage dose per milliliter.

The disappearance of iodide from plasma can be described by an exponential function. The data were fitted, together with the plasma volume at $t(0)$, individually to sums of n=1-3 exponentials (6)

$$Y(t)= A_1 \exp(\lambda_1{}^*t)+ A_2 \exp(\lambda_2{}^*t) + A_3 \exp(\lambda_3{}^*t)$$

using the program DIMSUM, whereby A_i coefficients are expressed as percentage dose per milliliter and exponents λ_i are expressed per minute (16).

Absolute iodide uptake after 24 h.

After measurement the rats were placed in metabolic cages to collect urine.

Twenty-four hours after injection of the ^{125}I the rats were killed by bleeding and perfusion with saline under ether anesthesia. The maternal thyroid, mammary gland, placentas and fetuses were removed. The fetal thyroid was collected by excising that part of the trachea containing the thyroid.

All radioactivities measured (i.e., maternal and fetal thyroid, plasma, urine, mammary gland, placentas and fetuses minus thyroid at 24 h) were expressed as percentages of the injected dose.

The assumption can be made that, during a steady state situation the specific activity of iodine (ratio of ^{125}I to ^{127}I) in urine is the same as that in the plasma from which it originated (28). The absolute iodide uptake (AIU) by the maternal and fetal thyroids, mammary gland, placentas, and fetuses minus thyroid as well as the plasma inorganic iodide (PII) can be calculated after determination of the specific activity in urine (1).

$$AIU = \frac{\% \text{ dose after 24 hr}}{\% \text{ dose urine/ng iodide urine}} \quad (= ng/ 24 \text{ hr.}).$$

$$PII = \frac{\% \text{ dose/ml plasma}}{\% \text{ dose urine/ng iodide urine}} \quad (= ng/ml).$$

Radioiodide clearance by the thyroid (C_{Th}) and by the kidneys (C_R) is calculated from the increment between 120 and 240 min divided by the radioactivity in a plasma sample collected simultaneously (1).

$$C_{Th} = \frac{\% \text{ dose/min in thyroid}}{\% \text{ dose/ml plasma}} \quad (= ml/min).$$

$$C_R = \frac{\% \text{ dose urine}}{\% \text{ dose/ml plasma} * 1440 \text{ min}} \quad (= ml/min).$$

Statistical analysis

All data are expressed as means ± SE. Data were analyzed using the Statistical Package for Social Sciences (25). All data were subjected to one-way analysis of variance and statistical differences between groups were determined using the least significant difference method.

RESULTS

Marginal iodine deficiency had no effect on either the bodyweight of the rats or the number of fetuses. No significant alterations were found for plasma TSH. Plasma T_4 and T_3 did not change significantly during marginal iodine deficiency. Pregnancy resulted in a decrease in plasma T_4 and T_3 in NID as well as MID rats (Table 1).

Table 1: Body weight and plasma thyroid hormone concentrations.

	NID, C (n=13)	NID, P (n=10)	MID, C (n=8)	MID, P (n=8)
Body weight, g	229 ± 4	300 ± 5*	235 ± 4	304 ± 6*
# fetuses		11 ± 1		11 ± 1
T_4, nM	31.4 ± 0.6	21.2 ± 1.5*	29.5 ± 2.4	16.9 ± 2.0*
T_3, nM	0.55 ± 0.04	0.49 ± 0.03*	0.69 ± 0.06	0.47 ± 0.04*
TSH, ng/ml	0.51 ± 0.09	0.48 ± 0.07	0.75 ± 0.05	0.65 ± 0.09

Values are means ± SE. NID and MID, normal and marginal iodine dose, respectively; C, control rats; P, near-term pregnant rats; T_4, thyroxine; T_3, 3,5,3'-triiodothyronine; TSH, thyrotropin. * $P < 0.05$, P vs. C.

Feed intake and urinary iodide excretion

At the start of the experiments, the marginally iodine-deficient groups received potassium perchlorate for 2 days. The effect of this treatment on urinary iodide excretion is shown in Fig. 1. Potassium perchlorate treatment from *day 0* to *day 2* resulted in an increase in iodide excretion. Within 1 wk after perchlorate treatment urinary iodide excretion had decreased to 0.4 µg/day; it remained constant during the rest of the experimental period.
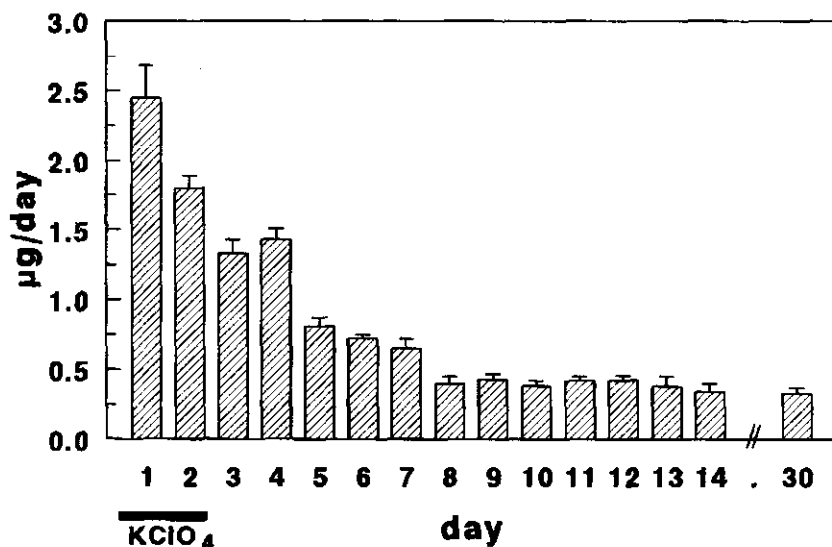
Figure 1: Effect of $KClO_4$ treatment on the 24-h urinary iodide excretion in marginally iodine-deficient nonpregnant rats.

The mean feed intake and urinary iodide excretion for the four groups of rats are shown in Fig. 2. No effect of MID on feed intake was found. During pregnancy feed intake and urinary iodide excretion increased .
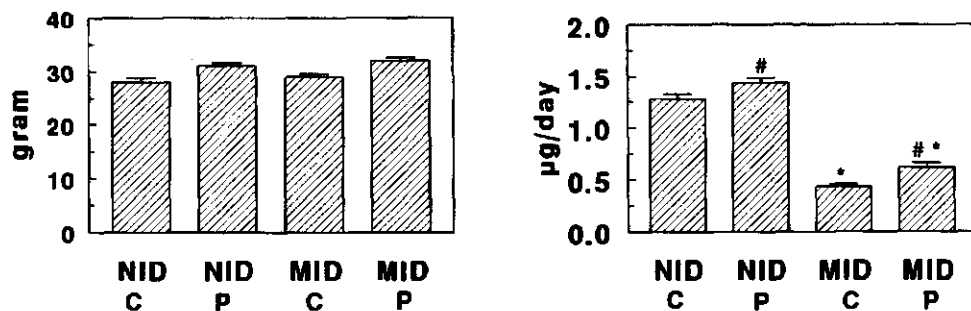

Figure 2: Effect of marginal iodine deficiency and pregnancy on the daily feed intake (A) and urinary iodide excretion (B). $P<0.05$: # pregnant (P) vs. control (C);
* marginal iodine dose (MID) vs. normal iodine dose (NID).

Thyroidal ^{125}I uptake

Table 2 shows the data on thyroidal ^{125}I uptake. The weight of the thyroid increased significantly in both marginally iodine-deficient groups; pregnancy had no effect on the weight of the thyroid.

Table 2: Weight of thyroid and parameters of uptake of radioiodide by thyroid.

	NID, C (n=17)	NID, P (n=23)	MID, C (n=15)	MID, P (n=15)
Thyroid, mg	17.3 ± 1.8	17.4 ± 1.0	27.3 ± 1.5@	23.5 ± 1.1@
Uptake				
4-h, %dose	21.2 ± 1.7	13.4 ± 1.1*	52.3 ± 4.2@	35.7 ± 3.8*@
A, %dose	26.9 ± 2.2	16.0 ± 1.2*	51.3 ± 9.5@	42.2 ± 4.4*@
γ, min	166 ± 20	139 ± 9	125 ± 16	112 ± 15

Values are means ± SE. A, maximum %dose taken up by the thyroid; γ, time constant.
$P < 0.05$: * P vs. C; @ MID vs. NID.

The 4-h ^{125}I uptake by the thyroid increased in C and P MID rats. Pregnancy induced a decrease in ^{125}I uptake by the thyroid in both NID and MID rats. The 4-h ^{125}I uptake is a direct measurement of the thyroid; A and γ are the results of fitting the data to the one-exponential function %dose=A [1-exp(-t/γ)]. Two- and three-exponential functions did not yield satisfactory results. The time constant (γ) of the ^{125}I uptake was unchanged in all groups. The mean fitted thyroidal ^{125}I uptake is given in Fig. 3, where we also see that the thyroidal ^{125}I uptake was lower in P rats and increased in the MID groups.

Perchlorate discharge test

No discharge of radioiodide from the thyroid was found for any of the four groups. The radioactivity of the thyroid remained unchanged (results not shown).

Iodide kinetics in plasma

Table 3 shows the parameters of iodide kinetics in plasma. No significant changes were found except for A_1 in pregnant rats.
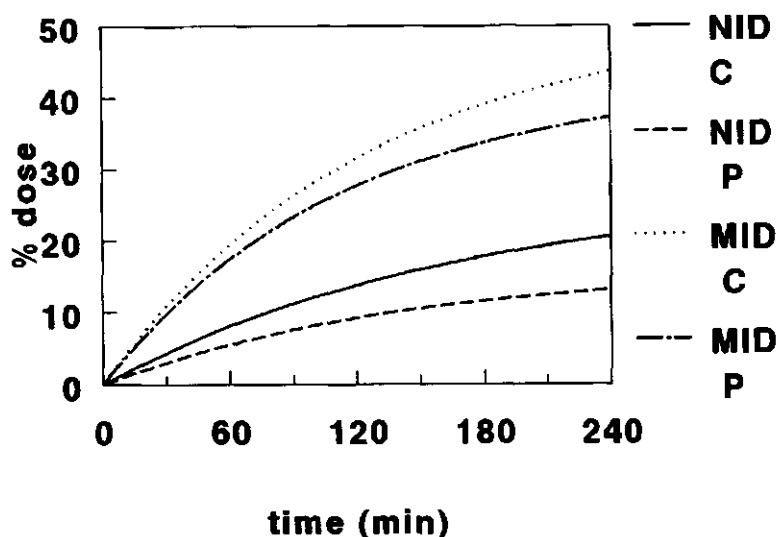
Figure 3: Fitted 4-h thyroidal radioiodide uptake based on mean maximal %dose taken up (*A*) and time constant (γ) values.

AIU after 24 h.

Table 4 shows the specific activity of urine and the results of calculations of the AIU by the maternal thyroid, PII, C_{Th} and C_R . The urinary specific activity was un-changed during pregnancy, but marginal iodine deficiency caused an increase in specific activity. The AIU by the thyroid was decreased by pregnancy, while marginal iodine deficiency had no effect on the AIU. PII was decreased in the MID groups, whereas pregnancy had no effect. Unidirectional iodide C_{Th} was increased in the MID groups. C_R was increased by pregnancy, whereas MID resulted in a decrease in C_R in C and near-term P rats (Table 4).

The AIU values at 24 h by the maternal thyroid, placentas, fetal thyroids, remaining part of the fetuses and mammary gland are shown in Fig. 4. Whereas MID had no effect on the AIU by the maternal thyroid, a pronounced decrease was found for the fetal thyroids and the remaining part of the fetuses. The amount of iodide taken up by the mammary gland was also decreased by MID, whereas no significant effect was found for the placentas.

Table 3: Parameters of iodide kinetics in plasma.

	NID, C (n=13)	NID, P (n=10)	MID, C (n=8)	MID, P (n=5)
A_1, %dose/ml	7.05 ± 0.42	4.92 ± 0.13*	7.01 ± 0.16	5.34 ± 0.22*
λ_1, 1/min	-1.78 ± 0.13	-2.32 ± 0.24	-2.47 ± 0.13	-2.28 ± 0.21
A_2, %dose/ml	1.14 ± 0.15	1.19 ± 0.13	1.12 ± 0.07	1.22 ± 0.16
λ_2, 1/min	-0.068 ± 0.014	-0.085 ± 0.013	-0.059 ± 0.004	-0.072 ± 0.009
A_3, %dose/ml	0.806 ± 0.067	0.762 ± 0.049	0.837 ± 0.055	0.767 ± 0.069
λ_3, 1/min	-0.0019 ± 0.0003	-0.0022 ± 0.0007	-0.0025 ± 0.0003	-0.0034 ± 0.0007

Values are means ± SE. $P < 0.05$, * P vs. C.

Tabel 4: Urinary specific activity, absolute iodide uptake by thyroid at 24 h, plasma inorganic iodide, and clearance of iodide from plasma by thyroid and kidneys.

	NID, C (n=7)	NID, P (n=12)	MID, C (n=5)	MID, P (n=7)
Urine, % dose/ng	0.022 ± 0.003	0.023 ± 0.003	0.034 ± 0.005	0.037 ± 0.005@
AIU, µg/ 24 h	1.29 ± 0.09	0.87 ± 0.16	1.57 ± 0.35	0.89 ± 0.14*
PII, ng/ml	24.5 ± 4.6	19.9 ± 2.4	11.7 ± 3.6@	9.11 ± 2.3@
C_{Th}, ml/min	0.079 ± 0.014	0.079 ± 0.015	0.367 ± 0.172@	0.164 ± 0.044
C_R, ml/min	0.149 ± 0.010	0.228 ± 0.040	0.081 ± 0.019@	0.173 ± 0.032*

Values are means ± SE. AIU, absolute iodide uptake; PII, plasma inorganic iodide; C_{Th} and C_R, clearance of iodide from plasma by thyroid and kidneys. $P < 0.05$; * P vs. C; @ MID vs. NID.
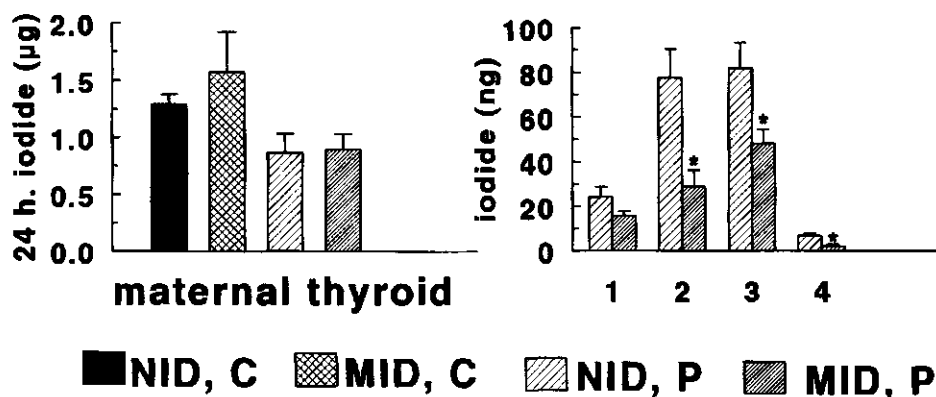
Figure 4: Absolute iodide uptake by the maternal thyroid and feto-placental compartment; 1. placentas; 2. fetal thyroids; 3. fetuses minus thyroids; 4. mammary gland (1 g). * $P<0.05$; NID,P vs. MID,P.

DISCUSSION

The process by which the thyroid gland adapts to an insufficient iodine supply is to increase the trapping of iodide. When iodine intake is low, adequate secretion of thyroid hormones may still be achieved by marked modifications of thyroid activity. The thyroid is stimulated by an increased thyroid blood flow (3) and TSH (19, 23). The T_3-to-T_4 ratio of iodothyronines secreted by the thyroid is increased during iodine deficiency, especially because of a decrease in T_4 production (23). Marginal iodine deficiency was induced in our rats by feeding them a MID diet. Within 2 wk of the start of the MID diet, urinary iodide excretion remained at the same level. So, at the moment of measurement, at least 4 wk after start of the MID diet, the rats were in a steady state. The fact that only a marginal iodine-deficient state was achieved, and not a severe iodine-deficient state, situation is demonstrated by the unchanged plasma T_4 and T_3 levels. Despite a slight increase in plasma TSH, the weight of the thyroid had already increased by 50%. The unchanged number of fetuses during pregnancy also demonstrates that the induced iodine deficiency was not severe (8).
In this study we were able to measure thyroidal radioiodide uptake continuously. For the kinetic analysis of radioiodide uptake in the human thyroid a three-compart-

mental model is used (3, 14, 30). In this model, a plasma iodide pool and an inorganic and an organic thyroidal iodide pool can be distinguished. We tried to fit our data to this model; however, our data could only be fitted to a one-exponential function. Therefore, we assume that, even in euthyroid rats, iodide transport from plasma into the thyroid is unidirectional because of an extremely avid iodine organification. This idea is confirmed by the total absence of a perchlorate discharge in all rats, meaning that there is no efflux of inorganic iodide from the thyroid (17).

The thyroidal uptake of radioiodide was stimulated by marginal iodine deficiency. The kinetic analysis shows that the time constant for thyroidal radioiodide uptake was not affected by iodine deficiency, meaning that the time needed to achieve the maximum effect remained the same. However, the AIU by the thyroid remained normal. This was achieved by an increased C_{Th} resulting from the increased thyroid volume and thyroidal blood flow (3).

The urinary specific activity was increased, whereas the radioiodide activity in plasma was not altered by marginal iodine deficiency. This resulted in a PII that was significantly lower, such as is found in humans residing in areas of iodine deficiency (5) and in 10-day-old iodine deficient rats (31).

Our observations emphasize the need for caution in interpretation of results obtained by studies measuring the thyroidal radioiodide uptake only. We have demonstrated that a decrease or increase in radioiodide uptake does not automatically mean that the AIU has changed.

Also during normal pregnancy changes in iodide metabolism are found. The urinary iodide excretion was higher for P than C rats of the NID as well as MID groups. This can be explained by the increased feed intake and the increase in C_R. Galton (11) also found an increased urinary iodide excretion for pregnant rats during the last days of gestation (11).

At the end of gestation the percentage radioiodide taken up by the thyroid was significantly decreased. This has also been reported for rats with a normal iodine intake (9, 11, 15). Because the urinary specific activity was not altered by pregnancy, this also resulted in a decrease in the absolute iodide uptake by the maternal thyroid at the end of gestation in NID as well as MID rats. It seems that the thyroid of the near-term P rat has less iodide available for the production of thyroid hormones. However, no change in thyroidal hormone production is found at the end of gestation (27). This would seem to imply that the use of iodide in the thyroid of the near-term P rat is more efficient or that the thyroglobulin stores are depleted.

The difference in thyroidal iodide uptake between C and near-term P rats cannot be attributed entirely to the increase in urinary iodide excretion. We suggest that the remaining part of the iodide is used by the mammary glands and the feto-placental compartment. For lactating rats it has been shown that, 4 h after injection, the mammary glands contain as much radioiodide as the thyroid, and it was suggested that during pregnancy radioiodide was already being transported to the mammary glands (15). The latter was contradicted by our measurements. One gram of the mammary gland had only taken up 6.5 ng iodide at 24 h; this is less than 1% of the thyroidal uptake.

The placenta possesses a mechanism for actively transporting iodine (21). There-fore, it is to be expected that a certain amount of radioiodide is transported to the fetal compartment. Our results, collected 24 h after injection, show that the fetal thyroid is capable of concentrating iodide on *day 20* of pregnancy. The thyroidal region of the fetus contained as much iodide as the rest of the fetus. However, the total amount of iodide in placentas and fetuses could not explain the decreased uptake of iodide by the maternal thyroid. Therefore, the feto-placental compartment and the mammary glands are not the only factors responsible for the difference in maternal thyroidal iodide uptake.

During marginal iodine deficiency the AIU of the maternal thyroid remained normal. However, there was already a tendency toward a shift in thyroid hormone synthesis; T_4 decreased and T_3 remained normal. In contrast to the maternal thyroid no com-pensation, by an increased thyroid clearance for the decreased PII concentration was found for the fetal thyroid. The AIU by the fetal thyroid was decreased by 50%. This pattern can lead to lower T_4 availability for the fetus. This is supported by kinetic studies of marginally iodine-deficient, near-term pregnant rats showing that the maternal transfer of T_4 and T_3 to the fetuses is decreased during marginal iodine deficiency (unpublished data). As long as the increase in type II deiodinase is suf-ficient to maintain normal T_3 values in the fetal brain, problems need not be expected (18). If this is not achieved, defects in brain development will occur.

In conclusion: During pregnancy the AIU by the maternal thyroid gland is decreased. Marginal iodine deficiency does not affect the maternal AIU. The low availability of iodide was compensated by increased activity of the maternal thyroid, whereas fetal thyroidal uptake of iodide decreased by 50%. The fetus is apparently not able to regulate its iodide metabolism in case of marginal iodine deficiency. Therefore, this level of marginal iodine deficiency, despite the near euthyroid status of the mother, already has an effect on the availability of iodide for the fetus. This can mean that

fetal T_4 production is markedly diminished at a time when it is of eminent importance for the normal development of many organs, especially the brain.

ACKNOWLEDGEMENTS

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CHAPTER 5

EFFECTS OF MARGINAL IODINE DEFICIENCY ON THYROID HORMONE

PRODUCTION, DISTRIBUTION AND TRANSPORT IN NONPREGNANT AND

NEAR-TERM PREGNANT RATS.

P.M. Versloot, J.P. Schröder-van der Elst, D. van der Heide, and L. Boogerd

ABSTRACT

During pregnancy maternal thyroid hormones are of great importance for normal development of the central nervous system of the fetus. Iodine deficiency of the mother can result in an impaired development of the fetal brain. In large areas of the world the iodine intake is moderately low. To study the effects of marginal iodine deficiency (MID) on the production, distribution, and transport of thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) in nonpregnant and near-term pregnant rats we performed kinetic experiments (three-compartment analysis). Despite unchanged plasma T_4 and T_3 during MID, the production and plasma clearance rates of T_4 decreased, while these values for T_3 increased in nonpregnant rats. Hepatic deiodinase type I activity increased during MID. It appears that during MID rats are able to maintain their euthyroid status. The pronounced increase in transport of T_4 from plasma to the fast pool observed in normal pregnant rats did not occur during MID. The alterations in T_3 metabolism due to MID in pregnant rats were the opposite of those found for nonpregnant rats.

Conclusion: Marginal iodine deficiency affects maternal thyroid hormone metabolism, thus influencing the availability of maternal T_4 for the fetuses.

INTRODUCTION

In large areas of the world iodine intake is insufficient. Severe iodine deficiency can result in miscarriage, stillbirth, and congenital anomalies as well as the more familiar goiter, cretinism, impaired brain function and hypothyroidism in children and adults (15).

In rats iodine deficiency can be induced by prolonged administration of a low iodine diet. This treatment results in a markedly increased weight of the thyroid. The T_3-to-T_4 ratio in the thyroid as well as the T_3-to-T_4 ratio secreted by the thyroid increased progressively with the duration of iodine deficiency (12, 23). In the iodine-deficient rat plasma T_4 is decreased and plasma thyrotropin (TSH) increased while circulating T_3 remains normal (26). Since T_3 in the brain is generated mainly by local conversion from T_4 (8), normal circulating T_3 levels, in combination with low plasma T_4, are not sufficient to maintain euthyroidism in the brain (22).

Pregnancy also influences the thyroid hormone status. In humans normal pregnancy

is accompanied by a rise in the serum levels of total T_4 and T_3. However, due to a rise in T_4-binding globulin the free T_4 and T_3 levels decrease during pregnancy (11, 32). In the rat normal pregnancy also results in a decrease in plasma T_4 and T_3 (5, 29). This leads to reduced concentrations of T_4 and T_3 in the maternal tissues, except for T_3 in the brain (5). The clearance of T_4 from plasma is increased markedly which can be a result of the transport of T_4 to the fetal compartment (29).

Thyroid hormones are known to play an important role in brain maturation. Their absence during fetal development leads to irreversible brain damage. Studies of pregnant rats on a low iodine diet by the Madrid group revealed that when iodine deficiency is severe enough to cause very low maternal plasma T_4 levels, fetal plasma and tissues will suffer a shortage of T_4 and T_3 both before and after onset of fetal thyroid function (9, 21). During normal pregnancy mainly T_4 is transported from mother to fetus (4). In severely hypothyroid pregnant rats only T_4 can mitigate fetal T_4 and T_3 deficiency (3). In the fetal brain T_4 is necessary for the local generation of T_3 from T_4 by cerebral type II deiodinase (ID-II) (21).

Most experimental studies are performed during severe iodine deficiency. However, in large populations in the world the iodine intake is only marginally iodine-deficient. Data about the effects of marginal iodine intake on thyroid hormone metabolism are lacking. Therefore, we induced an iodine deficiency in rats such marginal that growth and reproduction outcome were not affected. The aim of this study was to determine the effects of marginal iodine deficiency on thyroid hormone metabolism in nonpregnant and near-term pregnant rats. T_4 and T_3 production, distribution, and transport were examined by performing a kinetic study using the three-compartment model of distribution and metabolism developed by DiStefano et al. (6, 7). In this model three compartments can be distinguished: 1) the plasma; 2) tissues that exchange T_4 and T_3 with plasma at a fast rate, i.e. the fast pool; and 3) the slow exchanging pool, the slow pool. Liver and kidney are presumed to be the main components of the fast pool, whereas skin, muscle, and brain belong to the slow pool (6, 7).

By means of this model we have already described the production, distribution, and transport of T_4 and T_3 in near-term pregnant rats on a normal iodine diet; we suggested that T_4 is transported very rapidly from plasma to the feto-placental compartment (29). In this study we will concentrate on the effects of marginal iodine deficiency on maternal thyroid hormone metabolism. Does a marginal iodine deficiency affect the availability of thyroxine for the feto-placental compartment ?

MATERIAL AND METHODS

Animals and diet

Three-month-old female Wistar rats (CPB/WU, Iffa Credo, Brussels) were used. The animals were individually housed at 22 °C, with alternating 14-h light and 10-h dark periods. The rats were fed a semi-synthetic American Institute of Nutrition (AIN) diet (2). The dry feed was mixed with distilled water (60 % dry weight- 40 % water) with the addition of potassium iodide: 55 ng (normal iodine diet; NID) or 2.9 ng (marginal iodine diet; MID) per gram of feed. The iodine-deficient groups received 1% $KClO_4$ in their drinking water during the first two days of the MID. $KClO_4$ was given to accelerate thyroidal depletion of total iodine stores.

The rats were mated after at least two regular estrus cycles. The day that sperm appeared in the vaginal smear was taken as day 0 of gestation. The gestational period of the rat is 22 days.

Design of the study

MID treatment was started at least two weeks before mating for the pregnant groups and minimally five weeks before measurements for the nonpregnant groups. Surgery (insertion of the cannula) was conducted one week before measurements.

There were four groups of rats:

1. nonpregnant rats on a normal diet ; NID-C.
2. pregnant rats on a normal diet ; NID-P.
3. nonpregnant rats on a marginal iodine-deficient diet ; MID-C.
4. pregnant rats on a marginal iodine-deficient diet ; MID-P.

Daily feed intake and urinary iodide excretion were determined for all rats. The mean feed intake was 30 gram, resulting in a calculated daily iodide intake of 1.3 µg for the NID groups and 66 ng for the MID groups.

Kinetic experiments were performed on day 19 of gestation. After the kinetic experiment the animals were killed and maternal and fetal liver and brain were collected.

The experiments were approved by the University Committee on Animal Care and Use of the Agricultural University of Wageningen.

Kinetic and analytical protocols

$[^{125}I]T_4$ and $[^{131}I]T_3$ (specific activity 80 and 130 Mbq/µg (2200 and 3500 µCi/µg), respectively) were prepared in our laboratory (2, 18). $Na^{125}I$ and $Na^{131}I$ were obtained from Amersham (Arlington Heights, IL); $L-T_3$ and 3,5-L-diiodothyronine, the respective substrates for labelling, were purchased from Sigma (St. Louis, MO). Purity of the tracers was assessed by means of HPLC.

Via a cannula inserted into the right jugular vein (24) the rats received a 400 µl bolus injection of a solution of 3700 Bq (10 µCi) $[^{125}I]T_4$ and 3700 Bq (10 µCi) $[^{131}I]T_3$ in saline containing heparin (0.3 U/ml; Organon, Tilburg, The Netherlands), ticarcillin (0.4 mg/ml; Ticarpen, Beecham S.A., Heppignies, Belgium) and 5 % normal rat serum. Ticarcillin is added to solutions containing labelled iodothyronines to prevent eventual bacterial infections and reduce artefactual deiodination, as described by Van Doorn (8).

From the canula in the jugular vein blood samples of 0.2 ml were taken at 1, 4.5, 10, 23, 44, 115, 202 and 315 min; 0.4 ml. were drawn at 465, 600, 900, 1200 and 1440 min, being the optimal time schedule according to DiStefano et al. (6, 7).

Fifty to hundred microliters plasma (in duplicate) were used to count the total ^{131}I and ^{125}I activities. The plasma samples were extracted with ethanol/ammonia (25 %)(197:3 v/v) with 0.1 mM propylthiouracil (PTU). The dried extracts were dissolved in 0.1 ml 0.2 M ammonia containing carrier T_4, T_3 and KI (1 mg/10 ml) and subjected to HPLC to separate the iodothyronines, according to the method described by Schröder-van der Elst and van der Heide (27).

The endogenous concentrations of T_4 and T_3 in plasma were measured by means of a specific rat RIA (14) of samples taken before the kinetic experiment was performed.

Plasma TSH was measured by the specific RIA developed for the rat by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health (USA). Reference preparation RP-2 was used as a standard.

Calculations

The percentage doses of $[^{131}I]T_3$ and $[^{125}I]T_4$ per ml plasma were calculated from the total radioactivities and the $[^{131}I]T_3$ and $[^{125}I]T_4$ distributions on the HPLC chromatogram. The percentage doses per ml, together with the plasma volume at t(0) (16, 29), were fitted individually to sums of n=1 to 3 exponential:

$Y(t)= A_1 \exp(\lambda_1 {}^*t) + A_2 \exp(\lambda_2 {}^*t) + A_3 \exp(\lambda_3 {}^*t)$

using the program DIMSUM, whereby A_i is expressed in %dose/ml, λ_i in min^{-1} (20).

To calculate the parameters of production, distribution and metabolism the program MAMPOOL (19) was used. The sum of the three exponential functions was fitted (by weighted least squares regression analysis) to the data collected for each individual rat and, together with plasma T_4 and T_3 levels, substituted into the three-compartment model, according to the kinetic T_4 and T_3 studies of DiStefano et al. (6, 7). All data are expressed as mean ± SE. Statistical analysis was performed by the Student's t-test using the Statistical Package for Social Sciences (SPSS) (28).

Analytical determinations

Urinary iodide was determined as described by Sandell and Kolthoff (25).
Mitochondrial and cytosolic fractions from liver and brain were obtained by differential centrifugation, according to the separation scheme described by van Doorn (8).
Liver and brain mitochondrial alpha-glycerophosphate dehydrogenase (α-GPD) was measured by means of the method described by Garrib and McMurray (10).
Cytosolic malic enzyme (ME) was determined for liver and brain according to the method of Hsu and Lardy (16).
The determination of type I deiodinase (ID-I) activity in liver homogenates was performed by the method described by Janssen et al. (17).
Protein was determined by the bicinchoninic acid (BCA) method (Pierce Europe, Oud Beijerland) using BSA as standard.

RESULTS

Urinary iodide excretion

The effect of treatment with 1 % $KClO_4$ for two days is demonstrated in Fig. 1a. Within a week after perchlorate treatment urinary iodide excretion had decreased to 0.4 µg/day; it remained constant during the rest of the experimental period. Fig. 1b shows the daily urinary iodide excretion four weeks after the start of the diet. MID resulted in a sharp decrease in urinary iodide excretion. No effect of pregnancy on urinary iodide excretion was present in NID and MID rats.
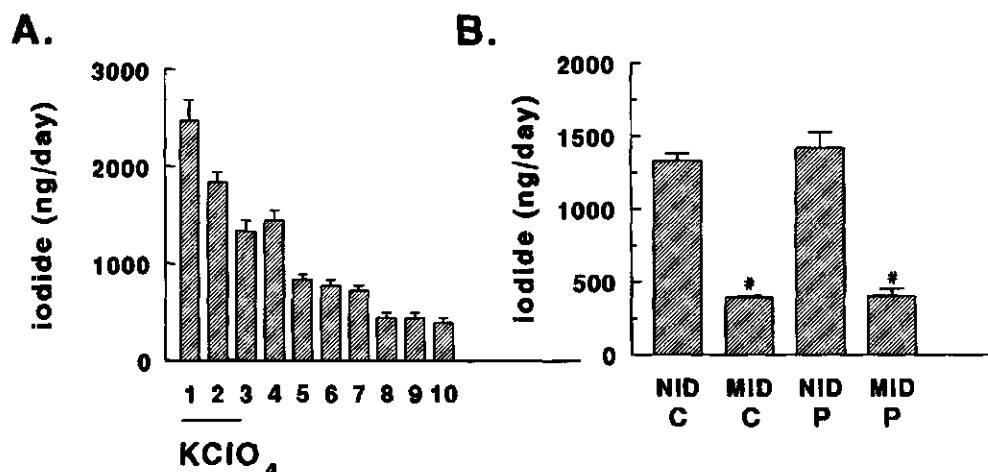
Figure 1: The effect of KClO$_4$ treatment on the 24 h urinary iodide excretion (A) and the daily urinary iodide excretion (B). Values are means ± SEM. $P < 0.05$, # MID vs. NID.

Body weight and plasma hormones

Table 1 shows the body weight (BW) and plasma thyroid hormone and TSH levels. Marginal iodine deficiency had no significant effect on maternal BW and the number of fetuses. During pregnancy the plasma T$_4$ and T$_3$ decreased; plasma TSH did not change significantly. This applied for NID as well as MID rats. During marginal iodine deficiency plasma T$_4$ and T$_3$ values were unchanged in nonpregnant as well as in pregnant rats; plasma TSH was increased significantly in nonpregnant rats only.

Table 1: Body weight and plasma thyroid hormone concentrations.
Values are means ± S.E.M.

	NID-C	MID-C	NID-P	MID-P
	(n=6)	(n=9)	(n=8)	(n=6)
Body wt (g)	211 ± 6	229 ± 6	304 ± 6*	323 ± 9*
No. of fetuses			11 ± 1	11 ± 1
T$_4$ (nmol/l)	33.8 ± 2.2	34.6 ± 3.0	22.5 ± 1.5*	17.2 ± 1.7*
T$_3$ (nmol/l)	0.62 ± 0.04	0.73 ± 0.06	0.53 ± 0.06	0.44 ± 0.04*
TSH (ng/ml)	0.28 ± 0.08	0.75 ± 0.10@	0.49 ± 0.06	0.65 ± 0.09

$P < 0.05$: * P vs. C; @ MID vs. NID.

T_4 kinetics

The effect of pregnancy on the fractional turnover and transport rates of T_4 was similar for NID and MID rats; T_4 disappeared more quickly from all three pools in pregnant rats. No effects of marginal iodine deficiency on the kinetic parameters of T_4 were found (data not shown).

The mean results of distribution volumes, pool sizes, and transport rates of T_4 are summarized in Table 2.

Table 2: Parameters of distribution, volumes, pool sizes, and rates of transport of T_4 (expressed per 100 g body weight). Values are means ± S.E.M.

	NID-C	MID-C	NID-P	MID-P
PCR (ml/h)	1.38 ± 0.06	0.93 ± 0.07[@]	1.73 ± 0.04[*]	1.79 ± 0.17[*]
PR (pmol/h)	47.2 ± 4.2	31.9 ± 3.4[@]	38.6 ± 2.5	30.4 ± 3.0
Vtotal (ml)	17.9 ± 0.6	16.1 ± 0.5	18.1 ± 0.8	15.9 ± 1.5
Vp (ml)	4.9 ± 0.1	4.5 ± 0.1	4.9 ± 0.1	4.7 ± 0.2
V2 (ml)	4.3 ± 0.3	4.5 ± 0.3	4.0 ± 0.3	3.6 ± 0.7
V3 (ml)	8.7 ± 0.6	7.1 ± 0.5	9.2 ± 0.5	7.6 ± 1.0
Qtotal (pmol)	615 ± 51	546 ± 34	421 ± 32[*]	274 ± 35[*] [@]
Qp (pmol)	166 ± 12	156 ± 11	110 ± 7[*]	82 ± 8[*][@]
Q2 (pmol)	145 ± 12	152 ± 12	89 ± 9[*]	65 ± 17[*]
Q3 (pmol)	303 ± 33	239 ± 18	207 ± 17[*]	127 ± 16[*][@]
TRpf (pmol/h)	973 ± 114	1392 ± 363	2574 ± 317[*]	1250 ± 249[@]
TRfp (pmol/h)	952 ± 115	1376 ± 363	2563 ± 313[*]	1233 ± 250[@]
DRfo (pmol/h)	23.6 ± 2.1	16.0 ± 1.7[@]	19.8 ± 1.3	15.4 ± 1.4
TRps (pmol/h)	162 ± 23	108 ± 20	188 ± 32	124 ± 44
TRsp (pmol/h)	138 ± 21	92 ± 18	169 ± 31	109 ± 45
DRso (pmol/h)	23.6 ± 2.1	16.0 ± 1.7[@]	19.8 ± 1.3	15.4 ± 1.4

PCR, plasma clearance rate; PR, production rate; V, plasma equivalent distribution volume; Q, pool quantity; TRpf, TRfp, TRps, and TRsp, rates of transport between plasma and the fast and slow pools respectively, in each direction; DRfo and DRso, disposal rate from fast and slow pool respectively; % PF and %PS, fraction of T_4 in plasma transported unidirectionally to the fast and slow tissue pools respectively; p, plasma; 2, fast pool; 3, slow pool.
$P < 0.05$: [*] P vs. C; [@] MID vs. NID.

In pregnant rats the plasma clearance rate of T_4 is increased. A decreased plasma clearance rate due to marginal iodine deficiency was found for nonpregnant MID rats but not pregnant MID rats.

The production rate of T_4 remained unchanged during pregnancy. Marginal iodine deficiency resulted in a decrease in T_4 production, which was significant for nonpregnant rats only.

The distribution volumes of T_4 were not affected by pregnancy or marginal iodine deficiency.

The total amount of T_4 decreased in the pregnant groups. This decrease was found in all three pools. Marginal iodine deficiency induced a further decrease in T_4 pool sizes for the pregnant rats; for nonpregnant rats no effect of marginal iodine deficiency was found.

The transport of T_4 changed only in pregnant rats on a normal iodide diet. Transport to the fast pool and vice versa were more than doubled. This effect of pregnancy was not found in the MID group. The disposal of T_4 decreased in both nonpregnant and pregnant rats.

The transit times for the three pools and the total residence time for T_4 are shown in Table 3. In pregnant rats the transit times for T_4 in plasma and the fast pool decreased. The total residence time also decreased. Marginal iodine deficiency results in an increase in the total residence time for T_4 in nonpregnant rats only.

Table 3: Transit times and total residence time for T_4. Values are means ± S.E.M.

	NID-C	MID-C	NID-P	MID-P
Transit time, min				
Plasma	9.5 ± 0.8	9.1 ± 1.5	3.7 ± 0.9*	4.5 ± 1.1*
Fast pool	9.7 ± 1.1	10.1 ± 1.8	3.5 ± 1.2*	4.2 ± 1.5*
Slow pool	127 ± 15	166 ± 24	99 ± 23	154 ± 90
Total residence time, min	789 ± 30	1079 ± 73@	616 ± 20*	560 ± 86

$P < 0.05$: * P vs. C; @ MID vs. NID.

T_3 kinetics

For T_3 almost no significant alterations were found in the kinetic parameters. Only in pregnant MID rats a decrease of the transport rate from plasma to the fast pool, due to marginal iodine deficiency, was found (data not shown).

Table 4 shows the distribution volumes, pool sizes, and transport rates of T_3.

Table 4: Parameters of distribution volumes, pool sizes, and transport rates of T$_3$ (expressed per 100 g body wt). Values are means ± S.E.M.

	NID-C	MID-C	NID-P	MID-P
PCR (ml/h)	21.7 ± 2.1	36.6 ± 5.4[@]	26.1 ± 2.8	24.9 ± 2.4
PAR (pmol/h)	13.4 ± 1.5	27.6 ± 5.0[@]	13.3 ± 1.1	10.3 ± 0.4*[@]
PR (pmol/h)	14.6 ± 1.7	32.2 ± 6.5[@]	14.6 ± 1.2	11.4 ± 0.5*[@]
Vtotal (ml)	209 ± 20	278 ± 21[@]	194 ± 15	224 ± 39
Vp (ml)	4.8 ± 0.1	4.8 ± 0.6	4.9 ± 0.1	4.8 ± 0.2
V2 (ml)	28.6 ± 2.1	35.1 ± 5.1	33.1 ± 4.3	24.9 ± 3.4
V3 (ml)	176 ± 19	238 ± 21	180 ± 27	194 ± 37
Qtotal (pmol)	128 ± 11	216 ± 24[@]	111 ± 13	95 ± 18*
Qp (pmol)	3.0 ± 0.2	3.4 ± 0.3	2.6 ± 0.3	2.1 ± 0.2*
Q2 (pmol)	17.5 ± 1.0	24.4 ± 2.9	17.2 ± 2.5	10.6 ± 1.4*[@]
Q3 (pmol)	108 ± 11	176 ± 25[@]	92 ± 14	83 ± 17*
TRpf (pmol/h)	214 ± 15	256 ± 30	229 ± 34	100 ± 20*[@]
TRfp (pmol/h)	207 ± 15	242 ± 28	222 ± 34	95 ± 20*[@]
DRfo (pmol/h)	6.7 ± 0.8	13.8 ± 2.5[@]	6.6 ± 0.6	5.1 ± 0.2*[@]
TRps (pmol/h)	53 ± 7	81 ± 13	56 ± 11	44 ± 4*
TRsp (pmol/h)	55 ± 7	85 ± 13	58 ± 11	47 ± 5*
DRso (pmol/h)	8.0 ± 0.9	18.5 ± 4.0[@]	8.0 ± 0.6	6.3 ± 0.3*[@]

PCR, plasma clearance rate; PAR, plasma appearance rate; PR, production rate; V, plasma equivalent distribution volume; Q, pool quantity; TRpf, TRfp, TRps, and TRsp, rates of transport between plasma and the fast and slow pools respectively, in each direction; DRfo and DRso, disposal rate from fast and slow pool respectively; % PF and %PS, fraction of T$_3$ in plasma transported unidirectionally to the fast and slow tissue pools respectively; p, plasma; 2, fast pool; 3, slow pool.
$P < 0.05$: * P vs. C; [@] MID vs. NID.

The plasma clearance rate of T$_3$ increased in the nonpregnant MID group only. Pregnancy resulted in a decrease in T$_3$ production in MID rats. The effects of marginal iodine deficiency on the production of T$_3$ were conflicting. In nonpregnant MID rats we found an increase, while in pregnant MID rats the T$_3$ production decreased slightly.

The transport of T$_3$ from plasma to the fast pool and vice versa decreased in pregnant MID rats only. The disposal of T$_3$ increased in nonpregnant MID rats. Marginal iodine deficiency induces in pregnant rats even a decrease in the disposal of T$_3$.

In pregnant MID rats the increased transit time for T_3 in plasma was the only change in transit times and total residence time found for T_3 (Table 5).

Table 5: Transit times and total residence time for T_3. Values are means ± S.E.M.

	NID-C	MID-C	NID-P	MID-P
Transit time, min				
Plasma	0.67 ± 0.01	0.76 ± 0.22	0.57 ± 0.04	0.93 ± 0.16[@]
Fast pool	4.99 ± 0.36	6.64 ± 1.35	4.78 ± 0.66	7.23 ± 1.22
Slow pool	114 ± 15	122 ± 25	95 ± 10	99 ± 23
Total residence time, min	607 ± 76	544 ± 95	513 ± 52	553 ± 94

$P < 0.05$: [*] P vs. C; [@] MID vs. NID.

Enzymes

Figure 2 shows the α-GPD activity in liver and brain. The same pattern is found for liver (2a) and brain (2b). The α-GPD activity decreased in the pregnant groups; marginal iodine deficiency had no effect.

In the fetal liver and brain no differences in α-GPD activity were found between the NID and MID rats (data not shown).
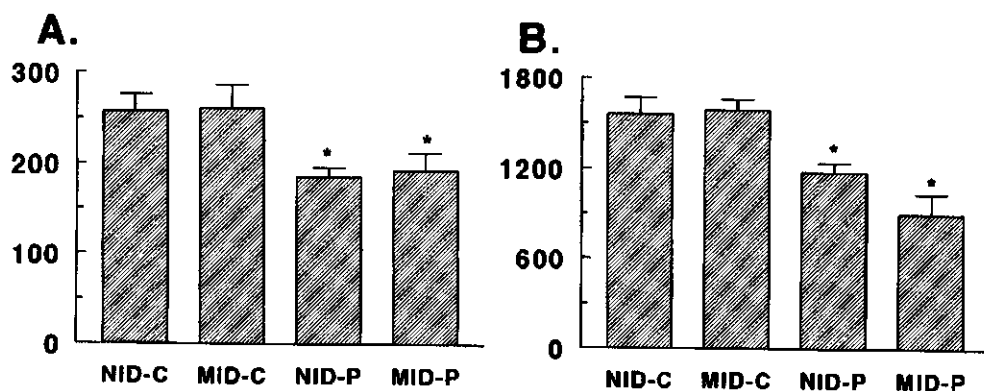

Figure 2: α-GPD in maternal liver (A) and maternal brain (B).
Values are means ± SEM. $P < 0.05$; [*] P vs. C.

The data on ME are shown in Fig.3. No effect of pregnancy or marginal iodine deficiency was found in the liver. In the brain however, ME activity increased in pregnant rats.

Pregnancy and marginal iodine deficiency both increase ID-I activity in the liver (Fig.4); this increase is additive.
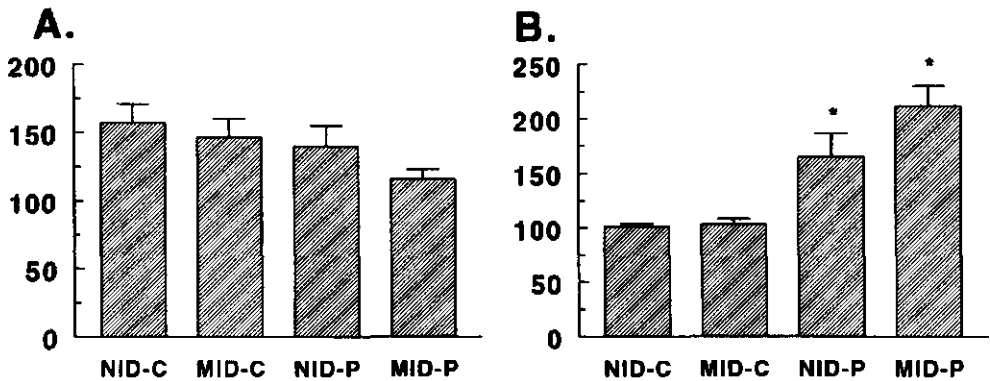

Figure 3: Malic enzyme in maternal liver (A) and maternal brain (B).
Values are means ± SEM. $P < 0.05$; * P vs. C.
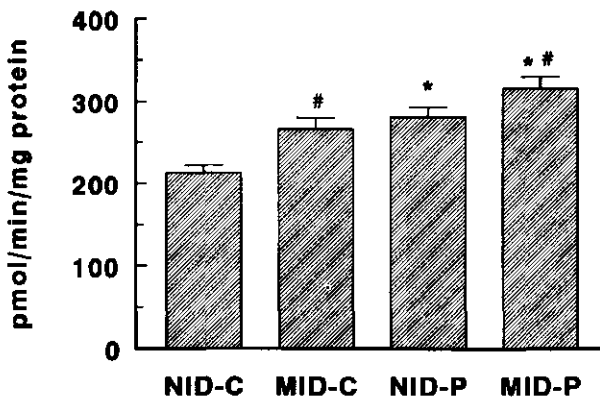

Figure 4: Deiodinase type I in liver. Values are means ± SEM.
$P < 0.05$; * P vs. C, # MID vs. NID.

DISCUSSION

The aim of this study was to determine whether a marginal iodine deficiency will
affect thyroid hormone production, distribution, and transport in nonpregnant as well
as near-term pregnant rats. During fetal growth thyroid hormones are necessary for
development of the central nervous system. It has been shown that a moderately low
iodine supply is sufficient for the daily needs of healthy adult subjects. During preg-
nancy, however, the iodine supply becomes relatively insufficient, due to enhanced
maternal requirements (13) and increased renal clearance (1).

Many animal studies have been performed to study the effect of iodine deficiency on
the development of fetuses (9, 21), but no information is available on the effects of
iodine deficiency on maternal thyroid hormone metabolism and the availability of
maternal thyroid hormones during pregnancy. The effects of iodine deficiency on
thyroid hormone secretion have only been described for nonpregnant rats (12, 23).
In most experimental studies severe iodine deficiency was induced, while in our
study the rats were marginally iodine-deficient. This condition is much more common
in iodine-deficient areas.

Severe iodine deficiency is characterized by an increase in thyroid weight, low
serum T_4, normal or slightly elevated serum T_3 and high TSH (9, 22, 26). During
pregnancy decreased reproductive competence has been reported (9).   In our
laboratory, rats receiving the same treatment as described for this study showed an
increase in thyroid weight (30). Neither retarded growth nor decreased number of
fetuses was observed, so the iodine deficiency achieved must have been marginal.

During marginal iodine deficiency plasma T_4 and T_3 values remained unchanged, but
plasma TSH increased sligthly. Even under these moderate conditions T_4 and T_3
production and their plasma clearance rates had already changed. The kinetic
experiment revealed a decreased production of T_4 in nonpregnant MID rats. The
unaltered plasma T_4 might possibly be explained by the decreased plasma clearance
rate, resulting in an increased mean residence time for T_4 in the body.

The production of T_3 increased, but plasma T_3 was not affected by marginal iodine
deficiency. We attribute this to the increased plasma clearance rate.

Janssen *et al.* (17) found no effect of iodine deficiency on hepatic ID-I activity, even
though T_4 in their animals was lower. In the brain an increase in ID-II was found in
iodine-deficient rats (17). In our study marginal iodine deficiency induced an in-
crease in hepatic ID-I activity. Therefore, the increased T_3 production might possibly

be explained by enhanced T_4 to T_3 conversion in the liver.

We suggest that rats are able to maintain their euthyroid status during marginal iodine deficiency. The alterations in T_4 and T_3 production are compensated by changes in the plasma clearance rates, resulting in normal thyroid hormone values in plasma and tissues. This is confirmed by the unchanged levels of the thyroid hormone-sensitive enzymes α-GPD and malic enzyme in liver and brain.

The alterations in thyroid hormone metabolism due to pregnancy are in agreement with previous findings for iodine-sufficient rats (29). There is a difference in the effects of marginal iodine deficiency on the production and metabolism of T_4 during pregnancy and in the nonpregnant situation.

During pregnancy T_4 production also decreased as a result of marginal iodine deficiency, but the plasma clearance rate remained unchanged. This resulted in a decrease in the total amount of T_4 in the body. Striking is the effect of marginal iodine deficiency on the transport of T_4 to the fast pool in pregnant rats. During normal pregnancy the transport of T_4 to the fast pool and vice versa is markedly increased. We suggested that this is caused by the fetal compartment (29). However, in marginally iodine-deficient near-term pregnant rats no increase in transport to the fast pool was found. This could indicate that during marginal iodine deficiency the transport of T_4 to the fetal compartment is already diminished. This is exceedingly important, because it is mainly T_4 that is transported from mother to fetuses. The absence of maternal T_4 during fetal development can cause damage to the central nervous system (4). However, we did not find any effect on α-GPD activity in the fetal brain during marginal iodine deficiency. This might possibly be explained by an increase in fetal ID-II; during severe iodine deficiency the fetal brain is protected from hypothyroidism by an increase in the activity of ID-II (21).

The alterations in T_3 metabolism due to marginal iodine deficiency found in pregnant rats are the exact opposite of the effects in nonpregnant rats. During pregnancy production and the plasma clearance rate of T_3 decrease slightly; these parameters increase in nonpregnant rats.

Despite a decrease in plasma T_4 and T_3 hepatic ID-I is increased at the end of gestation. This had already been described earlier (5, 29). Just as in nonpregnant rats a further increase of ID-I is found during marginal iodine deficiency.

The transport of T_3 from plasma to the fast pool and vice versa was decreased by fifty percent in marginally iodine-deficient pregnant rats. This implies that, despite the markedly increased ID-I activity, locally produced T_3 is either decreased or does

not enter the circulation.

The present results demonstrate that under these conditions marginal iodine intake influences maternal thyroid hormone metabolism. The total amount of T_4 in the mother is decreased and less T_4 is transported to the fetuses. So, even a marginal iodine deficiency seems to affect the availability of maternal T_4 for the fetuses. Eventually this will cause fetal hypothyroidism, resulting in impaired fetal brain development.

ACKNOWLEDGEMENTS

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CHAPTER 6

MATERNAL THYROXINE AND 3,5,3'-TRIIODOTHYRONINE KINETICS IN NEAR-TERM PREGNANT RATS AT TWO DIFFERENT LEVELS OF HYPOTHYROIDISM.

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ABSTRACT

Thyroid hormones are extremely important for development of the fetal central nervous system. Thyroidectomy results in severe hypothyroidism. In this study two levels of maternal hypothyroidism were reached by administration of different amounts of thyroxine (T$_4$) and 3,5,3'-triiodothyronine (T$_3$) to thyroidectomized pregnant rats. We examined the production, distribution and transport of T$_4$ and T$_3$ by performing a kinetic experiment (three-compartment analysis) with intact and thyroidectomized near-term pregnant rats which received either very low (Tx+lowTH) or normal (Tx+TH) doses of T$_4$ and T$_3$. Despite administration of normal doses of thyroid hormones plasma TSH was still elevated in the Tx+TH rats, meaning that these rats were still mildly hypothyroid. The Tx+lowTH rats were markedly hypothyroid, the plasma T$_4$ and T$_3$ levels being very low. In the mildly hypothyroid rats the transport of T$_4$ from plasma to the fast pool and *vice versa* was decreased compared with intact near-term pregnant rats. This could imply that much less T$_4$ is transported to the feto-placental compartment. Liver type I deiodinase was decreased, resulting in lowered plasma T$_3$ values. In the markedly hypothyroid rats all pools and rates of transport of T$_4$ and T$_3$ were greatly decreased. In conclusion, even mild hypothyroidism, despite normal plasma T$_4$ values, results in significant changes, especially in maternal T$_4$ transport. We suggest that even mild maternal hypothyroidism will have a negative effect on the availability of maternal T$_4$ for fetuses.

INTRODUCTION

Thyroid hormones are extremely important for fetal development, especially of the central nervous system. In man, the children of mothers who were hypothyroid during pregnancy show a high incidence of behavioral and neurological disorders (14).

Rat studies have demonstrated that maternal hypothyroidism during pregnancy has irreversible effects on brain development, as indicated by a decrease in the activity of various cell marker enzymes in the brain of the progeny (8). The influence of maternal hypothyroidism on fetal development is particularly dramatic during the first half of the gestational period (2). However, even after the fetal thyroid starts to function, on day 17.5-18 of gestation, maternal thyroid hormones contribute to the

fetal iodothyronine pool (16). The effects of maternal thyroidectomy, resulting in
severe hypothyroidism, on fetal development and thyroid hormone levels have been
studied extensively by the Madrid group (17, 19, 22). They demonstrated a decrease
in the number of fetuses and the fetal body weight of thyroidectomized dams (17, 19,
22). Maternal plasma thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) levels decreased
markedly, while thyrotropin (TSH) was highly elevated in thyroidectomized rats. T_4
and T_3 concentrations were decreased in both liver and brain tissue (17, 18, 20).
The concentrations of T_4 and T_3 in embryonic tissues from thyroidectomized dams
were undetectable before the onset of fetal thyroid function (19). At the end of
gestation, on day 21, fetal plasma TSH was elevated (2) and the plasma T_4 and T_3
levels of the fetuses of thyroidectomized dams were similar (22) or even slightly
elevated (17, 20) compared to those of intact dams. Total thyroidal T_4 and T_3 levels
in fetuses from thyroidectomized dams had decreased markedly by the end of gesta-
tion (22). The fetal liver T_4 concentration was decreased, whereas such a change
was not found for hepatic T_3. The deiodinase type I (ID-I) activity in fetal liver had
decreased by 50% (22). No effect of maternal thyroidectomy on T_4 and T_3 concentra-
tions in fetal brain on day 20 was found; in contrast, an increase in the cerebral T_4
and T_3 concentrations on day 21 was reported (17, 19).
Euthyroidism can be restored in thyroidectomized rats by administration of both T_4
and T_3 (6). If the amount of thyroid hormones administrated is less than the amount
normally produced, the animal will still be hypothyroid. In the present study two
levels of thyroid hormone status were achieved by the administration of different
amounts of T_4 and T_3 to thyroidectomized pregnant rats.
The aim of our study was to examine the effects of a mild and a marked hypothyroid
state on maternal thyroid hormone metabolism in the near-term pregnant rat. T_4 and
T_3 metabolism, distribution, and transport were examined by performing kinetic
studies using the three-compartmental model of distribution and metabolism de-
veloped by DiStefano (3, 4). In this model three compartments can be distinguished:
1) the plasma; 2) tissues that exchange T_4 and T_3 with plasma at a fast rate, the fast
pool; and 3) tissues which exchange at a slow rate, the slow pool. Liver and kidney
are considered to be the main components of the fast pool, whereas skin, muscle
and brain belong to the slow pool (3, 4). Previously we suggested that in normal
near-term pregnant rats T_4 is transported very rapidly from plasma to the feto-pla-
cental compartment (25). In the present study we compared the transport of maternal
thyroid hormones to the feto-placental compartment during different levels of hypo-

thyroidism. The mildly hypothyroid rats are of special interest; will there already be an effect on maternal thyroid hormone metabolism, and is the maternal contribution of thyroid hormones to the fetal compartment affected ?

MATERIAL AND METHODS

Animals and diet

Female Wistar rats (CPB/WU, Iffa Credo, Brussels) weighing 150 g were rendered hypothyroid by means of ^{131}I thyroid ablation (a single intraperitoneal injection of 15 Mbq (0.4 mCi) Na[131]I). One week before and one week after radiothyroidectomy the rats received iodide-free American Institute of Nutrition (AIN) food (1). Subsequently iodide-rich AIN food (40 µg KI per g food) was given for 5 days to wash the ^{131}I out of the animals' system. During the rest of the experiment the animals received AIN food (dry powder mixed with distilled water (60% dry weight - 40 % water)) containing 55 ng iodide/g food.

Intact, age-matched controls also consumed AIN food with 55 ng iodide/g food.

Hypothyroidism was demonstrated by undetectable plasma T_4 levels six weeks after radiation. No sooner than two months after radiothyroidectomy the rats were mated. Until mating the rats received 0.25 µg T_3 daily. Mating was confirmed by the presence of sperm in a vaginal lavage the following morning, called day 0 of gestation.

Design of the study

Three groups of rats were used:

1. Intact: intact, pregnant euthyroid rats.
2. Tx+TH: pregnant radiothyroidectomized (Tx) rats on high T_4 and T_3 (0.25 µg T_3 and 2.5 µg T_4 per day added to the AIN diet).
3. Tx+low TH: pregnant radiothyroidectomized (Tx) rats on low T_4 and T_3 (0.01 µg T_3 and 0.1 µg T_4 per day added to the AIN diet).

T_4 and T_3 were purchased from Sigma (St. Louis, MO, USA).

Kinetic experiments were performed on day 19 of gestation. Twenty-four hours after the kinetic experiment the animals were killed; maternal blood, liver, brain and pituitary were collected.

The experiments were approved by the University Committee on Animal Care and Use of the Agricultural University of Wageningen.

Kinetic and analytical protocols

$[^{125}I]T_4$ and $[^{131}I]T_3$ (specific activity 80 and 130 Mbq/µg (2200 and 3500 (µCi/µg), respectively) were prepared in our laboratory (11, 26). $Na^{125}I$ and $Na^{131}I$ were obtained from Amersham (Arlington Heights, IL, USA); $L-T_3$ and 3,5-L-diiodothyronine, the respective substrates for labelling, were purchased from Sigma (St. Louis, MO, USA). Purity of the tracers was assessed by means of HPLC.

Via a cannula inserted into the right jugular vein (21) the rats received a 400 µl bolus injection of a solution of 3700 Bq (10 µCi) $[^{125}I]T_4$ and 3700 Bq (10 µCi) $[^{131}I]T_3$ in saline containing heparin (0.3 U/ml; Organon, Tilburg, The Netherlands), ticarcillin (0.4 mg/ml; Ticarpen, Beecham S.A., Heppignies, Belgium) and 5% normal rat serum. Ticarcillin is added to solutions containing labelled iodothyronines to prevent eventual bacterial infections and reduce artefactual deiodination, as described by Van Doorn et. al. (5).

Blood samples of 0.2 ml were taken at 1, 4.5, 10, 23, 44, 115, 202 and 315 min; 0.4 ml. were drawn at 465, 600, 900, 1200 and 1440 min, being the optimal time schedule according to DiStefano et al. (3, 4).

Fifty to hundred microliters plasma (in duplicate) were used to assess the total ^{131}I and ^{125}I activities. The plasma samples were extracted with ethanol/ammonia (25%)(197:3 v/v) containing 0.1 mmol/l propylthiouracil (PTU). The dried extracts were dissolved in 0.1 ml 0.2 mol/l ammonia containing carrier T_4, T_3 and KI (1 mg/10 ml) and subjected to HPLC to separate the iodothyronines, according to the method described by Schröder-van der Elst and van der Heide (23).

The endogenous concentrations of T_4 and T_3 in plasma were measured by means of a specific rat RIA (9) of samples taken via the canula directly before the kinetic experiments were performed.

Plasma TSH was measured by the specific RIA developed for the rat by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health (USA). Reference preparation RP-2 was used as a standard.

Calculations

The percentage doses of $[^{131}I]T_3$ and $[^{125}I]T_4$ per ml plasma were calculated from the total radioactivities and the $[^{131}I]T_3$ and $[^{125}I]T_4$ distributions on the HPLC chromatogram. The percentage doses per ml, together with the plasma volume at t(0) (3), were fitted individually to sums of n=1 to 3 exponential:

$$Y(t) = A1 \exp(\lambda1^*t) + A2 \exp(\lambda2^*t) + A3 \exp(\lambda3^*t)$$

using the program DIMSUM, whereby Ai is expressed in %dose/ml, λi in min^{-1} (15). To calculate the parameters of production, distribution and metabolism the program MAMPOOL (12) was used. The sum of the three exponential functions was fitted (by weighted least squares regression analysis) to the data collected for each individual rat and, together with the plasma T$_4$ and T$_3$ levels, substituted into the three-compartment model, according to the kinetic T$_4$ and T$_3$ studies of DiStefano *et al.* (3, 4). All data are expressed as means ± S.E.M. Data were analyzed using the Statistical Package for Social Sciences (SPSS)(24). All data were subjected to one-way analysis of variance, and statistical differences between groups were determined by means of the modified least significant difference method.

Analytical determinations

Mitochondrial fractions from liver and brain were obtained by differential centrifugation, according to the separation scheme described by van Doorn (5).

Liver and brain mitochondrial alpha-glycerophosphate dehydrogenase (α-GPD; EC 1.1.2.1) was measured by means of the method described by Garrib and Mc Murray (7).

The determination of type I deiodinase (ID-I) activity in liver homogenates was performed by the method described by Janssen *et al.* (10).

Protein was determined by the bicinchoninic acid (BCA) method (Pierce Europe, Oud Beijerland, the Netherlands) using bovine serum albumin as standard.

RESULTS

Body weight and plasma hormones

Table 1 shows the body weight (BW) and plasma thyroid hormone levels. At the start of pregnancy all rats had a similar BW. Hypothyroidism affects the BW of the near-term pregnant rat. Tx+lowTH rats had a significantly lower BW. The BW of the fetuses of Tx+lowTH mothers was also decreased. In this group the number of viable fetuses was decreased, while more dead and resorbed fetuses were found.

Plasma T$_4$ is markedly decreased in the Tx+lowTH group, while in the Tx+TH group a normal T$_4$ level was reached.

Plasma T$_3$ is decreased in both Tx groups; no difference was found between the Tx+lowTH and Tx+TH groups.

Plasma TSH was highly elevated in both Tx groups. In Tx+lowTH rats the plasma TSH level was higher than in Tx+TH rats.

Table 1: Body weight and plasma thyroid hormone concentrations in the intact, Tx+TH and Tx+lowTH groups. Values are means ± S.E.M.

	Intact group (n=8)	Tx+TH group (n=7)	Tx+lowTH group (n=8)
Body wt on day 0 (g)	226 ± 3	229 ± 4	228 ± 5
Body wt on day 19 (g)	299 ± 7	300 ± 3	252 ± 5*#
No. of fetuses	11 ± 1	9 ± 1	7 ± 2
Fetal body wt (g)	4.24 ± 0.07	4.14 ± 0.06	3.38 ± 0.19*#
T_4 (nmol/l)	23.6 ± 1.6	30.6 ± 3.1	4.0 ± 0.9*#
T_3 (nmol/l)	0.50 ± 0.06	0.28 ± 0.05@	0.21 ± 0.01*
TSH (ng/ml)	0.56 ± 0.08	4.57 ± 0.61@	7.83 ± 0.32*#

$P < 0.05$: @ Tx+TH vs. Intact, * Tx+lowTH vs. Intact, # Tx+lowTH vs. Tx+TH.

T_4 kinetics

The disappearance curves for T_4, based on the mean A and λ values for the three groups, are shown in Fig. 1. The slope of the regression lines for plasma is decreased in Tx+lowTH rats, meaning that T_4 disappeared from plasma more slowly in this group. The fractional turnover rates for all three pools were decreased (data not shown). As a result the transport between plasma and the fast pool in Tx+TH rats was 10 % of that for intact controls and only 1% in the Tx+lowTH group.

For T_4, distribution volumes, pool sizes, and transport rates are summarized in Table 2. The plasma clearance rate was decreased in both Tx groups, but no additional decrease was found for Tx+lowTH rats. The calculated rate of production of T_4 in the Tx+TH group was similar to that found for intact rats. The low 'T_4 production' in the Tx+lowTH group resulted in an equivalent decrease in the amount of T_4 in all three pools. The distribution volumes of T_4 remained unchanged.

The rate of transport of T_4 from plasma to the fast pool and *vice versa* was markedly decreased in both Tx groups, an additional decrease was found for Tx+lowTH rats. The transport of T_4 between plasma and the slow pool was only decreased in the Tx+lowTH group. It also appeared that in these rats an increasing fraction of T_4 was transported unidirectionally from plasma to the slow tissue pool.

The transit times in all three pools are increased in both Tx groups, but no significant change in the total residence time was found (results not shown).

Table 2: Parameters of distribution, volumes, pool sizes, and rates of transport of T$_4$ (expressed per 100 g body weight) in the intact, Tx+TH and TX+lowTH groups. Values are means ± S.E.M.

	Intact group	Tx+TH group	Tx+lowTH group
PCR (ml/h)	1.64 ± 0.04	1.19 ± 0.07[@]	1.26 ± 0.09*
PR (pmol/h)	38.5 ± 2.5	35.6 ± 2.7	4.7 ± 1.0*[#]
Vtotal (ml)	17.6 ± 1.0	16.6 ± 0.8	17.4 ± 2.4
Vp (ml)	4.9 ± 0.1	5.0 ± 0.1	4.8 ± 0.2
V2 (ml)	3.8 ± 0.4	3.8 ± 0.7	2.4 ± 0.5
V3 (ml)	8.9 ± 0.6	7.8 ± 0.3	10.2 ± 2.6
Qtotal (pmol)	437 ± 32	519 ± 78	64 ± 14*[#]
Qp (pmol)	116 ± 6	156 ± 20	19 ± 5*[#]
Q2 (pmol)	88 ± 9	122 ± 33	9 ± 3*[#]
Q3 (pmol)	207 ± 17	239 ± 30	36 ± 9*[#]
TRpf (pmol/h)	2542 ± 390	794 ± 177[@]	33 ± 8*
TRfp (pmol/h)	2523 ± 389	776 ± 177[@]	31 ± 8*
DRfo (pmol/h)	19.3 ± 1.2	17.8 ± 1.3	2.4 ± 0.5*[#]
TRps (pmol/h)	195 ± 36	143 ± 43	16 ± 4*[#]
TRsp (pmol/h)	176 ± 35	125 ± 43	13 ± 4*
DRso (pmol/h)	19.3 ± 1.2	17.8 ± 1.3	2.4 ± 0.5*[#]
% PF	92.9 ± 0.8	85.0 ± 2.3	67.2 ± 10.0*
% PS	7.1 ± 0.8	15.0 ± 2.3	32.8 ± 10.0*

PCR, plasma clearance rate; PR, production rate; V, plasma equivalent distribution volume; Q, pool quantity; TRpf, TRfp, TRps, and TRsp, rates of transport between plasma and the fast and slow pools respectively, in each direction; DRfo and DRso, disposal rate from fast and slow pool respectively; % PF and %PS, fraction of T$_4$ in plasma transported unidirectionally to the fast and slow tissue pools respectively; p, plasma; 2, fast pool; 3, slow pool.
$P < 0.05$: [@] Tx+TH vs. intact, * Tx+lowTH vs. intact, [#] Tx+lowTH vs. Tx+TH.

Figure 1:

T_4-disappearance curves obtained with a three exponential model.

(A) plot of log y(t) (in % dose/ml) 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.

(B) plot of log [y(t) - A_3 exp(λ_3*t)] against time, with the least squares regression line on the curve, giving estimates of coefficient A_2 and exponent λ_2.

(C) plot of log [y(t) - A_3 exp(λ_3*t) - A_2 exp(λ_2*t)] against time, giving estimates of coefficient A_1 and exponent λ_1.
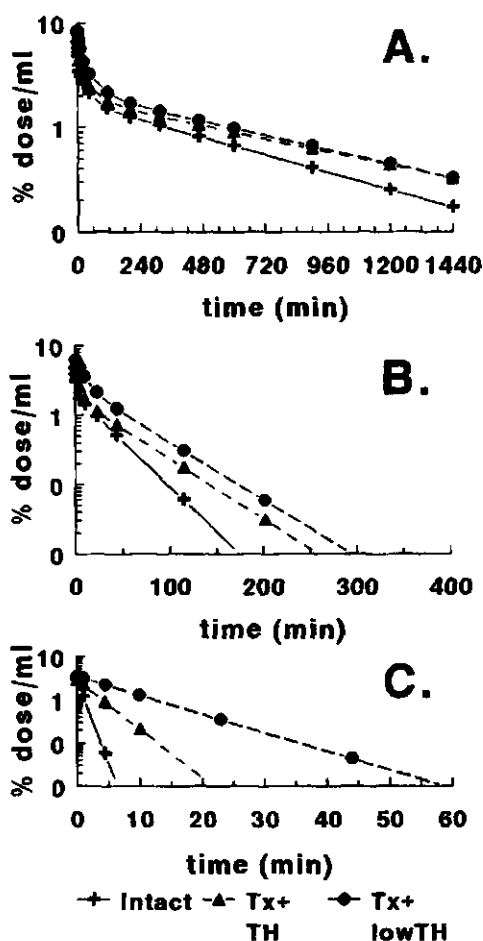
T_3 kinetics

The kinetic parameters for T_3 are significantly different from intact rats only in the Tx+lowTH group. The fractional rate of turnover of T_3 in plasma and the transport rates from plasma to the tissue pools were decreased (data not shown).

Most changes found in the distribution, metabolism and transport of T_3 (Table 3) were similar for the Tx+TH and Tx+lowTH groups. The plasma clearance rate for T_3 was decreased. The plasma appearance rate and the production rate for T_3 were diminished in all Tx rats.

Table 3: Parameters of distribution, volumes, pool sizes, and rates of transport of T_3 (expressed per 100 g body weight) in the intact, Tx+TH and TX+lowTH groups. Values are means ± S.E.M.

	Intact group	Tx+TH group	Tx+lowTH group
PCR (ml/h)	25.0 ± 2.4	17.6 ± 1.3[@]	16.8 ± 1.9*
PAR (pmol/h)	12.2 ± 1.2	4.9 ± 0.9[@]	3.6 ± 0.5*
PR (pmol/h)	13.5 ± 1.1	5.5 ± 1.0[@]	4.2 ± 0.8*
Vtotal (ml)	201 ± 20	173 ± 13	138 ± 7*
Vp (ml)	4.8 ± 0.1	5.4 ± 0.6	5.6 ± 0.5*
V2 (ml)	28.3 ± 3.0	28.1 ± 2.7	17.3 ± 1.3* #
V3 (ml)	200 ± 37	139 ± 11	115 ± 7*
Qtotal (pmol)	115 ± 19	49 ± 9[@]	29 ± 2*
Qp (pmol)	2.4 ± 0.3	1.5 ± 0.2[@]	1.1 ± 0.1*
Q2 (pmol)	13.8 ± 1.3	7.9 ± 1.3[@]	3.6 ± 0.3* #
Q3 (pmol)	99 ± 19	44 ± 12[@]	24 ± 2*
TRpf (pmol/h)	205 ± 27	115 ± 21[@]	50 ± 6*
TRfp (pmol/h)	199 ± 27	112 ± 20[@]	48 ± 6*
DRfo (pmol/h)	6.1 ± 0.6	2.5 ± 0.5[@]	1.8 ± 0.3*
TRps (pmol/h)	53.3 ± 13.4	16.5 ± 2.6[@]	10.3 ± 0.8*
TRsp (pmol/h)	54.9 ± 13.8	16.9 ± 2.7[@]	10.6 ± 0.9*
DRso (pmol/h)	7.4 ± 0.6	3.0 ± 0.6[@]	2.5 ± 0.5*
% PF	80.5 ± 3.4	86.7 ± 1.5	81.8 ± 2.2
% PS	19.5 ± 3.4	13.3 ± 1.5	18.2 ± 2.2

PCR, plasma clearance rate; PAR, plasma appearance rate; PR, production rate; V, plasma equivalent distribution volume; Q, pool quantity; TRpf, TRfp, TRps, and TRsp, rates of transport between plasma and the fast and slow pools respectively, in each direction; DRfo and DRso, disposal rate from fast and slow pool respectively; % PF and %PS, fraction of T_3 in plasma transported unidirectionally to the fast and slow tissue pools respectively; p, plasma; 2, fast pool; 3, slow pool.
$P < 0.05$: [@] Tx+TH vs. Intact, * Tx+lowTH vs. Intact, # Tx+lowTH vs. Tx+TH.

The total distribution volume as well as the distribution volume of the tissue pools was reduced in the Tx+lowTH group only. In all pools the amount of T_3 had decreased in both Tx groups. The transport of T_3 in all directions was reduced.

No significant changes, except an increase in the transit time for plasma (0.6±0.06 min and 1.2±0.15 min for intact and Tx+lowTH rats respectively) , were found for the

transit times and the total residence time of T_3.

Enzyme activities

The α-GPD activities in liver and brain are shown in Fig. 2. Hepatic α-GPD activity was reduced in the Tx+TH group; an even stronger decrease was found for the Tx+lowTH group. No effect of hypothyroidism on α-GPD activity in the brain could be demonstrated.

Deiodinase type I (ID-I) activity (in mOD/min/mg protein) was diminished in all Tx rats; 267±13, 147±16, and 73±8 for intact, Tx+TH and Tx+lowTH rats respectively. Thus the strongest reduction was found in the Tx+lowTH rats.

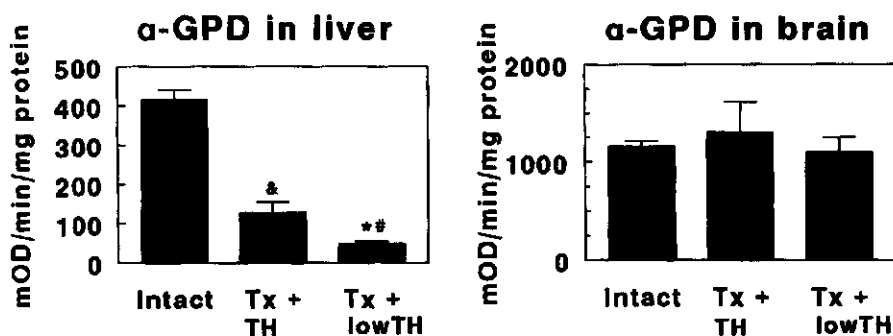**Figure 2**: α-GPD in maternal liver and maternal brain in the three groups studied (intact, Tx+TH, Tx+lowTH). $P < 0.05$; &Tx+TH vs. intact; * Tx+lowTH vs. intact; #Tx+lowTH vs. Tx+TH.

DISCUSSION

Thyroidectomy resulted in a lower increase in bodyweight during pregnancy. This is due not only to the diminished weight of the mother but also to reduction of the fetal compartment. Treatment of the Tx animals with T_4 and T_3 (Tx+TH) normalized the bodyweights of mother and fetus.

Since the plasma thyroid hormone and TSH levels are not normal, the Tx+TH group is certainly not euthyroid; we describe them as mildly hypothyroid. Tx animals which received a very low dose of T_4 and T_3 (Tx+lowTH) were markedly hypothyroid, the levels of plasma T_4 and T_3 being very low.

Hypothyroidism is known to influence thyroid hormone metabolism. In this study we concentrated on the effects of hypothyroidism on maternal thyroid hormone metabolism. The most important information about the effects of hypothyroidism on the availability of maternal thyroid hormones for fetuses will be obtained from the mildly hypothyroid group; does this state already have an effect on maternal-fetal transport?

Despite normal plasma T_4 values in the mildly hypothyroid group the transport of T_4 from plasma to the fast pool, compared to intact near-term pregnant rats, was already strongly reduced; in markedly hypothyroid rats almost no T_4 was transported to the fast pool. Previously we suggested that in pregnant rats the fast pool consists of the liver, the kidney (3, 4), and the feto-placental compartment. In intact rats pregnancy results in a pronounced increase in the transport of T_4 from plasma to the fast pool (25).

It is unlikely that the total decrease in the transport of T_4 to the fast pool in mildly hypothyroid rats can be explained solely by a decrease in the transport of T_4 to the liver and kidney. This implies, therefore, that even in a mildly hypothyroid situation the transport of maternal T_4 to the placenta can also be diminished. This is very important, because T_4 plays a major role in the development of the fetal central nervous system. Only maternal T_4 is able to mitigate fetal cerebral T_4 and T_3 deficiency before the fetal thyroid starts to function (18).

The plasma T_3 level in the mildly hypothyroid group was not normal. The amount of T_3 added was less than the amount of T_3 produced daily in intact rats (0.25 µg vs. 0.60 µg per day). However, since the plasma T_4 level was normal, it was to be expected that T_3 would also be produced locally. T_3 produced locally in the liver contributes in particular to the plasma T_3 level (5). However, in the hypothyroid rat the local production of T_3 is decreased in the liver (5). This was also demonstrated by the decrease in ID-I activity in the liver; this can possibly be explained by the regulation of hepatic ID-I activity by T_3 (13). Therefore, even in a mildly hypothyroid condition with normal plasma T_4 levels, the T_3 level in the liver will be reduced; this hypothesis is supported by the decrease in hepatic α-GPD activity.

For non-pregnant thyroidectomized rats receiving an infusion of T_4 and T_3 it has been reported that in the event of normal plasma T_4 levels and a reduced plasma T_3, hepatic ID-I and cerebral ID-II activities will be normal (6). However, despite the administration of T_4 in an amount which equals the amount normally produced, local T_3 production was decreased in our near-term pregnant rats. It appears, therefore,

that in near-term pregnant rats the effect of mild hypothyroidism is stronger than in non-pregnant rats.

Marked hypothyroidism in both non-pregnant and near-term pregnant rats results in highly reduced pools and diminished rates of transport of T_4 and T_3.

In conclusion, even mild hypothyroidism results in significant alterations in maternal thyroid hormone metabolism, despite normal plasma T_4 levels. In particular the transport of T_4 from plasma to the fast pool will be reduced. We speculate that this results in a decrease in the availability of maternal T_4 for the fetuses. This could affect the development of the fetal brain, which depends mainly on maternal T_4 for normal T_3 values.

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CHAPTER 7

GENERAL DISCUSSION
AND CONCLUSION

GENERAL DISCUSSION AND CONCLUSION

Thyroid hormone metabolism is influenced by several (patho)physiological condi-
tions. During pregnancy thyroid hormone metabolism is also altered. The aim of this
study was to investigate the effects of pregnancy on maternal thyroid hormone
metabolism, i.e.   - T_4 and T_3 secretion by the thyroid

                   - peripheral production of T_3

                   - T_4 and T_3 concentrations in plasma and tissues

                   - distribution of T_4 and T_3

                   - interpool transport of T_4 and T_3

Since iodine is an essential element of the synthesis of thyroid hormones we also
studied changes in iodide uptake by the thyroid during normal pregnancy and during
marginal iodine deficiency.

7.1. NORMAL PREGNANCY

Iodide uptake by the thyroid is regulated by TSH. In the near-term pregnant rat the
plasma TSH level remains unchanged or is slightly elevated (3, 4, 6). During preg-
nancy the iodide uptake, which is slightly decreased, does not seem to be regulated
by TSH only. Another factor which affects iodide uptake by the thyroid is the thyr-
oidal blood flow (1). We did not measure this parameter, but no changes were found
in either the clearance of iodide from plasma by the thyroid or plasma inorganic
iodide. We suggest that less iodide is taken up by the maternal thyroid simply be-
cause less iodide is available: at the end of gestation the mother has to compete
with the fetuses for her iodine supply. Iodine can be transported through the pla-
centa from mother to fetuses (7); on day 19 of gestation the fetal thyroid is capable
of concentrating iodine. The decreased availability of iodide for the maternal thyroid
is not so dramatic that thyroid hormone production by the thyroid is affected. The
thyroid is still able to maintain thyroid hormone synthesis at a normal level.
Despite the unaltered thyroidal production of T_4, the plasma T_4 concentration is
decreased at the end of gestation which demonstrates that altered plasma concen-
trations are not always the result of changes in production rate. In the pregnant rat
the transport of T_4 from plasma increases drastically. Most likely T_4 is also trans-
ported to the feto-placental compartment, which is a developing compartment that

must be filled at the end of gestation. With the three-compartment model it is not possible to distinguish between maternal tissues and the feto-placental compartment. Therefore, we could only speculate about changes in transport to the feto-placental compartment in our kinetic studies. However, for the normal pregnant rat we also have data from steady-state experiments. These data show that the tissue-to-plasma ratio for T_4 decreased in all maternal tissues in the near-term pregnant rat. Since plasma T_4 decreased, the amount of T_4 in the tissues diminished even more. In the kinetic study the amount of T_4 in the tissue pools of near-term pregnant rats remained constant. This implies that one part of the tissue pools consists of non-maternal organs, i.e. placentas plus fetuses. Therefore, despite a normal thyroidal production less T_4 is available for the maternal organs which results in decreased tissue T_4 concentrations, as well as a decrease in the tissue T_3 concentration in most organs. Even an increase in deiodinase activity, ID-I as well as ID-II (3), would not lead to normal tissue T_3 levels, as has been demonstrated by the steady-state experiments.

7.2. MARGINAL IODINE DEFICIENCY DURING PREGNANCY

In the normal situation the mother shares the available iodide and T_4 with her fetuses, at the cost of her own supply. However, during marginal iodine deficiency (MID) the amount of available iodide is reduced. By means of an increase in thyroidal clearance, resulting from an increase in thyroid volume and thyroidal blood flow (1), the mother is able to maintain a normal absolute iodide uptake by the thyroid. Just as during normal pregnancy these processes do not seem to be regulated by TSH, because plasma TSH remains unchanged. In the fetuses the absolute iodide uptake is decreased during MID; the fetal thyroid is not able to compensate for the decrease in plasma inorganic iodide. This can possibly result in a diminished thyroid hormone production by the fetal thyroid. During maternal hypothyroidism fetal plasma TSH is elevated (2). However, the effect of MID on fetal TSH is unknown.
Both the production of T_4 by the maternal thyroid and the amount of T_4 in plasma and the tissue pools are only slightly decreased in the near-term pregnant rat. The mother is able to maintain the euthyroid state during MID. Therefore, it appears that MID will not affect the availability of T_4 for the fetuses. However, the transport of T_4 is seriously altered during MID. In particular interpool transport between plasma and

the fast pool is decreased. This could mean that less T_4 is transported to the fetuses. Since data on fetal thyroid hormones are not available it is difficult to speculate on the consequences for fetal development. The fetal brain is able to avoid T_3 deficiency by increasing ID-II activity (6). However, if there is insufficient substrate, i.e. T_4, this will not result in normal T_3 levels. This might be the situation during MID, because both the maternal and fetal supplies of T_4 are decreased.

7.3. MATERNAL HYPOTHYROIDISM

During maternal hypothyroidism only the maternal thyroid function is affected. The availability of iodide for the fetuses is normal or perhaps even increased. We have described a moderate situation, meaning that T_4 and T_3 are available, but normal plasma concentrations had not yet been reached; plasma T_4 was normal, whereas plasma T_3 was decreased. Despite the normal plasma T_4, the transport of T_4 to the fast pool, including the feto-placental compartment, was reduced to 30 % of that found for intact animals. This means that even in a mild hypothyroid state the availability of maternal T_4 for the fetuses is diminished.

7.4. IN CONCLUSION

During normal pregnancy the production of T_4 by the thyroid remains unchanged. However, this T_4 has to be distributed between the mother and her fetuses. This results in a decreased availability of T_4 for the maternal organs, leading indirectly to a deficiency of T_3 in the maternal organs.

During marginal iodine deficiency and maternal hypothyroidism the mother maintains the same thyroidal status in all organs as during normal pregnancy. This occurs at the expense of the availability of iodide and T_4 for the fetuses, which in the long run could lead to impaired development of the fetal central nervous system.

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CHAPTER 8

SUMMARY
SAMENVATTING

SUMMARY

Thyroid hormones, thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3), are produced by the thyroid gland. To synthesize thyroid hormones the thyroid needs iodide. The uptake of iodide as well as the production and secretion of T_4 and T_3 by the thyroid gland is regulated by thyrotropin (TSH), which is produced by the pituitary. However, most of the biologically active form, T_3, is produced from T_4 via monodeiodination in peripheral tissues. This reaction is catalyzed by the deiodinases, type I (ID-I) in liver and kidney, and type II (ID-II) in the central nervous system and brown adipose tissue (BAT). T_4 and T_3 concentrations differ in the various tissues, like the contribution of T_3 produced locally from T_4. A large portion of the T_3 produced in the liver enters the circulation, whereas T_3 produced in the brain and cerebellum is mainly used locally.

The production, distribution and transport of thyroid hormones are influenced by several (patho)physiological conditions. In this study we concentrated on the effects of pregnancy on maternal thyroid hormone metabolism. It is well known that thyroid hormones are very important for normal fetal development, especially of the central nervous system. During development thyroid hormones produced by the mother, mainly T_4, contribute to the fetal thyroid hormone pools before and also after onset of fetal thyroid function. Insufficient production of maternal thyroid hormones during pregnancy can result in permanent brain damage in the offspring. At the end of gestation the concentrations of T_4 and T_3 in maternal plasma and tissues have decreased. In order to gain more insight into the effects of pregnancy on the production, distribution, and transport of thyroid hormones in the mother we performed kinetic experiments with T_4 and T_3 using nonpregnant and near-term pregnant rats (chapter 2). A bolus injection of $[^{125}I]T_4$ and $[^{131}I]T_3$ was given, and blood samples were taken at regular times during the next twenty-four hours. Physiological parameters of the production, interpool transport, distribution and metabolism of T_4 and T_3 were estimated by means of a three-compartment model. According to this model three compartments can be distinguished: 1. the plasma; 2. the fast pool; and 3. the slow pool. Liver and kidney are considered to be the main components of the fast pool, whereas skin, muscles and brain belong to the slow pool. In the near-term pregnant rat the production and distribution of T_4 remained unchanged. The transport of T_4 from plasma to the fast pool was more than tripled, whereas transport to the slow pool remained constant. We suggest that in the near-term pregnant rat

available T_4 was distributed between the maternal and fetal compartments by means of very fast transport. This hypothesis is based on the fact that it seems unlikely that the transport of T_4 to maternal liver and kidney, which are considered to be the main components of the fast pool, will have increased that much in the near-term pregnant rat. This was confirmed by the results of steady-state, double isotopic experiments using nonpregnant and near-term pregnant rats (chapter 3). In this study, the rats received a continuous simultaneous infusion of $[^{125}I]T_4$ and $[^{131}I]T_3$ in order to achieve equilibrium in all tissues. With this method it was possible to calculate the T_4 and T_3 concentrations, the relative contributions of plasma-derived vs. locally produced T_3, the thyroidal T_4 and T_3 secretion rates, and the plasma-to-tissue ratios for T_4 and T_3. Indeed, the transport of T_4 to liver and kidney, as well as almost all other maternal organs, was diminished. Since the production of T_4 remained unchanged this implies that T_4 is transported to another compartment, i.e. the feto-placental compartment. This compartment was not measured in these studies. The plasma appearance rate for T_3 remained constant in the near-term pregnant rat. This was accomplished by an increase in the secretion of T_3 by the thyroid and a decrease in locally produced T_3. Less T_3 was transported from plasma to liver, kidney, BAT and pituitary. ID-I activity in liver, and ID-II activity in the brain both increased during pregnancy. However, this did not result in an increase in the local conversion of T_4 to T_3 in these tissues. In the liver the contribution of T_3 produced locally remained constant, while in the brain even a decrease was found. The insufficient availability of T_4 in maternal tissues, as demonstrated by the lower T_4 concentrations, might explain the discrepancy between deiodinase activities and the local production of T_3. The transport of T_4 to the feto-placental compartment resulted indirectly in a deficiency of T_3 in the maternal organs. We can conclude that pregnancy affects maternal thyroid hormone metabolism. The mother has to share the available thyroid hormones, especially T_4, with the fetuses.

Iodide is an essential element for the synthesis of thyroid hormones. In rats the fetal thyroid is capable of producing thyroid hormones on day 18 of gestation. Iodide is transported across the placenta from the maternal to the fetal circulation. In chapter 4 we assessed iodide uptake by the maternal thyroid, while the iodide uptake by the fetal thyroid was estimated. We measured the in vivo uptake of ^{125}I by the thyroid continuously. By using the specific activity of iodide in the urine we were able to calculate the absolute iodide uptake in the thyroid. Pregnancy resulted in a decrease in the absolute thyroidal iodide uptake. On day 20 of pregnancy the fetal thyroid is

already capable of concentrating iodide. However, the difference in absolute iodide uptake by the maternal thyroid, compared to nonpregnant controls, cannot fully be explained by the transport of iodide to the fetal compartment and/or the mammary glands. The decrease in iodide uptake by the maternal thyroid has no impact on the thyroidal production of thyroid hormones.

Iodine deficiency can lead to disturbed physical and mental development. In large populations in the world iodine intake is marginally deficient. For this reason a marginal iodine deficiency, instead of the more common severe iodine deficiency, was induced in our rats. We used this model to study the effects of marginal iodine deficiency on iodide metabolism (thyroidal iodide uptake; chapter 4) and thyroid hormone metabolism (kinetic experiments; chapter 5) in near-term pregnant rats. The absolute iodide uptake by the maternal thyroid was not affected by marginal iodine deficiency. The decreased plasma inorganic iodide was compensated by an increase in thyroidal clearance. A similar compensation was not found for the fetus; the uptake of iodide by the fetal thyroid decreased by 50 % during marginal iodine deficiency. During this marginal iodine deficiency plasma T_4 and T_3 remained constant in nonpregnant as well as near-term pregnant rats. The production rate and the plasma clearance rate for T_4 were both decreased. No effects of marginal iodine deficiency on pool sizes and transport rates were found for nonpregnant rats. In the near-term pregnant rat marginal iodine deficiency resulted in a marked decrease in the transport of T_4 from plasma to the fast pool. For T_3 an increase in the production rate and plasma clearance rate was found for nonpregnant, marginally iodine-deficient rats, while these parameters were slightly decreased in near-term pregnant rats. Marginal iodine deficiency induced a 50 % decrease in the interpool transport rates of T_3 between plasma and the fast pool in near-term pregnant rats. The hepatic activity of ID-I was increased as a result of marginal iodine deficiency in nonpregnant as well as near-term pregnant rats.

On the basis of the results of thyroid hormone studies in normal pregnant rats (chapter 2 and 3) we suggest that during marginal iodine deficiency less maternal T_4 is available for the fetal compartment. Together with the lower uptake of iodide by the fetal thyroid this can lead to diminished levels of thyroid hormone of maternal and fetal origin in the fetal organs. In this case, marginal iodine deficiency will have a negative effect on fetal development, especially of the brain.

Another situation which irreversibly affects fetal brain development is maternal hypothyroidism. Two different levels of hypothyroidism were induced in female rats,

by giving thyroidectomized rats two different doses of T_4 and T_3. The effects of hypothyroidism on maternal thyroid hormone metabolism in near-term pregnant rats (kinetic experiment, chapter 6) were studied. Plasma T_4 and T_3 levels were very low severely hypothyroid animals, whereas only plasma T_3 was decreased in the mildly hypothyroid group. Even during this mild hypothyroidism profound alterations in the transport rates of T_4 were found compared to intact, pregnant rats. The transport of T_4 from plasma to the fast pool was decreased. Therefore, it appears that even during mild hypothyroidism the transport of T_4 to the feto-placental compartment is affected.

In conclusion: Pregnancy seriously affects the maternal thyroid hormone status. Despite an unchanged thyroidal production of T_4, all maternal T_4 tissue levels are decreased. Less T_4 is available for the mother because of the transport of T_4 to the feto-placental compartment. Indirectly this results in a T_3-deficiency in most maternal organs. During marginal iodine deficiency and maternal hypothyroidism the transport of maternal T_4 to the feto-placental compartment is diminished, whereas during marginal iodine deficiency the availability of iodine for fetal thyroid hormone syn-thesis is also decreased. Eventually this can result in impaired development of the fetal central nervous system.

SAMENVATTING

Schildklierhormonen, thyroxine (T_4) en 3,5,3'-triiodothyronine (T_3), worden geproduceerd door de schildklier. Voor de vorming van schildklierhormoon heeft de schildklier jodide nodig. Zowel de opname van jodide als de productie en uitscheiding van schildklierhormoon door de schildklier worden gereguleerd door het schildklier stimulerend hormoon (TSH). Dit hormoon wordt geproduceerd door de hypofyse. Echter, het grootste deel van de biologisch actieve vorm van schildklierhormoon, het T_3, wordt, door middel van een monodejoderingsreactie, uit T_4 gevormd in de perifere weefsels. Voor deze reactie zijn dejoderende enzymen verantwoordelijk; type I monodejodase (ID-I) in de lever en nieren, en type II monodejodase (ID-II) in het centraal zenuwstelsel en bruin vetweefsel. De concentratie van T_4 en T_3 in de diverse weefsels verschilt, evenals de bijdrage van de hoeveelheid T_3 welke lokaal is gevormd uit T_4. Een groot deel van de T_3, welke lokaal in de lever is gevormd, wordt afgegeven aan de circulatie, terwijl lokaal gevormd T_3 in de hersenen en het cerebellum voornamelijk lokaal gebruikt wordt.

De productie, de verdeling en het transport van schildklierhormoon worden beïnvloed door verschillende (patho)fysiologische omstandigheden. In deze studie hebben we ons geconcentreerd op de effecten van zwangerschap op het schildklierhormoon-metabolisme van de moeder. Het is algemeen bekend dat schildklierhormoon van groot belang is voor een normale ontwikkeling van de foetus, met name van het centraal zenuwstelsel. Tijdens deze ontwikkeling levert schildklierhormoon van de moeder, zowel voor als na de start van de foetale schildklierfunctie, een bijdrage aan de foetale schildklierhormoonpools. Een ontoereikende productie van schildklierhormoon door de moeder tijdens de zwangerschap kan resulteren in permanente hersenbeschadiging van het nageslacht. In de rat zijn de concentraties van T_4 en T_3 in het plasma en de weefsels van de moeder aan het einde van de dracht verlaagd. Om meer inzicht te verkrijgen in de effecten van zwangerschap op de productie, de verdeling en het transport van T_4 en T_3 in de moeder, hebben we in niet-drachtige ratten en ratten aan het einde van de draagtijd experimenten uitgevoerd waarbij het kinetische gedrag van T_4 en T_3 is bepaald (hoofdstuk 2). De ratten ontvingen een eenmalige injectie van $[^{125}I]T_4$ en $[^{131}I]T_3$, waarna gedurende 24 uur, op vastgestelde tijdstippen bloedmonsters werden genomen. Fysiologische parameters van productie, transport, verdeling en metabolisme van T_4 en T_3 werden geschat met behulp van een drie-kompartimentenmodel.

In dit model kunnen drie kompartimenten worden onderscheiden: 1. het plasma; 2. een snel kompartiment; en 3. een langzaam kompartiment. Lever en nieren worden beschouwd als de belangrijkste componenten van het snelle kompartiment, terwijl de huid, spieren en hersenen tot het langzame kompartiment behoren. In de drachtige rat zijn, aan het einde van de dracht, de produktie van de verdeling van T_4 niet veranderd. Het transport van T_4 van het plasma naar het snelle kompartiment was meer dan verdrievoudigd, terwijl het transport van het plasma naar het langzame kompartiment constant gebleven was. Wij veronderstellen dat in de rat, aan het einde van de dracht, het beschikbare T_4, door middel van een zeer snel transport, wordt verdeeld tussen de moeder en de foetussen. Deze hypothese is gebaseerd op het feit dat het onwaarschijnlijk lijkt dat in de drachtige rat het transport van T_4 naar de lever en de nieren van de moeder, welke als belangrijkste componenten van het snelle kompartiment beschouwd worden, in deze mate is toegenomen. Dit wordt bevestigd door de resultaten van studies waarbij ratten continu een infuus met $[^{125}I]T_4$ en $[^{131}I]T_3$ ontvingen totdat in alle organen een evenwichtssituatie bereikt was. Dit houdt in dat de verhouding radioactief hormoon en endogeen hormoon in het hele lichaam gelijk is. Met behulp van deze methode is het mogelijk om de concentraties van T_4 en T_3, de relatieve bijdrage van T_3 afkomstig uit het plasma ten opzichte van lokaal gevormd T_3, de sekretie van T_4 en T_3 door de schildklier en de plasma/weefsel ratios in de verschillende weefsels te berekenen. En inderdaad, het transport van T_4 naar lever en nieren, en bijna alle andere organen was verminderd. De onveranderde produktie van T_4 wijst erop dat T_4 wordt getransporteerd naar een ander kompartiment. Wij gaan ervan uit dat dit de placentas plus de foetussen zijn. In onze experimenten kon dit kompartiment niet gemeten worden. De snelheid waarmee T_3 in het plasma verschijnt blijft constant in de drachtige rat. Dit wordt bereikt door middel van een toename in de sekretie van T_3 door de schildklier en een afname in de hoeveelheid lokaal gevormd T_3. De hoeveelheid T_3 die vanuit het plasma naar de lever, nieren, bruin vet en hypofyse getransporteerd wordt is afgenomen. Zowel de ID-I activiteit in de lever als de ID-II activiteit in de hersenen is toegenomen tijdens de dracht. Echter, dit heeft geen toename van de lokale omzetting van T_4 naar T_3 in deze weefsels tot resultaat. In de lever blijft de bijdrage van lokaal gevormd T_3 gelijk, terwijl in de hersenen zelfs een verlaging wordt gevonden. Dit kan verklaard worden doordat de beschikbaarheid van T_4 in de weefsels van de moeder ontoereikend is. Het transport van T_4 naar de placentas plus foetussen resulteert indirekt in een tekort aan T_3 in de organen van de moeder.

We kunnen concluderen dat zwangerschap het schildklierhormoonmetabolisme van de moeder beïnvloedt. De moeder moet de beschikbare schildklierhormonen, vooral T_4, met de foetussen delen.

Voor de synthese van schildklierhormoon is jodide een onmisbaar element. In de rat is de foetale schildklier vanaf dag 18 van de dracht in staat om schildklierhormoon te produceren. Jodide wordt via de placenta van de bloedsomloop van de moeder naar de foetale bloedsomloop getransporteerd. In hoofdstuk 4 hebben we de opname van jodide door de schildklier van de moeder gemeten, terwijl een schatting is gemaakt van de opname van jodide door de foetale schildklier. De *in vivo* opname van ^{125}I door de schildklier werd gedurende vier uur onafgebroken gemeten. Door gebruik te maken van de specifieke activiteit van jodide in de urine waren we in staat om de hoeveelheid jodide opgenomen door de schildklier te berekenen. Zwangerschap had een kleine afname van de hoeveelheid jodide welke door de schildklier van de moeder werd opgenomen tot resultaat. Op dag 20 van de dracht was de foetale schildklier in staat om jodide te concentreren. Het verschil in opgenomen hoeveelheid jodide door de schildklier van de moeder, vergeleken met de niet-drachtige situatie, kan echter niet volledig worden toegeschreven aan het transport van jodide naar de foetussen en/of de melkklieren. De daling in de opname van jodide door de schildklier van de moeder heeft geen gevolgen voor de productie van schildklierhormoon door de schildklier.

Jodiumdeficiëntie kan een verstoorde fysische en mentale ontwikkeling tot gevolg hebben. In grote populaties in de wereld is de inname van jodium marginaal. Daarom is in onze experimenten gekozen voor het toebrengen van een marginale jodium-deficiëntie, in plaats van de veel vaker gebruikte ernstige jodiumdeficiëntie. We hebben de effecten van marginale jodiumdeficiëntie op het metabolisme van jodide (opname van jodide door de schildklier; hoofdstuk 4) en het metabolisme van T_4 en T_3 (kinetiek experimenten; hoofdstuk 5) in de drachtige rat, aan het einde van de draagtijd, bestudeerd. De hoeveelheid jodide opgenomen door de schildklier van de moeder werd niet beïnvloed door marginale jodiumdeficiëntie. De afname in de jodideconcentratie in het plasma werd gecompenseerd door een toename in de klaring door de schildklier. Een dergelijke compensatie werd niet gevonden in de foetussen; de opname van jodide door de foetale schildklier was gehalveerd tijdens marginale jodiumdeficiëntie. Zowel in drachtige als niet-drachtige ratten bleven de concentraties van T_4 en T_3 in het plasma onveranderd tijdens marginale jodiumdeficiëntie. Zowel de snelheid waarmee T_4 werd geproduceerd als de snelheid waarmee T_4 uit het plasma werd geklaard namen af.

In niet-drachtige ratten werden geen effecten van marginale jodiumdeficiëntie op de hoeveelheid T_4 in de diverse kompartimenten en de snelheden van het transport van T_4 gevonden. Marginale jodiumdeficiëntie resulteerde in de drachtige rat in een sterke daling van het transport van T_4 van het plasma naar het snelle kompartiment. Voor T_3 werd een toename in de snelheid van productie en de snelheid van klaring uit het plasma gevonden in niet-drachtige, maginaal jodiumdeficiënte rat, terwijl deze parameters lichtelijk waren afgenomen in de drachtige rat. Marginale jodiumdeficiëntie had een afname van 50 % in de snelheid van transport van T_3 tussen het plasma en het snelle kompartiment in de drachtige rat tot gevolg. De activiteit van ID-I in de lever was toegenomen tijdens marginale jodiumdeficiëntie in zowel niet-drachtige als drachtige ratten.

Gebaseerd op de resultaten van de schildklierhormoonstudies in normale, drachtige ratten (hoofdstuk 2 en 3) veronderstellen wij dat tijdens marginale jodiumdeficiëntie de hoeveelheid T_4 van de moeder, welke beschikbaar is voor de foetussen, verminderd is. Samen met de daling in de opname van jodide door de foetale schildklier, kan dit leiden tot verlaagde schildklierhormoonniveaus, afkomstig van zowel moeder als foetus, in de foetale organen. In dit geval zal marginale jodiumdeficiëntie negatieve effecten hebben op de ontwikkeling van de foetussen, in het bijzonder van de hersenen.

Hypothyreoïdie van de moeder is een andere situatie waarbij de ontwikkeling van de foetale hersenen irreversibel beïnvloed wordt. Door vrouwelijke ratten waarvan de schildklier met behulp van ^{131}I kapot is gestraald twee verschillende dosissen T_4 en T_3 toe te dienen, werden twee verschillende niveaus van hypothyreoïdie geïnduceerd. Het effect van hypothyreoïdie op het metabolisme van T_4 en T_3 in de drachtige rat wordt beschreven in hoofdstuk 6. In de diep hypothyreote dieren waren de concentraties van T_4 en T_3 in het plasma erg laag, terwijl in de milde hypothyreote dieren alleen de concentratie van T_3 in het plasma verlaagd was. Zelfs tijdens deze milde vorm van hypothyreoïdie werden, in vergelijking met intacte, drachtige dieren, verregaande veranderingen in de snelheden van transport van T_4 gevonden. Het transport van T_4 van het plasma naar het snelle kompartiment was afgenomen. Dus, het lijkt erop dat zelfs tijdens een milde hypothyreoïdie het transport van T_4 naar de placentas plus foetussen is aangetast.

Concluderend: zwangerschap heeft veranderingen in de schildklierhormoonstatus van de moeder tot gevolg. Ondanks een onveranderde productie van T_4 door de schildklier is in alle organen van de moeder de concentratie van T_4 verlaagd. Door het transport van T_4 naar de placentas plus foetussen is minder T_4 beschikbaar voor de moeder zelf.

Dit resulteert indirect in een tekort aan T_3 in de meeste organen. Zowel bij marginale jodiumdeficiëntie als bij milde hypothyreoïdie van de moeder is het transport van T_4 naar de placentas plus foetussen verminderd. Tijdens marginale jodiumdeficiëntie is ook de beschikbaarheid van jodide voor synthese van schildklierhormoon door de foetale schildklier verlaagd. Deze veranderingen kunnen in de foetus uiteindelijk een aantasting van de ontwikkeling van het centrale zenuwstelsel tot gevolg hebben.

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Petra

Curriculum Vitae

Petronella Maria (Petra) Versloot is geboren op 5 maart 1968 te Wymbritseradeel, Friesland. In 1986 behaalde zij haar VWO diploma aan het Han Fortmann College te Heerhugowaard en begon haar studie Biologie aan de Universiteit van Amsterdam. Na het propedeatisch examen (cum laude) werd gekozen voor de bovenbouw studierichting Medische Biologie. Specialisaties tijdens de studie waren fysiologie en endocrinologie. Ook werd de artikel 9 bevoegdheid voor het werken met proefdieren behaald. Tijdens de doctoraal fase zijn afstudeervakken uitgevoerd bij de vakgroepen Experimentele Kindergeneeskunde en Anatomie en Embryologie van het Academisch Medisch Centrum te Amsterdam. Het doctoraal examen werd behaald in februari 1991, waarna zij werkzaam is geweest als Onderzoeker in Opleiding bij de vakgroep Fysiologie van Mens en Dier van de Landbouwuniversiteit te Wageningen. Het onderzoek bij deze vakgroep heeft geleid tot dit proefschrift.