Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow

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Abstract

Dietary polyunsaturated fatty acid (PUFA) intake in humans can affect the incidence of a variety of diseases including coronary heart disease. Feeding high PUFA diets to cows can alter the PUFA content of milk for human consumption. PUFAs supply the precursors for prostaglandin (PG) synthesis and PGs in turn influence many aspects of reproduction. This study examined the effects of a control (CONT), a high n-6 PUFA diet (derived from protected soya) and a high n-3 diet (derived from protected linseed) on uterine PG synthesis in the lactating dairy cow.

Endometrial explants obtained on days 15–17 of the oestrous cycle were cultured for an initial 42 h *in vitro* in fully defined medium (basal production) and then challenged with control medium, oxytocin (OT; 20 or 200 nM) or calcium ionophore A23187 (CaI; 10 µM). PGF_{2α}, PGE₂ and 6-keto-PGF_{1α} were measured in the spent medium. The experiments were repeated using tissue from two groups of cows, nine in Experiment 1 (three cows per diet) and seven in Experiment 2 (four CONT and three n-6).

Results of the two experiments were consistent. The basal concentrations of all three PGs were significantly lower (>50% reduction) in the n-6-fed group in comparison with CONT and n-3 groups. The n-3 diet did not alter basal PGF_{2α} and PGE₂ but increased 6-keto-PGF_{1α}. The n-6 diet also inhibited the ability of the tissue to respond to both OT and CaI, with significant reductions in the stimulated levels of all three PGs. In contrast, the n-3 diet only had minor effects; it did not alter the response to OT but did reduce the long-term response to CaI at 24 h post treatment.

In conclusion, dietary PUFA intake can inhibit PG production in bovine endometrial explants, with a more pronounced effect following n-6 rather than n-3 supplementation. These data suggest that a high n-6 diet reduces the endometrial capacity to produce PGs and may therefore have implications for the control of luteolysis and other PG-mediated events such as ovulation.

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Introduction

The n-3 polyunsaturated fatty acids (PUFAs) are believed to ameliorate chronic human diseases such as coronary heart disease, atherosclerosis and inflammation (Holman 1978, Lands 1986, 1992, Burr *et al.* 1989, Fischer 1989, Dolecek & Grandits 1991). There are two families of PUFAs termed n-3 and n-6, based on the position of the first double bond along the hydrocarbon chain. Among them, linoleic acid (LA; 18:2 n-6) and α-linolenic acid (ALA; 18:3 n-3) are essential fatty acids which have to be acquired from the diet. LA is the major fatty acid in most plant oils including soybean oil whereas ALA predominates in forage lipids and in linseed. Since desaturation of fatty acids does not occur at positions greater than Δ9 (Cook 1996), ALA and LA cannot be endogenously

synthesized in ruminants and metabolic conversion can occur only within the same PUFA family. In animals, metabolic enzymes can desaturate and elongate ALA to form other members of the n-3 family, notably eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). Similarly, LA can be converted to form the n-6 family including γ-linolenic acid (GLA; 18:3 n-6), dihomo-γ-linolenic acid (DGLA; 20:3 n-6), arachidonic acid (AA; 20:4 n-6) and docosapentaenoic acid (22:5 n-6) (Bezard *et al.* 1994). Alternatively, these longer chain PUFAs can be obtained directly from the diet, with fish oil a major source of EPA and DHA.

The UK Department of Health (1994) has advised a change in human diet such that foods rich in n-3 PUFAs form a larger component than at present, including an increase in the n-3:n-6 PUFA ratio. This report also

recommended that there should be no further increase in intakes of n-6 PUFA. Milk products are a major source of dietary fat and an increased proportion of PUFAs in the milk can be achieved by altering PUFAs in the feed (Wrenn *et al.* 1976, Schingoethe *et al.* 1996, Mansbridge & Blake 1997). Dietary fats including PUFAs may also be fed to lactating cows to increase the energy density of the diet and thus reduce the energy deficit which occurs in early lactation (Staples *et al.* 1998).

Supplemental dietary PUFAs may alter reproductive performance both by changing the energy status and by providing precursors for the synthesis of prostaglandins (PGs) (for reviews see Staples *et al.* 1998, Abayasekara & Wathes 1999, Mattos *et al.* 2000). AA is the precursor for the production of the 2 series PGs (such as $\text{PGF}_{2\alpha}$ and PGE_2) via the action of cyclo-oxygenase (COX), but COX can also convert DGLA into 1 series PGs (such as $\text{PGF}_{1\alpha}$ and PGE_1 ; Kinsella *et al.* 1990) and EPA into 3 series PGs (such as $\text{PGF}_{3\alpha}$ and PGE_3 ; Sargent 1997). The 1 and 3 series PGs are believed to be less biologically active than the 2 series (Gurr & Harwood 1991, Lands 1992) but may be produced at the expense of 2 series PGs, depending on the proportions of the various precursors present in the membrane phospholipids. This, in turn, is affected by diet.

The 2 series PGs regulate many physiological processes including the vascular, immune and endocrine systems. They have been implicated in the process of reproduction, including ovulation (Espey 1980), follicular development (Wallach *et al.* 1975), corpus luteum function including luteolysis (Abayasekara *et al.* 1995, Poyser 1995, Wathes & Lamming 1995) and parturition (Challis 1980). As well as providing precursors or substrates, n-3 and n-6 PUFAs interact and compete with each other for incorporation into phospholipids and as substrates for metabolic enzymes (especially desaturase and COX) (Olsen *et al.* 1986, Abayasekara & Wathes 1999, Mattos *et al.* 2000). Changes in the amounts of PUFAs or their ratios may affect production of PGs in the reproductive system in both cows (Thatcher *et al.* 1994) and humans (Graham *et al.* 1994).

The release of AA from phospholipid storage pools and the availability of COX protein are both rate-limiting steps in PG synthesis. Free AA for PG production can be generated by the direct action of phospholipase A₂ (PLA₂) or the co-ordinated action of phospholipase C (PLC) and diacyl glycerol lipase generating two second messengers, inositol triphosphate (which increases intracellular calcium; $[\text{Ca}^{2+}]_i$) and diacylglycerol (DAG) (which activates protein kinase C; PKC). The subsequent conversion of AA to PG is dependent on the presence of COX. There is evidence that oxytocin (OT) can activate all these mechanisms to generate PGs in the ruminant uterus: e.g. PLA₂ (Lee & Silvia 1994, Danet-Desnoyers *et al.* 1995), PLC and DAG (Flint *et al.* 1986, Silvia *et al.* 1994), PKC (LaFrance & Goff 1990, Kim & Fortier 1995), increased $[\text{Ca}^{2+}]_i$ and COX-2 mRNA (Asselin *et al.* 1997). The calcium

ionophore A23187 (CaI) promotes calcium flux across membranes and activates PLA₂. It can also stimulate PG synthesis in the bovine endometrium (Danet-Desnoyers *et al.* 1995, Tysseling *et al.* 1998).

This study investigated the effects of feeding isoenergetic diets that differed in their n-3 and n-6 PUFA content on uterine PG synthesis in the dairy cow. This was examined by measuring basal, OT and CaI stimulated PG production in endometrial explants from cows on different diets, cultured in defined medium.

Materials and Methods

All reagents were from Sigma Chemical Co. (Poole, Dorset, UK) or BDH Merck Ltd (Lutterworth, Leics, UK) unless otherwise stated. All animal experiments were performed under the Animal (Scientific Procedures) Act 1986.

Animals

Two experiments were performed, both with lactating Holstein/Friesian dairy cows. Experiment 1 utilised nine mature cows which had had no previous fertility problems either in the current or previous lactation. Experiment 2 involved seven first lactation cows which were at least 4 weeks post partum at the start of the trial. In both cases, ovarian cyclicity before and during the trial was monitored by $\times 3$ weekly milk progesterone analysis (Bulman & Wood 1980).

Dietary treatments

Cows on both trials were fed isoenergetic and isonitrogenous diets which varied in their PUFA content (Table 1). These were individually fed twice daily *ad libitum* as a total mixed ration. Any food uneaten from the previous day was removed. Control diets (CONT) contained a low level of n-3 and n-6 PUFAs, the high n-3 diet (n-3) contained an extra 243 g/day n-3 PUFAs which were provided from LinPreme (Borregaard UK Ltd, Warrington, Cheshire, UK) and the high n-6 diet (n-6) contained an extra 250–270 g/day n-6 PUFAs from SoyPreme (Borregaard UK Ltd). LinPreme and SoyPreme are non-enzymatically browned full fat linseed or soya products respectively (Abel-Caines *et al.* 1998). This process protects the lipid fraction of the oil seeds from ruminal degradation. Cows also received 150 g/day of a mineral vitamin supplement containing the anti-oxidant vitamin E (450 mg/cow/day). Cows were housed throughout with water freely available and were milked twice daily.

Experimental protocols

Experiment 1 The study was a 3×3 factorial design such that each dietary treatment (CONT, n-3 or n-6) was

Table 1 Constituents and composition of the diets. The amounts given are in kg/cow per day unless otherwise indicated

	Experiment 1			Experiment 2	
	CONT	n-3	n-6	CONT	n-6
Grass silage	17.9	17.9	17.9	40.8	40.8
Maize silage	16.7	16.7	16.7	—	—
Wheat	1.9	1.5	1.7	4.0	3.0
Sugar beet pulp	1.0	1.2	1.2	3.0	3.0
Rapeseed meal	1.5	1.0	1.2	2.4	0.5
Soya 48	—	—	—	0.75	—
Soypass	1.3	1.0	—	—	—
Megalac	0.65	0.3	0.3	—	—
LinPreme	—	1.25	—	—	—
SoyPreme	—	—	2.8	—	3.0
Dry matter (DM)	15.8	15.7	15.7	18.6	18.0
Metabolisable energy (MJ/day)	191.6	191.4	191.0	210.4	210.9
Protein (g)	2699	2654	2649	3184	3129
% oil in DM	2.9	5.5	5.9	2.7	5.6
n-3 PUFA (g)	113.0	356.3	147.8	198.3	234.6
n-6 PUFA (g)	141.3	178.3	390.7	117.3	388.9

given to three cows for 4 weeks. The diet was introduced on day 1. On day 4, oestrous cycles were synchronised using a progesterone-releasing intravaginal device (PRID; Sanofi Animal Health, Watford, Herts, UK) inserted for 7 days followed by an injection of 2 ml Estrumate i.m. (PGF analogue; Schering Plough Ltd, Harefield, Middx, UK) on the day of PRID removal. Animals were killed at the local abattoir by captive bolt on day 15 of the induced cycle and the reproductive tracts were transported to the laboratory on ice.

Experiment 2 Cows used for this trial were part of a larger experiment for which the *in vivo* results will be reported separately. Four cows received the CONT diet and three cows received the n-6 diet for a period of four oestrous cycles (about 86 days). Beginning at least 4 weeks post partum, the dietary treatments were introduced and oestrous cycles were synchronised by a PRID inserted for 10 days followed by 2 ml Estrumate at PRID removal. After the cows had experienced two and a half normal cycles (based on milk progesterone profiles), Estrumate (2 ml, i.m.) was administered again in the mid-luteal phase to re-synchronise the start of the fourth cycle. The cows were slaughtered on days 16–17 of the fourth cycle for harvest of the tissues.

Tissue culture

Endometrial explants were cultured in defined medium following a method described previously (Leung & Wathes 2000). Briefly, the uterus was washed with 70% (v/v) ethanol and placed on to a sterile metal dish in a laminar flow hood. Strips of intercaruncular endometrium (com-

prising luminal epithelium, glandular epithelium and stromal tissue) were separated from the uterus by manual dissection and transferred into serum-free Dulbecco's minimal essential medium/F12 1:1 nutrient mix (Gibco Life Technologies, Paisley, Strathclyde, UK) containing 50 000 units/l penicillin, 50 mg/l streptomycin, 1.125 g/l bovine serum albumin and 1.125 g/l sodium bicarbonate. The endometrial strips were chopped with a McIlwain mechanical tissue chopper (McIlwain Laboratory Engineering, Guildford, Surrey, UK) into 1 mm³ cubes. The chopped endometrium was blotted and weighed to provide 140–160 mg tissue per dish, which was placed on the top of a metal grid (30 mm by 30 mm by 0.5 mm), cushioned with lens tissue, inside a Petri dish (50 mm by 15 mm single dent) containing 6 ml of the above medium supplemented with ITS (5 mg/ml insulin, 5 mg/ml transferrin and 5 µg/ml selenium; Sigma Chemical Co.). There were four replicate dishes per treatment and these were incubated in a humidified incubator at 37 °C with 5% CO₂. The medium was changed at 2 h (Experiment 1 only), 18 h and 42 h for the measurement of basal PGs. At 42 h, the tissue was challenged with (i) control culture medium (CM), (ii) OT (Intervet (UK) Ltd, Cambridge, Cambs, UK) at 20 nM in Experiment 1 and 200 nM in Experiment 2 or CaI (A23187, Sigma) at 10 µM. At 2, 6 and 24 h after the start of the challenge, the medium was harvested and replaced with the same challenge medium. Medium was then stored at –20 °C until analysis. Tissue morphology in the explants is well preserved for at least 72 h in culture using this technique (authors' unpublished observations). The challenge doses were selected to give maximal responses

in terms of PG production following preliminary experiments over the range 2–2000 nM (OT) or 0.1–100 µM (CaI) (data not shown).

PG radioimmunoassays (RIA)

PGE₂ in the tissue culture medium was determined using a method reported previously (Higgins & Lees 1984) with some modifications. Briefly, the standard curve (range 0.08–10 ng/ml) was prepared in pH 7.4 Tris buffer (0.05 M) containing 0.1% gelatin and 0.01% sodium azide. The culture medium was diluted in buffer ($\times 20$ –100) without prior extraction. One hundred microlitres of standards or diluted samples were mixed with 100 µl anti-PGE₂ serum (diluted to give 50% binding with the total counts (TC), 100 µl tritiated tracer (8000 c.p.m.; Amersham International plc, Amersham, Bucks, UK) in LP4P tubes (Life Sciences International Ltd, Southampton, Hants, UK) in duplicate. After overnight incubation at 4 °C, 200 µl dextran-coated charcoal suspension containing 0.4% dextran (T-70; Amersham Pharmacia Biotech, Uppsala, Sweden) and 2% neutralized charcoal was added to all tubes except the TC. They were incubated at 4 °C for 10 min and centrifuged at 2000 g for 10 min. The supernatant was removed into 6 ml scintillation vials containing 4 ml scintillant (Ultima gold; Packard Bioscience BV, Pangbourne, Berks, UK) and counted for 2 min. The concentrations of PGE₂ were calculated using a semi-logarithmic plot. The limit of detection was 2 pg/tube and the coefficient of variation (Co-Var) was 3.5% ($n=6$) for the intra-assay and 6.3% for the interassay ($n=6$) variation.

The RIAs for PGF_{2α} and 6-keto-PGF_{1α} were carried out using the same procedures as described for PGE₂, but using the standards, antisera and tracers for PGF_{2α} and 6-keto-PGF_{1α} respectively. The limit of detection was 1 pg/tube for PGF_{2α} and 8 pg/tube for 6-keto-PGF_{1α}. The intra-assay Co-Var values for PGF_{2α} and 6-keto-PGF_{1α} were 4.1% and 6.5% respectively and the interassay Co-Var values were 9.6% for PGF_{2α} and 3.8% for 6-keto-PGF_{1α}. The antisera to all three PGs were a kind gift from Professor N L Poyser (University of Edinburgh, Edinburgh, UK) and their cross-reactivities have been reported previously (Poyser 1987).

Statistical analysis

The results are corrected as ng/ml/h for 150 mg tissue and are quoted as the mean $\pm$ standard error (S.E.). Statistical comparisons between CONT, n-3 and n-6 were carried out using a 2-way analysis of variance (ANOVA) for basal PGs and a 3-way ANOVA for challenged PGs using SPSS 9.0 software package (SPSS Inc., Chicago, IL, USA) via a general linear model routine. This method took account of the differences of treatments, time and challenges and their associated interactions. Where a significant difference

($P<0.05$) was achieved, Fisher's LSD multiple comparisons were carried out to investigate the differences between the dietary groups or interactions at an individual error rate of 0.05.

Results

Information from the milk progesterone assays (not shown) confirmed that all cows responded appropriately to the synchronisation procedure and were therefore in the late-luteal phase at the time of tissue collection on day 15 (Experiment 1) or days 16–17 (Experiment 2).

Basal PG production

The production of PGF_{2α}, PGE₂ and 6-keto-PGF_{1α} by the endometrial explants in the first 42 h before the OT or CaI challenge is regarded as the basal level. In Experiment 1, the cultured tissue produced significant amounts of all three PGs in a time-related fashion and this pattern differed between the three treatment groups (Fig. 1). In the CONT group, the concentration of PGF_{2α} increased significantly from 18 to 42 h ($P<0.05$), in the n-3 group the concentrations of PGF_{2α} did not differ between culture times ($P>0.05$) and in the n-6 group, the concentrations of PGF_{2α} at 18 and 42 h were about half of those at 2 h ($P<0.05$). PGE₂ production in all three dietary groups was low at 2 h but increased significantly at 18 and 42 h ($P<0.05$). 6-keto-PGF_{1α} did not change with time in the CONT group ($P>0.05$), but in the n-3 and n-6 groups it was significantly lower at 42 h than at 2 and 18 h ($P<0.05$).

The ANOVA also illustrated that there were significant differences in the basal concentrations of PGs between the dietary groups at particular time-points ($P<0.01$, Fig. 1). Concentrations of PGF_{2α}, PGE₂ and 6-keto-PGF_{1α} were all significantly lower in the n-6 dietary group than in the CONT and n-3 group at 18 and 42 h ($P<0.05$) and this inhibitory effect produced by the n-6 PUFAs on PGs was greater than 50%. Compared with the CONT group, the n-3 dietary group did not alter PGF_{2α} and PGE₂ production over a 2–42 h culture period ($P>0.05$) but it increased 6-keto-PGF_{1α} production at 2 and 18 h ($P<0.05$).

In Experiment 2, the basal PGF_{2α} concentrations in the n-6 group tended to be lower than in the CONT group at both 18 and 42 h ($P=0.11$). Dietary treatment with n-6 PUFAs inhibited basal PGE₂ production significantly when analysed overall ($P<0.01$) and significant difference was achieved at 42 h ($P<0.05$, Fig. 2). 6-keto-PGF_{1α} was not measured in this experiment.

The generation of uterine PGs in response to the challenges of OT and CaI

In Experiment 1, an OT challenge given 42 h after the start of culture stimulated the production of PGF_{2α} and

Figure 1 Basal PG (A: PGF_{2α}; B: PGE₂; C: 6-keto-PGF_{1α}) production by endometrial explants obtained from cows in Experiment 1 fed diets with differing PUFA contents, CONT, high n-3 and high n-6. There were three cows per diet and four replicates per cow. The medium was changed at 2, 18 and 42 h after the start of culture and values are expressed as the means ± S.E. The values labelled with different letters are significantly different ($P < 0.05$).

6-keto-PGF_{1α} significantly at 2 h compared with explants challenged with CM ($P < 0.05$) and the effect of OT on PGF_{2α} output was still significant after 6 h. OT did not significantly increase PGE₂ output. CaI stimulated the production of all three PGs at 2 and 6 h and the effect on PGE₂ and 6-keto-PGF_{1α} was still significant at 24 h. Peak values (ng/ml/h) were achieved at 2 h following the challenges. Compared with the OT challenge, the effect of CaI caused a more sustained release, with concentrations of PGF_{2α} significantly higher at 6 h and of PGE₂ and 6-keto-PGF_{1α} at 6 and 24 h ($P < 0.05$) (Figs 3, 4 and 5).

The ANOVA for the overall effects showed that the feeding of different PUFA diets caused significant alterations in the PG responses to both OT and CaI ($P < 0.01$). The generation of the three tested PGs was significantly inhibited in the n-6 compared with the CONT group at most time-points ($P < 0.05$ – 0.01 , Figs 3, 4 and 5). Individ-

Figure 2 Basal PG (A: PGF_{2α}; B: PGE₂) production by endometrial explants obtained from cows in Experiment 2 fed diets with differing PUFA contents, CONT ($n = 4$) or high n-6 ($n = 3$). There were four replicates per cow. The medium was changed at 18 and 42 h after the start of culture and values are expressed as the means ± S.E. * $P < 0.05$, significant difference of the n-6 diet in comparison with the control diet at the same time-point.

ual comparisons showed that concentrations of PGF_{2α} in the n-6 group were significantly lower than in the CONT group at 2 h after CM, OT and CaI challenges, at 6 h after CaI challenge and at 24 h after CM and CaI challenge ($P < 0.05$). The n-6 diet inhibited uterine PGE₂ generation significantly at 2 h in the CM- and OT-treated tissues, at 6 h in CM-treated tissues and at 24 h in CM- and CaI-treated tissues ($P < 0.05$). Generation of 6-keto-PGF_{1α} was significantly reduced at 2 h post CM and OT stimulation and at 24 h post CM and CaI stimulation ($P < 0.05$).

In contrast, the n-3 treatment only had minor effects on uterine PG generation. A statistically significant decrease in overall PGF_{2α} generation in the n-3 group was detected but the difference was very small (overall means for n-3 and CONT 1.69 ± 0.13 ng/ml/h vs 2.21 ± 0.14 ng/ml/h, $P < 0.05$). Compared with the CONT group for values in sequential culturing time-courses and different challenges, the n-3 diet did not alter PGF_{2α}, PGE₂ or 6-keto-PGF_{1α} production when the tissues were

Figure 3 PGF_{2α} production by endometrial explants obtained from cows in Experiment 1 fed diets with differing PUFA contents, CONT, high n-3 or high n-6. There were three cows per diet and four replicates per cow. The explants were cultured for 42 h and then challenged with (i) CM, (ii) OT (20 nM) or (iii) Cal (10 μM). Values are expressed as the means ± S.E. Medium was changed at (A) 2, (B) 6 and (C) 24 h after the start of challenge. **P* < 0.05, significant difference of the n-3 or n-6 diet in comparison with the control diet at the same time-point. Bars with different letters indicate overall differences between times and in response to OT or Cal (*P* at least < 0.05).

challenged with CM or OT over a period of 2–24 h (*P* > 0.05). In the tissues stimulated with Cal, n-3 PUFA feeding inhibited PGF_{2α} at 6 and 24 h and PGE₂ and 6-keto-PGF_{1α} at 24 h (*P* < 0.05) (Figs 3, 4 and 5).

Experiment 2 confirmed the above results that an n-6 PUFA-enriched diet suppressed uterine PG production stimulated by OT and Cal (Figs 6 and 7). Compared with the CONT group, PGF_{2α} production was significantly inhibited at 2 h and 6 h following OT and Cal challenges (*P* < 0.05). PGE₂ generation was significantly inhibited at 2 and 6 h following CM, OT and Cal challenges and at 24 h following Cal (*P* < 0.05). The results also confirmed the difference in responsiveness over time to OT and Cal. There was a significant stimulation of PGF_{2α} but not PGE₂

Figure 4 PGE₂ production by endometrial explants obtained from cows in Experiment 1 fed diets with differing PUFA contents, CONT, high n-3 or high n-6. There were three cows per diet and four replicates per cow. The explants were cultured for 42 h and then challenged with (i) CM, (ii) OT (20 nM) or (iii) Cal (10 μM). Values are expressed as the means ± S.E. Medium was changed at (A) 2, (B) 6 and (C) 24 h after the start of challenge. **P* < 0.05, significant difference of the n-3 or n-6 diet in comparison with the control diet at the same time-point. Bars with different letters indicate overall differences between times and in response to OT or Cal (*P* at least < 0.05).

in response to OT at 2 h only, whereas responsiveness to Cal was still present at 6 h (PGF_{2α}; Fig. 6) and 24 h (PGE₂; Fig. 7).

Discussion

In the rumen, the activity of bacterial flora can hydrogenate PUFAs biologically to reduce the amount of PUFAs reaching the duodenum and therefore being incorporated into adipose tissue and milk. The resistance of PUFAs to biohydrogenation in the rumen can be greatly increased by chemical or physical treatment of PUFAs, such as calcium

Figure 5 6-keto-PGF_{1α} production by endometrial explants obtained from cows in Experiment 1 fed diets with differing PUFA contents, CONT, high n-3 or high n-6. There were three cows per diet and four replicates per cow. The explants were cultured for 42 h and then challenged with (i) CM, (ii) OT (20 nM) or (iii) Cal (10 μM). Values are expressed as the means ± S.E. Medium was changed at (A) 2, (B) 6 and (C) 24 h after the start of challenge. **P* < 0.05, significant difference of the n-3 or n-6 diet in comparison with the control diet at the same time-point. Bars with different letters indicate overall differences between times and in response to OT or Cal (*P* at least < 0.05).

salts of long-chain fatty acids (Klusmeyer *et al.* 1991). In the present study the n-3 and n-6 PUFAs were enriched by non-enzymatically browned full fat liseed (LinPreme) or soya (SoyPreme) respectively to protect the PUFAs from hydrogenation in the rumen (Abel-Caines *et al.* 1998). The predominant PUFAs in linseed are ALA, which make up 47% of the triglycerides and 18% of the phospholipid fatty acids (EI-Shattory 1976). In soya oil, LA comprises more than 80% of PUFAs and the ratio of LA to ALA is >7 (Homayoun *et al.* 1988). The PUFAs in the non-treated feed components comprising the rest of the diet would be expected to suffer damage in the rumen so that the PUFAs from LinPreme or SoyPreme respectively represent the effective PUFAs received by the cows.

Figure 6 PGF_{2α} production by endometrial explants obtained from cows in Experiment 2 fed diets with differing PUFA contents, CONT (*n* = 4) or high n-6 (*n* = 3). There were four replicates per cow. The explants were cultured for 42 h and then challenged with (i) CM, (ii) OT (200 nM) or (iii) Cal (10 μM). Values are expressed as the means ± S.E. Medium was changed at (A) 2, (B) 6 and (C) 24 h after the start of challenge. **P* < 0.05, significant difference of the n-6 diet in comparison with the control diet at the same time-point. Bars with different letters indicate overall differences between times and in response to OT or Cal (*P* at least < 0.05).

In ruminants, luteolysis is achieved by OT stimulation of pulsatile PGF_{2α} secretion from the endometrium (for review see McCracken *et al.* 1999). The main regulatory factor is the timing of up-regulation of oxytocin receptors (OTR) in the uterine epithelium (Wathes & Lamming 1995). In the cow, OTR up-regulation occurs between days 15 and 17 of the oestrous cycle (Jenner *et al.* 1991, Mann & Lamming 1994, Robinson *et al.* 1999). In the present study, we used an endometrial explant system to study PG synthesis. The explants contained luminal and glandular epithelium and stroma. We have previously shown that, in bovine endometrium removed in the late-luteal phase, OTR up-regulation occurs spontaneously between 24 and 48 h in culture and is confined to the luminal epithelial cells (Leung & Wathes 2000).

Figure 7 PGE₂ production by endometrial explants obtained from cows in Experiment 2 fed diets with differing PUFA contents, CONT ($n=4$) or high n-6 ($n=3$). There were four replicates per cow. The explants were cultured for 42 h and then challenged with (i) CM, (ii) OT (200 nM) or (iii) Cal (10 μ M). Values are expressed as the means $\pm$ S.E. Medium was changed at (A) 2, (B) 6 and (C) 24 h after the start of challenge. * $P<0.05$, significant difference of the n-6 diet in comparison with the control diet at the same time-point. Bars with different letters indicate overall differences between times and in response to OT or Cal (P at least <0.05).

Other workers have shown that the epithelium secretes more PGF_{2 α} than the stroma, which secretes predominantly PGE₂; while OT stimulates release of both PGs from the epithelium it has no effect on stromal cells (Danet-Desnoyers *et al.* 1994, Asselin *et al.* 1996). COX-2 mRNA is also confined to the luminal epithelial cells (Charpigny *et al.* 1997), whereas evidence using a non-specific COX antibody suggests that COX-1 is also present in the stroma (Boos 1998). Our results on OT and Cal stimulation of endometrial PGs are consistent with previous reports (see Introduction). The action of OT was probably confined to the epithelial cells present in the system thus explaining the lack of an effect on PGE₂. In contrast, Cal would have been able to influence PG generation from both epithelial and stromal cell types, thus

contributing to the more sustained response. The loss of responsiveness to OT from 6 to 24 h after challenge is also consistent with previous work showing a time-dependent reduction in both OT-binding sites and mRNA in human myometrial cells exposed to OT for up to 20 h (Phaneuf *et al.* 1997). The reduction in the basal production of PGs from 2 to 24 h after challenge could reflect (i) a reduction in available PLA₂ and/or COX, (ii) a reduction in available precursor or (iii) greater metabolism of PGs during the longer interval between collection times. These different possibilities were not explored in the current experiment.

A number of previous studies have demonstrated inhibitory effects of n-3 PUFAs on 2 series PG production (for reviews see Bezard *et al.* 1994, Staples *et al.* 1998, Abayasekara & Wathes 1999, Mattos *et al.* 2000). The n-3 PUFAs, notably EPA and DHA, can suppress PG production at various stages. These include (i) inhibiting AA synthesis during the LA desaturation and elongation processes in the liver (Sprecher 1981, Chen & Nilsson 1993, Bezard *et al.* 1994), (ii) competing with n-6 PUFAs, especially AA, for incorporation into phospholipids in cell membranes to decrease the available AA for COX (German *et al.* 1988, Raederstorff & Moser 1992) and (iii) competitively blocking COX activity (German *et al.* 1988, Larsen *et al.* 1997).

In the present study, the effect of the n-3 PUFA diet on uterine PG production was limited. It did not significantly alter either the basal or OT-challenged PGF_{2 α} or PGE₂ generation at any time-point. The only difference to the control diet was that tissues from n-3 fed cows were less able to sustain a prolonged release of PGF_{2 α} , PGE₂ or 6-keto-PGF_{1 α} in response to a challenge with Cal. The likely reason for the less pronounced response in comparison with previous reports may be the difference in the sources of the n-3 PUFAs. Most previous studies utilised fish oil (which contains predominantly EPA and DHA; Lambert *et al.* 1996) for the dietary supplement or pure EPA and DHA for *in vitro* incubation. In the present study, however, the n-3 PUFAs in the diet were provided from linseed in which the predominant n-3 PUFA is ALA. EPA and DHA are broad and potential inhibitors for 1 and 2 series PG production and their inhibitory effects are greater than their precursor, ALA (Yang & Williams 1978, Garg *et al.* 1988, 1990). Although ALA can be converted into different n-3 PUFAs, including EPA and DHA (Bezard *et al.* 1994), the amounts of these longer chain PUFAs generated may be small. Nevertheless, inhibitory effects on PG generation were still observed when the explants were subjected to a prolonged challenge.

The present study did, however, demonstrate a significant inhibitory effect of dietary n-6 PUFAs on *in vitro* PG production by endometrial explants of bovine uterus. The production of PGF_{2 α} , PGE₂ and 6-keto-PGF_{1 α} was significantly inhibited both before and after OT or Cal challenges and the same results were repeated in two

experiments using separate groups of cows. Several possible explanations for similar inhibitory effects have been suggested by previous investigations. In the present study, the n-6 PUFAs were enriched using a protected form of soya bean in which LA predominates. A high level of LA in the diet may inhibit the synthesis of AA, which is the substrate for 2 series PGs and/or the activity of COX enzyme which catalyses PG synthesis. It was reported that the AA level had decreased in most phospholipids in rats fed diets high in LA (Zevenbergen *et al.* 1988). Oldick *et al.* (1997) illustrated that infusion of a fat source rich in LA (17%) into the abomasum of lactating dairy cows led to a significant attenuation in 13,14 dihydro-15 keto-PGF_{2α} (PGFM; a metabolite of PGF_{2α}) in peripheral plasma in response to OT injection on day 15 of a synchronised oestrous cycle. Inhibition of COX by LA was reported by Pace-Asciak & Wolfe (1968) and LA was also suggested to act as a natural inhibitor of uterine PG synthesis during the maternal recognition of pregnancy in cows (Thatcher *et al.* 1994).

Another mechanism for inhibition of PG synthesis by n-6 PUFAs could be a non-selective inhibition of fatty acid synthesis. In mice fed maize oil (rich in n-6 PUFAs) or beef tallow diets (containing saturated fatty acids), fatty acid synthesis was inhibited in all tissues examined including interscapular brown adipose tissue, epididymal white adipose tissue, the liver and the carcass; the inhibition in the maize oil group was greater than in the beef tallow group (Van den Brandt & Trayhurn 1981). It is also possible that a high level of LA may compete with AA for distribution and incorporation into the membrane phospholipids, thus decreasing available AA and leading to a drop in PG synthesis. This explanation was suggested by Graham *et al.* (1994) in a study using endometrium from women with regular menstrual cycles who received a 6-month dietary supplement of either GLA or EPA. They demonstrated that the ability of the endometrium to take up ¹⁴C-AA *in vitro* and production of PGF_{2α} and PGE₂ all decreased following the GLA and EPA diets. In the present study, the cows continuously received ALA- or LA-enriched diets for a period of 28 days (Experiment 1) or four oestrous cycles (approximately 80 days, Experiment 2). This would alter the distribution and incorporation of PUFAs between and within n-3 and n-6 families.

In contrast, a variety of other studies have shown that increasing the supply of n-6 PUFAs can lead to enhanced PG production via increased availability of AA. In humans receiving short-term diets rich in AA, increased phospholipid levels of AA, urinary PGI₂ and thromboxane (TX) A₂ (a PG produced by platelet COX) were observed (Sinclair & Mann 1996). When rats were fed an AA-rich diet, PGE₂ production from leukocytes was enhanced (Peterson *et al.* 1998) and in rats fed an n-6 PUFA-supplemented diet (provided by safflower oil), the AA content of plasma lipids and blood synthesis of TXB₂ was

significantly increased (Croft *et al.* 1984). Quoc & Pascaud (1996) reported that the dietary administration of GLA (from evening primrose oil) in rats increased the *in vitro* production by the aorta of PGE₁ derived from dihomogLA, although it did not significantly influence the production of AA and PGE₂ by the aorta or the TXB₂ level in serum. Differences between experiments may relate to varying n-6:n-3 PUFA ratios in the respective diets.

The data presented therefore show that a high n-6 PUFA diet derived from soya can inhibit both basal and stimulated uterine PG synthesis *in vitro*, whereas a high n-3 linseed-derived diet has a less pronounced effect but may also be inhibitory to sustained PG release. The relevance of this to the *in vivo* situation is uncertain. In cows, inhibition of PG synthesis in the late-luteal phase by LA could potentially improve conception rates by reducing the incidence of early embryo loss at this stage of the cycle (Thatcher *et al.* 1994, Mattos *et al.* 2000). Human populations on a high fish diet have extended pregnancies which might be related to a decreased ability of the reproductive tract to up-regulate PG synthesis sufficiently at term (Olsen *et al.* 1986, 1992). Paradoxically, *in vivo* challenge of the cows used in Experiment 2 during an earlier oestrous cycle found no effect of the diet on the PGFM response to an OT challenge on days 15 and 16 of the cycle, but there was a slightly enhanced response on day 17 in the n-6 fed cows in comparison with the controls (R S Robinson, P G A Pushpakumara & D C Wathes, unpublished observations). The reasons for this discrepancy are unclear. *In vivo* there would be a continual supply of more LA for AA synthesis from the blood, but conversely rapid utilisation of LA and AA for PG production during luteolysis might prevent a local build up in the tissues. *In vitro*, in contrast, both the supply and rate of utilisation of these precursors may be more limited and this could influence the dynamics of the reactions.

In conclusion, the present study in dairy cows demonstrated that a diet supplemented with n-6 PUFAs (predominantly LA) decreased uterine PG production significantly *in vitro*. This inhibitory effect was considerably greater than that produced by the diet supplemented with n-3 PUFAs (predominantly ALA). This may have implications for the fertility of both cattle and human populations consuming high n-6 diets.

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