REVISTA ESPAÑOLA DE FISIOLOGIA, 41, 311-316. 1985

Effect of the Hypothyroidism on the Levels of Different Metabolites in Pregnant Rats

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(Received on September 10, 1984)

P. LOPEZ-LUNA and S. MORALES. *Effect of the Hypothyroidism on the Levels of Different Metabolites in Pregnant Rats.* Rev. esp. Fisiol., 41, 311-316. 1985.

The effect of gestational hypothyroidism on some maternal and foetal metabolites were studied on pregnant rats after 24 h fasting. Thyroidectomy induced a decrease in body weight in the pregnant rats. Their foetuses showed lower weights than the controls. No difference was found in circulating glucose levels in the thyroidectomized pregnant rats, although an increase was found in their foetuses. Hepatic glycogen and total serum lipids decrease in hypothyroid pregnant rats. Cholesterol concentration increases as a result of hypothyroidism. However, foetuses coming from thyroidectomized mothers show an increase in their total lipid levels. Hypothyroidism in pregnant rats affects foetal development and metabolic changes are greatly manifested in the starved condition.

Key words: Hypothyroidism, Pregnant, Rats.

During pregnancy, profound metabolic and hormonal changes occur in order to adapt maternal tissues to foetal growth (20).

The influence of maternal thyroid hormones on the foetus has not been clearly established (3) although it is proved that the concentration of circulating foetal thyroid hormones is independent of maternal concentrations in plasma, since placental transfer of thyroid hormones is minimal (8, 24, 28).

The metabolic turnover is known to be altered in hypothyroidism (11), but in the fed status an equilibrium can be established as a consequence of parallel changes taking place in anabolic and catabolic pathways (1).

The influence of maternal hypothyroidism upon the foetus could be mainly due to changes in the metabolic substrates reaching the foetus (16).

Any changes in metabolic parameters during pregnancy are enhanced in the fasting condition (10) and there exists the possibility that maternal hypothyroidism could modify such a response to food withdrawal. To study this possibility, in the present work we have determined the influence of gestational hypothyroidism on some maternal and foetal metabolites after 24 h fasting.

Materials and Methods

Female Wistar rats weighing 190-200 g were used. They were mated and day 0 of gestation was considered to be the day that spermatozoids were detected in vaginal smears. On the first day of gestation the animals were either surgically thyroidectomized following the technique by ZARROW (36) or sham operated, the latter being considered as controls.

Non pregnant rats of similar age were subjected to the same surgical procedures.

Body weight of the animals in each group was controlled from the first day until the moment they were sacrificed. All animals were killed by guillotine after 24 h starvation, precisely on day 21 of gestation in pregnant rats.

Blood was collected into ice-cold tubes, and after centrifugation in the cold, serum aliquots were kept frozen at —20°C until use. Liver portions were immediately removed after decapitation and kept at —20°C for posterior determination of glycogen.

Foetuses were removed from the uterus, weighed and bled by decapitation, blood being collected into capillary tubes and centrifuged at 10,000 r.p.m. Sera from each litter were pooled and aliquots were used for analyses.

Serum determinations were accomplished according to the techniques proposed by the following authors: TRINDER (32) for glucose, SCHOENHEINER (29) for cholesterol and ZOLLNER and KIRSCH (37) for total lipids.

Liver samples were digested with KOH and glycogen purified by ethanol precipitation according to GOOD *et al.* (12). Glucose was measured after acid hydrolysis (32).

Statistical comparisons between the groups were performed according to Student's «t» test.

Results

Body Weights. In pregnant rats thyroidectomized at day 1 of gestation a decrease in body weight gain could be observed during pregnancy (43.98±1.56) relative to the controls (56.97 ± 3.35) ($p < 0.001$). This decrease in body weight gain was observed in the thyroidectomized virgin animals as well (9.40±2.08) which is significative compared to controls (21.35 ± 3.79) ($p < 0.02$).

At day 21 of gestation foetal weight was lower in the hypothyroid mothers (2.36 ± 0.04 g) than in the controls (3.31 ± 0.04 g) ($p < 0.001$) The size of the litter was not affected by maternal hypothyroidism.

Circulating Glucose Levels. Serum glucose levels were lower in thyroidectomized virgin rats than in their controls. In pregnant animals serum glucose was lower than in virgins but no significant difference was found between thyroidectomized versus control mothers. Foetuses from thyroidectomized mothers showed higher serum glucose levels than those from controls (Table I).

Liver Glycogen. Hypothyroid animals either virgin or pregnant presented low hepatic glycogen concentrations as compared with controls. When values from virgin and non thyroidectomized pregnant animals were compared it was observed that during gestation the storage of hepatic glycogen was increased ($p < 0.02$) (Table I).

Circulation Levels of Total Lipids and Cholesterol. Hypothyroidism did not alter serum total lipid levels in virgin rats but cholesterol levels were significantly increased. On day 21 of gestation serum lipids appeared much higher than the levels found in virgin rats. In contrast to virgin rats, in pregnant rats, hypothyroidism decreased serum total lipid concen-

Table I. *Effects of hypothyroidism in the pregnant rat.*

Rats were thyroidectomized on the day after mating and were killed on day 21 of gestation after 24 h starvation. Sham-operated animals were used as controls. The results are means ± S.E.M. (n = 8-10). Statistical comparison between thyroidectomized and their controls are denoted by the P values, and comparison between thyroidectomized virgin and thyroidectomized mothers are shown by asterisks: * $p < 0.001$; absence of asterisks represents no significant difference (N.S.).

	Glucose (mg/100 ml)	p	Liver glycogen (mg/100 ml)	p	Total lipids (mg/100 ml)	p	Cholesterol (mg/ml)	p
Virgin								
Control	135.12 ± 5.24	< 0.05	0.25 ± 0.007	< 0.001	338.06 ± 29.52	N.S.	0.64 ± 0.02	< 0.001
Thyroidectomized	116.09 ± 6.34		0.06 ± 0.002		290.00 ± 15.98		0.77 ± 0.02	
Pregnant								
Control	90.50 ± 1.66	N.S.	0.30 ± 0.025	< 0.001	1120.36 ± 78.88	< 0.05	0.59 ± 0.02	< 0.001
Thyroidectomized	86.60 ± 3.16*		0.06 ± 0.001		813.36 ± 127.45*		0.78 ± 0.01	
Foetuses								
From controls	25.42 ± 1.37	< 0.001	Not determined		360.48 ± 7.18	< 0.01	0.76 ± 0.03	N.S.
From thyroidectomized	43.41 ± 2.80				411.07 ± 10.70		0.70 ± 0.03	

tration whereas there was a significant increase in plasma cholesterol. No difference was found in this latter parameter when control pregnant animals were compared with control virgins.

Foetuses from hypothyroid mothers showed an increase in serum total lipids while they showed no difference in cholesterol levels when compared to their controls (Table I).

Discussion

Maternal hypothyroidism during the first half of the rat gestation period caused alterations in the foetus-mother relationship, which lasted until the end of the gestation even though the mother was turned euthyroid for the second half of this period (4). In the thyroidectomized pregnant rat foetal body weight diminished indicating the need of thyroid hormones by the mother in order to maintain a suitable environment for foetal growth.

During gestation, a decrease in maternal blood glucose levels was observed, and this can be interpreted in terms of the known enhanced rate of utilization of this metabolite by the foetus, which is higher than its rate of incorporation to the maternal blood (15). LORENZO *et al.* (23) have shown that in the last days of gestation the adipose tissue presents an increased lipogenesis, for which it is necessary a higher supply of substrates that contributes to the decrease in glucose levels in pregnant animals.

In the gestation-hypothyroidism interaction no significant changes in serum glucose were observed, which is in accordance with the results presented by HENDRICH *et al.* (14). Nevertheless, foetuses from hypothyroid mothers were hyperglycemic with regard to their controls. This fact can account for the decrease in hepatic glycogen found in these animals by several authors (4, 14).

The rapid decrease in liver glycogen in either pregnant or non-pregnant thyroidectomized rats could be due to their having difficulty in increasing the synthesis of glucose in a higher degree than the utilization of this substrate. It is well established that glucose consumption is diminished in both fed and fasted hypothyroid rats (6, 22, 26, 30, 31).

Thyroid hormones play an important role in regulating plasma triglyceride and cholesterol concentrations (9). Hypothyroidism in humans results in a mild-to-moderate hypercholesterolemia often accompanied by high or very high plasma triglyceride concentrations (13, 34). However, it has been recently demonstrated that in the rat, hypothyroidism-induced hypercholesterolemia is accompanied by significant decreases in plasma triglyceride concentrations (5, 7), and therefore for these parameters hypothyroidism appears to be species specific.

No variation was observed in total lipid concentrations in non-pregnant hypothyroid rats. However, the serum concentration increases during pregnancy both in control and hypothyroid animals.

Transport of maternal lipids to the foetus in the rat is lower than that observed in other mammals (18, 21). However, the total lipid concentration found in rat foetal serum in our experiments is high, even higher in those foetuses from hypothyroid mothers. This fact has also been noted in newborns of several species including man (35).

The origin of these lipids is not sufficiently clear; some foetal tissues show a high lipogenic activity in order to provide for the foetal needs during some periods of development (19, 27, 33).

In both pregnant and non-pregnant hypothyroid rats cholesterol levels are increased. The absence of thyroid hormones inhibit hepatic mechanism that extract cholesterol from circulation.

It is a proved fact that total cholesterol levels increase during pregnancy in hu-

mans reaching its peak value during the 33-36 week (25). However, in pregnant rats this parameter does not change despite the fact that their foetuses present total cholesterol values higher than those found in their mothers, which is in accordance with the data presented by JOHANSSON (17).

The different values in lipids might reflect different lipolytic enzymes activities in the maternal and foetal serum (2).

Our data show that the influence of maternal hypothyroidism on its offspring could be exerted through changes in the availability of metabolic substrates to the foetus, which are more noticeable in the starved condition. Further studies are required to determine whether the response to food deprivation is affected in the hypothyroid mother or the differences observed are also present in the fed condition.

Acknowledgements

The authors want to express their gratitude to Prof. E. Herrera for critical suggestions when reading the original version of the manuscript.

Resumen

Se estudia en rata gestante tras 24 h de ayuno, el efecto del hipotiroidismo sobre algunos metabolitos. La tiroidectomía provoca disminución en el peso corporal de las ratas y de sus fetos. No se observan modificaciones en los niveles circulantes de glucosa en la rata, estando aumentados en sus fetos. Tanto el glucógeno hepático como los lípidos totales séricos disminuyen en la rata, mientras que aumenta el colesterol. En cuanto a los fetos se observa un incremento en sus niveles lipídicos totales. El hipotiroidismo en la rata gestante afecta al desarrollo fetal y tras un ayuno de 24 h tienen lugar profundos cambios metabólicos.

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