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). PGF2α, PGE2 and 6-keto-PGF1α 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 PGF2α and PGE2 but increased 6-keto-PGF1α. 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.

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 fat 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 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 PGF$_{3\alpha}$ and PGE$_{3}$; Kinsella et al. 1990) and EPA into 3 series PGs (such as 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 A$_2$ (PLA$_2$) or the co-ordinated action of phospholipase C (PLC) and diacyl glycerol lipase generating two second messengers, inositol triphosphate (which increases intracellular calcium; [Ca$^{2+}$]) 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. PLA$_2$ (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 [Ca$^{2+}$], and COX-2 mRNA (Asselin et al. 1997). The calcium ionophore A23187 (Ca$^+$) promotes calcium flux across membranes and activates PLA$_2$. 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 isonenergetic 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 Ca$^+$ 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 isonenergetic 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 rumenal 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 $3 \times 3$ factorial design such that each dietary treatment (CONT, n-3 or n-6) was
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 (comprising 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 Cal (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.

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

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
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<td></td>
<td>CONT  n-3</td>
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<td>2649</td>
<td>3184</td>
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<td>390.7</td>
<td>117.3</td>
<td>388.9</td>
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*Z. Cheng and others*
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)**

PGE\(_2\) 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 (× 20–100) without prior extraction. One hundred microlitres of standards or diluted samples were mixed with 100 µl anti-PGE\(_2\) 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 4 ml scintillant (Ultima gold; Packard Bioscience BV, Pangbourne, Berks, UK) and counted for 2 min. The concentrations of PGE\(_2\) 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\(_{2\alpha}\) and 6-keto-PGF\(_{1\alpha}\) were carried out using the same procedures as described for PGE\(_2\), but using the standards, antisera and tracers for PGF\(_{2\alpha}\) and 6-keto-PGF\(_{1\alpha}\) respectively. The limit of detection was 1 pg/tube for PGF\(_{2\alpha}\) and 8 pg/tube for 6-keto-PGF\(_{1\alpha}\). The intra-assay Co-Var values for PGF\(_{2\alpha}\) and 6-keto-PGF\(_{1\alpha}\) were 4·1% and 6·5% respectively and the interassay Co-Var values were 9·6% for PGF\(_{2\alpha}\) and 3·8% for 6-keto-PGF\(_{1\alpha}\). 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 ± 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\(_{2\alpha}\), PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) 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\(_{2\alpha}\) increased significantly from 18 to 42 h (P<0·05), in the n-3 group the concentrations of PGF\(_{2\alpha}\) did not differ between culture times (P>0·05) and in the n-6 group, the concentrations of PGF\(_{2\alpha}\) at 18 and 42 h were about half of those at 2 h (P<0·05). PGE\(_2\) 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\(_{1\alpha}\) 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\(_{2\alpha}\), PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) 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\(_{2\alpha}\) and PGE\(_2\) production over a 2–42 h culture period (P>0·05) but it increased 6-keto-PGF\(_{1\alpha}\) production at 2 and 18 h (P<0·05).

In Experiment 2, the basal PGF\(_{2\alpha}\) 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 PGE\(_2\) production significantly when analysed overall (P<0·01) and significant difference was achieved at 42 h (P<0·05, Fig. 2). 6-keto-PGF\(_{1\alpha}\) 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\(_{2\alpha}\) and
6-keto-PGF1α significantly at 2 h compared with explants challenged with CM (P<0.05) and the effect of OT on PGF2α 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-PGF1α 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 PGF2α significantly higher at 6 h and of PGE2 and 6-keto-PGF1α 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). Individual comparisons showed that concentrations of PGF2α 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-PGF1α 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 PGF2α 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 PGF2α, PGE2 or 6-keto-PGF1α production when the tissues were
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\(_{2\alpha}\) at 6 and 24 h and PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) 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\(_{2\alpha}\) production was significantly inhibited at 2 h and 6 h following OT and Cal challenges \( (P<0.05) \). PGE\(_2\) 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\(_{2\alpha}\) but not PGE\(_2\) in response to OT at 2 h only, whereas responsiveness to Cal was still present at 6 h (PGF\(_{2\alpha}\); Fig. 6) and 24 h (PGE\(_2\); 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.
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 liseded (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.

In ruminants, luteolysis is achieved by OT stimulation of pulsatile PGF$_{2\alpha}$ 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).
Dietary PUFAs and uterine prostaglandin production

Other workers have shown that the epithelium secretes more PGE2 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 (Chargigny 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 CaI 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, CaI 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 2 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 & Wathe 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 PGF2α 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 PGF2α, PGE2 or 6-keto-PGF1α in response to a challenge with CaI. 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 PGF2α, PGE2 and 6-keto-PGF1α was significantly inhibited both before and after OT or CaI challenges and the same results were repeated in two
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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$_2$$\alpha$ (PGFM; a metabolite of PGF$_{2\alpha}$) 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 $^{14}$C- AA in vitro and production of PGF$_{2\alpha}$ and PGE$_2$ 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 PGI$_2$ and thromboxane (TX) A$_2$ (a PG produced by platelet COX) were observed (Sinclair & Mann 1996). When rats were fed an AA-rich diet, PGE$_2$ 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 TXB$_2$ 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 PGE$_1$ derived from dihomo-GLA, although it did not significantly influence the production of AA and PGE$_2$ by the aorta or the TXB$_2$ level in serum. Differences between experiments may relate to varying n-6:n-3 PUFAs 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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References


Abayasekara DRE, Sheldrick EL, Flick-Smith HC & Flint APF 1995 Role of protein kinase C in the inhibitory action of trophoblast interferons on expression of the oxytocin receptor in sheep endometrium. Endocrine 3 151–158.


German JB, Lokesh B & Kinsella JE 1988 The effect of dietary fish oils on eicosanoid biosynthesis in peritoneal macrophages is influenced by both dietary N-6 polyunsaturated fats and total dietary fat. Prostaglandins, Leukotrienes and Essential Fatty Acids 34 37–45.


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Raederstorff D & Moser U 1992 Influence of an increased intake of linoleic acid on the incorporation of dietary (n-3) fatty acids in phospholipids and on prostanooids in rat tissues. Biochimica et Biophysica Acta 1165 194–200.


Mann GE & Lamming GE 1994 Use of repeated biopsies to monitor endometrial oxytocin receptors in cows. Veterinary Record 135 403–405.


