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Expression of cholesterol packaging and transport genes in human and rat placenta: impact of obesity and a high-fat diet

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Abstract

Evidence suggests that sub-optimal maternal nutrition has implications for the developing offspring. We have previously shown that exposure to a low-protein diet during gestation was associated with upregulation of genes associated with cholesterol transport and packaging within the placenta. This study aimed to elucidate the effect of altering maternal dietary linoleic acid (LA; omega-6) to alpha-linolenic acid (ALA; omega-6) ratios as well as total fat content on placental expression of genes associated with cholesterol transport. The potential for maternal body mass index (BMI) to be associated with expression of these genes in human placental samples was also evaluated. Placentas were collected from 24 Wistar rats at 20-day gestation (term = 21–22-day gestation) that had been fed one of four diets containing varying fatty acid compositions during pregnancy, and from 62 women at the time of delivery. Expression of 14 placental genes associated with cholesterol packaging and transfer was assessed in rodent and human samples by quantitative real time polymerase chain reaction. In rats, placental mRNA expression of *ApoA2*, *ApoC2*, *Cubn*, *Fgg*, *Mttp* and *Ttr* was significantly elevated (3–30 fold) in animals fed a high LA (36% fat) diet, suggesting increased cholesterol transport across the placenta in this group. In women, maternal BMI was associated with fewer inconsistent alterations in gene expression. In summary, sub-optimal maternal nutrition is associated with alterations in the expression of genes associated with cholesterol transport in a rat model. This may contribute to altered fetal development and potentially programme disease risk in later life. Further investigation of human placenta in response to specific dietary interventions is required.

Introduction

Maternal nutrition can have a profound impact on fetal development and future physiological function and metabolic health.[1] A number of dietary perturbations, including maternal undernutrition and low-protein diets, have been associated with increased risk of obesity and cardiovascular disease in the adult offspring.[2,3] In the context of the growing epidemic of obesity, focus has shifted towards understanding the effects of nutritional excess and obesity on offspring programming of disease. Studies have consistently demonstrated that these exposures are associated with a substantial increase in the risk of poor metabolic health in the offspring in both humans[4] and animal models.[5] There is emerging evidence from animal studies, however, that maternal high-fat diets also have the potential to program metabolic outcomes in the offspring independent of the effects of maternal obesity. In addition, these effects appear to depend not only on the amount of fat in the diet[6] but also on the fatty acid composition.[7,8] The majority of studies to date that have investigated the effects of a maternal high-fat diet have utilised diets high in saturated fat. However, due to changes in population-level patterns in dietary consumption,[9,10] attention has now shifted towards the roles of polyunsaturated fats within the diet.

The mechanisms underlying this early life programming of obesity and metabolic disease are not completely understood. However, as the sole interface between the mother and the fetus, structural and functional changes within the placenta have been implicated as playing a key role.[11] Cholesterol is present in every cell of the human body and an adequate supply is therefore critical for supporting normal fetal development. As the precursor for all steroid hormone synthesis, cholesterol also plays an important role in placental function. During pregnancy, the fetus obtains cholesterol via endogenous synthesis as well as transfer across the placenta from the maternal circulation, disturbances to either of these processes have negative impacts on fetal growth, cell proliferation, metabolism and the organisation of tissues.[12,13] The

endogenous synthesis of cholesterol appears to be most critical for the developing fetus, as defects in this pathway are known to be lethal.[14] Sub-optimal maternal contribution of cholesterol across the placenta, however, has been associated with lower birthweight[15,16] and microcephaly[16] in humans, highlighting the importance of this exogenous cholesterol supply.

Transport of cholesterol across the placenta is a complex process in both humans and rodents.[17,18] Briefly, the majority of cholesterol circulates the body in the form of HDL, LDL and VLDL cholesterol, which are associated with specific structural apolipoproteins (*ApoA2*, *ApoB* and *ApoC2*, respectively). The layer of trophoblast cells, located closest to maternal circulation, take in LDL and VLDL through their respective receptors. HDL cholesterol can be taken up via a specific receptor (scavenger receptor class B type 1; *SR-B1*) or by binding to proteins such as megalin and cubilin (*Cubn*). Once within the cell, cholesterol is hydrolysed into free cholesterol, bound to sterol carrier proteins and then transferred to the basolateral membrane where it passes through the fetoplacental endothelium. The processes governing cholesterol efflux from the endothelial layer are poorly understood, although it has been shown that exogenous cholesterol is secreted into fetal circulation, through association with various transporters,[19] where it is repackaged into fetal lipoproteins. This process is facilitated by microsomal triglyceride transfer protein (*Mttp*). The finding that the placenta expresses and secretes its own apolipoproteins such as *ApoB*[20] also raises the possibility that cholesterol is repackaged into HDL, LDL and VLDL cholesterol within the placenta itself.

In addition to its critical role in fetal growth and development, there is emerging evidence that alterations in placental cholesterol transfer capacity may also be a contributing factor to metabolic programming. In a previous study,[21] we showed providing rats with a low-protein diet until day 13 of gestation, a dietary treatment previously associated with programming of obesity, hypertension and glucose intolerance in adult offspring,[22,23] resulted in increased placental expression of a number of genes associated with cholesterol and lipoprotein transport and metabolism in the rat.

Given the similarity in the metabolic phenotype induced by maternal obesity/nutritional excess and low-protein diets, we hypothesised that programming of health and disease is driven by perturbations of a small set of common 'gatekeeper' processes[24,25] and changes in placental cholesterol transfer and metabolism may be common mechanisms underlying metabolic programming by different dietary exposures. Therefore, the aim of this current study was to investigate the effect of a high maternal dietary omega-6: omega-3 fatty acid ratio, associated with decreased placental weight,[26] against a lower ratio as well as total fat intake, on the expression of genes associated with cholesterol and lipoprotein transport, known to be affected by maternal diet, in the mature placenta of the rat. We also aimed to investigate whether placental expression of these same genes differed according to maternal body mass index (BMI) in a cohort of pregnant women.

Materials and methods

Animal experiments and sample collection

This paper reports data from the analysis of placentas that were collected as part of a previous study.[26] Virgin female Wistar rats ($n = 24$; 75–100 g; Charles River, UK) were housed on wood shavings in individually ventilated cages under a 12 h light/12 h dark cycle at a temperature of 20–22 °C and had *ad libitum* access to food and water throughout the experiment. Female rats were allowed to acclimatise to the unit for 1–2 weeks, during which time they were fed on standard laboratory chow (2018 Teklad Global 18% Protein Rodent Diet, Harlan Laboratories, UK). After acclimatisation, a tail vein blood sample was taken from each animal for the determination of fatty acid status. The rats were then randomly assigned to one of four dietary groups designed to provide either a high (9:1, high LA) or low (1:1.5 low LA) ratio of linoleic acid (LA) to alpha-linolenic acid (ALA), achieved by altering the amounts of flaxseed and sunflower oil included in the fat component of the feed. The levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were comparable in all diets, achieved by adjusting the amounts of coconut (SFA source) and macadamia (MUFA source) oils in the diets. For each level of LA, diets containing either 18% or 36% fat by weight were developed. This resulted in four experimental diets: high LA (18% fat), high LA (36% fat), low LA (18% fat) and low LA (36% fat) ($n = 5$–7 per dietary group). The list of ingredients and final fatty acid composition of the four experimental diets have been published previously.[26]

All animal procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986 under Home Office licence and were approved by the Animal Ethics Committee of the University of Nottingham, UK. Animals were pair housed from the start of the experiment until mating, after confirmation of conception animals were individually housed until completion of the experiment. Animals were maintained on their allocated diet for a 4-week 'feed-in' period after which they were mated. Conception was confirmed by the presence of a semen plug and this was recorded as day 0 of pregnancy. Female rats remained on their respective diets until day 20 of gestation (full term = 22 days) at which time rat dams were euthanised by CO_2 asphyxiation and cervical dislocation and fetuses by cervical dislocation and exsanguination. All fetuses were weighed and sexed via measurement of anogenital distance. Placentas from male fetuses were collected for analysis and a tail sample from the fetus was collected for sex-genotyping by polymerase chain reaction (PCR) for the SRY gene.[27] Any samples found to be female or inconclusive ($n = 5$) were not included in placental gene expression analysis. Full details of maternal weight gain, food intake and the effect of the diets on fetal and placental weight are published elsewhere.[26]

Human placental sample collection

Ethical approval for the study was obtained from the Derbyshire Research Ethics Committee (Ref: 09/H0401/90). Placental samples were obtained from patients attending the Department of Obstetrics and Gynaecology, Royal Derby Hospital, Derby, UK. Patients provided informed, written consent prior to undergoing elective caesarean section at term gestation (>37 weeks), indications for which were maternal request, previous elective section or breech presentation while cases with diabetes, hypertension and pre-eclampsia were excluded. Placentae, once checked by the midwife and with the cord clamped, were transported to the lab within 20 minutes of delivery, where placental villous samples were taken midway between the cord insertion site and placental periphery and frozen at −80 °C prior to extraction of RNA. Participants were stratified based on a BMI measurement taken during an antenatal clinic appointment, resulting in three groups of women: BMI <25 kg/m^2 ($n = 20$), BMI 25–35 kg/m^2 ($n = 21$) and BMI >35 kg/m^2 ($n = 21$).

Sample preparation and PCR

RNA was isolated from 20 to 25 mg of crushed snap-frozen human or rat placental tissue using the Roche High Pure Tissue kit

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(Roch Diagnostics Ltd., Burgess Hill, UK) according to manufacturer's instructions. RNA concentration was determined using a Nanodrop 2000 (Thermo Fisher Scientific, Loughborough, UK) and RNA quality was evaluated by agarose gel electrophoresis. cDNA was synthesised using a RevertAid™ reverse transcriptase kit (Thermo Fisher Scientific, Loughborough, UK) with random hexamer primers.

Gene targets were chosen based on our previous data[21] where RNASeq analysis of day 13 rat placentas revealed differential expression of 91 genes in response to maternal protein restriction. Ingenuity pathway analysis identified eight pathways that were significantly affected ($P < 0.001$), six of which were closely related functionally with a strong emphasis of cholesterol uptake and efflux across the placenta. Genes were selected based upon the ingenuity analysis (*ApoA2, ApoB, ApoC2, Ttr, Fgg, serpin G1* and *Rbp4*). Additional genes were chosen that were shown to be differentially expressed in the protein restricted condition (*Vil1, Gpc3, Prf1, Cubn, Mttp*) but not associated with pathways identified by ingenuity analysis. *Tagln* and *Tbp* gene targets were also chosen as preliminary RNASeq analysis suggested that they may be sensitive to maternal dietary factors, resulting in 14 target genes for analysis. Real-time PCR primers were designed using Primer Express software (version 1.5; Applied Biosystems) from the RNA sequence, checked using BLAST (National Centre for Biotechnology Information) and were purchased from Sigma (UK). The primer sequences can be found in supplementary material Table S1. Real-time PCR was performed on a Lightcycler 480 (Roche, Burgess Hill, UK) using the 384 well format. Each reaction contained 5 µl of cDNA with the following reagents: 7.5 µl SYBR green master mix (Roche), 0.45 µl forward and reverse primers (final concentration 0.3 µM each) and 1.6 µl RNase-free H_2O. Samples were pre-incubated at 95 °C for 5 min followed by 45 PCR amplification cycles (denaturation, 95 °C for 10 s; annealing, 60 °C for 15 s; elongation, 72 °C for 15 s). Transcript abundance was determined using a standard curve generated from serial dilutions of a pool of cDNA made from all samples. Expression was normalised against the expression of cyclophilin A, which was not significantly different between experimental groups.

Statistical analysis

Data are presented as mean ± SEM. Data were analysed using the Statistical Package for Social Sciences (Version 24, SPSS Inc.). For animal data, the effect of maternal dietary fatty acid ratio and maternal dietary fat content on placental expression of target genes was assessed using a two-way ANOVA, with dietary fat level and LA:ALA ratio as factors. Human data were analysed using a one-way ANOVA with maternal BMI as a factor. Possible co-variates were identified and corrected for within the analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Rat placenta

The results of the gene expression analyses in the rodent placentas are shown in Table 1. Expression was measured for 13 genes (*ApoB* excluded); however, *Tbp* expression was not detectable in any of the samples (data not shown) and so could not be included in the analysis.

Placental *Mttp* mRNA expression was significantly increased (3 fold; $P < 0.01$) in dams consuming a high LA compared to a low LA diet, independent of total dietary fat content (Table 1). There was a significant interaction between the effects of maternal total dietary fat and LA content in relation to mRNA expression of *ApoA2*, *ApoC2*, *Cubn*, *Fgg*, *Rbp4* and *Ttr* ($P < 0.05$), such that expression of these genes was significantly increased in rats consuming the high LA diets, but only when dietary fat content was also high (36% fat w/w). The magnitude of this effect was greatest for *Cubn* (30-fold increase in the high LA (36% fat) group when compared to the low LA (36% fat) group). A similar pattern was observed for other key cholesterol-transport and metabolism genes in the placenta (*Gpc3*, $P = 0.072$; *Vil1*, $P = 0.054$), with 1.5–7 fold higher expression in placentas of dams exposed to a high LA diet, but only when dietary fat content was also high. Placental expression of *Prf1*, *SerpinG1* and *Tagln* was not affected by either maternal dietary fatty acid ratio or fat content.

Table 1. Rat placental gene expression at day 20 of gestation

	High LA (18% fat)	High LA (36% fat)	Low LA (18% fat)	Low LA (36% fat)
*ApoA2**	0.75 ± 0.24	3.24 ± 1.13	0.52 ± 0.08	0.33 ± 0.20
*ApoC2**	0.72 ± 0.27	2.91 ± 1.00	0.56 ± 0.08	0.34 ± 0.21
*Cubn**	0.81 ± 0.35	2.92 ± 1.15	0.35 ± 0.06	0.10 ± 0.04
*Fgg**	0.76 ± 0.30	2.97 ± 1.09	0.36 ± 0.06	0.12 ± 0.05
Gpc3	0.96 ± 0.28	1.46 ± 0.43	0.56 ± 0.09	0.50 ± 0.09
Mttp	0.66 ± 0.25[a]	2.12 ± 0.77[a]	0.28 ± 0.05[b]	0.22 ± 0.09[b]
Prf1	1.65 ± 0.42	0.73 ± 0.14	1.17 ± 0.12	1.15 ± 0.31
*Rbp4**	0.69 ± 0.29	2.34 ± 1.17	0.48 ± 0.12	0.13 ± 0.05
SerpinG1	1.03 ± 0.26	1.00 ± 0.13	0.84 ± 0.21	1.20 ± 0.33
Tagln	0.95 ± 0.13	0.87 ± 0.06	0.67 ± 0.04	1.09 ± 0.17
*Ttr**	0.65 ± 0.22	2.43 ± 0.81	0.52 ± 0.08	0.32 ± 0.17
Vil1	1.23 ± 0.42	2.67 ± 0.97	0.51 ± 0.10	0.54 ± 0.22

Different superscript letters indicate significant differences between groups ($P < 0.01$).
*Indicates a significant interaction effect of maternal dietary LA:ALA ratio and total dietary fat content on placental gene expression ($P < 0.05$).

Human placenta

The sociodemographic and clinical characteristics of the women who provided placental samples for this study are summarised in Table 2. The average age, parity and gestation length of women within this study were similar between women in the three BMI groups; however, the birthweights of infants born to women with a BMI >25 kg/m^2 were significantly greater than those of women in the normal BMI range.

The results of the gene expression analyses in the human placentas are shown in Table 3. All 14 genes were measured and detected in these samples. Women with a BMI in the 25–35 kg/m^2 range (overweight to obese) exhibited a 2–6 fold higher mRNA expression of *ApoB* in their placental samples compared to women with a BMI either <25 or >35 kg/m^2. The expression of *Rbp4* was significantly lower in placentas obtained from women with a BMI >25 kg/m^2 compared to those with a BMI <25 kg/m^2 (85% downregulation, $P = 0.001$). A similar pattern was also observed for *Ttr*, with 71%–88% downregulation of expression in women with a BMI >25 kg/m^2, although this was not statistically significant ($P = 0.053$). The mRNA of other placental genes was not different between BMI groups.

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Table 2. Human participant characteristics

BMI (kg/m^2)	<25	25–35	>35
Age (years)	34.29 ± 1.28	33.55 ± 1.07	31.25 ± 1.04
BMI	21.88 ± 0.36	29.70 ± 0.70	40.10 ± 1.07
Parity	1.05 ± 0.11	0.75 ± 0.14	1.20 ± 0.19
Gestation length (weeks)	38.63 ± 0.23	38.68 ± 0.18	38.65 ± 0.20
Birthweight (g)*	3236 ± 88	3537 ± 112	3565 ± 108
Sex (% male)	45	45	65
N	21	20	20

Data are shown as mean ± SEM for N observations per group.
*ANOVA indicated that, with adjustment for gestational age, birthweight was influenced by maternal BMI ($P = 0.021$).

Table 3. Human placental gene expression

	BMI < 25	BMI 25–35	BMI > 35
ApoA2	1.04 ± 0.21	1.22 ± 0.27	0.53 ± 0.06
*ApoB***	0.34 ± 0.06	2.19 ± 0.55	1.04 ± 0.25
ApoC2	0.90 ± 0.15	1.02 ± 0.25	0.76 ± 0.13
Cubn	1.22 ± 0.21	1.15 ± 0.15	0.86 ± 0.09
Fgg	0.48 ± 0.14	0.82 ± 0.41	0.65 ± 0.42
Gpc3	0.97 ± 0.20	1.30 ± 0.20	0.83 ± 0.19
Mttp	0.57 ± 0.08	1.23 ± 0.37	0.42 ± 0.08
Prf1	1.14 ± 0.19	0.85 ± 0.10	1.03 ± 0.17
*Rbp4***	0.50 ± 0.13	0.07 ± 0.01	0.08 ± 0.01
SerpinG1	1.45 ± 0.24	0.90 ± 0.07	0.90 ± 0.11
Tagln	0.97 ± 0.11	1.04 ± 0.10	1.11 ± 0.10
Tbp	1.02 ± 0.11	1.16 ± 0.11	1.00 ± 0.07
Ttr	1.92 ± 0.90	0.23 ± 0.08	0.56 ± 0.20
Vil1	1.14 ± 0.20	1.82 ± 0.33	1.01 ± 0.18

**Indicates a significant effect of maternal BMI on placental gene expression ($P < 0.01$).

Discussion

This experiment aimed to test the hypothesis that maternal diet, specifically, fatty acid composition and quantity, and obesity would influence the expression of genes associated with cholesterol uptake and transport in rat and human placenta. The results of the rat studies suggested clear effects of maternal dietary fat content and composition, such that maternal consumption of a higher-fat, high LA maternal diet was associated with increased expression of key genes associated with these pathways, suggesting enhanced cholesterol transport to the fetus in this group. In the human study, however, only two of these genes were differentially expressed in placentas from women in different BMI categories, suggesting that maternal obesity had a limited impact on placental cholesterol transport at the level of gene expression.

Within this study, we have shown that exposure to a high LA, high-fat diet resulted in increased expression of genes involved in the formation of apolipoproteins, cholesterol uptake and cholesterol repackaging. These differences were not observed when either the fat content and/or the fatty acid ratio was altered, suggesting a strong interaction between these variables. Importantly, the effects observed on the placental gene expression profiles show striking resemblances to our previous findings[21] where upregulation of these genes was observed in the placentas of dams exposed to a low-protein diet. These data suggest that, not only do these differences persist to a late stage placenta (day 20), but, despite different dietary interventions, the similarities in results suggest a common mechanism of action. The upregulation of key genes in the rat placenta observed in this study suggests a state of increased cholesterol uptake and efflux. While the potential impacts of this in the current study are not clear, it has been demonstrated in previous studies that exposure to excess cholesterol during fetal development can be associated with adverse outcomes. In rodent models, maternal hypercholesterolaemia has been associated with growth restriction,[28] altered liver development[29] and atherosclerosis.[30,31] In humans, maternal hypercholesterolaemia has been associated with the development of fatty streaks in fetal arteries and cholestasis during pregnancy is associated with programming of an overweight, insulin resistant phenotype in the child.[32,33] It will therefore be important in future studies to determine the longer term consequence of the changes in placental gene expression for the postnatal offspring.

Based on the substantial impact of maternal high-fat high LA feeding, a dietary pattern commonly observed in the modern Western diet, we extended our study to determine if there was any evidence to suggest an effect of maternal obesity on cholesterol transfer in the human. There were, however, relatively few differences in the expression of key genes associated with different BMI categories in human placental samples, although there were some subtle differences in the expression of three genes (*ApoB*, *Rbp4* and *Ttr*) between BMI categories. *Ttr* is a protein that binds to and transports *Rbp4*. In the bound state, *Rbp4* is protected from glomerular filtration and so levels of these two proteins are often correlated. As such, the similar patterns of expression across these two genes observed in this study were anticipated. What was surprising, however, was our finding that women with a BMI above the normal range (>25 kg/m^2, overweight or obese) exhibited decreased placental expression of these genes since elevated levels of circulating *Rbp4* have been associated with many of the co-morbidities linked to obesity including hypertension,[34] insulin resistance and type 2 diabetes.[35,36] It is important to note, however, that these observations have all been associated with circulating levels of *Rbp4*, whereas we measured gene expression in the placenta. There is limited literature evaluating the role of *Rbp4* within the placenta during pregnancy, particularly in association with maternal obesity. It may be, however, that in obese mothers, placental expression is reduced to compensate for the increased maternal circulating levels and therefore avoiding fetal exposure to high quantities of *Rbp4*. Further experimentation is required to determine expression of *Rbp4* and *Ttr* in both the mother, fetus and the placenta throughout pregnancy and their association with maternal obesity.

Placental *ApoB* expression was increased in women whose BMI was above the normal range (>25 kg/m^2). A study by Dubé and colleagues[37] showed increased circulating *ApoB* concentrations in new-born infants of obese mothers, compared to mothers of normal weight, in the absence of any difference in maternal circulating concentrations. It is therefore possible that the high *ApoB* concentrations in infants of obese mothers may have been the result of increased placental *ApoB* expression. If this abnormal lipoprotein profile is present in the offspring and persists through childhood, it may contribute to increased risk of cardiovascular disease in later life.

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One key limitation of the current study is that direct measurements of cholesterol transport or measurement of the genes of interest at the level of protein were not analysed, and so care must be taken when extrapolating the findings to functional outcomes. Further to this, human participants within this study were stratified based on BMI whereas the animal experiments utilised specific dietary interventions. There are many factors that can affect BMI and, although nutrition is a key element, there is still a huge variety of nutritional habits that can lead to individuals resulting in similar BMIs. As such, assumptions cannot be made about specific nutrient intakes of the women based on this data. Finally, it is important to note that, while there are many similarities between placental physiology and function in the rat and humans, there are some key differences. Of particular importance to this study is the difference in circulating progesterone levels at the end of pregnancy. In rodents there is a dramatic decrease in circulating progesterone,[38] whereas in humans, progesterone levels are increased or at least maintained at the time of parturition.[39] Progesterone is a key steroid produced from cholesterol within the placenta and has been shown to regulate the expression of some genes including *Rbp4* in other tissues.[40] As such, careful consideration of the differences in placental hormone production, particularly steroid hormones, between the two species should be made when drawing comparisons.

In conclusion, this study aimed to elucidate whether differences in placental expression of genes involved in cholesterol transport and efflux were associated with altered maternal nutrition in a manner similar to our previous observations in the low-protein model. We demonstrated that exposure to high levels of omega-6 as part of a high-fat diet elicited a similar pattern of placental gene expression, suggesting an increase in cholesterol transport across the placenta. This highlights the potential for a common mechanism by which sub-optimal maternal nutrition during pregnancy alters placental function, and potentially fetal development, resulting in increased risk of disease in later life. We then carried out a preliminary study which aimed to establish if similar alterations were observed in human placentas. Although BMI was associated with some changes in expression, these observations were not consistent and further experimentation is required on placental samples where the specific nutrient intake of the participants is known.

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S2040174419000606

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the Derbyshire Research Ethics Committee (Ref. 09/H0401/90). The authors assert that all animal procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986 under Home Office licence and were approved by the Animal Ethics Committee of the University of Nottingham, UK.

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