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Maternal Diabetes Mellitus, a Rat Model for Nonthyroidal Illness: Correction of Hypothyroxinemia with Thyroxine Treatment Does Not Improve Fetal Thyroid Hormone Status

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ABSTRACT

Maintenance of normal maternal thyroxinemia prevents severe triiodothyronine (T_3) deficiency of the fetus with primary thyroid failure (1). We have studied whether thyroxine (T_4) would also protect the fetal brain when maternal hypothyroxinemia is caused by nonthyroidal illnesses. We have used the streptozotocin-induced diabetes mellitus pregnant rat as a model of maternal nonthyroidal illness. We measured the effects of diabetes mellitus, and of correction of the ensuing maternal hypothyroxinemia with T_4 as compared to insulin, on maternal body weight, the outcome of pregnancy, glucose, insulin, T_4, T_3, reverse T_3, and thyrotropin levels in the maternal and fetal circulation, as well as T_4 and T_3 concentrations in tissues, and iodothyronine deiodinases in liver, lung, and brain. The diabetic mothers showed changes in thyroid hormone status typical of nonthyroidal illnesses. Thyroid hormone status of the fetuses was severely affected: the total T_4 and T_3 pools decreased to one-third of normal values. T_4 and T_3 concentrations in the fetal brain were lower than normal and the expected increase in 5′-deiodinase activity was not observed. Although insulin treatment avoided or mitigated these changes, the low cerebral T_3 did not improve with T_4 treatment of the maternal hypothyroxinemia. Several findings indicated that treatment of the severely ill dams with T_4 was actually harmful for the outcome of pregnancy. These negative effects were observed without the expected increase in the maternal or fetal T_3 pools.

INTRODUCTION

THYROXINE (T4) and 3,5,3′-triiodothyronine (T_3) concentrations are very low in all tissues of rat fetuses from dams with methimazole (MMI)-induced primary thyroid failure. If the maternal hypothyroxinemia is avoided by treatment with T_4, the brain is preferentially protected against the deficiency of T_3 until birth (1): fetal brain T_3 reaches normal concentrations, despite lower than normal fetal serum T_4 and increased TSH levels. T_4 is by far the main source of cerebral T_3 in the rat fetus (1,2); changes in circulating T_3 hardly affect cerebral T_3 within a physiological range of serum T_3 levels. Both the capacity of cerebral type II 5′-iodothyronine deiodinase (5′D-II) to respond to low fetal serum T_4 with a marked increase in its activity and the amount of T_4 transferred from the mother to the fetus play crucial roles in maintaining T_3 homeostasis in the brain of the hypothyroid fetus. This protective effect of the maternal T_4 would explain the lack of major brain damage of most hypothyroid fetuses at birth, in contrast with the irreversible central nervous system damage observed in the neurological cretin. The mother of the latter is severely hypothyroxinemic, and does not offer any protection to the fetal brain, which is therefore markedly T_3 deficient before birth, despite the expected increase in 5′D-II activity (3). Both in the neurological cretin (4) and in the experimental model of rats fed a diet with a very low iodine content (3), maternal circulating T_3 is normal, but this does not improve fetal brain T_3 concentrations unless the maternal hypothyroxinemia is also corrected.

These findings have drawn attention to the importance of maintaining normal maternal T_4 levels during pregnancy, even when normal T_3 levels might prevent the appearance of clinical hypothyroidism. We already cautioned (1), however, against extrapolation of this conclusion to other situations where changes in maternal and fetal thy-

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roid hormone status might not be due to primary thyroid failure or an inadequate iodine supply, but to other causes, such as "nonthyroidal" illness. This might interfere with the transfer of T_4 from the mother to the fetus, the responses of fetal deiodinating enzymes, or other mechanism(s) contributing to fetal cerebral T_3 homeostasis. Moreover, the possibility exists that treatment with T_4 might actually be harmful in such conditions, as the decreased hormonal secretion by the thyroid is considered as a protective mechanism against energy loss and excessive catabolism (5–7).

Diabetes mellitus is considered as a nonthyroidal disease leading to alterations of thyroid hormone status typical of the so called "low T_3 syndrome" (5–8). The major alterations in thyroid hormone economy are a reduction in the TSH stimulation of the thyroid gland, probably caused by "central" hypothyroidism, and in the peripheral generation of T_3 from T_4 (8). The injection of streptozotocin (STZ) in rats is frequently used to obtain an experimental model for the study of nonthyroidal illness, by inducing diabetes mellitus.

In the STZ-diabetic adult rat, as in other situations of nonthyroidal illness, both the thyroidal secretion of T_4 and T_3 and the extrathyroidal monodeiodination of T_4 to T_3 are clearly impaired, as a consequence of which circulating T_4 and T_3 are very low, as well as the concentrations of both iodothyronines in most tissues (5–10). To our knowledge little is known about the possible influence of maternal diabetes on the thyroid hormone status of the fetus, except for a preliminary study from our group (11) showing that STZ-induced maternal diabetes mellitus also affects fetal thyroid hormone economy, causing a decrease of T_4 and T_3 in plasma and most fetal tissues, brain included, with possible impairment of the normal response of 5'D-II to low T_4 concentrations. These alterations were mitigated or avoided by appropriate treatment of the dams with insulin.

The present study has been undertaken to assess the potential benefits for the fetus of the correction of maternal hypothyroxinemia when caused by nonthyroidal illness. The underlying cause of the maternal hypothyroxinemia might not be readily ascertained or treated, and treatment with T_4 might be attempted to normalize maternal T_4 levels, in view of the beneficial effects of this treatment for fetal brain T_3 in case of primary thyroid failure (1). Diabetes mellitus was used as a model of nonthyroidal illness. As will be seen, T_4 treatment, without correction of the diabetic state, is of no benefit for fetal tissues, brain included, and actually appears to be harmful both for the mother and for the outcome of pregnancy.

MATERIALS AND METHODS

Experimental design

Female Wistar rats were used for this study. The guidelines for humane treatment of animals were followed in compliance with the principles outlined in "The care and use of animals" and the study was approved by the committee of our Institute. They were maintained at 22°C with 12-h periods of light and darkness and fed *ad libitum* with a standard diet (18 g protein, 39 g carbohydrate, 2.5 g lipid, and 4.5 g cellulose/100 g plus salt and vitamin mixtures, with an estimated caloric content of 2.54 kcal/g). They were mated with normal males and the morning of appearance of the vaginal plus was considered as day 0 of gestation. Twenty-four pregnant rats were divided into four groups. One group served as normal pregnant controls (C). At day 7 of gestation, the other three groups of rats (D groups) were injected into the femoral vein with 4.5 mg/100 g body weight (BW) of streptozotocin (STZ) dissolved in 50 mM citrate buffer, pH 4.5 (12). From the three groups of D rats, one was left without further treatment (D group), a second group was treated with insulin (D+Ins), and a third group with T_4 (D + T_4). The group of D+Ins dams was injected sc once daily with 0.5 U bovine insulin/100 g BW/day from 9 to 20 days of gestation (dg). The group of D+T_4 dams was implanted under the dorsal skin with Alzet 2ML2 osmotic minipumps (Alza Co. Palo Alto, CA) delivering at a constant rate 2.4 μg T_4/100 g BW/day from 9 to 21 dg. The infusion of T_4 was carried out as already described (1) with modifications: T_4 (free acid form, Sigma Chemicals Co, St. Louis, MO) was dissolved in the minimum volume of 0.05 N NaOH and then taken to the required volume in 50% propylene glycol.

At 21 dg all dams were anesthetized with ether, bled, and perfused with 40–50 mL of 0.05 M phosphosaline buffer, pH 7.4, as described (1). Maternal plasma, liver, brain, heart, lung, and mamma were obtained and frozen. The uterus was dissected out and carefully rinsed and blotted free of maternal blood. The fetuses were then dissected out, bled, separated from the placenta, weighed, and immediately placed on ice. The fetal brain, liver, and lung were dissected out and quickly frozen on dry ice; the thyroid, adhering to the trachea, was withdrawn and frozen. The placentas were separated, weighed, and divided into the basal (maternal) and labyrinthine (fetal) sides with blunt forceps and frozen rapidly, as described (1,13).

Determination of thyroid hormone concentrations

Thyroid hormones were determined by RIAs after extraction and purification of plasma and tissues (14–17). In brief, methanol is added to the still frozen tissue sample and homogenized with tracer amounts of [^{131}I]T_4 and [^{125}I]T_3 being added to each homogenate. This is followed by extraction of more than 90% of the endogenous and added iodothyronines using chloroform–methanol (2:1). The iodothyronines are then backextracted into an aqueous phase, and purified by passing this aqueous phase through Bio-Rad AG 1 × 2 resin columns. After a pH gradient, the iodothyronines are eluted with 70% acetic acid, which is then evaporated to dryness and dissolved in RIA buffer. Each extract is extensively counted to determine the recovery of the [^{131}I]T_4 and [^{125}I]T_3 added to each sample during the initial homogenization process. Average recovery is 50–60% for [^{131}I]T_4 and 60–70% for [^{125}I]T_3. 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. Concentrations are then calculated using the amounts of T_4 and T_3 found in the respective RIAs, the individual recovery of the

[^{131}I]T_4 and [^{125}I]T_3 added to each sample during the initial homogenization process, and the weight of the tissue sample submitted to extraction.

Maternal samples were processed individually. Plasma from different fetuses were pooled to obtain 300–400 μL aliquots. Fetal tissues were pooled (2 to 3 organs per pool) for the determination of T_4 and T_3. Pools were always obtained from fetuses of the same litter.

Percentage of circulating "free" T_4 and T_3

The method described by Mendel et al. (18) was used, with modifications. High specific activity [^{125}I]T_4 or [^{125}I]T_3 (approximately 300,000 cpm) was added in a 5 μL volume to 300 μL of plasma, and incubated at room temperature for 1 h. A 280-μL aliquot of each was submitted to ultrafiltration using Microcon 10 microconcentrators (Amicon Division, W.R. Grace and Co, Beverly, MA) and a 20 min centrifugation at 14,000 rpm. A measured volume of each ultrafiltrate was added to 0.5 mL bovine serum and submitted to precipitation with 10% trichloroacetic acid (TCA) and centrifuged, the pellet being washed twice with the same solution of TCA. The washed pellet was counted and its radioactivity calculated as percentage of the initial added tracer, submitted to the same TCA precipitation and washing procedure. This percentage of "free" T_4 (% FT_4) or "free" T_3 (% FT_3) and the T_4 and T_3 concentrations determined by RIA were used to calculate the concentrations of free T_4 (FT_4) or free T_3 (FT_3), respectively.

Iodothyronine 5'- and 5-deiodinase activities

Before each assay [^{125}I] rT_3 (3,3',5'-triiodothyronine) or [^{125}I]T_4 was purified by paper electrophoresis to separate the iodide. Iodothyronine 5'-deiodinase (5'D) activity was assayed as described (17,19), using 2 mM DTT and 400 or 200 nM rT_3 for maternal and fetal liver, respectively, and 2 nM rT_3 and 20 mM DTT for maternal and fetal lung. Maternal and fetal brain 5'D-II activity was assayed (2,19) using 2 nM T_4 + 1 μM T_3 and 20 mM DTT in the presence of 1 mM 2-*N*-propyl-6-thiouracil (PTU). The $^{125}I^-$ released was separated by ion exchange chromatography on Dowex-50W-X2 columns equilibrated in 10% acetic acid. The production of equal amounts of iodide and 3',3-T_2 was checked in some assays. The protein content was determined by the method of Lowry et al. (20), after precipitation of the homogenates with 10% TCA to avoid interferences from DTT in the colorimetric reaction.

Other determinations

rT_3 concentrations in maternal and fetal plasma, and in placental extracts, were determined by RIA, as previously described (13,21).

Maternal and fetal plasma glucose levels were determined by the glucose oxidase method (22) using 10–25 μL of plasma. Insulin levels in maternal and fetal plasma were measured using the specific RIA adapted for rat insulin with reagents supplied by Novo BioLabs (Denmark). We used rat insulin as standard, anti-porcine antiserum, and human ^{125}I-labeled insulin as antigen.

TSH was determined in 200-μL aliquots of maternal plasma using the immunoreactants for RIA kindly supplied by the National Institutes of Health (Bethesda, MD), and made available through the Rat Pituitary Agency of the National Institutes of Diabetes, Digestive and Kidney Diseases. Concentrations are expressed in weight equivalents of the rat TSH RP-3 reference preparation (23).

Drugs and reagents

T_4, T_3, 3,5-diiodothyronine (3,5-T_2), PTU, and DTT were obtained from Sigma Chemical Co. (St. Louis, MO). rT_3 and 3',3-T_2 were obtained from Henning Berlin GMBH (Berlin, Germany).

High specific activity [^{131}I]T_4, [^{125}I]T_3, [^{125}I]T_4, and [^{125}I]rT_3 (3000 μCi/μg) were synthesized in our laboratory (14,24) and used for highly sensitive T_4, T_3, and rT_3 RIAs, as recovery tracers for extraction, and as substrates for 5'-deiodinases.

Statistical analysis

After testing for homogeneity of variance using Bartlett's procedure, data were submitted to one-way analysis of variance. Square root or logarithmic transformations usually ensured homogeneity of variance when this was not achieved with the raw data. Significant differences among groups were assessed using the protected least significant difference (LSD) test. All statistical calculations were performed as described by Snedecor and Cochran (25). The SE appearing in the figures is the mean standard error calculated by ANOVA, and used for the identification of statistically significant differences between groups by the LSD test. For the sake of clarity, the ± SE is shown in figures only on the C value bar.

RESULTS

Degree of maternal illness

Figure 1 shows the insulin and glucose concentrations in the maternal plasma (M-plasma) from the different groups,

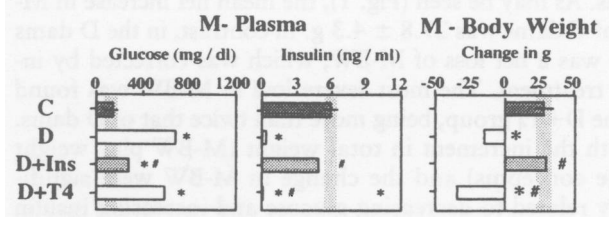

FIG. 1. Mean circulating insulin and glucose concentrations are shown for the dams from the different groups, as well as the calculated mean changes in the BW of the mother herself (M−BW) between 7 and 21 days of gestation, calculated as described in the Results section, by subtracting the weight of the conceptus from the total body weight. In this and the following figures, the SE shown only on the C value bar is the mean standard error calculated by ANOVA. The shaded area corresponds to the mean C value ± SE. An asterisk (*) identifies statistically significant differences versus C dams; number sign (#) identifies statistically significant differences versus the D group. Other statistically significant differences are not identified for the sake of clarity.

TABLE 1. MEAN (± SEM) INCREMENTS IN TOTAL BODY WEIGHT (BW) BETWEEN 7 AND 21 DAYS OF GESTATION, MEAN NUMBER OF FETUSES/ DAM, BW OF FETUSES (F-BW), AND WEIGHTS OF THE PLACENTA (TOTAL, MATERNAL AND FETAL SIDES), AT 21 DAYS OF GESTATION, OF NORMAL (C) AND STREPTOZOTOCIN-INJECTED DAMS (D), AND OF D DAMS TREATED WITH INSULIN (D + INS), OR INFUSED WITH T_4 (D + T_4)

Group	*Increment in total weight (g)*	*Number of fetuses/dam*	*F-body weight (mg)*	*Placental weight (mg)*	*M-placental weight (mg)*	*F-placental weight (mg)*
C	100.0 ± 2.5	12.4 ± 1.0	4872 ± 32	510.6 ± 7.9	154 ± 9	339 ± 11
D	31.6 ± 8.8[a]	10.0 ± 1.3	3196 ± 66[a]	568.1 ± 12.2[a]	116 ± 4[a]	361 ± 11
D + Ins	71.3 ± 10.6[a,b]	10.8 ± 1.8	4097 ± 65[a,b]	531.2 ± 14.6[b]	136 ± 5	326 ± 11
D + T_4	−11.3 ± 3.8[a,b]	6.7 ± 1.5[a]	2746 ± 92[a,b]	655.0 ± 40.4[a,b]	165 ± 28[b]	432 ± 66[a,b]

[a]Statistically significant differences ($p < 0.05$) versus C group.
[b]Statistically significant differences ($p < 0.05$) versus D group.

at 21 dg. Circulating glucose levels were very high in the STZ-injected dams, which did not receive insulin, whether or not they were infused with T_4. Insulin decreased significantly in the D dams; the infusion of T_4 resulted in insulin levels that were even lower than those of D dams. The injection of insulin affected both the insulin and glucose levels in the maternal circulation: Normal levels of insulin, as measured 24 h after the last injection, were found in the D+Ins group, with circulating glucose being somewhat higher than C values, although markedly decreased as compared to D animals.

Figure 1 also shows the calculated change in the body weight of the pregnant rats, free of the conceptus (M-BW), between 7 and 21 dg. The actual change of the dam plus conceptus appears in Table 1, as well as the number of fetuses per litter and the body weights of the fetuses (F-BW). The weights of the total placenta, as well as those of the maternal side (M-Placenta) and fetal side (F-placenta) are also shown in Table 1. The change in M-BW was calculated by substracting the weight of the conceptus from the measured change in total weight. The weight of the conceptus was calculated for each animal from the sum of the weights of all the fetuses and placentas in each dam. Although extraembryonic fluids and membranes had not been collected, the sum of the fetal and placental weights appears to be a reasonable approximation to the total weight of the conceptus. As may be seen (Fig. 1), the mean net increase in M-BW of C dams was 27.8 ± 4.3 g. In contrast, in the D dams there was a net loss of M-BW, which was corrected by insulin treatment. The most severe loss in M-BW was found for the D+T_4 group, being more than twice that of D dams.

Both the increment in total weight (M-BW plus weight of the conceptus) and the change in M-BW were significantly related to decreasing glucose and increasing insulin concentrations in the maternal circulation, and appeared to be good indices of the degree of maternal illness. The closest fit was found for the change in M-BW versus the logarithm of the M-plasma insulin levels ($n = 24$; $r = 0.76$, $p < 0.001$).

Effects of maternal illness on the outcome of pregnancy

No reproductive abnormalities were observed in the control (C) group, whereas reabsorbed fetuses were found in many D dams. Treatment with insulin prevented these abnormalities, whereas they were most frequent in the D+T_4 dams. Either the number of fetuses per litter or the F-BW (or both) were decreased in the diabetic animals, the lowest number and F+BW being found for the D+T_4 group. Treatment with insulin increased F-BW, but normal weights were not achieved. Observed changes in placental weights caused by the maternal diabetic state were greatest in the D+T_4 dams.

In summary, the outcome of pregnancy was affected by the maternal diabetic state and illness. This was prevented, or at least ameliorated, with the administration of 0.5 U of insulin/100 g BW/day, and was actually worsened by the infusion of T_4 (2.4 μg/100 g BW per day) into the D dams.

Effects on thyroid hormone status of the mothers

The concentrations of T_4, T_3, rT_3, and TSH in the maternal plasma are shown in Figure 2, as well as the circulating % FT_4, FT_4, % FT_3, and FT_3. Figure 3 shows the concentrations of T_4 and T_3 in the liver, lung, brain, heart, and mammary tissue.

Mean concentrations of T_4, T_3, and rT_3 in the maternal circulation and of T_4 and T_3 in most tissues studied (Figs. 2 and 3) were lower in the D dams as compared to the C mothers, the differences being statistically significant except for M-brain T_4 and M-mamma T_3. Circulating TSH (Fig. 2) was also lower in the D animals.

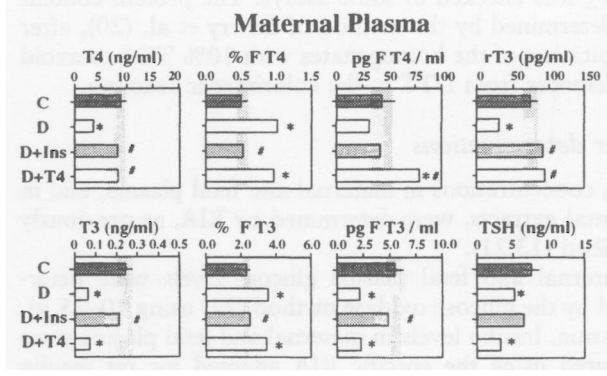

FIG. 2. The mean concentrations of T_4, % FT_4, and F T_4, T_3, % FT_3, and F T_3, as well as rT_3 and TSH in the maternal circulation are shown for the different groups of dams. See the legend to Figure 1 for the meaning of the shaded area and of the asterisk and #.

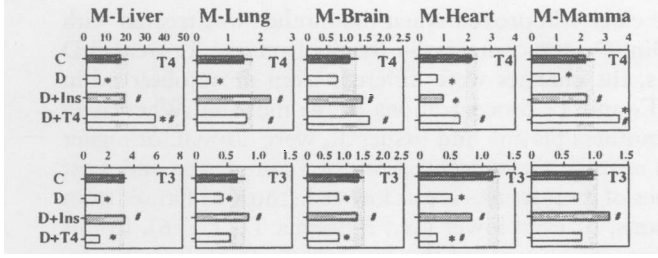

FIG. 3. The upper panels show the concentrations of T_4 in liver, lung, brain, heart, and mamma of the dams and the bottom panels the corresponding T_3 concentrations, given in ng/g.

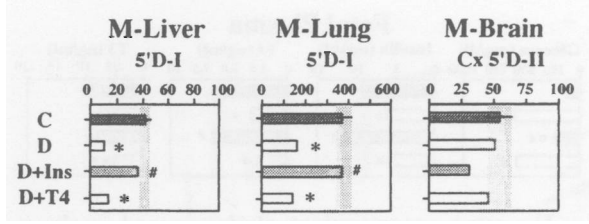

FIG. 4. The activity of the outer-ring 5'D-deiodinases are shown: type I for liver (pmol I^-/min/mg protein) and lung (fmol I^-/h/mg protein) and type II for Cx (fmol I^-/h/mg protein). The meaning of the shaded areas, asterisk, and # is the same as in the legend to Figure 1.

The circulating % FT_4 was increased in the diabetic dams (Fig. 2) to twice normal values of the normal pregnant dams. This increase was comparable to the decrease in total circulating T_4, as a result of which the mean total amount of circulating FT_4, although lower in D as compared to C dams, was not statistically different from that of the C mothers. The % FT_3 also increased in D as compared to C dams, but not to the extent that it could compensate for the decrease in circulating total T_3, and the FT_3 concentration was lower than that of C dams.

Treatment with insulin resulted in normal circulating T_4, T_3, rT_3, and TSH concentrations, and normal % FT_4, FT_4, % FT_3, and FT_3. Thus, the maternal hypothyroxinemia caused by the diabetic state was avoided in the insulin-treated D dams. Treatment with insulin also reversed the effects of the diabetic state on the concentrations of T_4 and T_3 in maternal tissues, with the exception of cardiac T_4.

The amount of T_4 infused into the D rats effectively prevented maternal hypothyroxinemia near term (Fig. 2). As the % FT_4 was markedly increased by the uncorrected diabetic state, the FT_4 levels actually increased 2-fold as compared to normal C rats. When the marked decrease in available T_4 of the D dams was avoided by the infusion of T_4, circulating rT_3 concentrations also increased to normal values and the rT_3/T_3 ratio increased 3-fold both with respect to the C and D groups. On the contrary, total T_3 and FT_3 concentrations did not increase with the infusion of T_4, and were as low as in D dams. The infusion of T_4 into the D rats did not alter their very low circulating TSH. In the T_4-infused diabetic animals, the concentration of T_4 in the maternal tissues increased as compared to that of D dams. Normal concentrations were reached in most tissues, with higher than normal levels being reached in liver and mammary tissue. As already observed for circulating T_3, the concentration of T_3 in most tissues did not improve with the infusion of T_4, with T_3 concentrations in the heart being actually lower than those of D dams.

5'D-I activity in the liver and lung was decreased in the diabetic dams (Fig. 4), a finding consistent with the decreased hepatic and pulmonary thyroid hormone concentrations. Despite the decreased plasma T_4 concentrations, no significant change was observed in the 5'D-II activity of the cortex, a finding consistent with the lack of decrease in cerebral T_4 concentration. Treatment with insulin normalized liver and lung 5'D-I activities. On the contrary, the mean 5'D-II activity in the maternal cortex decreased with insulin treatment, but the difference with respect to both D and C dams was not statistically significant. With T_4 treatment the activities of liver and lung 5'D-I and of cortex 5'D-II were the same as those of D animals.

Effects on thyroid hormone status of the placenta

Figure 5 shows the concentrations of T_4, T_3, and rT_3 in the M- and F-placenta. As described for these iodothyronines in the M-plasma, their concentrations decreased in the D dams, and improved with insulin treatment, although C levels were not always reached.

Treatment of the D dams with T_4 increased the concentrations of T_4 and rT_3 to normal values in the F-placenta, whereas T_3 remained as low as in D dams. These changes were similar to those observed in the M-plasma. On the contrary in the M-placenta, T_4 and rT_3 levels did not reach normal values and were lower than expected from the changes in M-plasma T_4 and rT_3 values, whereas T_3 was normal and higher than expected from the changes in M-plasma T_3.

Effects in the fetus

Figure 6 shows the glucose and insulin concentrations in F-plasma, as well as the corresponding T_4 and T_3 concentrations. F-plasma glucose was markedly elevated, and insulin decreased, in the D progeny. Both were clearly ameliorated by treatment of the mothers with insulin, whereas the infusion of T_4 had no effect.

The concentrations of T_4 and T_3 in the fetal circulation were decreased in the D group, with T_4 returning to nor-

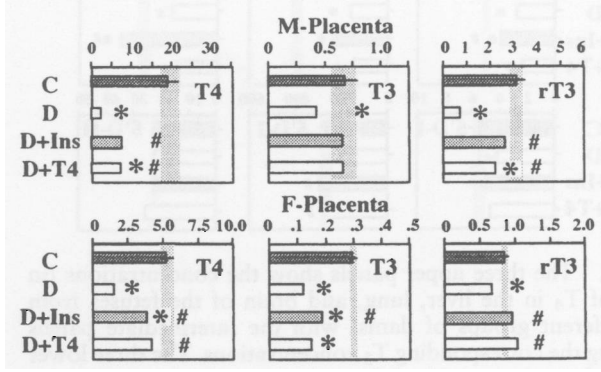

FIG. 5. The concentrations of T_4, T_3, and rT_3 in the maternal and fetal placenta are shown for the different groups of dams in ng/g. The meaning of the shaded areas, asterisk, and # is the same as indicated in the legend to Figure 1.

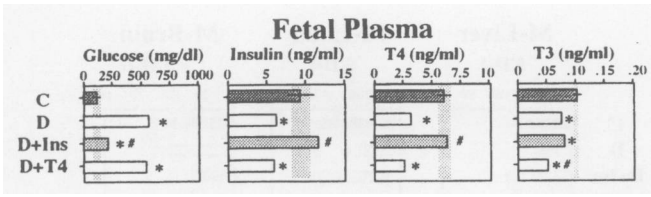

FIG. 6. The mean concentrations of glucose and insulin in the fetal circulation are shown, as well as the T_4 and T_3 levels. The meaning of the shaded areas, asterisk, and # is the same as indicated in the legend to Figure 1.

mal fetal values with insulin treatment of the mothers. Treatment of the D dams with T_4 did not improve fetal T_4 concentrations and T_3 levels were actually lower than those of D fetuses.

The concentrations of T_4 and T_3 in different F-tissues were decreased in the D group (Fig. 7). Treatment of the mothers with insulin usually resulted in improved T_4 and T_3 concentrations in most F-tissues, as compared to D fetuses, whereas infusion of T_4 into the D dams did not improve the low T_4 and T_3 concentrations of T-tissues, with the exception of a modest increase of F-brain T_4.

The 5'D activity in F-liver and F-lung was slightly decreased as compared to C fetuses (Fig. 7), being restored to normal both with insulin and T_4 treatment of the mothers. 5'D-II activity in the F-brain remained unchanged.

Comparison of the changes in thyroid hormone status observed in the dams and in their fetuses

Changes in thyroid hormone status of the fetuses from the D and insulin-treated dams were qualitatively similar to those observed for their mothers: the concentrations of T_4 and T_3 decreased in the circulation and in tissues, and

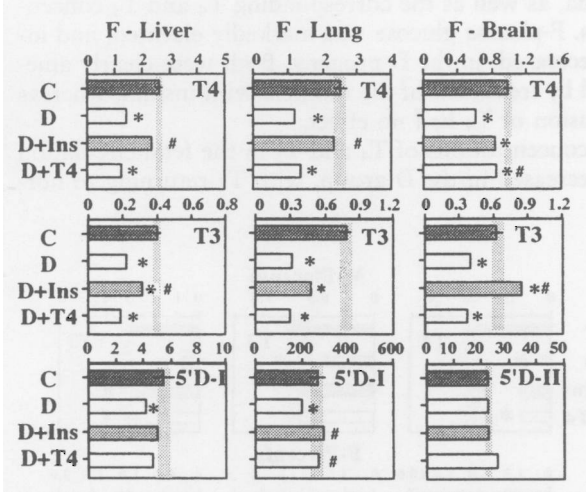

FIG. 7. The three upper panels show the concentrations (in ng/g) of T_4 in the liver, lung, and brain of the fetuses from the different groups of dams, with the intermediate panels showing the corresponding T_3 concentrations. The three lower panels show the activity of the outer-ring 5'D, type I for liver (pmol I^-/min/mg protein) and lung (fmol I^-/h/mg protein), and of type II for Cx (fmol I^-/h/mg protein). The meaning of the shaded areas, asterisk, and # is the same as indicated in the legend to Figure 1.

were clearly improved when the mother was treated with insulin. On the contrary, in fetuses from the T_4-treated D dams, the changes were different from those observed in the T_4 and T_3 concentrations of the mothers. Whereas in the mothers plasma and tissues T_4 were normal, or higher than normal, the concentrations of T_4 in plasma and most tissues of their fetuses are as low as in those of fetuses from D dams, or even lower (i.e., F-plasma T_3, Fig. 6). In this respect, the changes of the concentrations of T_4 in the fetuses from D+T_4 dams were similar to those observed for T_4 levels in the M-placenta.

There were very good correlations between indices of the fetal and the maternal diabetic state, as assessed both from the circulating glucose and the insulin concentrations. The F−BW (Table 1) was also affected by the diabetic condition of the mothers, an effect that was ameliorated, but not totally corrected, by the administration of insulin to the D mothers. On the contrary, treatment with T_4 resulted in even smaller fetuses than those of D mothers. The F−BW appeared to be related to other indices of the outcome of pregnancy, such as the number of viable fetuses per litter ($n = 24$; $r = 0.66$; $p < 0.001$).

DISCUSSION

Effects of STZ-induced diabetes mellitus and T_4 treatment on the mother

Alterations in thyroid hormone status have been extensively described for nonpregnant adult rats, treated with STZ to induce diabetes mellitus. These are considered typical of the alterations of thyroid function and thyroid hormone metabolism observed in "nonthyroidal illness" (5,6,10). At present they are considered as beneficial adaptive responses in situations of limited availability of intracellular energy, when a decrease in T_3-dependent catabolic effects is desirable (5–7,26), although this idea is being reconsidered (26). The two best known mechanisms involved in these responses are a decreased thyroidal secretion of both T_4 and T_3, which would lower the pool of T_4 available for extrathyroidal generation of T_3, and a decrease in the activity of enzymes involved in the extrathyroidal generation of T_3 from T_4.

The sequence of events leading to the first of these mechanisms appears to involve decreased release of hypothalamic TRH (27–29), decreased secretion of TSH (27–28,30–33), and a decreased sensitivity of the thyroid to TSH (32). The normal feedback mechanism is superseded, and TSH levels actually decrease, despite the lower levels of circulating total and/or free T_4 and T_3. Indeed, a return to normal secretion of TSH, accompanied by normalization of the thyroidal release of hormones, is considered as an index that the illness is remitting, or that the metabolic alterations are under control. In the nonpregnant rat with STZ-induced diabetes mellitus these mechanisms are fully operative, and the decreased thyroidal production of the iodothyronines is quantitative very important (10).

In the present study diabetes mellitus was induced during pregnancy. The changes in circulating total and free T_4 and T_3, together with decreased TSH, and the low T_4 and

T_3 levels found in all the tissues studied in the present D dams are in agreement with the changes described for the nonpregnant diabetic rats (10), and for patients dying from different nonthyroidal illnesses (34), indicating that 2 weeks after the injection of STZ, the adaptive mechanisms involving decreased thyroidal secretion of thyroid hormones are fully operative.

With respect to the second mechanism, the direct measure of 5'D-I activities in the liver and lung of the pregnant D rats of the present study has confirmed that generation of T_3 from T_4 is decreased. Such a decrease was also previously described for both nonpregnant (9,35,36) and pregnant (11) diabetic rats, as well as a decreased expression of 5'D-I mRNA (35). In contrast, in agreement with results in nonpregnant diabetic rats (37), 5'D-II activity in the cerebral cortex of the pregnant D rats was not changed. In other experimental situations not involving diabetes, however, a decrease in circulating T_4 is accompanied by an increase in 5'D-II activity of the cortex. This response was not observed in the D dams, possibly because cerebral T_4 was not decreased.

Although treatment with T_3 has been recently found to be beneficial after coronary artery bypass surgery (38,39), it is suspected that treatment with T_4 is of no benefit to patients with nonthyroidal illness (40,41). Even so, we had not foreseen that treatment of the D dams with T_4 would actually markedly worsen their condition. Their circulating insulin levels were actually lower than those of D dams. They clearly lost more body weight, and the outcome of their pregnancy was very poor; their litters were the smallest, both as regards the number and weight of the fetuses, and the number of resorptions the greatest. This deterioration could be explained if the administration of T_4, in a dose sufficient to compensate for the decreased thyroidal secretion of hormone, increased the amount of substrate available to 5'D-I, and more T_3 might be generated as compared to that of D dams, despite the decreased activity of the enzyme. The ensuing increase of the extrathyroidal T_3 pool would then be expected to aggravate T_3-dependent catabolic events, further decreasing the availability of intracellular energy (41).

This possible explanation is not, however, supported by present findings, as there was no evidence whatsoever that the T_3 pools increased: T_3 concentrations in plasma and tissues were actually as low as in D dams, or even lower (i.e., heart T_3 levels). This was an unexpected finding that suggests several possible explanations. More T_3 might actually have been generated and its deiodination increased, the steady state levels of T_3 remaining the same. T_4 may be having a catabolic effect per se, either through nonnuclear mechanism(s) of action, or by binding to nuclear receptors, which are not saturated by T_3 because of the decreased availability of T_3. Further experiments are necessary to clarify this unexpected effect of the infusion of T_4 into D dams.

Effects of STZ-induced maternal diabetes mellitus and T_4 treatment on the fetus

Present results confirm and extend our preliminary observations on the effects of maternal STZ-induced diabetes mellitus on fetal thyroid hormone status, as studied at 20 dg (11). The observed alterations in fetal thyroid hormone status are likely to result from the altered carbohydrate metabolism of their mothers, and from the ensuing alteration of carbohydrate metabolism in the fetus, and not from a direct destructive action of the STZ on the fetal pancreas. Maternal hyperglycemia results in overstimulation and finally exhaustion of the fetal pancreas (42). When maternal hyperglycemia is corrected with insulin treatment, fetal glycemia and insulinemia become normal. Considering that the placenta is impermeable to maternal insulin (43), normalization of fetal insulinemia indicates that the fetal pancreas had not been damaged by the injection of STZ to their mothers.

The thyroid hormone concentrations were lower in plasma and tissues that were obtained from fetuses of D dams, most of these alterations improving when the maternal (and fetal) hyperglycemia improved with insulin treatment of the mother. Despite the fact that treatment of the dams with T_4 ensured a normal maternal thyroxinemia, with FT_4 concentrations being actually increased, T_4 concentrations in the M- and F-placenta were not normal, although higher than in the placentas from D dams. In fetal plasma and tissues, T_4 concentrations were as low as in fetuses from D dams, except for a slight increase in the F-brain, which did not, however, reach normal concentrations. These findings contrast with those obtained in other models of maternal–fetal hypothyroxinemia caused by primary thyroid failure (1). The same dose of T_4 as used for the present study, when infused into MMI-treated dams, clearly increased the concentrations of T_4 in the M- and F-placenta, as well as in F-plasma and F-tissues, above those of MMI fetuses from untreated dams. Maintenance of normal T_4 levels of the MMI-treated dams ensured normal T_3 concentrations in the fetal brain (1). Two main factors were implied in this protective effect of maternal T_4, namely, a relatively minor increase in the amount of T_4 available to the fetal brain, and a marked increase in cerebral 5'D-II activity. No such beneficial effects resulted from the correction of the maternal hypothyroxinemia by treatment of D dams with T_4. Treatment of the D dams with T_4 did not improve the concentrations of T_3 either in the F-plasma or F-tissues, the brain included, despite the small increase in F-brain T_4. The lack of ameliorating effects on F-brain T_3 might be explained by the observed lack of response of 5'D-II activity in the F-brain of the D fetuses. Moreover, the infusion of T_4 into the D dams had clear negative effects on the degree of illness of the mothers, and on the general development of the litter and individual fetuses.

Unfortunately, the present results suggest that prevention of the maternal hypothyroxinemia, which would be relatively easy to accomplish, would be of little benefit, and might actually be harmful for the development of the fetus. Adequate control of the diabetes mellitus appears to be of prime importance.

CONCLUSIONS

Present results show that maternal diabetes mellitus, and possibly maternal nonthyroidal illnesses compromising intracellular energy availability, results in severe impairment

of the thyroid hormone status of the fetus. This includes low cerebral concentrations of T_3 during a very critical period of brain development. Although maternal diabetes as a cause of maternal hypothyroxinemia would be easily recognized, and controlled with insulin, other causes of maternal hypothyroxinemia might not always be evident, or might not be promptly controlled. Correction of the maternal hypothyroxinemia by treatment with T_4 might be attempted to protect the fetal brain (1). Unfortunately, the present results indicate that in such cases prevention of the maternal hypothyroxinemia might be of little benefit. If the present results are relevant for man and for causes of maternal hypothyroxinemia other than maternal diabetes, it would appear that correction of the illness is necessary to protect the brain, as compensatory mechanisms usually involved in maintaining cerebral T_3 homeostasis might not be operative, and correction of maternal hypothyroxinemia, without adequate control of the illness is of no benefit, and might actually be harmful. It would appear advisable to take the present tentative conclusions into consideration when faced with maternal hypothyroxinemia caused by nonthyroid illness, and not by primary thyroid failure.

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