Congenital Hypothyroidism, as Studied in Rats

Crucial Role of Maternal Thyroxine but Not of 3,5,3'-Triiodothyronine in the Protection of the Fetal Brain

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Abstract

To study the protective effects of maternal thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) in congenital hypothyroidism, we gave pregnant rats methimazole (MMI), an antithyroid drug that crosses the placenta, and infused them with three different doses of T_4 or T_3. The concentrations of both T_4 and T_3 were determined in maternal and fetal plasma and tissues (obtained near term) by specific RIAs. Several thyroid hormone-dependent biological end-points were also measured. MMI treatment resulted in marked fetal T_4 and T_3 deficiency. Infusion of T_4 into the mothers increased both these pools in a dose-dependent fashion. There was a preferential increase of T_3 in the fetal brain. Thus, with a T_4 dose maintaining maternal euthyroidism, fetal brain T_3 reached normal values, although fetal plasma T_4 was 40% of normal and plasma TSH was high. The infusion of T_3 into the mothers increased the total fetal extrathyroidal T_3 pool in a dose-dependent fashion. The fetal T_4 pools were not increased, however, and this deprived the fetal brain (and possibly the pituitary) of local generation of T_3 from T_4. As a consequence, fetal brain T_3 deficiency was not mitigated even when dams were infused with a toxic dose of T_3. The results show that (*a*) there is a preferential protection of the brain of the hypothyroid fetus from T_3 deficiency; (*b*) maternal T_4, but not T_3, plays a crucial role in this protection, and (*c*) any condition which lowers maternal T_4 (including treatment with T_3) is potentially harmful for the brain of a hypothyroid fetus. Recent confirmation of transplacental passage of T_4 in women at term suggests that present results are relevant for human fetuses with impairment of thyroid function. Finding signs of hypothyroidism at birth does not necessarily mean that the brain was unprotected in utero, provided maternal T_4 is normal. It is crucial to realize that maintainance of maternal "euthyroidism" is not sufficient, as despite hypothyroxinemia, the mothers may be clinically euthyroid if their T_3 levels are normal. (*J. Clin. Invest.* 1990. 86:889–899.) Key words: cretinism • placenta • 5' deiodinase • TSH • hypothyroxinemia

Introduction

For years the mammalian placenta has been considered virtually impermeable to the thyroid hormones, L-thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) (1, 2). Thus, thyroid hormones would not be required for normal embryonic development before onset of fetal thyroid function (3, 4). It has, however, been shown that rat and human embryonic tissues contain T_4 and T_3 before onset of fetal thyroid function, and that both are of maternal origin (5–8). The nuclear receptor for T_3 is also found in human and rat brain early in pregnancy (7, 9). The adverse effects of maternal hypothyroxinemia on the central nervous system (CNS) might be related to the thyroid hormone deficiency of the developing embryo (5, 8, 10–12). In neurologic endemic cretinism the degree of the developmental disorders has been correlated to with that of maternal hypothyroxinemia (13).

Even when transfer of maternal thyroid hormones early in pregnancy is accepted (14), it is believed that maternal transfer would not be necessary once the fetal thyroid is active; placental inner ring deiodinations would prevent the iodothyronines from reaching the fetus (2, 15). The fetal pituitary-thyroid axis functions with a great degree of autonomy: thyroid failure is accompanied by low T_4 and elevated serum TSH levels. This, however, does not necessarily mean total independence from maternal thyroid status. Transfer of maternal thyroid hormones might still continue, and mitigate the effects of thyroid hormone deficiency until birth.

We have shown in rats that both T_4 and T_3 continue to be transferred from the mother to the fetus near term, when the fetal thyroid is impaired (16, 17). The infusion of T_4 to the mother (16) mitigated both T_4 and T_3 deficiency of the methyl-mercapto-imidazole-2-thiol (MMI)-treated fetus. The brain actually attained normal concentrations of T_3, at present considered as the intracellularly active iodothyronine (18). In contrast, the infusion of T_3 to the mother did not mitigate fetal cerebral T_3 deficiency, although it increased the concentrations of T_3 in other fetal tissues (17).

These previous studies did not clarify several points of interest: (*a*) the range of maternal doses of T_4 that would ensure normal T_3 levels in the fetal brain; (*b*) whether, or not, a supraphysiological maternal supply of T_4 would result in excessive concentrations of T_3 in the fetal brain, and (*c*) whether or not, normalization of fetal brain T_3 would eventually be attained with higher doses of maternal T_3 than previously used. Such information may well be relevant to babies with congenital hypothyroidism. It has been recently shown by Vulsma et al. (19) that T_4 is transferred from the mother to the hypothyroid baby up to birth: T_4 levels in cord-blood from babies with total organification defect ranged from 2.7 to 5.4 μg/dl, namely 25 to 50% of normal values, then decreasing with a

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1. *Abbreviations used in this paper:* α-GPD, α-glycerophosphate dehydrogenase; BAT, brown adipose tissue; Cx, cerebral cortex; 5' D-II, type II 5'-iodothyronine deiodinase; MMI, methimazole 1-methyl-mercapto-imidazole-2-thiol; PTU, 6-*N*-propyl-2-thiouracil

half-life of disappearance of 3.6 d. Thus, in contrast with previous widespread opinion, maternal thyroid hormones might protect the human hypothyroid fetus, and especially its brain, at least up to birth.

The present paper reports results obtained in fetuses from rats treated with MMI, and infused with T_4 or T_3, at three different dose levels of each.

Methods

Experimental design. Female rats of a Wistar strain were used. They were mated with normal males, the day of appearance of a vaginal plug being considered as day 0 of gestation (8). They were divided into eight different groups (Table I), comprising three dams each: seven of the eight groups were given 0.02% MMI as drinking water, starting on the morning of the 14th day of gestation (MMI dams). The eighth group (C dams) did not receive MMI. At 15 d of gestation, all the dams were implanted with osmotic minipumps delivering at a constant rate either infusion solvent (dams of the C group and of one MMI group), T_4 (three MMI + T_4 groups) or T_3 (three MMI + T_3 groups). The doses of T_4 and T_3 are shown in Table I. The dams were killed at 21 d of gestation, and perfused as previously described in detail (8), except that 6-*N*-propyl-2-thiouracil (PTU) was not added to the perfusion medium to avoid possible interferences with the measurements of outer-ring iodothyronine deiodinase activities. We used a total of 24 dams and their 223 fetuses.

Continuous infusion of T_4 and T_3. We used 2-ml Alzet osmotic minipumps (Alza Corp., Palo Alto, CA), implanted under the dorsal skin (16). The pumps delivered 5 μl/h for 14 d. The minipumps implanted into the C and MMI dams contained only the infusion solvent, namely phosphosaline buffer, pH = 7.4, containing 5% serum from thyroidectomized rats. T_4 or T_3 in free acid form (Sigma Chemicals Co., St. Louis, MO) were dissolved in a small volume of 0.05 N NaOH and then diluted with infusion solvent. Small amounts of [^{131}I]T_4 or [^{125}I]T_3 (20–30,000 cpm per pump), prepared as described further on, were added to the T_4 or T_3 solutions, respectively. The radioactivity of the pumps was determined before implanting them, and at the end of the experiment. This permitted us to control that the delivery rate in vivo had been the one specified by the manufacturers. Performance of the pumps was always satisfactory.

The T_4 and T_3 doses indicated in Table I are referred to 100 g of body weight (BW) at 15 d of gestation, when the pumps were prepared and implanted. The amount of hormone delivered daily remained constant until the end of the experiment, but the dose per 100 g BW was decreasing progressively, as the weight of the dam plus concepta increased from 193±5 to 275±7 g between 15 and 21 d of gestation. The doses, as referred to 100 g BW at 21 d of gestation, are shown in brackets.

Table I. Experimental Design: Groups of Dams and Their Treatment Schedules

Group	MMI* (14–21)‡	Infusate (15–21)‡
C	–	Solvent
MMI	+	Solvent
MMI+ 1.8 μg T_4	+	1.8 μg T_4/100 g BW/d§ (1.3 μg T_4)
MMI+ 2.4 μg T_4	+	2.4 μg T_4/100 g BW/d (1.7 μg T_4)
MMI+ 3.6 μg T_4	+	3.6 μg T_4/100 g BW/d (2.5 μg T_4)
MMI+ 0.5 μg T_3	+	0.5 μg T_3/100 g BW/d (0.35 μg T_3)
MMI+ 1.5 μg T_3	+	1.5 μg T_3/100 g BW/d (1.05 μg T_3)
MMI+ 4.5 μg T_3	+	4.5 μg T_3/100 g BW/d (3.15 μg T_3)

* 0.02% in drinking water.
‡ Days of gestation.
§ Calculated according to the BW at 15 d of gestation, and thus decreasing per 100 g BW as the dams neared term. The daily dose, calculated according to the BW at 21 d of gestation is shown in brackets.

Preparations of samples. Maternal plasma, liver, heart, and brain were excised. The fetuses were bled, separated from the placenta and immediately placed on ice. The thyroid, adhering to the trachea, was obtained under the dissecting microscope, and kept frozen. The liver, brain, heart, and lung were dissected out, weighed and frozen rapidly on dry ice. The interscapular brown adipose tissue (BAT) pads were also dissected out and used for a parallel study on fetal BAT 5'-iodothyronine deiodinase activity (20). The rest of the fetus, referred to here as the carcass (whole embryo minus the blood, trachea, thyroid, liver, lung, brain, heart, and BAT) was stored frozen. The fetal and maternal sides of each placenta were separated with blunt forceps, and stored frozen for a separate study (Calvo, R., et al., manuscript in preparation).

The thyroid glands were processed individually. Plasma from different fetuses were pooled, so that 300–400-μl aliquots were obtained for extraction and determination of T_4, T_3, and TSH. Carcasses were processed individually, livers were pooled from two, brains from two to three, lungs and hearts from four to five fetuses to obtain ~ 0.5 g of tissue per pool (except for 0.1–0.15 g per pool of cardiac tissue). Tissues that were pooled were obtained from fetuses of the same litter. For most tissues there were two individual samples or pools/dam, and thus six pools for each of the eight experimental groups. All samples of a given tissue were processed in the same analytical run.

Determination of T_4 and T_3 concentrations. All samples, including maternal and fetal plasmas, were extracted and processed as described in detail (8, 16). In brief, homogenization in methanol is followed by extraction in chloroform-methanol, back-extraction into an aqueous phase, and purification of this phase through Bio-Rad AG 1 × 2 resin columns (Bio-Rad Laboratories, Richmond, CA). The iodothyronines are eluted with 70% acetic acid, which is then evaporated to dryness. RIA buffer is added, and the samples are submitted to highly sensitive RIAs for the determination of T_4 and T_3, the limits of sensitivity being 2.5 pg T_4 and 1.5 pg T_3/tube. Each sample is processed in duplicate or triplicate at two or more dilutions. Results are then calculated using individual recovery data obtained after addition of [^{131}I] T_4 and [^{125}I] T_3 during the initial homogenization process. The amounts of tracers added are such that the radioactivities carried over into the RIA tubes are too low to interfere with the determinations.

The total T_4 and T_3 contents of the fetal thyroids were determined in methanol extracts of proteolytic digests of whole glands (16).

The synthesis of the high specific activity ^{125}I- and ^{131}I-labeled tracers used for individual recovery calculations and of the labeled iodothyronines used as antigens for the T_4 and T_3 RIAs was carried out as described previously (8, 16).

Other determinations. Plasma levels of thyrotrophic hormone (TSH) were measured using immunoreactants kindly supplied by the Rat Pituitary Agency of the National Institutes of Arthritis, Diabetes & Digestive and Kidney Diseases (NIADDK) of the National Institutes of Health (Bethesda, MD), as previously described (21). Results are expressed in weight equivalents of the NIAMDDK-rTSH-RP-2 reference preparation.

The activity of intramitochondrial α-glycerophosphate dehydrogenase (α-GPD; EC 1.1.99.5) was measured in mitochondrial preparations from individual fetal livers as described by Lee and Lardy (22), with minor modifications.

The activity of type II 5'-iodothyronine deiodinase (5' D-II) in maternal cerebral cortex (Cx) and in fetal brain was determined as described (23, 24), using [^{125}I] T_4 (2 nM) in the presence of 20 mM DTT and 1 mM PTU, 100–200 μg protein/tube and a 1-h incubation at 37°C.

Statistical analysis. Mean values (±SEM) are given. Data from the eight experimental groups were submitted to one-way analysis of variance, after testing for homogeneity of variance using Bartlett's proce-

dure for groups of unequal size. Square root or logarithmic transformations usually ensured homogeneity of variance when this was not found with the raw data. Significance of differences between groups was assessed using the protected LSD (least significance difference) test, and considered significant when $P < 0.05$. All these calculations were performed as described by Snedecor and Cochran (25). Whenever it is stated that a variable in a group is increased, or decreased, as compared to another group, it is implied that the difference between the mean values was statistically significant. Statistically significant differences of data from T_4- or T_3-infused MMI-treated dams and those of the C and MMI-treated animals are identified in Tables that appear in the Appendix.

Results

Infusion doses maintaining euthyroidism in MMI-treated dams

Maternal plasma and tissue T_4 and T_3 concentrations are shown in the left-hand panels of Figs. 1 and 2, and biological end-points of thyroid hormone action, such as circulating TSH, cerebral cortex 5′ D-II and liver α-GPD activities in the left-hand panels of Fig. 3. In all the figures results corresponding to C or MMI-treated dams are shown as shaded areas, which enclose the mean value±SEM. The results corresponding to T_4 (or T_3) infused MMI-treated dams are represented as dose-response curves.

As may be seen, MMI treatment for 7 d decreased the levels of both T_4 and T_3 in plasma and all maternal tissues studied.

CHANGES IN THE CONCENTRATIONS OF T_4 (FIG. 1)

In dams infused with T_4. Dose-response relationships were found between the T_4 dose and T_4 levels in maternal plasma and tissues. The changes in tissues, however, were not necessarily the same as in plasma. Concentrations of T_4 in the heart and liver of MMI-treated dams infused with the 1.8-μg T_4 dose (for the sake of simplification, doses given henceforth refer to the daily dose per 100 g BW per d, at 15 d of gestation, even if this is not explicitly stated each time) were normal (that is, comparable to those of C dams), whereas in brain they were higher, and in plasma lower. Plasma T_4 was comparable to C values in dams infused with the 2.4-μg T_4 dose, whereas T_4 levels were higher than normal in all the tissues studied. Although the concentrations of T_4 exceeded C values in all maternal samples with the 3.6-μg T_4 dose, they did not increase above those of nonpregnant females of similar age (26), except for the brain.

In dams infused with T_3. Doses ranging from 0.5 to 4.5 μg T_3 either had no effect on T_4 levels in plasma and tissues of the MMI-treated dams, or actually decreased them, especially when the highest T_3 dose was used.

CHANGES IN THE CONCENTRATIONS OF T_3 (FIG. 2)

In dams infused with T_3. The dose-response relationships between the T_3 dose and T_3 levels were not the same for tissues and plasma. T_3 levels in the brain of dams infused with 0.5 μg T_3 did not attain C values, although T_3 levels were normal in plasma and heart, and elevated in the liver. Infusion of the 1.5-μg T_3 dose resulted in normal T_3 in plasma and brain, but liver and heart levels were higher than in C dams. The highest T_3 dose, namely 4.5 μg T_3, increased T_3 levels in all maternal samples both above C values and above those of normal nonpregnant female rats (26).

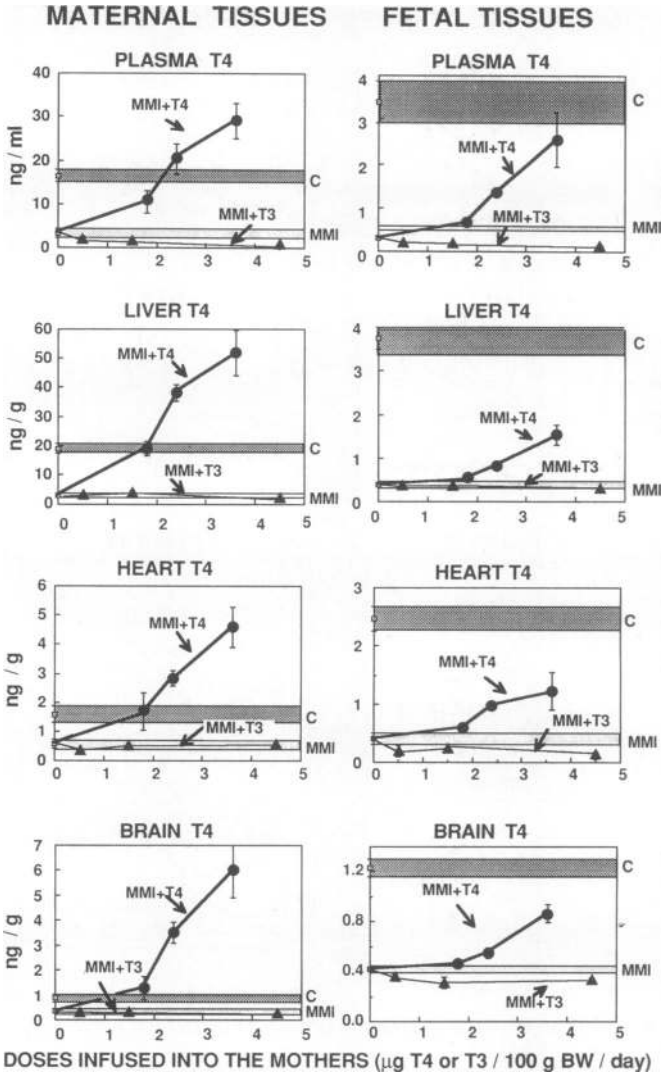

Figure 1. The left panels show the changes in the concentrations of T_4 in maternal plasma and tissues, as a function of the dose of T_4, or T_3, which was infused into the MMI-treated dams. The right panels show the changes in the plasma and tissues of their fetuses. The circles and thick lines correspond to the T_4-infused groups, the triangles and thin lines correspond to the T_3-infused groups. The SEM values are shown as vertical lines above and below the means, unless smaller than the size of the circles or triangles used as data points. The darker shaded area corresponds to the T_4 concentration in plasma and tissues from C dams, or their fetuses (±SEM); the lighter shaded area to the T_4 levels in the MMI-treated dams, or their fetuses. Statistically significant differences are summarized in Table AI of the Appendix.

In dams infused with T_4. T_3 levels in plasma and tissues increased with increasing doses of T_4. A 1.8-μg T_4 dose normalized brain T_3, but was inadequate to maintain normal T_3 levels in plasma, heart and liver. The 2.4-μg T_4 dose resulted in normal T_3 in plasma and all tissues studied, brain included. T_3 levels exceeded C values in the plasma and liver of the dams infused with 3.6 μg of T_4, but not in the brain and heart.

It therefore appears that no single dose of either T_4 or T_3 alone is able to maintain normal concentrations of both T_4 and T_3 in all tissues of MMI-treated dams.

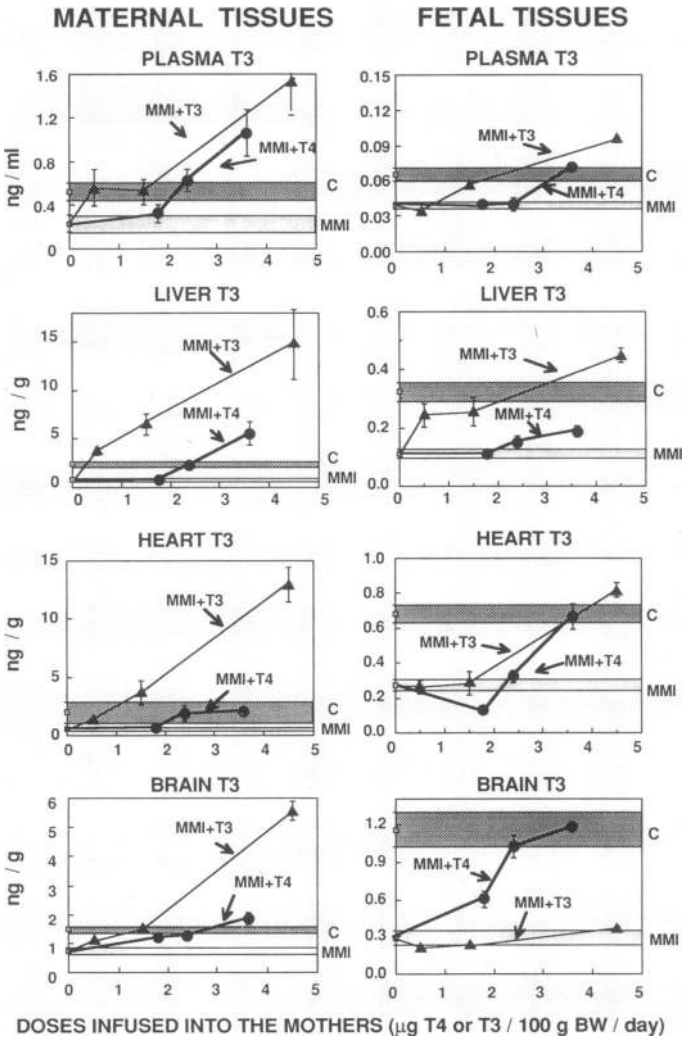

Figure 2. The left panels show the changes in the concentrations of T_3 in maternal plasma and tissues, the right panels those in their corresponding fetuses. Data are represented as indicated in the legend to Fig. 1. Statistically significant differences are recorded in Table AII of the Appendix.

EFFECTS ON END-POINTS OF THYROID HORMONE ACTION (FIG. 3)

Plasma TSH levels were increased fourfold by the 7-d treatment with MMI. This increase was avoided by the concomitant infusion of the dams with either T_4 or T_3, even at the lowest dose. 5′ D-II activity increased in the cerebral cortex of the MMI-treated dams. Infusion of T_4, but not of T_3, prevented this increase in dose-dependent fashion. 5′ D-II was inversely correlated to cerebral T_4 (but not to T_3): $y = 31.0\ x^{-1.016}$, where y = fmol/h per mg protein, and x = ng T_4/g, with $r = 0.98$. Mean hepatic α-GPD activity was lower in the MMI-treated as compared to the C dams, but the difference was not statistically significant. (This might be related to the finding that liver α-GPD activity is already quite low in normal pregnant rats at 21 d of gestation, as compared to age-paired nonpregnant rats [26].) The α-GPD activity was increased above MMI and C levels by the infusion of 3.6 μg T_4, or of 1.5 or 4.5 μg T_3. Activity was linearly related to hepatic T_3 (but not to T_4): $y = 0.78 + 0.69x$, where y = Δ OD/min per 100 mg protein, and x = ng T_3/g, with $r = 0.98$. The α-GPD activity usually found in non-pregnant female rats (26) was only exceeded with the highest T_3 dose.

In conclusion, a daily dose of 2.4 μg T_4 was not excessive for the MMI-treated dams. A daily dose of 1.5 μg T_3 appeared excessive for some tissues, but not for the brain, whereas 4.5 μg T_3 was a supraphysiological dose for all maternal tissues studied here.

Effects of maternal treatments on the fetuses

Though data are not shown here, none of the treatments (MMI for 7 d, with or without concomitant infusion of saline, T_4 or T_3) affected the number of fetuses per litter. No dose-dependent effects were observed on the body and organ weights of the fetuses.

EFFECTS OF MMI ON THE FETAL THYROID

Treatment with MMI effectively blocked fetal thyroid function: total T_4 contents in proteolytic digests were 35.3±1.0 and 0.22±0.02 ng/gland in fetuses from C and MMI-treated dams, respectively, the T_3 contents being 1.695±0.061 and 0.086±0.001 ng/gland. Thyroidal T_4 and T_3 did not increase with the infusion of T_4 or T_3 into the MMI-treated dams. Hyperemic enlarged glands were observed in all the MMI-treated fetuses, irrespective of maternal T_4 or T_3 infusions.

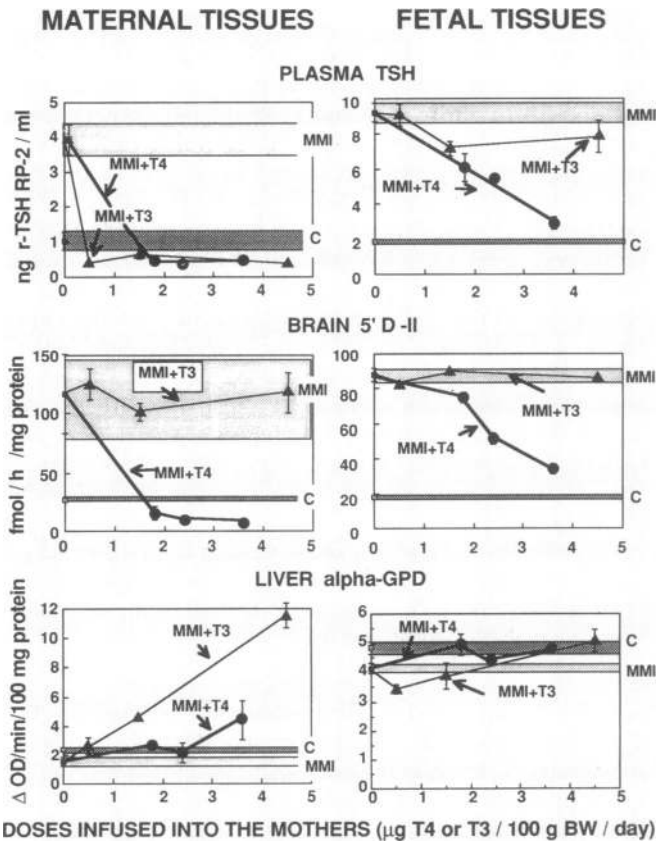

Figure 3. The left panels show the changes in plasma TSH, cerebral 5′ D-II, and hepatic α-GPD in dams, the right panels those observed in their fetuses. Data are represented as indicated in the legend to Fig. 1. Statistically significant differences are recorded in Table AIII of the Appendix section.

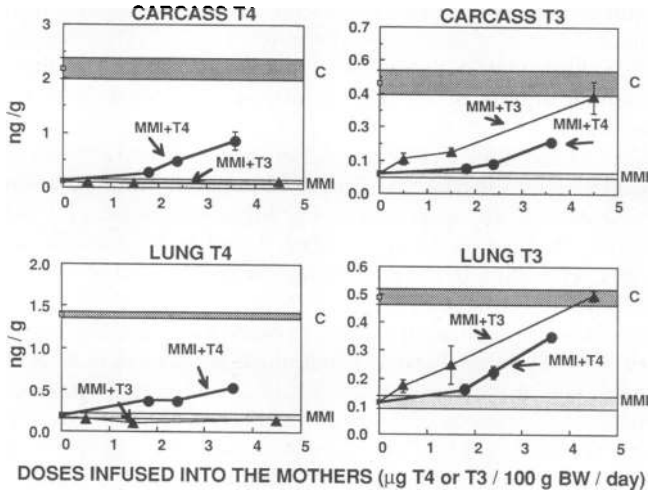

Figure 4. Fetal tissues. The left panels show the concentrations of T_4, the right panels those of T_3, in fetal lung and carcass. Data are shown as indicated in the legend to Fig. 1. Statistically significant differences are recorded in Table AIV of the Appendix.

EFFECTS ON FETAL EXTRATHYROIDAL T_4 AND T_3 CONCENTRATIONS

T_4 and T_3 decreased in plasma and tissues of fetuses from MMI-treated dams, as shown in the right panels of Figs. 1 and 2, and in Fig. 4. Data for fetuses from T_4- or T_3-infused dams are shown as dose-response curves.

Changes in the concentrations of T_4 (Figs. 1 and 4)

In fetuses from dams infused with T_4. The infusion of T_4 mitigated the effect of MMI on T_4 levels in fetal plasma and tissues, even at the lowest (1.8 μg) dose. Normal (C) values, however, were not reached even with the highest dose, namely 3.6 μg T_4: the T_4 concentrations closest to C values were found in plasma and brain, the lowest in lung.

In fetuses from dams infused with T_3. The T_4 concentrations in plasma, brain, liver, heart, carcass, and lung of fetuses from MMI-treated dams were either unchanged by the infusion of the dams with T_3, or actually decreased further, especially with the 4.5-μg T_3 dose.

Changes in the concentrations of T_3 (Figs. 2 and 4)

In fetuses from dams infused with T_3. Dose-response relationships were observed between the T_3 level in a fetal tissue and the dose of T_3 infused into the mother, the degree of change being tissue specific. Normal T_3 levels were reached with the 1.5-μg T_3 dose in plasma and liver, but not in heart, lung, or carcass. Normal fetal T_3 levels were only reached in carcass and lung with the highest dose, namely 4.5 μg T_3, although T_3 levels in plasma, liver, and heart clearly exceeded C values.

Changes in fetal brain T_3 are in marked contrast with those in fetal plasma and other tissues: T_3 concentrations were not increased above the MMI levels even with the infusion of 4.5 μg T_3, which raised fetal plasma T_3 above C levels.

In fetuses from dams infused with T_4. The infusion of the two lower doses of T_4 hardly affected T_3 levels in most fetal tissues, brain excepted. With the 3.6-μg T_4 dose normal fetal T_3 levels were reached in plasma and heart, but not in other tissues.

The changes in cerebral T_3 were striking: with the lowest T_4 dose, T_3 concentrations in the brain were almost 50% of control values, despite the fact that this dose did not increase T_3 in fetal plasma and other fetal tissues above MMI levels. Brain T_3 levels were the same as those of normal fetuses with the 2.4-μg T_4 dose, and did not exceed them in fetuses from dams receiving the highest dose. Thus, brain T_3 was normal in fetuses in which both plasma T_3 and T_4 were still low: T_4 was ~ 40% of C values, and T_3 was in the MMI range.

FETAL BRAIN T_4 AND T_3 VERSUS TOTAL EXTRATHYROIDAL POOLS

The total extrathyroidal T_4 and T_3 pools (Fig. 5, *left*) were calculated by adding the amounts of each hormone contained in plasma, fetal organs and carcass (The mean concentration values and the mean organ weights were used to calculate the T_4 [or T_3] contents in each tissue. The mean total blood volume was calculated as 16.8% of body weight, based on data obtained in rat fetuses near term [27]. Data for BAT were from Obregon et al. [20].): The values obtained for normal fetuses were taken as 100%. The cerebral T_4 and T_3 contents are represented in similar fashion in the right-hand panel of Fig. 5. Comparison of both panels stresses the finding that brain T_3 was normal in fetuses from MMI-treated dams infused with doses of T_4 which were insufficient to avoid T_4 and T_3 deficiency of the fetus as a whole. T_3 deficiency of the fetus could be prevented by the infusion of a supraphysiological dose of T_3, but this had no mitigating effect on fetal brain T_3 deficiency.

EFFECTS ON FETAL END-POINTS OF THYROID HORMONE ACTION (FIG. 3)

Both fetal plasma TSH and fetal brain 5′ D-II increased markedly with MMI treatment. Both increases were avoided in a dose-dependent fashion by the concomitant infusion of T_4 into the mothers, but not by the infusion of T_3. Both endpoints were inversely related to the respective T_4 concentrations. The relationship between cerebral 5′ D-II and T_4 was $y = 25.9\ x^{-1.17}$ (with y = fmol/h per mg protein, and x = ng T_4/g brain), $r = 0.97$, and was quite similar to the one found for the cerebral cortex of their mothers.

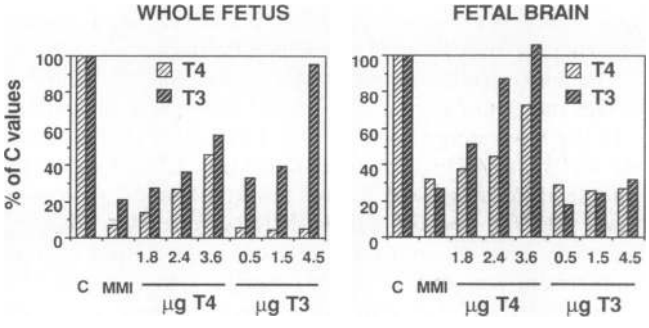

Figure 5. The left panel shows the total extrathyroidal pools of T_4 (light-hatched bars) and of T_3 (dark-hatched bars) in fetuses from C and MMI-treated dams, and from T_4 or T_3-infused MMI-treated dams. The extrathyroidal pools corresponding to C fetuses (11.19 ng T_4 and 2.42 ng T_3) are taken as the 100% values. The right panel shows the amounts of T_4 and T_3 in the fetal brain. The values corresponding to C fetuses (0.221 ng T_4 and 0.208 ng T_3) are taken as the 100% values.

Fetal plasma TSH and T_4 were inversely related, $y = 8.99 \cdot 10^{-0.196x}$ (where y = ng TSH/ml, and x = ng T_4/ml), $r = 0.98$. In contrast with findings in the mothers, fetal plasma TSH and T_3 levels were not inversely related. Plasma TSH in fetuses from T_3-infused dams was as high as in fetuses from dams on MMI alone, even with the highest T_3 dose, which increased fetal plasma T_3 above C levels.

Changes in α-GPD activities in the fetal liver were not as clearly thyroid hormone-dependent as in the maternal liver, although the infusion of T_3 into the MMI-treated dams appeared to have a dose-related effect.

Discussion

Maintainance of maternal euthyroidism: T_4 versus T_3. Infusion of either T_4 or T_3 alone does not normalize both T_4 and T_3 in plasma and all tissues. The 2.4-μg T_4 dose increased T_3 in maternal plasma and tissues to normal levels and also reversed several biological end-points of thyroid hormone action to normal values, although T_4 concentrations were higher than in normal pregnant dams. Gray and Galton (28) found that daily subcutaneous injections of 2 μg T_4/100 g BW maintained thyroidectomized pregnant rats euthyroid. They increased the dose with increasing BW, so that near term it was actually very similar to the present 2.4 μg T_4 infusion dose, which was 1.7 μg T_4/100 g BW per d by 21 d of gestation. The present MMI-treated dams on this dose were considered equivalent to euthyroid C dams. So were the MMI-treated dams receiving the 0.5- to 1.5-μg T_3 doses, although it must be kept in mind that they differed from normal euthyroid dams in an important respect, namely, that they have very low T_4 pools and are thus deprived of substrate for regulatory mechanisms involving local generation of T_3 from T_4 (29).

Transfer of T_4 and T_3 from the mother to the conceptus. Comparison of changes in T_4 and T_3 levels in maternal versus fetal samples (fetal brain T_3 excluded) confirms that both iodothyronines cross the rat placenta near term (16, 17). The fetal T_4 and T_3 levels are related to the dose of the parent iodothyronine infused into the mothers, but the slopes of the dose-response curves are lower for fetal, as compared to maternal, plasma and tissues. Transfer is, therefore, not free, but limited. It is quite likely that the placenta plays an important role in this, as previously proposed by others (2) and reported elsewhere (Calvo, R., et al., in preparation).

Present results contradict the notion that the placenta avoids transfer of any T_4 or T_3 from the mother to the fetus (2, 15). We have indeed recently found that at 21 d of gestation the net maternal contribution represents 17.5±0.9% of the total fetal extrathyroidal T_4 pool (30) (Normal dams were infused with high specific activity [^{125}I] T_4, plus KI to block ^{125}I recycling, from 11 to 21 d of gestation, at which time isotopic equilibrium was reached and the specific activity of the [^{125}I] T_4 was determined in maternal and fetal tissues. Comparison of these values allows calculation of the net maternal contribution to fetal extrathyroidal T_4 [30].) Moreover, preliminary data indicate that transfer of T_4 is not increased, in absolute amounts, by MMI-induced fetal thyroid failure (30).

Infusion of T_4 versus T_3 into the mother; differential effects on fetal thyroid hormone economy. Present results, as summarized in Fig. 5, show that infusion of T_4 into the mothers mitigates both the T_4 and T_3 deficiency of the fetus as a whole, and is especially beneficial for the fetal brain. In contrast, maintainance of maternal euthyroidism with T_3 deprives the fetus of regulatory mechanisms that require T_4 for the intracellular supply of T_3 (29), as already indicated for their mothers.

The consequences of an inadequate supply of T_4 are more dramatic for the fetal than for the adult brain. Administration of a dose of T_3 which was clearly supraphysiological for the mother did not increase fetal brain T_3 above MMI levels, despite restoration of the total extrathyroidal T_3 pool to normal values.

Thus, the fetal brain appears to depend on local conversion of T_4 to T_3 for its T_3 supply to a much greater extent than in adult rats, as previously discussed (17, 23). This had been documented for neonatal rats 2 wk after birth by Silva and Matthews (31). The fetal brain is exclusively dependent on local generation of T_3 from T_4, although in the mothers a certain proportion of intracerebral T_3 is derived from plasma T_3. For non-pregnant adult rats almost half the T_3 bound to nuclei would be derived from plasma T_3 according to Crantz et al. (32), about 33% for cortex and 50% for cerebellum according to van Doorn et al. (33). Dickson et al. (34) have shown that the choroid plexus of adult rats accumulates T_4, but not T_3, in vitro. This structure appears to be important in the transfer of the iodothyronines into the brain. Present results suggest that the difference between the uptake of T_4 and T_3 might be even greater during fetal development, and/or that alternative pathways for the entry of the iodothyronines into the brain, which would account for uptake of T_3 from plasma in adult rats, have not yet developed.

T_4 and T_3 concentrations were not measured in the fetal pituitaries because of technical difficulties. The dose-response curves obtained for fetal plasma TSH, however, suggest that the changes in T_4 and T_3 levels in the pituitary would resemble those observed in the fetal brain. It is possible that the contribution of plasma T_3 to intrapituitary T_3 content is lower during fetal life than it is in adults and depends, as is the case for the brain, mostly on local conversion of T_4 to T_3 for its intracellular supply of T_3. The rat pituitary is apparently not sensitive to T_3 until 5 d postnatally (35), although Knobil and Josimovich (36) found some limited effects of very high doses of T_3 on the fetal pituitary, as measured by an antigoiter assay.

The lack of participation of plasma-derived T_3 in the cerebral (and possibly, hypophyseal) supply of T_3 during fetal life is not, however, a general characteristic of tissues with high 5′ D-II activities. T_3 concentrations in fetal BAT (20) not only increased with the infusion of 2.4 and 3.6 μg T_4 into the dams, but also when the 4.5-μg T_3 dose was infused.

Maternal thyroxinemia and the preferential protection of the fetal brain from T_3 deficiency. We wish to stress that a normal maternal thyroxinemia (dams on the 2.4-μg T_4 dose) results in the preferential protection of the brain in fetuses which would be detected as congenitally hypothyroid on the basis of their plasma T_4 (only 40% of normal), and their elevated plasma TSH. "Clinical" signs of congenital hypothyroidism might be detected in such fetuses near term (or in the newborns), but this would not necessarily mean that the brain had also been T_3 deficient in utero, provided maternal thyroxinemia was normal. A relatively minor degree of maternal hypothyroxinemia (dams on the 1.8-μg T_4 dose) would no longer be adequate for total protection: T_3 was only 50% of normal. In contrast, a slightly increased maternal thyroxin-

emia (dams on the 3.6-μg T_4 dose), did not result in excessive fetal brain T_3. Extrapolation of the dose-response curves for fetal brain T_4 concentrations (Fig. 1) and 5′ D-II activity (Fig. 3) suggests that normal brain T_4 levels and 5′ D-II activities might not be reached until the dose infused into the dams increased to 5–6 μg T_4/100 g BW/d considering that brain 5′ D-II is regulated by the concentration of T_4, not by T_3 levels in the physiological range (37). It seems unlikely that fetal brain T_3 levels would become excessive until the maternal supply of T_4 exceeded these doses, which are approximately double the dose ensuring maternal euthyroidism. This point, however, remains to be investigated.

Mechanisms involved in the preferential protection of the fetal brain from T_3 deficiency. The mechanisms which lead to a preferential protection during this important period of neurogenesis are not yet well defined. The ability of fetal brain 5′ D-II to increase four- to fivefold in response to hypothyroidism (22) obviously plays an important role. It is also possible that a decrease in inner-ring iodothyronine deiodinase activity contributes to the increase in cerebral T_3: activity of this enzyme is high in fetal brain and, contrary to 5′ D-II, decreases with hypothyroidism (38). Cerebral uptake of T_4 might also be increased, as suggested indirectly by the concentrations of the iodothyronines in plasma and tissues. Although plasma T_4 and T_3 in C fetuses are only 20.7% and 12.5%, respectively, of the maternal levels, and fetal liver T_4 and T_3 are 19.8% and 13.2% of those in maternal liver, fetal brain T_4 and T_3 are 140% and 77% of maternal cerebral levels. The brain to plasma T_4 ratio was almost seven times higher in the brain of C fetuses (0.364) than in the maternal brain (0.054), whereas liver to plasma T_4 ratios were very similar for C dams (1.16) and their fetuses (1.11). Previous studies carried out in 2-wk-old rat pups (29) showed that the neonatal brain responds to hypothyroidism with an increase in cerebral conversion of T_4 to T_3, in T_3 residence time, and in T_3 uptake.

Conclusions regarding the main aims of this study. The findings presented here show that a normal maternal supply of T_4 is essential for the preferential protection of the fetal brain from T_3 deficiency. A 50% increase in the maternal supply of T_4 above the "physiological" dose does not result in excessive brain T_3. The fetal brain is entirely dependent on T_4, and not on plasma T_3, for its intracellular supply of T_3 (23). Thus, unless maternal hypothyroxinemia is corrected, maintainance of maternal euthyroidism with T_3 is of no benefit for the fetal brain, although T_3 deficiency is mitigated in other tissues. Even doses of T_3 high enough to be in the toxic range for the mothers do not increase fetal brain T_3 above hypothyroid levels.

Clinical implications. The recent report by Vulsma et al. (19) regarding maternal transfer of thyroid hormones in man lends greater relevance to present findings in the rat as a model for human congenital hypothyroidism. Transfer of T_4 from the mother to the hypothyroid fetus at term is enough to ensure T_4 levels at birth that are 25–50% of normal. Despite older evidence showing maternal transfer of the iodothyronines (39–41), the more recent consensus was that it would be negligible in quantitative terms (1, 2, 42, 43). This notion was partly due to the lack of effect of increasing doses of T_3 on cord-blood TSH (44, 45). Present results in rats suggest that T_3 was actually transferred to the fetus, but that at this stage of development the pituitary is not yet as sensitive to plasma T_3 as in adults.

Maternal transfer of thyroid hormones is not sufficient to compensate completely for the lack of fetal thyroid function: babies with congenital thyroid failure are often born with signs of hypothyroidism, such as the absence of ossification centers that are present at birth in normal babies (4, 46–48). Moreover, most of their central nervous system (CNS) damage may be prevented by prompt postnatal treatment (49–51). From these observations it has been concluded (4, 15) that transfer is physiologically irrelevant and the human brain does not require thyroid hormone for normal development until after birth. The latter conclusion, however, does not take into consideration that not all of the CNS damage is prevented by immediate and proper postnatal treatment (49–52). The hypothesis also contrasts with evidence in species, such as the rat, where thyroidectomy at a stage of brain development grossly equivalent to that of a human fetus at mid-gestation results in profound effects on many phases of brain maturation that occur in utero in man (for reviews, and references of previous reviews, see 52–54). Moreover, Stein et al. (55) have recently found decreased abundance of EGF and Mβ5 tubulin isomer mRNAs in the cerebral cortex of congenitally hypothyroid mice on the day of birth, clearly showing thyroid hormone dependence of the brain during fetal life.

Present results afford an alternative interpretation, namely that thyroid hormones are important for phases of human brain development occurring in utero. Their supply in early pregnancy is of maternal origin, and later of combined fetal and maternal origins. Maternal transfer of T_4 could still provide the fetal brain with sufficient T_3 to avoid major irreversible CNS damage if the fetal thyroid is impaired. Cerebral T_3 deficiency would supervene after birth, and would be prevented by prompt T_4 therapy. The fetal brain might be protected by maternal T_4 even if transfer is not sufficient for other tissues. Signs of intrauterine hypothyroidism (i.e., delayed skeletal maturation) do not necessarily mean the brain was T_3 deficient before birth.

The degree of protection might, however, be variable and depend on maternal T_4 levels, and/or the permeability of the placenta. The hypothyroid fetus would be more severely affected, and the protection of the fetal brain less complete, when the degree of transfer is low. Several studies (49–52) have indeed shown that the babies with the poorest prognosis as regards their CNS development, despite prompt postnatal treatment, are those with the most severe retardation of bone maturation and with the lowest T_4 at screening.

The most severe CNS damage, possibly irreversible by the time of birth, would result from combined fetal and maternal hypothyroxinemia. In areas of marked iodine deficiency, where neurological cretins are born, pregnant women have very low levels of circulating total and free T_4 (13). The fetus is deprived of T_4 throughout pregnancy, initially because of maternal hypothyroxinemia, and later in gestation because maternal hypothyroxinemia continues and the fetal thyroid cannot produce enough T_4 due to the scarce supply of iodine (12, 13). Maternal levels of total and free T_3 are normal and may prevent the manifestation of clinical hypothyroidism in such women and in the neurological cretins born from them (56). But despite normal T_3 levels, the CNS was deprived of T_4 and, consequently, of locally generated T_3, during very important phases of development, which in man occur in utero. A single case reported in the United States with the full clinical picture of neurological cretinism, without iodine deficiency, was the

congenitally hypothyroid son of a hypothyroid mother (57). Carr et al. (58) described a previous case of combined fetal and maternal hypothyroidism, but treated the mother with high doses of desiccated thyroid, and treated the child from birth, apparently with success.

It is at present not possible to assess poor permeability of the placenta per se. But maternal hypothyroxinemia (low levels of "free" T_4) could be avoided by adequate controls during pregnancy. Treatment with T_3 should be avoided, as it might decrease maternal T_4 pools. Maintainance of T_4 levels in the upper range of normality (or even somewhat above it when fetal hypothyroidism is suspected) should be aimed at to protect the brain up to birth. Present comments might also be pertinent for thyrotoxic women on antithyroid drug treatment during pregnancy. In this condition the therapeutic goal is to achieve a euthyroid or slightly hyperthyroid mother and to prevent fetal hypothyroidism or hyperthyroidism (59). There is a general consensus that maternal hypothyroidism is to be avoided (43, 59–62), and present results strongly advocate maintainance of normal (or slightly elevated) maternal T_4. Whether this is to be ensured by treating with minimal doses of antithyroid compounds, thyroidectomy and T_4 replacement, or the regimen combining low doses of antithyroid drugs and T_4 supplementation has been, and still is, matter for debate (43, 59–62). The latter treatment, used by Selenkow et al. (60) with good results, apparently did not receive greater acceptance, because of the widespread belief that T_4 would not cross the placenta or benefit the fetus. We do not know whether treatment is also indicated for those pregnant women who have low levels of free T_4 due to causes not directly related to primary hypothyroidism (i.e., severe illness). Further studies are needed to ensure whether in such cases the mechanisms involved in the preferential protection of the fetal brain are operative, or affected by the condition of the mother.

In a recent editorial commenting the results of Vulsma et al. (19), Larsen (63) concludes: "It follows that if we ensure a euthyroid state during pregnancy and if parents and physicians try rigorously to provide adequate replacement therapy thereafter, it is not unrealistic to expect that even athyreotic infants whose condition is discovered by thyroid screening programs can reach their full intellectual potential." We fully agree with the general concepts underlying this conclusion, but also believe that present results strongly suggest that it is essential to define more precisely the maternal "euthyroid state during pregnancy." The degree of protection of the fetal brain is related to maternal thyroxinemia, and not to maternal "euthyroidism." Therefore, it is normal availability of maternal T_4 which has to be ensured, whether or not clinical euthyroidism is maintained by normal levels of T_3.

Appendix

Table AI.

	Maternal tissues								Fetal tissues							
	Plasma T_4 (ln x)		Liver T_4 (ln x)		Heart T_4 (ln x)		Brain T_4 (ln x)		Plasma T_4 ($x^{0.5}$)		Liver T_4 (ln x)		Heart T_4 ($x^{0.5}$)		Brain T_4 ($x^{0.5}$)	
Groups:	C	vs. MMI	C	vs. MMI	C	vs. MMI	C	vs. MMI	C	vs. MMI	C	vs. MMI	C	vs. MMI	C	vs. MMI
1.8 μg T_4	NS	*	NS	‡	NS	‡	*	‡	‡	‡	‡	§	‡	NS	‡	NS
2.4 μg T_4	NS	*	§	‡	*	‡	‡	‡	‡	‡	‡	‡	‡	§	‡	*
3.6 μg T_4	*	*	‡	‡	§	‡	‡	‡	‡	‡	‡	‡	§	§	‡	‡
0.5 μg T_3	*	NS	‡	NS	‡	NS	‡	NS	‡	NS	‡	NS	‡	NS	‡	NS
1.5 μg T_3	*	NS	‡	NS	‡	NS	§	NS	‡	*	‡	NS	‡	NS	‡	NS
4.5 μg T_3	*	*	‡	NS	§	NS	‡	NS	‡	§	‡	*	‡	*	‡	NS

Corresponds to Fig. 1. It identifies the statistically significant differences in the concentration of T_4 in plasma and tissues of MMI-treated dams (or of their fetuses) infused with T_4 or T_3, as compared both to the C and the MMI-treated groups. The transformation of the data required for homogeneity of variances is given in brackets: (—) indicates that the variances were homogeneous using the raw data. All T_4 values for the MMI-treated dams or their fetuses were significantly decreased versus C. NS, Not significantly different. * $P < 0.05$. § $P < 0.01$. ‡ $P < 0.001$.

Table AII.

	Maternal tissues								Fetal tissues							
	Plasma T_3 (—)		Liver T_3 (ln x)		Heart T_3 (ln x)		Brain T_3 ($x^{0.5}$)		Plasma T_3 (—)		Liver T_3 (ln x)		Heart T_3 (—)		Brain T_3 (ln x)	
Groups:	C	MMI	C	MMI	C	MMI	C	MMI	C	MMI	C	MMI	C	MMI	C	MMI
1.8 μg T_4	*	NS	‡	NS	§	NS	NS	*	‡	NS	‡	NS	‡	NS	‡	‡
2.4 μg T_4	NS	*	NS	‡	NS	§	NS	§	‡	NS	‡	NS	‡	NS	NS	‡
3.6 μg T_4	§	‡	§	‡	NS	‡	NS	‡	NS	‡	§	§	NS	‡	NS	‡
0.5 μg T_3	NS	NS	*	‡	NS	*	*	NS	‡	NS	NS	‡	‡	NS	‡	§
1.5 μg T_3	NS	NS	§	‡	*	‡	NS	‡	NS	*	NS	‡	‡	NS	‡	*
4.5 μg T_3	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	*	‡	NS	‡	‡	NS

Corresponds to Fig. 2. It identifies the statistically significant differences between the concentration of T_3 in plasma and tissues of MMI-treated dams (or of their fetuses) infused with T_4 or T_3, as compared to those of C and of MMI-treated groups. All T_3 values for the MMI-treated dams (or their fetuses) were significantly decreased versus C. * $P < 0.05$. § $P < 0.01$. ‡ $P < 0.001$.

Table AIII.

	Maternal tissues						Fetal tissues					
	Plasma TSH (—)		Brain 5′ D-II (ln x)		Liver α-GPD (—)		Plasma TSH ($x^{0.5}$)		Brain 5′ D-II (ln x)		Liver α-GPD (—)	
Groups:	C	MMI	C	MMI	C	MMI	C	MMI	C	MMI	C	MMI
1.8 μg T_4	NS	‡	NS	‡	NS	NS	‡	‡	‡	NS	NS	*
2.4 μg T_4	NS	‡	§	‡	NS	NS	‡	‡	‡	‡	NS	NS
3.6 μg T_4	NS	‡	‡	‡	*	§	*	‡	‡	‡	NS	NS
0.5 μg T_3	NS	‡	‡	NS	NS	NS	‡	NS	‡	NS	‡	*
1.5 μg T_3	NS	‡	§	NS	*	§	‡	*	‡	NS	§	NS
4.5 μg T_3	NS	‡	‡	NS	‡	‡	‡	NS	‡	NS	NS	§

Corresponds to Fig. 3. It identifies statistically significant changes in plasma TSH, cerebral 5′ D-II and hepatic α-GPD activities of MMI-treated dams (or of their fetuses) infused with T_4 or T_3, as compared to data from C and from MMI-treated groups. Values for MMI-treated dams (or their fetuses) were different from those of the C animals, except for maternal α-GPD activity. * $P < 0.05$. § $P < 0.01$. ‡ $P < 0.001$.

Table AIV.

	Fetal carcass				Fetal lung			
	T_4 (ln x)		T_3 (ln x)		T_4 ($x^{0.5}$)		T_3 (—)	
Groups:	C	MMI	C	MMI	C	MMI	C	MMI
1.8 μg T_4	‡	‡	‡	NS	‡	‡	‡	NS
2.4 μg T_4	‡	‡	‡	*	‡	‡	‡	*
3.6 μg T_4	‡	‡	‡	‡	‡	‡	§	‡
0.5 μg T_3	‡	*	‡	§	‡	NS	‡	NS
1.5 μg T_3	‡	‡	‡	‡	‡	§	‡	§
4.5 μg T_3	‡	‡	NS	‡	‡	NS	NS	‡

Corresponds to Fig. 4. It identifies statistically significant differences of the T_4, or T_3, concentrations in carcass and lung of fetuses from MMI-treated dams infused with T_4 or T_3, as compared to C and MMI-treated fetuses.
All differences between C and MMI-treated fetuses were significant. ‡ $P < 0.001$. * $P < 0.05$.

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