The effects of a high-fat, high-cholesterol diet on markers of uterine contractility during parturition in the rat

M J Elmes, D S-Y Tan, Z Cheng¹, D C Wathes¹ and S McMullen

Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonnington Campus, Loughborough, Leicestershire LE12 5RD, UK and ¹Reproduction and Development Group, Royal Veterinary College, Potters Bar, Hertfordshire AL9 7TA, UK

Correspondence should be addressed to M J Elmes; Email: matthew.elmes@nottingham.ac.uk

Abstract

Increasing levels of obesity within women of reproductive age is a major concern in the UK. Approximately, 13% of women aged <30 and 22% of 31- to 40-year-old women are obese. Obesity increases complications during pregnancy and the risk of caesarean section due to prolonged labour and poor uterine activity. The aim was to investigate whether a high-fat, high-cholesterol (HFHC) diet decreases markers of uterine contractility during parturition in the rat. Female Wistar rats were fed control (CON, n=10) or HFHC (n=10) diets for 6 weeks. Animals were mated and, once pregnant, maintained on their diet throughout gestation. On gestational day 19, rats were monitored continuously and killed at the onset of parturition. Body and fat depot weights were recorded. Myometrial tissue was analysed for cholesterol (CHOL), triglycerides (TAG), and expression of the contractile associated proteins gap junction protein alpha 1 (GJA1; also known as connexin-43, CX-43), prostaglandin-endoperoxide synthase 2 (PTGS2; also known as cyclooxygenase-2, COX-2) and caveolin-1 (CAV1) and maternal plasma for prostaglandin F2α (PGF2α) and progesterone. HFHC fed rats gained greater weight than CON (P<0.003) with significant increases in peri-renal fat (P<0.01). The HFHC diet increased plasma CHOL, TAG and progesterone, but decreased PGF2α versus CON (P<0.01, P<0.01, P=0.05 and P<0.02 respectively). Total CHOL and TAG levels of uterine tissue were similar. However, HFHC fed rats showed significant increases in PTGS2 (P<0.037), but decreases in GJA1 and CAV1 (P=0.059). In conclusion, a HFHC diet significantly increases body weight and alters lipid profiles that correlate with decreases in key markers of uterine contractility. Further work is required to ascertain whether these changes have adverse effects on uterine activity.

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Introduction

Obesity is a major public health concern, and its prevalence in women of reproductive age is increasing globally (WHO 2000). Currently, 13% of 21- to 30-year-old women and 22% of 31- to 40-year-old women in the UK are obese, with figures estimated to rise to 30 and 47% respectively by 2050 (FORESIGHT 2001). Obese women have a significantly higher risk of developing complications during pregnancy than those with a normal body mass index, and are at an increased risk of undergoing emergency caesarean section (Crane et al. 1997, Loverro et al. 2001, Cedergren 2004, Weiss et al. 2004) due to poor uterine activity and prolonged labour (Zhang et al. 2007).

Obesity correlates positively with plasma cholesterol (CHOL) concentrations (Gostynski et al. 2004), and raised serum CHOL in obese pregnant women (Ramsay et al. 2002) has been observed to play a key role in smooth muscle contraction (Babiychuk et al. 2004, Smith et al. 2005). Ex vivo manipulation of myometrial CHOL content reveals that decreasing and increasing CHOL increases and decreases the strength of myometrial contractions respectively, suggesting that high circulatory CHOL seen in many obese women could impair uterine activity during labour (Smith et al. 2005).

Uterine contractile activity is regulated by the key proteins caveolin-1 (CAV1), gap junction protein alpha 1 (GJA1; also known as connexin-43, CX-43) and prostaglandin-endoperoxide synthase 2 (PTGS2; also known as cyclooxygenase-2, COX-2). CAV1 is the critical structural component of caveolae (Okamoto et al. 1998), which are omega-shaped CHOL-rich invaginations of cell membranes that act as platforms for the coding of intracellular signals (Schlegel et al. 1998, Shaul & Anderson 1998). Although three different isoforms exist (CAV1–3; Okamoto et al. 1998), CAV1 controls activity of transduction pathways as Cav1 knockout mice exhibit impaired smooth muscle (aortic ring) vascular relaxation (Drab et al. 2001), and CHOL extraction reduces caveolae number increasing contractility of both human and rodent myometrium (Noble et al. 2006). High CHOL concentrations may increase caveolae number and CAV1 expression of
myometrial tissue, and may be a possible mechanism through which obesity leads to prolonged labour.

During labour, the uterus is primed to contract in response to stimuli through coordinated up-regulation of the contractile associated proteins (CAPs) GJA1 and PTGS2 (Challis & Lye 1994). GJA1 is the major myometrial gap junction that facilitates intracellular propagation of electrical impulses (Willecke et al. 2002) and synchronised myometrial contractions. As a consequence, GJA1 is up-regulated following the onset of labour and during delivery (Garfield et al. 1977, 1995, Chow & Lye 1994, Orsino et al. 1996). GJA1 plays a key role in parturition as its myometrial loss leads to defects in physiological coordination of uterine contractions, and labour is subsequently prolonged (Cluff et al. 2006, Doring et al. 2006). PTGS2 is responsible for the synthesis of the prostaglandins, PGF₂α and PGE₂, which regulate uterine activity during pregnancy and parturition (Zuo et al. 1994). PGE₂ causes uterine relaxation (Lopez Bernal et al. 1993), whereas PGF₂α stimulates the myometrial contractions that expel the foetus during labour (Challis et al. 1997). Uterine PTGS2 expression rises during late gestation but increases significantly with the onset of labour (Dong et al. 1996), and selective inhibition blocks PG production and delays induced labour in sheep (Scott et al. 2001) and mice (Gross et al. 2000).

Although current evidence suggests that myometrial contractility during labour may be adversely affected by obesity via CHOL-mediated mechanisms, no study to date has investigated this directly in an in vivo model. Therefore, the purpose of the present study was to utilise an animal model to test the hypothesis that a high-fat, high-cholesterol (HFHC) diet would lead to adverse lipid profiles and decrease expression of key markers of uterine contractility during parturition in the rat.

Results

Body and fat depot weights

Rats were fed either a control (CON) chow diet or a HFHC diet for 6 weeks prior to mating and throughout pregnancy. Feeding the HFHC diet to Wistar rat dams for 6 weeks prior to mating did not increase weight gain and therefore body weight at mating significantly when compared with controls (Fig. 1A and Table 1). However, pregnant rats maintained on the HFHC diet throughout gestation gained more weight than rats fed a CON diet, leading to a significant difference in body weight at the end of pregnancy (Fig. 1B and Table 1). Weighing of different fat depots revealed that only renal fat was affected by diet, with a significant weight increase in the HFHC fed rat dams (See Table 1).

Litter size and weight

Both groups of rats commenced delivery at a similar stage of gestation: controls took 22.2 ± 0.16 days and HFHC fed rats took 22.5 ± 0.23 days respectively. Litter size was not affected by maternal diet, and similarly average pup weights and total litter weights were not significantly different between pregnant rats fed either a CON or diet a HFHC diet (see Table 1).

Lipid profiles

Determination of total CHOL and triglyceride (TAG) concentration in the maternal plasma and uterine tissue from laboratory chow and HFHC fed rat dams produced contrasting results (Fig. 2). Circulating plasma total

Table 1 Measurements of body weight, litter size and weight and fat pad weights in control rats and those fed a high-fat, high-cholesterol (HFHC) diet.*

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>HFHC (n=7)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pregnancy weight gain (g)</td>
<td>132.67 ± 4.11</td>
<td>142.09 ± 4.79</td>
<td>0.403</td>
</tr>
<tr>
<td>End of pregnancy weight (g)</td>
<td>302.05 ± 6.21</td>
<td>321.37 ± 3.04</td>
<td>0.054</td>
</tr>
<tr>
<td>Litter size (n)</td>
<td>11.13 ± 1.09</td>
<td>10.14 ± 0.94</td>
<td>0.513</td>
</tr>
<tr>
<td>Average pup weight (g)</td>
<td>5.48 ± 0.15</td>
<td>5.41 ± 0.12</td>
<td>0.717</td>
</tr>
<tr>
<td>Total litter weight (g)</td>
<td>61.23 ± 5.70</td>
<td>54.46 ± 4.54</td>
<td>0.379</td>
</tr>
<tr>
<td>Renal fat (g)</td>
<td>2.77 ± 0.26</td>
<td>3.93 ± 0.35</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Gonadal fat (g)</td>
<td>7.15 ± 0.76</td>
<td>8.11 ± 0.71</td>
<td>0.41</td>
</tr>
<tr>
<td>Intrascapular fat (g)</td>
<td>0.93 ± 0.26</td>
<td>0.86 ± 0.17</td>
<td>0.829</td>
</tr>
</tbody>
</table>

*Except for the pre-pregnancy weight gain, all other measurements were taken immediately after birth of the first pup.
CHOL and TAG concentrations were almost three times higher in HFHC fed rat dams compared with controls (both P < 0.001). Uterine tissue levels of CHOL and TAG did not, however, differ between the two dietary groups.

**Uterine expression of CAPs**

Western blot analysis of GJA1, PTGS2 and CAV1 provided evidence that a HFHC diet can significantly alter the uterine expression of CAPs during parturition (Fig. 3). Rat dams fed the HFHC diet had significantly lower expression of GJA1 and CAV1 (both at the P < 0.059 level), but a higher expression of PTGS2 in comparison to controls (P < 0.037).

**Maternal plasma PGF2α**

After identifying that the HFHC diet caused a significant increase in uterine expression of PTGS2 during established labour, it was important to elucidate whether this increase would result in changes in PGF2α, which regulate myometrial contractions and help to expel the foetus during labour. Analysis of maternal plasma for PGF2α through RIA revealed that the feeding of a HFHC diet significantly reduced circulatory levels of PGF2α from 0.16 ± 0.022 ng/ml (observed in controls) to 0.086 ± 0.023 ng/ml; P < 0.02 (Fig. 4a).

**Maternal plasma progesterone**

With data already establishing that a HFHC diet decreases myometrial expression of key CAPs during labour, we wanted to determine whether maternal progesterone levels that can influence the expression of CAPs within the myometrium are altered by the HFHC diet. Analysis of maternal plasma for progesterone provided evidence that a HFHC diet fed prior to and during pregnancy was associated with dams starting labour with a significantly higher progesterone level of 33.9 ± 2.9 ng/ml compared with 27.3 ± 2.4 ng/ml, observed in controls; P = 0.05 (Fig. 4b).

**Discussion**

This study was the first to utilise a rat model to assess the effects of a HFHC diet on maternal lipid profiles and markers of uterine activity during parturition. The aim was to investigate whether dietary increases in lipid profiles would down-regulate key CAPs during labour in the rat, providing a possible mechanism behind the dysfunctional labour and poor myometrial activity commonly observed in obese women (Crane et al. 1997, Loverro et al. 2001, Cedergren 2004, Weiss et al. 2004, Zhang et al. 2007). Significant elevations in serum CHOL and TAGs are a normal occurrence during pregnancy (Piechota & Staszewski 1992, Toescu et al. 2004), but feeding a HFHC diet to rats 6 weeks prior to and during pregnancy significantly increased the circulatory levels of CHOL and TAGs when compared with
controls, mirroring the raised serum level of CHOL and TAGs observed in obese pregnant women (Ramsay et al. 2002). In contrast, and despite the increased circulating levels of CHOL and TAGs, feeding rats a HFHC diet did not increase incorporation of total CHOL levels within uterine tissue.

Previous studies investigating the effects of CHOL on uterine activity only manipulated tissue CHOL concentrations in an ex vivo setting, where myometrial strips were derived either from caesarean section biopsies in women (Zhang et al. 2007) or dissected from pregnant rats (Smith et al. 2005). Both studies provide evidence that CHOL content within myometrial tissue is important for normal uterine function during pregnancy. CHOL enrichment was inhibitory to both spontaneous and oxytocin-stimulated contractile force of the myometrium, and reversed following CHOL depletion. But an important omission that remains to be determined is whether uterine tissue from obese pregnant women who exhibit poor uterine activity during labour actually has increased incorporation of CHOL within their plasma membranes compared with women undergoing routine delivery. Within our model, the raised circulating CHOL concentrations induced by a HFHC diet were not reflected in uterine tissue. It is, however, possible that the CHOL could be in different pools within the cells from rats fed the HFHC diet or CON diet.

Despite the HFHC diet having no effect on the uterine tissue concentration of CHOL and TAGs during labour, significant differences in expression of key CAPs were evident. Feeding rats a HFHC diet decreased uterine expression of CAV1 and GJA1, but increased expression of PTGS2 significantly. Decreased expression of myometrial GJA1 during labour agrees with our original hypothesis, but the respective increase and decrease in PTGS2 and CAV1 expression were unexpected. Caveolae and their structural proteins CAVs (Okamoto et al. 1998) are extensive in myometrial smooth muscle cells increasing its surface area by almost 75% (Somlyo 1985). CAVs act as a scaffold to congregate multiple proteins involved in calcium signalling and CON of smooth muscle contractions, including calcium channels, calcium-binding proteins, calcium pumps (Darby et al. 2000) and calcium-sensitive potassium channels (Brainard et al. 2005). The calcium-sensitive potassium channel provides a strong repolarising current to buffer cell excitation in response to excitatory cell signals (Jackson 2000), and inhibition of this channel pharmacologically during late pregnancy induces myometrial cell depolarisation and enhanced uterine contraction (Anwer et al. 1993). Interestingly, uterine extraction of CHOL reduces caveolae number and CAV1 expression, resulting in increased contractility of human and rodent myomyometrium (Riley et al. 2003, Noble et al. 2006). This finding implies that decreased uterine expression of CAV1 following a HFHC diet in the context of this study may act to increase myometrial contractile activity during labour, suggesting that CAV1 expression is not adversely affected by the HFHC diet as hypothesised.

The HFHC diet was also associated with a reduced expression of the gap junction protein GJA1 within labouring uterine tissue. GJA1 gap junctions are specialised channels that allow direct intracellular transfer between coupled cells, thereby facilitating electrical communication and synchronous contractile activity (Willecke et al. 2002). GJA1 is expressed in extremely low levels in the non-pregnant uterus, but is increased significantly in abundance during parturition (Chow & Lye 1994, Orsino et al. 1996, Ou et al. 1997). GJA1 plays a key role in parturition, as extensive, but not full, loss of protein leads to uncoordinated contractile function of the murine uterus and prolonged labour (Doring et al. 2006). Similar findings are observed in women experiencing prolonged labour, as they exhibit significantly lower expression of GJA1 than women with routine deliveries. Mechanical stretch of the uterus is another key regulator of myometrial contractile activity. Two key studies reported increases in gap junction number following distension of the non-pregnant rat uterus with an intrauterine balloon (Wathes & Porter 1982, Manabe et al. 1985). Furthermore, uterine distension was shown to be a requirement for full expression of GJA1 and synchronous uterine contractility during labour (Garfield et al. 1995, Ou et al. 1997). Rapid foetal growth that occurs during the final stages of pregnancy could be the stretch-induced stimulus that enhances uterine expression of GJA1. Importantly, offspring weights and litter size (Table 1) at birth did not differ between rats fed the CON or HFHC diets, providing evidence that decreased uterine expression of GJA1 during labour in HFHC fed animals was not due to
differences in stretch-induced stimuli. The current findings suggest that exposure to a HFHC diet decreases uterine expression of GJA1, and may thus lead to uncoordinated uterine contractions and prolonged labour. Although the timing of delivery for the first pup was similar between CON and HFHC fed rats (both started to deliver at 22 days), this does not indicate whether uterine contractile strength or length of labour for delivery of whole litters was adversely affected.

PTGS2-derived PGE\(_2\) and PGF\(_{2\alpha}\) increase with the onset of labour (Zuo et al. 1994, Dong et al. 1996, Lye 1996), and are responsible for uterine relaxation (Lopez Bernal et al. 1993) and activation of contractile machinery respectively. Inhibition of PTGS2 blocks basal PG production and delays induced premature labour in sheep (Poore et al. 1999) and mice (Gross et al. 2000). The current finding that a HFHC diet increased PTGS2 expression in labouring uterine tissue was unexpected. However, recent research has provided evidence that saturated fatty acids induce expression of PTGS2 in macrophage cells (Lee et al. 2001), which infiltrate the myometrium and cervix with the onset of labour (Thomson et al. 1999, Osman et al. 2003). The HFHC diet fed to rats prior to and during pregnancy is high in saturated fat, and could explain the increased PTGS2 expression in their labouring uterine tissue. Despite this increase, there was no positive correlation with circulatory PGF\(_{2\alpha}\) concentrations, which were significantly reduced in rat dams fed the CON diet. Lower PGF\(_{2\alpha}\) concentrations could produce less forceful contractions during labour and could be due to decreased incorporation of unsaturated fatty acids (that are precursors for PG production) into the phospholipid membrane of the uterine tissue. Another possible reason for the discrepancy between PTGS2 expression and plasma PGF\(_{2\alpha}\) concentration may be that the HFHC diet inhibited PGF synthase (PGFS) activity, which converts PGH\(_2\) into PGF\(_{2\alpha}\).

With CHOL being an important precursor for steroid hormone production that plays key roles in the timing of labour and myometrial expression of CAPs, we determined the maternal plasma concentrations of progesterone between the two dietary groups. Our data revealed that rats exposed to a HFHC diet had significantly higher concentrations of plasma progesterone at labour onset when compared with controls. This associated decreased expression of the myometrial GJA1 with higher maternal progesterone concentrations is consistent with previously published studies. Administration of exogenous progesterone at term in the ewe not only inhibits the expression of myometrial CAPs but also blocks the onset of labour (Lye & Porter 1978). Furthermore, functional withdrawal of progesterone in the myometrium induces CAP expression and activates myometrial contractile activity (Lye et al. 1998). These data therefore provide some evidence to suggest that the adverse effects of a HFHC diet on myometrial contractile protein expression may be mediated through alterations in steroid synthesis.

In conclusion, a HFHC diet fed prior to and during pregnancy led to significant increases in circulatory CHOL, TAG and progesterone concentrations, which were associated with altered expression of CAPs in labouring uterine tissue. Contrary to our original hypotheses, expression of CAV1 and PTGS2 were decreased and increased respectively, which would be expected to increase contractility. In contrast, GJA1 expression and circulatory PGF\(_{2\alpha}\) concentrations were decreased, which we speculate could adversely affect co-ordination and strength of myometrial contractions during parturition in the rat. Further research is required: firstly, to determine whether these changes in uterine expression of CAPs following a HFHC diet lead to adverse physiological changes in myometrial contractions; secondly, whether these changes occur through key alterations in synthesis of steroid hormones of which CHOL is the precursor and finally whether the HFHC diet affects the expression and activities of PGFS and PGES.

Materials and Methods

Animals and experimental design

Within the animal facilities at the University of Nottingham, twenty virgin Wistar rat dams (Harlan Ltd, Belton, Leics, UK) weighing \(~ 60 \text{ g}\) were pair-housed and randomly assigned to be fed either a CON (standard laboratory chow, B&K Universal Ltd, Hull, UK, \(n=10\)) or a HFHC (\(n=10\)) diet for 6 weeks (HFHC diet details in Table 2). Rat dams were then mated naturally with Wistar stud males, and pregnancy was confirmed through the appearance of a semen plug on the cage floor. The pregnant rats were then housed individually and maintained on their CON or HFHC diets throughout gestation. At gestational day 20, hourly checks were made for signs of parturition, and following the birth of the first pup, each rat dam was immediately killed by CO\(_2\) asphyxiation and cervical dislocation. Maternal blood samples were collected by cardiac puncture and transferred to heparin tubes, centrifuged at

<table>
<thead>
<tr>
<th>Constituents</th>
<th>g/kg</th>
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<tbody>
<tr>
<td>Corn oil</td>
<td>100</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>Maize starch</td>
<td>218</td>
</tr>
<tr>
<td>Butter</td>
<td>295</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>20</td>
</tr>
<tr>
<td>Methionine</td>
<td>10</td>
</tr>
<tr>
<td>Choline</td>
<td>2</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2 Composition of the high-fat, high-cholesterol diet.
13 000 g at 4 °C for 10 min, and the plasma was retained for analysis of TAG, CHOL and PGF$_{2a}$. The uterus was dissected; foetuses were removed and separated from their foetal membranes and placentas and killed by destruction of the brain and decapitation. The uterus was immediately split into two horns, snap frozen and stored at −80 °C until subsequent analysis of TAG and CHOL content and expression of the CAPs, GJA1, CAV1 and PTGS2. In addition, renal, gonadal and intrascapular adipose tissue depots were carefully dissected and weighed to the nearest 0.1 mg and snap frozen in liquid nitrogen.

**Total CHOL and TAG assays**

Total CHOL and TAGs in the maternal plasma and uterine tissue were assayed through a commercially bought kit (ThermoTrace, Noble Park, Vic., Australia) according to the manufacturer’s instructions. Standard curves ranging from 0 to 5 mM and from 0 to 3.5 mM were produced for CHOL and TAGs respectively. On a 96-well plate, 200 µl of CHOL or TAG assay reagent was added to 10 µl of sample or standard in duplicate, and incubated for 15 min at 37 °C. The absorbance was then read at 550 nm (with a reference wavelength of 655 nm). The intra-assay coefficient of variation (CV) for plasma CHOL and TAG was 5.1 and 1% respectively. Determination of CHOL and TAG in uterine tissue was achieved following lipid extraction. In a mixture of hexane/isopropanol (3:2 v/v), 300 mg of uterine tissue was homogenised for 5 min. The contents were then centrifuged at 2000 g for 5 min at 25 °C. The resulting liquid phase was carefully removed and dried under liquid nitrogen for 1 h. The dried extract was then dissolved in 1 ml isopropanol and analysed using the same procedure as above and expressed in µM/mg tissue. The intra-assay CV for uterine CHOL and TAG was 2.5 and 2% respectively.

**Western blot analysis**

For analysis of uterine expression of GJA1, CAV1 and PTGS2, one frozen uterine horn was ground to a powder in liquid nitrogen and homogenised briefly for 30 s in ice-cold buffer containing 5 mM Tris, pH 7.4, 2 mM EDTA and protease inhibitor cocktail (Calbiochem, San Diego, CA, USA). Homogenates were then split into three parts for analysis of each protein. Homogenate for PTGS2 underwent centrifugation at 13 000 g, and both GJA1 and CAV1 were spun at 3500 g for 15 min at 4 °C, and the supernatants were extracted. Protein concentrations of each supernatant were determined using a Bio-Rad protein assay system (Bio-Rad) according to the manufacturer’s instructions. Samples were standardised to a concentration of 4 mg/ml with Laemmli’s sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.02% bromophenol blue and 150 mM dithiothreitol) and boiled for 3 min before equal protein quantities of each sample were separated by SDS-PAGE. Proteins were transferred to nitrocellulose membrane (Hybond-C extra, Amersham Bioscience) for probing with antibodies to PTGS2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; rabbit polyclonal antibody raised against amino acids 50–111 of PTGS2 of human origin), GJA1 (Cell Signalling, Danvers, MA, USA; rabbit polyclonal antibody raised against a synthetic peptide corresponding to residues of human GJA1) and CAV1 (Cell Signalling; rabbit MAB raised against a synthetic peptide corresponding to residues near the amino terminus of human CAV1). Membranes were incubated in blocking solution (5% dried skimmed milk in TBS with 1% Tween-20) prior to incubation with primary antibodies. HRP secondary antibody conjugated to rabbit IgG was used at a working concentration of 1:5000 (GE Healthcare, Amersham, UK). Bands were developed on high performance chemiluminescence film (Hyperfilm ECL, Amersham) using ECL reagent (GE Healthcare). Densitometric analysis of band intensity was performed using a Bio-Rad Gel Doc XR imaging system and Quantity One 1D analysis software.

**RIA for PGF$_{2a}$ and progesterone**

The concentration of PGF$_{2a}$ and progesterone in maternal plasma was quantified using established RIAs (Wathes et al. 1986, Leung et al. 2001). The tritiated tracers ([5, 6, 8, 9, 11, 12, 14, 15 (n)-3H]PGF$_{2a}$ and [1,2,6,7,16,17-3H]progesterone) were obtained from Amersham International, and standards were supplied by Sigma–Aldrich. The antisera against PGF$_{2a}$ and progesterone were kind gifts from Dr N L Poyser (University of Edinburgh, Edinburgh, UK) and Dr M Sauer (Veterinary Laboratory Agency, Weybridge, Surrey, UK) respectively. The concentrations of PGF$_{2a}$ and progesterone were calculated using a semi-logarithmic plot. The limit of detection for PGF$_{2a}$ was 1 pg/tube with an intra-assay CV value of 4.7%, and the limit of detection and intra-assay CV for progesterone were 0.22 ng/ml and 8.7% respectively.

**Statistical analysis**

All data were analysed using the Statistical Package for Social Science (Version 16; SPSS, Inc., Chicago, IL, USA). The effect of feeding the HFHC diet on measured outcomes was determined through use of one-way ANOVA. All data are expressed as the mean value with S.E.M., and $P<0.05$ was considered as statistically significant.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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