Maternal conjugated linoleic acid supplementation reverses high-fat diet-induced skeletal muscle atrophy and inflammation in adult male rat offspring


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Maternal conjugated linoleic acid supplementation reverses high-fat diet-induced skeletal muscle atrophy and inflammation in adult male rat offspring. Am J Physiol Regul Integr Comp Physiol 310: R432–R439, 2016. First published December 2, 2015; doi:10.1152/ajpregu.00351.2015.—A high-saturated-fat diet (HFD) during pregnancy and lactation leads to metabolic disorders in offspring concomitant with increased adiposity and a proinflammatory phenotype in later life. During the fetal period, the impact of maternal diet on skeletal muscle development is poorly described, despite this tissue exerting a major influence on life-long metabolic health. This study investigated the effect of a maternal HFD on skeletal muscle anabolic, catabolic, and inflammatory signaling in adult rat offspring. Furthermore, the actions of maternal-supplemented conjugated linoleic acid (CLA) on these measures of muscle phenotype were investigated. A purified control diet (CD; 10% kcal fat), a CD supplemented with CLA (CLA; 10% kcal fat), 1% total fat as CLA), a high-fat (HFD; 45% kcal fat from lard), or a HFD supplemented with CLA (HFCLA; 45% kcal fat from lard, 1% total fat as CLA) was fed ad libitum to female Sprague-Dawley rats for 10 days before mating and throughout gestation and lactation. Male offspring received a standard chow diet from weaning, and the gastrocnemius was collected for analysis at day 150. Offspring from HF and HFCLA mothers displayed lower muscular protein content accompanied by elevated monocyte chemotactic protein-1, IL-6, and IL-1β concentrations. Phosphorylation of NF-κBp65 (Ser536) and expression of the catabolic E3 ligase muscle ring finger 1 (MuRF1) were increased in HF offspring, an effect reversed by maternal CLA supplementation. The present study demonstrates the importance of early life interventions to ameliorate the negative effects of poor maternal diet on offspring skeletal muscle development.

ELEVATED PREPREGNANCY WEIGHT and excessive weight gain during pregnancy are most frequently linked to dietary habits that increase total caloric intake (43), a common feature of a diet rich in saturated fat (28). This excessive maternal nutrient intake has numerous adverse effects on both maternal and offspring health, independent of the postnatal diet (23). The detrimental impact to the health of offspring can be lifelong, particularly when combined with additional stressors into adulthood (23, 44). Although the actions of excessive dietary intake on infant health are complex, of particular interest is the role of saturated fat, which has been shown to elicit both inflammatory and metabolic dysfunction in both mother and offspring (37, 47).

The intrauterine environment is a major determinant of muscle mass due to muscle fiber number being set at the time of birth (8, 36). A reduction in muscle mass arising due to fewer muscle fibers persists even after compensatory or “catch-up” growth, since catch-up growth favors fat deposition over muscle development (12). Altered skeletal muscle function may be an important contributing factor to programmed obesity risk in offspring because skeletal muscle accounts for 40–50% of body mass and functions as a major site of metabolic activity, particularly glucose metabolism and fatty acid utilization, in addition to enabling locomotion and posture (32). Although the exact mechanisms are not well defined, there is evidence to demonstrate tissue-specific programming of skeletal muscle, including suppression of fetal myoblast cell-cycle activity (6, 8, 26, 49). Because maternal obesity can lead to placentral insufficiency and reduced fetal growth (16, 38), skeletal muscle development can be preferentially suppressed, leading to reduced muscle mass at birth (8), predisposing offspring to future risk for sarcopenia, obesity, and insulin resistance.

A maternal obesogenic environment has been shown to increase offspring adipogenesis and decrease myogenesis during fetal growth (49). Reduced activation of the mammalian target of rapamycin (mTOR) translation initiation signaling pathway, a key regulator of protein synthesis, was demonstrated to contribute to impaired skeletal muscle development in adult offspring of high-fat (HF)-fed mothers (39). However, maintenance of skeletal muscle mass is a net result of independent signaling pathways governing both protein synthesis and protein degradation (15). As well as suppressing muscle anabolism, chronic low-grade inflammation is a key stimulant of muscle catabolism through activation of the transcriptional regulator nuclear factor-κB (NF-κB). NF-κB is integral to the induction of a wide spectrum of proinflammatory cytokines and E3 ligases involved in the ubiquitin proteasome protein degradation pathway (9). Activation of this pathway has been reported in inflammatory-related states of muscle atrophy and more recently in obesity-associated diseases (3). While there is some evidence regarding the impact of maternal HF diets on offspring skeletal muscle impairment (5, 6, 26, 39), the role of intramuscular inflammation and associated catabolic pathways has not been comprehensively assessed.

The period of developmental plasticity during early life offers the ideal opportunity for novel interventions to mitigate
the negative actions of a poor maternal environment on offspring health (17). The anti-inflammatory actions of conjugated linoleic acid (CLA) supplementation have been widely demonstrated (24, 40). CLA is an n-6 polyunsaturated fatty acid composed of a heterogeneous mixture of geometric isomers of linoleic acid and is found in high quantities in the lipid fraction of meat and dairy products (4). Of the two most abundant CLA isomers, trans-10, cis-12 (t10c12) has been shown to modulate fat mass in rodent models, while cis-9, trans-11 (c9t11) exhibits anti-inflammatory effects by binding to peroxisome proliferator-activated receptors and modulating NF-κB activation (7, 30, 35). We have recently shown that CLA supplementation reduces inflammation in HF-fed mothers and improves fasting insulin concentrations in 24-day-old offspring (47). Furthermore, maternal CLA supplementation improves vascular function and lipid profiles and normalizes pubertal onset in the offspring of HF-fed mothers (20, 41). In the present study, our model of maternal HF feeding (23) was used to examine the potential therapeutic benefits of supplementation with a 50:50 mix of c9t11-t10c12 CLA on offspring skeletal muscle growth, inflammation, and markers of atrophy activation. We hypothesize that the anti-inflammatory effects of CLA may ameliorate metabolic inflammation in HF-fed mothers thereby preventing the adverse developmental programming effects on offspring skeletal muscle development.

METHODS

Animal experiments. Animal protocols for this study have been described in detail elsewhere (41, 47). In brief, Sprague-Dawley rats were housed in the same room under standard conditions (20–22°C, 55–65% humidity) and subjected to a 12:12-h light-dark cycle (7:00 A.M.–7:00 P.M.). Virgin female rats were randomly assigned to receive either 1) a purified control diet (CD, n = 6, 10% kcal from fat, 20% kcal from protein, 70% kcal from carbohydrate; Research Diets); 2) a purified high-fat diet (HF, n = 6, 45% kcal from fat, 20% kcal from protein, 35% kcal from carbohydrate); or 3) a CLA diet [CLA; n = 6, the purified CD supplemented with 1% total fat as CLA (Stepan Lipid Nutrition)] or 4) a purified high-fat-CLA diet [HFCLA; n = 5, the high-fat diet (HFCD supplemented with 1% total fat as CLA)] for 10 days before mating and throughout pregnancy and lactation. Diet composition has previously been detailed elsewhere (47). Female rats (90 days of age, n = 24) were time-mated using an estrous cycle monitor (EC40; Fine Science Tools, Foster City, CA). Food and water were provided ad libitum. Following birth, litters were randomly adjusted to eight pups (4 male, 4 female) to ensure standardized nutrition until weaning (day 21). Pups not allocated to litters were killed by decapitation. A minimum of five litters were assessed per maternal dietary group. Postweaning, male siblings were housed two per cage and maintained ad libitum on a standard chow diet (CD; Harlan Teklan, Oxon, UK) until day 150. Body weight and food intake were measured every 3rd day. Because of the confounds caused by the female estrous cycle, females were not examined in the present study. All animal work was approved by the Animal Ethics Committee (Approval R1069) of The University of Auckland.

Tissue collection and preparation. Before tissue collection at day 150, male offspring were fasted overnight. Animals were anesthetized with an intraperitoneal injection of pentobarbitone sodium (60 mg/kg) followed by decapitation. The gastrocnemius muscle was excised from the right hind limb and immediately snap-frozen in liquid nitrogen. All tissues were weighed and stored at −80°C until processed.

Triglyceride quantification. Extraction of lipids for triglyceride quantification was achieved by using a modified version of the Folch et al. method (14). Briefly, skeletal muscle was homogenized in 0.8 ml of 2:1 chloroform-methanol and rotated for 90 min at 4°C. Samples were mixed with 0.4 ml of methanol for 30 s and centrifuged at 2,400 g for 10 min, and 0.5 ml of 0.04% CaCl2 was added to the supernatant, which was then centrifuged at 2,400 g for 20 min. The upper phase was discarded, and the interphase was washed three times with 500 μl of 1:16:15 chloroform-methanol-water. The final wash was removed, and samples were mixed with 0.05 ml of methanol.

Protein extraction and quantification. Frozen skeletal muscle was weighed and homogenized in ice-cold modified RIPA lysis buffer (no. 20–188; Millipore, Billerica, MA) supplemented with a commercially available protease and phosphatase inhibitor cocktail (Halt Protease and Phosphatase Inhibitor Cocktail, no. 78442; Thermo Scientific, Waltham, MA) using a bead mill homogenizer (OMNI Ruptor; Omni International, Kennesaw, GA). Samples were rotated for 1 h at 4°C, and membrane fractions and cell debris were removed by centrifugation at 1,500 g for 10 min at 4°C. The total soluble protein concentration was determined using a BCA-protein kit according to the manufacturer’s protocol (Pierce BCA Protein Assay, kit no. 23225; Thermo Fisher Scientific, Rockford, IL).

Immunoblotting. Sample aliquots containing 20 μg of protein were suspended in 1× Laemmli buffer [10% glycerol, 2% SDS, 0.25% bromophenol blue, 400 mM dithiothreitol, 0.5 M Tris-HCl (pH 6.8)], boiled at 100°C for 5 min, and subjected to separation by SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) using the semidry Trans-Blot Turbo Transfer System (Bio-Rad). Membranes were incubated with blocking buffer [5% bovine serum albumin (BSA)-Tris-buffered saline-0.1% Tween 20 (TBST)] for 2 h at room temperature (RT), followed by overnight incubation at 4°C with primary antibodies in blocking buffer under gentle agitation. Samples were probed for phosphorylated (p)-Akt (Ser473), p-Akt (Thr308), p-Akt (Pan), p-eukaryotic initiation factor 4E-binding protein 1 (p4E-BP1; Thr377/S404), 4E-BP1, p70 S6 kinase (p70S6K), p-p70S6K (Thr389), p-p70S6K (Thr421/Ser422), p-eukaryotic initiation factor 4E-binding protein 1 (p4E-BP1; Thr377/S404), 4E-BP1, p70 S6 kinase (p70S6K), p-p70S6K (Thr389), p-p70S6K (Thr421/Ser422), S6 ribosomal protein (pS6), p-pS6 (Ser235/236), p-pS6 (Ser240/244), p-mTOR, p-mTOR (Ser2448), p-4E-BP1 (Ser65), p-4E-BP1 (Ser70), p-4E-BP1 (Ser246), p-4E-BP1 (Ser37/46), p-p70 S6 kinase (S6K1), p-S6K1 (Thr389), a mouse-specific β-actin (1:5,000 dilution), mouse-specific GAPDH (1:1,000 dilution), or mouse-specific tubulin (1:1,000 dilution). Abundance of total protein is presented normalized to GAPDH and constitutive loading controls that were loaded on every individual gel. Equal protein loading was determined by stripping and reprobing membranes for glyceraldehyde 3-phosphate dehydrogenase (GAPDH, ab9485, 1:10,000; Abcam). Abundance of total protein is presented normalized to GAPDH and phosphoproteins as phospho-to-total protein ratios.

RNA extraction and cDNA synthesis. RNA was isolated from the gastrocnemius muscle (15–25 mg) using the PureLink RNA Mini Kit according to the manufacturer’s protocol (Life Technologies). Total RNA concentration was measured using the NanoDrop 1000 Spectrophotometer (NanoDrop Spectrophotometer; Thermo Scientific). Total RNA (500 ng) was synthesized into single-stranded cDNA using the high-capacity cDNA Archive Kit (Applied Biosystems, Warrington, UK).

Gene expression analysis. Predesigned probes and Taqman Universal Mastermix were purchased from Applied Biosystems (ABI,
Auckland, New Zealand). mRNA expression was quantified using real-time PCR (Q-PCR) on an ABI 7900HT Fast RT-qPCR system using Sequence Detection System 2.4 software (Perkin Elmer Applied Biosystems). As a control for between-sample variability, mRNA expression was normalized to the geometric mean of three reference genes: Gapdh, hypoxanthine phosphoribosyltransferase (Hprr), and β-actin (Actb). The ΔΔCt method was used to calculate the relative expression of the gene of interest. Results are reported relative to the CD group (29).

Muscle cytokine analysis. Frozen skeletal muscle was homogenized in phosphate-buffered saline supplemented with a commercially available protease and phosphatase inhibitor cocktail (Halt Protease and Phosphatase Inhibitor Cocktail, no. 78442; Thermo Scientific). The total soluble protein concentration was determined using the BCA-protein kit as per the manufacturer’s protocol (Pierce BCA Protein Assay Kit, no. 23225; Thermo Fisher Scientific). Homogenates were analyzed for interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, IL-10, and monocyte chemotactic protein-1 (MCP-1)/CCR2 using commercial rat-specific ELISA kits according to the manufacturer’s instructions (Quantikine ELISA; R&D Systems, Minneapolis, MN). Lower limits of detection for these inflammatory markers and the coefficient of variation for these measurements were as follows: 21 pg/ml (4.5–8.8%) IL-6, 5 pg/ml (3.9–8.8%) IL-1β, 5 pg/ml (2.1–9.7%) TNF-α, and 10 pg/ml (3.0–9.5%) IL-10. Results were normalized to the amount of total soluble protein (pg/µg protein).

Statistical analysis. Statistical analysis was performed using SigmaPlot for Windows version 12.1 (Systat Software, San Jose, CA). Statistical significance was determined using two-way factorial ANOVA with maternal diet and maternal CLA as factors. Data were checked for normality of the residual error distribution and log10 transformed where appropriate. Pairwise comparisons were determined using the Holm-Sidak post hoc method. Graphs were generated using Prism software (GraphPad Software, La Jolla, CA). Data are shown as means ± SE. A probability of P < 0.05 was accepted as statistically significant.

RESULTS

Adult male offspring weights and muscle composition. There was a main effect for increased body weight for adult males born to dams fed the HF diet (P < 0.05). Furthermore, there was a main effect for decreased body weight in offspring born to dams supplemented with CLA (P < 0.05). However, post hoc analysis between individual groups failed to achieve statistical significance (Table 1). Absolute and relative (% of body wt) gastrocnemius muscle weight was not different between groups (Table 1). There was an overall decrease in the total protein content of the gastrocnemius muscle (P < 0.05) in response to maternal HF exposure, irrespective of CLA supplementation (Table 1). An overall decrease in intramuscular triglyceride content was observed with maternal CLA supplementation although post hoc analysis revealed no difference when comparing CD vs. CLA and HF vs. HFCLA when analyzed independently (Table 1).

Offspring skeletal muscle inflammation. There was an increase in intramuscular concentrations of MCP-1 with maternal HF diet (HF diet effect; P < 0.001). Both HF and HFCLA offspring had elevated concentrations of MCP-1 (CD 4.60 ± 0.51, CLA 4.42 ± 0.41, HF 6.60 ± 0.64, HFCLA 7.50 ± 0.87 pg/µg) (P < 0.05; Fig. 1A). There was a trend (P < 0.10) for an overall increase in mRNA expression of CD68 (HF diet effect, P = 0.08; Fig. 1B) in HF offspring groups. Although mRNA expression of the macrophage mannose receptor-1 did not change between groups (Fig. 1C), there was a main effect of maternal HF consumption to increase mRNA expression of Cdl1c. However, post hoc analysis revealed a significant increase in Cdl1c expression only in HFCLA offspring (P < 0.05; Fig. 1D).

NF-κB p65 (Ser536) phosphorylation was increased in HF offspring (main effect, P < 0.05) (Fig. 2A). Post hoc analysis indicated increased phosphorylation of NF-κB in HF offspring (P < 0.05, CD vs. HF) that was normalized in HFCLA offspring. There was also a main effect of HF for increased phosphorylation of IκBα (P < 0.05, Fig. 2B). Total IκBα and total NF-κB abundance were not influenced by maternal HF or CLA feeding.

Offspring from HFD mothers displayed increased muscle concentrations of IL-6 (CD 29.15 ± 0.18, CLA 5.23 ± 0.57, HF 58.51 ± 8.11, and HFCLA 53.24 ± 3.63 pg/µg) and IL-1ß (CD 5.23 ± 0.57, CLA 6.05 ± 0.94, HF 11.90 ± 1.78, and HFCLA 10.92 ± 1.60 pg/µg) (main effect, P < 0.05 in both cases) and reduced concentrations of IL-10 (CD 7.21 ± 0.83, CLA 8.13 ± 1.15, HF 5.52 ± 1.08, and HFCLA 6.34 ± 1.40 pg/µg) (main effect, P < 0.001). Post hoc analysis revealed that there were increased skeletal muscle concentrations of IL-6 (P < 0.001) and IL-1ß (P < 0.05) in HF and HFCLA offspring (Fig. 3A). In contrast, IL-10 was significantly reduced in both HF and HFCLA offspring (P < 0.001 in both cases). TNF-α was not detected.

mRNA expression showed a significant HF × CLA interaction for the IL-6 receptor (Il6r) and IL-10 receptor (Il10r) (P < 0.05 in both cases). Both the Il6r and the Il10r were increased in HF offspring (P < 0.05 in both cases) with no effect of maternal CLA supplementation (Fig. 3B). mRNA expression of Il10r did not differ between groups. However, there was a main effect of maternal CLA supplementation on increased gene expression of Tnfr1 (CLA effect, P < 0.05).

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<th>Table 1. Effect of maternal HF diet ± CLA supplementation on skeletal muscle parameters in adult male rat offspring</th>
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Values represent means ± SE, n, no. of litters/group. CD, control diet; CLA, CD supplemented with conjugated linoleic acid; HF, high fat; HFCLA, HF diet supplemented with CLA; NS, not significant. Data were obtained from offspring of CD-, CLA-, HF-, or HFCLA-fed dams at 150 days of age. *P < 0.05 for effect of fat.
Post hoc analysis showed that Tnfr1 expression was elevated in the CLA offspring compared with CD ($P < 0.05$).

**Ubiquitin proteosome system in offspring skeletal muscle.** There was a HF × CLA interaction for atrogin-1 ($Fbox32$), muscle ring finger 1 (MuRF1), and forkhead box O3a ($FOXO3a$) expression ($P < 0.05$ in all cases). MuRF1 ($Trim63$) gene expression was increased in HF offspring ($P < 0.01$). Maternal CLA supplementation normalized the expression of $Trim63$ ($P < 0.05$, HF vs. HFCLA) (Fig. 4A). In contrast, whereas the maternal HFD alone had no effect on offspring $Fbox32$ expression, there was decreased expression in HFCLA offspring ($P < 0.01$) (Fig. 4B). There was no difference between groups in $FOXO1$ expression (Fig. 4C). However, Foxo3 gene expression was increased in the CLA ($P < 0.05$) and HF ($P < 0.05$) groups (Fig. 4D).

**mTOR signaling pathway in offspring skeletal muscle.** No difference was observed in the phosphorylation of Akt at Thr$^{389}$ (data not shown) or Ser$^{473}$ (data not shown). There was a reduction in HF vs. CD offspring in p70S6K phosphorylation at Thr$^{421}$/Ser$^{424}$ ($P < 0.05$; Fig. 5A), whereas phosphorylation at Thr$^{389}$ of p70S6K did not differ (Fig. 5B); however, there was a trend toward a reduction in HF vs. CD offspring in rpS6 phosphorylation at Ser$^{240}$/244 ($P = 0.056$; Fig. 5C). Furthermore, there was reduced phosphorylation of rpS6 at Ser$^{235}$/236 in HF offspring (main effect, $P < 0.05$; Fig. 5D). Phosphorylation of mTOR (Ser$^{2448}$) did not differ between groups (data not shown). Similarly, phosphorylation at Thr$^{37}$/46 of the 4E-BP1 was not different across groups (data not shown).

**DISCUSSION**

Skeletal muscle plays important roles in locomotion and metabolic health. The period of in utero skeletal muscle development is critical, since there is no postnatal increase in the number of skeletal muscle fibers (8). Moreover, alterations to the perinatal environment that impact skeletal muscle growth establish long-lasting changes in metabolic profiles in offspring (6, 26). However, studies that assess the impact of maternal diet on skeletal muscle growth mainly focus on metabolic dysregulation, to date there are few studies evaluating the...
The influence of muscle inflammation and catabolism in offspring exposed to a maternal HFD. Therefore, this study aimed to investigate the impact of a maternal HF diet on inflammatory signaling responses and catabolic pathways in skeletal muscle of offspring and the potential beneficial effect of maternal CLA supplementation. It was demonstrated that a maternal HFD adversely impacts on protein content, intramuscular inflammation, and markers of protein synthesis/degradation in the gastrocnemius muscle from adult male offspring. Furthermore, maternal CLA supplementation partially reversed aspects of the negative programming responses of maternal HF feeding affecting offspring skeletal muscle.

We have previously reported that the CLA, HF, and HFCLA dams were heavier compared with CD dams during gestation and lactation (20, 47). Furthermore, male weanling HF offspring were significantly heavier and had reduced insulin sensitivity (47). Interestingly, male offspring of HF-fed dams have suppressed lean body mass and a reduction in myogenic signaling (39, 49), suggesting a preferential partitioning of energy storage toward fat mass, at the expense of muscle mass (12). Although the current study failed to demonstrate a reduction in the mass of the gastrocnemius muscle, total muscle protein content was reduced following maternal HFD. Furthermore, maternal HFD did not significantly elevate offspring intramuscular triglyceride content, yet CLA supplementation, regardless of diet, suppressed intramuscular triglyceride content. The t10c12 CLA isomer is known to promote its antiadipogenic effects by activating lipolytic pathways (7). Therefore, maternal CLA supplementation may have a beneficial impact on the skeletal muscle composition of adult offspring.

Although the relationship between obesity and chronic low-grade inflammation is well established (10, 33), the effects of excessive maternal weight gain and diet on the inflammatory state of the offspring are not fully understood. There is increasing evidence to support the infiltration of immune cells and activation of inflammatory signaling in the placenta (11, 52), which may be caused by the remodeling of adipose tissue during pregnancy, particularly during late gestation (51). We have previously demonstrated increased adipose expression of macrophage activation markers in pregnant dams fed a HFD (42). The present study found a significant increase in concent-

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**Fig. 3.** Skeletal muscle inflammatory profile in adult male offspring. A: protein concentration of interleukin (IL)-1β, IL-6, and IL-10 analyzed by Quantikine ELISA (R&D Biosystems). B: skeletal muscle mRNA expression of tumor necrosis factor receptor (TNFR) 1, IL1R, IL6R, and IL10R. *P < 0.05 for effect of fat and #P < 0.05 for effect of CLA; n = 5–6 litters/group. Data are expressed ±SE relative to the CD group.

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**Fig. 4.** Skeletal muscle atrophy markers in adult male offspring. Fold induction of atrophy-related genes in muscle of 150-day old adult male offspring. A: Trim63 (MuRF1); B: Fbxo32 (atrogin-1); C: FOXO1; D: FOXO3. *P < 0.05 for effect of fat and #P < 0.05 for effect of CLA; n = 5–6 litters/group. Data are expressed ±SE relative to the CD group.
trations of MCP-1 protein, a chemokine involved in the recruitment of macrophages, in HF and HFCLA offspring muscle. Moreover, there was a trend for CD68, a marker of macrophage activation, to be increased in HF but not HFCLA offspring. Further analysis revealed that, while expression of the anti-inflammatory macrophage marker MCR-1 was not different between groups, the inflammatory macrophage marker CD11c was upregulated in HFCLA offspring. Taken together, these results suggest that a maternal HF diet increases levels of inflammatory macrophages in male offspring skeletal muscle.

Given the increased skeletal muscle immune cell infiltration, activation of the NF-κB pathway, a major regulator of inflammatory responses, was examined. NF-κB acts as a transcriptional regulator of cytokines, interferons, and chemokines (2). In its inactive state, NF-κB is sequestered in the cytoplasm by the inhibitor of NF-κB (IκB). In response to cellular stress, IκB is phosphorylated and rapidly degraded, promoting phosphorylation and subsequent release of NF-κB (2). NF-κB p65 (Ser536) phosphorylation is a key event necessary for the nuclear translocation and transcriptional activation of NF-κB, which is often elevated in skeletal muscle in response to a HFD and obesity (3). Offspring from HFD dams exhibited increased NF-κB phosphorylation, an effect that was reversed by maternal CLA supplementation. Previous studies have demonstrated that treatment with CLA can reduce phosphorylation of NF-κB in dendritic cells (30). Although the offspring were not directly exposed to CLA, we propose that reductions in circulating inflammatory mediators in response to supplementation of a HFD with CLA during pregnancy may suppress in utero inflammatory signaling during fetal development.

Given that NF-κB acts as transcriptional regulator of many cytokines that play a central role in non-pregnancy-related obesity (3), intramuscular cytokine concentrations and gene expression were examined. There was increased intramuscular abundance of the proinflammatory cytokines IL-6, MCP-1, and IL-1β, accompanied by decreased protein concentrations of the anti-inflammatory cytokine IL-10 in HF and HFCLA offspring. We have previously demonstrated increased maternal plasma concentrations of IL-1β and TNF-α in HF dams, an effect that was prevented with CLA addition to the diet (47). Interestingly, despite the anti-inflammatory effects of maternal CLA supplementation on offspring NF-κB phosphorylation and dam cytokine concentrations, maternal CLA supplementation displayed no beneficial suppressive effect on cytokine expression in the offspring skeletal muscle. However, mRNA expression of the cytokine receptor Il6r was increased in HF but not HFCLA offspring. This suggests that, despite no effect of CLA on elevated muscle cytokines in offspring of mothers fed a HF diet, there was potentially a reduced sensitivity to cytokine
signaling in HFCLA offspring as a result of suppressed receptor expression.

Increased concentrations of intramuscular cytokines and activation of inflammatory signaling pathways may play a pivotal role in the initiation of protein catabolism leading to muscle atrophy. Epidemiological studies have reported a strong association between elevated expression of IL6 and IL1B genes and decreased muscle mass, strength, and cross-sectional area (13, 46, 50). Elevated concentrations of proinflammatory cytokines may contribute to skeletal muscle atrophy by both inhibiting protein synthesis and inducing protein breakdown (19, 21). While there are several pathways that contribute to muscle loss, most only contribute to breakdown on a subcellular scale and are of little importance in the accelerated breakdown of skeletal muscle (31). The ubiquitin proteasome pathway is the primary pathway responsible for regulating skeletal muscle protein degradation (27). In this pathway, enzymes link chains of ubiquitin to proteins, tagging them for degradation by the 26S proteasome (27). Expression of the muscle-specific E3 ligases MuRF1 and atrogin-1 has been shown to be upregulated 8- to 40-fold before the onset of atrophy (18, 27, 48). While atrogin-1 gene expression was not elevated in HF offspring, the expression of the FOXO family of transcription factors (22) was increased. FOXO3 can regulate transcription of atrogin-1 (45). In contrast to atrogin-1, a significant increase in MuRF1 gene expression in HF offspring was observed. MuRF1 is, in part, transcriptionally regulated by NF-κB (9, 34). The increase in phosphorylation of NF-κB p65 in HF offspring provides evidence to suggest a major role of NF-κB activation in the initiation of MuRF1 expression in HF offspring. Taken in the context of the current study, the normalized expression of MuRF1 in HFCLA offspring is likely attributable to the accompanying reduction in NF-κB p65 phosphorylation. Collectively these data suggest that maternal CLA supplementation may suppress NF-κB-mediated atrophy in HFCLA offspring.

The maintenance of skeletal muscle is regulated by both protein synthesis and protein degradation pathways; hence, the activity of the Akt/mTOR pathway (a major pathway involved in skeletal muscle protein synthesis) was examined. The mTOR signaling pathway increases protein synthesis by stimulating translation initiation and ribosome biosynthesis (25). The current study demonstrated no impact of maternal diets on Akt and mTOR phosphorylation, consistent with a previous maternal HF diet study (39). There was, however, reduced phosphorylation of p70S6K (Thr421/Ser424), the primary target for mTOR and translation initiation, in the HF offspring. This was not observed in the HFCLA offspring, indicating a possible protective effect of maternal CLA supplementation. The p70S6K downstream target S6 was unaltered by CLA supplementation, although there was an overall effect of maternal HF feeding to lower phosphorylation of rpS6 (Ser235/236). Taken together these data suggest a contributing role for suppressed muscle protein synthesis in the observed reduction of protein content in HF and HFCLA offspring.

Perspectives and Significance

The current study demonstrates that male offspring from HF and HFCLA mothers displayed altered skeletal muscle composition, intramuscular immune cell infiltration, and elevated inflammatory cytokine concentrations. Phosphorylation of NF-κB and components of the ubiquitin proteasome pathway were also increased in muscle of HF offspring, an effect that was ameliorated with maternal CLA supplementation. Collectively, the present study provides further evidence that a maternal HF diet negatively impacts on skeletal muscle development in offspring, predisposing the offspring to obesity and altered body composition. The results demonstrate the importance of early life interventions during the phase of developmental plasticity to reverse the negative effects of a poor maternal dietary environment. CLA may ameliorate some of the negative effects on skeletal muscle associated with a maternal HF diet. Furthermore, these results highlight the need for healthy weight management and regulation of dietary fat intake in women of childbearing age and those considering pregnancy.

REFERENCES


