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

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Abstract

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,* programmed 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 28d 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 to 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 2-fold higher than in chow fed mothers, $p=0.011$). The data strongly suggest that maternal hypercholesterolaemia programmes later susceptibility to atherosclerosis. This is consistent with previous observations in humans and animal models.

Introduction

Whilst 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 programme 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 aetiology[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 steps were taken to minimize animal suffering. Male and female mice (10-12 weeks) were maintained in a controlled environment (21^{o}C; 55% humidity) with a 12h 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 (135g/kg diet), corn oil (21.5g/kg diet) and tripalmitin (15g/kg diet). This diet contained 2g/kg cholesterol (standard chow was 0.31g/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 d17 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 4-9. Offspring were genotyped using a polymerase chain reaction assay before weaning at 28d 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 randomised 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 8 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^{o}C for 10min. The liver, adipose (perirenal and gonadal depots), kidneys, gastrocnemius muscle and abdominal aorta were dissected from each animal, weighed to the nearest 0.1mg 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.

Genotyping of transgenic mice

Genomic DNA was extracted from ear punches by standard procedures[20]. Polymerase chain reaction assay was performed on genomic ear DNA using primers spanning the ApoE*3 Leiden mutation (forward primer 5'

GCCCCGGCCTGGTACACTGC 3'; reverse primer 5' GGCACGGCTGTCCAAGGAGC 3') as described previously[13].

Measurement of plasma metabolites

Total circulating plasma cholesterol and TAG were assayed using commercially available kits (ThermoTrace, Noble Park, Vic, 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, Cambs, UK). Alternate sections of the aortic root (10 □m 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, Lancs, UK). Atherosclerotic lesions were analyzed and quantified using the method of Paigen *et al*.[21] using Image Pro-Plus software (Media Cybernetics, Inc., Bethesda, MD, USA) to determine 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 fifteen sections per animal[13].

Statistical Analysis

All data are presented as mean values ± standard error. Unless otherwise stated in the text, data were analysed 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[22]. 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[13,23,24]. *Post hoc* tests were not performed where ANOVA 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; Figure 1a), but pregnancy had no significant effect on maternal total plasma cholesterol levels (Figure 1b). Feeding a MHF diet did not impact on triglyceride levels but increased maternal total cholesterol 2-fold (p=0.011; Figure 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 per litter; MHF, 7.4 ± 0.6 pups per 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 28d postnatal age (Figure 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 (Figure 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 C57Bl/6J and ApoE*3 Leiden transgenic offspring fed the chow diet. There were no significant alterations in plasma triglyceride levels (Figure 3(a)) 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 3-fold higher cholesterol levels when placed on the atherogenic diet ($p<0.031$ compared to chow). There was no additional effect of the *in utero* exposure to the MHF diet (Figure 3(b)).

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 (Figure 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 Figure 5(a), with representative sections of aorta stained for lesions shown in Figure 5(b). C57Bl/6J animals 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 to 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 to the offspring of mothers fed a control diet. It is also clear that over-nutrition 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[25-30]. As in our 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.

Observations in humans have indicated the formation of atherosclerotic lesions in fetal vessels following exposure to maternal hypercholesterolaemia[31]. This has been supported by some studies using animals, indicating induction of hypercholesterolaemia during pregnancy is associated with the development of atherosclerosis in the offspring[32,33]. However, a number of inconsistencies are apparent within the animal literature. Palinski and Napoli[32] originally suggested that the increased susceptibility to atherosclerosis in offspring born to hypercholesterolaemic mice, was not associated with any changes in plasma lipids of the offspring. This work was performed in homozygous LDL- receptor deficient pregnant mice fed dietary cholesterol, which induced massive hypercholesteroaemia (25-30mmol/l). It should be noted that such levels of plasma cholesterol are only seen in humans suffering from familial hypercholesterolaemia. By contrast, Madsen *et al*., failed to demonstrate any increased atherosclerosis in the heterozygous offspring of homozygous ApoE knock-out females fed normal chow, despite exhibiting average plasma cholesterol levels of 10mmol/l[34]. Offspring were fed an atherogenic diet and developed hypercholesterolaemia (average of approximately 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)[35]. Thus the relative impact of maternal hypercholesterolaemia 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 (approximately 5mmol/l compared to 3mmol/l in chow fed animals)[34,35]. 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 to 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 and colleagues[32,33,36] 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, wildtype: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 (28d 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.,*[37] found that C-reactive protein was elevated in hypercholesterolaemic 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 prior to 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

hypercholesterolaemia as a driver of programmed disease is weakened by the observation of Alkemade and colleagues[38]. 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[38].

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[39,40]. Lipid metabolism, particularly lipogenesis, has been shown to be programmed through such resetting of epigenetic marks[41,42]. Grimaldi *et al.*[43] 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[44]. 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[44].

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[45] and Napoli's maternal hypercholesterolemia hypothesis[31,33,36,46]. 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 over-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.

Author contributions

Designed the experiments: AS, SLE, ET. Performed experiments: ET, KR, RA, SK. Performed data analyses: ET, SLE, AS. Wrote the manuscript: SLE, AS, ET. Edited the manuscript: SLE, AS, ET

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Conflict of interest

The authors have no conflicts of interest to declare.

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).

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Figure Legends

Figure 1. Maternal plasma triglyceride (a) and cholesterol (b) concentrations at day 17 gestation. MC, maternal chow; MHF, maternal MHF. 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 difference from mothers fed a maternal chow (MC) diet (P<0.01).

Figure 2. Body weight at 28d postnatal age (a) and after 14 weeks of postnatal feeding (b). MC, maternal chow diet; MHF, maternal MHF diet; Chow, postnatal chow diet; Athero, postnatal atherogenic diet. 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).

Figure 3. Plasma triglyceride (a) and cholesterol (b) concentrations in the offspring. MC, maternal chow; MHF, maternal MHF. 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 ($P<0.05$).

Figure 4. Liver weight. MC, maternal chow diet; MHF, maternal MHF diet; Chow, postnatal chow diet; Athero, postnatal atherogenic diet. 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* ($P<0.01$).

Figure 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 ($p<0.001$). *** Mean value was significantly different from ApoE*3 Leiden offspring from mothers fed a chow diet that were fed the same atherogenic postnatal diet ($P<0.001$).

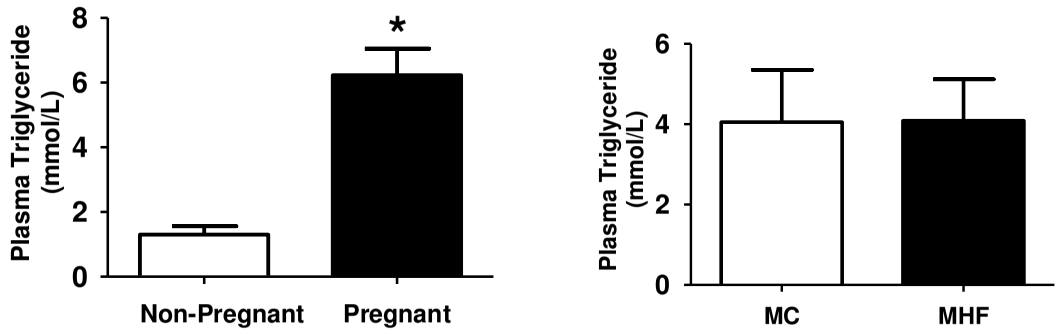
Table 1. Fatty acid composition of mouse diets

Fatty acid	Chow	MHF	Atherogenic Diet
C14:0	4.4	3.2	N.D
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

Data is shown as percentage of the total fatty acids present in the diet. Chow diet was 28g fat/kg; MHF 171.5 g fat/kg diet; Atherogenic diet 160 g fat/kg. N.D. Not detected
*derived from figures published in Tarling et al (2009)[47]

Figure 1.

(a)


Plasma Triglyceride (mmol/L)
8
6
4
2
0
*
Non-Pregnant
Pregnant
Plasma Triglyceride (mmol/L)
6
4
2
0
MC
MHF

(b)

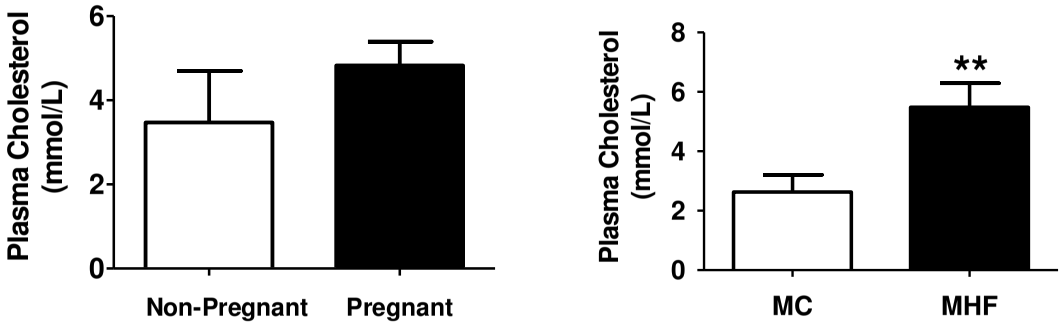
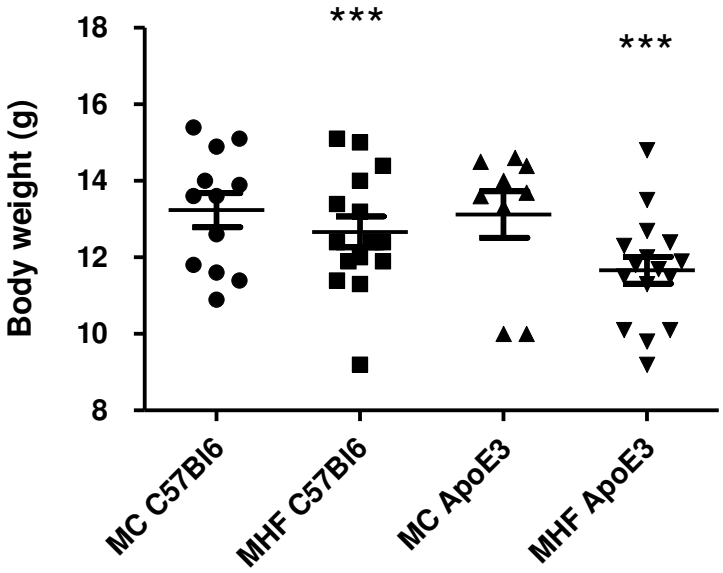

Plasma Cholesterol (mmol/L)
6
4
2
0
Non-Pregnant
Pregnant
Plasma Cholesterol (mmol/L)
8
6
4
2
0
**
MC
MHF

Figure 2.

(a)


***
***
18
16
14
12
10
8
Body weight (g)
MC C57Bl6
MHF C57Bl6
MC ApoE3
MHF ApoE3

(b)

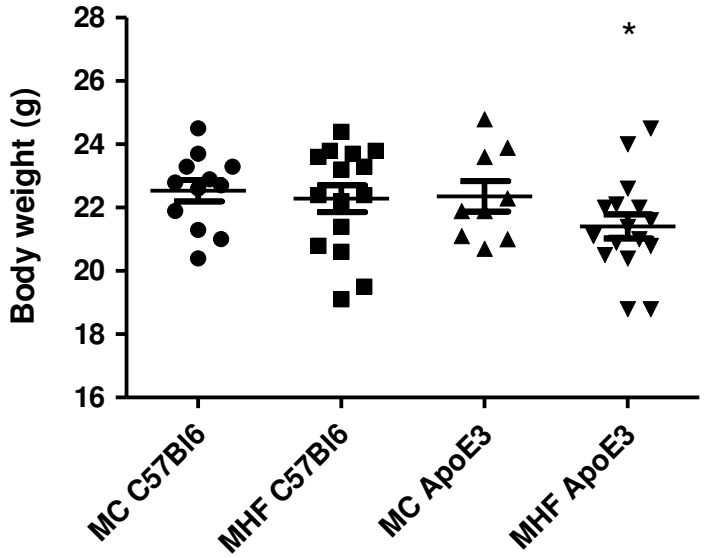
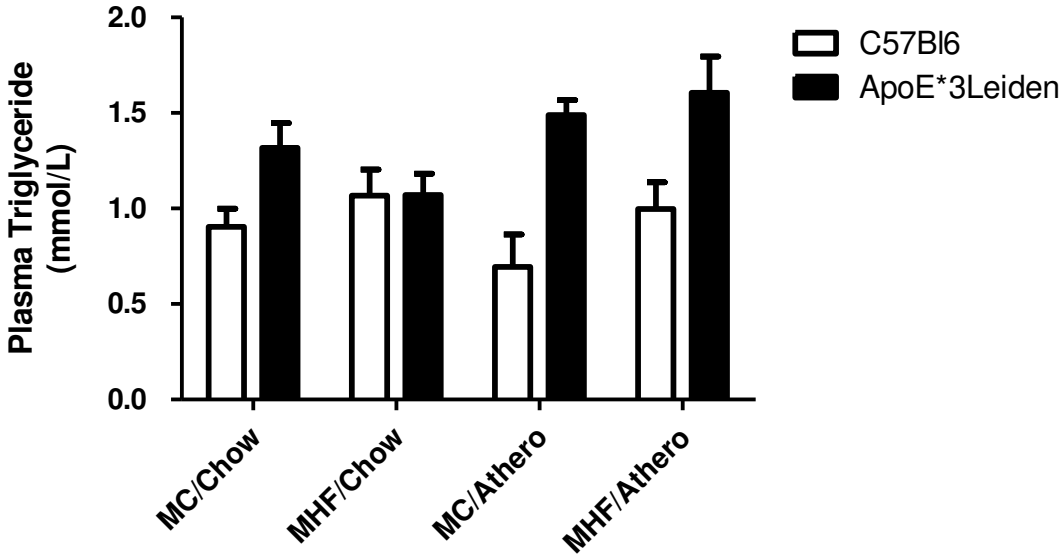

*
28
26
24
22
20
18
16
Body weight (g)
MC C57Bl6
MHF C57Bl6
MC ApoE3
MHF ApoE3

Figure 3.

(a)


Plasma Triglyceride (mmol/L)
2.0
1.5
1.0
0.5
0.0
MC/Chow
MHF/Chow
MC/Athero
MHF/Athero
C57Bl6
ApoE*3Leiden

(b)

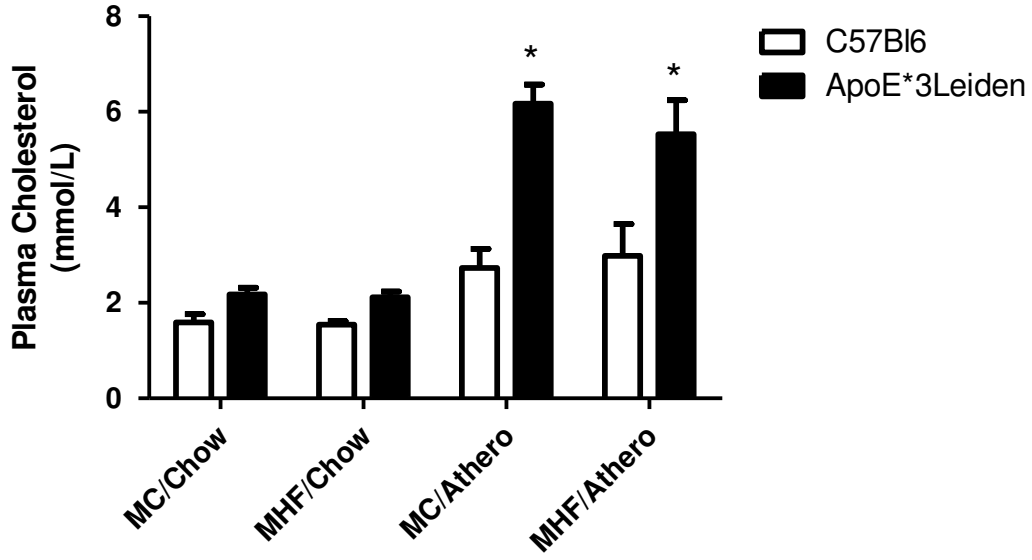

Plasma Cholesterol (mmol/L)
8
6
4
2
0
*
*
MC/Chow
MHF/Chow
MC/Athero
MHF/Athero
C57Bl6
ApoE*3Leiden

Figure 4.

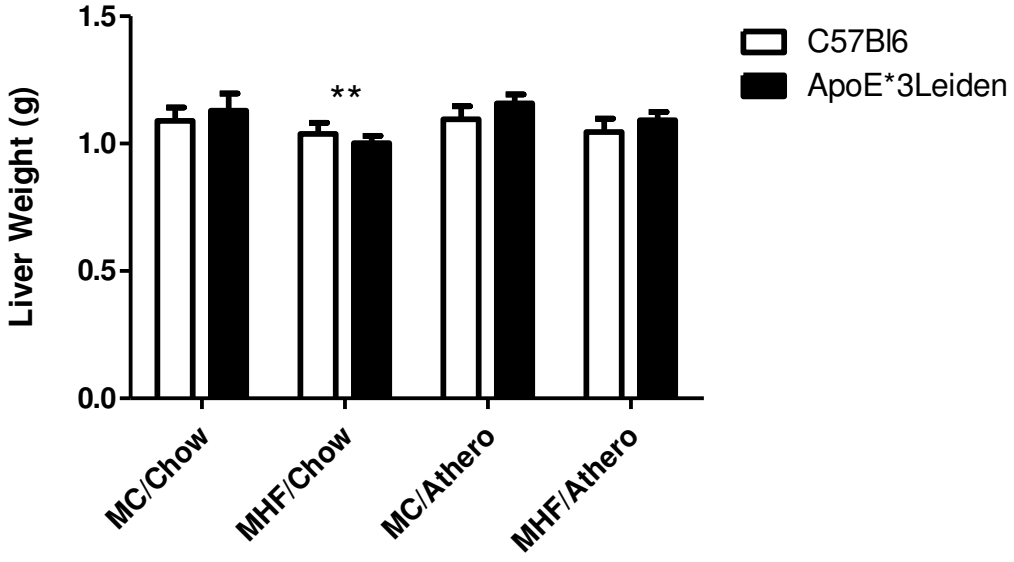
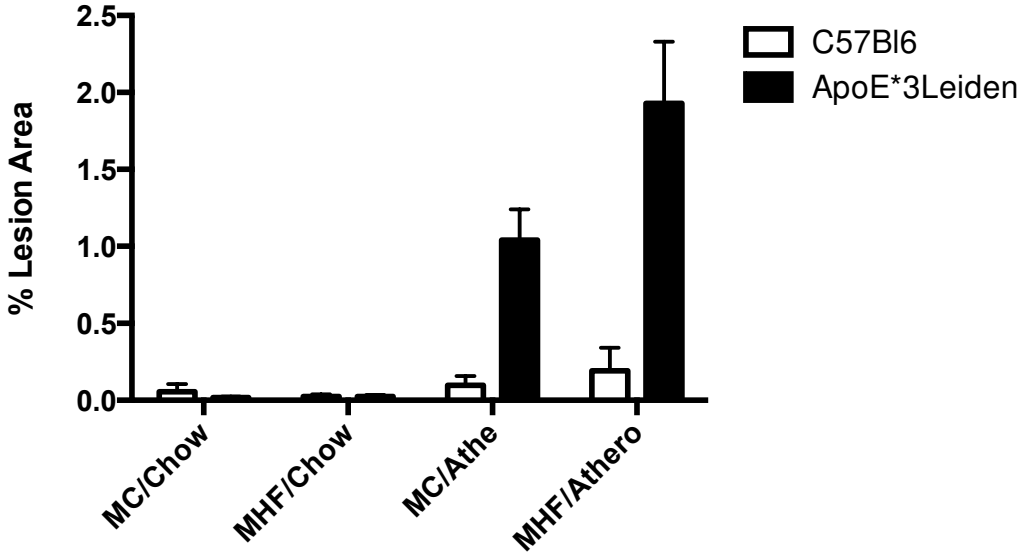
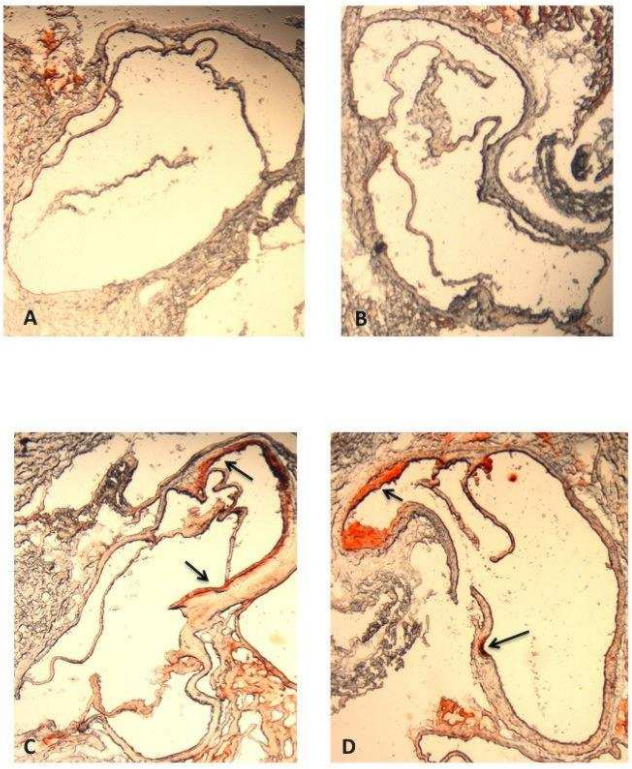

Liver Weight (g)
1.5
1.0
0.5
0.0
**
C57Bl6
ApoE*3Leiden
MC/Chow
MHF/Chow
MC/Athero
MHF/Athero

Figure 5.

(a)


% Lesion Area
2.5
2.0
1.5
1.0
0.5
0.0
MC/Chow
MHF/Chow
MC/Athe
MHF/Athero
C57Bl6
ApoE*3Leiden

(b)


A
B
C
D

Supplementary Figure 1. Growth of offspring from weaning/
Offspring were weaned onto either chow diet or atherogenic diet and growth was followed for 14 weeks.

Body weight was influenced by genotype (P=0.002), maternal diet (P=0.001) and interactions of maternal and postnatal diet (P=0.018) and genotype and maternal diet (P=0.015).

Supplementary Figure 1