Journal of Developmental Origins of Health and Disease (2016), **7**(3), 290–297.

doi:10.1017/S2040174416000027

ORIGINAL ARTICLE

Maternal high-fat feeding in pregnancy programs atherosclerotic lesion size in the ApoE*3 Leiden mouse

E. J. Tarling*, K. J. P. Ryan, R. Austin, S. J. Kugler, A. M. Salter and S. C. Langley-Evans*

School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK

Periods of rapid growth seen during the early stages of fetal development, including cell proliferation and differentiation, are greatly influenced by the maternal environment. We demonstrate here that over-nutrition, specifically exposure to a high-fat diet *in utero*, programed the extent of atherosclerosis in the offspring of ApoE*3 Leiden transgenic mice. Pregnant ApoE*3 Leiden mice were fed either a control chow diet (2.8% fat, $n = 12$) or a high-fat, moderate-cholesterol diet (MHF, 19.4% fat, $n = 12$). Dams were fed the chow diet during the suckling period. At 28 days postnatal age wild type and ApoE*3 Leiden offspring from chow or MHF-fed mothers were fed either a control chow diet ($n = 37$) or a diet rich in cocoa butter (15%) and cholesterol (0.25%), for 14 weeks to induce atherosclerosis ($n = 36$). Offspring from MHF-fed mothers had 1.9-fold larger atherosclerotic lesions ($P < 0.001$). There was no direct effect of prenatal diet on plasma triglycerides or cholesterol; however, transgenic ApoE*3 Leiden offspring displayed raised cholesterol when on an atherogenic diet compared with wild-type controls ($P = 0.031$). Lesion size was correlated with plasma lipid parameters after adjustment for genotype, maternal diet and postnatal diet ($R^2 = 0.563$, $P < 0.001$). ApoE*3 Leiden mothers fed a MHF diet developed hypercholesterolemia (plasma cholesterol two-fold higher than in chow-fed mothers, $P = 0.011$). The data strongly suggest that maternal hypercholesterolemia programs later susceptibility to atherosclerosis. This is consistent with previous observations in humans and animal models.

Received 2 October 2015; Revised 7 December 2015; Accepted 5 January 2016; First published online 2 February 2016

Key words: animal, developmental stage, fetus, small animals

Introduction

While the etiology of major disease states is influenced by a variety of factors, including genotype and environmental factors such as dietary pattern, susceptibility to chronic disease in adult life is influenced by the quality and quantity of nutrition experienced by the fetus during critical stages of development.[1–3] The developmental origins of adult health and disease hypothesis, established by the large number of studies reporting relationships between the risk of adult disease and early-life events,[4] is the basis of the 'programming' concept that disturbances to the normal fetal environment can result in irreversible changes to tissue structure, function and morphology.[1,5] These changes directly alter physiological functions and hence susceptibility to developing disease.[6,7]

Animal models of nutrient restriction are commonly used as tools to investigate early-life programming.[8] The feeding of a low protein diet during rat pregnancy, for example, has been shown to program hypertension and metabolic syndrome in the offspring.[9–12] The same protocol, with the atherosclerosis-prone ApoE*3 Leiden mouse, was found to increase the extent of atherosclerotic lesion formation.[13] However, over-nutrition is emerging as one of the major issues for pregnancy in developed countries. High weight gain in pregnancy is associated with poor pregnancy outcomes and long-term disease for babies exposed to this weight gain. The worldwide increase in the prevalence of being overweight and obese is increasingly impacting across all age groups in the population.[14,15] As a result, all developed countries are reporting high levels of obesity among women of childbearing age. The children of mothers who gain excessive weight in pregnancy are themselves at greater risk of increased adiposity and associated disease.[16] The effects of maternal over-nutrition on programming have primarily been modeled through feeding rodents high-fat diets during pregnancy,[17] or by inducing obesity in females before conception.[18] The programming effects of high-fat diets are remarkably similar to those observed with undernutrition suggesting a common etiology.[1] In the present study we aimed to assess the programming effects of feeding a high-fat diet, rich in saturated fat, during pregnancy, on the development of atherosclerosis in ApoE*3 Leiden transgenic offspring. We hypothesized that fetal exposure to a maternal high-fat diet would increase atherosclerotic lesion size in later life.

Methods

All experiments involving mice were performed in accordance with the Animals (Scientific Procedures) Act 1986 and subject to UK Home Office regulations. The work was approved by the University of Nottingham Animal Ethics Committee and covered by license PPL40/2435. Throughout the procedures

[a]Present address: David Geffen School of Medicine, UCLA, Los Angeles, CA, USA.

*Address for correspondence: Professor S. C. Langley-Evans, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK.
(Email Simon.Langley-Evans@nottingham.ac.uk)

steps were taken to minimize animal suffering. Male and female mice (10–12 weeks) were maintained in a controlled environment (21°C; 55% humidity) with a 12 h light–dark cycle. Animals were maintained on a standard laboratory chow diet (B&K Universal, Hull, UK) and had *ad libitum* access to food and water at all times. ApoE*3 Leiden mice were originally a gift from Dr Louis Havekes (TNO Pharma, The Netherlands) and the animals used in this study were obtained from the local Nottingham colony. Female ApoE*3 Leiden transgenic mice, on a C57Bl/6J background, were mated with wild-type C57Bl/6J males. The ApoE*3 Leiden transgene is lethal to homozygotes so this mating strategy was necessary to produce mice that were heterozygous for the transgene and would therefore be atherosclerosis prone.[19] All litters in the study contained a mixed population of wild-type and transgenic offspring.

Pregnant females were fed either a control (MC; 2.8% fat; $n = 12$) or a maternal high-fat (MHF; 19.4% fat; $n = 12$) diet. A further group of non-pregnant ApoE*3 Leiden mice (control: $n = 8$, MHF: $n = 12$) were fed the same diets to examine the impact of the two diets independently of pregnancy. The MHF diet was prepared by mixing the control chow with fat derived from beef dripping (135 g/kg diet), corn oil (21.5 g/kg diet) and tripalmitin (15 g/kg diet). This diet contained 2 g/kg cholesterol (standard chow was 0.31 g/kg). Feeding of the MHF diet commenced when females were housed with males for breeding. Pregnancy was confirmed by the appearance of a mating plug. Five to six mothers per group, together with parallel groups of non-pregnant females, were terminated at day 17 of pregnancy and maternal blood and liver were collected as described below. At birth all remaining animals were transferred to the same standard chow diet. Litters were not handled from birth to weaning to avoid losses, as C57 mothers are highly sensitive to handling stress. Variation in litter size was limited with the number of pups to litter spanning four to nine. Offspring were genotyped using a polymerase chain reaction assay before weaning at 28 days postnatal age. Previous work with this animal model[13] demonstrated that male ApoE*3 Leiden mice develop little or no atherosclerotic lesions when fed an atherogenic diet. Therefore, only female offspring were randomized to be fed either a chow diet (2.8% fat) or an atherogenic diet, again based on the control chow diet (15% cocoa butter, 40.5% sucrose and 0.25% cholesterol). The latter diet is designed to induce the atherosclerotic disease process. The fatty acid composition of each of the three diets used in this study are shown in Table 1. In the ApoE*3 Leiden mice cholesterol in the diet produces proportionate increases in circulating cholesterol.[19] In total eight groups of mice were available for study; MC/Chow, MHF/Chow, MC/Athero and MHF/Athero, for each of wild-type and ApoE*3 Leiden strains.

Our previous work with the ApoE*3 Leiden mouse model indicated that exposure of the animals to atherogenic diet for 3 months was sufficient to induce physiologically significant atherosclerotic lesions. After 14 weeks of postnatal feeding, animals were sacrificed using a rising concentration of carbon dioxide and were not fasted before cull. Whole blood was collected into vacutainers by heart puncture and plasma prepared by centrifugation at 13,000 ***g*** at 4°C for 10 min. The liver, adipose (perirenal and gonadal depots), kidneys, gastrocnemius muscle and abdominal aorta were dissected from each animal, weighed to the nearest 0.1 mg and snap-frozen in liquid N_2. Hearts and the aortic root were dissected from each animal and infused with OCT fixing compound (Miles Inc., Elkhart, IN, USA) and snap-frozen in OCT until sectioning.

Table 1. *Fatty acid composition of mouse diets*

Fatty acid	Chow	MHF	Atherogenic diet
C14:0	4.4	3.2	nd
C16:0	24.4	31.4	25.2
C18:0	10.5	16.7	36.13
C18:1	27.4	28.6	33.27
C18:2	27.9	12.8	2.94

nd, not detected; MHF, maternal high fat diet; Chow, postnatal chow diet.

Data is shown as percentage of the total fatty acids present in the diet. Chow diet was 28 g fat/kg; MHF 171.5 g fat/kg diet; Atherogenic diet 160 g fat/kg.

Source: Derived from figures published in Tarling *et al.* (2009)[20].

Genotyping of transgenic mice

Genomic DNA was extracted from ear punches by standard procedures.[21] Polymerase chain reaction assay was performed on genomic ear DNA using primers spanning the ApoE*3 Leiden mutation (forward primer 5' GCCCCGGCCTGG TACACTGC 3'; reverse primer 5' GGCACGGCTGTC CAAGGAGC 3') as described previously.[13]

Measurement of plasma metabolites

Total circulating plasma cholesterol and triacylglycerides (TAG) were assayed using commercially available kits (ThermoTrace, Noble Park, Victoria, Australia), according to the manufacturer's instructions. Assay linearity was 20 mmol/l for cholesterol and 10 mmol/l for TAG.

Histological analysis of the heart and aortic root

Frozen heart and aortic root samples were sectioned using a cryostat (Bright Instruments, Huntingdon, Cambridgeshire, UK). Alternate sections of the aortic root (10 µm thickness) were collected, stained with Oil Red O and imaged using a Nikon phase contrast 2 microscope and a Micropublisher 3.3 RTV camera (Q Imaging, St Helens, Lancashire, UK). Atherosclerotic lesions were analyzed and quantified using the method of Paigen *et al.*[22] using Image Pro-Plus software (Media Cybernetics, Inc., Bethesda, MD, USA) to determine

https://doi.org/10.1017/S2040174416000027 Published online by Cambridge University Press

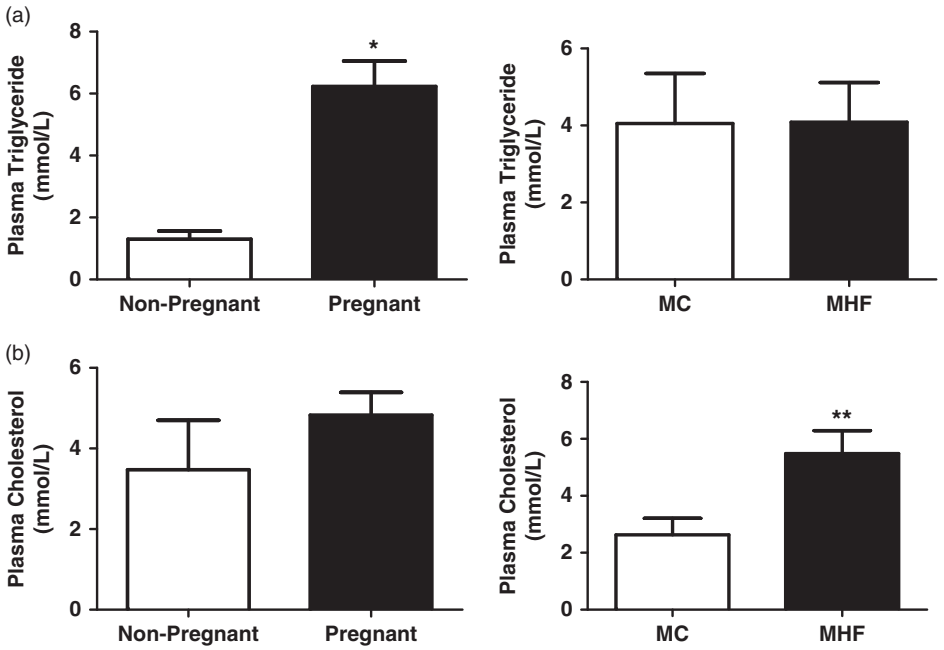

Fig. 1. Maternal plasma triglyceride (*a*) and cholesterol (*b*) concentrations at day 17 gestation. Data are means, with standard errors. For Non-Pregnant MC $n = 3$, Pregnant MC $n = 4$. For Non-Pregnant MHF $n = 4$, Pregnant MHF $n = 5$. (*a*) ANOVA indicated significant effects of pregnancy ($P = 0.02$). *Mean value was significantly different from Non-Pregnant. (*b*) ANOVA indicated significant effects of maternal MHF diet ($P = 0.011$). **Mean value was significantly different from mothers fed a maternal chow (MC) diet ($P < 0.01$). MC, maternal chow; MHF, maternal high fat.

the percentage of the total area of the aortic intima exhibiting atherosclerotic lesions. The average lesion area for each animal was calculated using data from 15 sections/animal.[13]

Statistical analysis

All data are presented as mean values ± standard error. Unless otherwise stated in the text, data were analyzed using a mixed-model analysis using SPSS (version 17.0; SPSS, Inc., Chicago, IL, USA). In the case of plasma TAG, cholesterol and mean atherosclerotic lesion area, maternal diet, postnatal diet and genotype were the fixed factors and the results adjusted for within-litter effects.[23] This adjustment removed the influence of having littermates within some of the groups and is an analytical approach we have used in our previous studies of programming.[13,24,25] *Post hoc* tests were not performed where analysis of variance indicated an interaction between groups. The primary outcome measure was atherosclerotic lesion area and the study was powered against this variable.

Results

Maternal weight gain during pregnancy was similar in the two groups of animals (Control; 13.99 ± 1.21, MHF; 15.01 ± 0.44 g, not significant). In comparison to non-pregnant mice, the pregnant mice exhibited increased (1.98-fold) maternal liver weight at day 17 gestation (non-pregnant Control; 0.95 ± 0.04, non-pregnant MHF; 0.95 ± 0.06 pregnant control; 1.24 ± 0.04 pregnant MHF; 1.33 ± 0.04 g, $P = 0.022$ when adjusted for body weight) and hypertriglyceridemia ($P = 0.02$; Fig. 1a), but pregnancy had no significant effect on maternal total plasma cholesterol levels (Fig. 1b). Feeding a MHF diet did not impact on triglyceride levels but increased maternal total cholesterol two-fold ($P = 0.011$; Fig. 1b).

Pregnant ApoE*3 Leiden mice fed control or a MHF diet gave birth to litters of similar size (control, 5.9 ± 0.8 pups/litter; MHF, 7.4 ± 0.6 pups/litter). The proportion of ApoE*3 Leiden mice produced was not significantly different between the two maternal diet treatments ($P > 0.05$ χ^2 test; control, 28.75% transgenic; MHF 25.0% transgenic). Maternal food intake was similar between groups ($P = 0.36$, Chow fed 2.88 ± 0.60 g/day; MHF fed 2.75 ± 0.20 g/day). Offspring were not weighed at birth to avoid maternal distress, but significant effects of maternal diet on body weight were apparent when the animals were weaned at 28 days postnatal age (Fig. 2a). Offspring that were exposed to a MHF diet during gestation were lighter ($P < 0.001$) than those from mothers fed a control chow diet, and this effect was independent of genotype. At the end of 14 weeks postnatal feeding offspring from MHF-fed mothers remained lighter ($P = 0.021$), although this effect was restricted to the ApoE*3 Leiden transgenic strain (Fig. 2b). The full growth trajectories of the offspring are shown in Supplementary Figure 1.

Figure 3 shows plasma triglycerides and total cholesterol levels after 14 weeks of postnatal feeding. Plasma cholesterol and triglyceride concentrations were similar in wild-type

https://doi.org/10.1017/S2040174416000027 Published online by Cambridge University Press

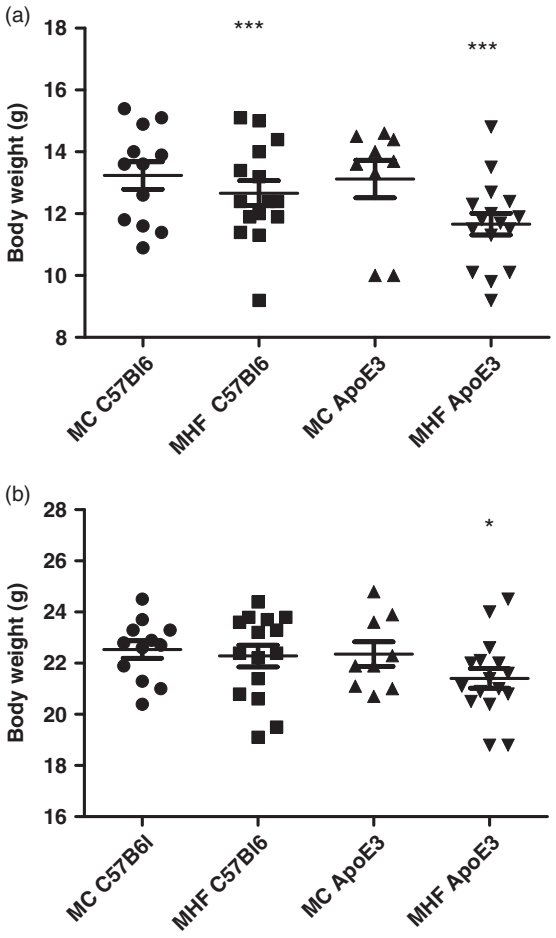

Fig. 2. Body weight at 28 days postnatal age (*a*) and after 14 weeks of postnatal feeding (*b*). Data are means with standard errors. For C57Bl/6J mice: MC Chow $n = 9$; MHF Chow $n = 9$; MC Athero $n = 8$; MHF Athero $n = 11$. For ApoE*3 Leiden mice: MC Chow $n = 7$; MHF Chow $n = 11$; MC Athero $n = 8$, MHF Athero $n = 8$. (*a*) ANOVA indicated significant effects of prenatal diet ($P < 0.001$). ***Mean value was significantly different from offspring (both C57Bl/6J and ApoE*3 Leiden) from mothers fed a chow diet. (*b*) ANOVA indicated significant effects of prenatal diet ($P = 0.001$), postnatal diet ($P = 0.001$) and genotype ($P = 0.038$). There were interactions of prenatal diet and postnatal diet ($P = 0.031$) and prenatal diet and genotype ($P = 0.021$). *Mean value was significantly different from C57Bl/6J offspring from mothers fed either a chow or a MHF diet, and from ApoE*3 Leiden offspring from mothers fed a chow diet ($P < 0.05$). MC, maternal chow diet; MHF, maternal high fat diet; Chow, postnatal chow diet; Athero, postnatal atherogenic diet.

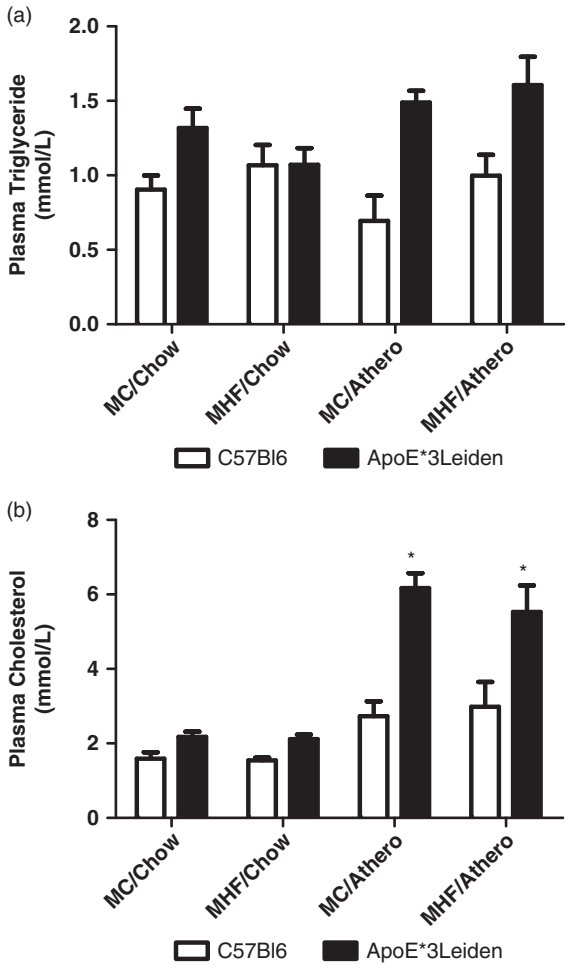

Fig. 3. Plasma triglyceride (*a*) and cholesterol (*b*) concentrations in the offspring. Data are means, with standard errors. For C57Bl/6J mice: MC Chow $n = 9$; MHF Chow $n = 9$; MC Athero $n = 8$; MHF Athero $n = 11$. For ApoE*3 Leiden mice: MC Chow $n = 7$; MHF Chow $n = 11$; MC Athero $n = 8$, MHF Athero $n = 8$. (*b*) ANOVA indicated significant effects of postnatal diet ($P < 0.001$) and genotype ($P = 0.004$). There was an interaction of postnatal diet and genotype ($P = 0.031$). *Mean value was significantly different from C57Bl/6J offspring from control and MHF-fed mothers fed a postnatal atherogenic diet ($P < 0.05$). MC, maternal chow; MHF, maternal high fat.

C57Bl/6J and ApoE*3 Leiden transgenic offspring fed the chow diet. There were no significant alterations in plasma triglyceride levels (Fig. 3a) although the ApoE*3 Leiden transgenic mice displayed a trend for elevated triglyceride concentrations ($P = 0.084$) when comparing mice fed atherogenic diet to those fed chow. ApoE*3 Leiden offspring developed three-fold higher cholesterol levels when placed on the atherogenic diet ($P < 0.031$ compared with chow). There was no additional effect of the *in utero* exposure to the MHF diet (Fig. 3b).

When animals were killed livers were dissected and carefully weighed. There was a significant effect of prenatal diet on liver size ($P = 0.007$), with animals being exposed to a MHF diet *in utero* displaying smaller livers (Fig. 4). However, this difference was no longer present when animals were fed an atherogenic diet (interaction of prenatal diet and postnatal diet, $P = 0.005$). These effects remained when liver weight was corrected for total body weight.

The extent of atherosclerotic lesions found in the aortic intima is shown in Fig. 5a, with representative sections of aorta stained for lesions shown in Fig. 5b. C57Bl/6J animals

https://doi.org/10.1017/S2040174416000027 Published online by Cambridge University Press

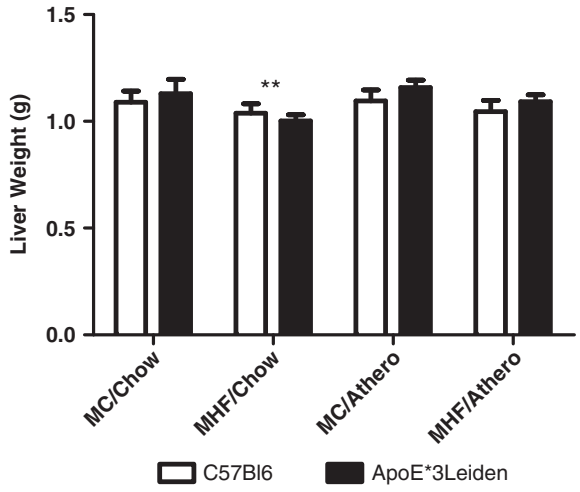

Fig. 4. Liver weight. Data are means with standard errors. For C57Bl/6J mice: MC Chow $n = 9$; MHF Chow $n = 9$; MC Athero $n = 8$; MHF Athero $n = 11$. For ApoE*3 Leiden mice: MC Chow $n = 7$; MHF Chow $n = 11$; MC Athero $n = 8$, MHF Athero $n = 8$. ANOVA indicated significant effects of prenatal diet ($P = 0.007$) and postnatal diet ($P = 0.05$). There was an interaction of prenatal and postnatal diets ($P = 0.005$). **Mean value was significantly different from offspring on a chow diet exposed to a chow diet *in utero* ($P < 0.01$). MC, maternal chow diet; MHF, maternal high fat diet; Chow, postnatal chow diet; Athero, postnatal atherogenic diet.

displayed no significant lesions, neither were there any significant effects of maternal or postnatal diets. The challenge of the atherogenic diet-induced lesion formation to a greater degree (1.9-fold) in ApoE*3 Leiden animals exposed to the MHF diet during gestation compared with those exposed to the control chow diet ($P < 0.001$). Although there was no significant effect of the MHF maternal diet on plasma cholesterol levels, the amount of cholesterol in the blood was directly correlated to the extent of atherosclerotic lesions in the aortic intima ($R = 0.750$, $P < 0.001$) in ApoE*3 Leiden mice.

Discussion

Human epidemiological studies and experiments using animal models have clearly demonstrated that maternal diet can have a significant impact on the susceptibility of the offspring to metabolic disease, including type 2 diabetes, hypertension and atherosclerotic cardiovascular disease.[1,6] Using the ApoE*3 Leiden mouse model we have previously shown that the female offspring of mother fed a low protein diet had increased susceptibility to atherosclerosis.[13] This was associated with increased plasma cholesterol, in response to an atherogenic diet compared with the offspring of mothers fed a control diet. It is also clear that overnutrition in pregnancy can also impact on the susceptibility of the offspring to metabolic disease, though it remains to be established whether this is a result of maternal obesity, changes in maternal carbohydrate or lipid metabolism or other factors.[26–31] As in our

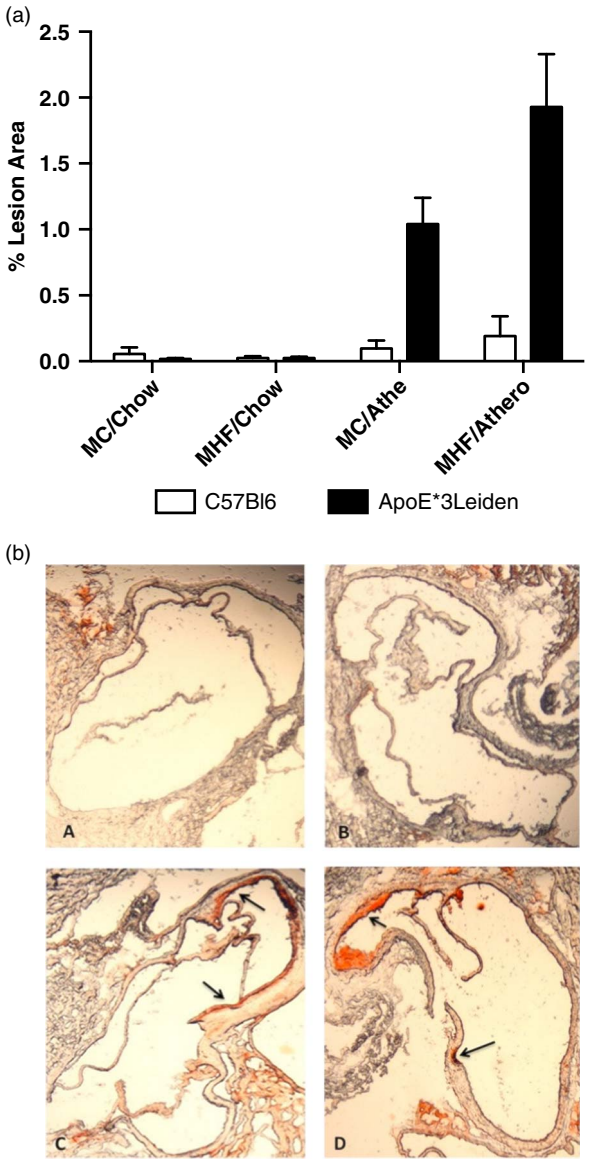

Fig. 5. (*a*) Area of aortic intima exhibiting atherosclerotic lesions in female ApoE*3 Leiden mice. (*b*) Examples of atherosclerotic lesions in equivalent sections from atherogenic diet-fed (A) wild-type offspring exposed to chow diet *in utero*, (B) wild-type offspring exposed to MHF diet *in utero*, (C) ApoE*3 Leiden offspring exposed to chow diet *in utero*, (D) ApoE*3 Leiden offspring exposed to MHF diet *in utero*. Arrows indicate positive Oil Red O staining for neutral lipid. Data are means with standard errors. For C57Bl/6J mice: MC Chow $n = 9$; MHF Chow $n = 9$; MC Athero $n = 8$; MHF Athero $n = 11$. For ApoE*3 Leiden mice: MC Chow $n = 7$; MHF Chow $n = 11$; MC Athero $n = 8$, MHF Athero $n = 8$. (*a*) ANOVA indicated significant effects of genotype ($P < 0.001$), prenatal diet ($P < 0.001$), postnatal diet ($P < 0.001$) and interactions of genotype, prenatal and postnatal diets ($P < 0.001$). MC, maternal chow diet; MHF, maternal high fat diet; Chow, postnatal chow diet; Athero, postnatal atherogenic diet.

previous work,[13] the inclusion of wild-type C57 mice in the study demonstrates the specificity of the prenatal–postnatal diet interaction to the mutated ApoE background.

https://doi.org/10.1017/S2040174416000027 Published online by Cambridge University Press

Observations in humans have indicated the formation of atherosclerotic lesions in fetal vessels following exposure to maternal hypercholesterolemia.[32] This has been supported by some studies using animals, indicating induction of hypercholesterolemia during pregnancy is associated with the development of atherosclerosis in the offspring.[33,34] However, a number of inconsistencies are apparent within the animal literature. Palinski and Napoli[33] originally suggested that the increased susceptibility to atherosclerosis in offspring born to hypercholesterolemic mice, was not associated with any changes in plasma lipids of the offspring. This work was performed in homozygous low density lipoprotein (LDL) – receptor deficient pregnant mice fed dietary cholesterol, which induced massive hypercholesteroaemia (25–30 mmol/l). It should be noted that such levels of plasma cholesterol are only seen in humans suffering from familial hypercholesterolemia. By contrast, Madsen *et al.*, failed to demonstrate any increased atherosclerosis in the heterozygous offspring of homozygous ApoE knockout females fed normal chow, despite exhibiting average plasma cholesterol levels of 10 mmol/l.[35] Offspring were fed an atherogenic diet and developed hypercholesterolemia (average of ~12 mmol/l) and atherosclerosis independently of maternal diet. However, another study in ApoE knockout mice showed that chow-fed heterozygous offspring of homozygous ApoE knockout females (mated with wild-type males), had increased plasma cholesterol and more atherosclerosis than those born of wild-type females (mated with homozygous ApoE knockout males).[36] Thus, the relative impact of maternal hypercholesterolemia on plasma lipids and development of atherosclerosis in the offspring remains unclear.

In the present study we aimed to extend previous work and hypothesized that fetal exposure to a maternal high-saturated fat/moderate-cholesterol diet would increase atherosclerotic lesion size in later life. In heterozygous ApoE*3 Leiden pregnant mice, such a diet induced a more modest increase in plasma cholesterol than seen in ApoE knockout animals (~5 mmol/l compared with 3 mmol/l in chow-fed animals).[35,36] Although it would have been interesting to profile blood lipids over pregnancy to see how changes developed over pregnancy and between genotypes, this was not possible within the current experiment. When the offspring were challenged with an atherogenic diet, animals exposed to a MHF diet *in utero* developed 1.9-fold greater lesioned area compared with animals exposed to a chow diet. Interestingly, this was independent of any differences in plasma lipids in the offspring, thus supporting the original premise of Napoli *et al.*[33,34,37] that other factors are involved in such programming.

The ApoE*3 Leiden mouse is a unique research tool in that the atherogenic diet is an absolute requirement for the development of atherosclerotic lesions, thereby mirroring the etiology of the human disease.[13,19] The increased susceptibility to atherosclerosis in ApoE*3 Leiden offspring when fed the atherogenic diet would appear to be a specific effect of the maternal metabolic and endocrine response to the MHF diet fed during pregnancy, as the period of feeding was insufficient to produce maternal obesity. This is confirmed by the finding that maternal weight gain was similar in mice fed chow and MHF diet. The most likely explanation of the observations is that atherosclerotic lesions are already forming in the ApoE*3 Leiden fetuses during development, as there is no clear metabolic effect of the diet later in life (offspring did not exhibit dyslipidaemia). It is evident that the programming of atherosclerosis in the ApoE*3 Leiden offspring is a very specific effect of the maternal diet. There was no effect of the MHF diet on litter size, birth weight, male:female ratio, wild type:transgenic ratio or postnatal survival. Food intake and growth rates were comparable between the groups (data not shown). ApoE*3 Leiden animals exposed to a MHF diet during pregnancy were lighter at weaning (28 days postnatal age) than their relative controls ($P < 0.001$) and this difference persisted after 14 weeks feeding an atherogenic diet ($P = 0.021$).

The cause of this increase in susceptibility to diet-induced atherosclerosis has not been fully identified, but in humans Liguori *et al.*[38] found that C-reactive protein was elevated in hypercholesterolemic women, suggesting that increased inflammation may drive fetal lesions. Normal pregnancy is an inflammatory state and this could be exacerbated by diets rich in pro-inflammatory lipids. Such a possibility could be further investigated by examining maternal cytokine profiles, and additionally by assessing offspring inflammatory markers and circulating cholesterol before feeding the atherogenic diet. The current observation that the prenatal diet impacts upon liver weight may also suggest that there is some programming of a hepatic phenotype, which in itself could impact upon lipid metabolism and development of atherosclerosis. The case for maternal hypercholesterolemia as a driver of programed disease is weakened by the observation of Alkemade *et al.*[39] Offspring of ApoE deficient ($ApoE^{+/-}$) fed a 1% cholesterol diet, did not exhibit fetal lesions or develop spontaneous atherosclerosis, but formed more severe plaques were atherosclerosis was induced with a carotid cuff.[39]

There is currently considerable interest in the possible effects of maternal diet upon the fetal epigenome. Resetting of epigenetic marks, such as DNA methylation, can have a long-term effect upon gene expression and the response to dietary or environmental challenges.[40,41] Lipid metabolism, particularly lipogenesis, has been shown to be programed through such resetting of epigenetic marks.[42,43] Grimaldi *et al.*[44] reported that non-coding RNAs regulate endothelial function, lipid metabolism and inflammatory responses and that this may contribute to the regulation of gene expression by cholesterol and hence the development of atherosclerosis. ApoE deficient offspring of hypocholesterolaemic ApoE knockout mice developed more pronounced atherosclerosis when fed an atherogenic diet postnatally.[45] This is associated with differential epigenetic patterning in the vasculature. Vascular smooth muscle cells and endothelial cells in the carotid arteries had altered methylation of histones (3Me-K4-H3, 3Me-K9-H3 and 3Me-K27-H3) in response to atherogenic diet, dependent upon maternal cholesterol concentrations.[45]

This paper reports the novel findings of a preliminary study that investigated the impact of maternal high-fat feeding in programming long-term risk of atherosclerosis. As such it is observational in nature and was not powered or designed to consider mechanistic aspects of this programming. It is noteworthy that no previous study has delivered a full mechanistic understanding of the association between maternal diet and atherosclerosis in the offspring. There is now a need for further experiments to consider the hypothesis that high-fat feeding during fetal development induces the development of atherosclerotic lesions in fetal ApoE*3 Leiden mice. It would also be of interest to assess the impact of high-fat feeding on inflammatory markers in both mothers and offspring. Consideration should also be given to effects of the maternal diet upon gene expression in fetal life and upon expression of microRNAs and other epigenetic marks that regulate expression.

This study provides additional support for both Barker's developmental origins of adult disease hypothesis[46] and Napoli's maternal hypercholesterolemia hypothesis.[32,34,37,47] Our work also demonstrates that maternal hypercholesterolemia is an important factor that should be included in the assessment of the risk of atherosclerosis. With increasing focus on the long-term effects of maternal obesity and maternal qover-nutrition during pregnancy, we have demonstrated that relatively acute changes to maternal nutrition can have major and presumably lifelong effects upon health in the next generation.

Acknowledgments

The authors thank L. Havekes of TNO Pharma, Leiden, The Netherlands for supplying the original breeding stock of ApoE*3 Leiden mice and for permission to carry out the study. The authors acknowledge the support of R Plant and C Armett in the maintenance of the ApoE*3 Leiden colony. Authors' contributions: Designed the experiments: A.S., S.L.E., E.T. Performed experiments: E.T., K.R., R.A., S.K. Performed data analyses: E.T., S.L.E., A.S. Wrote the manuscript: S.L.E., A.S., E.T. Edited the manuscript: S.L.E., A.S., E.T.

Financial Support

This project was funded by a project grant from the Biotechnology and Biological Sciences Research Council. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

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 guides on the care and use of laboratory animals [UK Animals (Scientific Procedures) Act 1986] and has been approved by the institutional committee (University of Nottingham ethical review panel).

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S2040174416000027

References

1. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. *J Hum Nutr Diet*. 2015; 28(Suppl. 1), 1–14.
2. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev*. 2014; 94, 027–1076.
3. Mone SM, Gillman MW, Miller TL, Herman EH, Lipshultz SE. Effects of environmental exposures on the cardiovascular system: prenatal period through adolescence. *Pediatrics*. 2004; 113(Suppl. 4), 1058–1069.
4. Symonds ME, Mendez MA, Meltzer HM, *et al*. Early life nutritional programming of obesity: mother-child cohort studies. *Ann Nutr Metab*. 2013; 62, 137–145.
5. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull*. 2001; 60, 5–20.
6. Langley-Evans SC. Developmental programming of health and disease. *Proc Nutr Soc*. 2006; 65, 97–105.
7. Barker DJ, Thornburg KL, Osmond C, Kajantie E, Eriksson JG. Beyond birthweight: the maternal and placental origins of chronic disease. *J Dev Orig Health Dis*. 2010; 1, 360–364.
8. McMullen S, Mostyn A. Animal models for the study of the developmental origins of health and disease. *Proc Nutr Soc*. 2009; 68, 306–320.
9. Langley SC, Jackson AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin Sci*. 1994; 86, 217–222.
10. Swali A, McMullen S, Hayes H, *et al*. Cell cycle regulation and cytoskeletal remodelling are critical processes in the nutritional programming of embryonic development. *PLoS One*. 2011; 6, e23189.
11. McMullen S, Langley-Evans SC. Maternal low-protein diet in rat pregnancy programs blood pressure through sex-specific mechanisms. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288, R85–R90.
12. Elmes MJ, Gardner DS, Langley-Evans SC. Fetal exposure to a maternal low-protein diet is associated with altered left ventricular pressure response to ischaemia-reperfusion injury. *Br J Nutr*. 2007; 98, 93–100.
13. Yates Z, Tarling EJ, Langley-Evans SC, Salter AM. Maternal undernutrition programmes atherosclerosis in the ApoE*3-Leiden mouse. *Br J Nutr*. 2008; 10, 1–10.
14. Ogden CL, Carroll MD, Kit BK, Flegal KM. *Prevalence of Obesity Among Adults: United States, 2011–2012. NCHS Data Brief, No. 131*. 2013. National Center for Health Statistics: Hyattsville, MD.
15. World Health Organization. Obesity and overweight. WHO factsheet 311. Retrieved 28 January 2014 from http://www.who.int/mediacentre/factsheets/fs311/en/.
16. Oken E, Kleinman KP, Belfort MB, Hammitt JK, Gillman MW. Associations of gestational weight gain with short- and longer-term maternal and child health outcomes. *Am J Epidemiol*. 2009; 170, 173–180.

17. Taylor PD, McConnell J, Khan IY, *et al*. Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. *Am J Physiol*. 2005; 288, R134–R139.
18. Samuelsson AM, Matthews PA, Argenton M, *et al*. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension*. 2008; 51, 383–392.
19. Groot PH, van Vlijmen BJ, Benson GM, *et al*. Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. *Arterioscler Thromb Vasc Biol*. 1995; 16, 926–933.
20. Tarling EJ, Ryan KJ, Bennett AJ, Salter AM. Effect of dietary conjugated linoleic acid isomers on lipid metabolism in hamsters fed high-carbohydrate and high-fat diets. *Br J Nutr*. 2009; 101, 1630–1638.
21. Hogan B, Costantini F, Lacy E. *Manipulating the Mouse Embryo: A Laboratory Manual*. 1986. Cold Spring Harbor Laboratory Press: Cold Spring Habor, NY.
22. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment of atherosclerotic lesions mice. *Atherosclerosis*. 1987; 68, 231–240.
23. Festing MF. Design and statistical methods in studies using animal models of development. *ILAR*. 2006; 47, 5–14.
24. Erhuma A, Bellinger L, Langley-Evans SC, Bennett AJ. Prenatal exposure to undernutrition and programming of responses to high-fat feeding in the rat. *Br J Nutr*. 2007; 98, 517–524.
25. Erhuma A, Salter AM, Sculley DV, Langley-Evans SC, Bennett AJ. Prenatal exposure to a low-protein diet programs disordered regulation of lipid metabolism in the aging rat. *Am J Physiol*. 2007; 292, E1702–E1714.
26. Armitage JA, Lakasing L, Taylor PD, *et al*. Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. *J Physiol*. 2005; 565, 171–184.
27. Holemans K, Gerber R, O'Brien-Coker I, *et al*. Raised saturated-fat intake worsens vascular function in virgin and pregnant offspring of streptozotocin-diabetic rats. *Br J Nutr*. 2000; 84, 285–296.
28. Khan IY, Dekou V, Douglas G, *et al*. A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am J Physiol*. 2005; 288, R127–R133.
29. Khan IY, Taylor PD, Dekou V, *et al*. Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension*. 2003; 41, 168–175.
30. Chechi K, Cheema SK. Maternal diet rich in saturated fats has deleterious effects on plasma lipids of mice. *Exp Clin Cardiol*. 2006; 11, 129–135.
31. Akyol A, McMullen S, Langley-Evans SC. Glucose intolerance associated with early-life exposure to maternal cafeteria feeding is dependent upon post-weaning diet. *Br J Nutr*. 2012; 107, 964–978.
32. Napoli C, Glass CK, Witztum JL, *et al*. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. *Lancet*. 1999; 354, 1234–1241.
33. Palinski W, Napoli C. The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnany influence in utero programming and postnatal susceptibility to atherogenesis. *FASEB J*. 2002; 16, 1348–1360.
34. Napoli C, de Nigris F, Welch JS, *et al*. Maternal hypercholesterolemia during pregnancy promotes early atherogenesis in LDL receptor-deficient mice and alters aortic gene expression determined by microarray. *Circulation*. 2002; 105, 1360–1367.
35. Madsen C, Dagnaes-Hansen F, Møller J, Falk E. Hypercholesterolemia in pregnant mice does not affect atherosclerosis in adult offspring. *Atherosclerosis*. 2003; 168, 221–228.
36. Goharkhay N, Sbrana E, Gamble PK, *et al*. Characterization of a murine model of fetal programming of atherosclerosis. *Am J Obstet Gynecol*. 2007; 197, 416.e1– e5.
37. Napoli C, D'Armiento FP, Mancini FP, *et al*. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest*. 1997; 100, 2680–2690.
38. Liguori A, D'Armiento FP, Palagiano A, Palinski W, Napoli C. Maternal C-reactive protein and developmental programming of atherosclerosis. *Am J Obstet Gynecol*. 2008; 198, 281.e1–5.
39. Alkemade FE, Gittenberger-de Groot AC, Schiel AE, *et al*. Intrauterine exposure to maternal atherosclerotic risk factors increases the susceptibility to atherosclerosis in adult life. *Arterioscler Thromb Vasc Biol*. 2007; 27, 2228–2235.
40. Lillycrop KA, Hoile SP, Grenfell L, Burdge GC. DNA methylation, ageing and the influence of early life nutrition. *Proc Nutr Soc*. 2014; 73, 413–421.
41. Bogdarina I, Haase A, Langley-Evans S, Clark AJ. Glucocorticoid effects on the programming of AT1b angiotensin receptor gene methylation and expression in the rat. *PLoS One*. 2010; 5, e9237.
42. Ehara T, Kamei Y, Takahashi M, *et al*. Role of DNA methylation in the regulation of lipogenic glycerol-3-phosphate acyltransferase 1 gene expression in the mouse neonatal liver. *Diabetes*. 2012; 61, 2442–2450.
43. Cordero P, Gomez-Uriz AM, Campion J, Milagro FI, Martinez JA. Dietary supplementation with methyl donors reduces fatty liver and modifies the fatty acid synthase DNA methylation profile in rats fed an obesogenic diet. *Genes Nutr*. 2013; 8, 105–113.
44. Grimaldi V, Vietri MT, Schiano C, *et al*. Epigenetic reprogramming in atherosclerosis. *Curr Atheroscler Rep*. 2015; 17, 476.
45. Alkemade FE, van Vliet P, Henneman P, *et al*. Prenatal exposure to ApoE deficiency and postnatal hypercholesterolemia are associated with altered cell-specific lysine methyltransferase and histone methylation patterns in the vasculature. *Am J Pathol*. 2010; 176, 542–548.
46. Barker DJ, Thornburg KL. The obstetric origins of health for a lifetime. Clin Obstet Gynecol. 2013; 56, 511–519.
47. Napoli C, Witztum JL, Calara F, de Nigris F, Palinski W. Maternal hypercholesterolemia enhances atherogenesis in normocholesterolemic rabbits, which is inhibited by antioxidant or lipid-lowering intervention during pregnancy : an experimental model of atherogenic mechanisms in human fetuses. *Circ Res*. 2000; 87, 946–952.