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

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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 cyclo-oxygenase-2, COX-2) and caveolin-1 (CAV1) and maternal plasma for prostaglandin $F_{2\alpha}$ (PGF_{2 α}) 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 PGF_{2 α} 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

Figure 1 Body weight changes 6 weeks before mating (A) and during pregnancy (B) in rats fed either a control ($n=10$) or HFHC ($n=10$) diet. Values are means with S.E.M. represented by vertical bars. Weight gain prior to pregnancy was not dissimilar between dietary groups, however weight gain during pregnancy was significantly higher in HFHC fed animals ($P<0.003$).

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, $\text{PGF}_{2\alpha}$ and PGE_2 , which regulate uterine activity during pregnancy and parturition (Zuo *et al.* 1994). PGE_2 causes uterine relaxation (Lopez Bernal *et al.* 1993), whereas $\text{PGF}_{2\alpha}$ 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^a.

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

^aExcept for the pre-pregnancy weight gain, all other measurements were taken immediately after birth of the first pup.

Figure 2 The effects of feeding either a control ($n=8$) or HFHC ($n=7$) diet for 6 weeks prior to and during pregnancy on the concentrations of total cholesterol and TAG within maternal plasma and uterine tissue during parturition in the rat. Values are means with S.E.M. represented by vertical bars. Data were analysed by one-way ANOVA and significant differences between control and HFHC rats were at the $P<0.005$ level (*).

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 a 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 $\text{PGF}_{2\alpha}$

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 $\text{PGF}_{2\alpha}$, which regulate myometrial contractions and help to expel the foetus during labour. Analysis of maternal plasma for $\text{PGF}_{2\alpha}$ through RIA revealed that the feeding of a HFHC diet significantly reduced circulatory levels of $\text{PGF}_{2\alpha}$ 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

Figure 3 The effects of feeding a control ($n=8$) or HFHC ($n=7$) diet for 6 weeks prior to and during pregnancy on uterine protein expression of contractile associated proteins (A), CAV1 (B) and PTGS2 (C) during parturition in the rat. Following densitometry, data were analysed by one-way ANOVA and P values for CAV1 and PTGS2 between control and HFHC fed animals were $P<0.059$, $P<0.059$ and $*P<0.037$ respectively.

Figure 4 The effects of feeding a control ($n=8$) or HFHC ($n=7$) diet for 6 weeks prior to and during pregnancy on the circulatory levels of (a) $\text{PGF}_{2\alpha}$ and (b) progesterone. Values are means with S.E.M. represented by vertical bars. Data were analysed by one-way ANOVA and significant differences between control and HFHC rats were at the $P<0.02$ level ($\text{PGF}_{2\alpha}$) and $P=0.05$ (progesterone).

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 myometrium (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 PGE₂ and PGF_{2α} 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_{2α} concentrations, which were significantly reduced in rat dams fed the CON diet. Lower PGF_{2α} 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_{2α} concentration may be that the HFHC diet inhibited PGF synthase (PGFS) activity, which converts PGH₂ into PGF_{2α}.

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_{2α} 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 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 until birth at 22 days of gestation. Daily food intake and weight gains were recorded prior to and during pregnancy. 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 CO₂ asphyxiation and cervical dislocation. Maternal blood samples were collected by cardiac puncture and transferred to heparin tubes, centrifuged at

Table 2 Composition of the high-fat, high-cholesterol diet.

Constituents	g/kg
Corn oil	100
Casein	200
Maize starch	218
Butter	295
Sucrose	100
Cellulose	50
Vitamin mix	5
Mineral mix	20
Methionine	10
Choline	2
Cholesterol	10

13 000 g at 4 °C for 10 min, and the plasma was retained for analysis of TAG, CHOL and $\text{PGF}_{2\alpha}$. The uterus was dissected; fetuses 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 $\mu\text{M}/\text{mg}$ 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 the 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 $\text{PGF}_{2\alpha}$ and progesterone

The concentration of $\text{PGF}_{2\alpha}$ and progesterone in maternal plasma was quantified using established RIAs (Wathes *et al.* 1986, Leung *et al.* 2001). The tritiated tracers ($[5, 6, 8, 9, 11, 12, 14, 15 \text{ (n)}^{-3}\text{H}]\text{PGF}_{2\alpha}$ and $[1,2,6,7,16,17^{-3}\text{H}]\text{progesterone}$) were obtained from Amersham International, and standards were supplied by Sigma-Aldrich. The antisera against $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ and progesterone were calculated using a semi-logarithmic plot. The limit of detection for $\text{PGF}_{2\alpha}$ 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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