Effects of conjugated linoleic acids on prostaglandin secretion by bovine endometrial epithelial cells in vitro

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Objective—To determine the effects of 2 conjugated linoleic acid (CLA) isomers (cis-9, trans-11 and trans-10, cis-12) on synthesis of prostaglandin (PG) E_2 and $F_{2\alpha}$ and expression of prostaglandin H synthase-2 (PGHS-2) of adult and fetal bovine endometrial epithelial cells in vitro.

Sample—Primary cultures of endometrial epithelial cells obtained from 4 adult cows and 4 fetal bovine carcasses.

Procedures—Cells were exposed to 0, 50, 100, or 200µM cis-9, trans-11 or trans-10, cis-12 CLA isomers for 24 hours. Culture media collected before and after 6 hours of stimulation of cells with phorbol 12-myristate 13-acetate were assayed to detect PGE_2 and $PGF_{2\alpha}$ via ELISA. After stimulation, cells were collected for western blot analysis to quantify PGHS-2.

Results—Concentrations of $PGF_{2\alpha}$ and PGE_2 were significantly lower in culture media of adult and fetal endometrial epithelial cells exposed to any concentration of either CLA than they were in media of cells not exposed to CLAs. The trans-10, cis-12 CLA isomer seemed to decrease PG production more markedly than did the cis-9, trans-11 CLA isomer. Most concentrations of both CLAs significantly reduced culture media PGE_2:$PGF_{2\alpha}$ concentration ratios of cells. Exposure of cells to CLAs did not affect expression of PGHS-2 protein.

Conclusions and Clinical Relevance—Results of this study indicated CLAs significantly decreased $PGF_{2\alpha}$ and PGE_2 concentrations and PGE_2:$PGF_{2\alpha}$ concentration ratios for cultures of adult and fetal endometrial epithelial cells with no apparent effect on PGHS-2 expression. Similar effects in cows could have effects on maternal recognition of pregnancy and immune function. (*Am J Vet Res* 2013;74:491–498)

Conjugated linoleic acid is the general term for a group of positional and geometric isomers of the omega-6 essential fatty acid, linoleic acid (C18:2). Typically, the trans-10, cis-12 and cis-9, trans-11 CLA isomers have been used in studies in which effects of CLAs have been evaluated. Conjugated linoleic acids are naturally occurring fatty acids produced in the rumen of ruminants by the bacterium *Butyrovibrio fibrisolvens*, which isomerizes linoleic acid to form CLA. Foods produced from products of ruminants are the most common natural source of CLA in diets of humans. The cis-9, trans-11 and trans-10, cis-12 isomers of CLA are the predominant types of CLA in fat of ru-

ABBREVIATIONS	
BSA	Bovine serum albumin
CLA	Conjugated linoleic acid
HBSS	Hank's buffered salt solution
PG	Prostaglandin
PGHS	Prostaglandin H synthase
PMA	Phorbol 12-myristate 13-acetate
PPAR	Peroxisome proliferator–activated receptor
TBST	Tris(hydroxymethyl)aminomethane-buffered saline (0.9% NaCl) solution with Tween
TRIS	Tris(hydroxymethyl)aminomethane
WCM	Williams' complete medium

minants and compose > 80% of CLA isomers in dairy products.[1] Endogenous synthesis in mammary glands is the major source of cis-9, trans-11 CLA in milk fat of lactating cows; trans-11 C18:1 CLA produced in the rumen is the substrate.[2]

Conjugated linoleic acids are effective inhibitors of many types of cancer.[3] Conjugated linoleic acids (especially cis-9, trans-11 and trans-10, cis-12 isomers)

have other beneficial physiologic effects in animals, including activation of the immune system, reduction of body fat deposition and subsequent decreased risk of cardiovascular disease, and increased feed efficiency and growth.[1]

Addition of supplemental CLA to diets improves energy status of dairy cows fed energy-restricted diets via reduction of milk fat synthesis.[4] Feeding of diets with supplemental CLA also improves reproductive variables of dairy cows (eg, increased frequency of ovulation by 21 days after parturition and decreased number of days open [ie, not pregnant]).[4–6] Feeding of CLA to animals can affect the n-6 polyunsaturated fatty acid metabolic pathway for biosynthesis of eicosanoids. Linoleic acid obtained from dietary sources can be metabolized into gamma-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid (the substrate for production of 2-series PGs). Prostaglandins affect several reproductive processes, including ovulation, follicular development, corpus luteum function, preparturient cervical dilation, and parturition.[7] Conjugated linoleic acids can be metabolized to form conjugated trienes (C18:3 and C20:3) and 1 of 2 isomers of arachidonic acid (C20:4). These intermediate compounds may decrease synthesis of eicosanoids via competition for the desaturation and chain-elongation enzyme systems.[7] Results of other studies[7–9] indicate CLAs inhibit PGE_2 and $PGF_{2\alpha}$ synthesis. Inhibition of PG production is associated with anticarcinogenesis, and PGs can promote tumor development.[10,11]

Prostaglandin H synthase (cyclooxygenase), the enzyme that catalyzes the rate-limiting step of PG production from fatty acids, has 2 isoforms, one of which has constitutive expression and another that has induced expression.[12] Prostaglandin H synthase-1 (cyclooxygenase-1), which is present in most mammalian tissues, is constitutively expressed; this leads to continuous production of low quantities of PGs that maintain physiologic homeostasis of blood flow, gastric secretions, and blood platelet aggregation.[13] Prostaglandin H synthase-2 (cyclooxygenase-2) oxidizes arachidonic acid to form PGH_2, which is the precursor for all other 2-series PGs (including $PGF_{2\alpha}$ and PGE_2). During physiologically normal conditions, PGHS-2 is detectable in trace amounts. However, PGHS-2 expression can be induced by cytokines, bacterial endotoxins, growth factors, and phorbol esters,[14] which leads to high production of PGE_2. Results of another study[1] indicate CLAs affect many cellular pathways, and different isomers of CLA can have different effects on such pathways.

The objective of the study reported here was to determine the effects of 2 CLA isomers (cis-9, trans-11 and trans-10, cis-12) on expression of PGHS-2 and production of PGE_2 and $PGF_{2\alpha}$ by fetal and adult bovine endometrial epithelial cells in vitro. Because fetal bovine endometrial epithelial cells grow more rapidly and are viable for a higher number of cell culture passages versus bovine endometrial epithelial cells from adult animals (adult cells), such cells may be useful for research. However, similarities of function between adult and fetal bovine endometrial cells should be determined before widespread use of fetal cells in research can be advocated. Therefore, another objective of the study reported here was to compare results for fetal and adult bovine endometrial epithelial cells regarding constitutive and CLA-induced PG secretion.

Materials and Methods

Samples—Adult bovine endometrial epithelial cells were prepared from endometrial biopsy samples obtained from 4 clinically normal adult (age range, 3 to 5 years) dairy cows in the luteal phase of the estrous cycle; cows were from a university-owned herd. Fetal bovine endometrial epithelial cells were prepared from tissues of reproductive tracts of 4 bovine fetuses in approximately the last month of gestation (crown rump length, 75 to 90 cm) that were obtained from gravid uteri of bovine carcasses at a local abattoir. The procedure was approved by the Cornell University Institutional Animal Care and Use Committee.

Cell culture media—A mixture of WCM and Williams' medium E[a] was reconstituted in accordance with manufacturer's instructions; 2mM L-glutamine,[a] 1% insulin-transferrin-selenium-X,[a] 0.01 μg of epidermal growth factor/mL,[a] 100 U of penicillin/mL, 100 μg of streptomycin/mL, 250 ng of amphotericin B/mL,[a] and a protein source were added to the cell culture medium. The protein source was BSA for media used during experiments in which cells were treated with CLAs, and the protein source was 10% heat-inactivated charcoal and dextran-stripped fetal bovine serum[b] for media used during establishment of primary cultures and differential trypsinization of cells for removal of fibroblasts.

Stock CLA isomer solutions were prepared in 100% ethanol. The CLA isomers (cis-9, trans-11 and trans-10, cis-12) were diluted further in WCM containing BSA fraction V as a carrier (final concentration of CLAs, 0 to 200μM). Emulsification was performed via incubation of medium with CLA isomers at 37°C on a rocking platform in a 50-mL tube for at least 6 hours.

Preparation of fetal bovine cells—Primary cultures of fetal bovine endometrial cells were established via a modification of a method of other investigators.[15] Reproductive tracts of bovine fetuses in approximately the last month of gestation (crown rump length, 75 to 90 cm) were obtained from gravid uteri of cows at a local abattoir within 30 minutes after slaughter and transported on ice in calcium- and magnesium-free HBSS[c] with 500 U of penicillin/mL, 500 μg of streptomycin/mL, and 1.25 μg of amphotericin B/mL.[a] Excess soft tissue was removed from fetal reproductive tracts, and uterine horns were dissected from uterine tubes and ovaries. Each uterine horn was separated from the contralateral uterine horn and the uterine body via dissection at the bifurcation of the horns. A sterile hemostat was inserted into the lumen of each horn and clamped to the fascia at the cranial aspect of the horn. Each uterine horn was then inverted by pulling the cranial aspect through the lumen. Uterine horns were then placed in a sterile 50-mL centrifuge tube containing 25 mL of calcium- and magnesium-free HBSS with 0.3% trypsin-EDTA[a] and incubated for 1 hour on a rocking platform at 37°C. Then, the surfaces of uterine horns

were gently scraped with sterile forceps and rinsed with 10 mL of HBSS; fluid was collected in the 50-mL centrifuge tubes. Tissue remnants were discarded, and 2 mL of FBS was added to each centrifuge tube to inactivate the trypsin. Tubes were centrifuged at 500 $\times$ g for 15 minutes, and supernatant was discarded. Cell pellets were gently resuspended in WCM, pooled, diluted to 10^4 cells/well, and plated in accordance with the manufacturer's instructions.[d] Cell culture medium was completely removed via aspiration and replaced 24 hours after cell seeding and then every 72 hours until cell monolayers were 90% confluent. Experiments were repeated for cells obtained from each of the 4 bovine fetuses.

Preparation of adult bovine cells—Primary cultures of adult bovine endometrial cells were established with cells obtained via endometrial biopsy of cows; culture conditions had been previously optimized for primary explants of bovine endometrial tissue.[16] Briefly, uterine endometrial biopsy samples were collected from Holstein cows in the luteal phase of the estrous cycle via passage of biopsy forceps[e] through the cervix into the uterine horn ipsilateral to the corpus luteum by use of transrectal manipulation. The open jaws of the biopsy forceps (dimensions, 2 $\times$ 1 mm) were pressed against the endometrium, the jaws were closed, and tissue samples (total weight of endometrial biopsy samples from each cow, approx 100 mg) were removed. Endometrial biopsy samples were transported to a laboratory in calcium- and magnesium-free HBSS[c] with 500 units of penicillin/mL, 500 μg of streptomycin/mL, and 1.25 μg of amphotericin B/mL.[a] Endometrial biopsy samples were minced into 1-mm cubes; tissue explant cubes were placed in T25 flasks (5 to 10 tissue explant cubes/flask) in a minimal volume of WCM. During the following 3 to 5 days, an additional 1 to 2 mL of WCM was added to culture flasks when a visible corona of cells had begun to form around explants. Explants and culture media were removed from flasks when the corona of cells around each explant was 5 to 8 mm in diameter, and 5 mL of fresh WCM was added to each flask. Culture medium was changed every 72 hours until cells in cultures were 90% confluent. Experiments were repeated for cells obtained from each of the 4 adult cows.

Selective trypsinization of bovine cells—When adult and fetal bovine endometrial cells were approximately 90% confluent, cultures were trypsinized with 0.05% trypsin-EDTA and rinsed with calcium- and magnesium-free HBSS to remove fibroblasts; by use of this method, fibroblasts detached from culture flasks without detachment of more strongly adherent epithelial cells. Trypsin was then inactivated via addition of WCM with fetal bovine serum (10%). Epithelial cells were cultured until they reached 90% confluence. Trypsin EDTA (0.05%) was added, and cultures were rinsed to remove remaining fibroblasts. Then, 0.5% trypsin EDTA was added to flasks to dislodge epithelial cells. Epithelial cells were diluted in WCM, added to 24-well culture dishes (approx 10^4 cells/well), and grown to confluence. Live cell numbers were determined via Trypan blue staining with a hemocytometer.

CLA treatment of bovine cells—When epithelial cells were confluent, they were washed twice with calcium- and magnesium-free Dulbecco PBS solution. Then, cells of each type were incubated at 37°C for an additional 24 hours in WCM containing fatty acid–free BSA[c] with or without 1 of the 2 CLA isomers (cis-9, trans-11 or trans-10, cis-12; CLA concentration range, 0 to 200μM). Supernatant was removed and stored at –20°C until PG assays were performed. Cells were washed twice with calcium- and magnesium-free Dulbecco PBS solution to remove CLA isomers, and 1 mL of WCM with 0.5% BSA and 100 mg of PMA/mL was added. Cell cultures were incubated at 37°C for 6 hours, and supernatant was collected and stored at –20°C until PG assays were performed. Cell pellets were collected in RNA stabilization reagent[f] and stored at –80°C until analyzed via Western blotting for detection of PGHS-2 protein. Experiments were repeated for cells obtained from each of the 4 adult cows and each of the 4 bovine fetuses.

Quantification of $PGF_{2\alpha}$ and PGE_2 via ELISA—Concentrations of $PGF_{2\alpha}$ and PGE_2 in tissue culture supernatants collected before and after incubation of cells with PMA were determined with commercially available ELISA kits[g] in accordance with the manufacturer's instructions. Each tissue culture supernatant sample was assayed in duplicate. The mean of the duplicate values was used for statistical analysis.

Quantification of PGHS-2 via western blot analysis—Western blot analysis was performed to quantify PGHS-2 protein in adult and fetal endometrial epithelial cells. Immediately prior to lysis of cells, the following protease inhibitors were added: 25mM β-glycerophosphate,[c] 5mM benzamidine,[c] 200μM phenylmethylsulfonyl fluoride,[c] 1μM leupeptin,[c] and 1μM pepstatin.[c] Cells were homogenized in 2$\times$ radio-immunoprecipitation buffer (40mM TRIS-HCl, 274mM NaCl, 20% glycerol, 2% tergitol-type nonyl phenoxypolyethoxylethanol-40, 0.2% SDS, 1% Na-deoxycholate, and 1mM EDTA; pH, 8) with a pellet pestle. Cell lysates were centrifuged (1,500 $\times$ g, 10 minutes, 4°C), supernatant was collected, and total protein was quantified.[g] Proteins were denatured in loading buffer (40% SDS, 10% 1M TRIS, 20% glycerol, and 0.013% bromophenol blue) at 95°C for 3 minutes. Lysates were loaded in lanes of 6% SDS polyacrylamide gels (approximately 30 μg of total protein/lane). Prestained protein markers[h] were loaded in a lane of each gel as molecular weight standards. Gels were electrophoresed (ie, SDS-PAGE) at 150 V. The separated proteins were transferred at 4°C to membranes[i] by use of a transfer tank[j] operating at 200 mA. Membranes were incubated with blocking buffer (1% nonfat dried milk powder and 1% BSA in TBST buffer, 10mM TRIS-HCl, 150mM NaCl, and 0.5% Tween-20; pH, 7.6) at 4°C for 60 minutes. Membranes were incubated with polyclonal rabbit anti-human PGHS-2 primary antibody[k] overnight (dilution, 1:1,000 in blocking buffer). Membranes were washed 3 times for 10 minutes each in TBST and then incubated with secondary antibody[l] (dilution, 1:5,000 in blocking buffer) for 2 hours at room temperature (approx 25°C). Then, the membranes were washed 4 times for 15 min-

utes each in TBST, and a chemiluminescence reagent containing luminol[m] was applied. The membranes were exposed to autoradiography film,[n] film was scanned to a computer, the signal intensity for protein bands was quantified with software,[o] and protein quantity was expressed in arbitrary units.

Statistical analysis—Concentrations of $PGF_{2\alpha}$ and PGE_2 were analyzed with a commercially available statistical software program[p] with a completely randomized design via the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} is the dependent variable, μ is the overall mean concentration value, T_i is the CLA treatment effect, and ε_{ij} is the residual error value.

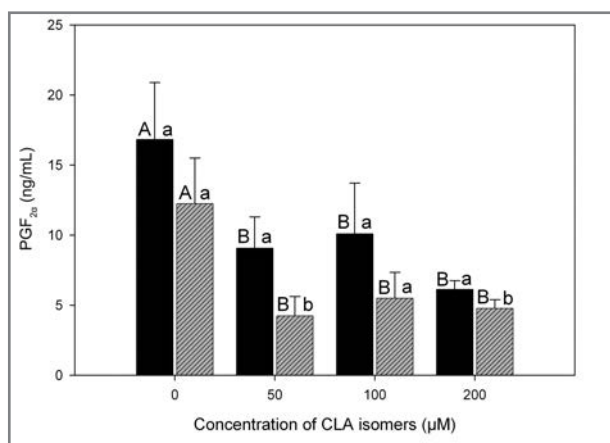Normal distribution of data was determined via visual evaluation of the distribution of the residual val-

ues. Responses of fetal and adult bovine endometrial epithelial cells to CLAs were determined via calculation of correlation coefficients for PG production for each experimental condition (to determine whether different cell types responded similarly to CLAs). When treatment effects were detected ($P < 0.05$), Bonferroni corrections were used to compare mean values between groups. Values of $P < 0.05$ were considered significant.

Results

The $PGF_{2\alpha}$ and PGE_2 concentrations in culture media of adult and fetal bovine endometrial epithelial cells incubated for 24 hours with cis-9, trans-11 and trans-10, cis-12 CLA before PMA stimulation were significantly lower than concentrations in culture media of such cells that were not incubated with CLAs (**Figures 1–4**). Most concentrations of CLAs also decreased the PGE_2:$PGF_{2\alpha}$ concentration ratio in culture media of unstimulated adult and fetal bovine endometrial epithelial cells (**Table 1**). The trans-10, cis-12 CLA iso-

Figure 1—Mean ± SD concentration of $PGF_{2\alpha}$ in cell culture media of endometrial epithelial cells of 4 adult cows with or without exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]) without PMS stimulation. [A,B]Among doses of a CLA isomer, values with different capital letters are significantly ($P < 0.05$) different. [a,b]Between CLA isomers within a dose, values with different lowercase letters are significantly ($P < 0.05$) different.
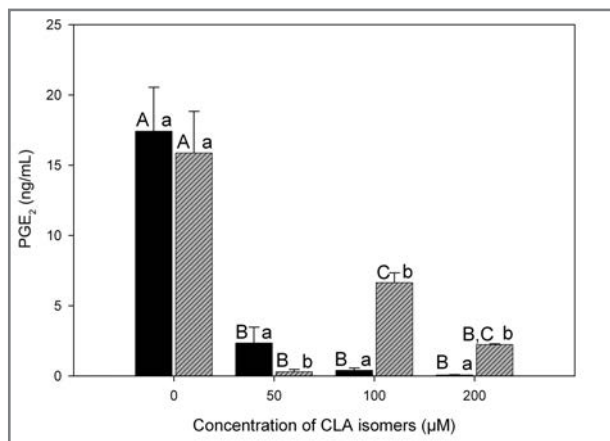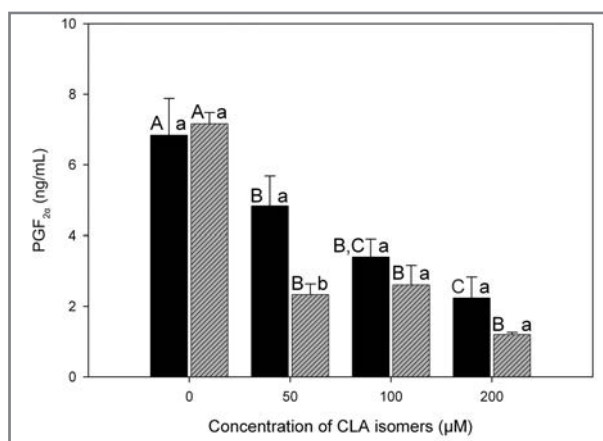

Figure 3—Mean ± SD concentration of $PGF_{2\alpha}$ in cell culture media of endometrial epithelial cells of 4 bovine fetal carcasses with or without exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]) without PMS stimulation. *See* Figures 1 and 2 for remainder of key.

Figure 2—Mean ± SD concentration of PGE_2 in cell culture media of endometrial epithelial cells of 4 adult cows with or without exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]) without PMS stimulation. [A–C]Among doses of a CLA isomer, values with different capital letters are significantly ($P < 0.05$) different. *See* Figure 1 for remainder of key.

Figure 4—Mean ± SD concentration of PGE_2 in cell culture media of endometrial epithelial cells of 4 bovine fetal carcasses with or without exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]) without PMS stimulation. *See* Figures 1 and 2 for remainder of key.
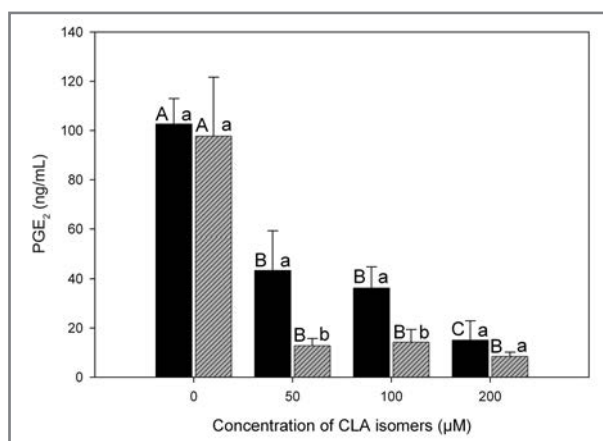
Table 1—Effect of various concentrations of 2 CLA isomers (cis-9, trans-11 and trans-10, cis-12) on cell culture media PGE2:PGF$_{2a}$ concentration ratios for PMA-stimulated or unstimulated endometrial epithelial cells obtained from 4 adult cows or 4 bovine fetal carcasses.

CLA isomer†	PMA stimulation and cell type	CLA concentration (µM)*						Orthogonal contrasts		
		0	50	100	200	SE‡	P value§	Linear‖	Quadratic¶	100µM vs 200µM#
9,11	Unstimulated fetal cells	48.40[a]	30.29[b,c]	38.17[a,b]	25.23[c]	3.93	< 0.01	< 0.01	NS	< 0.05
10,12	Unstimulated fetal cells	44.03[a]	22.16[b]	22.08[b]	18.61[b]	3.5	< 0.01	< 0.01	< 0.01	NS
9,11	Unstimulated adult cells	0.44[a]	0.10[b]	0.02[c]	0.01[c]	0.3	< 0.05	< 0.01	< 0.01	NS
10,12	Unstimulated adult cells	0.51[a]	0.02[b]	0.41[a]	0.14[b]	0.05	< 0.01	< 0.01	NS	< 0.01
9,11	Stimulated fetal cells	6.88[a]	5.85[a,b]	4.81[b,c]	3.34[c]	0.5	< 0.01	< 0.01	NS	NS
10,12	Stimulated fetal cells	6.51[a]	3.82[b]	3.63[b,c]	3.03[c]	0.2	< 0.01	< 0.01	< 0.01	NS
9,11	Stimulated adult cells	1.49[a]	1.07[b,c]	1.32[a,b]	0.80[c]	0.11	< 0.01	< 0.01	NS	< 0.01
10,12	Stimulated adult cells	1.61[a]	0.58[b]	1.66[a]	1.37[a]	0.11	< 0.01	NS	NS	NS

*Data are mean values of cell culture media PGE2:PGF$_{2a}$ concentration ratios. †Cells were exposed to cis-9, trans-11 CLA (9,11) or trans-10, cis-12 CLA (10,12). ‡The SE values are the pooled standard error of means for that row. §Values of P were calculated by use of general linear models. ‖Values are P values for the comparisons performed by use of orthogonal linear contrasts. ¶Values are P values for the comparisons performed by use of orthogonal quadratic contrasts. #Values are P values for the comparisons between 100 and 200µM CLA groups performed by use of orthogonal contrasts.
[a–c]Within a row, values with different superscript lowercase letters are significantly ($P < 0.05$) different.
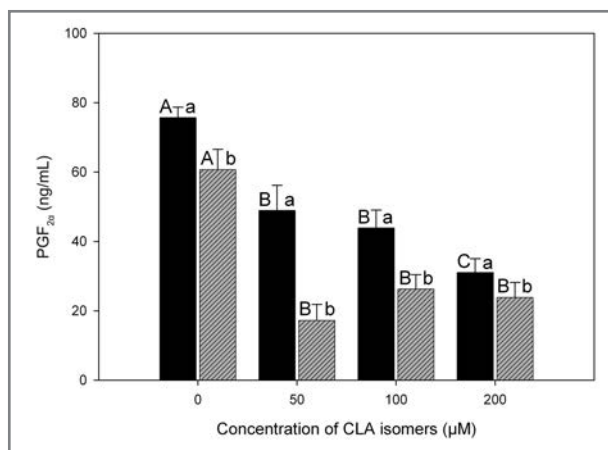NS = Not significantly ($P < 0.05$) different.

Figure 5—Mean ± SD concentration of PGF$_{2a}$ in cell culture media of endometrial epithelial cells of 4 adult cows stimulated with PMA without exposure or after exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]). See Figures 1 and 2 for remainder of key.
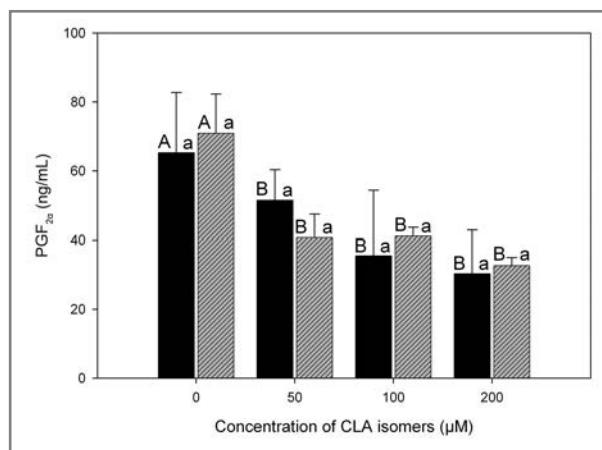

Figure 7—Mean ± SD concentration of PGF$_{2a}$ in cell culture media of endometrial epithelial cells of 4 bovine fetal carcasses stimulated with PMA without exposure or after exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]). See Figure 1 for remainder of key.
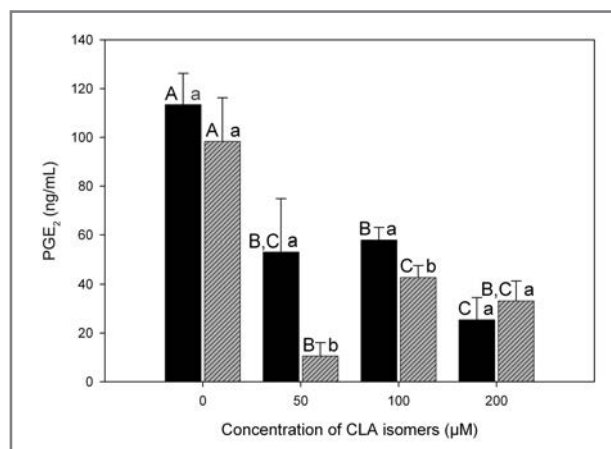

Figure 6—Mean ± SD concentration of PGE$_2$ in cell culture media of endometrial epithelial cells of 4 adult cows stimulated with PMA without exposure or after exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]). See Figures 1 and 2 for remainder of key.

mer seemed to decrease cell culture media PGF$_{2\alpha}$ and PGE$_2$ concentrations more markedly than did the cis-9, trans-11 CLA isomer.

Similarly, PGF$_{2\alpha}$ and PGE$_2$ concentrations in culture media of adult and fetal bovine endometrial epithelial cells incubated with cis-9, trans-11 and trans-10, cis-12 CLA after PMA stimulation were significantly lower than concentrations in culture media of such cells that were not incubated with CLAs (**Figures 5–8**). Most concentrations of CLAs also decreased the PGE$_2$:PGF$_{2\alpha}$ concentration ratio in culture media of PMA-stimulated adult and fetal bovine endometrial epithelial cells (Table 1). Similar to results for cells that were not stimulated with PMA, trans-10, cis-12 CLA seemed to decrease cell culture media PGF$_{2\alpha}$ and PGE$_2$ concentrations more markedly than did cis-9, trans-11 CLA isomer for PMA-stimulated cells.

The CLA isomers had no significant effect on amount of PGHS-2 protein in PMA-stimulated adult or fetal bovine endometrial epithelial cells (data not shown). Qualitatively, the fetal and adult-derived cells
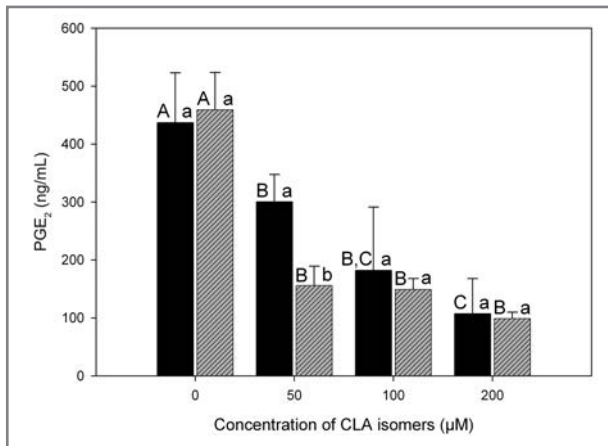
Figure 8—Mean ± SD concentration of PGE$_2$ in cell culture media of endometrial epithelial cells of 4 bovine fetal carcasses stimulated with PMA without exposure or after exposure to various concentrations of CLA isomers (cis-9, trans-11 [black bars] or trans-10, cis-12 [gray diagonal-striped bars]). *See* Figures 1 and 2 for remainder of key.

had similar biological behavior; for both cell types, PG secretion decreased in the presence of CLA isomers and PGE$_2$:PGF$_{2\alpha}$ ratios changed in similar directions, although the concentration values of PGs were different. Fetal bovine cells seemed to have higher culture media PGE$_2$:PGF$_{2\alpha}$ concentration ratios than did adult bovine cells because of higher PGE$_2$ production.

Discussion

The most important finding of the present study was that CLAs decreased PGF$_{2\alpha}$ and PGE$_2$ production by bovine endometrial epithelial cells without changing PGHS-2 protein expression. This result suggested that CLAs affected PG synthesis via a mechanism other than reduced availability of enzymes involved in PG production; presumably, CLAs competed with arachidonic acid as substrates for such enzymes. This finding of the present study is consistent with findings of other investigators[17] that treatment of phorbol-stimulated immortalized bovine endometrial cells with 100µM cis-9, trans-11 CLA or trans-10, cis-12 CLA causes a decrease in PGF$_{2\alpha}$ synthesis without a change in PGHS-2 mRNA or protein expression. Those investigators[17] did not detect any differences in effects between the 2 CLA isomers at a concentration of 100µM, and results indicated CLAs decreased PGF$_{2\alpha}$ production of cells by approximately 20%. The lower response of cells to CLA detected in that other study[17] may be attributable to differences between responses of immortalized cells in that study and cells of primary cultures in the present study.

Results of another study[18] indicate PGHS-2 expression in cells of a human prostate cancer cell line is not affected by exposure to any evaluated concentration (25 to 150µM) of trans-10, cis-12 CLA and PGHS-2 expression is only slightly affected by the highest evaluated concentration (150µM) of cis-9, trans-11 CLA. In contrast, other authors[19,20] reported that CLAs reduce PGHS-2 expression in cells of the RAW 264.7 cell line (derived from mouse macrophages). Investigators in one of those studies[19] evaluated effects of mixed isomers of CLA, and investigators in the other study[20] evalu-

ated effects of cis-9, trans-11 and trans-10, cis-12 CLA isomers; results of that study[20] indicate trans-10, cis-12 CLA has more potent effects on PGHS-2 expression of cells than does cis-9, trans-11 CLA. Those authors concluded that PGHS-2 expression is regulated at transcriptional and posttranscriptional levels. The trans-10, cis-12 CLA isomer downregulates PGHS-2 mRNA and protein expression in murine cells in vitro and in mice in vivo in a concentration-dependent manner, and effects of the cis-9, trans-11 CLA isomer on PGHS-2 expression in murine cells and mice are similar only at a concentration of 100µM.[20] Results of another study[18] indicate PGHS-2 protein expression in human prostate cancer cells is only downregulated by cis-9, trans-11 CLA at a concentration of 150µM. In agreement with results of the present study, those other investigators[18] found that cis-9, trans-11 CLA; trans-10, cis-12 CLA; and a mixture of equal concentrations of those CLA isoforms did not have effects on PGHS-1 or PGHS-2 mRNA or protein expression at any other concentration evaluated. Results of that study[18] also indicate that cis-9, trans-11 CLA downregulates 5-lipoxygenase mRNA expression in human prostate cancer cells. In contrast, the trans-10, cis-12 CLA isomer did not have inhibitory effects on gene expression of enzymes evaluated in that study; those results indicate differences between the evaluated CLA isomers regarding effects on eicosanoid synthesis. Results of these studies suggest differences among tissues and species regarding responses to CLA isomers.

Results of the present study suggested that CLA (up to a concentration of 200µM) had no apparent adverse effects on growth of fetal and adult bovine endometrial epithelial cells in culture, as determined via subjective assessments and isolation of similar amounts of protein from treated and untreated cells. These results were similar to results of another study[19] in which concentrations of CLA similar to those evaluated in the present study had no adverse effects on growth of RAW 264.7 macrophages exposed to lipopolysaccharide. Although results of a recent study[21] indicate high concentrations of CLA cause damage to bovine mammary gland cells, findings of another study[22] indicate the apoptotic effects of CLA isomers are dose and time dependent.

The effects of CLA isomers on PG production may be attributable to several mechanisms. Results of another study[19] indicate that high concentrations of CLA significantly reduce the amount of arachidonic acid in cellular lipids but do not change the linoleic acid content of cellular lipids, suggesting that CLA-mediated alteration of the fatty acid composition of cellular lipids could have an effect on fatty acid metabolism and eicosanoid synthesis. In that study,[19] increasing concentrations of CLA caused increases in the amount of CLA (cis-9, trans-11 and trans-10, cis-12 isomers) in cellular lipids and concomitantly caused a decrease in the concentrations of other fatty acids in RAW 264.7 macrophages. In addition, CLAs can activate PPARs and induce expression of PPAR-responsive genes.[23] Other authors[24] have suggested that PPARγ is responsible for some of the biological effects of CLA. Investigators in other studies[19,20] have suggested that inhibitory effects

of CLA are mediated, at least in part, via modulation of NF-κB activation.

Results of the present study suggested different CLA isomers seemed to have different effects on production of $PGF_{2\alpha}$ and PGE_2. These differences were attributed to the different cellular pathways affected by these CLA isomers.[18] Results of another study[25] indicate that trans-10, cis-12 CLA reduces lipid synthesis in mammary glands of cows via inhibition of proteolytic activation of sterol response element-binding protein-1 and subsequent reduction in transcriptional activation of lipogenic genes. Results of another study[18] indicate the trans-10, cis-12 CLA isomer seems to have greater effects on human prostate cancer cells than do other CLA isomers; those effects are preferentially mediated via modulation of genes involved in apoptosis and cell cycle control. In contrast, cis-9, trans-11 CLA elicits cellular effects via regulation of genes involved in arachidonic acid metabolism and subsequent eicosanoid synthesis.[18]

Results of the present study indicated CLA treatment of fetal bovine endometrial epithelial cells decreased the culture media PGE_2:$PGF_{2\alpha}$ concentration ratio. In contrast to these results, findings of another study[19] indicated CLAs increased the PGE_2:$PGF_{2\alpha}$ concentration ratio in culture media of RAW 264.7 macrophages. Since both $PGF_{2\alpha}$ and PGE_2 are produced from PGH, the specific concentrations of these PGs are dependent on availability of PGH and the specific PGF and PGE isomerase and synthase activity. Authors of that other study[19] determined that 2 mechanisms may cause increased PGE_2 concentrations and PGE_2:$PGF_{2\alpha}$ concentration ratios. Inhibition of PGF synthase by CLA may cause accumulation of arachidonic acid in cells, leading to increased synthesis of PGE; alternately, CLA may stimulate PGE synthase directly.[19] Also, because results of another study[26] indicate bovine placental cells can convert $PGF_{2\alpha}$ to PGE_2, CLA may induce conversion of $PGF_{2\alpha}$ to PGE_2. Similar to results of the present study, results of that other study[26] indicate CLA treatment of bovine placental cells causes a decrease in culture media PGE_2 and $PGF_{2\alpha}$ concentrations and the PGE_2:$PGF_{2\alpha}$ concentration ratio.

Prostaglandins have effects on many physiologic processes. Luteolysis during the estrous cycle in nonpregnant animals is mediated by $PGF_{2\alpha}$ produced by cells of the endometrium. Modulation of $PGF_{2\alpha}$ secretion may allow successful signaling by an embryo for maternal recognition of pregnancy, with resultant increases in pregnancy rates.[27] The balance between PGE_2 and $PGF_{2\alpha}$ concentrations also influences immune responses. Specifically, PGE_2 suppresses innate immune responses such as release of nitric oxide and secretion of interferon gamma by activated human T cells in vitro.[28] High PGE_2 concentrations may also influence the concentrations of Th1 and Th2 cytokines in favor of a Th2 response,[29–31] thereby influencing uterine defense mechanisms in a manner that could limit responses to bacterial pathogens. In the present study, both evaluated isomers of CLA reduced PGE_2:$PGF_{2\alpha}$ concentration ratios in adult and fetal endometrial epithelial cells with or without phorbol stimulation. This result suggested that CLAs may influence immune responses; further

investigation regarding this finding may be warranted. A decreased PGE_2:$PGF_{2\alpha}$ concentration ratio may also have other negative effects, such as retention of fetal membranes.[32] Although administration of CLA to cows during the dry (ie, nonlactating) period has not been reported to cause an increased prevalence of retained fetal membranes to the authors' knowledge, such effects may develop.

Results of the present study and those of another study[32] indicated unstimulated fetal bovine endometrial epithelial cells had higher production of PGE_2 relative to $FGF_{2\alpha}$ than did adult bovine cells; these findings were likely attributable to the immature state of fetal cells. Nevertheless, changes in PG concentrations and concentration ratios in response to CLAs were consistent between fetal and adult cells in the present study; these findings suggested that fetal bovine endometrial epithelial cells, which are easier to grow in culture and more amenable to undergoing multiple subculture passages than are cells of adult animals, may be useful in research. Uteri of bovine fetuses do not have developed uterine glands; therefore, endometrial epithelial cells from such uteri are derived solely from luminal tissues. The methods used in this study did not allow distinction between glandular and luminal epithelium, but we assumed that most or all cells were of luminal origin. The biopsy method used to collect adult endometrial cells (without observation of the biopsy sites) and the small size of the fetal reproductive tracts made it impossible to determine with certainty whether tissues were obtained from caruncular areas, intercaruncular areas, or a combination of these uterine areas. Cells collected from caruncular and intercaruncular uterine areas might respond differently to CLAs, which would have confounded our results. The concentrations of CLAs used in this study ranged from physiologic to supraphysiologic concentrations,[33] but effects were detected even at the lowest CLA concentrations evaluated; this finding suggested that such effects may be physiologically important.

Results of this study indicated that treatment of unstimulated and PMA-stimulated adult and fetal bovine endometrial epithelial cells with either CLA isomer (cis-9, trans-11 or trans-10, cis-12) significantly decreased $PGF_{2\alpha}$ and PGE_2 production in vitro with no apparent effect on PGHS-2 activity. Responses of fetal bovine cells were similar to those of cells collected from adult cows. However, fetal bovine endometrial epithelial cells had higher culture media PGE_2:$PGF_{2\alpha}$ concentration ratios than did cells obtained from adult cows.

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l. Goat Anti-Rabbit IgG Horseradish Peroxidase Conjugate, Bio-Rad Laboratories, Hercules, Calif.
m. Perkin-Elmer, Boston, Mass.
n. Kodak X-OMAT Blue Autoradiography film, Rochester, NY.
o. ImageJ, version 1.29x, National Institutes of Health, Bethesda, Md. Available at: rsbweb.nih.gov/ij/index.html. Accessed Apr 6, 2006.
p. Stata/IC, version 10.1 for Windows, Stata Corp LP, College Station, Tex.

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