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The effect of supplementation with n-6 polyunsaturated fatty acids on 1-, 2- and 3-series prostaglandin F production by ovine uterine epithelial cells

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Abstract

Linoleic acid (LA, 18:2*n*-6) has variously been found to increase or inhibit synthesis of 2-series prostaglandins (PGs), derived from arachidonic acid (AA, 20:4n-6). γ-linolenic acid (GLA, 18:3*n*-6) containing oils are promoted to women for a variety of reproductive problems. Little is known concerning their actual effects on reproduction. We investigated the effects of LA, GLA and AA supplementation (25–100 μM) on basal and oxytocin (OT) stimulated production of 1-, 2- and-3 series PGs by uterine epithelial cells isolated from non-pregnant ewes, used as a model system to study endometrial PG production. PGF isomers were measured using radioimmunoassays following separation by high performance chromatography (HPLC). OT challenge increased the proportion of $PGF_{2\alpha}$ in relation to $PGF_{1\alpha}$ and $PGF_{3\alpha}$ in control medium. LA supplementation decreased all PGF isomer production and reduced responsiveness to OT. GLA increased both absolute and proportional $PGF_{1\alpha}$ production and slightly enhanced $PGF_{2\alpha}$ generation. AA increased $PGF_{2\alpha}$ generation and raised its isometric proportion. Both GLA and AA increased overall PGF output significantly but prevented the cells from responding to OT. These results suggest that consumption of LA and GLA are likely to differentially alter both uterine PG metabolism and responsiveness to OT. This may have implications for the control of a variety of reproductive processes.

Keywords: Polyunsaturated fatty acid; Prostaglandin isomer; Epithelial cell; Uterus; Ewe

1. Introduction

Most vegetable oils contain high concentrations of n-6 polyunsaturated fatty acids (PUFAs), predominantly as linoleic acid (LA) [1]. LA can be metabolised into γ-linolenic acid (GLA, 18:3*n*-6) by Δ6 desaturase. The action of elongase adds two carbons to GLA to become dihomo-γ-linolenic acid (DGLA, 20:3*n*-6), which can be further desaturated into arachidonic acid (AA, 20:4*n*-6) by Δ5 desaturase. Eicosanoids are oxygenated metabolites of the 20-carbon *n*-6 PUFAs AA and DGLA and the n-3 PUFA eicosapentaenoic acid (EPA, 20:5*n*-3) and include prostaglandins (PGs), thromboxanes, leukotrienes and lipoxins. The initial step in the formation of PGs from PUFAs is catalysed by the cyclooxygenase enzymes COX-1 and COX-2. When acting on AA, the preferred substrate [2–4] this gives rise to PGH_2 which can in turn can be converted into a variety of 2-series PGs, of which $PGF_{2\alpha}$ and PGE_2 predominate in uterine tissues [5]. However other *n*-3 and *n*-6 18–22 carbon PUFAs can also be oxygenated by both COX isoenzymes [2–4], which catalyse the conversion of DGLA into 1-series PGs and of EPA into 3-series PGs. Due to the limited activity of Δ6 desaturase, only a small fraction of dietary LA consumed is converted to GLA. Consumption of GLA should bypass the limitations of the normal metabolic pathway and thus increase the production of LA metabolites, such as 1 series PGs, which are believed to be both anti-inflammatory and anti-carcinogenic [6]. Less is known about the 3 series PGs, such as $PGF_{3\alpha}$ and PGE_3, but they are generally believed to have lower biological activity compared to their 1- and 2-series homologues [7].

Many previous studies have investigated the control of PG production by the ruminant uterus. In both cattle and sheep luteolysis is caused by pulsatile $PGF_{2\alpha}$ secretion from the

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endometrium [5,8]. Luteolysis in the non-pregnant ewe begins on approximately day 14 after oestrus and progresses rapidly through days 16–17. The main regulatory factor in the ewe is the timing of up-regulation of oxytocin (OT) receptor (OTR) in the uterine epithelium [8]. The same process is thought to play a modulatory role in the cow [9]. In both species, OT can increase endometrial $PGF_{2\alpha}$ generation via various mechanisms. These include activating phospholipase (PL) A_2 [10,11], PLC and DAG [12,13], protein kinase C [14,15] and intracellular calcium [16], and inducing COX-2 expression [10,16]. The effect of OT on COX-2 expression is probably mediated indirectly via PLC generated AA release [12], as AA can itself up-regulate COX expression in the ruminant uterus [17]. In the non-pregnant state, $PGF_{2\alpha}$ is principally produced by the epithelial cells and PGE_2 by stromal calls [18,19]. COX-2 is localized exclusively to the uterine epithelial cells and COX-1 mRNA was either reported as low [19] or not detectable in these cells [20].

As the proportions of different PUFAs in cell membranes reflect the amounts consumed in the diet, it follows that the relative amounts of 1-, 2- and 3-series PGs produced by COX activation can also be influenced by dietary factors [1]. This in turn is likely to influence female reproductive processes since PGs are key signalling molecules in the mechanisms leading to ovulation, luteal regression, menstruation and parturition [5,21,22]. In this investigation, we have used cells collected from the ovine uterus as a model system to determine how consumption of either LA or GLA may affect uterine PG production. In humans, LA comprises approximately 10% of the total fatty acid intake in a typical Western diet [1]. GLA containing oils such as evening primrose and borage oil are widely promoted as health products [6]. In our previous work, we have shown that in vitro supplementation of LA to endometrial cells isolated from maternal intercotyledonary endometrium in late pregnant ewes caused a significant reduction of 2 series PG production [23,24]. Similarly, when cyclic dairy cows were fed a dietary supplement containing a high LA concentration, PG production by uterine endometrial tissues collected in the late luteal phase was significantly reduced in comparison with that obtained from cows on a control diet [25]. On the other hand, feeding a similar diet high in LA to late pregnant ewes increased endometrial and placental PG production [26]. This was associated with an approximate 50% increase in AA concentrations in blood and caruncular endometrium [27], suggesting that in this case effective metabolism of LA to AA had occurred, thus providing more precursor.

All of these previous studies into uterine PG production in sheep and cattle have, however, focused solely on 2-series PGs, by measuring unseparated (pooled) PGs using antibody binding assays, such as RIAs and enzyme immunoassays. Due to the structural similarity of the isomers, most of the available antibodies to PGF isomers always possess cross-reactivity to each other and the accuracy of measurement can be compromised.

The aim of the present study was thus to compare the effects of supplementation with the different n-6 PUFAs LA, GLA and AA on the production of $PGF_{2\alpha}$ and its isomers $PGF_{1\alpha}$ and $PGF_{3\alpha}$ by uterine endometrial epithelial cells isolated from non pregnant ewes, as a model system for uterine PG production. The PGF isomers were measured individually following separation on HPLC, as described previously [28]. We also present the pooled values measured by a standard $PGF_{2\alpha}$ RIA, to provide a comparison with earlier work. The study focussed solely on PGF production, as this is the major product of ovine uterine epithelial cells [16,29].

2. Materials and methods

All reagents were supplied by Sigma Chemical Co. (Poole, Dorset, UK) or BDH Merck Ltd. (Lutterworth, Leics, UK) unless otherwise stated. All culture media contained 50,000 units/l penicillin and 50 mg/l streptomycin. Culture medium and challenges were sterilised before use by passage through a 0.20-μm filter (Nalge Nunc International, Rochester, NY14602-0365, USA or Millipore Company, Bedford, MA 01730, USA).

2.1. Animals

The experiment was carried out using cells obtained from 18 mature ewes with animals synchronised in batches of 2–3 ewes. During the breeding season, 5 batches of ewes were brought into oestrus by i.m. injection of 500 μl Estrumate (0.1% cloprostenol, Schering Plough Ltd., Welwyn Garden City, UK) in the mid-luteal phase and the ewes were killed on days 1–2 of the following cycle using an overdose of pentabarbitone. Three batches of ewes were also used during seasonal anoestrus. These animals were given an intravaginal sponge containing 60 mg medroxyprogesterone acetate (Veramix, Pharmacia and Upjohn Animal Health Ltd., Northants, UK) for 12 days followed by injections of oestradiol-17β (each 50 μg i.m. in 0.5 ml sterilised corn oil) at 0, 24 and 36 h after sponge removal. Animals were killed 48 h after sponge removal (equivalent to days 1–2 of the natural oestrous cycle). These two systems produced equivalent results (data not shown).

2.2. Cell isolation and culture

Uterine epithelial cells were isolated using a method described previously with modification [30]. Briefly, the uterus was removed *post mortem* and placed in a laminar flow hood to maintain sterility. The uterine lumen was rinsed by clamping the cervical end, injecting 20 ml Hank's Balanced Salt Solutions (HBSS) to the oviductal end, the whole tract was inverted several times and the solution was drained from the oviductal end. Twenty ml of digestive solution containing 4 mg/ml dispase II (Boehringer Mannheim Corporation, Indianapolis, IN, USA) and 0.05 mg/ml DNAse I (Roche Molecular Biochemicals, GmbH, Mannheim, Germany) dissolved in 50:50 HBSS and pancreatin 4×NF (Gibco Life Technologies, Paisley, UK) was then injected into the horn through the oviductal end. The oviductal ends were clamped to prevent leakage and the uterus was incubated in a humidified incubator at 39 °C with 5% CO_2 for 150 min while palpating and inverting every 15 min. The digestive solution containing the cells was then drained from the oviductal end into a sterilised beaker, the uterus was flushed twice with 10 ml HBSS and the pooled solution was filtered through a 100-μM mesh to remove undigested tissue. The filtrates were then re-suspended with 50 ml HBSS containing 10% fetal calf serum (FCS) in a 50 ml falcon vial to remove the enzyme solution. The vial was centrifuged at 100×*g* for 10 min, the supernatant was discarded and the cells were washed 3 times with 20 ml HBSS containing 10% FCS. The isolated epithelial cells from the 2–3 ewes per batch were then pooled and re-suspended in Dulbecco's minimal essential medium/F12 1:1 nutrient mix (DMEM/F12 medium, Gibco Life Technologies, Paisley, Strathclyde, UK) containing antibiotics and 10% FCS. About 200,000 cells in 2 ml of the above medium were allocated into each well of a 24-well IWAKI Microplate (Scitech DIV, Asahi Techno Glass, Japan). Culture medium was changed every 48 h for 7–8 days to allow the cells to grow