Maternal diet high in linoleic acid alters offspring fatty acids and cardiovascular function in a rat model

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Abstract

Linoleic acid (LA), an essential n-6 fatty acid (FA), is critical for fetal growth and development. We investigated the effects of maternal high LA (HLA) diet on offspring cardiac development and how it relates to circulating FAs and cardiovascular function in adolescent offspring, and the ability of the postnatal diet to reverse any adverse effects. Female Wistar Kyoto rats were fed low LA (LLA; 1.44% energy from LA) or HLA (6.21% energy from LA) diets for 10 weeks before pregnancy, and during gestation/lactation. Offspring, weaned at postnatal day 25 (PN25), were fed LLA or HLA diets and euthanised at PN40 (n = 6-8). Maternal HLA diet decreased circulating total cholesterol and HDL-cholesterol in females and decreased total plasma n-3 FA in males, while maternal and postnatal HLA diets decreased total plasma n-3 FA in females. Alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA) were decreased by postnatal but not maternal HLA diets in both sexes. Maternal and postnatal HLA diets increased total plasma n-6 and LA, and a maternal HLA diet increased circulating leptin, in both male and female offspring. Maternal HLA decreased slopes of systolic and diastolic PVRs, and increased cardiac *Colla1*, *Col3a1*, *Atp2a1* and *Notch1* in males. Maternal and postnatal HLA diets left-shifted the diastolic PVR in female offspring. Coronary reactivity was altered in females, with differential effects on flow repayment after 10–20 sec occlusions. In conclusion, maternal HLA diets impact lipids, fatty acids and cardiac function in offspring, with postnatal diet modifying fatty acids and cardiac function in the female offspring.

1. Introduction

Cardiovascular disease (CVD) is the most common non-communicable disease globally, with an estimated 17.8 million death in 2017 ⁽¹⁾. The World Health Organization (WHO) has focused the impact of lifestyle, such as unhealthy diet, physical inactivity and psychological stress in the development of CVD and stated that three quarters of all CVD related mortality may be avoided by the lifestyle modifications ⁽²⁾. The aetiology of this global health problem is complex and may commence in early life or childhood. Epidemiological studies demonstrate that atherosclerosis can have its origin in childhood, and that this major risk factor for CVD can persist into adulthood ^(3; 4). Further, childhood CVD risk factor clustering is reported to be predictive of CVD in adulthood ⁽⁵⁾. Both epidemiological data and animal model studies provide evidence of the fetal origin of CVD risk ^(6; 7; 8). For example, intrauterine growth restriction (IUGR) that occurred in response to the Dutch famine increased the risk of death from CVD in adult life in affected offspring ⁽⁹⁾. The effects of maternal environment on *in utero* development is termed as fetal programming or developmental origin of health and disease (DOHaD) ^(10; 11). Adverse maternal conditions such as overnutrition ⁽¹²⁾, obesity ⁽⁸⁾, hypoxia ⁽¹³⁾ and protein restriction ⁽¹⁴⁾ during pregnancy are also associated with development of cardiovascular dysfunction in later life of offspring.

Linoleic acid (LA; 18:2n-6; cis, cis-9,12-octadecadienoic acid) is the most abundant n-6 polyunsaturated fatty acid (PUFA) in modern human diets. It can be elongated and desaturated to produce arachidonic acid (AA, 20:4n-6) ⁽¹⁵⁾, a longer chain n-6 PUFA. AA is a precursor of eicosanoid mediators such as prostaglandins, thromboxanes and leukotrienes, which influence inflammatory processes ⁽¹⁶⁾ and are essential for the normal metabolic function of cells and tissues. When these eicosanoid mediators are in excess, they promote inflammation and chronic disease ⁽¹⁵⁾. In recent years, worldwide dietary guidelines have encouraged replacing animal based saturated fatty acid (SFA) with plant based PUFA, the most abundant being LA ⁽¹⁷⁾. As a result, the current intake of LA is significantly higher than required to prevent deficiency ⁽¹⁷⁾. The excess in LA

may decrease the ability of the n-3 PUFA α -linolenic acid (ALA), to produce longer chain anti-inflammatory n-3 PUFAs such as eicosapentaenoic acid (EPA, 20:5n-3) and DHA, as both are metabolised by the same enzymes, with the enzymes that metabolise LA and ALA having a higher enzymatic affinity for LA ⁽¹⁸⁾. A recent meta-analysis and analysis of recovered data from a past clinical trial found that substitution of dietary LA for SFA increases coronary heart disease (CHD), CVD and all-cause mortalities ⁽¹⁹⁾. However, there is evidence from randomised control trials that a high LA and low SFA diet reduces cardiac events and mortality in post-infarct patients ⁽²⁰⁾, suggesting the conflicting role of LA in CVD risk.

At this time, there is significant research which describes the effect of a high LA diet on cardiac health. Emerging research has indicated that LA is critical for normal development ⁽²¹⁾. Cardiac development commences *in utero* and perturbations during this time impacts cardiovascular function in later life. Despite the increased consumption of LA in Western cultures, including in women of reproductive age, there is paucity of data investigating the role of maternal diet high in LA on the cardiovascular system and incidence of CVD on offspring development. The fetal and early life exposure to a suboptimal maternal diet programs CVD risk due to altered cardiac development, with additional studies also demonstrating an important role for the postnatal environment. Development of the cardiomyocyte commences prior to birth, with a full complement of binucleated cardiomyocytes present at birth ⁽²²⁾. Following this period of hyperplasia, postnatal cardiomyocytes undergo hypertrophy ⁽²³⁾. Therefore, two critical windows of development of the heart occur both before and after birth in humans. In rodents the post-weaning period is also important for development, with a critical period in adolescent rats, between 5 and 9 weeks of life where perturbations have the capacity to influence final cardiac development and maturation ⁽²⁴⁾. Rodent studies also support a role for the post-weaning diet on disease risk, with a study demonstrating that a post-weaning obesogenic diet can exacerbate the detrimental effects of maternal diet-induced obesity on offspring body weight gain ⁽²⁵⁾. Thus, in addition to investigating the effects of a high LA maternal diet *in utero* on offspring CVD risk, it is important to identify any effects of a post-weaning diet on cardiovascular function. It is evident that the risk of development of the CVD increases

with ageing, however, emerging research suggests an increasing incidence of CVD in young adults ⁽²⁶⁾. Early markers of cardiomyopathy, such as left ventricular hypertrophy and left ventricular dysfunction are frequently observed during childhood and adolescence ⁽²⁷⁾ and may represent early heart damage associated with the later development of CVD.

In the current study, our aim was to investigate the effects of an elevated maternal LA diet on offspring heart function, and the ability of the postnatal diet to reverse or exacerbate any adverse phenotype. Further, we aimed to characterise any molecular changes that may contribute and the ability of the post-weaning diet to reverse any deleterious cardiovascular changes. Since it is well established that programmed disease outcomes are sexually dimorphic, we aimed to analyse male and female offspring outcomes. Therefore, the hypothesis of this study was that a maternal diet high in LA would program cardiovascular sex-specific deficits that will be reversed by a low LA diet following weaning.

2.Methods

2.1 Ethical statement

Ethical approval for all experimental protocols was granted by the Griffith University Animal Ethics Committee (NSC/01/17/AEC) prior to experimentation. All experiments were conducted humanely. Rats were housed in accordance to the Australian Code of Practice for Care and Use of Animals for Scientific Purpose, following the ARRIVE Guidelines for Reporting Animal Research.

2.2 Experimental design, animals and experimental diet

Eight-week-old female Wistar Kyoto (WKY) rats were purchased from the Australian Resource Centre (ARC, WA, Australia). Rats were housed in individually ventilated cages under 12 hours light-dark cycle at a temperature of 20–22°C and provided with standard food pellets during acclimatisation and tap water *ad libitum* throughout the study. After a week for acclimatization, female rats were randomised to consume either

a control low LA (LLA: 1.44% of energy from LA, n = 8) or high LA (HLA: 6.21% of energy from LA, n = 8) diet for 10 weeks. The minimum requirement for LA in the rodent diet is between 1-1.5% ⁽²⁸⁾. The composition of the custom diet has been previously reported ⁽²¹⁾. These diets were matched for n-3 PUFA and total fat content. The HLA diet used is comparable to the diet consumed in Western society ⁽²⁹⁾. After eight weeks of dietary exposure, vaginal impedance was measured daily for at least two estrous cycles using a rodent vaginal impedance reader (Muromachi Kikai Co. Ltd., Japan). Rats were considered ready for mating after 10 weeks of dietary exposure and when vaginal impedance was greater than $4.5 \times 10^3 \Omega$ and at this time were placed with a Wistar Kyoto male rat overnight. Male rats were maintained on standard chow, but during mating (~8 hours), the male rats consumed the similar diet as female rats. After rats gave birth, offspring were left unhandled to minimise stress and weaned at postnatal day 25 (PN25). From weaning, offspring were housed with one or two other litter mates of the same sex and fed either LLA or HLA diets (n=6-8/ litter/ sex). Thus, offspring were exposed to the maternal diet until weaning at PN25 before being placed on a either LLA or HLA for 15 additional days. The offspring were euthanised at postnatal day 40 (PN40). Rats were terminally anaesthetised with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) administered once and monitored until abolishment of reflexes. A thoracotomy was performed, and hearts rapidly excised into ice-cold fluid for Langendorff heart perfusion (details below). After perfusion experiments, hearts were weighed and fixed and frozen for gene analysis. Blood samples were placed in EDTA tubes and centrifuged at 5000 x g for 10 min to separate plasma, which was then stored at -80°C until analysis.

2.3 Quantification of fatty acids in plasma

Concentrations of fatty acids in plasma were measured using gas chromatography (GC) ⁽³⁰⁾. The samples were analysed by the South Australian Health and Medical Research Institute, following the method described in ⁽³¹⁾. Briefly, 50 µl of plasma sample was spotted onto Whatman SG81 ion exchange paper (1.5 cm x 1.5 cm) and dried in air at room temperature for ~30 mins. The dried spot was then soaked in acetone for 10 mins, then placed into a scintillation vial. The spotted plasma was added to 2 ml of 1% (v/v)

H₂SO₄ in anhydrous methanol in a 6 ml sealed scintillation vial, and heated at 70 °C for 3 hrs. The resultant fatty acid methyl esters (FAME) were extracted into n-heptane for gas chromatography analysis ⁽³¹⁾. The fatty acid content was expressed as total lipid fatty acids as %.

2.4 Biochemical analysis of plasma glucose and lipids

Plasma glucose and lipid profile were assessed using an automated chemistry analyser (Integra 400 plus, Roche Diagnostics, North Ryde, Australia) in EDTA anticoagulated plasma. All biochemistry assays were performed using Roche certified assay kits, which were calibrated using Calibrator for Automated Systems reagent. Quality control material (PeciControl ClinChem Multi 1 and 2; Roche Diagnostics) was run prior to sample analysis to ensure accuracy of results. All analyses were performed in duplicate. Leptin concentration in offspring plasma was estimated by ELISA following manufacture's guidelines (Mouse/Rat Leptin Quantikine ELISA Kit, R&D Systems, USA). The intra-assay coefficient of variation was <3.0%.

2.5 Langendorff heart perfusion

Hearts were isolated from male and female rat and perfused in a manner similar to that recently detailed for adult mouse hearts ⁽³²⁾. Briefly, following removal of hearts via thoracotomy and placement in ice-cold perfusion fluid, the aorta was cannulated for perfusion in a Langendorff mode at an aortic pressure of 80 mmHg. A modified Krebs-Henseleit buffer was used, and contained (in mM): NaCl, 119; NaHCO₃, 22; KCl, 4.7, MgCl₂, 1.2; KH₂PO₄, 1.2; EDTA, 0.5; CaCl₂, 1.85; D-(+)-glucose, 11; and Na⁺-pyruvate, 2 (all from Sigma Aldrich, St. Louis, MO, USA). Buffer was equilibrated with 95% O₂/5% CO₂ to maintain pH at 7.4 at 37°C. A fluid-filled plastic balloon inserted in the left ventricle (LV) and connected to a pressure transducer was used to monitor contractile function, while an in-line Doppler flow probe measured coronary flow (Transonic Systems Inc., Clifton, NJ, USA). Data were recorded using a 4-channel MacLab system (ADInstruments Pty Ltd: Bella Vista, Australia). Following initial preparation, the hearts were perfused within a 10-mL water jacketed chamber (37°C),

with perfusate and chamber temperatures measured via needle thermistors to ensure a constant temperature of 37°C was maintained.

2.6 Coronary functional assessment

After 20 min stabilisation to assess baseline function, the LV balloon was deflated to limit contractile workload, thus O₂ demand. After 10 min hearts were subjected to 10 or 20 sec occlusions (5 min recovery periods between), with coronary flow changes monitored throughout. Peak hyperaemic flows were measured, and % flow repayment over 3 min reperfusion (total reperfusion flow - pre-occlusion flow / flow debt x 100%) determined.

2.7 Left ventricle pressure-volume relationships (PVRs)

Following coronary analysis, left ventricle (LV) end-diastolic and systolic PVRs were assessed via stepped increases in LV balloon volume ⁽³²⁾. The ventricular balloon volume was initially adjusted to yield a zero systolic pressure over a 10 min stabilisation period. Balloon volume was then increased in 2.65 µL increments using a 500 µL calibrated syringe (Hamilton Co; Reno, NV, USA), with function assessed after 2 min at each volume. Experiments were terminated when end-diastolic pressure exceeded 30 mmHg. Hearts were then removed from the perfusion apparatus, blotted and weighed and the LV dissected and frozen in liquid nitrogen before storage at -80°C until later analysis. The PVR data were analysed to extract information on both LV volumes generating specific systolic and diastolic pressures, and slopes of PVRs (rate of rise in systolic or diastolic pressure per unit volume change). The linear slope of the systolic PVR (between 0 and 25 µl/g LV volumes) provides a measure of LV inotropic state (Δ pressure / Δ volume). The curvilinear diastolic PVRs were analysed to extract distinct early and late slopes: the initial slope at low LV volumes primarily reflects compliance (conversely stiffness) of intracellular elements (predominantly myofibrillar apparatus), with the extracellular matrix having negligible influence on passive tension over normal loads or stretch ⁽³³⁾. The final slope at abnormally high stretch or load

reflects additional contributions from extracellular elements such as the extracellular matrix.

2.8 Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from cardiac tissues using the RNeasy® mini kit (Qiagen) following the manufacturer's guidelines. Quantification and evaluation of RNA purity was carried out on a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Reverse transcription of RNA to synthesise complementary DNA was performed using the iScript gDNA clear cDNA synthesis kit (Biorad) following manufacturer's guidelines. Quantitative PCR was performed using QuantiNova SYBR® green master mix (Qiagen) following manufacturer's guidelines, in line with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines⁽³⁴⁾. PCR initial heat activation was run for 2 min at 95°C, then qPCR reactions were run for 40 cycles of 95°C for 5 sec (denaturation) and 60°C for 10 sec (combined annealing/ extension) using StepOne™ real-time PCR systems (Applied Biosystems). Gene expression was quantified using the $2^{-\Delta\Delta C_q}$ method normalised to the geometric mean of β -actin and β -2 microglobulin as reference genes. Each sample was tested in duplicate for the average of Cq value. The reference genes were stable across the treatment groups. All the primers used for this study were KiCqStart™ predesigned primers from Sigma- Aldrich: Leptin receptor (*Lepr*; NM_012596), *Myh6* (XM_006251951.2), *Myh7* (XM_017599642.1), *Atp2a1* (NM_058213.1), *Nppa* (NM_012612.2), *Nppb* (NM_031545.1), *Notch1* (NM_001105721.1), *Col1a1* (NM_053304.1) and *Col3a1* (NM_032085.1).

2.9 Statistical analysis

All data were analysed using GraphPad Prism 8.3.1 (GraphPad software, San Diego, US). One male and one female offspring from each litter were analysed. The sample size (n) represents the number of litters, and one male and one female were used from each litter. Only one male and one female were used from each litter for each

experiment to remove potential litter effects, as recommended in guidelines on experimental design in DOHaD studies ⁽³⁵⁾. In essence, each rat in the same litter is a replicate, and thus only one male and one female is analysed for each parameter.

Data were analysed to determine if they were normally distributed. Data were analysed separately for males and female with each sex analysed by two-way analysis of variance (ANOVA), with maternal and postnatal diet as the factors. Specific comparisons were made using Tukey's post-hoc multiple comparison test. Data are presented as mean $\pm$ standard error of the mean (SEM). P-values < 0.05 were considered evidence of significant differences.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3. Results

3.1 Effect of maternal and postnatal diets high in LA on offspring body weight and organ weights

In both sexes, there were different interaction effects of maternal and postnatal diets (Table 1, n = 6-8) In female offspring, a maternal LLA diet followed by a postnatal LLA diet, or a maternal HLA diet followed by a postnatal HLA diet, led to increased body weight (effect of interaction $p=0.02$). In offspring of both sexes, neither a maternal nor postnatal diet HLA altered weight of brain, left kidney and right kidney (Table 1, n = 6-8).

3.2 Effect of maternal and postnatal diets high in LA on circulating glucose, lipids and leptin

The levels of glucose, triglyceride, total cholesterol (TC), HDL-cholesterol (HDL-C) and TC/ HDL-C were unchanged in the plasma of male offspring (Table 2, n = 5-8). However, maternal HLA diet decreased TC (effect of maternal diet $p=0.01$) and HDL-C (effect of maternal diet $p=0.03$) levels in female offspring (Table 2, n = 5-8). Circulating leptin in offspring was elevated by a maternal diet high in LA but not by postnatal diet

in both sexes. Leptin levels in female offspring from LLA dams fed a HLA postnatal diet were reduced, and the maternal diet affected the influence of the postnatal diet in female offspring, indicative of an interaction effect (effect of maternal diet $p=0.001$, effect of interaction $p=0.01$) (Table 2, $n = 5-8$).

3.3 Effect of maternal and postnatal diets high in LA on offspring plasma fatty acid composition

There was no difference in total plasma SFA across dietary groups in both male and female offspring (Table 3 and Table 4, $n = 5-8$). Total plasma trans FA was unchanged among the groups in male offspring (Table 3), however maternal HLA diet increased trans FA in female offspring (Table 4, effect of interaction $p=0.04$). Both maternal and postnatal HLA diet significantly decreased total plasma mono-unsaturated fatty acid (MUFA) in both male (Table 3, effect of maternal diet $p=0.003$, effect of postnatal diet $p=0.0005$, effect of interaction $p=0.0002$) and female (Table 4, effect of maternal diet $p<0.0001$, effect of postnatal diet $p<0.0001$, effect of interaction $p<0.0001$) offspring. In male offspring, only postnatal HLA diet decreased total plasma n-3 PUFA (Table 3, effect of postnatal diet $p=0.0007$), however, in female offspring, both maternal and postnatal HLA diet decreased total plasma n-3 PUFA (Table 4, effect of maternal diet $p=0.001$, effect of postnatal diet $p<0.0001$). Interestingly, plasma ALA and EPA were significantly decreased by postnatal HLA diet but not by maternal diet in either sex. In male offspring, plasma docosapentaenoic acid (DPA) was significantly increased by a maternal HLA diet (Table 3, effect of maternal diet $p=0.02$), however in females the level of plasma DPA was significantly decreased by a postnatal HLA diet (Table 4, effect of postnatal diet $p=0.002$). As expected, both maternal and postnatal diets high in LA increased the levels of total plasma n-6 and LA in both sexes. Interestingly, offspring from HLA dams fed with LLA had a higher concentration of total plasma n-6 FA, suggesting that a LLA diet postnatally does not reverse the effect of high maternal LA on plasma n-6 levels. Plasma AA, a derivative of LA, was unchanged across the dietary groups, however the ratio of AA/DHA was significantly increased in the plasma by both maternal and postnatal HLA diets in both sexes. Further, maternal and postnatal diets high in LA increased the ratio of LA/ALA in the plasma of both male and female

offspring. Maternal and postnatal diets high in LA decreased the plasma DHA/ DPA ratio in male but not female offspring.

Further, in males, 24:0 SFA was significantly increased by the maternal HLA diet (Table 3, effect of maternal diet $p = 0.0366$). 14:0 and 22:0 SFA were significantly increased by postnatal HLA diets (Table 3, effect of postnatal diet $p=0.00157$ and $p=0.0093$, respectively). 15:0 SFA was significantly increased by both the maternal and postnatal HLA diets (Table 3, effect of maternal and postnatal diet $p=0.0049$ and $p = 0.0049$ respectively). 16:0 SFA had an interaction effect for the HLA diet (Table 3, $p = 0.0075$). 20:0 SFA was significantly altered by HLA diet (Table 3, effect of maternal diet $p=0.0005$, effect of postnatal diet $p=0.0224$, effect of interaction $p=0.0224$). For MUFA, 20:1n-9 was significantly reduced by postnatal HLA diet (Table 3, effect of postnatal diet $p=0.0257$), 18:1n-7 was affected by both maternal and postnatal HLA (Table 3, effect of maternal diet $p=0.0024$, effect of postnatal diet $p=0.0019$) and 16:1n-7 and 18:1n-9 were affected by maternal and postnatal HLA diet, with an interaction effect. trans fat t18:1n-7 had an interaction effect for the HLA diets (Table 3, effect of interaction $p= 0.0469$). Other n-6 PUFA were also altered, with 18:3n-6 affected by both maternal and postnatal HLA diet (Table 3, effect of maternal and postnatal diet $p=0.0306$ and $p = 0.0127$ respectively). 20:2n-6 was affected by maternal and postnatal diet and an interaction effect. Finally, total n-9 and n-7 FA were affected by maternal and postnatal diet and an interaction effect.

In females, 20:0 SFA was significantly increased by maternal HLA (Table 4, effect of maternal diet $p = 0.019$), while there was an interaction effect for 16:0 SFA and 18:0 SFA (Table 4, effect of interaction $p = 0.0034$ and 0.0237 respectively). For MUFA, 16:1n-7, both maternal and postnatal HLA diet had an effect (Table 4, effect of maternal diet $p<0.0001$ and effect of postnatal diet $p =0.0077$). 18:1n-7, 18:1n-9 and 20:1n-9 were affected by maternal, postnatal as well as an interaction effect. trans fat t18:1n-9 had an interaction effect for the HLA diets (Table 4, effect of interaction $p= 0.0123$). t18:1n-7 had an effect from both maternal and postnatal HLA diets (Table 4, effect of maternal $p=0.0005$, effect of postnatal $p = 0.0317$). Other n-6 PUFA were also altered, 18:3n-6 had a maternal effect, postnatal effect and an interaction (Table 4). 20:2n-6 was also affected by the maternal and postnatal HLA diets (Table 4, effect on maternal and

postnatal $p < 0.0001$, respectively). Finally, total n-9 and n-7 FA were affected by maternal and postnatal diet and an interaction effect.

3.4 Effect of maternal and postnatal diets high in LA on cardiovascular function of adolescent offspring

3.4.1 Baseline function

Baseline contractile function in *ex vivo* hearts did not differ, regardless of maternal and postnatal diet (Figure 1, n = 5-8). However, interaction between maternal and postnatal diet (combination of the two factors) in female rats significantly affected the baseline coronary flow rate (Figure 1D, n = 5-8).

3.4.2 Systolic and diastolic PVRs

The PVR data were interrogated to assess slopes of relationships, and LV volumes generating specific diastolic and systolic pressures. Systolic curves did not differ noticeably with maternal or postnatal diets in either sex. This was confirmed by lack of change in LV volumes yielding 100 or 120 mmHg systolic pressures (Table 5, n = 5-8). However, a maternal HLA diet resulted in a fall in the initial slope of the systolic PVR in male offspring (Figure 2A, n = 5-8) effect of maternal diet $p = 0.02$). No changes in LV volumes yielding 10 or 20 mmHg diastolic pressures (or diastolic pressure at a LV volume of 250 $\mu\text{l/g}$) were evident in male offspring (Table 5, n = 5-8). However, the initial slope of the diastolic PVR in male hearts was moderately reduced by a maternal HLA diet in male hearts (Figure 2C, n = 5-8, effect of maternal diet $p = 0.005$). In contrast to males, a maternal followed by postnatal HLA diet substantially shifted the diastolic PVR to the left in female offspring (Table 5, n = 5-8). This resulted in significant falls in LV volumes yielding 10 or 20 mmHg diastolic pressure, and a rise in diastolic pressure at a LV volume of 250 $\mu\text{l/g}$ in females (Table 5, n = 5-8, effect of maternal diet $p = 0.03$, effect of postnatal diet $p = 0.03$). An interaction was also evident

between maternal and postnatal diet in terms of diastolic parameters in female rats (Table 5, n = 5-8, effect of interaction $p=0.02$).

3.4.3 Coronary vascular reactivity

Peak coronary flows after 10 or 20 sec occlusions were comparable in male rat hearts, regardless of maternal or postnatal diet (Figure 3, n = 5-7). Debt repayment was substantially less after 20 vs 10 sec occlusion in both male (Figures 3C, 3G, n = 5-7) and female rats (Figures 3D, 3H, n = 5-7). There appeared to be no diet effects on flow repayment in males (Figures 3C, 3G, n = 5-7). Maternal HLA diet increased debt repayment after 10 sec in female rats with interactive effect of maternal and postnatal diet (Figure 3D, n = 5-7, effect of maternal diet $p=0.002$, effect of interaction $p=0.03$). Postnatal HLA diet decreased debt repayment after 20 sec in female offspring from dams fed with LLA diet demonstrating the interactive effect of maternal and postnatal diet (Figure 3H, n = 5-7, effect of postnatal diet $p=0.02$, effect of interaction $p=0.03$).

3.5 Effect of maternal and postnatal diets high in LA on cardiac gene expression in adolescent offspring

We measured the expression of various genes involved in cardiac hypertrophy and cardiac fibrosis to investigate potential molecular responses and remodelling in response to maternal or postnatal diets high in LA. Cardiac expression of myosin heavy chain 6 (*Myh6*) and myosin heavy chain 7 (*Myh7*) genes were not different between any dietary group (Table 6, n = 4-7). Male offspring born to HLA mothers fed with LLA postnatal diet showed elevated expression of natriuretic peptide type A (*Nppa*) compared to male offspring born to LLA mothers (Table 6, n = 4-7), effect of postnatal diet $p=0.04$, effect of interaction $p=0.04$). However, the gene expression of *Nppa* was unchanged across dietary groups in female offspring (Table 6, n = 4-7). There were no changes in expression of natriuretic peptide type B transcript (*Nppb*) in male offspring (Table 6, n = 4-7), however a high LA diet postnatally significantly upregulated the expression of *Nppb* in female offspring from dams fed with LLA diet and

downregulated its expression in offspring from dams fed with a HLA diet demonstrating an interaction between maternal and postnatal diet (Table 6, n = 4-7, effect of maternal diet p=0.04, effect of postnatal diet p=0.04, effect of interaction p=0.007). Cardiac fibrosis is a hallmark of cardiac hypertrophy; therefore, we further investigated the relative expression of genes related to fibrosis. Maternal diet high in LA significantly upregulated expression of collagen type I alpha 1 (*Col1a1*; effect of maternal diet p=0.04) and collagen type III alpha 1 (*Col3a1*; effect of maternal diet p=0.03) transcripts in male offspring (Table 6, n = 4-7), however the expression of these fibrosis related genes remained unchanged in female offspring (Table 6, n = 4-7). Further, we assessed gene expression of ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 1 (*Atp2a1*) which is important in calcium signalling in the heart. The expression of *Atp2a1* was significantly increased in male offspring of mothers fed a HLA diet (effect of maternal diet p=0.03), however expression was unchanged in female offspring (Table 6, n = 4-7). The Notch pathway is also involved in cardiac hypertrophy and regulates fibrotic and regenerative repair in the heart; we therefore measured the expression of the *Notch1* gene. Interestingly, a high LA maternal diet upregulated expression of *Notch1*, while a high LA postnatal diet downregulated its expression in male offspring (Table 6, n = 4-7, effect of maternal diet p=0.01, effect of postnatal diet p=0.03). In female rats, maternal and postnatal diet have an interactive effect in the expression of *Notch1* (Table 6, n = 4-7, effect of interaction p=0.04).

4. Discussion

Global dietary recommendations regarding LA, and its cardiovascular effects, remain debated ⁽³⁶⁾, while consumption is increasing worldwide ⁽¹⁷⁾. Further, *in utero* development is important in determining CVD risk factors ⁽⁷⁾, and there is paucity of data regarding effects of a maternal diet high in LA on offspring cardiac development or of the impacts of a subsequent change in postnatal diet. Recent studies have shown that maternal and postnatal diet supplemented with conjugated linoleic acid (CLA) affect cardiac lipid profile and serum lipoxygenase metabolite of PUFAs in rat offspring with mammary tumours ^(37; 38). Our study demonstrated that a maternal diet high in LA

programmed cardiovascular sex-specific deficits that were not reversed by a low LA diet in the male. For females, the post-weaning low LA diet able to restore the functional changes, in the absence of changes to the gross heart weight.

To our knowledge, this is the first study to elucidate the roles of both maternal and postnatal LA intake on offspring cardiovascular health.

4.1 Plasma lipid profile and leptin changes in female offspring

A key finding of this study is that a maternal diet high in LA significantly decreased both total cholesterol and HDL cholesterol in the plasma of female but not male offspring, regardless of LA amounts in the postnatal diet. In our previous study we reported that a maternal diet high in LA before pregnancy and during gestation decreased maternal plasma total cholesterol, HDL cholesterol and LDL cholesterol ⁽²¹⁾. A systemic review of randomised control trials demonstrated that increasing n-6 FA reduces serum total cholesterol, with little or no difference in HDL or LDL cholesterol ⁽³⁹⁾. One of the proposed mechanisms behind the protective effect of LA in CVD is its total cholesterol and LDL cholesterol lowering properties ⁽⁴⁰⁾, however, in the current study a maternal HLA diet also decreased HDL cholesterol in female offspring, which may limit cardiovascular benefit.

Maternal HLA diet increased the leptin levels in the plasma of both male and female offspring in this study and had an interaction effect in females. These changes occurred in the presence of body weight changes in the female, but an absence in the male. We have previously demonstrated that maternal leptin is reduced in response to an elevated LA diet in the absence of changes to body weight ⁽²¹⁾. Circulating leptin levels have a contradictory role in cardiovascular health, showing both protective and deleterious effect ⁽⁴¹⁾. Further, as changes in *Lepr* expression was associated with functional changes in males only, this suggests that altered cardiac *Lepr* expression does not mediate the changes in cardiac function in this model.

4.2 Diet affects offspring plasma fatty acid composition in a sex specific manner

Maternal and postnatal diets high in LA have significant impacts on offspring plasma fatty acid composition. Despite matching total n-3 PUFA across the diets ⁽⁴²⁾, a

significant effect of diet on total n-3 PUFA, ALA and other longer chain n-3 PUFAs in offspring plasma was evident. ALA and LA share enzymes for metabolism, and high LA will compete with ALA for its conversion, potentially reducing DHA and EPA production from ALA ⁽¹⁸⁾. This may have important effects on physiology, as long chain n-3 PUFAs such as EPA and DHA have anti-inflammatory characteristics and are beneficial for cardiovascular health ⁽⁴³⁾.

We observed effects of maternal and offspring diet on offspring plasma fatty acid composition, with some changes sex specific. Male offspring weaned on a HLA diet had lower levels of total n-3 FA irrespective of maternal diet, however total n-3 FA in female offspring was decreased with both maternal and postnatal diets high in LA. Whereas in both male and female offspring a postnatal diet high in LA decreased ALA and EPA, while DHA levels were reduced in both sexes with maternal and postnatal diets high in LA. High concentrations of ALA, EPA and DHA in serum lipids are associated with lower risk of death from CHD in men and women ⁽⁴⁴⁾. The decreased levels of total n-3 FA along with ALA, EPA and DHA in this study suggest an increased CVD risk profile in offspring exposed to HLA either pre- or postnatally. Both maternal and postnatal diets high in LA considerably upregulated total n-6 PUFA and LA in both male and female offspring, as expected. It is important to note that offspring from HLA dams have increased total n-6 PUFA and LA levels even if weaned onto a LLA diet, suggesting a dominant impact of maternal over postnatal LA intake. A consideration in interpreting this data, is that the data was derived from postnatal exposure which was short, and a longer exposure may induce more severe effects that could interact with aging to exacerbate disease. Notwithstanding this, ~2 week exposure to a HLA diet did alter some aspects of cardiometabolic function.

Concentrations of AA remained unchanged across the dietary groups studied. However, the AA/DHA ratio increased in male and female offspring in response to both maternal and postnatal HLA diets. The relationship between the AA/DHA ratio and coronary events is controversial ^(45; 46). Some studies show a positive correlation between AA:DHA and coronary events, while others find no such association ^(46; 47). Recent analysis suggests that a higher serum AA/EPA is associated with a greater risk of CVD in the general Japanese population, however no such association is observed for the AA:DHA ratio ⁽⁴⁶⁾. Interestingly, the DHA/DPA ratio is specifically decreased by

maternal and postnatal diets high in LA in male offspring only, suggesting that both postnatal and maternal LA intake can reduce conversion of DPA to DHA (in a sexually dimorphic manner).

In addition, there the HLA diet altered n-7 and n-9 FA. There have been a few studies that have suggested that n-7 FA are protective for CVD, and that they reduce the effects of CVD risk factors⁽⁴⁸⁾. It is believed that this occurs via the restoration of normal metabolic function. Further, n-9 PUFA negatively impacts cardiovascular function, due to its ability to promote inflammation and endothelial dysfunction⁽⁴⁹⁾. Both n-7 and n-9 studies have been limited in their scope, with most of the research focused on n-3 and n-6 imbalances and their effects on cardiovascular health. Further studies thus need to be undertaken to explore the biological and prognostic properties of both n-7 and n-9 and how they relate to CVD.

4.3 Sex specific cardiovascular changes

We assessed intrinsic cardiac and coronary functionality in isolated perfused hearts. There was no difference in baseline contractile function among groups. However, a combination of HLA diets in dams and offspring resulted in a shift in the diastolic PVR to lower LV volumes in female offspring, increasing diastolic pressures at LV volumes above ~200 μ l/g. This pattern suggests the change is extracellular in nature potentially due to extracellular rather than myofibrillar elements. Interestingly, in male offspring a maternal HLA diet decreased the initial slopes of systolic and diastolic PVRs, indicating moderately reduced inotropic state coupled with a shift in diastolic compliance largely independent of postnatal diet. This dominant effect of maternal diet on cardiac function is supported by a recent study in mice, which showed that maternal diet-induced obesity programmes cardiac dysfunction in male offspring independently of post weaning diet⁽⁵⁰⁾. Coronary reactivity changes also support a dimorphic impact of a HLA maternal diet, in female offspring.

4.4 Cardiac gene expression changes and potential relevance to function

Maternal and postnatal diet have interactive effect in the heart weight in male offspring. *Nppa* and *Nppb* are biomarkers of cardiac hypertrophy, though they may not be mechanistically involved in cardiac remodelling⁽⁵¹⁾. A maternal HLA diet increased *Nppa* expression in male offspring fed a LLA diet. Conversely, expression of *Nppb* was upregulated by a HLA postnatal diet in female offspring of dams fed a LLA diet, vs downregulated in female offspring of dams fed a HLA diet. The potential relevance of these sexually dimorphic responses to heart function is unclear, though hint at a potential maladaptive response to high LA.

Cardiac fibrosis distorts the architecture of the myocardium and leads to cardiac dysfunction⁽⁵²⁾. *Col1a1* and *Col3a1* expressions are increased in male offspring of dams fed a high LA diet. Collagen is the primary extracellular protein in the heart⁽⁵³⁾, and these increases in collagen I and III transcripts suggest a maternal HLA diet may selectively promote fibrosis in male offspring. A maternal diet high in LA upregulated cardiac expression of *Atp2a1/Serca1* in male offspring independently of postnatal diet, which may be associated with later cardiac dysfunction as the transition from hypertrophy to heart failure is often associated with dysregulation of sarcoplasmic reticulum calcium ATPase⁽⁵⁴⁾. Increased *Atp2a1/Serca1* increases rates of contraction and relaxation, thus is not predicted to contribute to the shift in the systolic PVR slope in males. However, this may reflect an adaptation, as supported by studies of heart failure⁽⁵⁵⁾.

Additionally, a maternal diet high in LA increased expression of *Notch1* in male offspring. Maternal and postnatal diet have interactive effect on *Notch1* expression in female offspring. *Notch1* prevents the uncontrolled hypertrophic growth of cardiomyocytes during cardiac adaptation by inhibiting the transcription of *GATA4*⁽⁵⁶⁾. However, this potentially adaptive response was negated by a postnatal diet high in LA. *Notch* signalling is upregulated following infarction, potentially contributing to remodelling responses, and is also implicated in cardioprotective responses⁽⁵⁷⁾. The current study demonstrated an up regulation of *Notch1* in male offspring, and an interaction affect in females where the maternal diet altered the effects of the postnatal diet. This finding suggests that the maternal HLA diet may contribute to remodelling and stress resistance supports prior studies that suggest male offspring may be more sensitive to nutrient supply during pregnancy^(58; 59). However, it is possible that the

transcriptional modifications observed here are yet to substantially influence myocardial architecture and function, which may require development beyond the 40 day timepoint assessed here. Thus, further investigations should be focused on the long-term health outcomes to offspring born to mothers consuming an elevated LA diet.

A potential limitation in this study is a lack of a control group, which would be consuming standard rat chow. Standard rat chow has 1.3% linoleic acid and 0.3% α -linolenic acid (https://www.specialtyfeeds.com/new/wp-content/uploads/2016/06/meat_free_rm.pdf). The LLA diet used in this study has 1.44% linoleic acid and 0.36% α -linolenic acid. Fat concentration in both of our speciality diets is matched (9% in LLA and HLA vs 4.8% in standard chow). However some researchers consider 11% as the fat content in standard chow e.g. ⁽⁶⁰⁾. Thus there may be an effect due to the elevated fat (assuming 4.8%). However as the aim of this study was to investigate the effects of a maternal diet with low (control) linoleic acid vs high linoleic acid concentrations, and the components of the diet were matched apart from the linoleic acid content, the control group consuming standard chow is unlikely to be different from the LLA group.

5. Conclusion

We have shown that both a maternal and a postnatal diet high in LA influence offspring plasma fatty acids, lipid profiles and cardiac function in a sex specific manner. These direct functional changes are associated with sex specific changes in fibrogenic gene expression in male offspring in response to a HLA maternal diet. Further studies in adult offspring are warranted to explore how maternal and early postnatal intake of LA affect cardiovascular phenotype in adulthood.

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Conflict of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors contributions

Nirajan Shrestha, James Cuffe, Andrew McAinch, John Headrick and Deanne Hryciw designed the study; Nirajan Shrestha, Simone Sleep, Tessa Helman, Olivia Holland and John Headrick collected the data; all authors analyzed data; Nirajan Shrestha, Simone Sleep and Tessa Helman wrote the first draft of the manuscript, all authors revised the manuscript. All the authors read and approved the final manuscript

References

1. Roth GA, Abate D, Abate KH *et al.* (2018) Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* **392**, 1736-1788.
2. Nishida C, Uauy R, Kumanyika S *et al.* (2004) The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public health nutrition* **7**, 245-250.
3. Berenson GS, Srinivasan SR, Bao W *et al.* (1998) Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *The New England journal of medicine* **338**, 1650-1656.
4. Gidding SS, McMahan CA, McGill HC *et al.* (2006) Prediction of coronary artery calcium in young adults using the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) risk score: the CARDIA study. *Arch Intern Med* **166**, 2341-2347.
5. Jaquith BC, Harris MA, Penprase B (2013) Cardiovascular disease risk in children and adolescents. *J Pediatr Nurs* **28**, 258-266.
6. Gademan MGJ, van Eijdsden M, Roseboom TJ *et al.* (2013) Maternal prepregnancy body mass index and their children's blood pressure and resting cardiac autonomic balance at age 5 to 6 years. *Hypertension (Dallas, Tex : 1979)* **62**, 641-647.
7. Roseboom TJ, van der Meulen JH, Osmond C *et al.* (2000) Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45. *Heart* **84**, 595-598.
8. Blackmore HL, Niu Y, Fernandez-Twinn DS *et al.* (2014) Maternal diet-induced obesity programs cardiovascular dysfunction in adult male mouse offspring independent of current body weight. *Endocrinology* **155**, 3970-3980.
9. Barker DJ, Osmond C, Simmonds SJ *et al.* (1993) The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ (Clinical research ed)* **306**, 422-426.
10. Hoffman DJ, Reynolds RM, Hardy DB (2017) Developmental origins of health and disease: current knowledge and potential mechanisms. *Nutr Rev* **75**, 951-970.
11. Barker DJ, Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* **1**, 1077-1081.
12. Wang J, Ma H, Tong C *et al.* (2010) Overnutrition and maternal obesity in sheep pregnancy alter the JNK-IRS-1 signaling cascades and cardiac function in the fetal heart. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **24**, 2066-2076.
13. Xu Y, Williams SJ, O'Brien D *et al.* (2006) Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in adult male offspring. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 1251-1253.
14. Elmes MJ, Haase A, Gardner DS *et al.* (2009) Sex differences in sensitivity to beta-adrenergic agonist isoproterenol in the isolated adult rat heart following prenatal protein restriction. *The British journal of nutrition* **101**, 725-734.
15. Whelan J, Fritsche K (2013) Linoleic acid. *Advances in nutrition (Bethesda, Md)* **4**, 311-312.
16. Innes JK, Calder PC (2018) Omega-6 fatty acids and inflammation. *Prostaglandins, leukotrienes, and essential fatty acids* **132**, 41-48.

17. Naughton SS, Mathai ML, Hryciw DH *et al.* (2016) Linoleic acid and the pathogenesis of obesity. *Prostaglandins & other lipid mediators* **125**, 90-99.
18. Choque B, Catheline D, Rioux V *et al.* (2014) Linoleic acid: between doubts and certainties. *Biochimie* **96**, 14-21.
19. Ramsden CE, Zamora D, Leelarthaepin B *et al.* (2013) Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ (Clinical research ed)* **346**, e8707-e8707.
20. Leren P (1970) The Oslo diet-heart study. Eleven-year report. *Circulation* **42**, 935-942.
21. Shrestha N, Cuffe JSM, Holland OJ *et al.* (2019) Elevated maternal linoleic acid reduces circulating leptin concentrations, cholesterol levels and male fetal survival in a rat model. *The Journal of physiology*.
22. Paradis AN, Gay MS, Zhang L (2014) Binucleation of cardiomyocytes: the transition from a proliferative to a terminally differentiated state. *Drug discovery today* **19**, 602-609.
23. Kreipke RE, Birren SJ (2015) Innervating sympathetic neurons regulate heart size and the timing of cardiomyocyte cell cycle withdrawal. *The Journal of physiology* **593**, 5057-5073.
24. Asif Y, Wlodek ME, Black MJ *et al.* (2018) Sustained cardiac programming by short-term juvenile exercise training in male rats. *The Journal of physiology* **596**, 163-180.
25. Howie GJ, Sloboda DM, Kamal T *et al.* (2009) Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *The Journal of physiology* **587**, 905-915.
26. Andersson C, Vasan RS (2018) Epidemiology of cardiovascular disease in young individuals. *Nature Reviews Cardiology* **15**, 230.
27. Mitsnefes MM (2005) Cardiovascular disease in children with chronic kidney disease. *Advances in chronic kidney disease* **12**, 397-405.
28. Choque B, Catheline D, Delplanque B *et al.* (2015) Dietary linoleic acid requirements in the presence of α -linolenic acid are lower than the historical 2 % of energy intake value, study in rats. *The British journal of nutrition* **113**, 1056-1068.
29. Jandacek RJ (2017) Linoleic Acid: A Nutritional Quandary. *Healthcare (Basel, Switzerland)* **5**.
30. Liu G, Muhlhausler BS, Gibson RA (2014) A method for long term stabilisation of long chain polyunsaturated fatty acids in dried blood spots and its clinical application. *Prostaglandins, leukotrienes, and essential fatty acids* **91**, 251-260.
31. Liu G, Gibson R (2020) A rapid method for the separation of the phospholipids from the neutral lipids in plasma. *Prostaglandins, leukotrienes, and essential fatty acids* **157**, 102096.
32. Kaakinen M, Reichelt ME, Ma Z *et al.* (2017) Cavin-1 deficiency modifies myocardial and coronary function, stretch responses and ischaemic tolerance: roles of NOS over-activity. *Basic research in cardiology* **112**, 24.
33. Linke WA, Popov VI, Pollack GH (1994) Passive and active tension in single cardiac myofibrils. *Biophysical journal* **67**, 782-792.
34. Bustin SA, Benes V, Garson JA *et al.* (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical chemistry* **55**, 611-622.

35. Dickinson H, Moss TJ, Gatford KL *et al.* (2016) A review of fundamental principles for animal models of DOHaD research: an Australian perspective. *Journal of developmental origins of health and disease* **7**, 449-472.
36. Marklund M, Wu JHY, Imamura F *et al.* (2019) Biomarkers of Dietary Omega-6 Fatty Acids and Incident Cardiovascular Disease and Mortality. *Circulation* **139**, 2422-2436.
37. Białek A, Jelińska M, Tokarz A (2015) Influence of maternal diet enrichment with conjugated linoleic acids on lipoxygenase metabolites of polyunsaturated fatty acids in serum of their offspring with 7, 12-dimethylbenz [a] anthracene induced mammary tumors. *Prostaglandins & other lipid mediators* **116**, 10-18.
38. Białek M, Białek A, Czauderna M (2020) Maternal and Early Postnatal Diet Supplemented with Conjugated Linoleic Acid Isomers Affect Lipid Profile in Hearts of Offspring Rats with Mammary Tumors. *Animals* **10**, 464.
39. Hooper L, Al-Khudairy L, Abdelhamid AS *et al.* (2018) Omega-6 fats for the primary and secondary prevention of cardiovascular disease. *The Cochrane database of systematic reviews* **7**, CD011094-CD011094.
40. Harris WS (2008) Linoleic acid and coronary heart disease. *Prostaglandins, leukotrienes, and essential fatty acids* **79**, 169-171.
41. Genoux A, Bastard JP (2020) Effects of leptin and adiponectin on the cardiovascular system. *Ann Biol Clin (Paris)*.
42. Shrestha N, Cuffe JSM, Holland OJ *et al.* (2019) Elevated maternal linoleic acid reduces circulating leptin concentrations, cholesterol levels and male fetal survival in a rat model. *The Journal of physiology* **597**, 3349-3361.
43. Swanson D, Block R, Mousa SA (2012) Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition (Bethesda, Md)* **3**, 1-7.
44. Erkkilä AT, Lehto S, Pyörälä K *et al.* (2003) n-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. *The American journal of clinical nutrition* **78**, 65-71.
45. Nishizaki Y, Shimada K, Tani S *et al.* (2016) Association between the docosahexaenoic acid to arachidonic acid ratio and acute coronary syndrome: a multicenter observational study. *BMC Cardiovasc Disord* **16**, 143-143.
46. Ninomiya T, Nagata M, Hata J *et al.* (2013) Association between ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cardiovascular disease: the Hisayama Study. *Atherosclerosis* **231**, 261-267.
47. Shoji T, Kakiya R, Hayashi T *et al.* (2013) Serum n-3 and n-6 polyunsaturated fatty acid profile as an independent predictor of cardiovascular events in hemodialysis patients. *Am J Kidney Dis* **62**, 568-576.
48. Yang ZH, Pryor M, Noguchi A *et al.* (2019) Dietary Palmitoleic Acid Attenuates Atherosclerosis Progression and Hyperlipidemia in Low-Density Lipoprotein Receptor-Deficient Mice. *Molecular nutrition & food research* **63**, e1900120.
49. Delgado GE, Krämer BK, Lorkowski S *et al.* (2017) Individual omega-9 monounsaturated fatty acids and mortality-The Ludwigshafen Risk and Cardiovascular Health Study. *Journal of clinical lipidology* **11**, 126-135.e125.
50. Loche E, Blackmore HL, Carpenter AA *et al.* (2018) Maternal diet-induced obesity programmes cardiac dysfunction in male mice independently of post-weaning diet. *Cardiovascular research* **114**, 1372-1384.

51. Calamaras TD, Baumgartner RA, Aronovitz MJ *et al.* (2019) Mixed lineage kinase-3 prevents cardiac dysfunction and structural remodeling with pressure overload. *American journal of physiology Heart and circulatory physiology* **316**, H145-h159.
52. Li L, Zhao Q, Kong W (2018) Extracellular matrix remodeling and cardiac fibrosis. *Matrix Biol* **68-69**, 490-506.
53. Xu Y, Williams SJ, O'Brien D *et al.* (2006) Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in adult male offspring. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 1251-1253.
54. Wilkins BJ, Molkentin JD (2004) Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochemical and biophysical research communications* **322**, 1178-1191.
55. Summerfield N, Peters ME, Hercock CA *et al.* (2010) Immunohistochemical evidence for expression of fast-twitch type sarco(endo)plasmic reticulum Ca²⁺ ATPase (SERCA1) in German shepherd dogs with dilated cardiomyopathy myocardium. *J Vet Cardiol* **12**, 17-23.
56. Ninh VK, El Hajj EC, Mouton AJ *et al.* (2018) Chronic Ethanol Administration Prevents Compensatory Cardiac Hypertrophy in Pressure Overload. *Alcohol Clin Exp Res*.
57. Gude N, Sussman M (2012) Notch signaling and cardiac repair. *Journal of molecular and cellular cardiology* **52**, 1226-1232.
58. Kuzawa CW, Adair LS (2003) Lipid profiles in adolescent Filipinos: relation to birth weight and maternal energy status during pregnancy. *The American journal of clinical nutrition* **77**, 960-966.
59. O'Tierney-Ginn P, Presley L, Minium J *et al.* (2014) Sex-specific effects of maternal anthropometrics on body composition at birth. *American journal of obstetrics and gynecology* **211**, 292.e291-299.
60. Buettner R, Parhofer KG, Woenckhaus M *et al.* (2006) Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *Journal of molecular endocrinology* **36**, 485-501.

Figure legends

Figure 1. Effect of maternal and postnatal diet high in linoleic acid on baseline functional parameters for Langendorff perfused hearts. Data were analyzed with two-way ANOVA with maternal and postnatal diet as two factors. Data are presented as mean $\pm$ standard error of the mean (SEM). $n = 5-8$. The sample size (n) represents the number of litters, and one male and one female were used from each litter. Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. LLA: low linoleic acid; HLA: high linoleic acid; LVDP: left ventricular diastolic pressure.

Figure 2. Effect of maternal and postnatal diet high in linoleic acid on offspring systolic PVR and slopes of diastolic PVR. A) Systolic PVR in male offspring. B) Linear slope of the systolic PVR in female offspring. C) Initial slope of the diastolic PVR in male offspring. D) Initial slope of the diastolic PVR in female offspring. E-F) Final slopes of the diastolic PVRs in male and female offspring. Data were analyzed with two-way ANOVA with maternal and postnatal diet as two factors. Data are presented as mean $\pm$ standard error of the mean (SEM). n= 5-8. The sample size (n) represents the number of litters, and one male and one female were used from each litter. LLA: low linoleic acid; HLA: high linoleic acid; PVR: pressure-volume relationship.

Figure 3. Effect of maternal and postnatal diet high in linoleic acid on offspring coronary reactivity after 10 sec and 20 sec occlusions. A-B) Peak coronary flow after 10 sec occlusions in male and female hearts. C) Flow repayment after 10 sec occlusions in male offspring. D) Debt repayment after 10 sec in female rats. E-F) Peak coronary flows

after 20 sec occlusions in male and female rat hearts. G-H) Debt repayment after 20 sec occlusions in male and female hearts. Data were analyzed with two-way ANOVA with maternal and postnatal diet as two factors. Data are presented as mean $\pm$ standard error of the mean (SEM). $n = 5-7$. The sample size (n) represents the number of litters, and one male and one female were used from each litter. Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. LLA: low linoleic acid; HLA: high linoleic acid.

Tables

Table 1: Effect of maternal and postnatal diet high in LA on body weight and organ weight of offspring

Parameters	LLA maternal diet		HLA maternal diet		p-values		
	LLA PN diet	HLA PN diet	LLA PN diet	HLA PN diet	Maternal diet	PN diet	Interaction
Male							
Body weight (gm)	113.3 $\pm$ 6.6	118.2 $\pm$ 5.1	115.8 $\pm$ 5.2	113.6 $\pm$ 5.5	ns	ns	ns
% Heart	0.45 $\pm$ 0.01	0.49 $\pm$ 0.02	0.46 $\pm$ 0.02	0.43 $\pm$ 0.01	ns	ns	ns
% Brain	1.51 $\pm$ 0.08	1.33 $\pm$ 0.06	1.44 $\pm$ 0.04	1.48 $\pm$ 0.07	ns	ns	ns
% Left Kidney	0.42 $\pm$ 0.01	0.42 $\pm$ 0.01	0.41 $\pm$ 0.01	0.40 $\pm$ 0.01	ns	ns	ns
% Right Kidney	0.42 $\pm$ 0.01	0.40 $\pm$ 0.01	0.40 $\pm$ 0.01	0.39 $\pm$ 0.01	ns	ns	ns
Female							
Body weight (gm)	104.5 $\pm$ 2.7 ^a	93.2 $\pm$ 2.9 ^b	99.4 $\pm$ 2.1	104.6 $\pm$ 2.1 ^a	ns	ns	0.02
% Heart	0.46 $\pm$ 0.01	0.52 $\pm$ 0.04	0.48 $\pm$ 0.02	0.47 $\pm$ 0.02	ns	ns	ns
% Brain	1.55 $\pm$ 0.04	1.66 $\pm$ 0.05	1.55 $\pm$ 0.04	1.59 $\pm$ 0.03	ns	ns	ns
% Left Kidney	0.42 $\pm$ 0.01	0.41 $\pm$ 0.01	0.41 $\pm$ 0.01	0.40 $\pm$ 0.01	ns	ns	ns
% Right Kidney	0.40 $\pm$ 0.01	0.42 $\pm$ 0.01	0.40 $\pm$ 0.01	0.41 $\pm$ 0.01	ns	ns	ns

Data are presented as mean $\pm$ standard error of the mean (SEM). Two-way ANOVA was performed for statistical analysis with maternal diet and postnatal diet as two

factors. Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. n= 6-8. The sample size (n) represents the number of litters, and one male and one female were used from each litter. LLA: low linoleic acid; HLA: high linoleic acid; PN: postnatal. Differences across the groups are denoted by different superscript letters, so that a is different than b.

Table 2: Effect of maternal and postnatal diet high in LA on plasma glucose level, lipids and leptin level in offspring

Parameters	LLA maternal diet		HLA maternal diet		p-values		
	LLA PN diet	HLA PN diet	LLA PN diet	HLA PN diet	Mater nal diet	PN diet	Interac tion
Male							
Glucose (mmol/L)	9.88 ± 0.40	10.84 ± 0.49	12.07 ± 0.63	11.68 ± 1.35	ns	ns	ns
Triglycerides (mmol/L)	0.51 ± 0.09	0.41 ± 0.05	0.38 ± 0.03	0.39 ± 0.04	ns	ns	ns
Total cholesterol (mmol/L)	2.50 ± 0.31	2.47 ± 0.03	2.34 ± 0.08	2.13 ± 0.07	ns	ns	ns
HDL-cholesterol (mmol/L)	1.81 ± 0.24	1.56 ± 0.13	1.61 ± 0.06	1.49 ± 0.05	ns	ns	ns
TC/ HDL-C ratio	1.39 ± 0.04	1.47 ± 0.06	1.45 ± 0.01	1.43 ± 0.02	ns	ns	ns
Leptin (ng/ml)	0.44 ± 0.01	0.41 ± 0.07	0.64 ± 0.03	0.60 ± 0.11	0.01	ns	ns
Female							
Glucose (mmol/L)	11.91 ± 1.37	9.99 ± 0.38	10.6 ± 0.27	10.36 ± 0.47	ns	ns	ns
Triglycerides (mmol/L)	0.47 ± 0.03	0.43 ± 0.01	0.42 ± 0.03	0.49 ± 0.03	ns	ns	ns
Total cholesterol (mmol/L)	2.56 ± 0.14	2.63 ± 0.07	2.49 ± 0.07	2.31 ± 0.06	0.01	ns	ns
HDL-cholesterol (mmol/L)	1.79 ± 0.04	1.84 ± 0.05	1.76 ± 0.05	1.65 ± 0.05	0.03	ns	ns
TC/ HDL-C ratio	1.43 ± 0.01	1.43 ± 0.01	1.42 ± 0.02	1.41 ± 0.01	ns	ns	ns
Leptin (ng/ml)	0.65 ± 0.11	0.23 ± 0.05 ^a	0.76 ± 0.09 ^b	0.92 ± 0.11 ^b	0.001	ns	0.01

Data are presented as mean ± standard error of the mean (SEM). Two-way ANOVA was performed for statistical analysis with maternal diet and postnatal diet as two

factors. Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. n= 5-8. The sample size (n) represents the number of litters, and one male and one female were used from each litter. LLA: low linoleic acid; HLA: high linoleic acid; PN: postnatal; HDL: high density lipoprotein; TC: total cholesterol. Differences across the groups are denoted by different superscript letters, so that a is different to b.

Table 3: Effect on maternal and postnatal diet high in LA on plasma fatty acid composition in male offspring

Parameter s	LLA maternal diet		HLA maternal diet		p-values		
	LLA PN diet	HLA PN diet	LLA PN diet	HLA PN diet	Mater nal diet	Postnat al diet	Interac tion
Male							
Total SFA	33.95 ± 0.35	33.36 ± 0.49	32.40 ± 0.31	33.40 ± 0.35	ns	ns	ns
Total trans FA	0.13 ± 0.03	0.13 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	ns	ns	ns
Total MUFA	22.67 ± 3.30 ^a	9.12 ± 0.37 ^b	10.21 ± 0.43 ^b	10.64 ± 1.74 ^b	0.0025	0.0005	0.0002
Total n-3 PUFA	3.85 ± 0.46	3.14 ± 0.06	3.60 ± 0.12	2.60 ± 0.17	ns	0.0007	ns
ALA	0.28 ± 0.03	0.20 ± 0.00	0.33 ± 0.03	0.20 ± 0.00	ns	0.0001	ns
EPA	0.28 ± 0.06	0.10 ± 0.00	0.16 ± 0.02	0.10 ± 0.00	ns	0.0007	ns
DPA	0.30 ± 0.04	0.31 ± 0.02	0.41 ± 0.02	0.34 ± 0.03	0.0200	ns	ns
DHA	3.07 ± 0.44	2.52 ± 0.07	2.70 ± 0.13	1.96 ± 0.15	0.0400	0.0050	ns
Total n-6 PUFA	39.38 ± 3.04 ^a	55.16 ± 0.20 ^b	53.63 ± 0.37 ^b	53.27 ± 1.39 ^b	0.0003	<0.0001	<0.0001
LA	12.27 ± 1.35 ^a	21.70 ± 0.92 ^b	23.33 ± 0.68 ^b	22.86 ± 1.08 ^b	<0.0001	0.0002	<0.0001
AA	25.80 ± 2.24	30.43 ± 0.67	28.21 ± 0.54	28.14 ± 1.97	ns	ns	ns
LA/ALA ratio	44.65 ± 3.93 ^a	103.0 ± 3.26 ^b	76.13 ± 5.60 ^c	99.89 ± 6.72 ^b	0.0100	<0.0001	0.0030
AA/DHA	8.87 ± 0.65	12.22 ± 0.26	10.58 ± 0.42	14.47 ± 0.62	0.0007	<0.0001	ns
DHA/DPA	10.53 ± 0.39	8.18 ± 0.67	6.61 ± 0.57	5.97 ± 0.64	<0.0001	0.0200	ns
14:0	0.40 ± 0.40	0.50 ± 0.50	0.44 ± 0.44	0.51 ± 0.51	ns	0.0157	ns

	0.00	0.04	0.03	0.03			
	0.18 ±	0.27 ±	0.27 ±	0.30 ±			
15:0	0.03	0.02	0.02	0.00	0.0049	0.0049	ns
	19.05 ±	17.07 ±	17.19 ±	17.89 ±			
16:0	0.89 ^a	0.28 ^b	0.16 ^b	0.39	ns	ns	0.0075
	0.27 ±	0.30 ±	0.30 ±	0.30 ±			
17:0	0.02	0.00	0.00	0.00	ns	ns	ns
	13.32 ±	13.71 ±	13.28 ±	13.50 ±			
18:0	0.91	0.37	0.23	0.65	ns	ns	ns
	0.20 ±	0.26 ±	0.30 ±	0.30 ±			
20:0	0.00 ^a	0.03 ^b	0.00 ^b	0.00 ^b	0.0005	0.0224	0.0224
	0.24 ±	0.35 ±	0.30 ±	0.34 ±			
22:0	0.02	0.04	0.00	0.02	ns	0.0093	ns
	0.25 ±	0.29 ±	0.30 ±	0.33 ±			
24:0	0.03	0.02	0.00	0.02	0.0366	ns	ns
	0.067 ±	0.00 ±	0.06 ±	0.08 ±			
t18:1n-9	0.03	0.00	0.02	0.03	ns	ns	ns
	0.07 ±	0.10 ±	0.13 ±	0.06 ±			
t18:1n-7	0.03	0.02	0.02	0.03	ns	ns	0.0469
	1.48 ±	0.56 ±	0.45 ±	0.44 ±			
16:1n-7	0.32 ^a	0.04 ^b	0.03 ^b	0.04 ^b	0.0007	0.0043	0.005
	18.85 ±	7.00 ±	8.16 ±	6.41 ±			
18:1n-9	2.81 ^a	0.33 ^b	0.41 ^b	1.29 ^b	0.003	<0.0001	0.009
	2.02 ±	1.30 ±	1.31 ±	1.13 ±			
18:1n-7	0.23	0.06	0.03	0.16	0.0024	0.0019	ns
	0.27 ±	0.20 ±	0.25 ±	0.23 ±			
20:1n-9	0.02	0.00	0.02	0.02	ns	0.0257	ns
Total n-9	19.10 ±	7.21 ±	8.40 ±	8.57 ±			
FA	2.81 ^a	0.34 ^b	0.42 ^b	1.49 ^b	0.0025	0.0003	0.0002
Total n-7	3.48 ±	1.87 ±	1.77 ±	1.80 ±			
FA	0.52 ^a	0.09 ^b	0.04 ^b	0.36 ^b	0.0043	0.01	0.0081
	0.38 ±	0.62 ±	0.60 ±	0.70 ±			
18:3n-6	0.05	0.02	0.02	0.11	0.0306	0.0127	ns
	0.15 ±	0.30 ±	0.30 ±	0.27 ±			
20:2n-6	0.03 ^a	0.02 ^b	0.00 ^b	0.02 ^b	0.003	0.003	<0.0001
	0.53 ±	0.44 ±	0.44 ±	0.43 ±			
20:3n-6	0.06	0.02	0.02	0.02	ns	ns	ns

Data are presented as mean ± standard error of the mean (SEM). Two-way ANOVA was performed for statistical analysis with maternal diet and postnatal diet as two factors. Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. n= 5-8. The sample size (n) represents the number of litters, and one male and one female were used from each litter. LLA: low

linoleic acid; HLA: high linoleic acid; PN: postnatal; FA: fatty acid; SFA: saturated fatty acid; MUFA: monosaturated fatty acid; PUFA: polyunsaturated fatty acid; ALA: α -linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; AA: arachidonic acid. Data are expressed as total lipid fatty acids as %. Differences across the groups are denoted by different superscript letters.

Table 4: Effect on maternal and postnatal diet high in LA on plasma fatty acid composition in female offspring

	LLA maternal diet		HLA maternal diet		p-values		
Parameters	LLA PN diet	HLA PN diet	LLA PN diet	HLA PN diet	Maternal diet	Postnatal diet	Interaction
Female							
Total SFA	34.27 $\pm$ 0.25	34.54 $\pm$ 0.22	34.03 $\pm$ 0.12	33.89 $\pm$ 0.29	ns	ns	ns
Total trans FA	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.18 $\pm$ 0.05	0.18 $\pm$ 0.05	0.0400	ns	ns
Total MUFA	16.17 $\pm$ 0.74 ^a	8.36 $\pm$ 0.18 ^b	9.13 $\pm$ 0.32 ^b	8.07 $\pm$ 0.30 ^b	<0.0001	<0.0001	<0.0001
Total n-3 PUFA	4.46 $\pm$ 0.14	3.60 $\pm$ 0.11	4.18 $\pm$ 0.11	3.01 $\pm$ 0.07	0.0010	<0.0001	ns
ALA	0.30 $\pm$ 0.04	0.20 $\pm$ 0.00	0.26 $\pm$ 0.02	0.23 $\pm$ 0.03	ns	0.0100	ns
EPA	0.30 $\pm$ 0.04	0.20 $\pm$ 0.00	0.27 $\pm$ 0.02	0.023 $\pm$ 0.03	ns	0.0100	ns
DPA	0.27 $\pm$ 0.02	0.20 $\pm$ 0.00	0.29 $\pm$ 0.02	0.20 $\pm$ 0.00	ns	0.0020	ns
DHA	3.70 $\pm$ 0.16	3.14 $\pm$ 0.09	3.51 $\pm$ 0.12	2.49 $\pm$ 0.08	0.0010	<0.0001	ns
Total n-6 PUFA	45.00 $\pm$ 0.49 ^a	53.42 $\pm$ 0.18 ^{b,c}	52.44 $\pm$ 0.39 ^b	54.81 $\pm$ 0.18 ^c	<0.0001	<0.0001	<0.0001
LA	13.53 $\pm$ 0.96	18.42 $\pm$ 0.41	19.66 $\pm$ 0.48	21.83 $\pm$ 0.63	<0.0001	<0.0001	ns
AA	30.03 $\pm$ 1.28	32.90 $\pm$ 0.29	30.81 $\pm$ 0.48	30.63 $\pm$ 0.59	ns	ns	ns
LA/ALA ratio	46.46 $\pm$ 2.54 ^a	101.6 $\pm$ 4.19 ^b	77.14 $\pm$ 3.97 ^c	97.91 $\pm$ 8.51 ^b	0.0200	<0.0001	0.0060
AA/DHA	8.16 $\pm$ 0.22 ^a	10.48 $\pm$ 0.25 ^b	8.86 $\pm$ 0.27 ^a	12.39 $\pm$ 0.30 ^c	0.0001	<0.0001	0.040

	13.55 ±	13.68 ±	12.66 ±	10.80 ±			
DHA/DPA	0.68	1.49	0.98	0.56	ns	ns	ns
	0.38 ±	0.38 ±	0.39 ±	0.44 ±			
14:0	0.02	0.02	0.03	0.04	ns	ns	ns
	0.20 ±	0.20 ±	0.20 ±	0.20 ±			
15:0	0.00	0.00	0.00	0.00	ns	ns	ns
	15.60 ±	14.86 ±	14.70 ±	15.11 ±			
16:0	0.16 ^a	0.14	0.19 ^b	0.15	ns	ns	0.0034
	0.30 ±	0.30 ±	0.30 ±	0.30 ±			
17:0	0.00	0.00	0.00	0.00	ns	ns	ns
	17.05 ±	18.02 ±	17.63 ±	16.90 ±			
18:0	0.40	0.27	0.29	0.38	ns	ns	0.0237
	0.20 ±	0.20 ±	0.24 ±	0.27 ±			
20:0	0.00	0.00	0.02	0.02	0.0019	ns	ns
	0.30 ±	0.30 ±	0.30 ±	0.30 ±			
22:0	0.00	0.03	0.02	0.00	ns	ns	ns
	0.30 ±	0.30 ±	0.30 ±	0.30 ±			
24:0	0.00	0.00	0.00	0.00	ns	ns	ns
	0.07 ±	0.00 ±	0.03 ±	0.06 ±			
t18:1n-9	0.02	0.00	0.02	0.02	ns	ns	0.0123
	0.05 ±	0.00 ±	0.11 ±	0.08 ±			
t18:1n-7	0.02	0.00	0.01	0.02	0.0005	0.0317	ns
	0.73 ±	0.56 ±	0.49 ±	0.44 ±			
16:1n-7	0.02	0.04	0.04	0.04	<0.0001	0.0077	ns
	13.57 ±	6.50 ±	7.20 ±	6.19 ±			
18:1n-9	0.69 ^a	0.15 ^b	0.28 ^b	0.27 ^b	<0.0001	<0.0001	<0.0001
	1.51 ±	1.08 ±	1.16 ±	1.16 ±			
18:1n-7	0.04 ^a	0.02 ^b	0.03 ^b	0.02 ^b	<0.0001	<0.0001	<0.0001
	0.25 ±	0.20 ±	0.20 ±	0.20 ±			
20:1n-9	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.0345	0.0345	0.0345
Total n-9	13.80 ±	6.66 ±	7.41 ±	6.40 ±			
FA	0.71 ^a	0.14 ^b	0.28 ^b	0.28 ^b	<0.0001	<0.0001	<0.0001
Total n-7	2.25 ±	1.60 ±	1.65 ±	1.60 ±			
FA	0.05 ^a	0.03 ^b	0.05 ^b	0.06 ^b	<0.0001	<0.0001	<0.0001
	0.40 ±	0.68 ±	0.63 ±	0.70 ±			
18:3n-6	0.00 ^a	0.03 ^b	0.03 ^b	0.02 ^b	0.0005	<0.0001	0.0023
	0.10 ±	0.20 ±	0.20 ±	0.27 ±			
20:2n-6	0.00	0.00	0.00	0.02	<0.0001	<0.0001	ns
	0.50 ±	0.44 ±	0.51 ±	0.47 ±			
20:3n-6	0.04	0.02	0.02	0.02	ns	ns	ns

Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. n= 5-8. The sample size (n) represents the number of litters, and one male and one female were used from each litter. LLA: low linoleic acid;

HLA: high linoleic acid; PN: postnatal; FA: fatty acid; SFA: saturated fatty acid; MUFA: monosaturated fatty acid; PUFA: polyunsaturated fatty acid; ALA: α -linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; AA: arachidonic acid. Data are expressed as total lipid fatty acids as %. Differences across the groups are denoted by different superscript letters.

Table 5: Effect of maternal and postnatal high LA diet on offspring left ventricular volume at systolic pressure and end-diastolic pressure.

Parameters	LLA maternal diet		HLA maternal diet		p-values		
	LLA PN diet	HLA PN diet	LLA PN diet	HLA PN diet	Maternal diet	PN diet	Interaction
Male							
LV volume	291 $\pm$	261 $\pm$	314 $\pm$	272 $\pm$			
EDP-10	17	30	15	38	ns	ns	ns
LV volume	340 $\pm$	334 $\pm$	375 $\pm$	329 $\pm$			
EDP-20	16	20	15	37	ns	ns	ns
	6.6 $\pm$	8.3 $\pm$	5.8 $\pm$	8.7 $\pm$			
EDP 250 μ l/g	1.4	2.4	1.1	4.3	ns	ns	ns
LV volume							
SysP-100	46 $\pm$ 13	74 $\pm$ 27	87 $\pm$ 19	60 $\pm$ 17	ns	ns	ns
LV volume	120 $\pm$	113 $\pm$	129 $\pm$	122 $\pm$			
SysP-120	27	23	18	29	ns	ns	ns
Female							
LV volume	298 $\pm$	320 $\pm$	328 $\pm$	238 $\pm$			
EDP-10	10	25 ^a	13 ^a	24 ^b	ns	ns	0.008
LV volume	354 $\pm$	351 $\pm$	378 $\pm$	282 $\pm$			
EDP-20	10 ^a	67	13 ^a	28 ^b	ns	0.009	0.01
	4.9 $\pm$	4.8 $\pm$	4.3 $\pm$	17.4 $\pm$			
EDP 250 μ l/g	1.5 ^a	1.2 ^a	0.9 ^a	4.8 ^b	0.03	0.02	0.02
LV volume							
SysP-100	32 $\pm$ 5	26 $\pm$ 7	29 $\pm$ 3	47 $\pm$ 14	ns	ns	ns
LV volume	123 $\pm$						
SysP-120	36	74 $\pm$ 22	79 $\pm$ 18	92 $\pm$ 19	ns	ns	ns

Table 6. Effect on maternal and postnatal diet high in LA on the expression of genes involved in leptin signaling, cardiac hypertrophy, fibrosis, calcium signaling and Notch pathway in offspring

<i>Lepr</i>	2.30 ± 0.87.	1.60 ± 0.45	2.27 ± 0.73	1.80 ± 0.94	ns.		
ns	ns						
<i>Genes related to cardiac hypertrophy</i>							
	0.52 ±	3.21 ±	1.44	1.87			
<i>Myh6</i>	0.20	1.36	±0.39	±0.87	ns	ns	ns
	0.29 ±	0.98 ±	0.93 ±	1.00 ±			
<i>Myh7</i>	0.12	0.40	0.29	0.52	ns	ns	ns
	4.41 ±	3.24 ±	4.64 ±	0.75 ±			
<i>Nppa</i>	2.41	0.99	1.91	0.13	ns	ns	ns
	0.93 ±	6.40 ±	1.79 ±	0.89 ±			
<i>Nppb</i>	0.38 ^a	1.89 ^b	0.55 ^a	0.15 ^a	0.04	0.04	0.007
<i>Genes related to cardiac fibrosis</i>							
	2.59 ±	1.95 ±	3.07 ±	1.35 ±			
<i>Col1a1</i>	0.91	0.67	0.89	0.29	ns	ns	ns
	1.74 ±	3.58 ±	2.93 ±	3.39 ±			
<i>Col3a1</i>	0.75	1.68	1.12	1.15	ns	ns	ns
<i>Genes related to calcium signaling and Notch pathway</i>							
	0.65 ±	2.00 ±	4.79 ±	2.37 ±			
<i>Atp2a1</i>	0.22	0.56	1.58	0.80	ns	ns	ns
	0.52 ±	1.62 ±	4.22 ±	1.26 ±			
<i>Notch1</i>	0.22 ^a	0.48	1.28 ^b	0.32	ns	ns	0.04

Data were analyzed with two-way ANOVA with maternal and postnatal diet as two factors. Data are presented as mean ± standard error of the mean (SEM). Mean values with different superscript letters are significantly different with Tukey's *post hoc* multiple comparison test. n= 4-7. The sample size (n) represents the number of litters, and one male and one female were used from each litter. LLA: low linoleic acid; HLA: high linoleic acid; MYH: myosin heavy chain 6; NPPA: natriuretic peptide type A; NPPB: natriuretic peptide type B; COL1A1: collagen type I alpha 1; COL3A1: collagen type III alpha 1; ATP2A1: ATPase sarcoplasmic/endoplasmic reticulum Ca²⁺ transporting 1. Differences across the groups are denoted by different superscript letters.