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Hypoglycemia and Glycogen Deficits in Fetuses of Hypothyroid Pregnant Rats (38891)

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(Introduced by Robert C. Little)

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Induction of maternal hypothyroidism has been reported to increase fetal mortality (1–5). The severity of this fetal effect and the degree of hypothyroidism appear to be dependent upon the procedure used to induce hypothyroidism. The thiouracils easily cross the placenta and impair fetal as well as maternal thyroid function (4, 6). Maternal thyroid irradiation with I^{131} can affect ovarian function (7) and presumably fetal development. Surgical thyroidectomy can interfere with parathyroid function and previous studies in which this technique has been utilized have in general not included parathormone replacement.

Severe thyroid dysfunction in the female blocks reproductive function (8) but of possibly greater concern is that degree of hypothyroidism which permits gestation to proceed to term. Hendrich and Galton have shown that hypothyroid rats have fewer and smaller fetuses (unpublished). Man and Jones have reported that the children of hypothyroid mothers show significantly impaired motor and mental performance at ages 8 mo and 4 yr (8, 9).

Carbohydrates are thought to represent the major fetal metabolic substrate in rats and humans (10–12) and the fetal R.Q. is thought to approximate 1.0 (10, 12). Consequently, alterations in fetal or placental carbohydrate metabolism could have obvious effects on development. Thyroid hormones are known to alter the metabolism of carbohydrates, proteins, and lipids in the nonpregnant adult and hence most likely also in the maternal system. The action of thyroid hormones on the maternal system has not been studied in detail. During gestation, the maternal and fetal metabolisms are intimately related either directly or indirectly (11). During the last few days of gestation, the level of fetal liver glycogen rises (13–15). Liver glycogen storage is not thought to be important in serum glucose homeostasis prior to birth but rather in preventing severe neonatal hypoglycemia (12), which can result in mental impairment and even death (12). These studies were designed to assess the effects of maternal thyroid deficiency on fetal development and to see if maternal hypothyroidism alters the supply of glucose to the fetus or the mechanisms whereby the fetus stores glycogen.

Materials and Methods. Virgin female Sprague–Dawley–Holtzman rats (180 g) were mated and the first day of pregnancy was considered to be the day spermatozoa were detected in the vaginal tract. A total of 124 animals were used in these experiments. On the first day of pregnancy, the animals were either surgically thyroparathyroidectomized (TPTX), sham operated, or not treated. Light ether anesthesia was used for the surgery. Thyroidectomy was performed after breeding because preliminary studies showed that the surgery per se did not alter the results whether it was performed prior to or just after breeding and after thyroidectomy cyclicity is altered (16, 17). No differences could be seen between untreated controls and sham-operated controls and, therefore, the two groups were combined for all statistics. All thyroidectomized animals were treated with 5 IU of parathormone/day plus 1% calcium lactate drinking water. Half of the thyroidectomized animals were created with 1.5 μg of L-thyroxine (T_4)/100 g body wt/day. This group served as parathyroidectomized controls to check for effects due to calcium imbalance. The animals were maintained on 12-hr light–dark cycles and at 75° during gestation and fed Purina Laboratory Chow and water ad lib. The animals were autopsied between 8:00 AM and 11:00 AM on the 22nd day of gestation.

At the time of autopsy, the animals were lightly anesthetized with ether and maternal blood was drawn by venal caval puncture and centrifuged at 5° to separate the serum. The liver was removed, weighed, and 1-g aliquots added to hot 30% KOH for digestion. The fetuses were counted, removed, weighed, bled by decapitation, and the livers removed and weighed. Due to the limited quantity, fetal serum within a litter was pooled and henceforth treated as one sample. Skeletal muscle was removed from the abdomen and back of the mothers and fetuses, weighed and digested in KOH for glycogen analyses. The fetal serum and liver samples were treated in the same manner as the maternal tissues. The placentas were removed, weighed, and aliquots taken for glycogen determinations.

Serum glucose levels were quantitated using Glytel ortho-toluidine reagent (Pfizer, Reproducibility within 2%). Glycogen determinations were done using a modification of the method of Good, Somogyi, and Kramer (18). The tissues were digested in 30% KOH in a boiling water bath for 1 hr and the glycogen was precipitated by adding 95% ethanol and allowing the samples to sit overnight. The samples were then centrifuged for 10 min at 2500 rpm and the supernatant decanted. The glycogen was then washed three times with ethanol, hydrolyzed with 1 *M* H_2SO_4, diluted with distilled water, and aliquots analyzed for glucose using Glytel reagent. The weights for glycogen are expressed in terms of milligrams of glucose. Serum calcium levels were measured by atomic absorption spectrophotometry and serum T_4's were quantitated using Harleco T_4 columns. Due to the limited quantity of fetal serum, single determinations were used for all serum measurements.

Results. The parathormone replacement and calcium lactate drinking water appeared to adequately compensate for any concomitant parathyroid damage as the mothers and fetuses maintained normal serum calcium levels regardless of the experimental treatments; and, for all parameters studied, TPTX + T_4 animals were statistically the same as the sham controls (Table I).

The weight gain of the hypothyroid animals was significantly less than normal (Table II). Earlier studies in which food consumption was measured in thyroidectomized, thyroxine-treated, and control animals have shown a significantly decreased consumption by hypothyroid rats. While the normal food-restricted animals showed slightly lower mean fetal weights, the fetuses of the hypothyroid mothers were much smaller than their pair-fed controls. It was concluded that the decreased fetal weight was only partially due to the voluntarily decreased food consumption of the hypothyroid animals. The hypothyroid mothers voluntarily decreased their food consumption 16%. Food restriction of 50% during the last 6 days of gestation has been shown to produce no significant changes in fetal or

TABLE I. SERUM CALCIUM AND THYROXINE LEVELS.

	Controls		Hypothyroid	
Index	I Sham $\bar{X} \pm$ SE $n = 20$	II TPTX + T_4 + PTH $\bar{X} \pm$ SE $n = 18$	III TPTX + PTH $\bar{X} \pm$ SE $n = 18$	Statistics[a]
Maternal serum Ca^{2+} (mg/100 ml)	11.3 ± .25	10.8 ± .43	10.6 ± .45	No significant differences
Fetal serum Ca^{2+} (mg/100 ml)	9.9 ± .52	9.8 ± .27	10.0 ± .96	No significant differences
Maternal total T_4 (μg/100 ml)	2.88 ± .23	3.07 ± .33	.23 ± .08	I, II > III $P < 0.01$
Fetal total T_4 (μg/100 ml)	2.80 ± .12	3.15 ± .38	2.81 ± .76	No significant differences

[a] Tukey's Honest Significant Difference.

TABLE II. EFFECT OF HYPOTHYROIDISM ON MATERNAL AND FETAL BODY WEIGHTS AND LITTER SIZE.

Index	Controls I Sham $\bar{X} \pm$ SE $n = 42$	Controls II TPTX + T_4 + PTH $\bar{X} \pm$ SE $n = 40$	Hypothyroid III TPTX, PTH $\bar{X} \pm$ SE $n = 42$	Statistics
Body weight gain (g)	150 ± 20	138 ± 23	98 ± 17	I, II > III $P < 0.01$
Average fetal weight (g)	5.72 ± .05	5.54 ± .10	4.52 ± .08	I, II > III $P < 0.01$
Number of live fetuses at day 22	11.24 ± .23	11.40 ± .36	9.60 ± .31	I, II > III $P < 0.01$
Number of resorptions	.35 ± .10	.48 ± .12	1.34 ± .20	III > I, II $P < 0.01$

TABLE III. EFFECT OF MATERNAL HYPOTHYROIDISM ON MATERNAL AND FETAL TISSUE WEIGHTS.

Index	Controls I Sham $\bar{X} \pm$ SE $n = 38$	Controls II TPTX ± PTH $\bar{X} \pm$ SE $n = 30$	Hypothyroid III TPTX ± PTH $\bar{X} \pm$ SE $n = 28$	Statistics
Maternal liver weight (g)	12.3 ± .2	12.0 ± .3	9.5 ± .2	I, II > III $P < 0.01$
Maternal liver weight/ body wt	.0307 ± .0010	.0326 ± .0007	.0280 ± .0004 (28)	I, II > III $P < 0.05$
Fetal liver weight (g)	.38 ± .004[a]	.38 ± .005[b]	.28 ± .005[c]	I, II > III $P < 0.01$
Fetal liver wt / Body weight	.0658 ± .0011	.0659 ± .0055	.0628 ± .0016	No significant differences
Average placental weight (g)	.59 ± .05	.57 ± .02	.49 ± .01	I, II > III $P < 0.01$

[a] $n = 114$ (three fetuses per mother).
[b] $n = 90$ (three fetuses per mother).
[c] $n = 84$ (three fetuses per mother).

placental weights (19) but a comparable food restriction during the entire course of pregnancy has been shown to result in permanent growth stunting of the progeny (20).

The livers of the hypothyroid mothers were smaller than normal even when expressed as a function of body weight (Table III). However, the smaller fetal livers in this group were proportional to the decreased fetal body weight.

There were no significant differences in maternal serum glucose concentrations (Table IV). Even though the maternal serum glucose concentrations in the hypothyroid rats were normal, fetal glucose levels in this group were significantly depressed. A very consistent glucose gradient from mother to fetus could be seen in almost all animals regardless of their treatment. At the 22nd day of gestation, the hypothyroid animals had significantly lower liver glycogen concentrations than either of the control groups (Table IV). Skeletal muscle glycogen concentration was also low being 26% below normal in the hypothyroid animals (Table IV). The fetuses of the hypothyroid mothers had significantly lower liver and muscle glycogen concentrations.

When total liver glycogen is analyzed, the difference between the experimental group and the controls is magnified due to the small livers of the hypothyroid animals.

TABLE IV. EFFECT OF HYPOTHYROIDISM ON MATERNAL TISSUE CARBOHYDRATE LEVELS.

Index	Controls I Sham $\bar{X} \pm$ SE n =38	Controls II TPTX, T_4+ PTH $\bar{X} \pm$ SE n = 30	Hypothyroid III TPTX, PTH $\bar{X} \pm$ SE n = 28	Statistics
Maternal serum glucose (mg/100 ml)	114 ± 2	114 ± 4	107 ± 3	No significant differences
Maternal liver glycogen concentration (mg/g)	22.3 ± 1.5	23.0 ± 1.9	14.7 ± 1.3	I, II > III $P < 0.01$
Maternal liver total glycogen (mg)	286 ± 18	278 ± 25	127 ± 16	I, II > III $P < 0.01$
Maternal liver total glycogen/maternal body weight (mg/g)	.730 ± .047	.798 ± .076	.373 ± .046	I, II > III $P < 0.01$
Maternal muscle glycogen concentration (mg/g)	4.04 ± 0.27	4.20 ± 0.32	3.00 ± 0.28	I, II > III $P<0.01$

TABLE V. EFFECT OF MATERNAL HYPOTHYROIDISM ON FETAL SERUM GLUCOSE, AND FETAL AND PLACENTAL GLYCOGEN LEVELS

Index	Controls I Sham $\bar{X} \pm$ SE n = 38	Controls II TPTX, T_4, PTH $\bar{X} \pm$ SE n = 30	Hypothyroid $\bar{X} \pm$ SE n = 28	Statistics
Fetal serum glucose (mg/100 ml)	93 ± 3	87 ± 3	72 ± 3	I, II > III $P < 0.01$
Fetal liver glycogen concentration (mg/g)	74 ± 3	75 ± 3	43 ± 3	I, II > III $P < 0.01$
Fetal liver total glycogen (g)	30.9 ± 1.7	28.9 ± 1.5	11.9 ± 0.7	I, II > III $P < 0.01$
Fetal liver total glycogen fetal body weight (mg/g)	5.33 ± .27	5.15 ± .28	2.63 ± .15	I, II > III $P < 0.01$
Fetal muscle glycogen concentration (mg/g)	14.19 ± 0.99	14.92 ± 1.09	8.98 ± .66	I, II > III $P < 0.01$
Placental glycogen concentration (mg/g)	1.86 ± .11	2.17 ± .15	2.29 ± .17	No significant differences
Placental total glycogen (mg)	1.07 ± .08	1.02 ± .11	1.20 ± .11	No significant differences

Even when total liver glycogen is expressed in terms of total body weight, the hypothyroid mothers show depleted glycogen storage. The fetuses of the hypothyroid mothers show markedly reduced total liver glycogen levels and when expressed on the basis of total body weight these fetuses have 51% less liver glycogen per gram of body weight.

Neither the placental glycogen concentration nor total glycogen were altered by hypothyroidism (Table V).

Discussion. We have found that maternal thyroidectomy on day 1 of pregnancy prevents the normal late gestation accumulation of glycogen in the liver and skeletal muscle of the fetal rat. The serum glucose levels of these fetuses are also depressed significantly. As glucose appears to be the major metabolic substrate for the fetus (12), these observations could represent a primary cause for the reduced fetal birth weight and the increased numbers of fetal resorptions. The

problem could be inadequate or inappropriate supply of nutrients to the fetus or abnormal or delayed placental and/or fetal enzyme maturation. These effects could have occurred as a direct result of disruptions of the thyroid system or as an indirect consequence of changes in the secretion or action of other hormones altered in hypothyroidism.

In the latter stages of gestation, the normal pregnant rat shows mobilization of the carbohydrate reserves accumulated during early gestation (21). The carbohydrate mobilization serves to supply the ever-increasing carbohydrate demands of the rapidly growing fetuses. The hypothyroid nonpregnant rat has been shown to be able to maintain normal or even high glycogen stores (22). However, the hypothyroid pregnant rat has significantly depressed glycogen reserves. Even though these reserves are low, she appears capable of adequate glucose mobilization and can maintain a normal serum glucose. The supply of glucose to the fetus does not appear normal. Glucose is thought to cross the placenta by facilitated diffusion (23) and a consistent maternal-to-fetal gradient was seen in almost all animals studied in this experiment. This observation is in accordance with most of the data published in both rats and humans (23, 24). However, even though maternal serum glucose was normal in the hypothyroid animals, fetal serum glucose was very low. The placentas of these animals were smaller than normal, and it appears likely that maternal thyroid hormones have a direct action on placental development and function. Placental glycogen storage was not altered in the hypothyroid animals but placental glycogen is relatively stable with respect to hormonal intervention. Hormones such as insulin and epinephrine which readily alter liver glycogen, do not change placental glycogen in normal animals (25, 26). The function of placental glycogen late in gestation is unknown and the levels appear relatively free from control by maternal or fetal intervention (25, 26).

The fetuses of the hypothyroid mothers had severely compromised glycogen stores. This may be merely a result of inadequate placental transport or it may be a result of changes in fetal glucose metabolism. Because of the association of thyroid hormones with maturation (27, 28) and also with the developmental formation of some enzymes in the fetal rat liver (29) there is always the possibility that the problem with the fetal liver glycogen storage is one of delayed maturation. However, the fetuses of the hypothyroid mothers had normal thyroxine levels. Therefore, if delayed maturation of enzyme systems did indeed occur, it would have to be the result of either altered substrate availability or altered nonthyroidal hormones. The inability of the fetuses of the hypothyroid mothers to accumulate glycogen in the liver and skeletal muscle not only is symptomatic of a present metabolic problem which could be the basis of the high number of fetal resorptions occurring but could also represent in itself a serious problem in neonatal life. Fetal liver glycogen is not thought to be important in fetal serum glucose homeostasis (25) but rather is stored to be used as a glucose reservoir during the early neonatal period when the transplacental glucose supply is lost (12). This glycogen represents the principal metabolic substrate during early neonatal life and is rapidly depleted then (12). It is thought that inadequate fetal glycogen storage can result in severe neonatal hypoglycemia which can result in brain damage or death (12). There are currently no data available that quantitate the relationship between fetal liver glycogen and neonatal hypoglycemia and the relationship is obviously a complex one. However, in induced intrauterine growth retardation (IUGR) in rats, liver glycogen is depressed (30, 31). One of the problems shown to occur in IUGR in humans is neonatal hypoglycemia (30) which is thought to be due in part to insufficient tissue glycogen as this is the major source of blood glucose immediately after birth (12). Raising fetal carbohydrate reserves by infusing the mother with excess glucose prior to delivery has been shown to improve statistically the Apgar scores in humans (32) further suggesting the importance of fetal carbohydrate reserves in late prenatal and early postnatal life. Furthermore, recent evidence has been obtained by Schulman *et al.* (33) that acute

fetomaternal hypoglycemia (levels not given) in sheep impairs net uptake of multiple amino acids by the fetal brain.

The fetuses of the hypothyroid mothers have normal thyroid hormone levels and, therefore, the changes seen in the fetal system are not a result of fetal hypothyroidism. The actions of the maternal thyroid hormones on metabolism and placental development and, therefore, the provision of nutrients to the fetus may well be direct ones. However, at this time one cannot discount the possibility that these effects are mediated indirectly by way of other maternal hormones whose secretion is altered by hypothyroidism. In fact, the secretion of insulin and growth hormone has been shown to be imparied in hypothyroidism (22, 34). These hormones have been shown also to act on the placenta (35, 36). Further studies are needed to clarify the role played by other hormones which could interact with thyroid hormones to produce these actions.

Summary. Maternal hypothyroidism, when induced by surgical thyroidectomy with parathyroid hormone replacement, results in fewer live fetuses and smaller fetuses at the 22nd day of gestation. The hypothyroid mother shows the ability to mobilize adequate amounts of glucose even at the expense of her own reserves but the supply of glucose to the fetus appears to be impaired. These fetuses have subnormal skeletal muscle and liver glycogen and are severely hypoglycemic. The impaired development of these fetuses may result from alterations of either transplacental carbohydrate transport or placentofetal carbohydrate metabolism.

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