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Vitamin A–Enriched Diet at Late Gestation Affects Intramuscular Fat Deposition in Beef Offspring

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Abstract: We aimed to assess the impact of vitamin A supplementation in beef cows at late gestation on intramuscular adipogenesis in the offspring. Thirty pregnant beef cows were randomly assigned to a control group (CON; $n = 15$) fed a diet containing 4.1 KIU of vitamin A per kilogram, and to a vitamin A–supplemented group (VITA; $n = 15$) fed a diet containing 12.2 KIU of vitamin A per kilogram. The treatment application occurred from 180 d of gestation until parturition. Calves were biopsied within 10 d of age to collect skeletal muscle samples for assessing gene expression and protein abundance of target genes/proteins related to adipogenesis. All calves were raised under the same conditions until slaughter at 479 ± 1.6 d of age. Cows from both treatments showed no differences ($P > 0.05$) in total gain and final body weight, although CON cows tended to exhibit greater ($P = 0.06$) dry matter intake. Skeletal muscle of newborns from the VITA group exhibited increased mRNA expression of retinoic acid receptor β (*RARβ*; $P = 0.05$), whereas no differences ($P > 0.05$) were observed in mRNA expression of markers for fibroadipogenic progenitor cells (*PDGFRα*) and adipogenesis (*ZFP423* and *PPARγ*). However, skeletal muscle of newborns from the VITA group showed greater protein abundance of DLK1 ($P < 0.01$) and PPARγ ($P = 0.03$) than those from the CON group. No differences ($P > 0.05$) among treatments were observed in the abundance of retinoid X receptor and PDGFRα. Repeated carcass ultrasound measurements of the offspring showed increased intramuscular fat content throughout all the evaluated stages of their post-natal life ($P < 0.05$), while no changes were observed within subcutaneous fat measurements ($P > 0.05$). Hot carcass weight, carcass yield and dressing percentage, and kidney pelvic heart (KPH) fat percentage were not affected by treatment ($P > 0.05$). These findings suggest that vitamin A supplementation during late gestation enhances intramuscular adipogenesis in offspring.

Key words: adipogenesis, early life development, fetal programming, skeletal muscle, marbling
Meat and Muscle Biology 8(1): 17646, 1–12 (2024) **doi:10.22175/mmb.17646**
Submitted 23 December 2023 Accepted 5 April 2024

Introduction

Intramuscular adipose tissue, commonly known as marbling, plays a pivotal role in enhancing both the tenderness and flavor of meat (Dodson et al., 2010). Its accumulation is influenced by the extent of intramuscular adipocyte hyperplasia and hypertrophy (Harris et al., 2018). Increased adipocyte hyperplasia leads to an augmented number of sites for lipid deposition, thus contributing to cell hypertrophy later in life and consequently increasing marbling (Harris et al., 2018). Therefore, approaches that increase intramuscular adipocyte hyperplasia may be an effective way to enhance the efficiency of marbling deposition later in life.

The developmental process of skeletal muscle in utero involves the interplay of myogenesis, adipogenesis, and fibrogenesis, originating from a shared

pool of mesenchymal progenitor cells (Du et al., 2010; Santos et al., 2022). These processes are intricately regulated, being nutritionally influenced pre- and postnatally (Du et al., 2013; Costa et al., 2021). Studies have highlighted that nutritional interventions during late gestation (Duarte et al., 2014; Moisá et al., 2015; Costa et al., 2022) and in the neonatal stage (Santos et al., 2023) can modify intramuscular adipogenesis. Collectively, these studies suggest that early life may be a strategic timepoint of development to trigger the differentiation of mesenchymal progenitor cells to undergo adipogenic lineage, thus increasing the intramuscular fat cell population.

In this sense, vitamin A has been suggested as a potential feed nutrient that can be used to help improve intramuscular adipogenesis at early stages of life (Harris et al, 2018; Maciel et al., 2022). Retinoic acid, a principal metabolite derived from vitamin A, functions as a ligand, exhibiting its molecular influence through binding to specific receptors (Wang et al., 2018). These receptors include retinoic acid receptors (RARα, RARβ, and RARγ) and retinoid X receptors (RXRα, RXRβ, and RXRγ) (Chambon, 1996). The binding of retinoic acid with these receptors initiates a cascade of molecular events, where the retinoic acid–receptor complex binds to retinoic acid response elements located on target gene loci (de The et al., 1990). This interaction ultimately regulates the expression of genes associated with various cellular processes, including adipogenesis (Wang et al., 2016). Moreover, it has been shown that retinoic acid promotes adipogenic commitment (Dani et al., 1997; Bost et al., 2002) and the expression of key factors involved in adipogenesis such as Zfp423, PPARγ, and FABP4 (Reichert et al., 2011). In livestock, vitamin A administration at birth enhanced marbling content in beef cattle (Harris et al., 2018; Maciel et al., 2022; Yu et al., 2022) being associated with increased expression of Zfp423 (Harris et al., 2018) and angiogenesis (Maciel et al., 2022; Yu et al., 2022). In mice, vitamin A supplementation during pregnancy was able to increase the population of fibroadipogenic progenitor cells ($PDGFR\alpha^{+}$) and beige adipogenesis in the offspring (Wang et al., 2017a).

Therefore, we hypothesized that maternal supplementation of vitamin A during late gestation affects intramuscular fat development in the offspring and ultimately increases marbling deposition at slaughter. The objective of this study was to evaluate the effects of vitamin A–enriched diet at late gestation on early intramuscular adipogenesis and marbling deposition in the offspring.

Material and Methods

Animal care protocol

The experiment was divided into 2 phases. The first phase refers to a gestational feeding trial conducted from December 2021 to March 2022. The second phase refers to the postnatal development of calves from birth to slaughter (March 2022–August 2023). Animal trials were conducted the Ontario Beef Research Centre (University of Guelph, Guelph, ON) and the slaughters at University of Guelph Meat Laboratory in accordance with Canadian Council of Animal Care guidelines and approved by the University of Guelph (Guelph, ON) Animal Care Council (Animal Utilization Protocol #4730).

Late gestation feeding trial

A total of 30 Angus-Simmental cross bred multiparous cows averaging 705 ± 12 kg of body weight (BW) and 12 ± 0.8 mm of subcutaneous fat thickness were used. All cows were from the Ontario Beef Research Centre (University of Guelph, Guelph, ON), and underwent to a fixed-time artificial insemination using 8 sires with similar genetic merit). Only cows pregnant with male calves were used to avoid confounding effects with between the dietary treatments and calf sex. From insemination to 180 d of gestation, all cows were managed as a single herd and fed the same diet. Cows were housed in pens (5.9 × 13.4 m^2; $n = 6$ cows/pen) and all animals within a pen had access to 3 Insentec feed bunks (Hokofarm Group, Marknesse, Netherlands). At 180 d of gestation cows were randomly assigned to one of 2 experimental treatments: CONTROL, where cows were fed a basal diet with 4.1 KIU of vitamin A/kg; and VITA, where cows were fed the same basal diet enriched with pure vitamin A to 12.2 KIU of vitamin A/kg (Table 1). Vitamin A was sourced from DSM (ROVIMIX®) and its inclusion rate was chosen based on the pre-existing level of vitamin A in the control diet and supplementing 3 times the amount of vitamin A in the supplemented group ensuring a substantial disparity between the 2 groups, as well as to compensate the losses due to microbial metabolism as suggested by Rode et al. (1990). Rations were formulated according to NASEM (2016) and cows were fed at 100% maintenance requirements for pregnant mature beef cows to avoid confounding effects between feed intake and vitamin A supplementation. Cattle were weighed every 28 d, and the BW was used to adjust the feed intake, dry matter

Table 1. Ingredients and chemical composition of the experimental diets

	Vitamin A	Control
Ingredient Composition		
Straw, %	35.7	35.7
Rye triticale silage, %	62.6	62.6
Mineral mixture,[1] %	0	1.7
Vitamin A–enriched mineral mixture,[2] %	1.7	0
Chemical Composition		
Dry matter, %	52.9	52.9
Crude protein, %	7.7	7.7
Total digestible nutrients, %	51.8	51.8
Vitamin A, KIU/kg	12.2	4.1

[1]Soy hulls–21.8%; wheat shorts–20.7%; fine salt–18.5%; monocalcium phosphate–15.7%; limestone calcium bicarbonate–7.9%; magnesium oxide–5.2%; sulphur–2.8%; vitamin E–2.2%; tallow–2.1%; FFM ruminant micro PRX–1.5%; Availa ZN 120–0.75%; selenium 200–0.54%; organic selenium 0.23%.

[2]Soy hulls–21.8%; wheat shorts–20.7%; fine salt–18.5%; monocalcium phosphate–15.7%; limestone calcium bicarbonate–7.9%; magnesium oxide–5.2%; sulphur–2.8%; vitamin E–2.2%; tallow–2.1%; FFM ruminant micro PRX–1.5%; Availa ZN 120–0.75%; vitamin A 50 000 GVF–0.58%; selenium 200–0.54%; organic selenium 0.23%.

intake (DMI) allowance was 1.7% of BW. The feed intake data was cleaned using the IntakeInspectR package, version 2.1.4 Innes et al., (2023). Animals from both groups were fed the experimental diets from 180 d of gestation until parturition. After parturition, the cow-calf pair was managed as a single herd and kept under the same dietary and environmental conditions until weaning.

Newborn skeletal muscle tissue sampling

At 10 d of age, calves were submitted to a muscle biopsy sampling, and tissue was collected from the right side of *Longissimus* muscle located between the 12th and 13th ribs. The biopsy site was initially shaved and sanitized with 4% chlorohexidine and 70%-ethanol alcohol. Following the sanitization, the biopsy site was anesthetized with 2% lidocaine HCl (6 mL). A 4 cm incision was then created using a sterile blade 10 scalpel across the skin and subcutaneous fat (when present). Next, muscle tissue was excised by using an 8-mm biopsy punch and rinsed with sterile phosphate-buffered saline solution. Visible connective (epimysium) and subcutaneous fat tissue (when present) were trimmed off, and the muscle sample was immediately snap-frozen, powdered in liquid nitrogen, and then stored at −80°C until analysis of gene expression and protein abundance of intramuscular adipogenesis markers.

RNA extraction and gene expression analysis

Total RNA was extracted from 0.1 g of tissue using Trizol® (InvitrogenTM, Thermo Fisher Scientific®, Oregon, USA) following the manufacture's recommendations. The total RNA was quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific®, Waltham, MA, USA), ensuring an optimal 260/280 ratio between 1.8 and 2.0, and the integrity was assessed in a 1% agarose gel. The RNA samples were reverse transcribed into cDNA using the GoScriptTM Reverse Transcription System Kit (Promega, Madison, WI, USA). The primers (Table 2) for amplification of target and endogenous genes were designed using PrimerQuest Software (PrimerQuest–design qPCR assays | IDT [idtdna.com]) with sequences obtained from GenBank (GenBank Overview [nih.gov]). Real-time quantitative PCR was performed in the thermal cycler QuantStudio 3 (Applied Biosystems, Foster City, CA, USA) using the SYBR Green detection method (Applied Biosystems, Foster City, CA, USA) and GoTaq® qPCR Master Mix Kit (Promega, Madison, WI, USA). Results are expressed relative to GAPDH and were calculated according to the methods described by Pffafl (2001).

Protein extraction and Western blotting analysis

Total protein was extracted from 0.1 g of tissue in 1 mL of lysis buffer (10 mM of Tris HCl [pH 7.6], 150 mM of NaCl, 1% of Triton X-100, 0.5% sodium

Table 2. List primers for relative mRNA expression by RT-qPCR

Gene Symbol	NCBI Accession Number	Primer
RARγ	NM_001130756.1	F: TCTTAGCACTGCCCCTCAGA
		R: ATGGCCCCCTTTTTGGTCTC
RARβ	XM_024986387.1	F: TACAGGCTTTTAGCTGGCTTGT
		R: TCCACCCAACTCCATCAAACTC
DLK1	NM_174037.2	F: TCCGCTGCCGTTGCC
		R: TGCCGCCGTTGAGGC
PDGFRα	NM_001192345.3	F: GGCAGGTGATGCTTTGGGA
		R: GGTGGGGCTTGCATTGAGA
ZFP423	NM_001101893.1	F: GCGACTACTGTGAGGACACG
		R: CTCGTCGACAAAGACCTCGG
C/EBPα	NM_176784.2	F: GCCCCCGATGAGCAGC
		R: GATGCCGCCCAGAGGC
PPARγ	NM_181024.2	F: ACTTTGGGATCAGCTCCGTG
		R: AACCATCGGGTCAGCTCTTG
GAPDH	NM_001034034.2	F: CGTGTCTGTTGTGGATCTG
		R: GTCCTCAGTGTAGCCTAGAA

Table 3. List of antibodies used in Western blotting analysis

Antibody	Source	Dilution	Manufacturer	Catalog Number
Primary Antibodies				
PGDFRα	Rabbit Polyclonal IgG	1:2000	Thermo Fisher Scientific®	500–2694
DLK1	Mouse Monoclonal IgG	1:2000	Thermo Fisher Scientific®	MA515915
RXRα	Rabbit Polyclonal IgG	1:2000	Thermo Fisher Scientific®	PA585395
PPARγ	Rabbit Polyclonal IgG	1:2000	Thermo Fisher Scientific®	PA3821A
α-tubulin	Mouse Monoclonal IgG	1:5000	Thermo Fisher Scientific®	200–301–880
Secondary Antibodies				
HRP	Goat anti-Mouse IgG	1:5000	Thermo Fisher Scientific®	31430
HRP	Goat anti-Rabbit IgG	1:5000	Thermo Fisher Scientific®	A16096

deoxycholate, 1% sodium dodecyl sulfate [SDS], and 1% of protease inhibitor cocktail mammalian cells and tissues [Sigma-Aldrich®]), and the lysate was sonicated. Total protein content was estimated using the Bradford protein assay (Bio-Rad, Hercules, CA, USA) and stored at −80°C. The proteins were separated using a 10% SDS-PAGE gel loaded with 40 µg of protein per sample, transferred to a 0.45 µm Nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) using a semi-dry Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, CA, USA), and blocked for 1 h at room temperature with 2% bovine serum albumin (BSA, Sigma Aldrich®) in 1x Tris-Buffered Saline (TBS1x; 50mM Tris-HCL, pH 7.5; 150mM NaCl; Sigma Aldrich®). Subsequently, the membranes were incubated for 12 h at 4°C with the primary antibodies (Table 3) diluted in blocking solution. After 12 h of incubation, membranes were washed 3 times for 15 min with Tris Buffered Saline and 0.1% Tween® (TBSt) and incubated with the secondary antibody (Table 3) diluted in blocking solution for 1 h at room temperature. Membranes were then washed with TBSt, revealed by ECL Plus Western Blotting Detection System (GE HealthCare, Buckinghamshire, Ul) and the images generated by ChemiDoc XRS+ System (Bio-Rad, Hercules, CA, USA). The α-tubulin was used as a housekeeping protein to normalize the abundance of the target proteins.

Offspring performance

At birth the offspring from both treatments were weighed, submitted to a castration procedure within 24 h after birth and kept with the dams under the same environmental and nutritional conditions until weaning (216 ± 0.9 d old). The birth and weaning weights were used to calculate the average daily gain (ADG) of the calves during the suckling phase, BW gain from birth to weaning divided by days of age at weaning. During the suckling phase, only cows had access to feed via the Insentec bins while calves only consumed their dam's milk. After weaning, the steers were managed as a single herd and fed the same growing diet composed of straw (16.5%), hay (31.9%), corn silage (9.9%), haylage (38.5%), and mineral mixture (3.2%) for 100 d. The weaning weight and the initial BW at the finishing phase were used to calculate the ADG during the growing phase. At the end of the growing phase, steers were moved to finishing barn of Ontario Beef Research Centre (University of Guelph, Guelph, ON) and were fed the same finishing diet composed of corn silage (14%), high moisture corn (56%), corn grain (20%), soybean meal (8%), and mineral mixture (2%) for 90 d prior to slaughter (479 ± 1.6 d of age). Steers were housed in pens (5.9 × 13.4 m^2; $n = 6$ steers/pen) with access to 3 Insentec feed bunks within their pen allowing monitoring of their individual DMI. The feed intake data was cleaned using the IntakeInspectR package, version 2.1.4 (Innes et al., 2024).

Carcass ultrasound evaluation of the offspring

Carcass ultrasound measurements of the offspring were taken at approximately 280, 330, 380, 410, 440, and 480 d of age. The measurements of the ribeye area and subcutaneous fat thickness were taken at *Longissimus dorsi* muscle between the 12th and 13th rib. A second measurement of subcutaneous fat was taken at the rump. Carcass subcutaneous fat thickness resulted from the average of measurements taken at *Longissimus dorsi* muscle and at the rump. Intramuscular fat percentage was also measured by ultrasound using a minimum of 4 independent images collected across the 11th to 13th ribs at a lateral position the animal's midline at a point three-fourths of the distance from the medial end of the *Longissimus dorsi* muscle. All images were

collected by using an ExaGo ultrasound (IMV Imaging, Bellshil, Scotland) equipped with a linear probe with 18 cm of length. Images were analyzed by using the Centralized Ultrasound Processing Lab (CUP LAB, Ames, IA, US) software (2023 version).

Offspring slaughter and meat sampling

At the end of the finishing phase, steers were slaughtered at the Canadian Food Inspection Agency (CFIA) inspected Meat Science Laboratory of the University of Guelph. Due to the capacity of the meat science laboratory, the animals were not slaughtered on the same day. However, the number of animals per treatment was balanced across slaughtering dates to avoid confounding effects with the experimental treatments. Steers were humanely handled and slaughtered (via captive bolt stunning, followed by exsanguination) using commercial industry standards under the inspection of CFIA agent. At the end of the slaughtering process, each carcass was split, and each side of the carcasses was chilled intact at 4°C. After 48-h postmortem chill period, the *longissimus* muscle area (LMA) was measured at the 12th rib on the left side of each carcass. *Longissimus* muscle areas were traced on transparencies and measured later with a planimeter. Next, *longissimus thoracis* rib portions anterior to the 12th and 13th rib interface was collected and had their position tracked until subsampling into steaks. The *Longissimus thoracis* samples were then cut into 2.5-cm thick steaks beginning at the posterior end (12th/13th rib interface) being the first steak (closest to the 12th/13th rib interface) used for instrumental tenderness assessment. Steaks were vacuum packaged and aged for 14 d at 4°C, then frozen prior to instrumental tenderness analysis.

Instrumental tenderness analysis

Steaks were thawed before being cooked to an internal temperature of 72 °C on a clamshell Garland Grill (Ed-30B: Garland Commercial Ranges LTD, Mississauga, Canada) set to a surface temperature of 105°C. Following cooking, samples were cooled in a refrigerator to an internal temperature of approximately 4°C. Eight 1.25-cm diameter cores running parallel to the muscle fibers were removed from each steak. Each core was sheared perpendicularly to the muscle fibers with a Warner–Bratzler blade using a TA-XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, USA) at a crosshead speed of 3.3 mm/s. The average peak force was recorded for each steak.

Statistical analysis

The data measured were analyzed according to the following base linear mixed model:

$$Y_{ikl} = \mu + D_i + S_k + b_1 iBW_{ikl} + b_2 iSFT_{ikl} + e_{ikl} \quad [Eq.\ 1]$$

where Y_{ikl} is the observed value; μ is the intercept; D_i is the fixed effect of the i^{th} level of Maternal dietary treatment, with $i = 1, 2$; S_k is the random effect of the k^{th} Sire, with $k = 1 \ldots 8$, assuming $S \sim N(0, \boldsymbol{I}\sigma_s^2)$, where $\boldsymbol{I}$ represents the identity matrix; b_1 is the partial regression coefficient for the covariate initial body weight (*iBW*) of the cow, where iBW_{ikl} represents the iBW of the l^{th} cow; b_2 is the partial regression coefficient for the covariate initial subcutaneous fat thickness (*iSFT*) of the cow, where $iSFT_{ikl}$ represents the iSFT of the l^{th} cow; and e_{ikl} is the random error associated with y_{ikl}, assuming $e \sim N(0, \boldsymbol{I}\sigma_e^2)$.

For traits measured at finishing and slaughter, an additional covariate was included in Equation [1] to account for the time the calf spent at these periods, respectively. For traits measured multiple times across time in the calves, the following mixed model was used:

$$Y_{ijkl} = \mu + D_i + T_j + (D * T)_{ij} + S_k + b_1 iBW_{ikl} + b_2 iSFT_{ikl} + b_3 wAge_{ikl} + e_{ijkl} \quad [Eq.\ 2]$$

Where all variables are as described in Eq. [1], except for T_j that is the fixed effect of the j^{th} level of time-point of measurement, with $j = 1 \ldots 6$; $(D * T)_{ij}$ is the fixed effect interaction between the i^{th} level of Maternal dietary treatment and the j^{th} level of time-point of measurement; b_3 is the partial regression coefficient for the covariate age of the progeny of the cow at weaning (*wAge*), where $wAge_{ikl}$ represents the wAge of the progeny of the l^{th} cow; and e_{ijkl} is the random error associated with y_{ijkl}, assuming $e \sim N(0, \boldsymbol{SP}\sigma_e^2)$, where ***SP*** represents a spatial power covariance structure, such that correlations amongst repeated records where a function of the age of the calves at each time point. Prior to final analyses, other covariance structures, such as compound symmetry, unstructured, and identity, were evaluated, but they were not used in the final analyses for showing greater Akaike information criterion values than ***SP***.

Prior to final analyses, residuals were evaluated for assumptions of the models used. For each analysis, data points were removed one at a time if assumptions were not met, such as absolute Studentized residuals greater than 3 and significant ($P < 0.01$) Shapiro-Wilk's test for normality.

Table 4. Performance of pregnant beef cows fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition

	Experimental Treatment		*P* Value
	Control	Vitamin A	
Initial body weight, kg	690 ± 22.2	711 ± 22.0	0.34
Initial subcutaneous fat thickness, mm	11.6 ± 1.4	11.8 ± 1.3	0.91
Initial ribeye area, cm^2	67.6 ± 1.7	67.6 ± 1.5	0.97
Final body weight, kg	710 ± 7.3	709 ± 7.1	0.91
Final subcutaneous fat thickness, mm	11.1 ± 0.4	10.2 ± 0.4	0.12
Final ribeye area, cm^2	66.2 ± 1.9	67.7 ± 1.7	0.57
Body weight gain, kg	5.20 ± 7.3	4.22 ± 7.1	0.91
Average dry matter intake, kg/d	9.9 ± 0.2	9.5 ± 0.1	0.06
Average vitamin A intake (KIU/d)	41.87 ± 2.3	116.79 ± 2.1	<0.01

Expected means were generated from the final models and separated using Tukey's test. All analyses were performed in R (R version 4.3.2; R Core Team, 2023), and differences were declared when $P \leq 0.05$.

Results

Animal performance

The cows from both treatments showed similar initial BW ($P = 0.28$). Despite the observed trend of increased DMI by the cows at the control group ($P = 0.06$) no differences were observed for BW gain ($P = 0.91$) and final BW ($P = 0.91$; Table 4). Moreover, vitamin A supplementation during late gestation did not influence calves' birth weight ($P = 0.62$), weaning weight ($P = 0.91$) and ADG from birth to weaning ($P = 0.41$; Table 5). Calves' performance at the backgrounding phase was not influenced by maternal treatments, final BW ($P = 0.64$), BW gain ($P = 0.21$), and ADG ($P = 0.21$) (Table 5). Furthermore, at the finishing phase, maternal supplementation with vitamin A at late gestation did not alter the offspring's final BW ($P = 0.36$), BW gain ($P = 0.95$), ADG ($P = 0.94$), and DMI ($P = 0.74$; Table 5).

mRNA expression and protein abundance of retinoic acid receptors in the skeletal muscle of calves

A greater mRNA expression of retinoic acid receptor β (*RARβ*) was observed in the skeletal muscle of the calves from the vitamin A group ($P = 0.05$; Figure 1), whereas the mRNA expression of retinoic acid receptor γ (*RARγ*; $P = 0.78$) and the protein abundance of retinoid X receptor ($P = 0.63$) were similar between treatments (Figure 1).

Table 5. Performance of the offspring born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition

	Experimental Treatment		*P* Value
	Control	Vitamin A	
Calving Phase			
Birth weight, kg	37.7 ± 1.5	38.7 ± 1.4	0.62
Weaning weight, kg	331 ± 7.9	330 ± 7.4	0.91
Average daily gain, kg/d	1.36 ± 0.03	1.33 ± 0.03	0.41
Background Phase			
Final body weight, kg	508 ± 9.7	516 ± 9.3	0.64
Average daily gain, kg/d	1.06 ± 0.03	1.10 ± 0.03	0.22
Dry matter intake, kg/d	9.29 ± 0.3	9.82 ± 0.2	0.17
Finishing Phase			
Final body weight, kg	702 ± 8.3	691 ± 7.4	0.36
Average daily gain, kg	1.92 ± 0.1	1.92 ± 0.1	0.94
Dry matter intake, kg/d	12.0 ± 0.3	12.1 ± 0.3	0.74

mRNA expression and protein abundance of fibroadipogenic and adipogenic markers in the skeletal muscle of calves

The mRNA expression ($P = 0.89$) and protein abundance ($P = 0.57$) of platelet-derived growth factor receptor alpha (*PDGFRα*) did not change between treatments (Figure 2). Similarly, the mRNA expression of *ZFP423* ($P = 0.73$) in the skeletal muscle of the calves was not affected by vitamin A supplementation of the cows at late gestation (Figure 3). However, a greater protein abundance of delta like non-canonical notch ligand 1 (DLK1; $P < 0.01$) was observed in the skeletal muscle of calves from vitamin A–supplemented cows (Figure 3). Regarding the peroxisome proliferator activated receptor (PPARγ), despite the lack of differences between treatments in mRNA expression ($P = 0.95$; Figure 4), a greater protein abundance of PPARγ was observed in calves born to dams supplemented with vitamin A at late gestation ($P = 0.03$; Figure 4).

Repeated measurements of carcass ultrasound in the offspring

Vitamin A supplementation at late gestation allowed an increase in intramuscular fat percentage in the offspring throughout all the evaluated stages

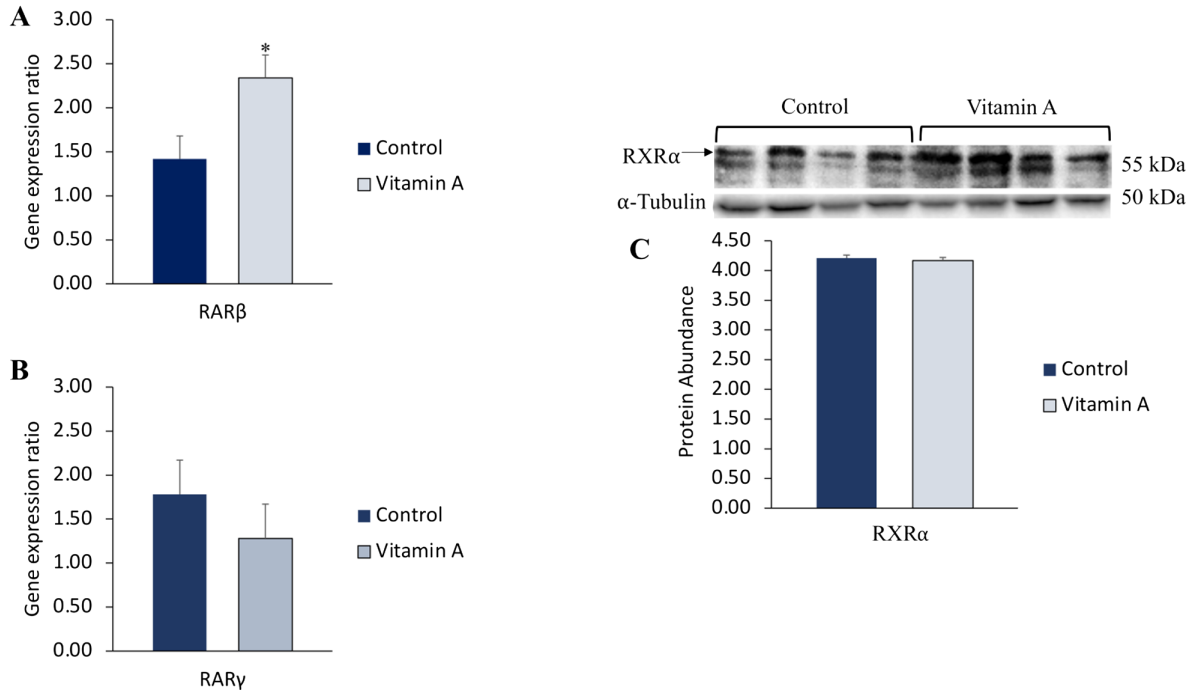

Figure 1. mRNA expression of retinoic acid receptor (RAR) β (panel A) and γ (panel B) and protein abundance of retinoid X receptor (RXR) α (panel C) in skeletal muscle of calves born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition. Differences (*) were considered when $P \leq 0.05$.

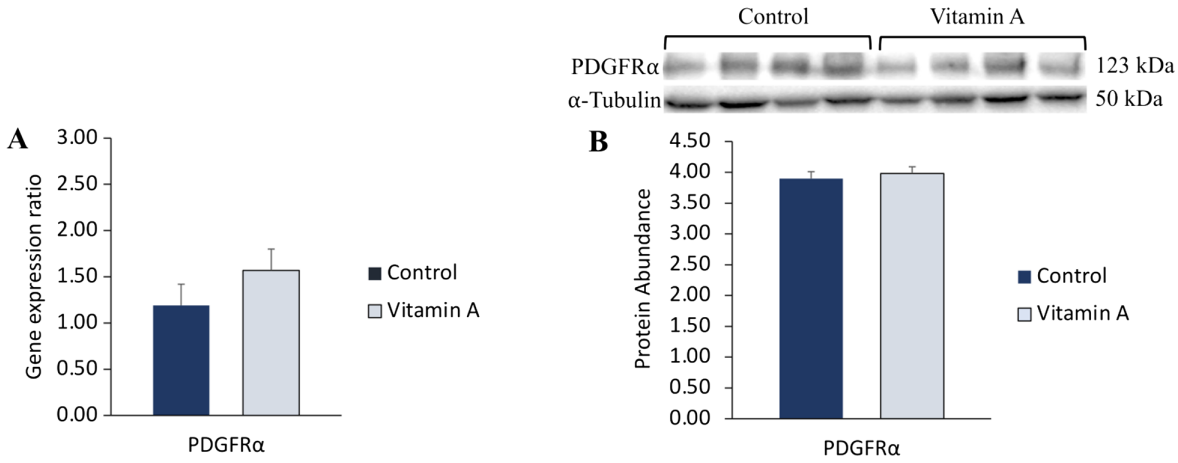

Figure 2. mRNA expression (panel A) and protein abundance (panel B) of platelet-derived growth factor α (PDGFRα) in skeletal muscle of calves born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition. Differences (*) were considered when $P \leq 0.05$.

of their post-natal life (Figure 5; $P \leq 0.05$). No effects of vitamin A supplementation at late gestation were observed in the subcutaneous fat thickness ($P > 0.05$; Figure 6) or on LMA ($P > 0.05$; Figure 7) throughout the offspring's life.

Carcass traits assessment

There were no effects of maternal vitamin A supplementation at late gestation on hot carcass weight (HCW; $P = 0.83$), and carcass dressing percentage ($P = 0.40$; Table 6). Similarly, there was no effect of vitamin A supplementation at late gestation of Kidney Pelvic Heart (KPH) fat in kilograms ($P = 0.72$) or percentage ($P = 0.69$) in the carcass of the offspring (Table 6). The carcass final temperature ($P = 0.56$) and pH_{24h} ($P = 0.89$) did not differ between calves born to dams fed the control diet or vitamin A–enriched diet (Table 6). However, beef from calves born to dams fed vitamin A–enriched diet tended to show greater

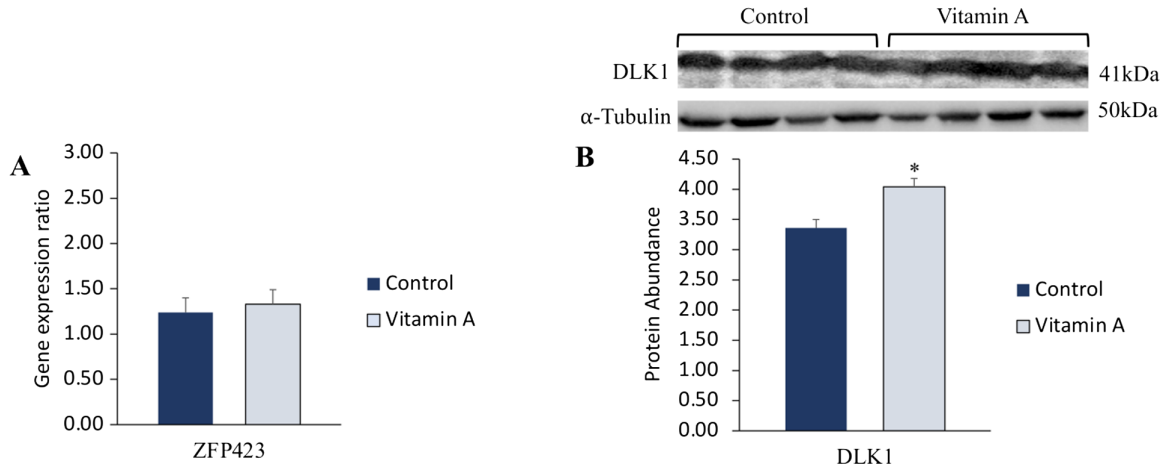

Figure 3. mRNA expression (panel A) of zinc finger protein 423 (ZFP423) and protein abundance (panel B) of delta like non-canonical notch ligand 1 (DLK1) in skeletal muscle of calves born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition. Differences (*) were considered when $P \leq 0.05$.

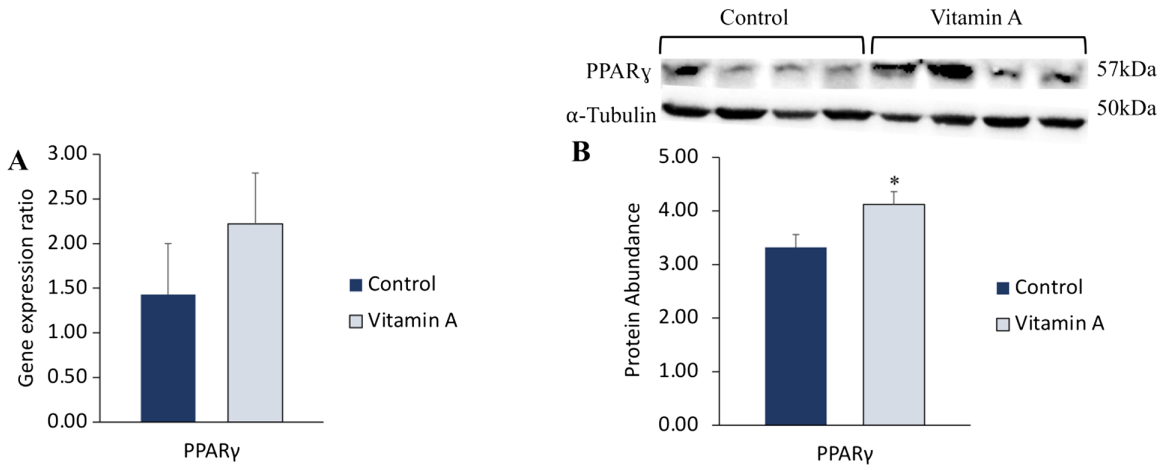

Figure 4. mRNA expression (panel A) and protein abundance (panel B) of peroxisome proliferator activated receptor gamma (PPARγ) in skeletal muscle of calves born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition. Differences (*) were considered when $P \leq 0.05$.

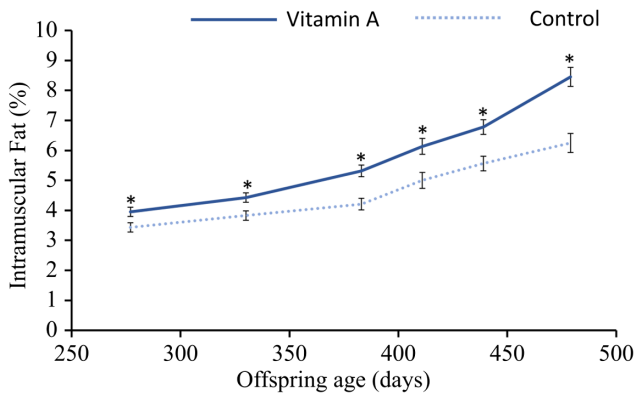

Figure 5. Intramuscular fat (IMF) percentage of offspring born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation until parturition. The %IMF was measured by ultrasound at different ages post-weaning until slaughter. Differences (*) were considered when $P \leq 0.05$.

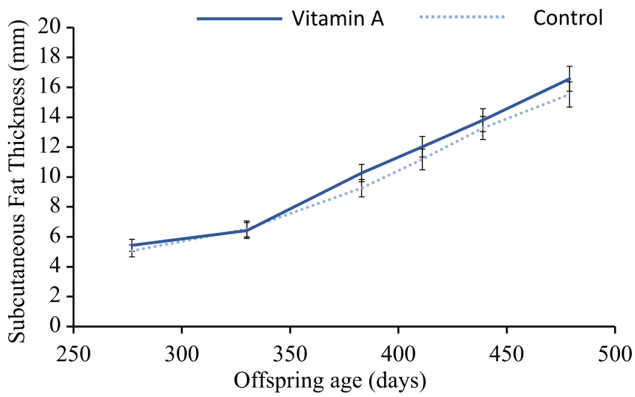

Figure 6. Subcutaneous fat thickness (SFT) of offspring born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation until parturition. The SFT was measured by ultrasound at different ages post-weaning until slaughter. Differences (*) were considered when $P \leq 0.05$.

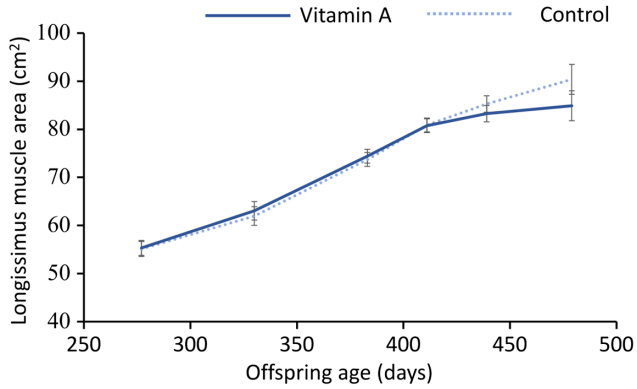

Figure 7. *Longissimus* muscle area (LMA) of the offspring born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation until parturition. The LMA was measured by ultrasound at different ages post-weaning until slaughter.

Table 6. Carcass traits and shear force of meat from the offspring born to dams fed either a control or vitamin A–enriched diet from 180 d of gestation to parturition

	Experimental Treatment		
	Control	Vitamin A	*P* Value
Hot carcass weight (kg)	397 ± 6.7	400 ± 6.4	0.83
Carcass dressing (%)	57.9 ± 0.4	57.6 ± 0.4	0.40
Carcass temperature$_{24h}$, °C	4.0 ± 0.3	4.2 ± 0.2	0.56
Carcass pH$_{24h}$	5.8 ± 0.04	5.8 ± 0.04	0.89
KPH[1] (kg)	15.2 ± 0.7	15.0 ± 0.7	0.72
KPH (%)	3.85 ± 0.2	3.72 ± 0.2	0.69
WBSF[2] (kg)	3.44 ± 0.22	4.01 ± 0.20	0.07

[1]KPH: kidney pelvic heart fat.

[2]WBSF: Warner-Bratzler shear force.

Warner-Bratzler shear force values compared to beef from calves born to dams from the control group ($P = 0.07$; Table 6).

Discussion

In the current study, our objective was to assess the impact of vitamin A supplementation administered during the third trimester of gestation on intramuscular adipogenesis. It was hypothesized that retinoic acid, derived from vitamin A metabolism, would increase intramuscular number of preadipocytes in the offspring. Consequently, our initial investigation focused on the mRNA expression of *RARγ* and *RARβ*, along with the abundance of retinoid X receptor as an indicator of availability of retinoic acid in the skeletal muscle of the offspring. Retinoic acid receptors bind with retinoid X receptors and form a heterodimer, RAR/RXR (Kurokawa et al., 1994), acting as an unliganded silent partner with retinoic acid receptor (Kurokawa et al., 1994). Notably, RARγ is highly expressed in human epithelial cells (Huang et al., 2014), while *RARβ* exhibits broader expression throughout the body (Huang et al., 2014). Our results revealed an increase in mRNA expression of *RARβ* in skeletal muscle of calves born to dams fed vitamin A–enriched diets, suggesting that maternal supplementation of vitamin A increased the level of retinoic acid in the skeletal muscle of the offspring.

Given the established role of retinoic acid in prompting adipogenic commitment (Harris et al., 2018), we speculated that the population of FAP cells would be increased due to the maternal supplementation with vitamin A, and this would be reflected by an increase in abundance of PDGFRα, a marker for FAP cells (Fitzgerald et al., 2023). A study done by Wang et al., 2017a showed that maternal vitamin A supplementation in mice increases PDGFRα progenitor cells. Contrary to what we have hypothesized, there were no differences within PDGFRα expression or protein abundance found between groups in the current study, suggesting that maternal vitamin A supplementation during late gestation may not define the population of FAP cells but may influence the further processes of adipogenesis.

Therefore, despite the lack of differences in PDGFRα, our results showed an increased in protein abundance for DLK1, a marker of preadipocytes (Gupta et al., 2010), due to vitamin A supplementation. DLK1 is a preadipocyte factor that prevents adipocyte differentiation, and during adipogenesis DLK1 expression decreases (Hudak & Sul, 2013). It has been found that retinoic acid induced the expression of DLK1 by activating CRABP-II and *RARγ* (Berry et al., 2012). During the later stages of adipocyte differentiation DLK1 is expressed is decreased. During differentiation of human fetal mesenchymal stem cells (MSC) into adipocytes, DLK1 expression rose early in the beginning of the differentiation process but decreased in the later stages of differentiation (Hudak & Sul, 2013). Increased DLK1 protein abundance indicates that maternal supplementation of vitamin A increases the development of preadipocytes within the offspring. Hence, due to the antagonism between the number of preadipocyte and mature adipocytes, the contrary abundance of DLK1 and PPARγ would be expected. Evaluating the effects of pre-weaning supplementation of heifers in a so-called marbling window, Santos et al. (2023) reported similar results, and suggested that FAP cells were being committed

and kept into the preadipocyte state. In our study, however, it appears that the state of preadipocytes were stimulated to differentiate, since the PPARγ abundance was also increased within the vitamin A group, suggesting that adipogenesis mediated by retinoic acids occurs in a more dynamic process. Mechanistically, it has been proposed that PPARγ binds with retinoid X receptor, forming a heterodimer that regulates the transcription of adipocyte target genes (Cao et al., 2017), which may explain the increase in the protein abundance of both RXRα and PPARγ in skeletal muscle of calves born to dams supplemented with vitamin A.

Due to the role of ZFP423 stimulating the expression of PPARγ and helping to induce the conversion of preadipocytes into mature adipocytes (Gupta et al., 2010), the increase in its mRNA expression in skeletal muscle of calves from the vitamin A group was expected. However, no changes were observed in mRNA expression of *ZFP423* or *PPARγ* in the skeletal muscle of calves from both treatments, suggesting that *ZFP423* in vivo may not have the same effects on adipogenesis regulation as it does in vitro (Gupta et al., 2010). There is a very close association between maternal nutrition and DNA methylation levels of *ZFP423* promoter, which consequently influence the extension of FAP cells determination (Yang et al., 2013). It was demonstrated that retinoic acids derived from vitamin A may disrupt the DNA demethylation in the promoter of *ZFP423* (Wang et al., 2017b). Moreover, Kim et al. (2019) showed that the positive or negative regulation of adipogenesis and cell proliferation in 3T3-L1 cell lineage are vitamin A dose-dependent. Collectively, our results suggest that retinoic acid did not impact the commitment of mesenchymal stem cells into FAP cells, however it did impact the commitment of FAP cells to undergo to adipogenesis.

Indeed, repeated ultrasound images taken throughout the offspring's life showed increased intramuscular fat deposition within the vitamin A group. During the early stages of life, there was increased production of preadipocyte and mature adipocyte cells, providing increased "area" for intramuscular fat development. Moreover, the offspring had significantly increased intramuscular fat deposition without increased visceral and subcutaneous fat. These results agree with the marbling window concept, the time period during early life development in which muscle and adipose tissue develop (Du et al., 2013), which allowed the increase in intramuscular fat deposition, without increasing other fat depots. Adipocyte hyperplasia begins around mid-gestation, and although this process is not limited to the pre-natal stages, hyperplasia potential decreases over time (Du et al., 2013). Moreover, fat deposition in various fat depots is dynamic and develops differently and is heavily regulated by internal signaling. Subcutaneous fat depots develop and fill with lipid first, followed by the other fat depots (Hausman et al., 2014). Furthermore, it has been shown that intramuscular fat depots are smaller and hypertrophied much less than subcutaneous fat and perirenal fats (Hausman et al., 2014), probably due to the reduced capacity of energy transfer compared with larger adipocytes from subcutaneous or visceral fat depots (Louveau et al., 2016). Nevertheless, similar to our results, the use of vitamin A at a young age in beef cattle has the potential to effectively increase marbling. Injections of vitamin A in beef calves increased intramuscular adipocytes and improved marbling without increasing the overall carcass fatness (Maciel et al., 2022; Yu et al., 2022). Vitamin A–treated animals also had more triglycerides within the *longissimus* muscle compared to the control (Yu et al., 2022), and the size of the subcutaneous fat cells was smaller within the vitamin A steers (Yu et al., 2022). Thus, the resulting progeny from maternal vitamin A supplementation during late gestation was able to produce high-quality beef without the production of excess waste fat. These findings are critical to improving beef production, as the propagation potential of intramuscular preadipocytes is lower compared to subcutaneous preadipocytes (Wan et al., 2009).

Regarding carcass characteristics and meat tenderness, maternal vitamin A supplementation at late gestation had no effects on offspring's HCW, carcass width, length, carcass yield, temperature, pH, and KPH. However, steaks from calves whose dams were supplemented vitamin A had greater values of Warner-Bratzler shear force (3.97 kg compared to the control group's 3.47 kg). Although meat tenderness is a multi-parameter sensory that is mainly influenced by the combination of collagen fibrils and intermolecular cross-linking in the connective tissue (Zhao et al., 2019), the intramuscular fat content may indirectly account for the establishment of this parameter. This occurs due to the disarrangement that intramuscular fat causes in the structure of intramuscular connective tissue, contributing to the increase in meat tenderness (Hocquette et al., 2010). Nevertheless, this mechanism essentially occurs in breeds with high marbled meat, where collagen is a source of variation in tenderness (Hocquette et al., 2010). However, beyond the differences between treatments, both groups have shear force values that are considered tender (Destefanis et al., 2008).

Conclusions

In summary, our findings indicate that supplementing vitamin A during late gestation enhances the early development of intramuscular fat in offspring. This effect is likely attributed to an increased commitment of FAP cells to the adipogenic lineage. The higher population of intramuscular preadipocytes at birth was associated with a consistently greater percentage of intramuscular fat throughout the animal's life, while no differences were observed in fat deposition in other areas of the carcass. To comprehensively understand the underlying mechanisms, further investigation into the impact of vitamin A on the commitment of bovine intramuscular FAP cells to adipogenesis is warranted.

Acknowledgments

We thank the Beef Cattle Research Council (BCRC; Grant Number POC.14.20), Ontario Ministry of Agriculture and Rural Affairs (OMAFRA; Grant Number 101829), and Natural Sciences and Engineering Council of Canada (NSERC; Grant Number 401862) for the funding support. We also thank the team of the Ontario Beef Research Centre (OBRC) and the Meat Science Laboratory of the University of Guelph for support on the data and sampling acquisition.

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