Maternal supplementation with conjugated linoleic acid in the setting of diet-induced obesity normalises the inflammatory phenotype in mothers and reverses metabolic dysfunction and impaired insulin sensitivity in offspring

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Abstract

Maternal consumption of a high-fat diet significantly impacts the fetal environment and predisposes offspring to obesity and metabolic dysfunction during adulthood. We examined the effects of a high-fat diet during pregnancy and lactation on metabolic and inflammatory profiles and whether maternal supplementation with the anti-inflammatory lipid conjugated linoleic acid (CLA) could have beneficial effects on mothers and offspring. Sprague–Dawley rats were fed a control (CD; 10% kcal from fat), CLA (CLA; 10% kcal from fat, 1% total fat as CLA), high-fat (HF; 45% kcal from fat) or high fat with CLA (HFCLA; 45% kcal from fat, 1% total fat as CLA) diet ad libitum 10 days prior to and throughout gestation and lactation. Dams and offspring were culled at either late gestation (fetal day 20, F20) or early postweaning (postnatal day 24, P24). CLA, HF and HFCLA dams were heavier than CD throughout gestation. Plasma concentrations of proinflammatory cytokines interleukin-1β and tumour necrosis factor-α were elevated in HF dams, with restoration in HFCLA dams. Male and female fetuses from HF dams were smaller at F20 but displayed catch-up growth and impaired insulin sensitivity at P24, which was reversed in HFCLA offspring. HFCLA dams at P24 were protected from impaired insulin sensitivity as compared to HF dams. Maternal CLA supplementation normalised inflammation associated with consumption of a high-fat diet and reversed associated programming of metabolic dysfunction in offspring. This demonstrates that there are critical windows of developmental plasticity in which the effects of an adverse early-life environment can be reversed by maternal dietary interventions. © 2015 Elsevier Inc. All rights reserved.

Keywords: Conjugated linoleic acid; Developmental programming; Inflammation; Impaired insulin sensitivity; Maternal obesity

1. Introduction

Maternal obesity is becoming an increasingly prevalent health issue for both mother and child. Obesity is strongly associated with pregnancy-related complications including gestational diabetes, pre-eclampsia, congenital abnormalities and fetal death [1]. Additionally, there is a large amount of research demonstrating that the early-life environment increases offspring susceptibility to obesity and related noncommunicable diseases (NCDs) during adulthood [2]. This concept, known as the developmental origins of health and disease hypothesis, describes the process whereby the fetus may undergo programmed alterations in organ structure and/or function arising as a consequence of a suboptimal in utero environment [3]. However, the mechanisms linking maternal obesity to metabolic dysfunction in offspring still remain poorly defined.

It has become clear that obesity is associated with metainflammation, a state of chronic subclinical inflammation characterised by increased circulating cytokines, and innate immune cell infiltration of insulin-sensitive peripheral tissues [4]. Similarly, insulin resistance is strongly associated with metainflammation and has been shown to be mediated by proinflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNFα) [5,6]. In the setting of maternal obesity, this inflammatory state extends to the placenta, which has negative implications for placental function and fetal development [7,8].

Lifestyle changes such as weight loss by dietary management and incorporation of exercise are often recommended during pregnancy; however, these are difficult to implement in humans. In animal models, pharmaceutical treatments that improve maternal metabolic and inflammatory status have beneficial effects on mother and offspring, but long-term safety and efficacy have not been verified [9,10]. Therefore, nutritional-based strategies pose promising avenues...
for the intervention of programmed adult offspring disease. Indeed, health benefits associated with consumption of anti-inflammatory omega-3 fatty acids in the context of obesity and inflammatory disease have previously been reported [11,12].

Conjugated linoleic acid (CLA) refers to a mixture of positional and geometrical isomers of linoleic acid (18:2n-6) and is naturally present in meat and dairy products from grass rather than grain-fed cattle [13]. Although up to 28 isomers have been identified, the cis-9,trans-11 (c9,t11) and trans-10,cis-12 (t10,c12) isomers are the most naturally occurring and bioactive isomers attributed to beneficial health effects, and a majority of CLA supplements primarily consist of both isomers [14]. The c9,t11 isomer is linked to anti-inflammatory and immunomodulatory effects in conditions such as inflammatory bowel disease and type 2 diabetes [15,16]. c9,t11-CLA binds and activates peroxisome proliferator-activated receptor PPARγ [17] and modulates nuclear factor κ-light-chain enhancer of activated B cells (NF-κB) activation [18], contributing to decreased pro-inflammatory cytokine production [19,20]. The t10,c12 isomer is responsible for modulation of body composition and reductions in fat mass [21]; however, some adverse effects including hyperinsulinemia and fatty liver have also been reported [22].

Despite reported anti-inflammatory, anti-adiposity and anti-diabetic effects of CLA, the impact of CLA supplementation in the context of pregnancy disorders and developmental programming of offspring metabolic dysfunction induced with high-fat diet has not been investigated. Therefore, in the present study, we investigated the effect of obesity induced with high-fat diet on inflammatory status during pregnancy and whether maternal supplementation with CLA can ameliorate maternal and offspring indices of inflammation, adiposity and metabolic function.

2. Materials and methods

2.1. Animal model

The following procedures were approved by the Animal Ethics Committee at the University of Auckland (Approval R1006). Virgin Sprague–Dawley rats were housed at 22°C with a 12: h light:12: h dark cycle. Animals were randomly assigned to one of four diets ad libitum, 10 days prior to and throughout gestation and lactation. We utilised our previously established model of maternal high-fat feeding during pregnancy and lactation, where we have previously shown that placental function is altered and offspring develop metabolic dysfunction independent of postnatal fat diet [23,24]. The assigned dietary groups were as follows (n=12/group; Research Diets, New Jersey, USA; Table 1 and Supplementary Table 1): (1) standard purified control diet (CD; 10% kcal from fat); (2) standard purified CD diet with addition of CLA (CLA; 10% kcal from fat, 13 total fat as CLA); (3) high-fat diet (HF; 43% kcal from fat); or (4) HF diet with addition of CLA (HFLA; 45% kcal from fat, 13 total fat as CLA). The CLA supplemented comprised 50% c9,t11-CLA and 50% t10,c12-CLA (Stean Lipid Nutrition, New Jersey, USA). Female rats (110±5 days) were time-mated using an estrous cycle monitor (EC-40; Fine Science Tools, San Francisco, USA). Day 1 of pregnancy was determined by detection of spermatozoa following vaginal lavage, after which dams were individually housed. A subcohort of pregnant dams (n=5–6/group) were killed at fetal day 20 (F20) to term is 22) by decapitation following isoflurane anaesthesia. Remaining dams continued to term (n=5–6/group). At the time of birth, all pups were weighed, sexed, and nose–anus length was measured. On postnatal day 2, litter size was randomly adjusted to 8 pups (4 males and 4 females) to ensure standardised nutrition until weaning (postnatal day 22). Unused pups were killed by decapitation. All dams and offspring were weighed every second day until weaning. Dams at the time of weaning and offspring at postnatal day 24 (P24) were killed by decapitation following intraperitoneal (ip) anaesthesia with pentobarbital (60 mg/kg; ip). Plasma samples were collected and profiled as detailed below. Plasma was collected in nonfasting animals at F20 to avoid adverse effects on the dam and fetus but was collected in the fasting state for both dams and offspring at P24.

2.2. Tissue and blood collection

Maternal tissues were dissected, weighed, snap frozen and stored at −80°C until analysis. Retropertitoneal adipose tissue (Rpad) from P24 male and female offspring was dissected and weighed. Trunk blood was collected in heparinised vacutainers (Becton Dickinson, Franklin Lakes, USA) and plasma was stored at −20°C until analysis.

2.3. Plasma analysis

Maternal plasma was analysed for insulin, leptin (Crystal Chem, Chicago, USA), IL-1β and TNFα (Quantikine ELISA; R&D Systems, Minneapolis, USA) by commercial rat-specific ELISAs according to manufacturer’s instructions. Maternal plasma was also analysed for aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), total cholesterol (TC), triglycerides (TAGs), lactate dehydrogenase (LDH), uric acid and glucose by Hitachi 902 autoanalyzer (Hitachi High Technologies Corporation, Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOME-IR) was calculated as follows: [fasting glucose (mmol/L)/fasting insulin (mU/L)]2.25 [25].

2.4. Gene expression analysis

RpadT RNA was extracted using the RNeasy Lipid Tissue Mini Kit according to manufacturer’s instructions (QIAGEN, Venlo, Netherlands) and liver RNA was extracted using TRI Reagent (Sigma-Aldrich, St. Louis, USA). mRNA was reverser transcribed using a high-capacity cDNA archive kit (Applied Biosystems, Warrington, UK). RT-qPCR analysis was performed on the ABI 7900HT Fast RT-qPCR System using Sequence Detection System 2.4 software to quantify mRNA expression of genes involved in lipid metabolism, glucose/insulin signalling and inflammation using TaqMan Fast Advanced Master Mix and predesigned TaqMan probes (Applied Biosystems, Warrington, UK; Supplementary Table 2). To control for variability between samples, the relative amounts of the genes were normalised to peptidylprolyl isomerase A and hypoxanthine phosphoribosyltransferase 1 expression. The comparative Ct method (2−ΔΔCt) was used to analyse data [26].

2.5. Statistical analysis

Data were graphed using Prism 6 software (GraphPad Software Inc., La Jolla, USA) and statistical analysis was performed using SigmaPlot 12.0 (Systat Software Inc., San Jose, USA). Growth and cumulative caloric intake curves were analysed by repeated-measures ANOVA. All other data were analysed by two-way factorial ANOVA, with maternal HF diet and maternal CLA supplementation as factors. Holm–Sidak post hoc tests where indicated for multiple comparisons testing between groups were performed. Differences between groups were considered significant at P<0.05. All data are presented as mean±SEM unless otherwise stated.

3. Results

3.1. Maternal weights and intakes

Animals consuming HF and HFLA diets had significant increases in body weight compared to CD animals during the pregestational feeding period (Fig. 1a), with significantly higher cumulative caloric intake in HF and HFLA animals compared to CD and CLA animals (Fig. 1b). Throughout pregnancy and lactation, CLA, HF and HFLA animals were significantly heavier compared to CD animals (Fig. 1c), with no significant differences in cumulative caloric intake between groups during pregnancy (Fig. 1d). Despite the overall significant differences in body weights throughout pregnancy, there were no significant differences in absolute maternal body weight at F20 (Table 2). At F20, there was a maternal HF diet effect on relative liver weights, which was significantly reduced in HF dams compared to CD and CLA dams and reduced in HFLA dams compared to CLA dams. As expected, a maternal HF diet significantly increased relative RpadT weights.

Table 1

<table>
<thead>
<tr>
<th>Macronutrient composition of diets.</th>
<th>CD (% kcal)</th>
<th>CLA (% kcal)</th>
<th>HF (% kcal)</th>
<th>HFLA (% kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>70</td>
<td>70</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
<td>10</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Lard</td>
<td>4.44</td>
<td>4.34</td>
<td>39.44</td>
<td>38.99</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>5.56</td>
<td>5.56</td>
<td>5.56</td>
<td>5.56</td>
</tr>
<tr>
<td>CLA (cis 18:2 fatty acids)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Total (% kcal)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Energy density (kcal/g)</td>
<td>3.8</td>
<td>3.8</td>
<td>4.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>
3.2. Maternal (F20) metabolic profile

Maternal metabolic data are presented in Table 2. Maternal CLA supplementation resulted in significant overall decreases in plasma AST, HDL and LDH. Following post hoc analysis, plasma HDL and LDH concentrations were lower in CLA groups compared to CD groups. Plasma TAG and leptin concentrations were increased overall with CLA supplementation with post hoc analysis showing an increase in TAG in CLA versus CD and HF groups. There was an overall effect of maternal HF diet on reduction of TC concentrations, with post hoc analysis showing a reduction in TC in HFCLA versus CLA. There was a significant interaction between maternal HF diet and CLA supplementation on TC/HDL ratio.

Table 2: Maternal body/organ weights and plasma profile at F20.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CD</th>
<th>CLA</th>
<th>HF</th>
<th>HFCLA</th>
<th>P values</th>
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<tr>
<td></td>
<td>Effect of maternal high fat</td>
<td>Effect of maternal CLA</td>
<td>Interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Liver (g/wt)</td>
<td>4.12±0.01</td>
<td>4.33±0.09</td>
<td>3.79±0.07</td>
<td>3.85±0.1</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>105.9±11</td>
<td>75.75±6</td>
<td>142.47±41</td>
<td>94.66±17</td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>0.15±0.03</td>
<td>0.14±0.03</td>
<td>0.21±0.03</td>
<td>0.09±0.03</td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.93±0.11</td>
<td>0.58±0.11*</td>
<td>1.00±0.11</td>
<td>0.82±0.12</td>
<td></td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>0.16±0.04</td>
<td>0.29±0.06</td>
<td>0.26±0.07</td>
<td>0.12±0.03</td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>2.15±0.14</td>
<td>2.4±0.14</td>
<td>1.89±0.14</td>
<td>1.93±0.16</td>
<td></td>
</tr>
<tr>
<td>TAG (mmol/L)</td>
<td>2.29±0.33</td>
<td>4.35±0.48</td>
<td>2.29±0.54</td>
<td>2.53±0.38</td>
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<tr>
<td>LDH (U/L)</td>
<td>2.44±0.48</td>
<td>4.02±0.48**</td>
<td>2.17±0.48</td>
<td>3.01±0.53</td>
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</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>30.42±3.2</td>
<td>28.4±1.3</td>
<td>37.82±4.9</td>
<td>27.41±2.1</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>8.64±1.68</td>
<td>12.37±1.68</td>
<td>10.33±1.68</td>
<td>14.00±1.84</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.88±0.19</td>
<td>6.76±0.19</td>
<td>8.01±0.84</td>
<td>7.48±0.27</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.19±0.53</td>
<td>2.95±0.58</td>
<td>2.59±0.53</td>
<td>1.48±0.58</td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean±S.E.M., n=5–6/group.
* P<.05 versus all other groups.
** P<.01 versus all other groups.
+ P<.05 versus CD.
- P<.05 versus CLA.
+ P<.05 versus HF.
plasma LDL concentrations with a reduction in LDL in the HFCLA versus HF groups but no difference between CD and CLA groups. A significant interaction was also present for LDL/HDL ratios with a CLA-induced increase in the LDL/HDL ratio in CLA versus CD groups but a decrease in the ratio in HFCLA versus HF groups. An effect was also observed in the TC/HDL ratio with an overall effect on increasing ratios with CLA supplementation; post hoc analysis revealed a significant effect of CLA in increasing the TC/HDL ratio in the CLA group versus all other groups. There were no overall effects of maternal HF diet or CLA supplementation on maternal plasma uric acid, glucose or insulin concentrations.

3.3. Maternal (F20) inflammatory profile

There were significant effects of a maternal HF diet and maternal CLA on circulating inflammatory cytokines IL-1β and TNFα in F20 dams (Fig. 2a and b), with a significant interaction between these factors in TNFα concentrations. These cytokines were significantly elevated in HF dams compared to all other groups.

3.4. Maternal (F20) adipose tissue gene expression

There were no significant changes in IL-1β and TNFα gene expression (data not shown). Expression of glucose transporter GLUT4, the main insulin-regulated glucose transporter in adipose tissue, was significantly decreased in HF groups (Supplementary Fig. 1a). Monocyte chemoattractant protein 1 (MCP-1) was significantly increased in HF and HFCLA groups (Supplementary Fig. 1c). However, there were no significant differences in PPARγ, a major regulator of adipogenesis (data not shown).

3.5. Maternal (F20) hepatic gene expression

Hepatic gene expression of IL-1β was significantly decreased by an HF diet, with post hoc analyses revealing lower expression in HF and HFCLA animals than CLA animals (Fig. 3a). No significant differences were observed in TNFα (data not shown). GLUT2, a facilitated glucose transporter in the liver, was significantly reduced in HF-fed groups (Fig. 3b). In response to CLA, CD36, an integral membrane protein involved in fatty acid transport, was significantly decreased (Fig. 3c). There was a significant decrease in fatty acid synthase (FASN), a key lipogenic marker in HF and HFCLA groups (Supplementary Fig. 1c). However, there were no significant differences in PPARα, a major regulator of adipogenesis.

3.6. Fetal weights (F20)

There was a significant effect of maternal HF diet on fetal body weight in both males and females, as well as a significant interaction between maternal HF and maternal CLA (Fig. 4a and b). Male fetuses from HF mothers had significantly lower body weights compared to CD, CLA and HFCLA mothers. Female fetuses from HF mothers had significantly lower body weights than fetuses from CD and CLA mothers.

3.7. Weanling weights and plasma profile

Male and female weanlings from P24 HF mothers displayed significant catch-up growth in the preweaning period, with significantly higher body weights in HF weanlings compared to CD, CLA and HFCLA weanlings (Fig. 5a and b). At P24, a maternal HF diet resulted in significantly increased insulin concentrations in HF and HFCLA groups compared to CD and CLA groups in males and greater in the HF group compared to CD and CLA groups in females.

Sex-specific differences in IL-1β and TNFα were observed. In males, there was a significant effect of maternal HF diet and an interaction between maternal HF and CLA supplementation in IL-1β concentrations, with post hoc analyses showing increased IL-1β in HF versus CD. There was no significant effect of maternal diet or CLA on TNF concentrations across male groups. However, in females, there were no differences in IL-1β concentrations between dietary groups. However, there was a significant decrease in TNFα in response to a maternal HF diet, with post hoc analyses showing a significant reduction in TNFα in response to the CLA weanling, with post hoc analyses showing increased TNFα in CLA and HFCLA weanlings compared to CD weanlings.

There were no significant differences in male and female glucose concentrations. However, in males, there was a significant effect of maternal CLA supplementation on plasma insulin concentrations. Post hoc analyses showed a significant increase in insulin concentrations in HFCLA weanlings compared to HF weanlings. In females, there was a significant interaction between a maternal HF diet and CLA supplementation in IL-1β concentrations, with post hoc analyses showing increased IL-1β in HF versus CD. There was no significant effect of maternal diet or CLA on TNF concentrations across male groups. However, in females, there were no differences in IL-1β concentrations between dietary groups.

There was no significant difference in leptin concentrations across male groups. In females, there were no differences in leptin concentrations between dietary groups. However, in females, there was a trend towards an increase in plasma leptin concentrations in the HF group versus all other groups but this did not reach statistical significance.

Fig. 2. Maternal inflammatory profile. At F20, a subcohort of dams (n=5–6/group) were culled, and plasma was analysed for (a) IL-1β and (b) TNFα. Data expressed as mean±S.E.M., where *P<0.05 versus all other groups.
3.8. Maternal (P24) body weights, plasma glucose and insulin homeostasis

In P24 dams (2 days postweaning), HF animals were significantly heavier than CD (Fig. 6a). There were significant effects of maternal HF diet and a significant interaction between maternal HF diet and CLA supplementation on plasma glucose. HF animals had significantly higher glucose compared to CD animals, with HFCLA significantly lower than HF animals (Fig. 6b). Maternal CLA supplementation had a significant effect on plasma insulin concentrations, with post hoc analyses revealing elevated insulin in HF groups compared to CLA and HFCLA groups (Fig. 6c). HOMA-IR values did not reach statistical significance; however, strong trends were observed for overall effects of maternal CLA supplementation ($P= .07$) and an interaction between

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Fig. 3. Maternal hepatic gene expression at F20. Liver mRNA expression of (a) IL-1β, (b) GLUT2, (c) CD36, (d) FASN and (e) DGAT1 determined by RT-qPCR ($n=5–6/group$). Data expressed as mean±S.E.M., where $^aP<.05$ versus HF and HFCLA and $^bP<.05$ versus CD and CLA.

Fig. 4. Fetal weights at F20. Dams were culled, and male (a) and female (b) fetuses were excised and weighed ($n=5–6$ litters/group). Data expressed as mean±S.E.M., where $^*P<.05$ versus all other groups and $^#P<.05$ versus CD and CLA.

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\[3.8.\text{Maternal (P24) body weights, plasma glucose and insulin homeostasis}

In P24 dams (2 days postweaning), HF animals were significantly heavier than CD (Fig. 6a). There were significant effects of maternal HF diet and a significant interaction between maternal HF diet and CLA supplementation on plasma glucose. HF animals had significantly higher glucose compared to CD animals, with HFCLA significantly lower than HF animals (Fig. 6b). Maternal CLA supplementation had a significant effect on plasma insulin concentrations, with post hoc analyses revealing elevated insulin in HF groups compared to CLA and HFCLA groups (Fig. 6c). HOMA-IR values did not reach statistical significance; however, strong trends were observed for overall effects of maternal CLA supplementation ($P=.07$) and an interaction between
maternal diet and CLA (P=.065) (Fig. 6d). However, post hoc analyses indicated that the HF group had significantly higher HOMA-IR compared to CD and HFCLA groups.

4. Discussion

It is now well recognised that unbalanced maternal nutrition can programme adult-onset NCDs [2]. Offspring from obese and undernourished mothers display remarkably similar phenotypes; this U-shaped relationship underscores the importance of balanced and adequate maternal nutrition as well as potential common programming mechanisms [27,28]. In humans, maternal obesity prior to or during pregnancy is strongly associated with an increased prevalence of obesity and features of metabolic syndrome in offspring, with onset evident in childhood [29]. These programmed events were formerly considered to represent a permanent alteration in developmental trajectory. However, emerging animal work suggests that targeted interventions during critical periods of developmental plasticity can potentially reverse programmed effects [30–33]. The present study investigated the effectiveness of maternal CLA supplementation in a validated model of maternal HF-diet-induced obesity [23], where offspring develop obesity and related metabolic dysfunction in adult life. The present study provides novel evidence that CLA reverses maternal HF-diet-related metaflammation and prevents programming of adverse offspring metabolic outcomes.

CLA has the capability to regulate gene expression as a potent ligand and activator of the PPAR group of nuclear receptors that

![Fig. 5. Preweaning growth curves and weanling HOMA-IR values.](image)

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of maternal high fat</td>
<td>Effect of maternal CLA</td>
</tr>
<tr>
<td>Males</td>
<td>CD</td>
</tr>
<tr>
<td>RpAT (%BW)</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>24.32±1.93</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>8.25±2.52</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.35±0.49</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.44±0.15</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>0.68±0.34</td>
</tr>
</tbody>
</table>

Females

| RpAT (%BW) | 0.11±0.05 | 0.12±0.05 | 0.33±0.05 | 0.19±0.05 | P=.01 | NS | NS | NS |
| IL-1β (pg/ml) | 28.08±3.98 | 29.86±3.56 | 29.26±3.56 | 28.19±3.98 | NS | NS | NS | NS |
| TNFα (pg/ml) | 14.85±1.47 | 10.17±1.32 | 8.64±1.32 | 7.97±1.48 | P=.01 | NS | NS | NS |
| Glucose (mmol/L) | 5.55±0.27 | 5.58±0.27 | 5.69±0.27 | 5.31±0.27 | NS | NS | NS | NS |
| Insulin (ng/ml) | 0.51±0.26 | 0.54±0.23 | 1.30±0.23 | 0.29±0.23 | P=.05 | NS | NS | NS |
| Leptin (ng/ml) | 0.56±0.19 | 0.59±0.17 | 0.87±0.17 | 0.46±0.17 | NS | NS | NS | NS |

Data expressed as mean±S.E.M.; n=5–6/group.

* P<.05 versus all other groups.

† P<.05 versus CD.

‡ P<.05 versus CD and CLA.

§ P<.05 versus HF.
regulate the expression of genes involved in adipogenesis, inflammation and lipid metabolism [17,20]. Although alterations in gene expression of adipose tissue PPARγ and hepatic PPARα were modest due to dependence on posttranslational modifications, further protein work is required to elucidate the impact of these results. The trend in increased relative RpAT mass in CLA and HFCLA groups compared to their respective isocaloric matched controls is likely due to the adipogenic promoting activity of CLA through activation of PPARγ. In humans, contrasting effects of CLA on inflammation have been reported. In healthy normal-weight volunteers, daily consumption of CLA enriched butter (1020±167 mg/day of CLA) for 8 weeks resulted in increased IL-10 (anti-inflammatory) and reduced NF-κB, TNFα, IL-2 and IL-8 in the serum [34]. However, in an 8-week double-blinded crossover study, CLA fortified yoghurt (2.7 g/day) had no effect on glycemic control, lipid profile, body composition or plasma concentration of TNFα, IL-6 or adiponectin in overweight hyperlipidemic men [35]. However, human studies widely vary in design, notably in the isomeric composition of CLA administered. In mice, c9,t11-CLA reduced adipose tissue inflammation (reduced TNFα, NF-κB and macrophage marker CD68), which resulted in increased insulin sensitivity [15]. The present study demonstrated that an obesogenic diet resulted in elevated circulating IL-1β and TNFα in late gestation dams, which was mitigated by CLA supplementation. We observed the highest percentage of RpAT in HFCLA dams; however, plasma IL-1β and TNFα concentrations were comparable to those of leaner control offspring. Therefore, CLA appears to be responsible for resolving plasma metainflammation in HFCLA dams in late gestation, independent of effects on RpAT mass and body weight.

Obesity during pregnancy contributes to a substantially increased risk of gestational diabetes [36], a condition associated with increased risk of macrosomia, hyperinsulinemia and impaired glucose control in offspring [37]. Additionally, it has been shown that even modest fasting hyperglycemia in mothers is associated with negative outcomes including increased neonatal adiposity and cord blood C-reactive protein [38]. Studies in nonpregnant states have shown that increased inflammation can predict later development of insulin resistance [39]. CLA has been identified as a promoter of insulin sensitivity in vitro and in animal models [15]; however, human clinical trial data remain inconclusive and thus use of CLA as a dietary supplement in humans is controversial. Riserus et al. demonstrated that supplementation with c9,t11 [40] and t10,c12 [41] isomers induced isomer-specific insulin resistance in obese men. Conversely, studies in overweight men have shown no changes to insulin sensitivity [35,42]. However, administration of a mixed isomer CLA supplement in young, healthy sedentary individuals improved insulin sensitivity [43]. Individual variance in response to CLA, participant metabolic health status and the form/dose of supplement may be responsible for these differences. Indeed, CLA’s effect on adipose tissue gene expression has been found to vary depending on a polymorphism in PPARγ2 in humans [44]. As seen in nonpregnant obesity, a maternal HF diet resulted in down-regulation of GLUT4 (adipose tissue) and GLUT2 (liver) gene expression similarly in HF and HFCLA groups. However, GLUT4 and GLUT2 translocation from the cell nucleus to cell membrane is critical for insulin-stimulated glucose uptake by cells and is heavily reliant on posttranslational modifications, similar to PPARγ. The observed differences in plasma glucose and insulin in F20 dams are inline with plasma metainflammation and trend towards decline in HFCLA dams compared to HF dams. However, plasma was collected in a nonfasting state at F20 to avoid confounding effects on fetuses, and therefore, it is unclear if CLA had significant effects on glucose and insulin homeostasis during gestation. Nevertheless, the potential insulin sensitising effects of CLA as an activator of PPARγ cannot be completely neglected and that this in combination with resolved metainflammation contributed to improved outcomes in HFCLA offspring.

CLA supplementation to an HF diet had a beneficial effect on LDL. However, divergent effects of CLA were observed in other blood lipid indices, with differences depending on the fat percentage of the maternal diet. HDL was reduced and LDL/HDL ratio, TC/TCHDL ratio and TAG were increased in the CLA group versus CD, with only modest changes in comparison in the HFCLA group versus CD. Kloss et al.
demonstrated that CLA supplementation had more beneficial effects on blood lipids when combined with a saturated fat diet rather than an unsaturated fat diet [45]. Therefore, the divergent effects of CLA may be explained by differences in the amount of saturated fat in the diets, with the HF and HFCLA diets having a larger quantity of lard than the CD and CLA diets. There were no detected alterations in hepatic SREBP-1c expression, indicating normal fatty acid biosynthesis in the liver. Adipose tissue and hepatic gene expression of FASN were similarly declined in HF and HFCLA groups, likely due to the increased fat supply from the diet decreasing the requirement for de novo lipogenesis. In insulin resistance, TAGs tend to be elevated due to increased production and impaired removal. Surprisingly, CLA supplementation decreased TAG in CLA and HFCLA groups despite declined hepatic DGAT1 expression in HF and HFCLA groups. However, hepatic CD36 expression was reduced in CLA and HFCLA groups, which may decrease hepatic fatty acid uptake and reduce excessive hepatic storage of TAG. These changes are important to note as the developing fetus is exposed to the maternal fatty acid profile in maternal circulation during in utero development. Therefore, although the effects of CLA on lipid metabolism appear mixed, there were no detrimental effects on insulin homeostasis of the mother or developing fetus.

Leptin is an adipokine that regulates satiety, metabolism and energy expenditure. Obesity is characterised by a state of leptin resistance and contributes to development of the metabolic syndrome [46]. Previous studies involving CLA supplementation tend to report decreased leptin concentrations; however, these are often associated with reductions in fat mass [47]. It has been demonstrated that it is the r10,c12 isomer that is responsible for decreasing leptin secretion from 3T3-L1 adipocytes [48,49]. Conversely, the r10,c12 isomer has also been shown to increase leptin gene expression in cultures from human stromal vascular cells from preadipocytes, with no effect from the c9,c11 isomer [50]. In accordance with nonpregnant states [51], circulating concentrations of leptin were representative of body weight in pregnant dams. During pregnancy, hormonal shifts occur to support a leptin-resistant state, which is further amplified by consumption of an HF diet [52]. Similarly, lactation is a leptin-resistant state [53], and maternal CLA supplementation during lactation has been shown to increase leptin concentrations, as seen in a pregnant state in the present study [54]. Catalano et al. demonstrated that, in obese mothers with elevated leptin, fetuses developed in utero insulin resistance, were heavier at birth and had elevated cord leptin compared to those from lean mothers [55]. Additionally, cord leptin concentrations were inline with fetal insulin resistance, implicating maternal obesity and related sequelae in the programming of insulin resistance. We have previously shown that offspring of obese mothers are hypoinsulinemic and hypoleptinemic at birth but display marked catch-up growth reflected in increased fat mass and hyperleptinemia in the prepubertal period [56]. In the present study, weaning plasma leptin correlated with both adiposity and insulin resistance and strongly trended towards significance in male offspring (P = .08). The elevated circulating leptin concentrations in HF offspring may indicate a predisposition to increased appetite, which may likely perpetuate throughout life.

Zhang et al. demonstrated that maternal CLA administration during lactation may mediate hypermethylination-induced programming in offspring, representing a potential programming mechanism of CLA [54]. Previous studies have reported that maternal CLA during gestation and lactation can have growth and development promoting effects in offspring [57], but findings remain inconsistent [58] and appear to be dependent on offspring sex and the dose/duration of exposure. The present study does not support increased growth of offspring from CLA and HFCLA mothers, evidenced by similar weights in both male and female offspring in utero and throughout weaning compared to CD offspring. However, male and female fetuses from HF dams were significantly smaller in utero compared to CD fetuses, inline with commonly reported low birth weight in clinical and experimental models of maternal obesity. In males, this effect was significantly normalised in HFCLA fetuses and a similar trend was observed in females. Whilst the literature tends to support an association between maternal obesity and macrosomic offspring [59], our group and others have previously shown in rats that maternal obesity is also associated with reduced fetal weights [60]. This is due in part, to placental insufficiency [24] and reduced placental blood flow to the fetus [61,62]. Although low birth weight remains a crude indicator of development, growth-restricted offspring tend to exhibit postnatal catch-up growth, later-life obesity and insulin resistance. This is likely due to fetal adaptations to the in utero environment, including altered organogenesis and metabolic function. Indeed, in the present study, male and female offspring from HF dams were heavier throughout the preweaning period and had poorer postweaning insulin sensitivity. This early sign of impaired glucose metabolism may represent a predisposition to insulin resistance. As our group has previously reported, programmed effects in offspring often display sex specificity [63]. In males, IL-1β was significantly elevated in HF offspring and partially normalised in HFCLA offspring. Surprisingly, in females, TNFα was significantly lower in HF and HFCLA compared to controls. However, male and female offspring from HFCLA dams were protected from programmed insulin resistance suggesting that CLA reversed the suboptimal in utero environment complicated by maternal obesity.

In the immediate postweaning period, P24 HF dams were significantly heavier than CD dams and displayed significant disturbances in glucose and insulin homeostasis. HF dams had significantly elevated insulin and glucose, which was completely rescued in dams on the HFCLA diet. These disturbances were likely not detected in F20 dams as plasma was collected in the fed state. Human studies have shown that inflammation can be predictive of insulin resistance and can occur prior to manifestation a phenotype [64]. The current study demonstrates that the insulin sensitivity in P24 dams mirrored the inflammatory profile in F20 dams. Therefore, CLA supplementation to dams consuming an obesogenic diet appears to confer immediate benefits during pregnancy demonstrated by reversal of inflammation and with postgestation prevention of insulin resistance. Women who have had gestational diabetes have a significantly increased risk of developing type 2 diabetes postpregnancy [65]. Therefore, CLA may represent a tool to prevent the development of type 2 diabetes in high-risk women. However, further research is needed to evaluate the optimal dose, isomeric composition and safety of long-term CLA usage in human populations.

In conclusion, we have shown that a maternal HF diet induces metainflammation in dams, which was reversed with CLA supplementation. Offspring from HF dams displayed intrauterine growth restriction, followed by catch-up growth and insulin resistance in the weaning period, all of which were reversed in offspring from dams supplemented with CLA. Since obesity was similarly induced in HF and HFCLA dams, we suggest that inflammatory mediators are, in part, contributing to the programmed effects observed in the offspring. Furthermore, the current study provides evidence that an adverse early-life environment can predispose offspring to later-life metabolic dysfunction and that there are critical windows of developmental plasticity in which the effects of an adverse early-life environment can be ameliorated. CLA during pregnancy is potentially beneficial for maternal long-term risk of subsequent type 2 diabetes development as well as reversal of programmed diminished insulin sensitivity after exposure to an HF diet in utero. CLA may represent a novel therapeutic agent for the prevention of maternal HF-diet-induced developmental programming of obesity and metabolic dysfunction. However, although our study demonstrated clear benefits of CLA supplementation to an HF diet on postweaning outcomes in offspring and dams, there are some potential adverse metabolic effects during gestation,
which are not fully understood, and as a result, the benefits of dietary
CLA intake during pregnancy merit further investigation.

Author Contributions
C.M.R. and M.H.V. designed research; S.A.S., X.D.Z., C.G. and C.M.R.
conducted research; S.A.S. and C.M.R. wrote manuscript and C.M.R.,
C.G. and M.H.V. critically evaluated the paper.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.

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