0013-7227/91/1291-0210$03.00/0
Endocrinology

Vol. 129, No. 1
Printed in U.S.A.

Maternal Hypothyroidism during the First Half of Gestation Compromises Normal Catabolic Adaptations of Late Gestation in the Rat*

BARTOLOME BONET AND EMILIO HERRERA

Departamento de Investigación, Hospital Universitario Ramón y Cajal, Universidad de Alcalá de Henares, 28034 Madrid, Spain

ABSTRACT. Female rats were mated and thyroidectomized on the same day. Some animals were kept without treatment and killed on day 12 or 21 of gestation (T). Others were subsequently treated daily with 1.8 μg L-T_4/100 g BW for either the first 12 days and then not treated from that time until day 21 [T+T_4(I+0)] or else not treated for the first 12 days and then treated from days 12–21 [T+T_4(0+II)]. A final group received treatment during the entire 21-day study [T+T_4(I+II)] and was used as the control. The net maternal body weight increased until day 12 of gestation in T+T_4(I+II) rats, but not in T animals. On day 21 net maternal body weight was significantly lower in T and T+T_4(0+II) than in T+T_4(I+II) rats. Lipoprotein lipase activity in the lumbar fat pads increased from days 0 to 12 of gestation and decreased on day 21, whereas in the heart the change was in the opposite direction, and these changes were greater in T+T_4(I+II) rats than in T rats. Incorporation of [U-^{14}C]glucose administered *in vivo* into liver [^{14}C]fatty acids or [^{14}C]glycogen was significantly lower in T rats than in T+T_4(I+II) on either the 12th or 21st day of gestation. The response of plasma triglyceride, glycerol, or β-hydroxybutyrate levels to 24 h of starvation was similar in 12-day pregnant rats regardless of whether they were treated with T_4, whereas on day 21 the change was greater in T+T_4(I+II) or T+T_4(I+0) animals than in T or T+T_4(0+II) animals. Results show that maternal hypothyroidism during the first half of gestation impaired the anabolic events occurring during this phase and compromised the normal catabolic response during late gestation even when T_4 treatment was restored. However, once maternal metabolic stores were built up normally during the first half of gestation, maternal hypothyroidism during late gestation did not affect the mother's normal metabolic adaptation, including the accelerated response to starvation. (*Endocrinology* **129:** 210–216, 1991)

MATERNAL hypothyroidism is known to affect the development of offspring adversely. Women living in areas of severe iodine deficiency maintain low serum T_4 levels during pregnancy (1), and this maternal hypothyroxinemia plays an important role in the broad spectrum of congenital abnormalities associated with severe iodine deficiency. Perinatal mortality and morbidity increase as maternal plasma T_4 decreases (2). In the rat, maternal hypothyroidism also alters fetal body and brain weights (3) and reduces the number of fetuses (4). It has been suggested that thyroid hormone deficiency in the developing embryo even before the onset of fetal thyroid function (5) contributes to the adverse effects of maternal hypothyroidism on the progeny.

Metabolic adaptations taking place in the mother during gestation may also be modified and contribute to altered fetal development. We have previously shown that thyroidectomized pregnant rats treated with substitutive doses of T_4 from day 12 of gestation, but no earlier, have smaller fetuses on day 21 than those receiving the T_4 treatment between days 0–12, but no later (6). During the first half of gestation, when fetal development is slow, the mother is in an anabolic condition where fat stores are accumulated for use in the later part of gestation when the rate of fetal accretion is maximal (7). The possibility, therefore, exists that maternal hypothyroidism during that first half of gestation could impair the anabolic changes in the mother addressed to building up her fat stores and limit the proper availability of substrates to sustain the normal catabolic events that support the rapid fetal development that takes place during late gestation. This alteration may be especially manifest during the periods of food restriction, when accelerated starvation is known to be an intrinsic condition of normal pregnancy (8). To test that hypothesis, the present study has repeated an experimental design we have previously used to determine TSH and growth parameters in fetuses (6) in which pregnant rats were thyroidectomized on day 0 of gestation and treated with exogenous

Received December 31, 1990.
Address all correspondence and requests for reprints to: Emilio Herrera, Ph.D., Departamento de Investigación, Hospital Ramón y Cajal, Ctra. Colmenar Km 9, 28034 Madrid, Spain.
* This work was supported by grants from the Fondo de Investigaciones Sanitarias de la Seguridad Social (no. 90/0253) and Dirección General de Investigación Científica y Técnica (no. PM88–0050) of Spain.

T_4 for different periods, but here it is the maternal metabolic condition under fed or 24-h fasted conditions at both midgestation (day 12) and late gestation (day 21) that is studied.

Materials and Methods

Animals and experimental design

Virgin female Wistar rats from our own colony, weighing 200–220 g, were housed in a temperature-controlled room (22 ± 1C) with a 12-h on-off light schedule and fed a Purina chow diet (Panlab, Barcelona, Spain). The care and handling of the animals throughout the study followed the current law for Animal Care of the European Common Market (Strasburg March 18, 1986). Animals were mated, and the same day that sperm appeared in vaginal smears (day 0 of gestation) they were surgically thyroidectomized (T) under ether anesthesia, with care taken to spare the parathyroids. The day after thyroidectomy animals were divided into six groups. Four groups received a daily ip injection of 1.8 μg L-T_4/100 g BW for different specified periods of time; the first 11 days of gestation were considered period I, and the second half of gestation (days 12–21) were considered period II. In group T+T_4(I+0), T rats received T_4 treatment for the first period and not for the second and were killed at the end of period II (day 21). Group T+T_4(0+II) received T_4 only during period II and were killed at the end of period II. In group T+T_4(I+II), the animals received T_4 in both periods and were killed at the end of period II. Finally, group T+T_4(I) animals received T_4 only during period I and were killed at the end of period I (day 12). The T_4 dose was chosen on the basis of our previous experience (9, 10) and because we have previously shown that it produced a euthyroid state in pregnant rats (6). Taking this into account, both T+T_4(I+II) and T+T_4(I) were considered as controls for T rats killed at 21 and 12 days, respectively, since we have also previously found that neither body weight nor endocrine parameters in these animals differ from those in intact pregnant animals (6). The two remaining groups of T rats did not receive T_4 treatment; one was killed on day 12 of gestation, and the other on day 21. One additional group of virgin rats was studied in parallel with the pregnant animals for purposes of comparison (V). Rats were always killed 24 h after the last T_4 treatment, and some of them were studied after a 24-h fasting period. After decapitation their blood was collected from the neck wound into dry heparinized tubes. The two uterine horns were immediatly dissected and weighed with their content to obtain the whole conceptus weight, which was subtracted from the mother's whole weight to obtain the net maternal body weight. The heart and lumbar fat pads were excised, immediately placed into liquid N_2, and stored at −80 C until assay for lipoprotein lipase (LPL) activity.

Processing and analysis of the samples

Aliquots of whole blood were deproteinized with $Ba(OH)_2$ and $Zn\ SO_4$ (11), and supernatants were used for glucose (12), β-hydroxybutyrate (13), and glycerol (14) determination. Aliquots of plasma were used for the analysis of triglycerides (15), RIA of insulin (16) was performed using a RIA kit specific for rats generously provided by Novo Industri/AS (Copenhagen, Denmark), and RIA of TSH was performed by a specific rat RIA kindly provided by the Rat Pituitary Hormone Distribution Program of the NIDDK. Plasma T_4 was determined with a highly sensitive and specific RIA (17). Intra- and interassay coefficients of variations for the RIA determinations were 5.1% and 9.3% for insulin, 6.2% and 11.9% for TSH, and 5.0% and 6.8% for T_4, respectively. LPL activity was measured in acetone/diethyl ether extracts in aliquots of the tissues kept in liquid N_2, as previously described (18). To avoid interassay variations, all LPL determinations from the same tissue were run in the same assay. The intraassay coeficient of variation was 5.6%.

Administration of [U-^{14}C]glucose

Some fed animals received 1 μCi [U-^{14}C]glucose (SA, 257 μCi/mmol; Radiochemical Center, Amersham, United Kingdom) dissolved in 250 μl saline 10 min before death via iv administration. Livers were removed and placed in liquid N_2. Aliquots of the liver were used for [^{14}C]glycogen determination (19) and lipid extraction and purification (20). Total lipid extracts were saponified and fractionated to measure ^{14}C-labeled fatty acids, as previously described (21).

Statistical analysis

The mean ± SEM are given. The significance of the difference between the means of two groups was obtained with Student's *t* test, and for more than two groups with Newman-Keuls test (22).

Results

Plasma T_4 and TSH levels

Plasma T_4 and TSH levels were measured as an index of the thyroid condition of the animals at the time of death. As shown in Fig. 1, on day 21 of gestation plasma T_4 levels were significantly lower in T and T+T_4(I+0) than in T+T_4(I+II) rats used as controls, whereas T_4 levels in T+T_4(0+II) did not differ from those in T+T_4(I+II). The oposite changes were found for plasma TSH levels.

Maternal net body weight gain during gestation

As shown in Table 1, the maternal net body weight gain (free of conceptus) during the first 12 days of gestation was higher in T rats treated with 1.8 μg T_4/100 g BW·day [T+T_4(I)] than in those that were untreated (T) or in age-matched virgin females (V). On day 21 of gestation, maternal net body weight gain in T rats receiving treatment for the entire period (I+II) was slightly but not significantly lower than that in V rats. In T animals net body weight was significantly lower than in either V or T+T_4(I+II) rats, and this was also the case when T rats received the T_4 treatment only during the second half of gestation [T+T_4 (0+II); Table 1]. On the contrary, as also shown in Table 1, when T rats received

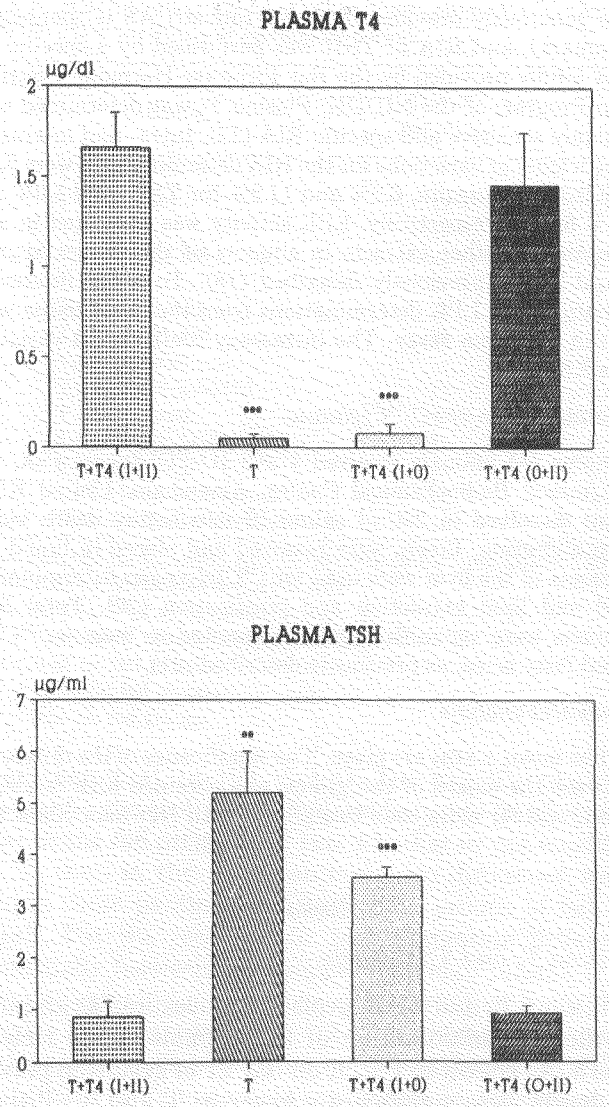

FIG. 1. Plasma concentrations of T_4 and TSH on day 21 in pregnant untreated T rats or rats treated with 1.8 μg T_4/100 g BW for different gestational periods. *Asterisks* correspond to the statistical significance with respect to data from T+T_4(I+II) dams (***, $P < 0.001$). Data are the mean ± SEM of 6–12 rats/group.

T_4 treatment from days 0–12, but not later [T+T_4(I+0)], their net body weight appeared higher than that in either V and T+T_4(I+II) control rats, although the difference was statistically significant only with the latter group ($P < 0.05$).

Plasma glucose and insulin levels

As also shown in Table 1, regardless of whether T_4 treatment was given, the plasma glucose concentration was lower in 12- and 21-day pregnant T rats than in virgin animals. Whereas no difference was found between T or T+T_4(0+II) rats and the T+T_4(I+II) controls, on day 21 the plasma glucose level was significantly lower in T+T_4(I+0) rats than in the T+T_4(I+II) control rats.

TABLE 1. Maternal net body weight increase and plasma glucose and radioimmunossayable insulin levels during gestation in untreated T rats or rats treated with 1.8 μg T_4/100 g BW for different gestational periods

Group	Day of gestation	Net BW increase (g)	Plasma glucose (mg/dl)	Plasma RIA insulin (μU/ml)
V		23.9 ± 2.5	94.0 ± 1.9	65.1 ± 6.0
T+T_4(I)	12	44.5 ± 2.9[a]	82.1 ± 3.0[b]	88.4 ± 7.0[c]
T	12	27.1 ± 4.3[d]	78.9 ± 4.0[b]	53.9 ± 3.0[d]
V		42.6 ± 4.0	93.5 ± 2.8	69.8 ± 5.4
T+T_4(I+II)	21	37.9 ± 4.3	78.1 ± 4.7[c]	49.6 ± 3.1[b]
T	21	21.2 ± 5.5[c,e]	74.5 ± 3.1[b]	49.7 ± 3.6[c]
T+T_4(I+0)	21	52.4 ± 4.8[e]	63.9 ± 2.6[a,e]	45.1 ± 2.9[b]
T+T_4(0+II)	21	15.9 ± 2.6[a,d]	68.8 ± 2.1[a]	44.8 ± 3.6[b]

Age-matched intact virgin rats (V) were used for comparison. Data are the mean ± SEM of 6–12 rats/group. T pregnant rats studied on day 12 of gestation were treated daily with T_4 [T+T_4(I)], and those studied on day 21 were treated from days 0–12 of gestation [T+T_4(I+0)], from days 12–21 [T+T_4(0+II)], or for the entire period [T+T_4(I+II)] or received no T_4.

[a] $P < 0.001$ *vs.* V.
[b] $P < 0.01$ *vs.* V.
[c] $P < 0.05$ *vs.* V.
[d] $P < 0.01$ *vs.* T+T_4(I) or T+T_4(I+II).
[e] $P < 0.05$ *vs.* T+T_4(I) or T+T_4(I+II).

On the contrary, insulin levels (determined by RIA) had increased on day 12 in rats receiving the T_4 treatment for the whole period and returned to below basal levels (day 0) on day 21 of gestation [T+T_4(I+II)], whereas in T animals insulin levels did not change significantly on day 12 of gestation compared to those in V animals, and this value was still stable on day 21. Treatment with T_4 or interruption of treatment during the first or second half of gestation did not modify these differences in plasma insulin levels on the 21st day of gestation in any of the groups compared to those in V rats.

LPL activity in lumbar fat pads and heart

As shown in Fig. 2, on day 12 of gestation in T+T_4(I+II) rats, there was a significant increase in lumbar fat pad LPL activity, which later declined to values significantly below those in virgin animals on day 21 of gestation. This effect was not modified even if the T animals receiving T_4 treatment did not receive T_4 after the 12th day of gestation [T+T_4 (I+0)]. In T rats the increase in adipose tissue LPL on day 12 was slightly smaller than that in T+T_4(I) animals (Fig. 2), whereas on day 21 those values also declined in this latter group, and no significant difference was observed between the different T pregnant animals. The change in LPL activity in the heart is the opposite of what occurs in adipose tissue (Fig. 2), since the activity in the heart declines on day 12 in T control rats receiving T_4 treatment for the entire period and increases on day 21 to the levels found

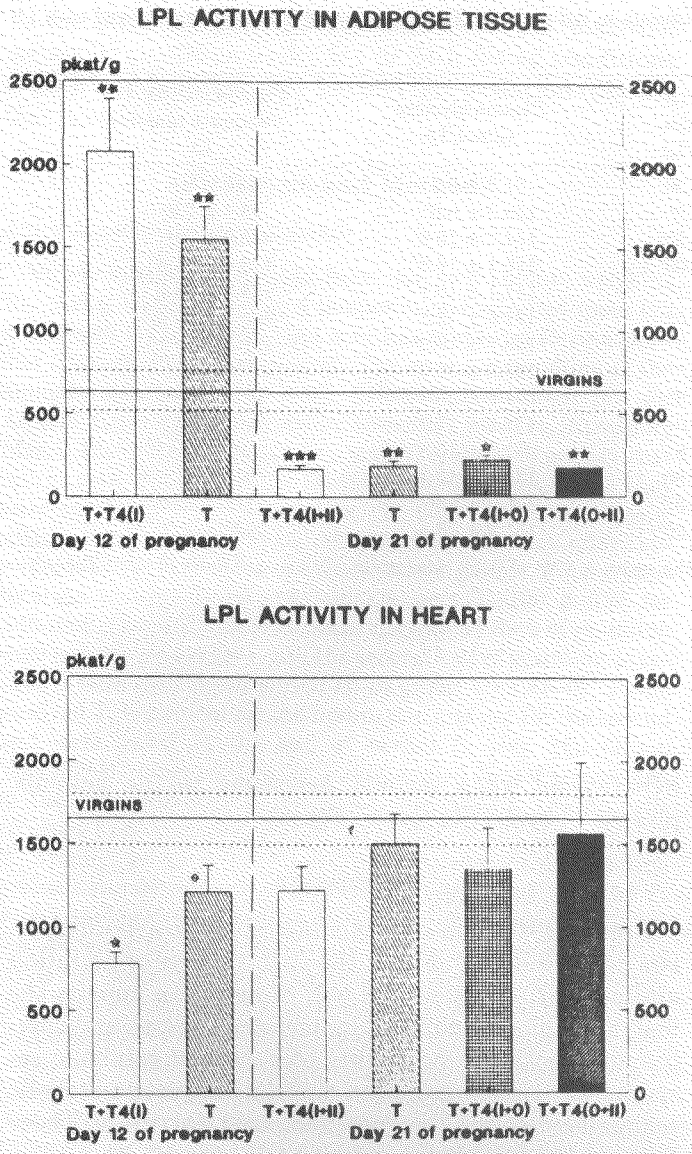

FIG. 2. LPL activity in lumbar fat pads and heart in T pregnant rats treated daily with T_4 (1.8 μg/100 g BW) for different gestational periods. The mean ± SEM values in virgin animals are shown by the *horizontal lines*. Comparisons *vs.* virgins are shown by *stars* (★, $P < 0.05$; ★★, $P < 0.01$; ★★★, $P < 0.001$), and those between treated and untreated groups by crosses (+, $P < 0.05$).

on day 0 of gestation (virgin animals). The drop in heart LPL activity on day 12 of gestation does not appear in T rats, so the difference in the mean values of the two groups at this gestational time is significant, although it disappears on day 21 independently of whether the animals received T_4 treatment from day 12 (Fig. 2).

In vivo utilization of [U-^{14}C]glucose

The *in vivo* conversion of [U-^{14}C]glucose to liver [^{14}C] fatty acids and [^{14}C]glycogen was studied in the T rats receiving or not receiving T_4 treatment, and results are summarized in Fig. 3. The synthesis of both fatty acids

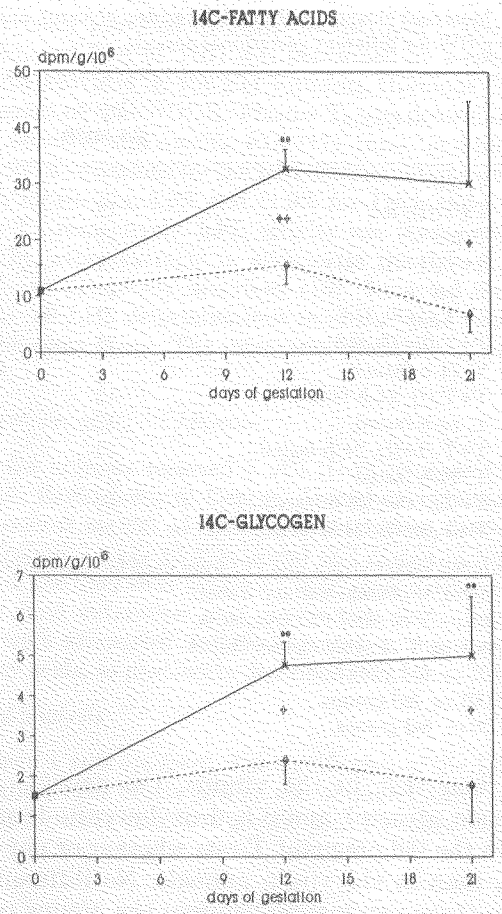

FIG. 3. Appearance of ^{14}C-labeled fatty acids and glycogen in liver 10 min after the iv administration of [U-^{14}C]glucose in T pregnant rats treated daily with T_4 (1.8 μg/100 g BW; X–X) or receiving no T_4 (◇- -◇). *Asterisks* correspond to the statistical significance with respect to 0 days of gestation, and *crosses* to the comparison between T_4-treated and untreated rats.

and glycogen was much lower in T rats than in T+T_4(I+II) control rats; it was already significantly lower on day 12 of gestation and remained so on the 21st day.

Effect of 24-h starvation on the plasma steady state concentrations of different metabolites

When a mother is hypothyroid, reduction of her body metabolic stores may impair her capacity to respond to starvation normally. To test this possibility, the steady state concentrations of plasma metabolites in fed and 24-h fasted animals were studied. Since the response to starvation is normally accelerated during late gestation (8), the group of intact virgin animals was studied in parallel for comparison. Values are summarized in Table 2, where it is shown that on day 12 of gestation fed T rats, whether or not treated with T_4, show lower plasma triglyceride and β-hydroxybutyrate levels than virgin animals, although their glycerol level is significantly

TABLE 2. Maternal plasma level of triglycerides, glycerol, and β-hydroxybutyrate in fed and 24-h fasted untreated T rats or rats treated with 1.8 μg T_4/100 g BW for different gestational periods

Group	Day of gestation	Plasma triglycerides (mmol/L)	Plasma glycerol (μmol/L)	Plasma β-hydroxybutyrate (μmol/L)
V, fed		2.87 ± 0.30	91.3 ± 9.9	87.8 ± 5.6
T+T_4(I), fed	12	1.03 ± 0.2[a]	148.2 ± 8.6[b]	48.7 ± 7.9[c]
T, fed	12	1.72 ± 0.10[a]	134.7 ± 10.2[a]	43.9 ± 9.0[c]
T+T_4(I+II), fed	21	5.72 ± 1.03[c]	156.1 ± 16.3[b]	170.6 ± 31.8[a]
T, fed	21	4.41 ± 0.7[a]	147.1 ± 10.1[b]	124.9 ± 26.8
T+T_4(I+0), fed	21	4.20 ± 0.5[a]	131.6 ± 12.3[a]	144.3 ± 26.0[a]
T+T_4(0+II), fed	21	4.35 ± 0.5[a]	139.1 ± 19.9[a]	130.4 ± 15.1[a]
V, 24-h fasted		2.19 ± 0.2	153.3 ± 16.2[d]	810 ± 98[e]
T+T_4(I), 24-h fasted	12	3.86 ± 0.8[a,f]	204.5 ± 23.3[f]	1066 ± 68[e]
T, 24-h fasted	12	3.32 ± 1.0	177.2 ± 13.2[f]	801 ± 113[e]
T+T_4(I+II), 24-h fasted	21	8.75 ± 0.8[c,f]	221.3 ± 10.8[a,f]	2002 ± 166[c,e]
T, 24-h fasted	21	3.50 ± 0.60	141.0 ± 19.2	918 ± 231[f]
T+T_4(I+0), 24-h fasted	21	7.13 ± 1.35[c,f]	178.0 ± 7.6[f]	2112 ± 233[b,e]
T+T_4(I+II), 24-h fasted	21	7.20 ± 1.20[c,f]	151.7 ± 16.4	1260 ± 223[e]

Age-matched intact virgin rats (V) were used for comparison. Data are the mean ± SEM of 6–12 rats/group. Groups are defined as in Table 1.
[a] $P < 0.05$ *vs.* V of same dietary status.
[b] $P < 0.01$ *vs.* V of same dietary status.
[c] $P < 0.001$ *vs.* V of same dietary status.
[d] $P < 0.01$ *vs.* fed groups.
[e] $P < 0.001$ *vs.* fed groups.
[f] $P < 0.05$ *vs.* fed groups.

higher than that in the virgins. On day 21, plasma triglyceride, glycerol, and β-hydroxybutyrate levels were higher in the fed pregnant T rats whether or not treated with T_4 than in the virgin animals. No difference was found in these parameters between the groups of fed T animals, but the response to 24-h starvation differed substantially between the groups. Although plasma triglyceride levels did not change with starvation in virgin animals, they increased in 12-day pregnant T rats whether treated [T+T_4(I)] or not treated with T_4 (T), although the difference between values in fed and not fed rats was not significant for the latter group. This change was much greater on day 21 of gestation in T+T_4(I+II), T+T_4(I+0), and T+T_4(0+II) rats, whereas the values did not change with starvation in the T animals. Plasma glycerol levels significantly increased with starvation in virgin rats and on day 12 of gestation in pregnant T rats regardless of whether they received T_4 treatment. These levels also increased with starvation on day 21 of gestation in the T+T_4(I+II) or T+T_4(I+0) animals, but they did not change significantly in 21-day pregnant T or T+T_4(0+II) rats. Starvation enhanced β-hydroxybutyrate levels in all groups, but the effect was much greater in the 21-day pregnant T+T_4(I+II) or T+T_4(I+0) rats than in any of the other groups studied (Table 2). It is remarkable that the effect of starvation on plasma β-hydroxybutyrate levels was much lower in the 21-day pregnant T or T+T_4(0+II) rats than in either the T+T_4(I+II) or T+T_4(I+0) groups despite the fact that the latter group, but not the T+T_4(0+II) group, was hypothyroid when studied, according to the animals' plasma T_4 and TSH levels (Fig. 1).

Discussion

Present results in rats demonstrate that hypothyroidism occurring only during the first 12 days of gestation and not during the second half of gestation impairs the maternal capacity to build up metabolic stores and compromises both the possibility of a normal catabolic response during late gestation and the adequate metabolic realignments necessary for accelerated starvation. These findings agree with the reduced fetal growth previously found by us in rats subjected to similar experimental conditions of hypothyroidism circumscribed to the first half of gestation (6).

A maternal hypothyroid condition is known to reduce the availability of thyroid hormones to the fetus (5, 23), and this effect could contribute to the reduced fetal growth in the T rats not receiving T_4 treatment for the whole gestational period (6). However, the mother's limited capacity to manifest normal metabolic adaptations during late gestation, rather than a deficiency of thyroid hormones, seems to be the major factor limiting normal fetal growth in those T rats receiving substitutive T_4 treatment from just the 12th day of gestation (6). This possibility is further sustained by the normal plasma TSH levels previously found in growth-retarded fetuses of T+T_4(0+II) mothers (6), which indicate that these fetuses are not thyroid deficient.

From previous studies we know that most of the increase in net maternal body weight during gestation corresponds to fat stores (7, 24). Here we show that this net maternal body weight increase is impaired on day 12 of gestation when the mother is hypothyroid up to that day, and the effect cannot be corrected even though animals are treated with T_4 from that time on. Increased maternal body weight gain during the second half of gestation in rats in which hypothyroidism is circumscribed to that gestational phase rather than to the first half indicates that the catabolic changes normally occurring during late gestation are decreased, thereby allowing maternal fat stores to be preserved. These data demonstrate the key role of the anabolic condition normally occurring in the mother during the first half of gestation, when fetal development is minimal, for ensuring both the normal catabolic changes that occur during late gestation and the proper availability of substrates to sustain normal fetal growth.

At midgestation, LPL activity decreases in the heart and increases in adipose tissue, as was seen in our control animals [$T+T_4(I+II)$]. These changes permit circulating triglycerides to be driven to and taken up by adipose tissue and, together with enhanced lipogenesis, probably contribute to the fat accumulation normally seen in the mother at this stage of gestation (7). We have seen here that some of these changes did not occur and others were milder in pregnant rats that were kept hypothyroid during the first half of gestation compared to those receiving T_4 treatment for the whole period. This together with their decreased liver glycogen synthesis indicates that the anabolic manifestations seen during the first half of gestation in control animals are greatly impaired in our hypothyroid rats.

The mechanism through which these negative effects are produced can not be justified by low thyroid hormone levels alone, since other endocrine disorders are known to accompany the hypothyroid condition. We have seen here that on day 12 of gestation plasma insulin levels in hypothyroid pregnant rats are much lower than those in euthyroid animals. Insulin is known to both positively modulate adipose tissue LPL activity and have a negative effect on the same enzyme in the heart (25, 26). This justifies not only the slightly decreased LPL activity found in the lumbar fat pads on day 12 of gestation in the hypothyroid rats compared to the euthyroid ones, but also the unchanged LPL activity in their heart, in contrast to the reduced activity found in the euthyroid animals.

Accelerated starvation is a normal feature during late gestation in both women (27) and rats (28). This change facilitates proper substrate availabiliy to the fetus under conditions of food deprivation. As an index of this, we have seen here that circulating triglycerides, glycerol, and β-hydroxybutyrate increase more in thyroidectomized pregnant rats receiving substitutive T_4 treatment for the entire period than in virgin control animals, although this response appeared impaired in pregnant rats that were kept permanently hypothyroid. This reduced response to starvation could be the result of either the thyroid hormone deficiency in itself or a secondary consequence of the reduction in available resources for mobilization. The starvation response was impaired during late gestation in pregnant rats that were hypothyroid during the first half of gestation but euthyroid during the second half [$T+T_4(0+I)$] and normal in those that were under the opposite experimental condition (euthyroid during the first half and hypothyroid during the second half of gestation, when the animals were studied [$T+T_4(I+0)$]. This indicates that an insufficient availability of metabolic resources, rather than the thyroid hormone deficiency itself, is the major factor that reduces the maternal capacity to respond to the fasting stimulus.

Since the metabolic response to starvation in 12-day pregnant rats appeared similar in hypo- and euthyroid animals and did not differ from that in virgin rats, the impaired response in certain groups during late gestation indicates that this effect is circumscribed to the accelerated starvation that occurs at this specific gestational period, when the rate of fetal accretion is maximal.

The present findings together with earlier ones (6) emphasize the importance of the anabolic changes occurring in the mother during early gestation in supporting the metabolic adaptations that take place during late gestation, which are mainly addressed to assuring the proper availability of substrates to the fetus even during periods of food deprivation. The results also show that maternal hypothyroidism during the first stages of gestation has negative effects on maternal structures, which last until late gestation even when substitutive thyroid hormone therapy is established. In addition to the potential direct negative effects on embryonal development of thyroid hormone deficiency during the first gestational stages, it is proposed that the effect of reduced maternal fuel stores may contribute to the pathogenic manifestations in the offspring of hypothyroid mothers.

Acknowledgment

The authors thank Ms. Carol F. Warren from the ICE of Alcalá de Henares University for her editorial assistance.

References

1. Choufoer M, van Rhijn M, Querido A 1965 Endemic goiter in Western New Guinea. II. Clinical picture, incidence and pathogenesis of endemic cretinism. J Clin Endocrinol Metab 25:385–402
2. Pharoah POD, Ellis SM, Ekins RP, Williams ES 1975 Maternal thyroid function, iodine deficiency and fetal development. Clin Endocrinol (Oxf) 5:159–166
3. Morreale de Escobar G, Pastor R, Obregon MJ, Escobar del Rey F

1985 Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues before and after onset of fetal thyroid function. Endocrinology 117:1890–1990
4. Porterfield SP, Hendrich CE 1975 The effects of maternal hypothyroidism on maternal and fetal tissue glucose 1-^{14}C incorporation in rats. Horm Res 6:236–246
5. Escobar del Rey F, Pastor R, Mallol J, Morreale de Escobar G 1986 Effects of maternal iodine deficiency on the L-thyroxine and 3,5,3′-triiodo-L-thyronine contents of rat embryonic tissues before and after onset of fetal thyroid function. Endocrinology 118:1259–1265
6. Bonet B, Herrera E 1988 Different response to maternal hypothyroidism during the first and second half of gestation in the rat. Endocrinology 122:450–455
7. Herrera E, Lasunción MA, Gómez-Coronado D, Aranda P, López-Luna P, Maier I 1988 Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am J Obstet Gynecol 158:1575–1583
8. Freinkel N 1965 Effects of the conceptus on maternal metabolism during pregnancy. In: Leibel SS, Wrenshall GA (eds) On the Nature and Treatment of Diabetes. Excerpta Medica, Amsterdam, pp 679–691
9. Llobera M, Seibel MJ, Herrera E 1978 Metabolic response to short periods of starvation in hypo- and hyperthyroid rats. Horm Metab Res 10:319–324
10. Seibel MJ, Llobera M, Herrera E 1978 Effects of glucose, insulin and adrenalin on glycerol metabolism in adipose tissue of hypothyroid rats. Mol Cell Endocrinol 10:307–318
11. Somogyi M 1954 Determination of blood sugar. J Biol Chem 160:69–73
12. Huggett AS, Nixon DA 1957 Use of glucose oxidase peroxidase, and *O*-dianisidine in determination of blood and urinary glucose. Lancet 2:368–379
13. Williamson DH, Mellanby T, Krebs HA 1962 A enzymatic determination of D-β-hydroxybutiric acid and acetonic acid in blood. Biochem J 82:90–96
14. Garland PB, Randle PJ 1962 A rapid assay for glycerol. Nature 196:987–988
15. Ramirez JI, Llobera M, Herrera E 1983 Method for triglyceride measurement in small amounts of plasma. Rev Esp Fisiol 39:327–332
16. Heding LG 1972 Determination of total serum insulin (IRI) in insulin-treated diabetic patients. Diabetologia 8:260–266
17. Obregon MJ, Pascual A, Morreale de Escobar G, Escobar del Rey F 1979 Pituitary and plasma thyrotropin, thyroxine and triiodothyronine after hyperthyroidism. Endocrinology 104:1467–1473
18. Llobera M, Montes A, Herrera E 1979 Lipoprotein lipase activity in liver of the rat fetus. Biochem Biophys Res Commun 91:272–277
19. Soley M, Chieri R, Herrera E 1983 Liver glucose, glycogen and lipid synthesis in fed and 24-hour fasted rats soon after a pulse of (3-^{14}C)pyruvate through the portal vein. Int J Biochem 15:45–49
20. Folch JM, Lees GH, Sloane-Stanley GH 1957 A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
21. Carmaniu S, Herrera E 1980 Comparative utilization *in vivo* of (U-^{14}C)glucose and (1-^{14}C)palmitate in the rat. Arch Intern Physiol Biochim 88:255–263
22. Snedecor GW 1956 Statistical Methods, ed 5. Iowa State University Press, Ames
23. Morreale de Escobar G, Calvo R, Obregon MJ, Escobar del Rey F 1990 Contribution of maternal thyroxine to fetal thyroxine pools in normal rats near term. Endocrinology 126:2765–2767
24. López-Luna P, Munõz T, Herrera E 1986 Body fat in pregnant rats at mid- and late gestation. Life Sci 39:1389–1393
25. Robinson D, Speake B 1989 Role of insulin and other hormones in the control of lipoprotein lipase activities. Biochem Soc Trans 17:40–42
26. Semenkovich CF, Wims M, Noe L, Etienne J, Chen L 1989 Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at posttranscriptional and posttranslational levels. J Biol Chem 264:9030–9038
27. Freinkel N 1980 Of pregnancy and progeny. Diabetes 29:1023–1035
28. Herrera E, Lasunción MA, Palacin M, Zorzano A, Intermediary metabolism in pregnancy: the first theme of the Freinkel era. Diabetes, in press