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Thyroid Response in Fetuses of Calorie-Restricted Pregnant Rats Given Goitrogen[1] (41667)

YASUNOBU EGUCHI, MASAKO YAMAMOTO, AND MELVIN LEE

Department of Anatomy, Azabu University School of Veterinary Medicine, Sagamihara, Kanagawa 229, Japan, and Division of Human Nutrition, School of Home Economics, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

Abstract. The growth of fetuses of 50% calorie-restricted (R) rats was retarded by approximately 1 day as compared with that of *ad libitum* fed control (C) rats. Nevertheless, the thyroid glands in such growth-retarded fetuses developed in proportion to the size of fetuses, as did the thyroid follicular cell height and the follicular diameter. Pregnant rats were treated with 40 mg propylthiouracil (PTU) each day for 2 days and autopsied on the third day in various gestational periods of Days 17–19, 18–20, 19–21, and 20–22. PTU given to C rats on Days 17 and 18 of gestation did not alter the fetal thyroid weight and histology on Day 19. When given on Days 18 and 19, or later, PTU caused a significant increase in the thyroid weight and follicular cell height. However, when given to R rats on Days 18 and 19, PTU did not influence the fetal thyroid. When given on Days 19 and 20, or later, PTU was effective in R rats. Thus, in fetuses of the R rats, the reciprocal relationship between the pituitary and the thyroid appears to be established with a 1-day delay as compared with the C rats, similar to the 1-day retardation in body weight gain.

Permanent growth retardation has been observed in the offspring of rats restricted in calories or in protein during gestation and lactation (1–4). The retardation involves delayed skeletal development (3) as well as a delay in the appearance of other developmental indicators, such as eye opening and sexual maturation (5). The retardation cannot be compensated for even when the offspring are allowed to feed *ad libitum* after weaning (1, 2).

Some endocrine impairment seems to be involved in the process of growth stunting in the offspring of the malnourished pregnant rats. For example, unilateral adrenalectomy of fetal rats on late gestational days, which normally results in hypertrophy of the remaining adrenal (6), does not do so if the mother is calorie malnourished (7). However, the level of circulating corticosterone in the malnourished mother rats is markedly elevated, and following maternal adrenalectomy, subsequent unilateral adrenalectomy of the fetus results in a certain degree of compensatory hypertrophy of the remaining fetal adrenal (7). This suggests that the increased amount of maternal corticosterone can cross the placenta, suppressing the fetal pituitary–adrenal system and affecting the corticosteroid-sensitive metabolic processes.

Under maternal protein deprivation throughout gestation, the thyroid tissues of the fetal and newborn rats show retarded morphogenesis (4), which may be involved in the physical growth impairment which has been reported. Hence, it is of interest to see whether a similar retardation of thyroid morphogenesis occurs in fetal rats as a consequence of maternal calorie restriction. The fetal pituitary–thyroid system can respond to maternally administered propylthiouracil (PTU) by exhibiting hypertrophy of the fetal thyroid (8). Therefore, it would be also interesting to see whether the feedback response of the system to goitrogen is retarded as a result of maternal malnutrition. The present study was designed to answer these questions, together with observation on the effect of fetal TSH injection, using gravimetric and histologic methods.

Materials and Methods. Female Sprague–Dawley rats were mated overnight with males of the same strain. The morning on which the presence of sperm was confirmed in the vaginal smear was regarded as Day 1 of gestation. Pregnant females were randomly assigned to

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control (C) or restricted (R) categories and caged individually. The C rats were fed Purina Laboratory Chow (Ralston Purina Co.) *ad libitum* throughout gestation. The R rats were fed 50% as much food as was consumed by C rats each day. The R rats consumed all of their food each day, usually within the first 2 hr.

Pregnant rats belonging to each category (C or R) were divided into two groups, PTU-treated (P) and nontreated (N). Consequently, there were four groups: CP, CN, RP, and RN. PTU-treated rats were given 40 mg PTU daily, by a stomach tube, on 2 consecutive days and autopsied on the third day. PTU was suspended in 1 ml water and administered on Days 17–18, 18–19, 19–20, or 20–21 of gestation. Nontreated rats were autopsied on Days 19, 20, 21, and 22 of gestation, as controls for corresponding PTU-treated rats, and also to establish the effects of calorie restriction on the fetal body weight, thyroid weight, and histology.

At autopsy, fetuses were quickly removed from the uterus and weighed. Both lobes of the thyroid were removed, weighed, and then fixed in Bouin's fluid. Each fixed thyroid was embedded in paraffin, sectioned serially at 7 μm, and stained with periodic acid Schiff (PAS) and hematoxylin. The average diameter of follicles and the average height of the follicular cells were determined as described by Eguchi and Morikawa (9).

In some pregnant rats (both C and R), TSH were injected into fetuses on Day 19 of gestation. TSH (Sigma Chemical Co.) was suspended in 0.025 ml peanut oil, at a dose of 0.1 IU, to form a long-acting depot. As controls, some littermate fetuses were given peanut oil alone. The injection was made as follows: A tiny opening was made in the uterine wall fetal membranes. A thread was inserted through the exposed fetal skin in two places and tied in a loose loop. The syringe needle was inserted through the loop and under the fetal skin. Following the injection, the needle was withdrawn and the loop of thread was pulled tight to prevent the injected material from leaking out. Autopsy was made on the next day, Day 20 of gestation. Only fetal body weight and thyroid weight were recorded in this series of experiments.

Statistical analyses of data were made with Duncan's new multiple range test for the series of PTU experiment and with Student's t test for the series of TSH injection.

Results. At each age, RN weighed less than did CN fetuses, as shown in Table I. The retarded body weight in RN became more marked as the age of fetuses approached the end of gestation, to the extent of about 1-day retardation in body weight.

The weight of the fetal thyroids was also less in RN than in CN, at any age (Table I). However, there appeared no marked difference in the ratio of thyroid weight/body weight between the two groups, CN and RN, on each observed gestational day, but on Day 21 there was a slight difference. Histologically, the height of the thyroid follicular cells varied from day to day in such a manner that on Days 19 and 20 the height was smaller in RN than in CN, but on Days 21 and 22 the situation tended to be reversed, though not significantly.

PTU given to pregnant rats caused some reduction in fetal body weight (Table I). In both groups (CP and RP) PTU given on Days 17 and 18 of gestation failed to alter either the fetal thyroid weight or cell height of the thyroid follicles, compared with the corresponding nontreated controls (CN and RN). When PTU was given on Days 18 and 19, the weight of CP fetal thyroids was significantly increased with a significant degree of heightening of follicular cells and a decrease in the amount of colloid stored in follicles, as compared with CN fetal thyroids. In contrast, the weights of RP fetal thyroids were not significantly altered, and there was only a slight heightening of follicular cells. The ratios of thyroid weight/body weight showed overall patterns similar to those of the absolute weight of the thyroid. When PTU was given on Days 19 and 20 or Days 20 and 21, it induced a highly significant increase in the weight of the fetal thyroids and height of the follicular cells with a marked decrease in the amount of colloid stored in follicles in both groups, CP and RP, compared with their controls. The follicular diameter was significantly enlarged in both groups, but only following PTU administration on Days 20 and 21.

TSH injected into fetuses on Day 19 induced a significant increase in the thyroid weight 1 day later in both groups, C and R (Table II).

TABLE I. CHANGES IN WEIGHT OF FETAL THYROID OF NORMAL CONTROL RATS (C) AND CALORIE-RESTRICTED RATS (R) GIVEN PROPYLTHIOURACIL (PTU) AT DIFFERENT EXPERIMENTAL PERIODS OF GESTATION

				Fetal body weight and thyroid weight				Thyroid follicle		
Control or restricted	Days of maternal treatment	Day at autopsy	Maternal treatment	No. of fetuses examined	Body wt mean ± SEM (g)	Thyr. wt mean ± SEM (mg)	Thyr. wt/Body wt mean ± SEM (mg/100 g)	No. of fetuses examined	Cell height mean ± SEM (μm)	Diameter mean ± SEM (μm)
C	17, 18	19	PTU (P)	12 (2)	1.41 ± 0.02^a	0.55 ± 0.02^a	38.7 ± 1.6^a	8	9.69 ± 0.16^a	23.7 ± 0.4^a
		19	None (N)	12 (2)	1.43 ± 0.04^a	0.56 ± 0.04^a	39.3 ± 2.5^a	8	9.73 ± 0.17^a	23.5 ± 0.2^a
R	17, 18	19	PTU (P)	12 (2)	1.20 ± 0.02^b	0.40 ± 0.02^b	33.3 ± 1.8^a	8	8.65 ± 0.09^b	20.5 ± 0.8^b
		19	None (N)	12 (2)	1.29 ± 0.01^c	0.46 ± 0.03^b	35.6 ± 2.3^a	8	8.85 ± 0.08^b	21.5 ± 0.5^b
C	18, 19	20	PTU (P)	12 (2)	2.49 ± 0.05^a	1.19 ± 0.05^a	47.9 ± 2.1^a	8	9.95 ± 0.25^a	25.8 ± 0.8^a
		20	None (N)	12 (2)	2.67 ± 0.03^b	0.95 ± 0.03^b	35.6 ± 1.4^b	8	9.03 ± 0.19^b	24.2 ± 0.3^a
R	18, 19	20	PTU (P)	18 (3)	1.58 ± 0.05^c	0.60 ± 0.03^c	37.8 ± 1.3^b	8	9.01 ± 0.06^b	23.3 ± 0.3^b
		20	None (N)	18 (3)	1.75 ± 0.04^d	0.62 ± 0.03^c	35.2 ± 1.5^b	8	8.67 ± 0.09^c	23.2 ± 0.2^b
C	19, 20	21	PTU (P)	12 (2)	4.53 ± 0.19^a	1.61 ± 0.10^a	35.7 ± 2.5^a	8	10.86 ± 0.18^a	30.1 ± 0.6^a
		21	None (N)	12 (2)	4.55 ± 0.08^a	1.02 ± 0.07^b	22.5 ± 1.0^b	8	8.99 ± 0.17^b	29.9 ± 0.7^{a}§
R	19, 20	21	PTU (P)	18 (3)	2.67 ± 0.05^b	1.12 ± 0.03^b	41.9 ± 1.2^c	12	11.80 ± 0.23^c	28.5 ± 0.6^{b}§
		21	None (N)	18 (3)	2.91 ± 0.09^b	0.78 ± 0.02^c	27.5 ± 1.2^d	12	9.43 ± 0.22^b	27.8 ± 0.6^b
C	20, 21	22	PTU (P)	12 (2)	4.78 ± 0.09^a	1.87 ± 0.06^a	39.4 ± 1.2^a	8	12.10 ± 0.14^a	30.6 ± 0.5^a
		22	None (N)	12 (2)	5.97 ± 0.08^b	1.13 ± 0.06^b	19.0 ± 0.8^b	8	8.83 ± 0.31^b	26.9 ± 0.2^b
R	20, 21	22	PTU (P)	18 (3)	3.62 ± 0.13^c	1.33 ± 0.06^c	32.7 ± 1.8^c	12	11.94 ± 0.23^a	31.2 ± 0.7^a
		22	None (N)	18 (3)	4.12 ± 0.07^d	0.80 ± 0.02^d	22.4 ± 0.7^b	12	9.55 ± 0.33^b	28.3 ± 0.4^b

Note. ( ): No of litters. Figures not sharing the same letter (*a, b, c, d*) are significantly different at $P < 0.05$. §: Not significantly different between the figures marked.

TABLE II. DATA FOR FETAL THYROIDS OF FETUSES SUBJECTED TO TSH INJECTION

Control or restricted	Day at treatment	Day at autopsy	Kind of treatment	Fetal body weight and thyroid weight: No. of fetuses	Body wt mean ± SEM (g)	Thyr. wt mean ± SEM (mg)	Thyr. wt/Body wt mean ± SEM (mg/100 g)
C	19	20	TSH	8 (3)	2.47 ± 0.09 ns	0.94 ± 0.04**	38.6 ± 2.5*
			Oil	8	2.25 ± 0.10	0.71 ± 0.04	31.6 ± 1.5
R	19	20	TSH	5 (3)	1.70 ± 0.07 ns	0.66 ± 0.02*	38.7 ± 1.4*
			Oil	5	1.78 ± 0.03	0.55 ± 0.04	31.1 ± 2.0

Note. ( ): No of litters. ns: not significant.
* $P < 0.05$.
** $P < 0.01$.

Discussion. The foregoing observations clearly show the growth stunting of fetuses of R rats, in agreement with the retarded growth seen in the progeny born under the similar conditions (1–3). The degree of stunting was progressively increased toward the end of gestation in a manner suggesting approximately a 1-day retardation in body weight gain, presumably owing to the increasing nutrient requirements for the R rats. Nevertheless, the thyroid glands in such growth-retarded fetuses developed as well as those of fetuses of a similar size of C rats, i.e., in terms of the ratio of thyroid weight/body weight and in the histologic variables of the follicular cell height and follicular diameter. Histologically on Days 19 and 20 of gestation, the height of follicular cells was slightly lower in fetuses of R rats than in fetuses of C rats, but the height was not significantly different between R and C on Days 21 and 22. Such a finding on days close to parturition is quite different from the result obtained with maternal protein-deficient feeding, in which both the follicular cell height and the follicular diameter of fetuses were significantly less than those of fetuses of normally fed control dams (4). This discrepancy may be due to the difference in experimental conditions, calorie restriction vs protein deprivation.

PTU is an effective agent for the formation of goiter. PTU blocks iodide organification in the thyroid (10) and acts first on the peripheral deiodation of thyroxine and later on the thyroxine synthesis (11). By a negative feedback mechanism, PTU induces an increased level of plasma TSH and an increased weight of the thyroid (11). In the present study, when PTU was given to C rats on Days 17 and 18 of gestation, the weight of the fetal thyroid was not increased. The height of follicular cells, which is considered an indication of the degree of response to TSH, was not altered. When PTU was given on Days 18 and 19 or later, it induced a significant increase in the weight of the fetal thyroids and the height of the follicular cells. This indicates that after Day 18 the fetal thyroid can be stimulated by endogenous pituitary TSH as a result of the negative feedback mechanism, in agreement with the previous report (8). In contrast, in the R rats, PTU does not induce an increase in the weight of the fetal thyroids during the experimental period Days 18–20, but induces only a slight increase in the height of follicular cells. Thus, the present results show that in fetal rats of the 50% calorie-restricted dams, the appearance of the reciprocal relationship between the pituitary and the thyroid is retarded by about 1 day, compared with fetuses of the *ad libitum* fed dams.

Since the untreated controls were not administered the PTU vehicle (water) it cannot be established that none of the effects was due to treatment stress. However, this explanation is unlikely, as the PTU and untreated control groups did not show differences on Days 17–19 of gestation.

Although the reciprocal relationship appears to be retarded by a day, the present study also reveals that the fetal thyroids of R rats on Day 19 can respond to injected TSH to a degree of significant increase in weight a day later. This means that the fetal thyroid itself can respond to TSH at this period.

Since the observed increase in the weight

of fetal thyroids following maternal PTU treatment is considered as a result of the negative feedback mechanism between the fetal pituitary and the fetal thyroid, it is apparently different from the finding of the fetal adrenals, though under a different condition in that no compensatory hypertrophy of the right fetal adrenal is induced following removal of the left fetal adrenal, also as a result of the negative feedback mechanism between the pituitary and the adrenal (7). This difference may be caused by the different placental permeabilities to thyroid hormones and corticoids. Thyroid hormones cross the placenta only to a limited degree (12), whereas corticoids can cross the placenta to suppress the fetal adrenals. That this is the case is shown by the fact that after maternal adrenalectomy of malnourished rats fetal compensatory adrenal hypertrophy can be inducted (7) in a manner similar to that seen under normal conditions (6).

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