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Effect of Fetal Thyroidectomy on Newborn Thermogenesis in Lambs

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ABSTRACT. We investigated the effect of the transient neonatal hyperthyroid state on thermogenesis at birth by measuring rectal temperature, plasma free fatty acids, plasma catecholamines, and *in vitro* brown adipose tissue respiration in thyroidectomized ($n = 6$) and sham operated ($n = 5$) fetal sheep. Surgery was performed at an average of 133 days of gestation followed by cesarean delivery at 146 days. Fetuses were delivered into a constant room temperature of 25° C. Serial measurements were made *in utero* before delivery and at timed intervals after birth. Serum 3,3′,5 triiodothyronine and thyroxine concentrations in the neonatal period were normal in sham operated and nondetectable in thyroidectomized fetuses. Rectal temperatures and serum free fatty acid levels were reduced in thyroidectomized newborns. Plasma epinephrine concentrations were increased and the hypothyroid neonates were acidotic when compared to control animals. *In vitro* basal and norepinephrine stimulated brown adipose tissue respiration were reduced in thyroidectomized compared to control animals. These results indicate that thyroid hormone deficiency impairs nonshivering thermogenesis in brown adipose tissue and leads to hypothermia despite augmented plasma epinephrine values. (*Pediatr Res* 21: 453–457, 1987)

Abbreviations

T_3, 3,3′,5 triiodothyronine
T_4, thyroxine
BAT, brown adipose tissue
FFA, free fatty acid
αGP, α glycerol phosphate
αGPD, α glycerol phosphate dehydrogenase
$(Bu)_2$ cAMP, dibutyryl cyclic adenosine monophosphate
NE, norepinephrine
GA, gestational age
RIA, radioimmunoassay
ANOVA, analysis of variance

During the transition from fetal to neonatal life there are profound changes in thyroid function resulting in markedly elevated plasma T_3 levels in the human newborn. Similar events occur in the fetal-neonatal lamb providing a model to study thyroid metabolism in the perinatal period (1). Previously, we hypothesized that these elevated T_3 concentrations play an important role in temperature regulation by the newborn adapting to the extrauterine environment (2). In support, we reported that thyroid hormone augments BAT thermogenesis in the newborn rabbit (3).

The neonatal "surge" in plasma T_3 levels is associated with parturition-induced elevations of plasma catecholamine levels which may be important in stimulating neonatal thermogenesis (4). In newborn rabbits, cold exposure to exogenously administered catecholamines increases metabolic rate, increases surface skin temperature over sites of BAT, and increases circulating FFA concentrations (5). Surgical removal of BAT leads to a reduction of these responses (6).

The present studies were conducted to determine whether thyroid hormone influences body temperature regulation directly or indirectly via catecholamine levels in the early neonatal period. We studied the response of fetal sheep thyroidectomized 2 wk prior to delivery compared to sham-operated control fetuses, measuring circulating thyroid hormone levels, rectal temperature, hemodynamic variables, plasma epinephrine and norepinephrine, and serum FFA concentrations in addition to *in vitro* oxygen consumption in BAT.

MATERIALS AND METHODS

Animal studies. Western, mixed breed, time-dated pregnant ewes were obtained from the Nebeker Ranch (Lancaster, CA) and acclimated to our laboratory conditions and feed. Initially, singleton pregnancies ($n = 3$) were studied; subsequently twin pregnancies ($n = 4$) were included to provide paired (twin) animals as a source of tissue for *in vitro* analysis of oxygen consumption.

At a mean (±SEM) 133 (± 1) days gestation (term = 148 days) either fetal thyroidectomy ($n = 6$) or sham operation ($n = 5$) was performed. When a twin pregnancy was used, both fetuses were handled in a similar manner. Ewes were fasted from the evening prior to surgery. During continuous ketamine infusion after establishing local anesthesia (lidocaine, 2%), a midline abdominal incision was followed by palpation of fetal parts and identification of the fetal head. A hysterotomy was performed over the fetal neck, and the uterine wall and fetal membranes were marsupialized to the fetal neck to avoid loss of amniotic fluid. The fetal neck was infiltrated with 1% lidocaine followed by dissection and complete removal of the thyroid gland. The incisions were closed and the ewes were treated for 3 days postoperatively with intramuscular oxacillin (4 g in divided doses, given daily).

Thirteen days later (GA = 146 ± 1 days) the ewes underwent spinal epidural anesthesia followed by a paramedian abdominal incision. A fetal hindlimb was identified and delivered from the uterus through a small incision held snugly around the limb with a purse string suture. Fetal skin was infiltrated with 1% lidocaine

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and the dorsalis pedis artery was dissected and catheterized. Fetal skin was closed, a fetal rectal temperature probe was placed, fetal parts were reduced into the amniotic cavity, and a period of 20 min was allowed for stabilization. Previously, we showed that during this time fetal plasma catecholamine levels return to baseline values (4). Twenty min after catheterization the fetus was delivered, the umbilical cord was ligated and divided, and the lamb was superficially dried. The ambient temperature in the delivery room was held constant at 25° C for the duration of the study. When a twin pregnancy was used the first twin was removed, killed with an overdose of barbiturate, and the above protocol was conducted on the second twin.

Blood samples were obtained prior to delivery and umbilical cord cutting, followed by collection of newborn blood samples at 15, 30, 60, 120, and 180 min of age. Blood samples were replaced with equal volumes of normal saline. Rectal temperature, pulse, mean arterial pressure, and respiratory rate were assessed at each sampling time.

Measurements of BAT respiration. BAT was isolated and oxygen consumption measured using our previously described methods (7, 8). Briefly, BAT was excised from the perirenal deposits of one of each twin animal ($n = 4$ thyroidectomized and 4 sham-operated fetuses) and placed in modified Krebs Ringer bicarbonate buffer at room temperature for transport to the laboratory. BAT cells were isolated and equally aliquoted into Warburg vessels which were placed on a Gilson differential respirometer (Gilson Medical Electronics, Middleton, WI). Basal oxygen consumption was measured, as well as cell respiration after addition of the following substances (final concentrations in parentheses): NE; (10^{-5}, 10^{-6}, 10^{-7} M), $(Bu)_2$ cAMP; (2, 4, and 8×10^{-3} M), α-GP; (2×10^{-2} M) and butyric acid; (2×10^{-3} M). Results are expressed as microliters of O_2 per 10^6 cells/h.

Mitochondrial enzyme activity. α GPD activity was determined in crude BAT mitochondrial fractions from one of each twin pair of animals using the method of Fried *et al.* (9). The change in optical density $\times 10^3 \cdot$ mg protein$^{-1} \cdot$ min^{-1} is defined arbitrarily as 1 U of enzyme activity. Tissue protein was determined by the method of Lowry *et al.* (10).

Measurement of circulating catecholamines, FFA, T_3, T_4, and blood gases. Epinephrine and NE concentrations were measured in plasma samples by radioenzymatic assay using the method of Peuler and Johnson (11). Serum fatty acids were determined colorimetrically by the method of Sardini and Goloni (12). Blood gases were determined using a Radiometer blood gas machine. Serum T_3 and T_4 concentrations were determined using our previously described RIA methods (1); the lower limit of sensitivity for T_3 was 10 ng/dl and for T_4 1 μg/dl.

Data analysis. Data were analyzed by two-way analysis of variance for assessment of possible differences between responses in the two groups of animals. Values are reported as mean ± SEM. Significance was defined as $p < 0.05$.

RESULTS

Newborn studies. Serial serum T_3 and T_4 values are shown in Figure 1. The mean serum T_3 concentration in control animals was 216 (± 56) ng/dl at 60 min. In thyroidectomized animals,

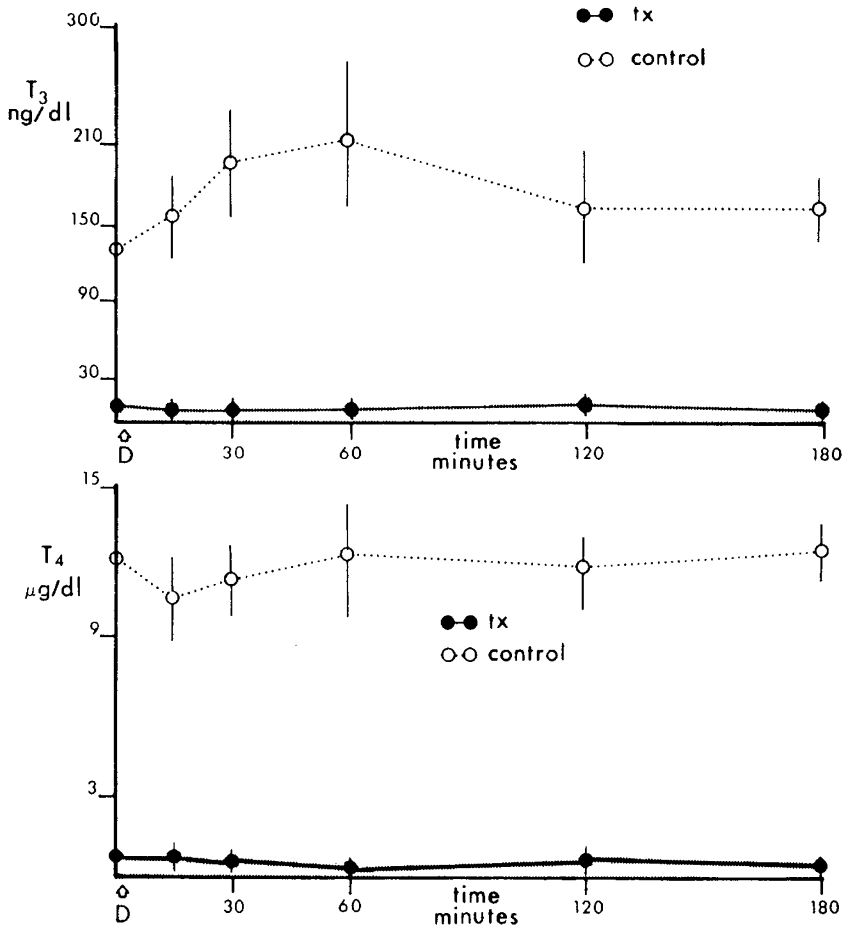

Fig. 1. Serum T_3 concentrations (*upper panel*) in thyroidectomized (Tx●) lambs were at the lower limits of detection (*gray hatch*) of the assay and significantly lower than T_3 concentrations in control (○) lambs ($p < 0.001$). Plasma T_4 levels (*lower panel*) in thyroidectomized lambs were at the lower limits of detection of the assay and significantly lower than T_4 values in control lambs ($p < 0.001$). *D* indicates time of umbilical cord cutting. Results recorded as mean ± SEM.

the T_3 levels were below the limit of detection (10 ng/dl) in all but one fetus; in that animal the T_3 peak value in serum was 46 ng/dl. The T_3 responses were significantly different in the two newborn groups by two-way ANOVA ($p < 0.001$). The mean serum T_4 value in control animals at 60 min was 12.2 (± 1.2) μg/dl. T_4 levels in thyroidectomized animals were below the limit of detection in all but the one fetus with the detectable serum T_3 level; in that animal, serum T_4 was 4.5 μg/dl 60 min after birth. The T_4 responses were significantly different between the two groups by two-way ANOVA ($p < 0.001$).

Serial serum FFA and temperature responses are shown in Figure 2. The mean plasma FFA level was 1117 (± 199) μEq/liter in control animals and 740 (± 257) μEq/liter in thyroidectomized animals by 120 min. The mean FFA response in the thyroidectomized animals was significantly less than that in control by two-way ANOVA ($p < 0.01$). Rectal temperature in control animals was 37.9 (± 0.05)° C while the temperature of thyroidectomized animals fell to 35.9 (± 1.3)° C at 180 min. The values in the thyroidectomized animals were lower than control animals by two-way ANOVA ($p < 0.25$).

Plasma epinephrine and NE responses are shown in Figure 3. The mean plasma epinephrine value was 0.34 (± 0.10) ng/ml in control animals and 1.46 (± 0.64) ng/ml in thyroidectomized lambs at 60 min ($p < 0.01$ by two-way ANOVA). NE values were similar in the two groups of animals at 60 minutes, 2.8 (± 0.8) ng/ml in the control and 2.6 (± 0.7) ng/ml in the thyroidectomized animals.

Thyroidectomized animals demonstrated a significant metabolic acidosis when compared with control animals. There were no differences in pulse rate, mean arterial blood pressure, respiratory rate, or the pO_2 or pCO_2 values between the two groups of animals (Table 1).

BAT respiration and mitochondrial enzyme activity. Twins used in this part of the experiment were comparable in thyroid status, sex, and birth weight to the animals used for the newborn studies. The average serum T_3 concentration in these animals was 27 (± 9) ng/dl in controls; values were undetectable in the thyroidectomized animals. Similarly, the average serum T_4 value was 11.2 (± 2.0) μg/dl in controls and undetectable in the thyroidectomized group.

BAT respiration data in these animals are shown in Table 2. Basal BAT oxygen consumption was greater in control animals [46 (± 2.5) μl $O_2/10^6$ cells/h] than in the thyroidectomized fetuses [31 (± 2.2) μl $O_2/10^6$ cells/h; $p < 0.002$]. A pronounced difference between the two groups of animals was seen during NE stimulation. Control BAT respiration increased 8-fold to 391 (± 52) μl $O_2/10^6$ cells/h in response to 10^{-6} M norepinephrine, whereas BAT from thyroidectomized animals demonstrated only a 4-fold increase in response to 10^{-6} M NE (144 ± 16 μl $O_2/10^6$ cells/h; $p < 0.002$). This significant difference in response was demonstrated at all three concentrations of NE used in the experiment. BAT respiration also was significantly decreased in thyroidectomized animals in the presence of dibutyryl cAMP, αGP, or butyric acid (Table 2).

BAT αGPD activities in the two groups of animals were also determined. The mean control value (638 ± 150 U) is comparable to that of the thyroidectomized animals (645 ± 117 U).

DISCUSSION

Although thyroid hormones are known to influence various physiologic responses in adult animals (13), the effects of thyroid hormones on metabolic events in the newborn are less clear. Previously, we were unable to demonstrate an effect of thyroid hormone on brain, liver, or kidney oxygen consumption or on αGPD or Na-K ATPase activities in these tissues in fetal or newborn sheep (7). The low serum thyroid hormone levels in the hypothyroid lambs in the present study were associated with relative hypothermia, suggesting impaired thermogenesis. BAT is the primary site of nonshivering (chemical) thermogenesis in

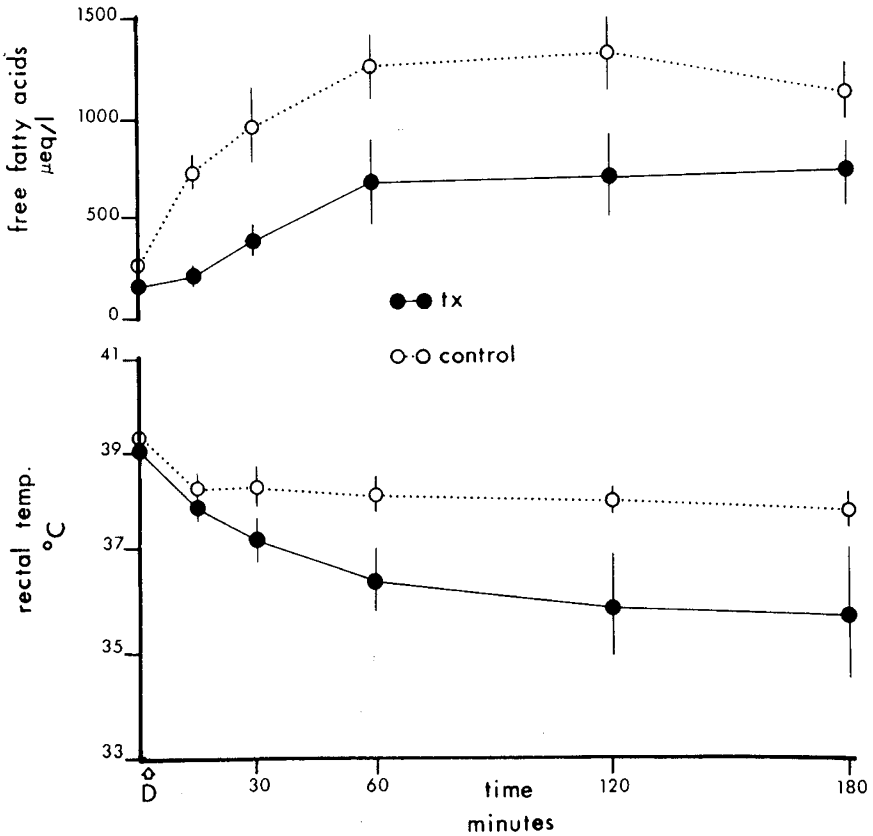

Fig. 2. Serum FFA and rectal temperature responses were significantly decreased after delivery in thyroidectomized (*Tx*) lambs relative to control animals ($p < 0.01$; $p < 0.025$). *D* indicates time of umbilical cord cutting. Results recorded as mean ± SEM.

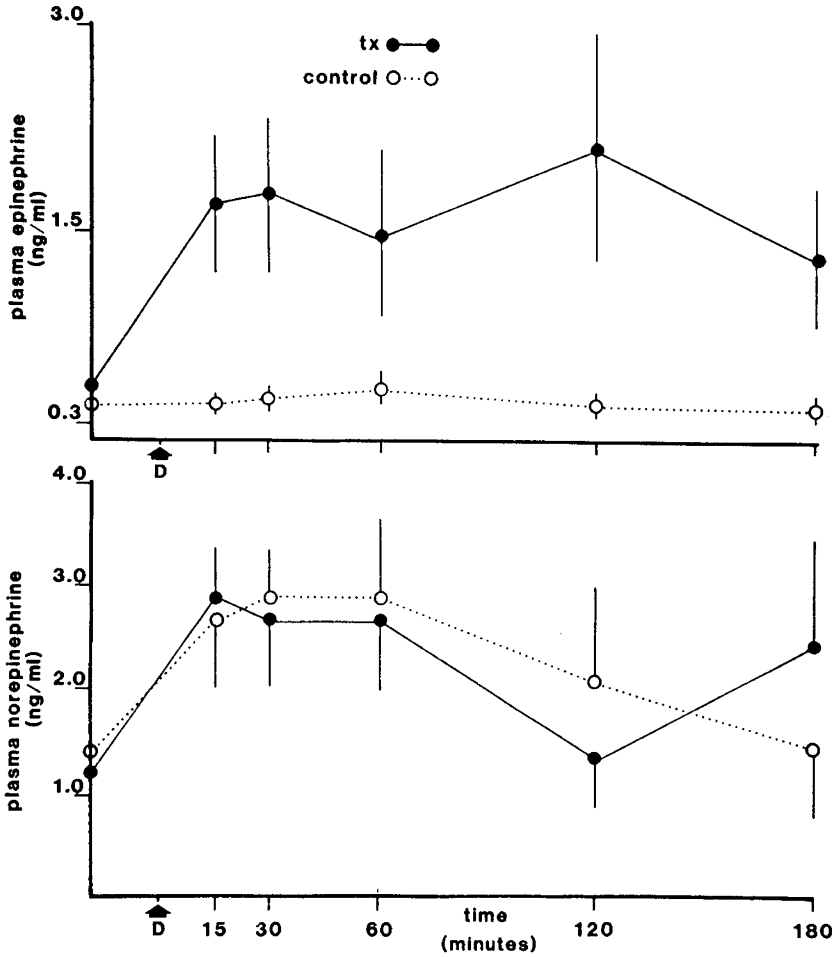

Fig. 3. The plasma epinephrine response after delivery (*upper panel*) was significantly increased in thyroidectomized (*Tx*) animals as compared to controls ($p < 0.01$). Plasma NE levels (*lower panel*) were similar in the two groups of animals. *D* indicates time of umbilical cord cutting. Results recorded as mean ± SEM.

Table 1. *Metabolic and biochemical responses in thyroidectomized (Tx) and control (C) lambs 60 min after umbilical cord cutting**

	Tx	C
Pulse	218 ± 41	223 ± 53
Mean arterial blood pressure	53 ± 11 torr	53 ± 11 torr
Respiratory rate	65 ± 3	59 ± 9
pH	7.13 ± 0.1	7.28 ± .03
pO_2	38 ± 4	40 ± 4
pCO_2	32 ± 5	30 ± 4

* Values recorded as mean ± SEM.

newborn animals and plays a critical role in postnatal temperature regulation of the newborn (2). The perirenal adipose tissue used in this study has the histologic appearance of brown fat, is free from white adipocytes, is adrenergically innervated and responds to both α- and β-adrenergic agonists.

Ovine perirenal BAT is similar to that from other species; the major difference being increased synthesis of ATP by ovine perirenal adipocytes (21–23). In newborn and adult rats, BAT thermogenesis is known to be affected by thyroid hormones (14, 23). In newborn rabbits, we reported that 3 days of treatment with thyroid hormones increased basal BAT respiration, as well as BAT mitochondrial αGPD activity, suggesting that thyroid hormones modulate BAT mitochondrial associated enzyme activity (3). BAT is the primary site of origin of circulating FFA during the newborn period; the FFA are released in association with stimulation of BAT thermogenesis. Moreover, *in vivo* cold exposure or norepinephrine infusion to the human newborn results in a marked increase in serum FFA levels (2). The hypothyroid, hypothermic lambs in the present study had lower FFA levels in serum than the control lambs, supporting the hypothesis of a perinatal role for thyroid hormone in BAT thermogenesis.

This conclusion is supported by the *in vitro* studies of oxygen consumption in brown adipocytes. The hypothyroid state was associated with lower basal BAT oxygen consumption as well as a pronounced defect in NE stimulated maximal oxygen consumption (Table 2). The observation that $(Bu)_2$ cAMP failed to correct the reduced respiration in hypothyroid derived BAT suggests that the defect resides, at least in part, distal to the β-adrenergic receptor. Previously we demonstrated that heart and lung β-adrenergic receptor binding is unaffected by thyroid status in the ovine fetus, becoming thyroid hormone responsive only in the postnatal period (15). In hypothyroid adult rats, agonist affinity for β-adrenergic receptor is decreased in adipose tissue, and the ability of agonists to elevate cAMP is significantly impaired (16, 17). We did not measure β-adrenergic receptor binding in BAT in the present study, and it is possible that this was reduced in the hypothyroid animals. However, the *in vitro* studies suggest that the reduced BAT responsiveness in our hypothyroid newborns was largely accountable through postreceptor mechanisms.

Earlier hypotheses suggested a thyroid hormone effect on mitochondrial respiration and mitochondrial enzyme activity related to the control of ion transport between extramitochondrial nicotinamide adenosine dinucleotide and the cytochrome system of the mitochondria via the glycerol phosphate cycle. Mitochondrial αGPD plays a pivotal role in this system, and αGPD activity doubles during the perinatal period in sheep (8, 20). The present observations that αGP substrate failed to in-

Table 2. *O_2 consumption in brown adipocytes isolated from control and thyroidectomized lambs under basal, stimulated, and substrate supplied conditions**

	BAT respiration (μl $O_2/10^6$ cells/h)				
	Basal $\dot{Q}$ O_2	NE-stimulated (10^{-6} M)	$(Bu)_2$ cAMP (4 mM)	αGP	Butyrate
Control	46.4 ± 2.5	391 ± 52	305 ± 34	104.4 ± 9	74 ± 7
Thyroidectomized	31.3 ± 2.2†	146 ± 16†	209 ± 35‡	74.6 ± 3§	53 ± 5‖

* Results are reported as mean ± SEM. $\dot{Q}$ O_2, oxygen consumption.
† $p < 0.002$.
‡ $p < 0.05$.
§ $p < 0.01$.
‖ $p < 0.03$

crease BAT respiration in the hypothyroid animals and that measured BAT αGPD activity was not reduced suggests that BAT αGPD is not affected by hypothyroidism. This is further supported by the decreased respiration demonstrated in hypothyroid BAT cells supplemented with butyrate since αGPD is the only enzyme involved in the metabolism of αGP which is not shared by butyrate metabolism.

These observations contrast with our earlier work in rabbits in which thyroid hormone supplementation of euthyroid fetuses was associated with increased αGPD activity and suggest that some of the thermogenic effects of thyroid hormone may be species specific (3). Data in newborn rats demonstrate that thyroid hormone deficiency has little effect on *in vivo* basal oxygen consumption, but decreases both nonshivering thermogenesis during a cold stress and the thermogenic response to exogenous NE (14, 18). Thyroid supplementation in amounts which raise basal oxygen consumption in these animals has no effect on the thermogenic response to cold but augments catecholamine-stimulated respiration (14, 18).

Plasma NE concentrations were similar in control and hypothyroid newborns in the present study, supporting the hypothesis that the thermogenic defect resides in BAT tissue. The observation of elevated epinephrine values was unexpected. The relative hypothermia and/ or acidosis demonstrated in the hypothyroid animals may account for the increased levels of epinephrine in these animals (19). However, secretion or clearance rates for catecholamines were not determined and the mechanism(s) for the increased epinephrine levels remains unclear. The observation that hypothyroid newborn animals were unable to elevate circulating FFA or rectal temperatures to levels seen in control animals despite augmented circulating levels of epinephrine again supports the hypothesis that the defect in thermogenesis is observed in these animals is within the BAT.

In summary, we demonstrated that thyroidectomy in near term ovine fetuses is associated with decreased rectal temperatures and reduced levels of circulating FFA in the neonatal period suggesting a defect in chemical thermogenesis. *In vitro* studies of BAT respiration demonstrate that fetal thyroidectomy is associated with a decrease in basal, NE, $(Bu)_2$ cAMP, αGP, and butyrate stimulated BAT $\dot{Q}$ O_2. No changes in BAT αGPD activity were demonstrated. These data suggest that normal thyroid function in the sheep plays an important role in neonatal temperature regulation through its effects on BAT thermogenesis.

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