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Altering n-3 to n-6 polyunsaturated fatty acid ratios affects prostaglandin production by ovine uterine endometrium

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ABSTRACT

Consumption of n-3 polyunsaturated fatty acids (PUFAs) is considered beneficial to health but effects on fertility remain uncertain. This study investigated the effect of n-3 PUFA supplementation on endometrial prostaglandin (PG) production. Ovine uterine endometrial cells were cultured to confluence in DMEM/F12 medium containing 10% foetal bovine serum. Stromal and epithelial cell populations were confirmed by immunocytochemistry. Cultures were supplemented with 0, 20 or 100 μM of α-linolenic acid (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA) with lipopolysaccharide (LPS) at 0 and 0.1 μg/ml, or different combinations of EPA with arachidonic acid (AA) in serum-free medium for 24 h. PGs were quantified using radioimmunoassay and PG-endoperoxide synthase (*PTGS*) isoforms, PGE and PGF synthase (microsomal *PGES1* and *PGFS*) mRNAs by qPCR. LPS increased PGE_2 production significantly without changing $PGF_{2\alpha}$ production, causing increased PGE_2:$PGF_{2\alpha}$ ratios. ALA and SDA increased PGE_2, $PGF_{2\alpha}$ and PGE_2:$PGF_{2\alpha}$ ratios ($P < 0.05$–0.01) while EPA alone did not affect PG generation. AA significantly stimulated *PTGS1* and *PTGS2* mRNA expression and PGE_2 and $PGF_{2\alpha}$ production ($P < 0.01$). The stimulatory effect of AA was attenuated by up to 80% ($P < 0.05$) when AA was combined with EPA. The PGE_2:$PGF_{2\alpha}$ ratio was not affected by AA or EPA alone, but increased when these two PUFAs were combined ($P < 0.05$). SDA and EPA decreased *PTGS1* mRNA expression ($P < 0.05$) but did not alter *PTGS2* expression. EPA and AA up-regulated *mPGES1* expression ($P < 0.05$) without affecting *PGFS* expression. Since AA is preferentially incorporated in uterine endometrium to produce 2-series PGs, alteration of PG production by EPA may affect many reproductive processes.

1. Introduction

A high intake of n-3 polyunsaturated fatty acids (PUFAs) can benefit many aspects of human health (Gil et al., 2012; Calder, 2013). Dietary and medical products enriched with n-3 PUFAs are thus widely promoted. To produce meat and milk with higher n-3 PUFAs, the diets rich in α-linolenic acid (ALA, 18:3n-3) and fish products were fed to food producing animals (Givens et al., 2006; Gulliver et al., 2012) and exogenous n-3 fatty acid desaturase (*fat-1*) genes converting n-6 to n-3 PUFAs were transgenically introduced into various mammalian species, including cows (Wu et al., 2012), sheep (Duan et al., 2012) and pigs (Zhang et al., 2012).

Both n-3 and n-6 PUFAs utilise the same enzyme systems for metabolism and PG production. ALA is metabolised into stearidonic acid (SDA, 18:4n-3) and eicosapentaenoic (EPA, 20:5n-3) while linoleic acid (LA, 18:2n-6) is converted into γ-linolenic acid (GLA, 18:3n-6),

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dihomo-γ-linolenic acid (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6). By the action of prostaglandin (PG)-endoperoxide synthase isozymes (*PTGS1* and *PTGS2*) and PGE and PGF synthases (PGES and PGFS), EPA is catalysed into 3-series PGs, such as PGE_3 and $PGF_{3\alpha}$, and DGLA and AA are converted into 1- and 2-series PGs, respectively, such as PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$. The PUFA supplementation may change the ratios of n-3 to n-6 and affect membrane phospholipid incorporation and metabolism of the PUFA families, and thus production of PGs (Huang et al., 1996; Calder, 2013).

Many reproductive processes involve inflammation, in which 2-series PGs, such as PGE_2 and $PGF_{2\alpha}$, are key signalling molecules (Challis et al., 2000; Jabbour et al., 2009). In both cattle and sheep luteolysis is caused by the pulsatile secretion of $PGF_{2\alpha}$ from the endometrium (Poyser, 1995; Wathes and Lamming, 1995). In the non-pregnant uterine endometrium $PGF_{2\alpha}$ is principally produced by epithelial cells and PGE_2 by stromal cells (Danet-Desnoyers et al., 1994; Charpigny et al., 1999). These PGs exert opposite effects on corpus luteum function: $PGF_{2\alpha}$ is a potent luteolysin (Horton and Poyser, 1976), whereas PGE_2 is luteotropic (Pratt et al., 1977). Supplementation with n-3 PUFAs may attenuate the inflammatory process via various mechanisms, including interrupting n-6 PUFA metabolism to reduce pro-inflammatory eicosanoids derived from AA and increasing production of "weak" eicosanoids (such as 3 – series PGs) and of anti-inflammatory resolvins and protectins from EPA and docosahexaenoic acid (DHA) (Wada et al., 2007; Calder, 2012, 2013). This will alter reproductive processes. When beef heifers were supplemented with rumen protected n-3 PUFA, many gene expression pathways associated with inflammation, lipid metabolism and reproduction in uterine endometrium were altered (Waters et al., 2012). It has been suggested that n-3 PUFA supplementation may benefit some aspects of reproduction (Gulliver et al., 2012). However *fat-1* transgenic mice with higher production of n-3 PUFAs suffered from serious reproductive disorders (Ji et al., 2009; Pohlmeier et al., 2011).

In the present study, we first examined the effects of different n-3 PUFAs on PG synthesis, then tested the hypothesis that altering the EPA:AA ratios may affect 2-series PG production by uterine endometrial cells.

2. Materials and methods

All reagents and consumables were supplied by Sigma Chemical Co. (Poole, Dorset, UK) or VWR International (Lutterworth, Leicestershire, UK) unless otherwise stated. All culture media contained 50,000 units/l penicillin and 50 mg/l streptomycin. All culture media were sterilised before use by passage through a 0.20 μm filter (Nalge Nunc International, Rochester, NY, USA).

2.1. Animals and experimental design

Two experiments were carried out using 20 cyclic ewes. In each experiment, 10 ewes were randomly grouped into 4 batches with 2 ewes in each of Batch 1 and 2, and 3 ewes in each of Batch 3 and 4. Oestrous cycles were synchronised by inserting intravaginal sponges containing 60 mg medroxyprogesterone acetate (Veramix, Pharmacia & Upjohn Animal Health Ltd, Northants, UK) for 12 days followed by an i.m. injection of 500 μl Estrumate (0.1% cloprostenol, Schering Plough Ltd., Welwyn Garden City, UK) at sponge withdrawal. Ewes showed behavioural oestrus 2 days later as confirmed by running with a vasectomised ram. Ewes were killed by captive bolt pistol on day 3 of the subsequent cycle for collection of the uterus. This timing of collection promotes optimum cell growth in vitro with both epithelial and stromal cell populations present to allow paracrine interactions. These experiments were performed under the UK Home Office (Scientific Procedures) Act 1986.

2.2. Cell isolation and culture

Uterine endometrial cells were isolated using trypsin-collagenase digestion as reported previously (Cheng et al., 2003). Strips of intercaruncular endometrium were separated from the uterus by manual dissection in a laminar flow hood under sterile conditions and put into serum-free Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F12 medium) (Sigma). The strips were then chopped into 1 mm^3 cubes using a McIlwain mechanical tissue chopper (McIlwain Laboratory Engineering, Guilford, Surrey, UK). The chopped tissue (about 40 g) was placed into a 200 ml sterile vial and mixed with 30 ml digestive solution containing 0.05% trypsin III (Roche Diagnostic GmbH, Mannheim, Germany), 0.05% collagenase II (Roche) and 0.11% fatty acid free bovine serum albumin (BSA, Sigma) in Hank's Balanced Salt Solution (HBSS, Sigma). This was allowed to settle for 10 min at room temperature, then the supernatant was removed and replaced with 60 ml fresh digestive solution. Following incubation for 90 min at 37 °C with manual mixing every 30 min, the cell suspension was filtered through a 100 μm mesh into 50 ml falcon vials. The suspension was re-suspended with supplemented HBSS containing 10% foetal bovine serum (FBS, Sigma) and 3 μg/ml trypsin inhibitor (Sigma) and centrifuged at $100 \times g$ and 10 °C for 10 min. The supernatant was removed and re-suspended with supplemented HBSS. Following two repetitions of the above washing procedures, the cell pellets containing mixed epithelial and stromal cells were re-suspended in DMEM/F12 medium containing 10% FBS to 0.25×10^5 cells/ml and plated in 24 well IWAKI micro plates (Scitech DIV, Asahi Techno Glass, Japan) at 2 ml per well. Following 20 h in culture, when the endometrial cells, including stromal and epithelial cells attached to the plate, the culture medium was changed to remove the other cells (such as red blood cells) and tissue debris. DMEM/F12 medium containing 10% FBS (2 ml/well) was changed every 48 h for 7–8 days to allow the cells to grow to confluence. The purification of the cell populations was confirmed by (1) cell morphology as previously described (Fortier et al., 1988) and (2) immunocytochemical staining with cell specific staining of cytokeratin and vimentin following the method described previously (Arnold et al., 2001). The results showed a monolayer of a mixed population of stromal (57%) and epithelial (43%) cells.