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Research article

Impact of nutritional restriction of pregnant rabbits on thyroid function ofoffspring

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Abstract

This study aims to examine the effect of maternal feed supplemented with high and low protein ratio on fetal growth thyroid functions during newborn life. Thirty pregnant females were divided into control, fed on standard diet, and two treated groups, fed on a diet supplemented with 70% (T1) and 240% (T2) of crude protein during the gestational period. Birth weight and body weight gain of newborns were recorded. Serum thyroid hormones (TT3, TT4, FT3, FT4 and TSH) concentrations of neonates were measured at birth, 7, 14, and 21 days. The results of neonate birth weight revealed significant increase of T1 group and significant decrease of T2 group compared with control. T1 group newborns showed significant decline of body weight gain during experimental periods, whereas T2 group showed no significant difference at first and second weeks and significant decrease at third week. The highest body weight, at the end of third week of the newborn life, was recorded in control group, whereas the lowest body weight was recorded in T1 group. **S**erum levels of TSH, TT3, FT3, TT4, and FT4 of T1 group were significantly lower among experimental groups, at all experimental periods, whereas those of T2 group were higher than that of T1 group but still lower than that of control group. In conclusion, postnatal growth in the rabbit is partly result from a relative increase in FT3 in neonate plasma at birth, also neonates are, particularly susceptible to exposure to a low-protein maternal feed during the gestational period.

Keywords: Hyperthyroidism, Hypothyroidism, Nutritional restriction, Rabbits,T3, T4.

Introduction

Nutrient restriction during pregnancy could affect fetal growth and development which could also affect postnatal growth, in which that will leads to accelerated intrauterine growth, but will increased adiposity in later life (1). The hypothalamic thyrotropin releasing hormone (TRH), pituitary thyroid stimulatory hormone (TSH) and thyroid hormones (THs) have roles in this mechanism. It has been mentioned that uterine nutritional and/or hormonal status influences postnatal growth (2, 3). Thyroid hormones (T3 and T4), secreted by thyroid

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gland, are part of the hypothalamic- pituitary-thyroid (HPT) axis and are involved in fetal development and postnatal growth because of their impact as energy homeostatic regulator and growth stimulator (4,5). On the other hand, leptin play an important positive effect by direct or indirect stimulatory role on releasing of TRH from the hypothalamus (6).

Epidemiological evidence showed that adverse intrauterine status during thyroid glandular differentiation result in programmed postnatal changes in thyroid functions (7). Conflicting results have been shown in other studies including sheep (8,9) and guinea pigs (10). As it has been shown that direct relationship was recorded between thyroid function status and skeletal muscles development (11,12), it has been reported that thyroid hormones regulate encoded genes in the skeletal muscles for protein and energy metabolism (13). On the other hand, thyroid hormones has an important role for the development and metabolism of the adipose tissue during the fetal stage (14,15) as well asneonatal thermogenesis (16).

The hypothesis of the present study was that maternal nutrient restriction, during gestational period, would give rise to post- natal hyperthyroidism in the offspring which could be accompanied by growth acceleration and increased adiposity. Therefore, the aim of the present study was to examine the effect of maternal feed supplemented with high and low protein ratio on fetal growth and development and their thyroid functions during newborn life.

Materials and methods

Ethical approval

The current experiment was accorded by the ethical guidelines and policies of the University of Al-Qadisiyah

Animals and experimental design:

Pregnant rabbits of local breeds (4 months aged and 1455±58 g of body weight) were housed at day light of 12L: 12D cycles and controlled temperature at 22-24 °C. Standard laboratory animal pellets (18% crude protein ratio and 2800 kilocalories energy) and drinking water was accessed ad libitum. Only females with success pregnancy were included in the experiment. Thirty pregnant females were divided into three equal groups. Control females were fed on standard feed throughout the gestational period, whereas treated groups were fed on a diet supplemented with 70% of crude protein (T1) and 240% of crude protein (T2) during the first and second stage of gestational period.

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Data and sample collection: Offspring were weighed at birth (0 days), 7, 14, and 21 day. Blood samples were collected from the jugular vein of neonates into non- heparinized tubes. Blood samples were kept on ice and centrifuged at room temperature at 5000 rpm for 10 minutes. Blood serum was harvested and stored at 20°C until assay.

Assessment of thyroid hormones: Serum thyroid hormones (TT3, TT4, FT3, FT4 and TSH) concentrations of neonates were measured at birth, 7, 14, and 21 days by ELISA according to the manufacturer's instructions (Ltd. Co., China).

Statistical analysis: All statistical analysis was carried out using the GraphPad Prism- Version 5 (SAS Institute, Inc., USA). Concentrations of hormones, birth body weight and body weight gain were analyzed using one way analysis of variance (ANOVA-I) to determine the effects of maternal nutrition during gestational period. Bonferroni-adjusted correlation analysis was used to calculate the ratio of TT3:TT4. Data were expressed as mean ± standard error of the mean. A probability of 5% ($P < 0.05$) was accepted as the level of significance (17).

Results

Body weight and body weight gain: The results of neonate birth weight, clarified in table (1), revealed significant differences ($p<0.05$) between the experimental groups, where T1 group recorded the highest birth weight and T2 group recorded the lowest birth weight in comparison with control group. In comparison with control, newborn of T1 group showed significant decline ($p<0.05$) of body weight gain during the first, second and third week of their life, whereas T2 group showed no significant difference ($p>0.05$) at first and second weeks and significant decrease ($p<0.05$) at third week. At the end of the third week of the newborn life, the highest body weight was recorded in control group, whereas the lowest body weight was recorded in T1 group.

Table (1): effect of maternal feed restriction on offspring birth weight and postnatal weight gain.

		Groups		
Parameter	Period	C	T1	T2
Birth weight (g)	1st d	34.55±3.85 b	48.26±6.77 a	27.55±4.54 c
Bogy weight gain (g)	7 d	63.33±7.56 b	51.22±8.11 c	74.39±7.66 a
	14 d	87.92±7.47 a	73.48±8.05 b	90.32±9.38 a

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	21 d	143.4±10.91 a	105.37±9.18 b	106.9±10.03 b
Body weight (g)	21 d	329.4±14.5 a	278.5±13.9 c	309.8±16.6 b

Values denote Mean ± S.E. C: control, pregnant rabbits supplemented with standard feed. T1: pregnant rabbits supplemented with high protein feed (240% of crude protein). T2: pregnant rabbits supplemented with low protein feed (70% of crude protein). Different letters dents significant difference ($p<0.05$) between groups.

Thyroid hormones: As illustrated in table (2), serum levels of TSH, TT3, FT3, TT4, and FT4 of T1 group were significantly lower (($p<0.05$) among experimental groups, at all experimental periods of the suckling newborn's life, whereas those of T2 group were higher than that of T1 group but still lower than that of control group.

Table (2): effect of maternal feed restriction on offspring serum thyroid hormone concentrations.

		Groups		
Parameter	Period	C	T1	T2
TSH conc. (x 0.01)(n.mole/L)	7 d	188.2±15.31 a	164.3±13.43 b	188.1±17.72 a
	14 d	195.3±13.96 a	152.8±16.49 c	190.3±13.55 b
	21 d	214.0±18.43 a	155.6±14.72 c	208.4±16.39 b
FT3 conc. (x 0.1)(n.mole/L)	7 d	10.5±1.12 a	6.7±0.73 b	6.6±0.83 b
	14 d	9.8±0.88 a	7.3±1.05 c	8.4±0.91 b
	21 d	11.8±0.95 a	7.8±1.11 c	8.9±0.88 b
TT3 conc. (x 0.1)(n.mole/L)	7 d	13.9±1.29 a	9.8±0.92 b	10.3±1.03 b
	14 d	13.4±1.13 a	10.2±0.96 c	11.8±1.07 b
	21 d	16.9±1.32 a	10.4±1.02 c	12.2±1.11 b
FT4 conc. (n.mole/L)	7 d	38.6±4.39 a	22.3±3.89 c	30.1±4.63 b
	14 d	39.5±5.31 a	27.9±4.65 c	33.6±3.98 b
	21 d	44.8±6.33 a	29.8±4.87 c	37.4±4.18 b
TT4 conc. (n.mole/L)	7 d	55.3±6.82 a	39.3±4.82 c	45.9±6.38 b
	14 d	68.7±8.77 a	48.8±6.49 c	55.4±5.82 b
	21 d	73.1±8.03 a	55.5±5.36 c	60.4±7.46 b
T3/T4 ratio	7 d	2.53±0.08 a	2.43±0.10 a	2.23±0.09 b
	14 d	2.08±0.07 b	2.09±0.09 b	2.13±0.12 a
	21 d	2.32±0.11 a	1.88±0.08 c	2.02±0.10 b

Values denote Mean ± S.E. C: control, pregnant rabbits supplemented with standard feed. T1: pregnant rabbits supplemented with high protein feed (240% of crude protein). T2: pregnant rabbits supplemented with low protein feed (70% of crude protein). Different letters dents significant difference ($p<0.05$) between groups.

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Discussion

In rabbits, maternal factors that affecting litter birth weight, during prenatal and postnatal periods, influences newborn growth and development, since the newborns spends considerable time with their mother during the suckling period (18,19). An adequate nutritional condition is known to be directly dependent upon the consumption of diets containing all the nutrition factors in proper amounts to meet specific requirements of the individual in all stages of life.

Although maternal compartment, particularly placenta, has an adaptive and protective role toward the developing fetus, but it seems that the early differentiation of fetal thyroid gland, protein levels in maternal feed during early gestation could be altered due to the reset of the physiology of the HPT axis and thus affect fetal thyroid development. It has been shown that protein restriction reduces placental nutritional transport before fetal growth retardation (20).

It has been hypothesized that adverse conditions during early fetal life may affect organ development and therefore potentially causing adult structural and functional deviations in later life (21). Previous suggestion mentioned that maternal nutrient restriction during early gestation affects placental development, which could then continue to influence fetal development through the rest of gestational period. Furthermore, placenta will get a compensatory adaptation to maintain permanent providing nutrient (22-24).

The present effect could be due to altered placental development which could be influence fetal growth and development, where it has been postulated that placental development is the first to be affected from nutritional restriction during early and/or mid-gestation (22-24). Furthermore, it has been suggested that a compensatory adaptation could be maintain constant nutrient supplies to the fetus in case of altered placental development due to maternal nutrient restriction in early gestation, in which placental overweight could be recorded in late pregnancy or at term after returning to normal feeding followed nutritional restriction during early gestation (25-26).

De Blasio (27) proposed that growth rate acceleration of offspring with low birth-weight could be dependent on circulating T3 and T4 levels. In the present study, it has been suggested that the low level of protein in the diet was improbable to have had any unfavorable effects to fetal differentiation and development, since no difference in conception rate and embryonic loss between T1 (high-protein dietary) and T2 (low-protein dietary) groups.

The present study reported increased serum FT3, the biologically active form of T3 (28), in offspring of T2 group. This elevation could be resulted from high conversion rate of T4 to T3 due to increased prenatal glucocorticoid–stimulation (29). Therefore, increased relative FT3 of neonates, at birth, may acts as a compensatory response to improve the thermogenic capacity of the lower birth weight. Same response was also observed in neonate rats born to dams fed on low- protein feed during gestation (30). This could be related to the increased levels of IGF2 as a compensatory response by the fetus to promote adipocyte development and thus thermogenic capacity (31). Furthermore, increased ratio of FT3 to TT3 in T1 group lighter birth weight newborns could be resulted from the high conversion level of T4 to T3 (29).

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On the other hand, decline of leptin may play a role in the enhancement of T4 to T3 conversion, since leptin has a positive regulatory effect of TT4 through pituitary TRH. Also the increased level of FT4, in T2 group, could be due to the decline of T4-binding proteins. It has been postulated that maternal leptin has an effective role, during the late gestation, where it affect the development of fetal hypothalamic and subsequently post-natal appetite (32). Therefore, it can be suggested that main differences of neonate growing between the studied groups could be related to the differences in milk production as well as neonate's appetite, since, as it has been shown that calves born to heifers fed on low-protein feed during early gestation spend longer suckling time (30,33). Furthermore, increased gut fill may in part explain the positive effect of low-protein maternal feeding during the gestational period on progression of body weight of neonates.

Previous studies reported a positive relationship between neonate birth weight and circulating concentrations of TH (34). It is known that the pre-partum surge in cortisol stimulates the deiodination of T4 to T3; however, this effect does not appeared at the first week of postnatal period, where the elevation in serum TT3, FT3, and FT4 concentrations at third week compared with first week may simply reflect the high sensitivity of TH concentrations to nutrient supply and energy intake (1,35).

In conclusion, the present study has reported that postnatal growth in the rabbit is partly result from a relative increase in FT3 in neonate plasma at birth. The present study also showed that neonates are particularly susceptible to exposure to a low-protein maternal feed during the gestational period.

Conflict of interest

The study has no conflict of interest.

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