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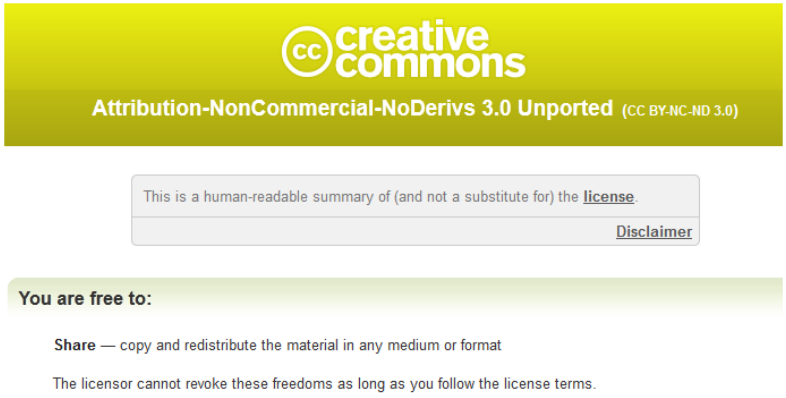

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Maternal endocrine adaptation throughout pregnancy to nutrient manipulation: Consequences for sexually dimorphic programming of thyroid hormones and development of their progeny☆

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ABSTRACT

Maternal nutrient restriction during critical windows of fetal development alters postnatal growth, often in a sexually dimorphic manner. Intrauterine growth restriction is frequently characterized by accelerated growth and increased adiposity in later life. Thyroid hormones are implicated as part of the mechanism involved in this scenario *via* their actions within the hypothalamic–pituitary–thyroid axis. We fed high (H = 240%) and low (L = 70%) levels of recommended daily crude protein intake during the first and second trimesters of gestation to beef heifers to investigate effects to their progeny's plasma concentrations of free and total triiodothyronine (FT3 and TT3) and thyroxine (FT4 and TT4) from birth until weaning at 191 days of age (n = 68). The study design was a two-by-two factorial. For male progeny, exposure to maternal diets low in protein during the first trimester of gestation resulted in greater FT4 at birth ($P < 0.05$) which was subsequent to lower concentrations of leptin in maternal plasma at 271 days of gestation compared with their high-protein–exposed counterparts. These same animals went on to have greater milk intake during the latter half of the lactation period ($P < 0.05$) and exhibited faster rates of average daily gain (ADG) relative to birth weight during this time ($P < 0.05$). For all progeny, independent of sex, exposure to low-protein maternal diets during the second trimester of gestation resulted in greater FT3 relative to TT3 at birth. Because FT3 at birth and 29 days was positively associated with ADG ($P < 0.05$) and ADG relative to birth weight ($P < 0.05$), it is proposed that FT3 plays an integral role in catch-up growth in the *bovine* as per other species. Protein intake during the first and second trimesters of gestation has a sexually dimorphic effect on progeny plasma thyroid hormone concentrations, and these changes are associated with altered milk intake and postnatal growth pathway.

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1. Introduction

Worldwide epidemiologic evidence indicates that the nutritional and hormonal environment *in utero* influences postnatal health and growth pathways [1,2]. The thyroid gland produces triiodothyronine (T3) and thyroxine (T4) which exist in circulation in the free (FT3 and FT4) or

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bound state. They form part of the hypothalamic–pituitary–thyroid (HPT) axis that plays a major role in fetal development and postnatal growth because of their metabolic and nonmetabolic mechanisms for stimulating growth and regulating energy homeostasis [3,4]. Leptin regulates the HPT axis *via* positive effects on thyrotropin-releasing hormone by direct and indirect pathways in the hypothalamus [5].

In the *bovine,* the fetal thyroid gland differentiates between 75 and 90 days of gestation (gd) after which it undergoes glandular proliferation [6]. Adverse intrauterine conditions during this critical time may, therefore, result in programmed effects on postnatal thyroid function. A single study in the *bovine* has explored this possibility, examining the effect of maternal nutrient intake during the late gestation on calf serum T3 at birth [7]. Limited studies in other species, including sheep [8,9] and guinea pigs [10], have produced conflicting results perhaps induced by differential time points of perturbations.

A direct relationship between fetal thyroid status and skeletal muscle development has been reported in sheep [11] and cattle [12]. Similarly in adults, genes responsible for protein and energy metabolism within skeletal muscle are regulated by thyroid hormones (THs) [13]. Furthermore, TH are critical to fetal adipose tissue development [14] and metabolism [15] and are required within brown adipose tissue for the initiation of neonatal thermogenesis [16]. It has been proposed that the accelerated growth rate of low-birth-weight offspring may also depend on circulating T3 and T4 [17].

In the present study, therefore, we investigated the effect of maternal nutrient intake during early and midgestation on the postnatal thyroid status of the progeny from birth until weaning at 191 days of age and their association with milk intake and preweaning growth. We hypothesized that maternal nutrient restriction during the first and second trimesters of gestation would result in increased thyroid activity in the offspring during the preweaning period and that this may be associated with the accelerated growth and later increased adiposity of these calves. Furthermore to this, we have reviewed our fetal growth, maternal metabolic and pregnancy hormones, and progeny milk intake data sets using repeated-measures multifactorial ANOVA that consider the effect of fetal sex. Previously, these data sets were analyzed using multifactorial general linear models that did not necessarily include fetal sex.

2. Materials and methods

All procedures were performed with the approval of The University of Queensland Animal Ethics Committee, approval number SVS/716/06/MLA/AACO.

2.1. Animals and diets

One hundred twenty composite breed beef heifers (mean age, 23 months and range, 21.6–24) were located in Queensland, Australia (28°52′S, 150°33′E) and individually stall fed throughout the experiment. They were acclimatized for 45 days to their environment and management practices, before being synchronized for timed artificial insemination. Estrus synchronization was achieved using a combination of intravaginal progesterone-releasing devices (EAZI-BREED CIDR cattle device; Pfizer Animal Health, Australia; 1.9-g progesterone), 1-mg estradiol benzoate intramuscular (Ciderol; Genetics Australia, Bacchus Marsh, Australia) and 25-mg prostaglandin intramuscular (Lutalyse; Pfizer Animal Health). Heifers were artificially inseminated with semen from one Senepol bull on the same day.

The study was a two-by-two factorial design with heifers initially stratified by body weight and genotype. From 0 to 93 gd (1T), groups were fed diets containing either 70% (low, L) or 240% (high, H) of National Research Council [18] recommended crude protein (CP) requirements (Table 1). From 94 to 180 gd (2T), half of the animals in each treatment group changed to the alternate group, giving rise to four treatment groups: high/high (HH), high/low (HL), low/high (LH), and low/low (LL). From 181 gd until term, all heifers were fed a standard diet (Table 1). Feed rations consisted of cottonseed meal (*Gossypium spp.*), cracked sorghum seed (*Sorghum spp.*), bambatsi hay (*Panicum coloratum*) or barley straw (*Hordeum spp.*), lime, and a vitamin and mineral premix. Water was provided *ad libitum*. The nutritional content was measured using a

Table 1
Details of high- and low-treatment group daily rations fed to dams during each trimester of gestation[a].

Item	Trimester 1 (Day 1–93)		Trimester 2 (Day 94–180)		Trimester 3 (Day 181 to term)
	High	Low	High	Low	All
Sorghum (kg)	0.65	1.56	1.00	1.20	1.13
Cotton seed meal (kg)	2.45	0.00	2.50	0.00	1.08
Bambatsi hay (kg)	7.88	2.73	5.79	0.00	0.86
Barley straw (kg)	0.00	5.14	2.21	7.58	7.14
Lime (kg)	0.07	0.02	0.12	0.06	0.08
Premix (kg)	0.07	0.06	0.10	0.10	0.10
DMI (kg)	9.95	8.64	10.51	8.10	9.39
Energy (MJ ME)	76.29	62.54	82.43	63.14	71.45
Energy (% NRC[b])	243	199	229	176	149
CP (kg)	1.37	0.41	1.40	0.38	1.06
CP (% NRC[b])	250	75	228	63	135
Gossypol content (g)	1.16	0	1.17	0	0.51

Abbreviations: CP, crude protein; DMI, Dry matter intake; NRC, National Research council.
[a] Data are presented on as fed basis per dam per day.
[b] Comparison of ration to NRC–recommended Nutrient Requirements of Beef Cattle (1996).

combination of wet chemistry and near infrared spectrophotometry (CASCO Agritech, Toowoomba, Queensland, Australia). Samples of cottonseed were analyzed for free gossypol by AOCS official method Ba 7 to 58 [19] (Table 1; [18]). Dams and their progenies grazed native pastures from calving to 191 days.

Ultrasound measures of *in utero* fetal growth were taken from 39 gd until term as reported [20]. Sex of progeny by treatment group was: HH = seven male and eight female, HL = nine male and nine female, LH = nine male and seven female, and LL = eight male and eleven female. Hereafter, all ages refer to the average age of progeny on the day of sampling. Male calves were castrated at 153 days. Calves were managed as previously described to 191 days [21].

2.2. Data and sample collection

Calves were weighed at birth (0 days), 15, 29, 65 days and then monthly until 191 days [21]. Jugular blood samples from calves were collected into tubes containing lithium heparin (Vacutainer; Becton Dickinson, Franklin Lakes, NJ, USA) within 5 minutes of birth and immediately after being brought in from grazing at 0800 on the same day as weighing. Samples were stored for 1 to 2 hours on ice and centrifuged at room temperature at 3000× *g* for 10 minutes (Hettich Universal Zentrifugen, Germany). Plasma was harvested and stored at −20 °C until assayed.

Additionally, placental and metabolic hormones were measured from maternal plasma samples taken at 28, 82, 179, and 271 gd as previously described [22].

2.3. Hormone and metabolite sample analyses

2.3.1. Insulinlike growth factors (IGF)

Plasma IGF-I and -II concentrations were measured at 28, 82, 179, and 271 gd in heifers and at 0, 29, 94, 191, 379, and 657 days in progeny by RIA after separation of IGF and their binding proteins (IGFBP) by size-exclusion high-performance liquid chromatography (HPLC) under acidic conditions, as previously described [13,23,24]. Samples were assayed in triplicate. Insulinlike growth factor I in HPLC fractions collected after injection of heifer plasma was assayed in 15 assays, with an interassay coefficient of variation (CV) of 7.9% and an intra-assay CV for extraction and assay of 14.5% for a pregnant cow quality control (QC) sample containing 59.8 ng/mL of IGF-I. Insulinlike growth factor II in HPLC fractions collected after injection of heifer plasma was assayed in nine assays, with an interassay CV of 5.5% and an intra-assay CV for extraction and assay of 25.2% for a pregnant cow QC sample containing 339.0 ng/mL of IGF-II.

Calf plasma IGF-I and -II was assayed as previously described [21]. The interassay CV for HPLC separation and RIA of IGF-I was 10.9% (n = 18 assays) and the intra-assay CV for extraction and assay was 22.0% for a calf QC sample containing 31.4 ng/mL of IGF-I. Interassay CV of IGF-II was 9.7% (n = 9), and intra-assay covariance for extraction and assay was 21.6% for a calf QC sample containing 78.0 ng/mL of IGF-II.

2.3.2. Leptin

Plasma leptin concentrations of heifers at 28, 82, 179, and 271 gd and at calving and their progenies at 153 days were measured as described previously [25] with the RIA previously developed by Blache et al. [26] and subsequently validated for cattle [27]. Intra-assay CVs were estimated using three QC standards containing 0.54 ng/mL (4.2%), 0.86 ng/mL (5.2%), and 1.85 ng/mL (4.8%) at a zero binding of 30%. The limit of detection was 0.05 ng/mL.

2.3.3. Progesterone

Plasma progesterone concentrations of heifers at 28, 82, 179, and 271 gd and at calving were assayed in duplicate by RIA using kits obtained from Beckman Coulter, Inc., USA, validated for the *bovine* [22]. The intra-assay and interassay CVs were 6.1% and 3.30%, respectively, with a sensitivity of 0.04 ng/mL (lowest standard).

2.3.4. Bovine pregnancy-associated glycoprotein (bPAG)

A monoclonal-based ELISA, as used by [28], was used to measure maternal bPAG in duplicate samples of plasma at 28, 82, 179, and 271 gd as described and validated [28]. Intra-assay and interassay CVs were 5.5% and 13.1%, respectively.

2.3.5. Thyroid hormones

Plasma TH (total T3 [TT3], TT4, FT3, and FT4) concentrations of calves were measured at birth, 29, 94, and 191 days by coated-tube RIA using kits previously used in the *bovine* [29–31]. Total T4 was assayed in 20-μL plasma by coated-tube RIA (IM1447; Immunotech/Beckman Coulter, Prague, Czech Republic) according to the manufacturer's instructions. Sensitivity of the assay defined by the lowest standard was 24 nM. The intra-assay CV was less than 10%. The interassay CVs across three assays were 16.6% at 74 nM and 4.5% at 148 nM.

Total T3 was assayed in 50-μL plasma by coated-tube RIA (IM1699; Immunotech/Beckman Coulter) according to the manufacturer's instructions. Sensitivity of the assay defined by the lowest standard was 0.77 nM. The intra-assay CV was less than 10%. The interassay CVs across three assays were 6.5% at 1.8 nM and 1.4% at 4.2 nM.

Free T4 was assayed in 25-μL plasma by coated-tube RIA (IM1363; Immunotech/Beckman Coulter) according to the manufacturer's instructions. Sensitivity of the assay defined by the lowest standard was 2.7 pM. The intra-assay CV was less than 10%. The interassay CV across three assays was 4.4% at 15.4 pM.

Free T3 was assayed in 100-μL plasma by coated-tube RIA (IM1579; Immunotech/Beckman Coulter) according to the manufacturer's instructions. Sensitivity of the assay defined by the lowest standard was 1.8 pM. The intra-assay CV was less than 10%. The interassay CV across three assays was 5.2% at 4.8 pM.

2.4. Milk production and calf intake

Estimation of milk production and calf intake using the weigh-suckle-weigh technique described by Beal et al. [32] was conducted at 65, 94, 123, 153, and 191 days. As previously described [33], measurements at two 6-hour

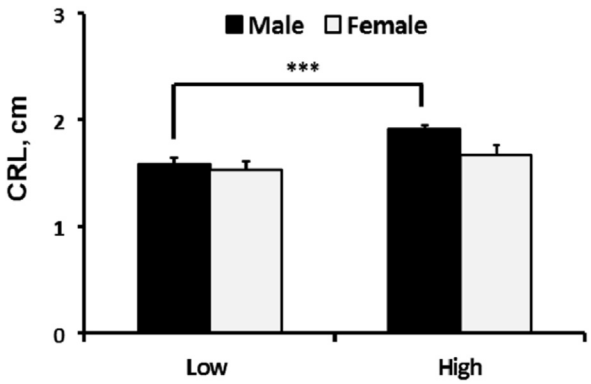

Fig. 1. Mean (±standard error of the mean) crown–rump length (CRL) of male and female fetuses from dams fed low (70% CP) or high (240% CP) protein diets during the first trimester of gestation. ***Denotes $P < 0.001$ between male progeny exposed to high- or low-protein diets.

intervals and at a 12-hour interval over a 24-hour period were obtained. The weigh-suckle-weigh technique assumes that the milk produced by the heifer is equal to that consumed by its calf. Consumption is measured by summation of the changes in the live weight of the calf during three supervised sucklings within a 24-hour period [34].

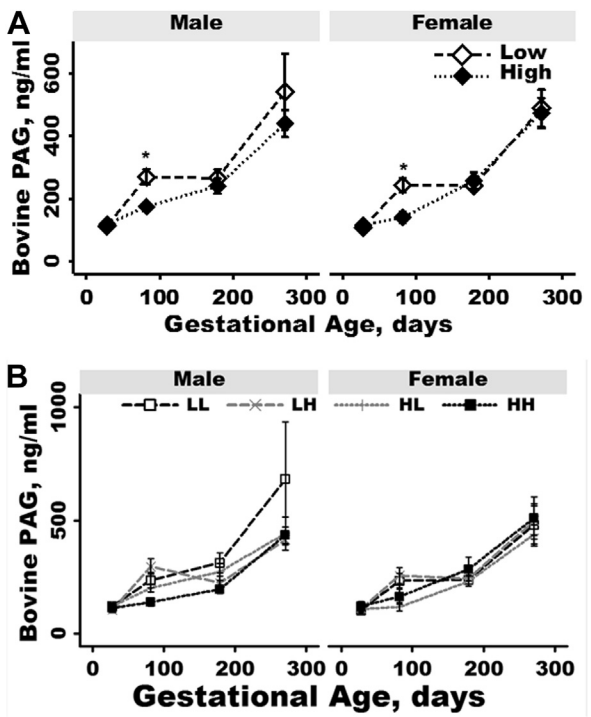

Fig. 2. (A) Maternal plasma bovine pregnancy-associated glycoprotein (bPAG) concentrations (mean ± standard error of the mean [SEM]) at 28, 82, 179, and 271 days of gestation (gd) by trimester one treatment group (low = 70%; ◇ and high = 240%; ◆) and fetal sex. *Denotes $P < 0.05$ between heifers fed a high- or low-protein diet at 95 days by fetal sex. (B) Maternal plasma bPAG concentrations (mean ± SEM) at 28, 82, 179, and 271 gd by trimester one and two treatment groups (LL, 70%: □; LH, 70%–240%: × ; HL, 240%–70%: +; and HH, 240%: ■) and fetal sex. HH, high protein during 1T and 2T; HL, high protein during 1T and low protein during 2T; LH, low protein during 1T and high protein during 2T; LL, low protein during 1 and 2T.

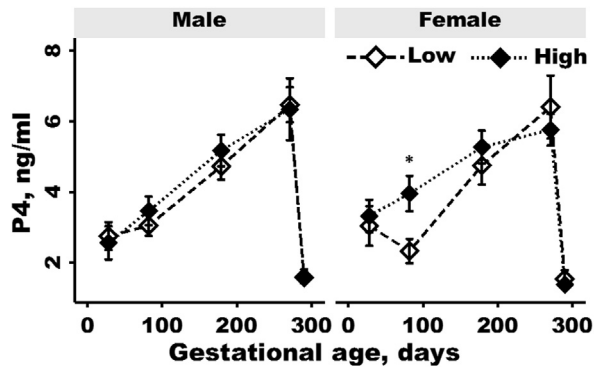

Fig. 3. Maternal plasma progesterone (P4) concentrations (mean ± standard error of the mean) at 28, 82, 179, and 271 days of gestation (gd) and parturition by trimester one treatment group (low = 70%; ◇ and high = 240%; ◆) and fetal sex. *Denotes $P < 0.01$ between heifers fed a high- or low-protein diet during 1T that were carrying a female fetus at 82 gd.

2.5. Statistical analysis

Concentrations of hormones, milk production, and fetal calf measures were analyzed using repeated-measures multifactorial ANOVA to determine the effects of maternal nutrition during 1T and 2T, progeny sex, and their interaction terms. Gestation length was included as a covariate to account for differences in age on the day of sampling. Bonferroni-adjusted correlation analyses were used to calculate the relationships between TH concentrations (TT3, TT4, FT3, and FT4) and their ratios (FT3:TT3, FT4:TT4, TT3:TT4, and FT3:FT4) with: (1) progeny body weight, average daily gain (ADG), and ADG relative to birth weight (fractional growth rate [FGR]) from birth until

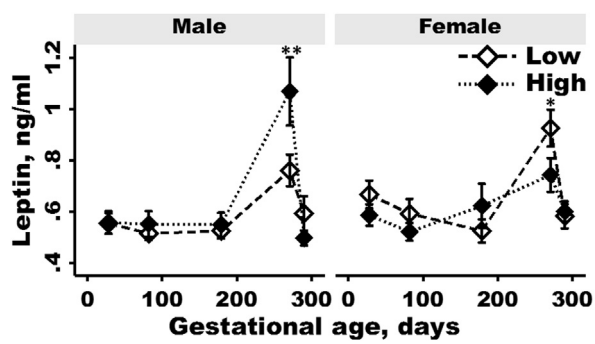

Fig. 4. Maternal plasma leptin concentrations (mean ± standard error of the mean) at 28, 82, 179, and 271 days of gestation (gd) and parturition by trimester one treatment group (low = 70%; ◇ and high = 240%; ◆) and fetal sex. *Denotes $P < 0.05$ between heifers fed a high- or low-protein diet during 1T that were carrying a female fetus at 271 gd. **Denotes $P < 0.01$ between heifers fed a high- or low-protein diet during 1T that were carrying a male fetus at 271 gd.

weaning and (2) between hormones and fetal growth measures. Data are presented as mean ± standard error of the mean. A probability of 5% ($P < 0.05$) was accepted as the level of significance, and trends reported at $P < 0.1$. Data were analyzed using Intercooled Stata 13 software (StataCorp, TX, USA).

3. Results

3.1. Fetal growth and placental hormone production

Low protein during the first trimester decreased fetal growth as measured by crown–rump length ($P < 0.001$) at 39 gd in male progeny (Fig. 1). This was associated with reduced dam plasma IGF-II concentrations ($P = 0.06$).

At 82 gd, maternal bPAG concentrations were greater ($P < 0.05$; Fig. 2A), independent of fetal sex, after exposure to low-protein diets during 1T. Concentrations of bPAG by group over gestation are shown in Figure 2B. Also at 82 gd, exposure to low-protein diets during 1T was associated with lower plasma progesterone concentrations for dams carrying a female fetus ($P < 0.01$; Fig. 3).

3.2. Leptin

Dams carrying a male fetus had lower plasma concentrations of leptin at 271 gd if they had experienced low-protein diets during 1T ($P < 0.01$, Fig. 4) with the opposite effect occurring in dams carrying female calves ($P < 0.05$).

3.3. Thyroid hormones

Average concentrations of free and total T3 and T4 for each treatment group by sex are given in Table 2. Ratios of FT3:TT3, FT4:TT4, TT3:TT4, and FT3:FT4 are given in Table 3. Plasma concentrations of free and total T3 and T4 from birth until 191 days according to nutritional treatment during 1T and sex are shown in Figure 5.

3.3.1. Total T3 and free T3

Progeny plasma TT3 (Fig. 5A) and FT3 (Fig. 5B) concentrations for male and female calves followed a similar pattern over time, increasing from birth to reach peak levels at 29 days to decline thereafter (time, $P < 0.001$). Plasma TT3 concentrations varied according to sex

Table 2
Plasma concentrations (mean ± standard error of the mean) of progeny total and free triiodothyronine (T3) and thyroxine (T4) according to their age, dam treatment group, and sex.

Parameter	Calf age (days)	Sex	Heifer nutritional treatment group			
			HH	HL	LH	LL
TT4 (nM)	0	M	75.3 ± 9.5	80.0 ± 10.8	111.6 ± 14.6	84.1 ± 13.0
		F	104.9 ± 15.5	93.7 ± 11.9	104.86 ± 17.9	90.2 ± 13.3
	28	M	79.1 ± 4.7	64.6 ± 7.1	70.0 ± 6.9	69.6 ± 3.9
		F	62.9 ± 6.4	85.0 ± 6.1	76.7 ± 6.2	71.3 ± 4.1
	94	M	68.4 ± 6.7	80.1 ± 5.3	76.1 ± 5.8	72.5 ± 5.1
		F	68.0 ± 7.9	76.9 ± 4.6	76.9 ± 7.1	71.3 ± 6.3
	191	M	71.0 ± 7.8	81.3 ± 4.5	85.2 ± 4.1	72.9 ± 4.5
		F	71.6 ± 3.1	85.6 ± 5.4	78.0 ± 1.9	75.7 ± 4.3
FT4 (pM)	0	M	11.9 ± 1.3*	14.1 ± 1.6*	16.2 ± 0.9‡	15.3 ± 1.1‡
		F	18.1 ± 2.0*	14.8 ± 2.3*	16.7 ± 1.7‡	16.5 ± 1.4‡
	28	M	16.3 ± 0.8	16.7 ± 0.7	18.3 ± 1.4	17.7 ± 1.1
		F	15.6 ± 0.7	19.2 ± 1.0	18.8 ± 0.9	17.5 ± 0.7
	94	M	13.2 ± 1.8	14.9 ± 1.1	13.5 ± 0.7	13.5 ± 0.6
		F	14.1 ± 0.8	16.0 ± 0.7	13.6 ± 0.9	13.3 ± 0.9
	191	M	12.2 ± 0.4	14.9 ± 0.9	14.7 ± 0.7	13.7 ± 0.3
		F	13.8 ± 0.6	14.7 ± 1.2	14.1 ± 1.2	13.1 ± 0.5
TT3 (nM)	0	M	1.8 ± 0.2	2.4 ± 0.3	3.5 ± 0.3	2.3 ± 0.5
		F	3.5 ± 0.6	2.0 ± 0.3	2.9 ± 0.5	2.8 ± 0.4
	28	M	2.7 ± 0.4	3.9 ± 0.4	4.0 ± 0.3	3.7 ± 0.2
		F	4.0 ± 0.2	4.3 ± 0.3	4.2 ± 0.4	4.5 ± 0.3
	94	M	3.4 ± 0.3	4.0 ± 0.2	3.7 ± 0.2	4.1 ± 0.3
		F	4.2 ± 0.3	4.1 ± 0.2	4.1 ± 0.2	3.8 ± 0.2
	191	M	3.5 ± 0.2	3.6 ± 0.1	3.5 ± 0.2	3.3 ± 0.2
		F	3.7 ± 0.1	3.5 ± 0.1	3.9 ± 0.2	3.6 ± 0.1
FT3 (pM)	0	M	4.33 ± 0.5	6.1 ± 0.9	6.7 ± 0.5	5.54 ± 0.8
		F	7.9 ± 1.0	5.2 ± 0.7	6.4 ± 0.6	7.2 ± 1.1
	28	M	11.1 ± 0.9	12.6 ± 1.5	12.3 ± 1.4	12.9 ± 1.8
		F	11.5 ± 1.1	14.7 ± 1.6	12.7 ± 1.5	11.7 ± 0.6
	94	M	7.7 ± 0.4	8.6 ± 0.5	8.3 ± 0.5	8.5 ± 0.4
		F	9.0 ± 0.7	8.7 ± 0.4	9.4 ± 0.8	8.7 ± 0.8
	191	M	5.7 ± 0.4	6.0 ± 0.2	5.5 ± 0.3	5.9 ± 0.4
		F	5.9 ± 0.3	5.7 ± 0.2	6.3 ± 0.3	5.9 ± 0.2

*,‡Values with different superscripts are significantly different ($P < 0.05$).
Abbreviations: F, female; FT3, free T3; FT4, free T4; HH, high protein during 1T and 2T; HL, high protein during 1T and low protein during 2T; LH, low protein during 1T and high protein during 2T; LL, low protein during 1 and 2T; M, male; TT3, total T3; TT4, total T4.

Table 3
Ratios (mean ± standard error of the mean) of progeny plasma total and free triiodothyronine (T3) and thyroxine (T4) according to their age, dam treatment group, and sex.

Parameter	Calf age (days)	Sex	Heifer nutritional treatment group			
			HH	HL	LH	LL
FT3:TT3 (1×10^{-3})	0	M	2.4 ± 0.2*	2.7 ± 0.3‡	1.9 ± 0.1*	3.2 ± 0.8‡
		F	2.4 ± 0.2*	2.8 ± 0.3‡	2.5 ± 0.3*	2.9 ± 0.4‡
	28	M	4.4 ± 0.6	3.4 ± 0.3	3.2 ± 0.4	3.4 ± 0.4
		F	2.9 ± 0.3	3.5 ± 0.5	3.0 ± 0.4	2.6 ± 0.1
	94	M	2.4 ± 0.3	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.2
		F	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.2	2.4 ± 0.2
	191	M	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.2
		F	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
FT4:TT4 (1×10^{-3})	0	M	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.04	0.2 ± 0.03
		F	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.02
	28	M	0.2 ± 0.03	0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.03
		F	0.3 ± 0.05	0.2 ± 0.01	0.3 ± 0.02	0.2 ± 0.01
	94	M	0.2 ± 0.04	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
		F	0.3 ± 0.07	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.02
	191	M	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
		F	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.7 ± 0.01
FT3:FT4 (1×10^{-3})	0	M	0.4 ± 0.05	0.4 ± 0.05	0.4 ± 0.05	5.54 ± 0.8
		F	0.4 ± 0.02	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.04
	28	M	0.7 ± 0.06	0.8 ± 0.08	0.7 ± 0.05	12.9 ± 1.8
		F	0.7 ± 0.05	0.8 ± 0.06	0.7 ± 0.05	0.7 ± 0.03
	94	M	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.02	8.5 ± 0.4
		F	0.6 ± 0.05	0.5 ± 0.03	0.7 ± 0.06	0.7 ± 0.06
	191	M	0.5 ± 0.02	0.4 ± 0.03	0.4 ± 0.02	5.9 ± 0.4
		F	0.4 ± 0.02	0.4 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
TT3:TT4 (1×10^{-3})	0	M	0.03 ± 0.003	0.03 ± 0.004	0.05 ± 0.022	0.03 ± 0.005
		F	0.04 ± 0.005	0.02 ± 0.003	0.03 ± 0.003	0.03 ± 0.005
	28	M	0.04 ± 0.006	0.06 ± 0.008	0.06 ± 0.008	0.06 ± 0.005
		F	0.07 ± 0.012	0.05 ± 0.003	0.06 ± 0.008	0.07 ± 0.005
	94	M	0.05 ± 0.007	0.05 ± 0.004	0.05 ± 0.004	0.06 ± 0.006
		F	0.07 ± 0.013	0.05 ± 0.002	0.06 ± 0.005	0.06 ± 0.009
	191	M	0.05 ± 0.006	0.04 ± 0.002	0.04 ± 0.002	0.05 ± 0.003
		F	0.05 ± 0.002	0.04 ± 0.003	0.05 ± 0.002	0.05 ± 0.003

*,‡Values with different superscripts are significantly different ($P < 0.05$).
Abbreviations: F, female; FT3, free T3; FT4, free T4; HH, high protein during 1T and 2T; HL, high protein during 1T and low protein during 2T; LH, low protein during 1T and high protein during 2T; LL, low protein during 1T and 2T; M, male; TT3, total T3; TT4, total T4.

($P < 0.001$), with female calves having greater plasma concentrations than males ($P < 0.01$) at 29 days if exposed to a low-protein maternal diet during 1T, and at 29 and 94 days if exposed to a high-protein maternal diet during 1T. Conversely, FT3 concentrations were similar for male and female calves between birth and 191 days with no apparent interaction with maternal nutrition ($P > 0.05$).

3.3.2. Total T4 and free T4

Contrasting patterns were observed between progeny plasma concentrations of TT4 (Fig. 5C) and FT4 (Fig. 5D) from birth to 191 days. Whereas FT4 plasma concentration followed a pattern similar to that observed for TT3 and FT3, peaking at 29 days followed by a decrease thereafter, TT4 showed the opposite. Concentrations of TT4 peaked at birth then declined by 29 days ($P < 0.05$) with levels then remaining relatively constant until 191 days (Fig. 5C). Females exposed to high-protein maternal diets during 1T had greater FT4 than their male counterparts from 0 to 95 days ($P < 0.05$). Exposure of male fetuses to low-protein maternal diets during 1T resulted in them having greater levels of FT4 at birth ($P < 0.05$; Table 2).

3.3.3. Ratios of free and total plasma T3 and T4

At birth, plasma FT3:TT3 ratio of all progeny was greater after exposure to low-protein maternal diets when compared with high-protein maternal diets during 2T ($P < 0.05$, Table 3).

Plasma TT3:TT4 ratio varied in an interaction between maternal nutrition during 1T, 2T, and sex ($P < 0.01$). Plasma TT3:TT4 ratio of female calves was greater than that of males at 29 and 94 days after exposure to high-protein maternal diets during either 1T or 2T ($P < 0.05$; Fig. 6).

3.4. Preweaning growth and milk intake

Milk intake from 65 to 191 days according to nutritional treatment group during 1T and sex is shown in Figure 7C and D. Males exposed to low-protein maternal diets during 1T had greater milk intake from 94 to 191 days than their female counterparts ($P < 0.05$), and FGR was positively related to milk intake at 191 days ($r = 0.19$; $P < 0.05$). Fractional growth rate from birth to 191 days varied in an interaction between sex, treatment group, and time hence FGR is displayed in Figure 7A and B by 1T nutritional treatment group and sex. Fractional growth rate of females

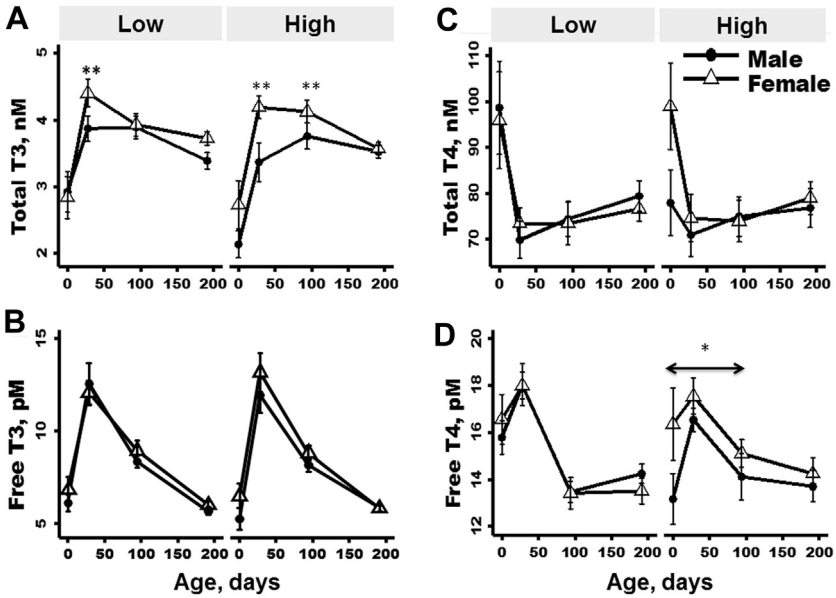

Fig. 5. Plasma concentrations (mean ± standard error of the mean) of total triiodothyronine (T3; A), free T3 (B), total thyroxine (T4; C), and free T4 (D) in male (●) and female calves (Δ) from birth to 191 days according to maternal treatment group during the first trimester of gestation. *Denotes $P < 0.05$ between male and female calves from birth to 95 days after exposure to high-protein maternal diets during 1T. **Denotes $P < 0.01$ between male and female calves at 29 days after exposure to low-protein maternal diets during 1T, and at 29 and 94 days after exposure to high-protein maternal diets during 1T.

was greater than that of males from 29 to 191 days after exposure to high-protein maternal diets during 1T ($P < 0.05$).

3.5. Associations of THs with preweaning growth

Fractional growth rate of all progeny after sampling was positively correlated with plasma FT3 concentration at birth ($r = 0.41$; $P < 0.05$) and 29 days ($r = 0.41$; $P < 0.05$). For male calves, FT4 and FT3 were positively correlated with their plasma leptin ($r = 0.62$ and 0.52; $P < 0.01$, respectively) from birth to 65 days after exposure to high-protein maternal diets during 1T. In this cohort of males at 95 days, their plasma IGF-I concentration was correlated to TT3 and FT3 ($r = 0.55$ and 0 0.74, $P < 0.01$, respectively).

4. Discussion

The present study forms the final in a series of experiments in which we investigated the effects of either high- or low-protein diets fed to heifers during the first two trimesters of gestation on phenotypic and metabolic changes to themselves and their progeny. To date, we have reported a positive effect of maternal dietary protein content on body weight, body condition score, and circulating concentrations of IGF-I and -II, total IGFBP, leptin, and urea in heifers during the first two trimesters of gestation [24]. Previous analyses of these and fetal growth data were performed using general linear models that included the effect of maternal genotype. Because the interaction between maternal genotype and treatment group produced only three and five animals per treatment group for one of the genotypes, we have reanalyzed the data without the inclusion of maternal genotype, but with the inclusion of fetal sex.

Fetal growth was affected by heifer nutrient intake by 39 gd, and there was evidence of preferential nutrient distribution to fetal body parts of developmental need when protein was restricted during early and midgestation [35]. This effect was sex specific, being present in male but not female fetuses. The receptivity of tissues associated with fetal nutrient transfer to maternal nutrient intake was highlighted by changes to umbilical cord diameter at 123 gd, and again at term by an increase in the number of cotyledons in the fetal placenta after feeding low-protein maternal diets during the second trimester of gestation [36]. Leptin may have played a signaling role in this because placental size was inversely associated with the circulating leptin [36]. Furthermore, placental function as determined by the ratio of calf weight to placental weight was increased in association with 1T gestational low protein as indicated by plasma bPAG and estrone sulfate concentrations, and in the second trimester as indicated by plasma bovine placental lactogen concentrations [22]. These changes in the second trimester were associated with a positive effect of heifer dietary protein intake on calf birth weight.

As well as phenotypic effects of heifer protein intake on progeny, sex-specific metabolic effects were evident, with an inverse relationship between plasma non esterified fatty acids of male calves at birth and maternal protein intake during the second trimester of gestation. The effect of maternal diet during the second trimester on birth weight was no longer apparent by 65 days postpartum; however, the reason for this was not fully explained by changes to progeny plasma IGF-I, -II or their total binding proteins [21].

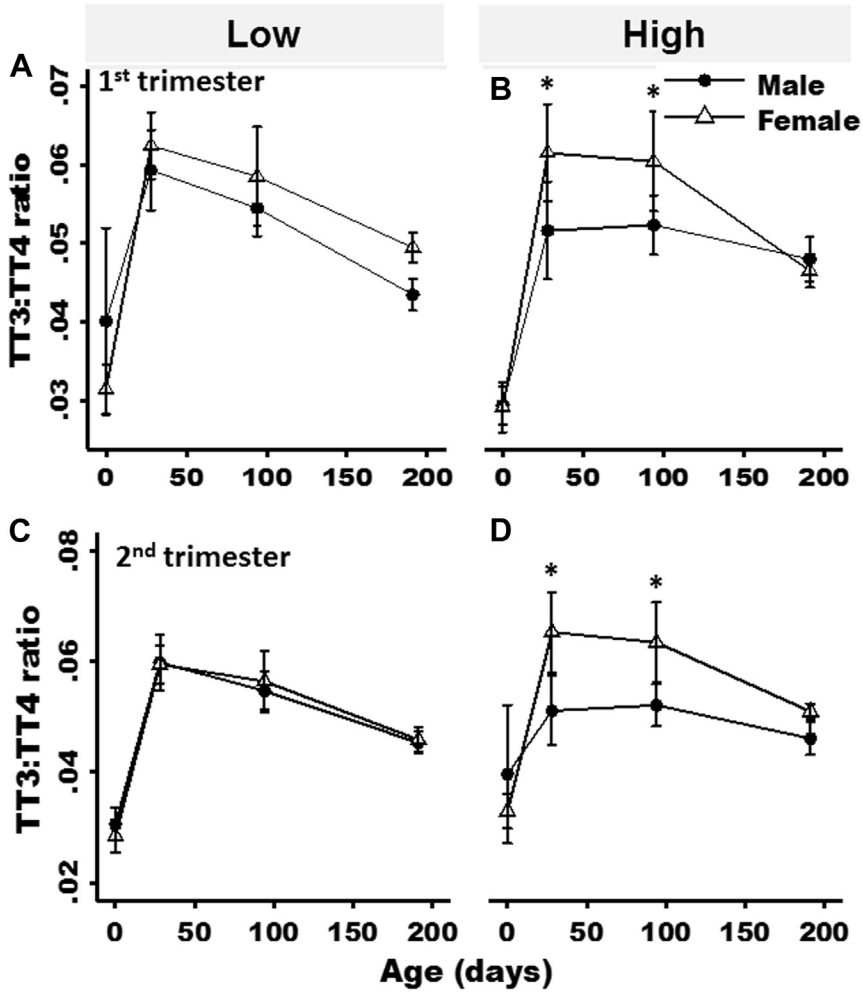

Fig. 6. Total triiodothyronine to total thyroxine ratio (mean ± standard error of the mean) of male (●) and female calves (Δ) between birth and 191 days from dams fed low- (70%, A and C) or high- (240%; B and D) protein diets during the first (A and B) and second trimesters (C and D) of gestation. *Denotes $P < 0.05$ between male and female calves at 29 and 94 days after exposure to maternal diets high in protein during 1T.

Subsequently, the effects of gestational diet on progeny phenotype were found to be more associated with maternal nutrition during the first rather than the second trimester and were sex specific. Male progeny exposed to low-protein maternal diets during the first trimester was heavier and leaner during the postweaning period and had greater cross-sectional areas of their *semitendinosus* and *longissimus dorsi* muscles when compared with their high-protein–exposed counterparts [21]. These changes were accompanied by greater *IGF-I* and *IGF-II* mRNA expression in their *semitendinosus* muscle [37] and lower *LEP* mRNA expression in their perirenal adipose tissue at 680 days [25]. In contrast, female progeny exposed to maternal diets low in protein during the first trimester of gestation were lighter and of smaller stature during the postweaning period which resulted in them having lighter carcasses at 680 days [21]. In addition, they had greater expression of *IGF-IIR* mRNA in their *semitendinosus* muscle [37] but decreased expression of *IGF-I* mRNA in perirenal adipose at 680 days when compared with their high-protein–exposed counterparts [25].

The aim of our present and final study, therefore, was to elucidate a possible role for THs in the mechanism underlying some of the sex-specific effects of maternal diet during the first and second trimesters of gestation on the fetal and postnatal development of the progeny.

The shorter crown–rump length of male fetuses from heifers fed low-protein diets to 39 gd coupled with a lesser concentration of IGF-II in maternal circulation during early gestation, may be evidence of the potential for a low-protein diet to affect the developing fetus because of changes in maternal metabolism during the very early gestation. However, as gestation proceeded, the possibility of an adaptive and protective role of the maternal compartment toward the developing fetus, away from potential detrimental effects of a low-protein external environment, was suggested by an increase in placental function as measured by maternal circulating bPAG

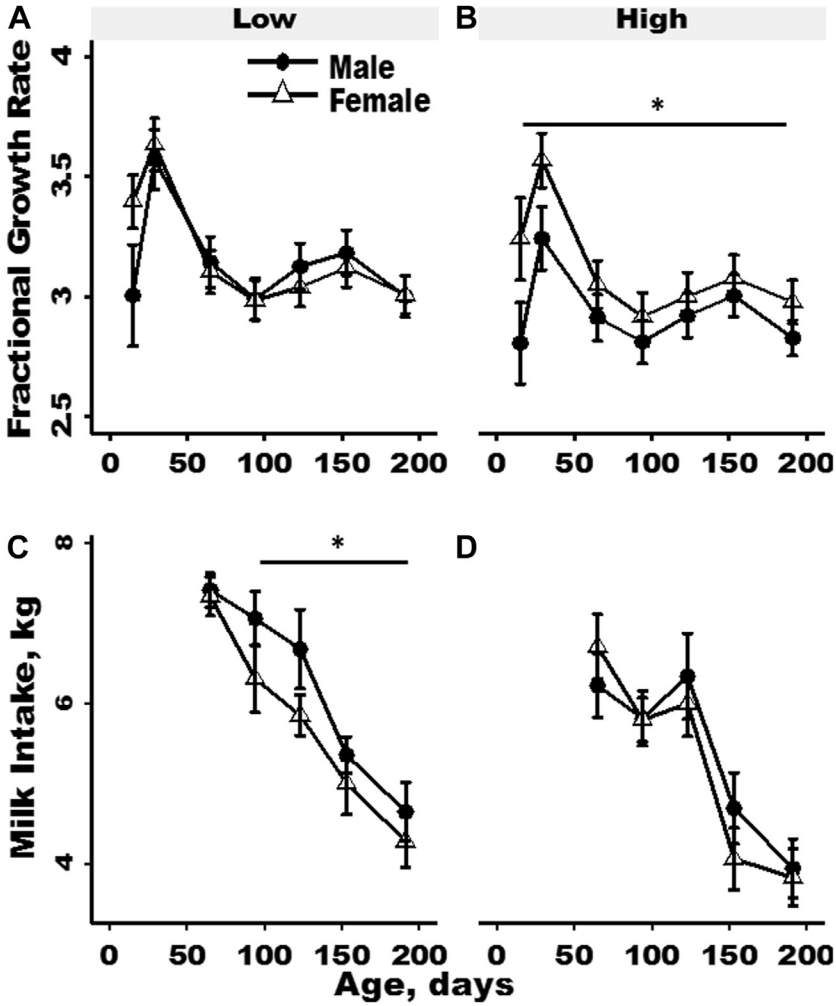

Fig. 7. Mean (± standard error of the mean) fractional growth rate (A and B) and milk intake (C and D) of male (●) and female calves (Δ) from birth to 191 days from dams receiving a low- (70%; A and C) or high- (240%; B and D) protein diet during the first trimester of gestation. *Denotes $P < 0.05$ between male and female calves after exposure to maternal diets high (fractional growth rate) or low (milk) in protein during 1T.

concentrations in male and estrone sulfate in both sexes at the end of the first trimester of gestation. Because the fetal thyroid gland differentiates between 75 and 90 gd, there was a window of opportunity for maternal protein intake during early gestation to have affected fetal thyroid development and thus the reset of the physiology of the HPT axis, should the adaptive ability of the maternal compartment not have been sufficient to protect against the external nutritional environment. Interestingly in the rat, such protein restriction reduces placental nutritional transport, even before fetal growth retardation [38].

4.1. Nutrition

As cotton seed meal was a major constituent of the high-protein diets, but was excluded from the low-protein diets, the gossypol levels were measured (Table 1). Previous studies have found a negative effect of gossypol on embryo cleavage and development and fetal skeletal development and calcium metabolism [39]. In our study, we consider that the comparatively low level of gossypol measured in the diets used (0.053% of dry matter) was unlikely to have had any detrimental effects to fetal development because there was no difference in conception rate between high- and low-protein dietary groups to suggest any associated embryonic loss and there were no detrimental effects to measure fetal skeletal development or calf skeletal measures at birth from feeding high-protein diets.

The high and low planes of maternal nutrition differed in both CP (3.3–3.6 fold) and energy content (1.2–1.3 fold), with both being above the recommended National Research Council energy requirements for heifers in the first and second trimesters. Therefore, the difference in CP between the high and low diets was much greater than that of energy. The degradable intake protein balance for the high and low planes of nutrition during the first trimester was 206 g/day and −345 g/day, respectively, and during the second trimester, 214 g/day and −464 g/day, respectively.

The negative degradable intake protein balance for the low plane of nutrition in both the first and second trimesters clearly reports that protein intake was restricted. Protein intake, especially arginine, is a determinant of placental angiogenesis and fetal growth [40]. We therefore consider that effects of maternal nutrition during the first and second trimesters of gestation on plasma TH in this study reflect the effects of protein intake rather than energy. The presence of a small amount of additional energy in high-protein diets was also a feature of the study by Larson et al. (2009).

4.2. Effect of maternal nutrient intake on plasma THs

The relatively increased FT3, the biologically active form of T3 [41], in calves exposed to low levels of maternal protein in T2 may have resulted from increased prenatal glucocorticoid–stimulated conversion of T4 to T3 [42], changes to TH binding proteins, or both. In the neonate, nonshivering thermogenesis is provided by brown adipose tissue and regulated by uncoupling protein-1 [43]. Expression of uncoupling protein-1 is upregulated by T3 [44] hence greater relative FT3 at birth may reflect a compensatory response to enhance the thermogenic capacity of these lower birth weight calves. Such a response is observed in rats born to mothers fed low-protein diets during gestation and includes enhanced thermogenesis, elevated core body temperature and basal metabolic rate [45]. This would be consistent with our previous suggestion that the increased expression of *IGF2* mRNA in the perirenal adipose tissue of these lighter birth weight calves was evidence of a compensatory response by the fetus to promote adipocyte development and thus thermogenic capacity [25]. Because receptor-saturating levels of T3 are required to induce the thermogenic potential of brown adipose tissue [46], increased ratio of FT3 to TT3 in these lighter birth weight calves could have resulted from increased prenatal glucocorticoid–stimulated conversion of T4 to T3 [42] without a concurrent change to TH-binding proteins. Furthermore, as per previous studies in sheep [17,47], the relatively increased FT3 may have contributed to the "catch-up growth" of these low-birth-weight calves by 65 days [21] because FT3 was positively correlated with ADG and FGR during this time.

Male progeny exposed to maternal diets low in nutrients during the first trimester of gestation had increased plasma concentrations of FT4 at birth subsequent to their dams having lower plasma leptin at 271 gd. Because leptin positively regulates TT4 *via* thyrotropin releasing hormone the most likely reason for greater FT4 is due to a reduction in T4-binding proteins; however, these were not measured because of financial constraints. Maternal leptin crosses the placenta during the late gestation and thus may affect development of the fetal hypothalamic regulatory network [48] and subsequent regulation of postnatal appetite. Interestingly, these same progenies had greater milk intake and higher FGR during the latter half of the preweaning period, an effect similar to that observed for intrauterine growth–restricted lambs [49]. Previously, we considered the measure of milk intake to reflect differences in milk production rather than differences in calf appetite. Recently, however, we have established that calves born to heifers fed low-protein diets during early gestational diets spend longer suckling their dam (Perry et al., unpublished data) and that this would be sufficient to increase milk output [50]. A similar hyperphagic state associated with low levels of leptin has been reported in male rats born to mothers fed low-protein diets during gestation [45]. Because this cohort of animals went on to be heavier once maternal differences in milk production were eliminated during the postweaning phase, further support is given to the distinct possibility that exposure to low concentrations of leptin in maternal circulation during the late gestation may have upregulated their appetite regulatory network *in utero*. Increased gut fill may in part explain why the positive effect of low-protein maternal diets fed during the first trimester of gestation on live weight of male progeny was lost at slaughter and not reflected in differences in carcass weight.

4.3. Postnatal ontogeny of THs

A prior study in cattle reports a breed-specific positive association between calf birth weight and circulating concentrations of TH [51], but such an association was not found in this study, perhaps influenced by the previously described dietary effect on birth weight in these animals [20] in which high protein in 2T increased birth weight. The decline in plasma TT4 after birth was consistent with the results of previous bovine studies [47,51]; however, the increase in TT3 was not. It is known that the prepartum surge in cortisol stimulates the deiodination of T4 to T3; however, this effect does not persist beyond the first day of postnatal life [51]. The surge in TT3, FT3, and FT4 at 29 days compared with plasma concentrations at birth may simply reflect the sensitivity of TH concentrations to nutrient supply and energy intake [52].

4.4. Conclusions

The present study has reported for the first time to our knowledge that postnatal catch-up growth in the *bovine* is partly attributable to a relative increase in FT3 in progeny plasma at birth, and we propose that this may form part of a greater mechanism to increase neonatal survival *via* enhanced thermogenesis. We have also shown that male progenies are particularly susceptible to exposure to a low-protein maternal diet during the first trimester of gestation from as early as 39 gd, and suggests that maternally derived leptin may act on the developing fetal hypothalamic regulatory network during the late gestation to stimulate postnatal appetite resulting in an enhanced growth pathway of the progeny. These significant findings are being further investigated with larger number of cattle in each cohort with preliminary reports validating the sexual dimorphic response to dietary perturbation reported here [53].

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