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Maternal Nonthyroidal Illness and Fetal Thyroid Hormone Status, as Studied in the Streptozotocin-Induced Diabetes Mellitus Rat Model*

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ABSTRACT

We have used the streptozotocin-induced diabetes mellitus pregnant rat as a model of maternal nonthyroidal illness. We measured the effects of different degrees of diabetes mellitus on maternal body weight, the outcome of pregnancy, circulating glucose, insulin, T_4, T_3, rT_3, and TSH in mother and fetus, T_4 and T_3 in maternal and fetal tissues, and iodothyronine deiodinases in liver, lung, and brain.

All of the changes in thyroid hormone status typical of nonthyroidal illnesses were observed in the mothers and were related to the degree of the metabolic imbalances. Most were controlled with a daily insulin dose of 0.5 U/100 g BW. Normalization of maternal placental T_4, however, required higher insulin doses than in other maternal tissues.

The number and body weight of the fetuses, their pituitary GH contents, and their thyroid hormone status were severely affected. The total extrathyroidal T_4 and T_3 pools decreased to one third of normal fetal values. T_4 and T_3 concentrations in the fetal brain were lower than normal, and the expected increase in type II 5′deiodinase activity was not observed. The low cerebral T_3 only improved with adequate insulin treatment of the dams.

It is concluded that maternal diabetes mellitus, and possibly other nonthyroidal illnesses that impair the availability of intracellular energy stores, may affect fetal brain T_3 when thyroid hormones are essential for normal development. (*Endocrinology* **138:** 1159–1169, 1997)

DIABETES mellitus leads to alterations of thyroid hormone status typical of other so called nonthyroidal illnesses (1–3). 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 (3). The injection of streptozotocin (STZ) in rats is frequently used to obtain an experimental model for the study of diabetes mellitus, often as a model of nonthyroidal illness.

In the STZ-diabetic adult rat, the alterations in the hypothalamo-pituitary-thyroid axis are numerous; hypothalamic and plasma TRH (4, 5), pituitary and plasma TSH, as well as TSH secretion rate are reduced (4, 6, 7), and the TSH response to TRH is decreased despite normal peripheral TSH metabolism (6). T_3 and T_4 production (8) and iodide uptake by the thyroid are diminished. There are also important structural changes in the thyroid gland and pituitary that are accompanied by marked alterations in their secretory activity. In addition, T_4 deiodination to T_3 in peripheral tissues is decreased (8–10). As a consequence of all of these changes, circulating levels of T_4 and T_3 are markedly reduced as are the concentrations of both iodothyronines in most tissues (8–10).

These alterations have been shown in the adult diabetic rat, but to our knowledge very little is known about the possible influence of maternal diabetes on the thyroid hormone status of the fetus. In a preliminary study (11) we have shown that STZ-induced maternal diabetes mellitus also affects fetal thyroid hormone economy, as studied at 20 days gestation, causing a decrease in T_4 and T_3 in plasma and most fetal tissues, including brain. These preliminary results also suggested that the normal response of 5′-deiodinase type II (5′D-II) to low T_4 concentrations was impaired in the fetal brain.

The present study has been undertaken to further define the effects of different degrees of maternal nonthyroidal illness, as induced by diabetes mellitus, on fetal thyroid hormone status and their prevention with adequate control of the maternal metabolic imbalances.

Materials and Methods

Experimental design

Female Wistar rats were used for this study. The guidelines for humane treatment of animals were followed, 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. They were mated with normal males, and the morning of appearance of the vaginal plug was considered day 0 of gestation. Thirty pregnant rats were divided into six groups. One group served as normal pregnant controls (C). At 7 days gestation (dg), the other five groups of rats were injected into the femoral vein with 4.5 mg/100 g BW STZ dissolved in 50 mM citrate buffer, pH 4.5 (12). One of the groups of STZ-treated dams was not injected with

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insulin and served as the long duration diabetes mellitus (D) group, as 14 days had elapsed since injection of the dams with STZ. A second group of STZ-injected pregnant rats received 1.5 U bovine insulin (Ultralente, Novo Nordisk, Bagsvaerd, Denmark)/100 g BW·day, sc, once daily from 9–15 days gestation and vehicle (Diluting Medium for Lente MC, Novo Nordisk) from 15–20 dg; this group is referred to as sD, for short duration diabetes mellitus, as the dams were left untreated only for the last 5 days. Three groups of rats were injected sc once daily with 0.5, 1.0, or 1.5 U bovine insulin/100 g BW·day from 9–20 dg (D+0.5 Ins, D+1.0 Ins, and D+1.5 Ins groups, respectively). Most results obtained in D and sD groups were very similar, so only results from D dams will be presented; differences between D and sD groups, if any, are indicated in the text, tables, or figure legends.

On the morning of 21 dg and 24 h after the last of the insulin injections, all dams were anesthetized with ether, bled, and perfused with 40–50 ml 0.05 M phosphosaline buffer, pH 7.4, as previously described (13).

Maternal plasma, liver, brain, lung, heart, and samples of mammary tissue 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. Two or three fetal thyroids and pancreas from each litter were fixed *in toto* by immersion in PBS containing 4% formaldehyde for morphological study. The rest of the fetus, referred to here as the carcass (whole embryo minus the blood, trachea, thyroid, liver, lung, brain, and heart) was stored frozen. The placentas were separated, weighed, and divided into the basal (maternal) and labyrinth (fetal) sides with blunt forceps and frozen rapidly, as previously described (14).

Determination of thyroid hormone concentrations

Thyroid hormone levels were determined by RIAs after extraction and purification of plasma and tissues (15). 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 added to each homogenate. This is followed by the addition of chloroform in a volume double that of methanol, centrifugation, and a further extraction of the pellet with chloroform-methanol (2:1). This extracts more than 90% of the endogenous and added iodothyronines. The iodothyronines are then back-extracted into an aqueous phase and purified by passing this aqueous phase through Bio-Rad AG 1 × 2 resin columns (Bio-Rad, Hercules, CA). 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. The samples are submitted to highly sensitive RIAs for the determination of T_4 and T_3; the limits of sensitivity are 2.5 pg T_4 and 1.5 pg T_3/tube. The cross-reactivities of the different iodothyronines and metabolites were as reported previously (15, 16). Each sample is processed in duplicate or triplicate at two or more dilutions. Concentrations were 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- to 400-μl aliquots. Fetal tissues were pooled (two or three organs per pool) for the determination of T_4 and T_3. Pools were obtained from fetuses of the same litter.

The fetal thyroids were pooled in groups of two or three, homogenized, and submitted to proteolytic digestion, followed by methanol extraction. The methanol extracts were submitted to evaporation to dryness in a microwave oven at maximum heat for 5–10 min; this prevents artifacts in the RIAs, presumably due to residual proteolytic activity transferred into the RIA tube. RIA buffer was added, and T_4 and T_3 were determined by RIA, as described above.

Percentage of circulating free T_4 and T_3

These percentages were determined by ultrafiltration of undiluted plasma samples, as described by Mendel *et al.* (17) with modifications. High specific activity [^{125}I]T_4 or [^{125}I]T_3 (~300,000 cpm) were added in a 5-μl volume to 300 μl 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 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, submitted to precipitation with 10% trichloroacetic acid (TCA), and centrifuged; the pellet was washed twice with the same solution of TCA. The washed pellet was counted, and its radioactivity was calculated as a 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 FT_4 and free FT_3, respectively.

Iodothyronine 5′- and 5-D activities

Before each assay, [^{125}I]rT_3 or [^{125}I]T_4 was purified by paper electrophoresis to separate the iodide. Iodothyronine 5′D-I activity was assayed as previously described (16), using 2 mM dithiothreitol (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 (5′D-I). Maternal and fetal brain 5′ D-II activities were assayed (18) 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-diiodothyronine (3′,3-T_2) was checked in some assays. The protein content was determined by the method of Lowry, after precipitation of the homogenates with 10% TCA to avoid interference from DTT in the colorimetric reaction (16).

5D activity was measured in maternal and fetal brain homogenates (19), incubating 20–50 μg protein in 100 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA with approximately 50,000 cpm inner ring labeled 5-[^{125}I]T_3, 50 nM T_3, 20 mM DTT, and 1 mM PTU for 60 min at 37 C. Radioiodide release was measured as described above. When necessary, inner ring labeled ([5-^{125}I]T_3 was repurified before use with disposable Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA) and methanol.

Other determinations

rT_3 concentrations in maternal and fetal plasma and in placental extracts were determined by RIA, as previously described (14).

Maternal and fetal plasma glucose levels were determined by the glucose oxidase method (20), using 10–25 μl plasma.

Insulin levels in maternal and fetal plasma were measured using the specific RIA adapted for rat insulin with reagents supplied by Novo Nordisk (Bagsvaerd, Denmark). We used rat insulin as standard, anti-porcine antiserum, and human ^{125}I-labeled insulin as antigen (18). A bovine insulin standard was used for the standard curve when the plasma was obtained from mothers injected with bovine insulin.

TSH was determined in 200-μl aliquots of maternal plasma, and GH was determined in fetal pituitaries, using the immunoreactants for RIA kindly supplied by the NIH (Bethesda, MD) and made available through the Rat Pituitary Agency of the NIDDK. Concentrations are expressed in weight equivalents of the rat TSH RP-3 and rat GH RP-2 reference preparations (21).

Drugs and reagents

T_4, T_3, 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 (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 (15) and used for highly sensitive T_4, T_3, and rT_3 RIAs, as recovery tracers for extractions, and as substrates for 5′-D.

Inner ring labeled 5-[^{125}I]T_3 (80 μCi/μg) was used as substrate for 5-D. It was provided by Drs. R. Thoma and H. Rokos from Henning Berlin.

Statistical analysis

After testing for homogeneity of variance using Bartlett's procedure for groups of unequal size, data were submitted to one-way ANOVA. Square root or logarithmic transformations usually ensured homogeneity of variance when this was not achieved with the raw data. Sig-

nificant differences among groups were assessed using the protected least significant difference test. All statistical calculations were performed as described by Snedecor and Cochran (22). The SE appearing in the tables and figures is the mean SE calculated by ANOVA and used for the identification of statistically significant differences between groups by the least significant difference test. For the sake of clarity, the ±SE is shown in figures only on the C value bar.

Possible interrelations among variables were tested by curve fitting of the individual data using Cricket Graph III for Macintosh (Computer Associates International, Inc., Islandia, NY), and the degree of fit was assessed from the corresponding value of r and the degrees of freedom.

Results

Experimental design

The aim of the present experimental design was to induce maternal diabetes mellitus of different duration and degree and to assess the effects of these different degrees of maternal nonthyroidal illness on fetal thyroid hormone status near term.

Degree of nonthyroidal illness: diabetic state and weight loss of the dams. Figure 1 shows the insulin and glucose concentrations in the maternal plasma (M-plasma) from the different groups at 21 dg. Insulin decreased significantly in all D dams. As expected, circulating glucose levels were very high in all STZ-injected dams that did not receive insulin. The injection of insulin affected both 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+0.5 Ins or D+1.0 Ins groups, with higher than normal values in the D+1.5 Ins dams. Circulating glucose was somewhat higher than C values in the D+0.5 Ins dams, although markedly decreased compared to those in D animals, and comparable to those in C dams in the D+1.0 Ins and D+1.5 Ins groups. The circulating glucose was inversely related to plasma insulin by a power function ($n = 30$; $r = 0.88$; $P < 0.001$).

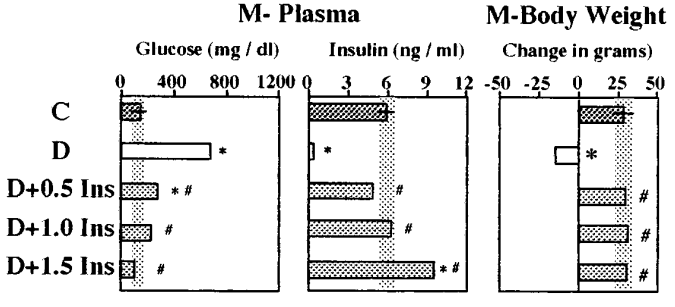

FIG. 1. The mean (±SE) circulating insulin and glucose concentrations in the plasma of dams from the different groups are shown. The *right panel* shows the difference between the change in total weight and the weight that can be attributed to the conceptus. This difference represents the change in the body weight of the mother herself (M-BW) between 7 and 21 dg. In this and the following figures, the SE shown only on the C value bar is the mean SE calculated by ANOVA. The *shaded area* corresponds to the mean C value ± SE. *, Statistically significant differences *vs.* C dams; #, statistically significant differences *vs.* the D group. Other statistically significant differences are not identified for the sake of clarity. The circulating insulin levels of sD dams (not shown) were very low, but somewhat higher than those of D dams, and the net loss in M-BW in sD dams was also less marked than that in D dams. For insulin or glucose values there was no statistically significant difference between the D+0.5 Ins and D+1.0 Ins dams, whereas insulin and glucose were different in the plasma of D+1.0 Ins *vs.* D+1.5 Ins animals. There were no statistically significant differences in the change in total body weight, weight of the conceptus, or maternal body weight among the three insulin-injected groups, with the exception of the weight of the conceptus in the D+0.5 Ins dams, which was greater than those in the D+1.0 Ins and D+1.5 Ins dams.

Figure 1 also shows the calculated change, between 7 and 21 dg, in the body weight of the pregnant rats, free of the conceptus (M-BW), namely the total measured weight of the dam minus the weight of the conceptus. The change in M-BW was calculated from data reported in Table 1, such as the change in total weight, the number of fetuses per litter, the body weights of the fetuses (F-BW), and the weights of the placentas. The weight of the conceptus was calculated for each animal from the sum of the weights of all 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 of the total weight of the conceptus. The change in M-BW was calculated by subtracting this calculated weight of the conceptus from the measured change in total weight of the animal. As shown in Fig. 1, the mean net increase in M-BW of C dams was 27.8 ± 4.3 g. In contrast, in all D dams, there was a net loss of M-BW, which was prevented by insulin treatment.

Both the increment in total weight and the change in M-BW were significantly related to decreasing glucose and increasing insulin concentrations in the maternal circulation and appear to be good indexes of the degree of maternal illness. The closest fit was found for the change in M-BW *vs.* the logarithm of the M-plasma insulin levels (Fig. 2).

Effects of maternal illness on the outcome of pregnancy. No reproductive abnormalities were observed in the normal (C) dams (Table 1). There were originally eight dams in the D group, four dams in the sD group, and four each in the three D+insulin groups. Whenever only reabsorbed fetuses or less than two apparently viable fetuses were found in the uterine cavity of these dams, they were excluded from the study. This reduced the number of dams in the D group. The proportion of dams in which such abnormalities were found was more frequent in the D groups. Treatment with insulin only appeared to effectively prevent these abnormalities in the D+0.5 Ins dams, which in these reproductive aspects were comparable to C animals.

The weight of the conceptus (not shown) was lower than normal in D groups. This was due to a smaller number of apparently viable fetuses, a decreased F-BW, or both. Treatment with insulin had variable effects; F-BW improved with respect to the D dams in the D+0.5 Ins and D+1.5 Ins groups, especially in the former, although C values were not reached, whereas both number and F-BW in the D+1.0 Ins group were as poor as in D dams.

The various treatments did not similarly affect the fetal and maternal sides of the placenta. Although mean F-placental weights tended to change in parallel with the total placental weight, the weight of the M-placenta decreased in D groups compared to that in C dams. In the insulin-treated animals, the weight of the M-placenta only increased to normal values in the D+0.5 Ins group and was actually smaller than normal in the D+ 1.5 Ins group.

In summary, the outcome of pregnancy was affected by

TABLE 1. Mean (±SEM) values of the increments in total body weight (BW) between 7–21 days gestation, number of fetuses per dam, body weights of fetuses (F-BW), and weights of the placenta (total, maternal, and fetal sides) at 21 days gestation, of normal (C) and streptozotocin-injected dams (sD and D), and of D dams treated with different daily doses of insulin

Group	No. of dams[a]	Increment in total wt (g)	No. of fetuses/ dam	F-BW (mg)	Placental wt (mg)	M-placental wt (mg)	F-placental wt (mg)
C	7 (7;*0*)	100.0 ± 2.5	12.4 ± 1.0	4872 ± 32	510.6 ± 7.9	154 ± 9	339 ± 11
D	7 (8;*5*)	31.6 ± 8.8[b]	10.0 ± 1.3	3196 ± 66[b]	568.1 ± 12.2[b]	116 ± 4[b]	361 ± 11
D + 0.5 Ins	4 (4;*0*)	71.3 ± 10.6[b,c]	10.8 ± 1.8	4097 ± 65[b,c]	531.2 ± 14.6[c]	136 ± 5	326 ± 11
D + 1.0 Ins	4 (4;*2*)	58.3 ± 7.6[b,c]	7.5 ± 1.0[b,d]	3141 ± 85[b,d]	482.6 ± 10.5[c,d]	126 ± 6[b]	317 ± 9
D + 1.5 Ins	4 (4;*3*)	65.3 ± 8.4[b,c]	8.8 ± 1.1[e]	3465 ± 150[b,c,e,f]	472.4 ± 10.4[b,c,e]	107 ± 8[b,e,f]	299 ± 15[b,c]

[a] The figures given are those of the number of dams included in the final evaluation of the results of the present study. When only reabsorbed embryos (or fewer than two apparently viable fetuses) were found in the uterine cavity, the dams were excluded from the study; their number minus that initially allotted to each treatment group is shown. This initial figure is given inside *parentheses* followed, in *italics*, by the number of dams in which reabsorbed fetuses were found.

[b] Statistically significant differences ($P < 0.05$) *vs.* C group. Although not shown, the F-BW of sD animals was significantly different.

[c] Statistically significant differences ($P < 0.05$) *vs.* D group. Although not shown, the F-BW of sD animals was significantly different.

[d] Statistically significant differences ($P < 0.05$) between D + 0.5 Ins and D + 1.0 Ins.

[e] Statistically significant differences ($P < 0.05$) between D + 0.5 Ins and D + 1.5 Ins.

[f] Statistically significant differences ($P < 0.05$) between D + 1.0 Ins and D + 1.5 Ins.

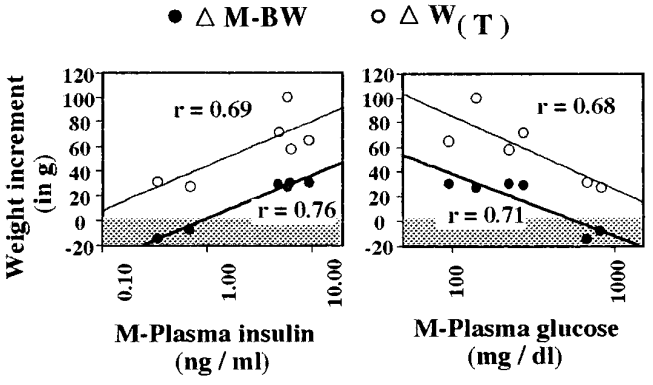

FIG. 2. The mean change in total weight ($\Delta W_{(T)}$) or in maternal body weight (ΔM-BW) are plotted against the mean maternal plasma insulin concentrations (*left panel*) or the mean maternal plasma glucose levels (*right panel*) on logarithmic scales. The r values shown correspond to the curve fit obtained using all individual paired values (n = 30 for each correlation) and were all statistically significant ($P < 0.001$). The closest fit of the data was found for the positive lineal correlation between the calculated change in maternal body weight and the logarithm of circulating insulin.

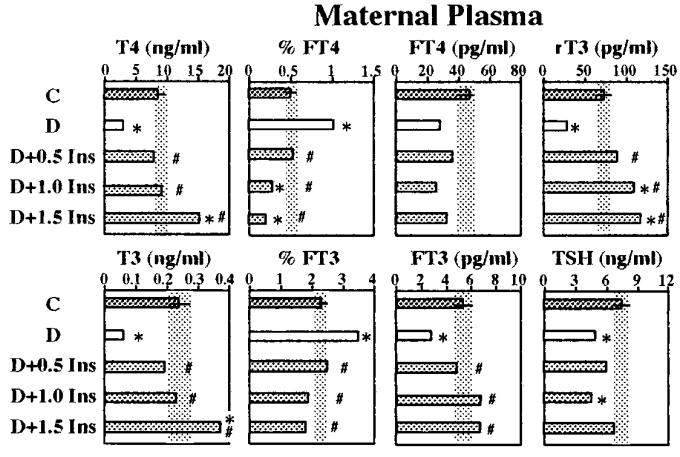

FIG. 3. The mean concentrations of T_4, % T_4, FT_4, T_3, % T_3, FT_3, rT_3, and TSH in the maternal circulation are shown for the various groups of dams. See Fig. 1 for the meaning of the *shaded area* and the symbols. Data for sD dams (not shown) were comparable to those for D dams, except for circulating TSH, which was still comparable to those for C dams. There were no statistically significant differences in the rT_3 and TSH concentrations among the three groups of insulin-injected dams. The T_4 and T_3 levels were the same for the D+0.5 Ins and D+1.0 dams, but were higher in the D+1.5 animals.

the maternal diabetic state and illness. The negative effects were best prevented with the administration of 0.5 U insulin/100 g BW·day.

Effects on thyroid hormone status of the mothers

The circulating concentrations of T_4, T_3, rT_3, and TSH as well as the % FT_4, % FT_4, % FT_3, and % FT_3 are shown in Fig. 3. Figure 4 shows the concentrations of T_4 and T_3 in the liver, lung, brain, heart, and mammary tissue. Iodothyronine deiodinase activities in some maternal tissues are shown in Fig. 5.

Effects of the diabetic state. Mean values of T_4, T_3, and rT_3 in the maternal circulation and of T_4 and T_3 in most tissues studied were lower in D dams than in C mothers, with the differences between C and D dams being statistically significant with few exceptions (brain T_4 and mammary gland T_3 concentrations).

Circulating % FT_4 was increased in the diabetic dams to twice the values normal for the pregnant dam. This increase was comparable to the decrease in total circulating T_4, as a result of which the mean circulating FT_4, although lower in D compared to C dams, was not statistically different from that in the C mothers. The % FT_3 also increased in D 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 in C dams.

5′D-I activity in the liver and lung were decreased in the diabetic dams (Fig. 5), 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 the cerebral T_4 concentration. The 5D-III activity of the cortex was not different in D and C dams.

Effects of insulin treatment of the D dams on their thyroid hormone status. The injection of insulin either normalized circulating T_4, T_3, and rT_3 concentrations or resulted in supraphysiologi-

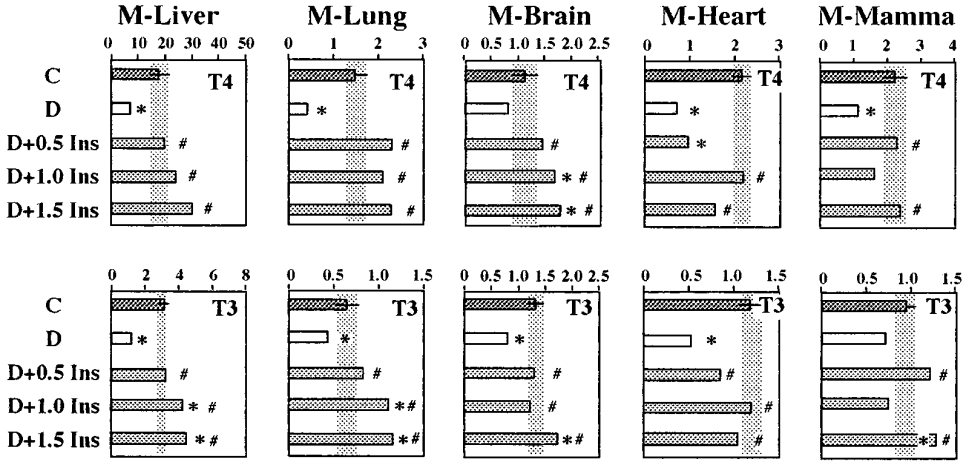

FIG. 4. The mean (±SE) concentrations (nanograms per g wet wt) of T_4 and T_3 are shown for liver, lung, brain, heart, and mammary tissue. The *shaded areas* and symbols are explained in Fig. 1. There were no statistically significant differences in the T_4 concentrations found in liver, lung, and brain of dams receiving the various insulin doses. The concentration of T_3 in these tissues was lower in the D+0.5 Ins dams than in the D+1.0 Ins and/or D+1.5 Ins mothers.

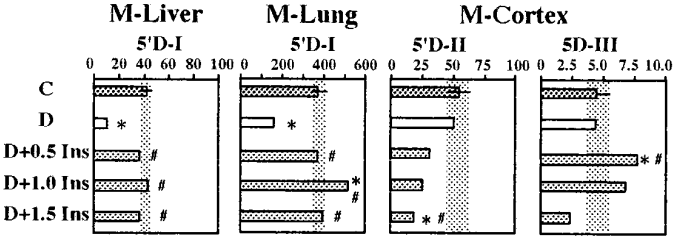

FIG. 5. The mean (±SE) activities of the outer ring 5′D, type I for liver (picomoles of I^- per min/mg protein) and lung (femtomoles of I^- per h/mg protein), type II for cortex (Cx; femtomoles of I^- per h/mg protein), and Cx inner ring 5D-III (picomoles of I^- per h/mg protein) are shown. The *shaded areas* and symbols are explained in Fig. 1. Although decreased in sD dams (not shown) compared to those in C dams, liver and lung 5′D-I activities were somewhat higher than those in D animals. There were no insulin dose-related differences in liver or brain 5′D activities. The activity in lung in D+1.0 Ins dams was higher than those in D+0.5 and D+1.5 Ins rats.

cal levels (Fig. 3). Thus, the maternal hypothyroxinemia caused by the diabetic state was avoided in all of the insulin-treated D dams. TSH concentrations improved with the injection of insulin, except in the D+1.0 Ins dams.

The effects of treatment with insulin depended on the dose used. In the D+0.5 Ins dams, all parameters of thyroid function that were affected by the diabetic condition were maintained within the normal range, with the exception of heart T_4 concentrations, which remained lower than normal. The effects in the two groups on the higher doses of insulin were more variable; higher than normal concentrations of the iodothyronines were often found in plasma and tissues. This occurred at different insulin doses, depending on the tissue and whether T_4 or T_3 concentrations were being considered.

The injection of insulin reversed to normal the decreased liver and lung 5′D-I activities or increased this activity to supraphysiological levels in the lung of the D+1.0 dams. On the contrary, the mean 5′D-II activity in the maternal cortex decreased with increasing insulin doses; the difference with respect to both D and C dams was significant in the D+1.5 Ins group. The 5D-III activity of the cortex increased in the D+0.5 Ins group above the values found in C and D dams, then decreased in an apparently insulin dose-dependent fashion.

Relationships between the degree of maternal illness and several indexes of maternal thyroid hormone status. To exclude differences related to the duration of the diabetic state, data from sD dams were not used for this evaluation. The changes in all circulating parameters of thyroid status (T_4, % FT_4, T_3, % FT_3, rT_3, and TSH) were closely correlated to the degree of maternal diabetes and illness, whether measured by the circulating glucose or insulin levels or the change in M-BW. When the parameters of thyroid hormone status were plotted against the above indexes of maternal metabolic imbalances, data fit linear functions, with values of r ranging from 0.77–0.85 ($P < 0.001$ in all cases).

The changes occurring in the activities of liver and lung 5′D-I were also closely related to the circulating glucose and insulin levels and the change in M-BW, with r values for the fit to linear functions ranging from 0.75–0.84 ($P < 0.001$). In contrast, no such relationships were found between the cerebral cortex 5′D-II and 5D-III activities and these indexes of the diabetic state.

Relationships between several indexes of maternal thyroid hormone status. Circulating TSH changed in parallel to plasma T_3 and not inversely, as expected if the negative feedback between the pituitary and thyroid was operative.

Although the patterns of changes in T_4 and T_3 concentrations in tissues appeared similar to those described for their respective circulating levels, we observed that the changes occurring in the different tissues could not be predicted from those in the corresponding plasma levels (total or free). Thus, for instance, the concentrations of T_3 in brain, heart, and mammary tissue of the D dams were at least 2-fold higher than expected from plasma T_3 or FT_3 levels.

Liver 5′D-I activity was fitted to a linear function of liver T_3 concentration, with an $r = 0.826$ ($P < 0.001$). The lung 5′D-I, cortex 5′D-II, and cortex 5D-III levels were more poorly fitted to linear functions when plotted against their tissue or plasma concentration of T_4 and T_3, with r values 0.60 or less.

Effects on the fetal compartment

Thyroid hormone status of the placenta. Figure 6 shows the concentrations of T_4, T_3, and rT_3 in the M- and F-placenta, which decreased in the D dams and returned to normal or

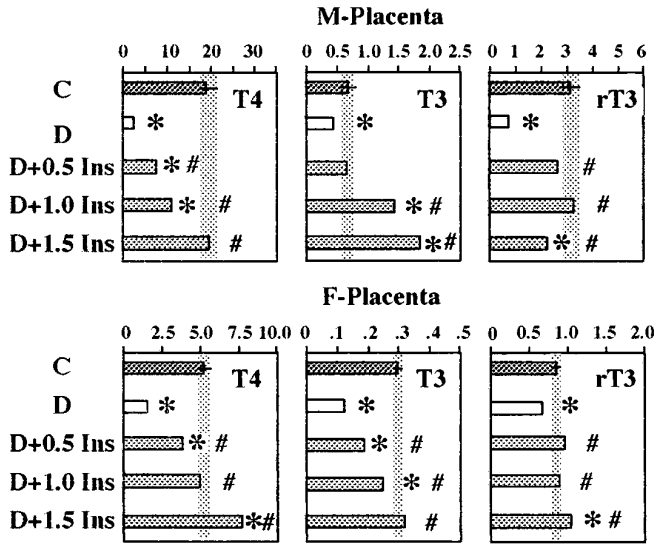

FIG. 6. The concentrations of T_4, T_3, and rT_3 in the maternal and fetal placenta are shown for the various groups of dams in nanograms per g. The *shaded areas* and symbols are explained in Fig. 1. The concentrations of T_4 and T_3 were significantly different in the dams receiving the three different insulin doses; rT_3 was higher or lower, respectively, in the M-placenta and F-placenta of the D+1.0 Ins dams than in the D+0.5 or D+1.5 Ins animals.

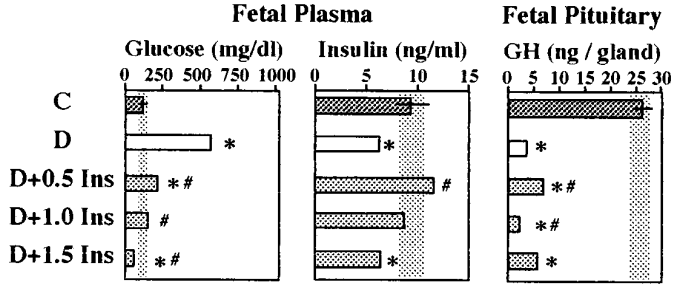

FIG. 7. The mean concentrations of glucose and insulin in the fetal circulation are shown as well as GH content of the fetal pituitary. The *shaded areas* and symbols are explained in Fig. 1. The glucose and insulin levels of the D+1.5 Ins fetuses were significantly lower than those of the D+0.5 Ins and D+1.0 Ins fetuses. The pituitary GH content was lower in the D+1.0 Ins fetuses than in those from dams injected with the lower and higher doses of insulin.

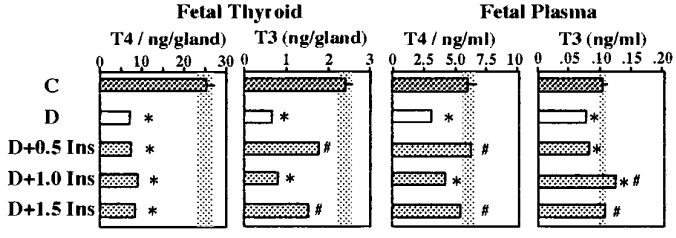

FIG. 8. Total T_4 and T_3 contents are shown for the fetal thyroids of the different experimental groups, as determined in proteolytic digests of the glands. Their corresponding concentrations in the fetal circulation are also shown. The *shaded areas* and symbols are explained in Fig. 1.

higher than normal values in D+Ins dams. There were quantitative differences between the M- and F-placenta with regard to the effects of diabetes and the various doses of insulin.

In the F-placenta, the changes in T_4 and rT_3 concentrations are comparable to those described in maternal circulating levels. On the contrary, in the M-placenta, T_4 and rT_3 concentrations were lower than expected from the circulating levels; the concentrations in the M-placenta of D dams decreased more in C dams than the observed change in the respective circulating levels and increased to only half the expected levels with insulin treatment. M-placenta T_4 was only normal in the D+1.5 Ins group, in which the circulating concentrations were clearly above C values. In contrast, T_3 concentrations in the M-placenta of D dams, whether treated with insulin or not, were higher than expected from T_3 circulating levels.

Thyroid hormone status of the fetus. Figure 7 shows the plasma glucose and insulin concentrations as well as the GH content of the F-pituitary. F-plasma glucose was markedly elevated in the progenies from D dams. Treatment of the mothers with insulin resulted in a dose-dependent decrease in F-plasma glucose; the fetuses from the D+1.5 Ins dams actually were hypoglycemic compared to those from normal dams, as measured 24 h after the injection of insulin into their mothers. The insulin levels in the fetal circulation were low in the fetuses from D dams, returning to normal in the D+0.5 Ins group, then decreasing in an insulin dose-dependent fashion. The pituitary GH content was lower in fetuses from D dams, with an ameliorating effect observed in fetuses from insulin-infused D dams (except in the D+1.0 Ins group), although pituitary GH content was far from normal values.

Total thyroidal T_4 and T_3 contents (Fig. 8) decreased in the fetuses from the diabetic mothers, with little improvement noted in the insulin-treated groups, except for a normalization of the T_3 content in the D+0.5 Ins and D+1.5 Ins fetuses. The T_4 and T_3 concentrations[1] in the fetal circulation (Fig. 8) decreased in the D groups and improved in the fetuses from insulin-treated dams, although not in an insulin dose-dependent fashion. The % FT_4 (not shown) increased from 0.126 ± 0.002% in C fetuses to 0.174 ± 0.016% in fetuses from D dams ($P < 0.01$). Unfortunately, there was not enough plasma to measure changes in % T_3 or changes in % T_4 in the other groups.

The T_4 and T_3 concentrations in different F-tissues (Fig. 9) decreased in tissues from D groups. Treatment of the mothers with insulin usually resulted in improved T_4 and T_3 concentrations in most F-tissues compared to those in D fetuses. However, in most tissues an insulin dose-dependent effect was not observed, and improvement was minimal in the D+1.0 Ins group.

T_4 and T_3 in the carcass (not shown) of fetuses from D mothers were lower (0.69 ± 0.06 ng T_4/g and 0.16 ± 0.01 ng T_3/g) than those in fetuses from C dams (1.71 ± 0.09 ng T_4/g and 0.33 ± 0.03 ng T_3/g; $P < 0.001$ for both T_4 and T_3). The total fetal extrathyroidal T_4 and T_3 pools were calculated (24); the T_4 pool decreased from 13.08 ng in C to 4.05 ng in D fetuses, and the T_3 pool decreased from 1.72 ng in C to 0.63

[1] Circulating TSH levels are not shown: due to the small amounts of plasma available for the different determinations, the plasma aliquots were one fourth the usual ones for the determination of TSH, and the levels in F-plasma from all groups were below reliable detection.

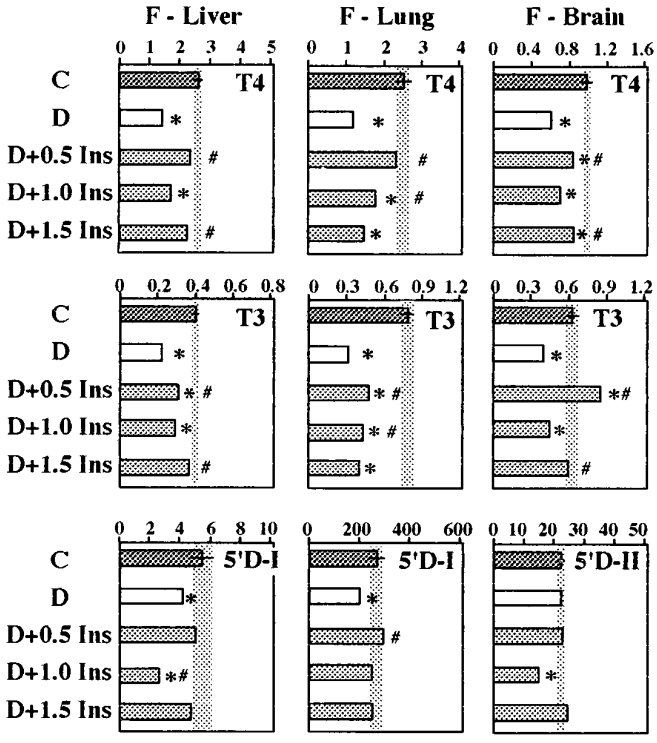

FIG. 9. The *three upper panels* show the concentrations (in nanograms per g) of T_4 in the liver, lung, and brain of the fetuses from the various groups of dams, with the *middle panels* showing the corresponding T_3 concentrations. The *three lower panels* show the activity of the outer ring 5′D, type I for liver (picomoles of I^- per min/mg protein) and lung (femtomoles of I^- per h/mg protein) and type II for cortex (Cx; femtomoles of I^- per h/mg protein). The *shaded areas* and symbols are explained in Fig. 1.

ng in D animals. The extrathyroidal pools of T_4 and T_3 in D fetuses were markedly reduced, to 31% and 36%, respectively, of the normal values.

In D fetuses, 5′D-activity was lower than normal in F-liver and F-lung (Fig. 9) and were restored to normal values in fetuses from insulin-treated dams, with the exception of F-liver in the D+1.0 Ins group, where it was actually reduced below D levels. 5′D-II activity in the F-brain was unchanged, except for a decrease in D+1.0 Ins fetuses. Fetal brain 5D-III activity (not shown) of normal fetuses was 6.09 pmol/h·mg protein and did not decrease significantly in fetuses from D dams, but increased slightly with insulin treatment of the mothers to 6.83 pmol/h·mg protein (similar results were observed regardless of the insulin dose).

Relationships between fetal and maternal thyroid hormone levels and between parameters of fetal thyroid hormone status

There was a very good correlation between the glucose concentrations in the fetal and maternal circulations ($n = 33$; $r = 0.96$; $P < 0.001$), but not between insulin levels. The latter was due to the finding that in the insulin-treated groups, insulin levels in M-plasma increased proportionally to the administered dose, whereas they decreased in F-plasma.

The F-BW (Table 1) was affected by the maternal diabetic state. This decrease was not totally corrected with any of the doses of insulin administered to the dams, but the closest to normal F-BW were observed for fetuses from the D+0.5 Ins mothers, and the lowest was found in fetuses from D+1.0 Ins dams. The F-BW was related to other indexes of the outcome of pregnancy, such as the number of viable fetuses per litter ($n = 29$; $r = 0.63$; $P < 0.001$). F-BW was clearly related to the GH content of the fetal pituitary (Fig. 8), with a good fit to a linear function of F-BW *vs.* the logarithm of the GH content ($n = 33$; $r = 0.87$, $P < 0.001$). With the exception of the F-plasma T_3 level, the T_4 and T_3 concentrations in F-tissues were more closely correlated to the F-BW and pituitary GH content than to the changes in maternal thyroid hormone status.

The total T_4 and T_3 contents in the thyroid gland of fetuses from the different treatment groups did not correlate with their changes in F-plasma or F-tissues. The changes observed in the concentrations of T_4 and T_3 in the F-tissues were similar to those in the corresponding hormone in F-plasma, as the F-tissue to F-plasma ratios (not shown) were usually the same as those in fetuses from C dams, except for T_3 in F-liver, F-lung, and F-brain in the D+1.0 Ins group, which was lower than expected from the F-plasma levels.

Changes in the thyroid hormone status of the fetuses from D groups were similar to those in their mothers. On the contrary, the effects of insulin treatment on T_4 and T_3 concentrations were different. Treatment of the D dams with insulin usually resulted in insulin dose-dependent changes, whereas there was no clear relationship between the insulin dose and fetal T_4 and T_3 concentrations. There was also no correlation between the changes in thyroid status of the fetuses and F-plasma insulin. As indicated above, there was a better correlation with F-BW and pituitary GH content.

The activities of 5′D-I in F-liver and F-lung and of 5′D-II and 5D-III in F-brain, either did not correlate with the T_4 and T_3 in F-plasma or in F-liver and F-lung, or did so poorly, with r values of 0.5 or less. No clearly significant correlations were found between these enzyme activities and the F-plasma glucose levels. Changes in the activities of 5′D-I in F-liver and F-lung and 5′D-II in F-brain were less marked than those in the corresponding maternal tissues.

Discussion

Present results confirm and extend our preliminary observations on the effects of maternal STZ-induced diabetes mellitus on fetal thyroid hormone status, as studied on 20 dg (11), which are likely to result from the altered carbohydrate metabolism of their mothers, and not from a direct destructive action of STZ on the fetal pancreas. The half-life of disappearance of STZ from the circulation is 5 h in rodents (23). The drug, injected at 7 dg, would no longer be present by the time the β-cells of the fetal pancreas develop in the rat (12.5–13.5 dg), long before the morphological structure of the β-cells is achieved on 18 dg (24). Moreover, when maternal hyperglycemia was mitigated with insulin, F-plasma insulin was normal, indicating that fetal β-cells were functional. As the placenta is impermeable to maternal insulin (25), this would not occur if the fetal pancreas had been directly affected by the drug. The pancreas of the hyperglycemic fetuses of the present D dams showed hyperplasia, hypertro-

phy, and degranulation of immunochemically identified insulin-positive β-cells (our unpublished data), confirming the findings of others (26) and indicating β-cell overstimulation and exhaustion.

A direct toxic effect of STZ on the placenta is also unlikely. Ultrastructural changes in the placenta have been described, but were less frequent when insulin was supplied (27), whereas a direct toxic effect would be independent from treatment with insulin. In a previous study (unpublished) in which the rats were treated with STZ before conception and maintained with insulin until midgestation, we found the same changes in the concentrations of both T_4 and T_3 in the M-placenta as those described here for dams treated with STZ during pregnancy; T_4 decreased to 16% of C values, and T_3 to 63%. The F-BW of the dams given STZ pregestationally was similarly affected as that of the present D fetuses, decreasing to 67% of C values.

The pregnant rat with diabetes mellitus as a model of maternal nonthyroidal illness

The changes in thyroid hormone status occurring in nonthyroidal illness are at present considered adaptive responses to a limited availability of intracellular energy, a situation in which a decrease in T_3-dependent catabolic effects would be beneficial (1, 2). Several mechanisms are involved in these responses, of which two are best known, namely 1) 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 2) a decrease in the activity of enzymes involved in the extrathyroidal generation of T_3 from T_4.

1) The sequence of events involves decreased release of hypothalamic TRH (4, 5), secretion of TSH (4, 6, 7), and sensitivity of the thyroid to TSH (6), which supersede the normal feedback mechanism; TSH decreases despite the lower levels of circulating total and/or free T_4 and T_3. Indeed, a return to a normal secretion of TSH and thyroidal release of hormones is considered an indication that the illness is remitting or that the metabolic alterations are under control. The present 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 of the tissues studied are in agreement with the changes described in the nonpregnant diabetic rats (8) and in patients dying from nonthyroidal illnesses (28).

2) Direct measurement of 5′D-I activities in the liver and lung has confirmed that generation of T_3 from T_4 is decreased in diabetic rats (11, 29, 30) and is consistent with a decreased expression of 5′D-I messenger RNA (29). 5′D-II activity in the cerebral cortex of D dams was not changed, possibly because cerebral T_4 was not decreased, and confirming the lack of change described in nonpregnant diabetic rats (10, 31).

Treatment with insulin: varying severity of maternal illness

The diabetic state of the dams improved. The best results were observed in the D+0.5 Ins group both with respect to parameters of diabetes mellitus and reproductive competence and with respect to thyroid hormone status, including circulating TSH and liver and lung 5′D-I activities.

The two higher doses of insulin used in the present study might well have been excessive; the glucose and insulin levels were normal 24 h after the last injection, suggesting daily periods of hypoglycemia. Most, but not all, changes in parameters of maternal thyroid hormone economy appeared to be insulin dose dependent.

Effects on the placenta

In diabetic women and rats, hyperglycemia increases placental weight and placental glycogen content (32). The placentas from the present D dams were clearly affected. It is very difficult to properly regulate placental function and fetal metabolism in diabetic mothers with insulin (33) administered once daily,[2] possibly because of the daily cycles of hyper- and hypoglycemia, or constant hypoglycemia. These were more likely to occur in the D+1.0 Ins and D+1.5 Ins dams. Indeed, the best results were observed in the D+0.5 Ins dams, which were not likely to have undergone prolonged periods of hypoglycemia.

The placenta plays a very important role in the supply of nutrients and in determining the metabolic status of the fetus. Posner *et al.* (34) suggested that insulin might promote substrate transport across the placenta, which has insulin receptors. The rat placenta is permeable to maternal T_4 and T_3 (13, 14) and is active in the local metabolism of both iodothyronines, as it contains iodothyronine deiodinase isoenzymes, with 5′D-II activity highest in the M-placenta (35), and 5-III highest in the F-placenta (36).

Although T_4 and T_3 decreased in the placenta of D dams and reversed to normal or supraphysiological levels with insulin treatment, the changes occurring in the M-placenta were quantitatively different from those found in M-plasma. T_4 concentrations decreased more than expected from the changes in circulating total T_4 or FT_4, in agreement with our previous report (11). The opposite was observed for the concentrations of T_3 in the M-placenta, which increased more with insulin treatment than expected from the circulating changes in total T_3 and FT_3. The differences in thyroid hormone status of the M-placenta compared to other M-tissues may well be related to the decreased uteroplacental blood flow caused by a significant reduction in the arterial blood velocity in the uterine artery, placenta, umbilical artery, and fetal aorta (37). The mechanisms, if any, regulating placental permeability to the iodothyronines and their metabolism in the M- and F-placenta are unknown at present, so that the possibility that their transfer is altered by the hyperglycemic state or the lack of insulin has not yet been studied. Changes in the activities of the different iodothyronine-deiodinating isoenzymes may also contribute to the observed concentrations, but were not defined in the present experiment.

Effects on fetal development and thyroid hormone status

The reproductive competence of the present STZ-induced diabetes mellitus pregnant rats was impaired, not only with regard to the decreased number of fetuses per litter and the increased frequency of resorptions, but also with respect to

[2] Administration of insulin by infusion with minipumps was not used for the present study, as despite repeated attempts with different insulin preparations, the number of rats in which the pump did not clot was limited, thus complicating the experimental approach considerably.

the development of their fetuses, as assessed by BW. In our study, the F-BW was significantly reduced in fetuses from all groups of STZ-injected dams, in agreement with the severity of the diabetes and with reports by others using models similar to the present one (12, 26) or with pregestational diabetes (38) and data of babies born from poorly controlled diabetic women (39–41). The growth impairment that exists in the adult rat with diabetes mellitus is attributed at least in part to decreased pituitary GH content and secretion (7) resulting from decreased GH messenger RNA and decreased GH transcription rate (42). Although the control of fetal growth is not usually attributed to fetal GH, but to insulin-like growth factors, the pituitary content of this hormone was lower in the fetuses from the D dams of the present study and was closely correlated to their F-BW.

The changes in F-plasma glucose levels were clearly related to those in the maternal circulation, as previously shown by others in rats (12) and sheep (43), as they have a limited capacity to handle the glucose coming from their hyperglycemic mothers. It is quite likely that the hyperglycemia of the D fetuses was responsible for exhaustion of an overstimulated fetal pancreas (26), resulting in decreased F-plasma insulin levels (11, 12). The thyroid hormone status of the fetus was clearly affected by the maternal diabetes mellitus, with regard to both intrathyroidal T_4 and T_3 contents and extrathyroidal pools; both were markedly reduced. Morphological parameters, such as the decreased area of epithelial cells, were consistent with a dormant thyroid in fetuses from D dams (our unpublished observations), as described for adult rats. This is in conceptual agreement with the finding that insulin is essential for the transcription of two thyroid-specific genes, namely the thyroglobulin and thyroid peroxidase genes (44, 45).

In fetuses from D dams, the concentrations of T_4 and T_3 were reduced in the circulation and all tissues studied, including the carcass, with the total extrathyroidal stores reduced to one third of C values. This might not only be due to decreased secretion by the fetal thyroid, but also to the decrease in maternal T_4 and T_3 pools available for transport (46) into the fetal compartment. A decreased availability of maternal hormones could be aggravated by effects of the maternal diabetes mellitus on M-placental function.

Whatever the relative roles played by the different mechanisms, the hyperglycemic fetuses presented all of the changes found in their mothers, including an increased % FT_4 and decreased liver and lung 5′D-I activities. 5′D-II activity in the fetal brain did not show the increase expected from the low F-plasma and F-brain T_4 (13–16), as a result of which cerebral T_3 concentrations were lower than those in normal fetuses.

In contrast, the effects of insulin treatment of the dams on fetal weight, circulating insulin levels, and thyroid hormone economy did not parallel those observed in their mothers. The different doses of insulin resulted in a normal increase in the M-BW in all three groups of insulin-treated D dams and dose-related increases in circulating insulin. The concentrations of T_4 and T_3 in M-plasma and most tissues also appeared to increase with increasing insulin doses. In contrast, a clear improvement in F-BW was only observed in the D+0.5 Ins group, the only group in which the number of fetuses per litter was normal and resorptions absent and in which F-plasma insulin levels were restored to normal values. Despite this improvement, a normal F-BW was not attained, in agreement with previous reports (11, 12). A normal fetal pituitary GH content was not attained with any of the insulin doses used in the present study. Although the fetal pituitary GH content was not measured, Erikson *et al.* (38) did not find normal F-BW when rat dams, injected with STZ weeks before the onset of pregnancy, were treated with insulin. We cannot at present explain these results and cannot exclude a direct effect of STZ, injected on day 7 of gestation into the mothers, on the fetal pituitary. This appears unlikely, considering that the decrease in pituitary GH in adult STZ-treated rats is corrected with insulin (7). Maternal hyperinsulinemia (47) and intermittent hypoglycemia (48) also affect fetal growth, and it is likely that such events accounted for the poor results obtained by us with the higher insulin doses, considering that Ultralente insulin was used in the present study.[3] F-BW in these groups was closely related to their pituitary GH content, not to F-plasma glucose or insulin levels. F-plasma insulin levels decreased with the increasing dose of insulin injected into their mothers and were as low as those in fetuses from D dams in the hypoglycemic fetuses of the D+1.5 Ins dams, possibly because of continuous overstimulation leading to exhaustion of their β-cells.

Most parameters of thyroid status improved or actually reverted to normal values in the fetuses from the D+0.5 Ins dams, including attainment of normal T_3 concentrations in the F-brain. The changes in T_4 and T_3 in the F-thyroid, F-plasma, and F-tissues did not appear to be dose dependent with respect to the insulin administered to their mothers, being often more closely related to the F-BW and pituitary GH content. If the latter parameters are taken as indexes of fetal metabolic abnormalities and intracellular energy availability, it would appear that if adequate control of the maternal diabetic condition were achieved, the alterations in fetal thyroid hormone economy, including brain T_3 levels, would be avoided.

Possible clinical relevance of the present findings

Fetal damage, abnormalities, and resorptions are well known hazards of diabetes in pregnancy, whether in human or experimental models (38, 49), especially when inadequately controlled, as evidenced by increased maternal β-hydroxybutyrate and triglyceride levels. Rizzo *et al.* (50) reported that diabetes mellitus during pregnancy affects the intellectual development and behavior of the progeny. They found a correlation between the alterations in lipid metabolism of pregnant woman and the intelligence quotient of their 2- to 3-yr-old children; the higher the levels of β-hydroxybutyrate and FFA, the lower the intelligence quotient of the offspring. Considering that if the present results are pertinent to man, brain T_3 might have been low during a

[3] We have no explanation regarding the very poor results obtained in the D+1.0 Ins fetuses, except that their mothers might have undergone a wider range of daily fluctuations between a hyperglycemic and a hypoglycemic state than the D+1.5 Ins dams, which might have been hypoglycemic throughout. Such fluctuations appear to be especially harmful for the placenta (47).

phase of development when thyroid hormones are of great importance for brain development, it is possible that alterations in fetal thyroid hormone status are contributing to this intellectual impairment. Unfortunately, although the present results would indicate that adequate control of the diabetes mellitus appears to be of prime importance to prevent the decrease in cerebral T_3, such control is not easily achieved.

Relevance of present findings for nonthyroidal illness other than diabetes mellitus

The diabetic dam displayed all of the changes in thyroid hormone status considered typical of nonthyroidal illness, changes that could be modulated and/or totally corrected with insulin. We cannot at present exclude that some of the effects observed in both the dams and the conceptus were specifically related to the shortage of insulin and would not be found in other models of nonthyroidal illness. However, the changes in the concentrations of T_4 in the circulation and tissues of nonpregnant STZ-induced diabetes mellitus and food-restricted rats have been shown to be related to energy availability, as measured by the changes in BW, and not specifically to the insulin levels (8). The same was found for the concentration of T_3 in brain, cerebellum, liver, and pituitary, whereas the T_3 level in brown adipose tissue, heart, muscle, and kidney were more specifically related to the availability of insulin. Although the present experimental design was not adequate to distinguish between the effects of energy shortage and the lack of insulin, the findings we report here may be relevant for other nonthyroidal illnesses in which intracellular energy availability is impaired.

Conclusions

The present results show that maternal diabetes mellitus and possibly maternal nonthyroidal illnesses compromising intracellular energy availability result in severe impairment of the thyroid hormone status of the fetus. This includes low cerebral concentrations of T_3 during a critical period of brain development. Correction of the illness is necessary to protect the brain, as compensatory mechanisms usually involved in maintaining cerebral T_3 homeostasis do not appear to be operative.

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References

1. **Wartofski L, Burman KD** 1982 Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome." Endocr Rev 3:164–217
2. **Tibaldi JM, Surks MI** 1985 Animal models of non-thyroidal disease. Endocr Rev 6:87–101
3. **Pittman CA, Suda AK, Chambers JB, McDaniel HG, Ray GY** 1979 Abnormalities of thyroid turnover in patients with diabetes mellitus before and after insulin therapy. J Clin Endocrinol Metab 48:854–860
4. **González C, Montoya E, Jolín T** 1980 Effect of streptozotocin diabetes on the hypothalamic-pituitary-thyroid axis in the rat. Endocrinology 107:2099–2103
5. **Rondeel JMM, de Greef WJ, Heide R, Visser TJ** 1992 Hypothalamo-hypophysial-thyroid axis in streptozotocin-induced diabetes. Endocrinology 130:216–220
6. **Pastor R, Jolín T** 1983 Peripheral metabolism and secretion rate of thyrotropin in STZ-diabetic rats. Endocrinology 112:1454–1459
7. **Ortíz-Caro J, González C, Jolín T** 1984 Diurnal variations of plasma growth hormone, thyrotropin, thyroxine and triiodothyronine in streptozotocin-diabetic and food-restricted rats. Endocrinology 115:2227–2232
8. **Schröeder van der Elst JP, van der Heide D** 1992 Effects of streptozotocin-induced diabetes and food restriction on quantities and source of T_4 and T_3 in rat tissues. Diabetes 41:147–152
9. **Bjorn-Hansen-Gotzsche LS, Gotzche OJ, Flyvbjerg A, Boye N** 1990 Early changes in thyroid hormone metabolism in the heart, liver, and brown adipose tissue during the induction of low T_3 syndrome in streptozotocin-diabetic rats. Acta Endocrinol (Copenh) 123:67–72
10. **Ortíz-Caro J, Obregón MJ, Pascual A, Jolín T** 1984 Decreased T_4 to T_3 conversion in tissues of STZ-diabetic rats. Acta Endocrinol (Copenh) 106:86–91
11. **Calvo R, Obregón MJ, Escobar del Rey F, Morreale de Escobar G** 1991 The effects of maternal diabetes mellitus on thyroid hormone economy of rat fetuses. In: Gordon A, Gross J, Hennemann G (eds) Progress in Thyroid Research. Balkema, Rotterdam, pp 813–816
12. **Herrera E, Palacín M, Martín M, Lasunción MA** 1985 Relationship between maternal and fetal fuels and placental glucose transfer in rats with maternal diabetes of varying severity. Diabetes [2] 34:42–46
13. **Calvo RM, Obregón MJ, Escobar del Rey F, Morreale de Escobar G** 1990 Congenital hypothyroidism, as studied in rats. Crucial role of thyroxine but not of triiodothyronine in the protection of the fetal brain. J Clin Invest 86:889–899
14. **Calvo R, Obregón MJ, Escobar del Rey F, Morreale de Escobar G** 1992 The rat placenta and the transfer of thyroid hormones from the mother to the fetus. Effects of maternal thyroid status. Endocrinology 131:357–365
15. **Morreale de Escobar G, Calvo R, Escobar F, Obregón MJ** 1994 Thyroid hormones in tissues from fetal and adult rats. Endocrinology 134:2410–2415
16. **Ruiz de Oña C, Morreale de Escobar G, Calvo R, Escobar del Rey F, Obregón MJ** 1991 Thyroid hormones and 5′-deiodinase in the rat fetus late in gestation: effects of maternal hypothyroidism. Endocrinology 128:422–432
17. **Mendel CM, Laughton CW, McMahon FA, Cavalieri RR** 1991 Inability to detect an inhibitor of thyroxine-serum protein binding in sera from patients with non-thyroidal illness. Metabolism 40:491–502
18. **Obregón MJ, Ruiz de Oña C, Hernández A, Calvo R, Escobar del Rey F, Morreale de Escobar G** 1989 Thyroid hormones and 5′-dediodinase in rat brown adipose tissue during fetal life. Am J Physiol 257:E625–E631
19. **Campos-Barros A, Köhler R, Muller F, Eravci M, Meinhold H, Wesemann W, Baumgartner A** 1993 The influence of sleep deprivation on thyroid hormone metabolism in rat frontal cortex. Neurosci Lett 162:145–148
20. **Hugget ASG, Nixon DA** 1957 Use of glucose oxidase, peroxidase and *o*-dianisidine in determination of blood and urinary glucose. Lancet 1:368–70
21. **Morreale de Escobar G, Calvo R, Escobar del Rey F, Obregón MJ** 1993 Differential effects of thyroid hormones on growth and thyrotropic hormone in rat fetuses near term. Endocrinology 132:2056–2064
22. **Snedecor GW, Cochran WG** 1980 Statistical Methods, ed 7. Iowa State University Press, Ames
23. **Schein PS, Loftus S** 1968 Streptozotocin: depression of mouse liver pyridine nucleotides. Cancer Res 28:1501–1506
24. **Portha B** 1990 Development of the pancreatic β cell: growth patterns and functional maturation. In: Cuezva JM, Pascual-Leone AM, Patel MS, Safford G (eds) Endocrine and Biochemical Development of the Fetus and Neonate. Plenum Press, New York, pp 33–43
25. **Knobil E, Yosimovich JB** 1958 Placental transfer of thyrotropic hormone, thyroxine, triiodothyronine and insulin. Ann NY Acad Sci 75:895–904
26. **Aerts L, van Assche FA** 1977 Rat fetal endocrine pancreas in experimental diabetes J Endocrinol 73:339–346
27. **Padmanabhan R, Al-Zuhair AG** 1990 Ultrastructural studies on the placentae of streptozotocin induced maternal diabetes in the rat. Z Mikrosk Anat Forsch 104:213–220
28. **Arem R, Wiener GJ, Kaplan SG, Kim HS, Reichlin S, Kaplan M** 1993 Reduced tissue thyroid hormone levels in fatal illness. Metabolism 42:1102–1108
29. **O'Mara BA, Dittrich W, Lauterio TJ, StGermain DL** 1993 Pretranslational regulation of type I 5′-deiodinase by thyroid hormones and in fasted and diabetic rats. Endocrinology 133:1715–1723
30. **Gavin L A, McMahon FA, Mueller M** 1981 The mechanism of impaired T_3 production from T_4 in diabetes. Diabetes 30:694–699
31. **Gavin LA, Cavalieri RR** 1986 Iodothyronine deiodination in the brain of diabetic rats: influence of thyroid status. J Endocrinol Invest 9:127–133
32. **Shafrir E, Barash V** 1991 Placental glycogen metabolism in diabetic pregnancy. Isr J Med Sci 27:449–461
33. **Copeland AD, Hendrich CE, Porterfield SP** 1990 Distribution of free amino-acids in streptozotocin-induced diabetic pregnant rats, their placentae and fetuses. Horm Metab Res 22:65–70
34. **Posner BE** 1974 Insulin receptors in human and animal placental tissue. Diabetes 23:209–217
35. **Kaplan MM, Shaw E** 1984 Type II iodothyronine 5′-deiodination by human and rat placenta *in vitro*. J Clin Endocrinol Metab 59:253–257
36. **Emerson CH** 1989 Role of the placenta in fetal thyroid homeostasis. In: Delange

F, Fisher DA, Glinoer D (eds) Research in Congenital Hypothyroidism, series A. Plenum Press, New York, vol 161:31–43
37. **Chartrel NC, Clabaut MT, Boismare FA, Schrub JC** 1990 Uteroplacental hemodynamic disturbances in establishment of fetal growth retardation in streptozotocin-induced diabetic rats. Diabetes 39:743–746
38. **Eriksson RSM, Thunberg L, Eriksson UJ** 1989 Effect of interrupted insulin treatment on fetal outcome of pregnant diabetic rats. Diabetes 38:764–772
39. **Pedersen J F, Molsted-Pedersen L** 1981 Early growth delay detected by ultrasound marks increased risk of congenital malformation in diabetic pregnancy. Br Med J 283:269–271
40. **Hill DJ** 1982 Fetal effects of insulin. Obstet Gynaecol Ann 11:133–149
41. **Rudolf MCJ, Sherwin RS, Markowitz R** 1982 Effect of intensive insulin treatment on linear growth in the young diabetic patient. J Pediatr 101:333–339
42. **Bedó G, Santisteban P, Jolin T, Aranda A** 1991 Expression of the growth hormone gene and the pituitary-specific transcription factor GHF-1 in diabetic rats. Mol Endocrinol 5:1730–1739
43. **Hay Jr WW, Sparks JW, Wilkening RB, Battaglia FC, Meschia G** 1984 Fetal glucose uptake and utilization as functions of maternal glucose concentration. Am J Physiol 246:E237–E242
44. **Santisteban P, Acebrón A, Polycarpou-Schwarz M, DiLauro R** 1992 Insulin and insulin-like growth factor I regulate a thyroid-specific nuclear protein that binds to the thyroglobulin promoter. Mol Endocrinol 6:1310–1317
45. **Aza-Blanc P, Di Lauro R, Santisteban P** 1993 Identification of a *cis*-regulatory element and a thyroid-specific nuclear factor mediating the hormonal regulation of rat thyroid peroxidase promoter activity. Mol Endocrinol 7:1297–1306
46. **Morreale de Escobar G, Calvo R, Obregón MJ, Escobar del Rey F** 1990 Contribution of maternal thyroxine to fetal thyroxine in normal rats near term. Endocrinology 126:2765–2767
47. **Ogata ES, Paul RI, Finley SL** 1987 Limited maternal fuel availability due to hyperinsulinemia retards fetal growth and development in the rat. Pediatr Res 22:432–437
48. **Sodoyez-Goffaux F, Sodoyez JC** 1976 Effects of intermittent hypoglycemia in pregnant rats on the functional development of the pancreatic B-cells of their offspring. Diabetologia 12:73–76
49. **Mills JL, Baker L, Goldman AS** 1979 Malformations in infants of diabetic mothers occur before the seventh gestational week. Diabetes 28:292–293
50. **Rizzo T, Metzger BE, Burns WJ, Burns K** 1991 Correlations between antepartum maternal metabolism and intelligence of offspring. N Engl J Med 325:911–916