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, T₄, T₃, rT₃, and TSH in mother and fetus, T₄ and T₃ 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 T₄, 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 T₄ and T₃ pools decreased to one third of normal fetal values. T₄ and T₃ 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 T₃ 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 T₃ 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 T₃ from T₄ (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 hypothalamic–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). T₃ and T₄ 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, T₄ deiodination to T₃ in peripheral tissues is decreased (8–10). As a consequence of all of these changes, circulating levels of T₄ and T₃ 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 T₄ and T₃ 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 T₄ 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...
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 (Ultracele, 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 d; 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 d (D=0.5 Ins, D=1.0 Ins, and D=1.5 Ins groups, respectively). Most results obtained in D and sD groups were 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 sample and homogenized, with tracers amounts of [131I]T4 and [125I]T3, 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 X 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 [131I]T4 and [125I]T3 added to each sample during the initial homogenization process. The samples are submitted to highly sensitive RIAs for the determination of T4 and T3, with a limit of sensitivity of 2.5 pg T4 and 1.5 pg T3/tube. The cross-reactivities of the different iodothyronines and metabolites were as previously described (15, 16). Each sample is processed in duplicate or triplicate at two or more dilutions. Concentrations were then calculated using the amounts of T4 and T3 found in the respective RIAs, the individual recovery of the [131I]T4 and [125I]T3, 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 T4 and T3. 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 with bovine胰蛋白酶 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 T4 and T3 were determined by RIA, as described above.

**Percentage of circulating free T4 and T3**

These percentages were determined by ultrafiltration of undiluted plasma samples, as described by Mendel et al. (17) with modifications. High specific activity [125I]T4 or [125I]T3 (~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 T4 (% FT4) or free T3 (% FT3) and the T4 and T3 concentrations determined by RIA were used to calculate the concentrations of FT4 and free FT3, respectively.

**Iodothyronine 5’- and 5-D activities**

Before each assay, [125I]rT3 or [125I]T4 was purified by paper electrophoresis to separate the iodide. Iodothyronine 5’-D activity was assayed as previously described (16), using 2 mM diithiothreitol (DTT) and 400 or 200 mM rT3, for maternal and fetal liver, respectively, and 2 mM T3 and 20 mM DTT for maternal and fetal lung (5’-D). Maternal and fetal brain 5’-D-II activities were assayed (18) using 2 mM T4, 1 mM T3, and 20 mM DTT in the presence of 1 mM 2-N-propyl-6-thiouracil (PTU). The [125I]T3 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-T2) 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).

5’D 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-[125I]T3, 50 mM T3, 20 mM DTT, and 1 mM PTU for 60 min at 37°C. Radioidode release was measured as described above. When necessary, inner ring labeled [5-125I]T3, was repurified before use with disposable Sep-Pak C18 cartridges (Waters Associates, Milford, MA) and methanol.

**Other determinations**

rT3 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, antiporcine antiserum, and human [125I]-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 immunoreagents 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**

T4, T3, 3,5,3-T2, PTU, and DTT were obtained from Sigma Chemical Co. (St. Louis, MO). rT3 and 3,3,5-T3, were obtained from Henning Berlin (Berlin, Germany). High specific activity [125I]T4, [125I]T3, [125I]T4, and [125I]rT3 (3000 μCi/μg) were synthesized in our laboratory (15) and used for highly sensitive T4, T3, and rT3 RIAs, as recovery tracers for extractions, and as substrates for 5’-D.

Inner ring labeled 5-[125I]T3 (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-
significant 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 (Computrac 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).

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of dams*</th>
<th>Increment in total wt (g)</th>
<th>No. of fetuses/dam</th>
<th>F-BW (mg)</th>
<th>Placental wt (mg)</th>
<th>M-placental wt (mg)</th>
<th>F-placental wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7 (7:0)</td>
<td>100.0 ± 2.5</td>
<td>12.4 ± 1.0</td>
<td>4872 ± 32</td>
<td>510.6 ± 7.9</td>
<td>154 ± 9</td>
<td>339 ± 11</td>
</tr>
<tr>
<td>D</td>
<td>7 (8:5)</td>
<td>31.6 ± 8.8h</td>
<td>10.0 ± 1.3</td>
<td>3196 ± 66</td>
<td>561.1 ± 12.2b</td>
<td>116 ± 4</td>
<td>361 ± 11</td>
</tr>
<tr>
<td>D + 0.5 Ins</td>
<td>4 (4:0)</td>
<td>71.3 ± 10.6bc</td>
<td>10.5 ± 1.8</td>
<td>4097 ± 65bc</td>
<td>531.2 ± 14.6j</td>
<td>136 ± 5</td>
<td>326 ± 11</td>
</tr>
<tr>
<td>D + 1.0 Ins</td>
<td>4 (4:2)</td>
<td>51.3 ± 7.6bc</td>
<td>7.5 ± 1.0gab</td>
<td>3141 ± 65d</td>
<td>482.6 ± 10.5cd</td>
<td>126 ± 6</td>
<td>317 ± 9</td>
</tr>
<tr>
<td>D + 1.5 Ins</td>
<td>4 (4:3)</td>
<td>63.3 ± 8.4c</td>
<td>8.8 ± 1.1i</td>
<td>3465 ± 150bc,ce</td>
<td>472.4 ± 10.4b,ce</td>
<td>107 ± 8b,ce</td>
<td>299 ± 15b,ce</td>
</tr>
</tbody>
</table>

Note: 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.

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

Effects on thyroid hormone status of the mothers

The circulating concentrations of T4, T3, rT3, and TSH as well as the % FT4, % FT3, % FT, and % FT, are shown in Fig. 3. Figure 4 shows the concentrations of T4 and T3 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 T4, T3, and rT3 in the maternal circulation and of T4 and T3 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 T4 and mammary gland T3 concentrations).

Circulating % FT4 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 T4, as a result of which the mean circulating FT4, although lower in D compared to C dams, was not statistically different from that in the C mothers. The % FT3 also increased in D compared to C dams, but not to the extent that it could compensate for the decrease in circulating total T3, and the FT3 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 T4 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 T4 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 T4, T3, and rT3 concentrations or resulted in supraphysiolog-
The injection of insulin reversed to normal the decreased status. The effect 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 T₄ 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 T₃ or T₄ 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 5'D-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 (T₄, %FT₄, T₃, %FT₃, rT₃, 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 5'D-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 T₃ and not inversely, as expected if the negative feedback between the pituitary and thyroid was operative.

Although the patterns of changes in T₄ and T₃ 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 T₃ in brain, heart, and mammary tissue of the D dams were at least 2-fold higher than expected from plasma T₃ or FT₃ levels.

Lung 5'D-I activity was fitted to a linear function of liver T₃ concentration, with an r = 0.826 (P < 0.001). The lung 5'D-I, cortex 5'D-II, and cortex 5'D-III levels were more poorly fitted to linear functions when plotted against their tissue or plasma concentration of T₄ and T₃, with r values 0.60 or less.

Effects on the fetal compartment

Thyroid hormone status of the placenta. Figure 6 shows the concentrations of T₄, T₃, and rT₃ in the M- and F-placenta, which decreased in the D dams and returned to normal or
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 T4 and rT3 concentrations are comparable to those described in maternal circulating levels. On the contrary, in the M-placenta, T4 and rT3 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 T4 was only normal in the D+1.5 Ins group, in which the circulating concentrations were clearly above C values. In contrast, T3 concentrations in the M-placenta of D dams, whether treated with insulin or not, were higher than expected from T3 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 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.

The T4 and T3 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 T3 content in the D+0.5 Ins and D+1.5 Ins fetuses. The T2 and T3 concentrations 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 % FT4 (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 % T3 or changes in % T4 in the other groups.

The T4 and T3 concentrations in different F-tissues (Fig. 9) decreased in tissues from D groups. Treatment of the mothers with insulin usually resulted in improved T4 and T3 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.

T4 and T3 in the carcass (not shown) of fetuses from D mothers were lower (0.69 ± 0.06 ng T4/g and 0.16 ± 0.01 ng T3/g) than those in fetuses from C dams (1.71 ± 0.09 ng T4/g and 0.33 ± 0.03 ng T3/g; P < 0.001 for both T4 and T3). The total fetal extrathyroid T4 and T3 pools were calculated (24); the T4 pool decreased from 13.08 ng in C to 4.05 ng in D fetuses, and the T3 pool decreased from 1.72 ng in C to 0.63

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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.
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 T₃ level, the T₄ and T₃ 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 T₄ and T₃ 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 T₄ and T₃ 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 T₃, 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 T₄ and T₃ 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 T₄ and T₃ 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 5′D-III in F-brain, either did not correlate with the T₄ and T₃ 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-

Fig. 9. The three upper panels show the concentrations (in nanograms per g) of T₄ in the liver, lung, and brain of the fetuses from the various groups of dams, with the middle panels showing the corresponding T₃ 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 T₄ and T₃ 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
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 T4 and T3 in the M-placenta as those described here for dams treated with STZ during pregnancy; T4 decreased to 16% of C values, and T3 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 non-thyroidal illness are at present considered adaptive responses to a limited availability of intracellular energy, a situation in which a decrease in T3-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 T4 and T3, which would lower the pool of T4 available for extrathyroidal generation of T3, and 2) a decrease in the activity of enzymes involved in the extrathyroidal generation of T3 from T4.

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 hypothalamic TRH (4, 5), secretion of TSH (4, 6, 7), and cerebral T4 was not decreased, and confirming the lack of change described in 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 T3 from T4 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 T4 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, 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 T4 and T3 (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 T4 and T3 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. T4 concentrations decreased more than expected from the changes in circulating total T4 or FT4, in agreement with our previous report (11). The opposite was observed for the concentrations of T3 in the M-placenta, which increased more with insulin treatment than expected from the circulating changes in total T3 and FT3. 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 gestational 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 T4 and T3 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 T4 and T3 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 T4 and T3 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 %FT4 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 T4 (13–16), as a result of which cerebral T3 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 T4 and T3 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. 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 T3 concentrations in the F-brain. The changes in T4 and T3 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 T3 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-hydroxbutyrate 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-hydroxbutyrate and FFA, the lower the intelligence quotient of the offspring. Considering that if the present results are pertinent to man, brain T3 might have been low during a

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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, the present results would indicate that adequate control of the diabetes mellitus appears to be of prime importance to prevent the decrease in cerebral T₃ 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 T₄ 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 T₃ in brain, cerebellum, liver, and pituitary, whereas the T₃ 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 T₃ 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 T₃ homeostasis do not appear to be operative.

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