Lipids (2015) 50:205–217
DOI 10.1007/s11745-014-3985-5

ORIGINAL ARTICLE

A Maternal High Fat Diet Has Long-Lasting Effects on Skeletal Muscle Lipid and PLIN Protein Content in Rat Offspring at Young Adulthood

Rebecca E. K. MacPherson · Laura M. Castelli · Paula M. Miotto · Scott Frendo-Cumbo · Amanda Milburn · Brian D. Roy · Paul J. LeBlanc · Wendy E. Ward · Sandra J. Peters

Received: 15 May 2014 / Accepted: 16 December 2014 / Published online: 1 January 2015

Abstract A maternal high fat diet (HFD) can have adverse effects on skeletal muscle development. Skeletal muscle PLIN proteins (PLIN2, 3 and 5) are thought to play critical roles in lipid metabolism, however effects of HFD on PLIN and lipases (HSL, ATGL, CGI-58) in mothers as well as their offspring have yet to be investigated. The primary objective of this study was to determine whether maternal HFD would influence skeletal muscle lipase and PLIN protein content in offspring at weaning (19d) and young adulthood (3mo). Female rats (28d old, $n = 9$/group) were fed control (CON, AIN93G, 7 % soybean oil) or HFD (AIN93G, 20 % lard) for 10 weeks prior to mating and throughout pregnancy and lactation. All offspring were weaned to CON [$n = 18$/group, 1 female and 1 male pup per litter were studied at weaning (19d) and 3mo of age]. There was no effect of sex for the main outcomes measured in plantaris, therefore male and female data was combined. Maternal HFD resulted in higher triacylglycerol content in pups at 3mo ($p < 0.05$), as well as in the dams ($p = 0.015$). Maternal HFD resulted in higher PLIN5 content in pups at weaning and 3mo ($p = 0.05$). PLIN2 and PLIN5 content decreased at 3mo versus weaning ($p < 0.001$). HFD dams had a higher PLIN3 content ($p = 0.016$). Diet had no effect on ATGL, CGI-58, or HSL content. In conclusion, exposure to a maternal HFD resulted in higher skeletal muscle lipid and PLIN5 content in plantaris of offspring through to young adulthood.

Keywords ADRP · OXPAT · Skeletal muscle · TIP47 · High fat diet · Maternal diet

Abbreviations

HFD	High fat diet
PLIN	Perilipin
ATGL	Adipose triglyceride lipase
HSL	Hormone sensitive lipase
CON	Control diet
ORO	Oil red O

Introduction

A high fat diet (HFD) leads to an abundance of circulating lipids as a result of lack of storage capacity in adipose tissue. As a consequence, lipids accumulate in ectopic tissues such as skeletal muscle [7, 46, 57, 60]. The proper storage of intramuscular lipids as triacylglycerols in lipid droplets is tightly regulated and plays a critical role in maintaining energy homeostasis and cellular function. To date, the exact mechanisms regulating how intramuscular lipids are stored in lipid droplets are unknown. Accumulating evidence indicates that a family of lipid droplet proteins, known as PLIN proteins (perilipin 1 through 5) [26], are likely involved in regulating skeletal muscle lipid droplet growth and development [17, 40]. However, most studies investigating the

R. E. K. MacPherson · L. M. Castelli · P. M. Miotto · S. Frendo-Cumbo · A. Milburn · B. D. Roy · P. J. LeBlanc · W. E. Ward · S. J. Peters
Faculty of Applied Health Sciences, Center for Bone and Muscle Health, Brock University, St. Catharines, ON, Canada

R. E. K. MacPherson (✉) · P. M. Miotto · S. Frendo-Cumbo · A. Milburn · B. D. Roy · W. E. Ward · S. J. Peters
Department of Kinesiology, Faculty of Applied Health Sciences, Centre for Bone and Muscle Health, Brock University, 500 Glenridge Ave.,, St. Catharines, ON L2S 3A1, Canada
e-mail: rm09aa@brocku.ca

L. M. Castelli · P. J. LeBlanc · W. E. Ward
Department of Health Sciences, Faculty of Applied Health Sciences, Brock University, St. Catharines, ON, Canada

roles of skeletal muscle PLIN proteins have focused on changes with exercise training [41, 48, 50, 51] and their potential role in regulating lipolysis [32–34], while few studies have investigated the effects of a HFD on skeletal muscle lipases and PLIN proteins [3, 7, 16].

Our understanding of PLIN proteins in skeletal muscle is limited, however studies in other tissues and in cell culture indicate that PLIN proteins are key regulators of lipid metabolism as they appear to be directly involved with the storage, mobilization, and utilization of fatty acids [11, 13, 17, 30, 31, 55]. Of the PLIN family three are expressed in skeletal muscle: PLIN2 formerly ADRP, PLIN3 formerly TIP47, and PLIN5 formerly OXPAT. Recent work indicates that skeletal muscle PLIN proteins enhance lipid droplet growth and expansion by directly interacting with lipases on the lipid droplet surface [33, 34, 42]. Further, studies in skeletal muscle indicate that these PLIN proteins are more highly expressed in scenarios where there is a higher intramuscular lipid content [41, 48–51, 65]. Due to a lack of studies investigating the specific roles of these PLIN proteins in skeletal muscle, it is necessary to extrapolate from findings utilizing other tissues types or models. Cell culture studies utilizing lipid loading and PLIN protein overexpression have indicated a role for PLIN2 [12, 21, 22, 25, 29], PLIN3 [64], and PLIN5 [15, 65] in both the development and growth of lipid droplets. As PLIN protein content appears to mirror the lipid content of the cell it is highly likely that these PLIN proteins would be sensitive to changes in dietary lipid content.

To date, only three studies have investigated the effects of high fat feeding on skeletal muscle PLIN protein content. Following 4 weeks of a HFD (40 % energy from lard and 5 % from soybean oil), there was an increased PLIN5 content with no change in PLIN2 or PLIN3 in mouse gastrocnemius [3]. In two additional studies, 8 weeks of HFD (45 % energy from fat) resulted in either significantly increased PLIN2 content in mouse tibialis anterior [7] or a trend towards an increase in mouse gastrocnemius [16]. These three studies indicate that there may be a temporal response in PLIN protein content to HFD, with PLIN5 content increasing early on and PLIN2 increasing at 8 weeks. In addition, this work also demonstrated that PLIN2 appears to be more sensitive to dietary unsaturated fatty acids compared to saturated [16]. Together these studies highlight the potential importance of skeletal muscle PLIN proteins in regulating lipid storage in response to high fat feeding, however further work is needed to clarify these findings.

In addition to the direct effects of HFD on skeletal muscle, HFD can have adverse effects on skeletal muscle development in offspring from high fat fed mothers. Maternal high fat feeding can cause obesity in dams and can modulate the intrauterine environment such that offspring are programmed to become obese [47, 63] and have metabolic disorders related to skeletal muscle health. Offspring, aged 21 days to adulthood, from obese rats have reduced muscle contractile capacity [4], impaired insulin signalling and mitochondrial capacity [5], and decreased GLUT4 content [52]. Therefore, there is potential for epigenetic modifications, which may predispose the offspring to obesity and metabolic disease as a result of HFD-induced changes in metabolism. However, the mechanisms involved in generating the above-mentioned changes in skeletal muscle are far from being completely understood, and an examination of changes in skeletal muscle PLIN in offspring from high fat fed dams has yet to be investigated. Further, another possible mechanism regulating the increased skeletal muscle lipid storage with a HFD is a reduction in lipolytic rate due to changes in the protein content of the lipases, adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL).

Based on emerging knowledge that maternal diet can modulate metabolism of offspring, the primary objective of this study was to determine if exposure of offspring to HFD in utero and throughout suckling modulated skeletal muscle lipid, PLIN, and lipase protein content at 3 months of age or young adulthood. We hypothesized that skeletal muscle lipid content would be higher in the offspring from the high fat fed dams and that this would be related to changes in PLIN protein and lipase content similar to changes seen in the high fat fed dams. This would indicate that a maternal HFD has long lasting effects on skeletal muscle lipid metabolism in offspring. A secondary objective was to determine the effect of a HFD on skeletal muscle lipid, PLIN, and lipase protein content on mothers fed continuous HFD since weaning. It was critical to establish the status of mothers first to determine the effects of the HFD on the dams and second to determine if these effects are carried into the offspring. This study showed that a maternal HFD resulted in higher PLIN5 content in pups at weaning and 3 months of age and an increased PLIN3 content in dams. Findings from this study provide novel information regarding potential adverse effects of maternal HFD on skeletal muscle health in offspring and insight into the potential roles of these PLIN proteins in skeletal muscle lipid storage.

Methods

Animals

Twenty-one day old female ($n = 18$) and male ($n = 6$) Wistar rats were purchased (Charles River, QC, Canada) for use in this experiment. Rats were housed within the Brock University Animal Facility, where they were

maintained on a 12:12-h light–dark cycle at 22 °C. All experimental procedures and protocols were approved by the Brock University Animal Care and Utilization Committee and conformed to all Canadian Council on Animal Care guidelines [18].

Experimental Design

Female rats (28 days old) were fed control (CON; AIN93G diet, 7 % soybean oil by weight; $n = 9$/group) or HFD (modified AIN93G diet, 20 % lard by weight; $n = 9$/group) ad libitum for 10 weeks at which time they were bred with age-matched males ($n = 6$) and continued on the same diet throughout gestation and lactation. Each male rat was bred with the same number of females from each intervention, ensuring equal paternal influence among groups. The male rats were fed a control AIN93G diet. The fatty acid composition of the two diets was measured by gas chromatography and is reported in another study that investigated bone health [37]. The HFD diet was modified to ensure that observed effects could be attributed to the higher fat content in the diet and not to lower levels of other nutrients. Specifically, the amount of protein, vitamins, and minerals in HFD was increased by a factor of 1.2 compared to the CON diet to account for the 1.2 fold higher energy content per kg of diet [37].

Litters were weaned at postnatal day 19 and 1 male and 1 female pup from each of the 9 litters per group were studied at weaning (postnatal day 19, $n = 18$ pups/group) and young adulthood (3 mo, $n = 18$ pups/group) to reduce potential litter effects [61]. All pups were fed CON until 3 months of age, regardless of whether their mothers were fed HFD or CON during pregnancy and lactation, to determine pre-weaning effects of a HFD to offspring. Offspring at both ages were anesthetized via intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt). Left and right plantaris muscles were isolated and removed. The left plantaris was snap frozen in liquid nitrogen for Western blotting analysis and the right plantaris was cut and mounted for histochemical analysis (see below). Dam and offspring omental, retroperitoneal, and parametrial fat pads were dissected, weighed and snap frozen for a measure of adiposity.

Western Blotting

Plantaris muscles were homogenized in a standard homogenization buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA; pH fixed to 6.8) using a 1:25 dilution of muscle to buffer with added protease (11836170001, Roach, QC), and phosphatase inhibitor (04906845001, Roach, QC, Canada). Protein concentration of the total homogenates was determined using a Bradford Assay (Bio-Rad Protein Assay Dye Reagent Concentrate; #500-0006, Bio-Rad, USA). SDS–polyacrylamide gel electrophoresis (8 or 10 % separating; 4 % stacking) was used to separate proteins (ATGL, CGI-58, HSL, PLIN2, PLIN3, and PLIN5) at 120 V for 1.5 h, and proteins were electroblotted onto polyvinylidene difluoride membranes (Amersham Biosciences, Piscataway, NJ, USA) for 1 h at 100 V followed by blocking in 2 (CGI-58) or 5 % (actin, PLIN2, PLIN5) fat-free milk in TBST, or 5 % BSA (PLIN3, ATGL) in TBST. Primary antibodies were diluted 1:1,000 in 2 (CGI-58) or 3 % (PLIN2, PLIN5) fat-free milk, or 1 (PLIN3) or 5 % BSA (ATGL) in TBST and incubated overnight at 4 °C. Secondary antibodies were diluted 1:10,000–20,000 in 2 (CGI-58) or 3 % (PLIN2, PLIN5) milk, or 1 (PLIN3) or 5 % (ATGL) BSA and incubated for 1 h. Blots of specific signals were visualized with enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA). The densities of the individual bands were integrated using Image J software (http://rsbweb.nih.gov/ij/). Blots were normalized to actin protein content and results are reported as the ratio of the density of the target protein to the density of actin in arbitrary units.

Antibodies

The following antibodies were used and have been used previously: anti-actin antibody (BD Biosciences, Mississauga, ON, 558623), PLIN2 (52 kDa) mouse monoclonal antibody (Progen Biotechnik, Heidelberg, Cat. No. 610102) [33, 41], PLIN3 (47 kDa) (ProSci Inc., Poway, California, Cat. No. 3883) [33, 41], PLIN5 (52 kDa) guinea pig polyclonal antibody (Progen Biotechnik, Heidelberg, Cat. Nos. GP34 and GP31) [8, 33, 36, 41], ATGL (54 kDa) rabbit monoclonal antibody (Cell Signalling Technology, #2439, Danvers, MA, USA) [1, 33], CGI-58 (42 kDa) rabbit polyclonal antibody (Novus Biologicals, NB110-41576, Oakville, ON, Canada) [1, 33, 56], and HSL (Cell Signalling Technology, 4107S, Danvers, MA, USA).

Histochemical Analysis

The muscle sections used for histochemical analysis were oriented for cross sections and mounted in embedding medium (Cryomatrix, Pittsburgh, PA, USA) on a piece of cork, which was plunged into 2-methylbutane cooled in liquid nitrogen. Following rapid freezing, the samples were stored at −80 °C until sectioning. Sectioning was completed with a cryotome (ThermoShandon, Runcorn, Cheshire, UK) optimally set at −20 °C. 10 µm thick sections were thaw mounted onto slides and stored at −80 °C until histochemical staining. For the examination of lipid droplets, oil red O (ORO; O0625; Sigma-Aldrich, St. Louis, MO, USA) was utilized [28, 53, 58, 59]. Briefly,

cryosections were fixed in 3.7 % formaldehyde for 1 h. Slides were then rinsed 3 times in deionized water for 30 s, and then immersed in the working solution of ORO for 30 min. Slides were rinsed three times in deionized water and cover slips were mounted using Prolong Gold antifade reagent (cat no. P36930, Invitrogen, Burlington, ON, Canada).

Image Capturing and Analyses

All sections were examined using a Nikon Eclipse 80i fluorescence microscope (Nikon Eclipse 80i; Chiyoda-ku, Tokyo, Japan). Digital images of the slides were captured with a digital camera (Retiga 1300, QImaging, Burnaby, BC, Canada) attached to the microscope. To visualize the ORO stain a TRITC (510–560 nm) excitation filter was used. Digitally captured images at 40× magnification were analyzed using imaging software (NIS-Elements AR 3.00; Nikon Instruments, Melville, NY, USA). An intensity threshold representing minimal values corresponding to lipid droplets was set manually and applied uniformly in all images. The lipid droplet fluorescent signals were quantified for each muscle fiber, a total of 60 ± 5 fibers were analyzed for each muscle cross section, which represented four fields of view for dams and 3 month old offspring and two fields of view for weaning offspring. Fiber area, as well as number and area of objects emitting a fluorescent signal, were recorded. Muscle fiber lipid droplet content was expressed as the fraction of the measured area that was stained per fiber [32, 58]. All measures were manually outlined and traced by investigators for each individual myocyte. The immunofluorescence method described here covers numerous fibers per muscle cross- section and, therefore, gives a good representation of the entire muscle. To test the reliability of the method in our hands, both intra-observer and inter-observer reliability were evaluated by two investigators. The intra-observer reliability involved the two investigators performing analysis of one image three times, at least 1 week apart. The inter-observer reliability involved two independent investigators (AM and RM) performing analysis for three separate images. These tests proved to be reliable with a coefficient of variation <5 % for both intra-observer and inter-observer reliability.

Triacylglycerol Fatty Acid Composition

Frozen plantaris muscle was homogenized in 10 volumes of Tris HCl buffer (pH 8.0) and used for lipid analysis. Total lipids from muscle homogenate were extracted [19] and triacylglycerols from each sample were separated using thin-layer chromatography [27]. Isolated triacylglycerols were methylated [35] and the fatty acid composition of each triacylglycerol was analyzed by gas chromatography [10]. Briefly, the fatty acid methyl esters were separated on a UFM-RTX WAX analytical column (Thermo Electron Corp., Milan, Italy) using gas chromatography (Trace GC Ultra, Thermo Electron Corp, Milan, Italy) fitted with a fast flame ionization detector, a split-splitless injector, and Triplus AS autosampler. Fatty acids were identified by retention time compared with a known standard (Supelco 37 component FAME mix, Supelco, Bellefonte, PA, USA) and absolute amounts of individual fatty acids were calculated with the aid of the internal standard, tridecanoic acid (13:0), added to the samples before the methylation process. Preliminary analyses indicated no detectable endogenous 13:0 in the samples analyzed (data not shown).

Table 1 Body weight and fat pad mass of offspring of dams fed control or high fat diet

Outcome	CON offspring		HFD offspring	
	Weaning	3 Months	Weaning	3 Months
Body Weight (g)	44.4 ± 1.4	411.7 ± 26.4	54.2 ± 2.1*	427.9 ± 30.7
Retroperitoneal (g)	2.6 ± 0.3	28.6 ± 2.1	4.2 ± 0.4*	31.7 ± 2.8
Omental (g)	8.2 ± 0.7	21.8 ± 1.0	9.6 ± 0.4	23.6 ± 1.9
Total fat pad (g)	10.6 ± 0.8	50.4 ± 2.6	13.8 ± 0.7*	55.3 ± 3.8

Values are expressed as means ± SEM ($n = 18$ per group)

There was a significant main effect for age for all outcomes ($p < 0.05$): * Significant difference from weaning control at same age ($p < 0.05$)

Con control diet, *HFD* high fat diet

Table 2 Body mass and fat pad mass of dams fed either control or high fat diet

Outcome	CON dams	HFD dams	*p* value Diet
Body mass (g)			
4 weeks old	82.9 ± 0.9	81.3 ± 2.1	$p > 0.05$
10 weeks old	323.7 ± 4.5	349.6 ± 9.7	$p = 0.028$
Fat pad weights (g)			
Retroperitoneal	4.9 ± 0.4	6.2 ± 0.6	$p = 0.104$
Omental	4.3 ± 0.5	5.5 ± 0.4	$p = 0.055$
Parametrial	6.0 ± 0.5	8.0 ± 0.9	$p = 0.068$
Total fat pad	15.2 ± 1.0	19.6 ± 1.9	$p < 0.05$

Values are expressed as means ± SEM ($n = 9$ per group)

Con control diet, *HFD* high fat diet

Statistics

A *t* test was used to compare body mass and fat pad mass of offspring at weaning and at 3 months of age, and for

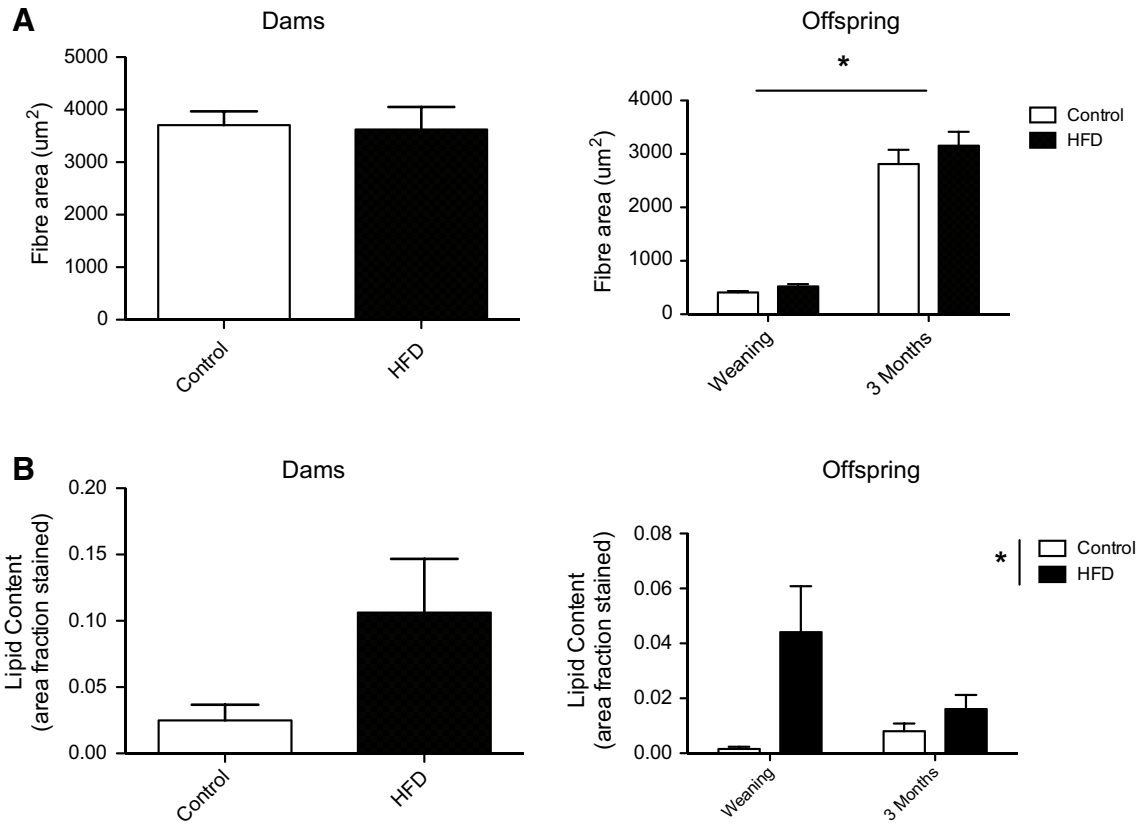


Fig. 1 **a** Skeletal muscle fibre area in dams (*left panel*) and offspring (*right panel*). Values are expressed as means of fibre area ± SEM. *Significantly higher than weaning ($p < 0.05$). **b** Intramuscular lipid content in dams (*left panel*) and offspring (*right panel*). Values are expressed as means of object fraction area ± SEM. *Significantly higher than control ($p < 0.05$) at weaning and 3 months

dams. A *t* test was also used for comparison of triacylglycerol composition of plantaris in 3 month old offspring and dams. Comparisons for lipid and protein content were performed using a two-way ANOVA (diet and age). Sexes were combined for statistical analysis because there were no significant difference between males and females for the main outcomes (PLIN and lipase protein content). Tukey's *post hoc* tests were performed when significance was detected. For outcomes measured in dams, analyses were performed using a t-test for normally distributed data (body and fat pad mass, mean lipid and protein content) or a Rank Sum test when data were not normally distributed (fiber area and lipid content). Statistical significance was set at $p < 0.05$. All data are expressed as means ± SEM.

Results

Offspring and Maternal Body and Fat Pad Mass

Offspring from dams fed HFD had a higher body mass at weaning as well as increased retroperitoneal and total fat pad mass. There were no significant differences in offspring body and fat pad mass at 3 months of age (Table 1). At the end of lactation, high fat feeding of dams resulted in a higher body mass [37], as well as omental and total fat pad mass (Table 2).

Fiber Area and Lipid Content

No significant differences between HFD and CON dams were found for the average muscle fiber area (CON: 3,706 ± 260 μm^2 and HFD: 3,622 ± 428 μm^2; Fig. 1a). Offspring fiber area did not differ between diets either at weaning or 3 months of age (weaning CON: 407 ± 29 μm^2 *vs* weaning HFD 521 ± 44 μm^2; young adult CON: 2,812 ± 258 μm^2 *vs* young adult HFD: 3,148 ± 254 μm^2; Fig. 1a). Fiber area of offspring at 3 months was significantly larger than weanlings ($p < 0.05$).

High fat feeding resulted in increased lipid content in the plantaris muscle of dams as well as pups, both at weaning and at young adulthood ($p < 0.05$; Fig. 1b). Figure 2 shows representative images of rat skeletal muscle cross sections viewed with an immunofluorescence microscope following incubation with ORO.

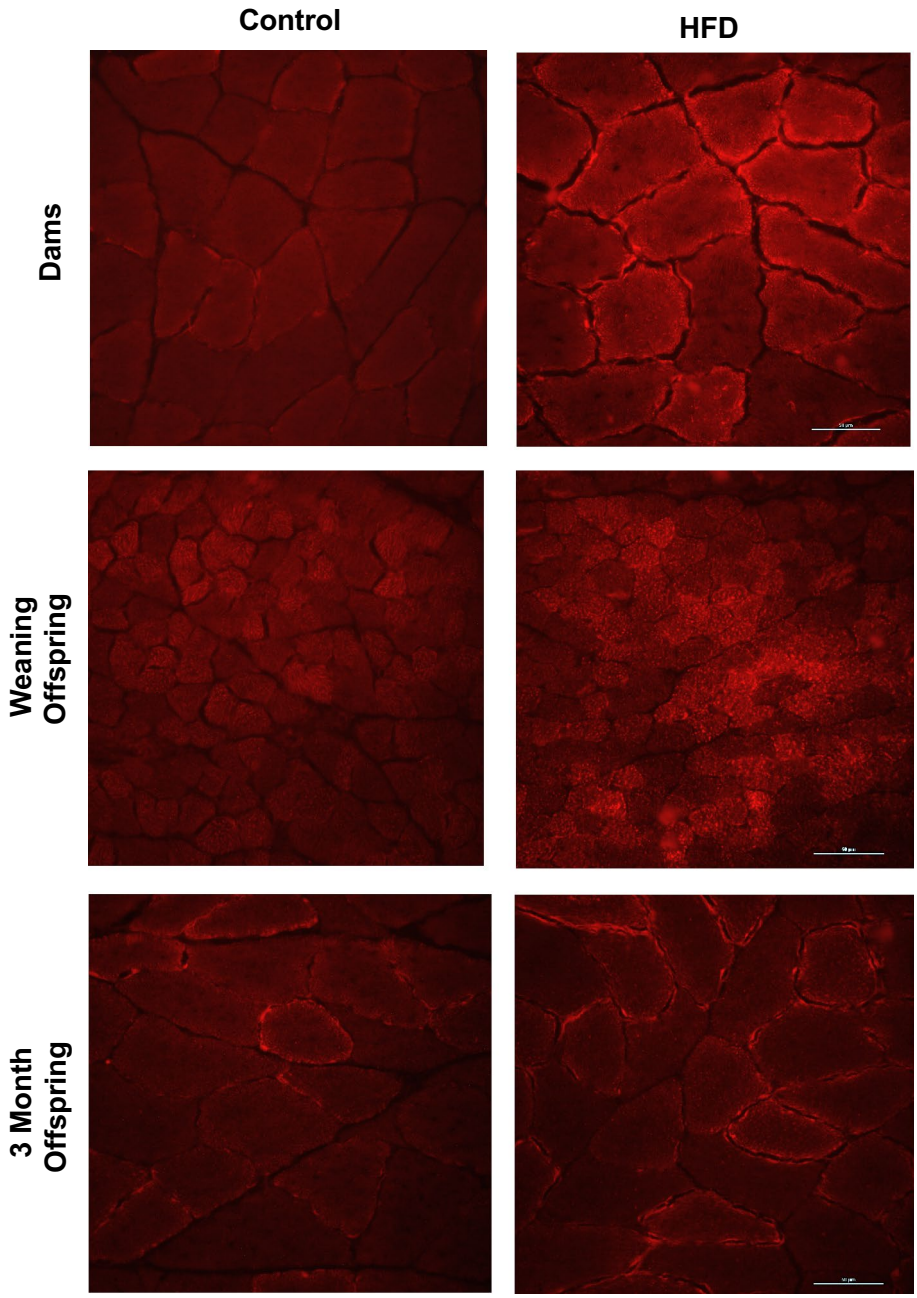


Fig. 2 Digitally captured images of one single field of view (×40 magnification) taken from a cross-section of plantaris muscle with Oil red O (ORO) staining (images on the *left* represent CON rats and images on the right represent HFD rats)

Triacylglycerol Fatty Acid Composition

To further determine if maternal high fat feeding resulted in changes in total triacylglycerol content and fatty acid composition lipid analysis was performed. In the dams, high fat feeding resulted in higher total triacylglycerol content. In the triacylglycerol fraction both saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents were higher in HFD compared to CON. There was no difference in total polyunsaturated fatty acid (PUFA) content between HFD and CON groups (Table 3).

Lipid analysis of 3 mo old offspring revealed that the plantaris muscle is responsive to maternal diet. In agreement with the oil red O results, the pups from the HFD dams had a higher total triacylglycerol content. Within the triacylglycerol fraction, there was a significant increase in SFA, MUFA, PUFA, *n*3 PUFA, and *n*6 PUFA in the pups from the HFD dams in comparison to the CON pups at 3 months (Table 4).

Table 3 Dam plantaris triacylglycerol fatty acid composition

Fatty acid (nmol/g muscle)	Treatment		*p* value
	CON dams	HFD dams	Diet
Total SFA	3.63 ± 0.88	7.56 ± 1.07	0.009
Total MUFA	3.14 ± 0.87	8.66 ± 1.50	0.006
Total PUFA	2.45 ± 0.80	2.91 ± 0.53	0.315
*n*3 PUFA	0.16 ± 0.07	0.11 ± 0.05	0.257
*n*6 PUFA	2.29 ± 0.73	2.80 ± 0.51	0.282
Total triacylglycerol	9.22 ± 2.53	19.13 ± 2.94	0.015

Data are expressed as means ± SEM ($n = 6$ or 7 per group)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *CON* control diet, *HFD* high fat diet

Table 4 Three month old offspring plantaris triacylglycerol fatty acid composition

Fatty acid (nmol/gram muscle)	Treatment		*p* value
	CON offspring	HFD offspring	Diet
Total SFA	6.38 ± 0.59	11.83 ± 2.09	0.009
Total MUFA	6.12 ± 0.68	12.52 ± 2.17	0.004
Total PUFA	3.98 ± 7.8	8.86 ± 1.66	0.004
*n*3 PUFA	0.36 ± 0.7	0.94 ± 0.22	0.008
*n*6 PUFA	3.62 ± 7.1	7.92 ± 1.46	0.004
Total triacylglycerol	16.48 ± 1.74	33.21 ± 5.85	0.005

Data are expressed as means ± SEM ($n = 15$ per group)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *CON* control diet, *HFD* high saturated fat diet

Muscle Lipolytic Enzyme Protein Content

In order to determine if the increased lipid content in both the dams as well as the pups was due to decreased lipase content, HSL, ATGL and CGI-58 protein content were measured. High fat feeding did not result in any significant changes in plantaris HSL, ATGL, or ATGL co-activator CGI-58 in offspring either at weaning or 3 months of age. Similar results were found in the dams for HSL ($p = 0.73$), ATGL ($p = 0.07$), and CGI-58 ($p = 0.47$) (Fig. 3a–c).

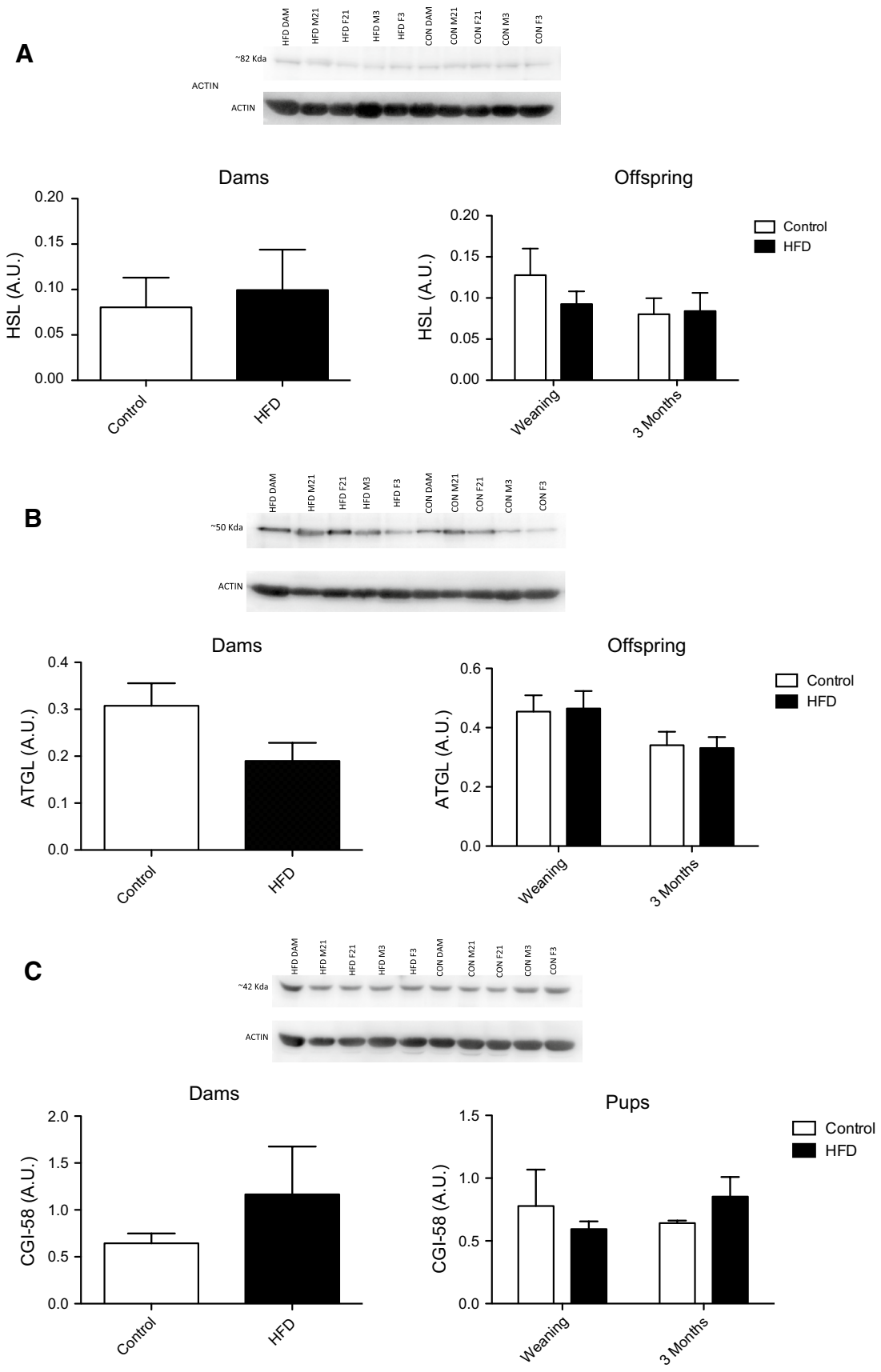
Muscle PLIN Protein Content

High fat feeding resulted in no significant difference in PLIN2 content between pups at weaning or young adulthood, or in the dams (Fig. 4a). Pups at young adulthood had higher PLIN2 content in comparison to at weaning ($p < 0.05$). There was no significant effect of diet or age on PLIN3 content in offspring. PLIN3 content was higher in dams fed HFD compared to control (Fig. 4b; $p < 0.05$). PLIN5 content was significantly higher in offspring from HFD dams at weaning and young adulthood ($p = 0.05$). High fat feeding resulted in no significant difference in PLIN5 content between dams (Fig. 4c).

Discussion

This study investigated the effects of a maternal HFD on skeletal muscle lipid, PLIN, and lipase protein content of offspring at weaning and 3 months of age as well as dams. Our results demonstrate for the first time that a maternal HFD leads to higher PLIN5 content in offspring at weaning and this effect persists at 3 months of age despite offspring being fed a CON diet from weaning onwards. These changes in PLIN5 protein content were accompanied by higher skeletal muscle triacylglycerol content in the offspring. Further, this study provides interesting and novel results in regards to developmental changes in PLIN protein expression, as both PLIN2 and PLIN5 protein content decreased from weaning to 3 months of age while PLIN3 content remained the same. Together, the findings from the current study contribute to the growing body of work implicating a role for PLIN proteins in skeletal muscle lipid storage as well as evidence that maternal nutrition may influence skeletal muscle metabolic function.

At weaning, offspring from the HFD dams were heavier and had increased fat pad mass, however this did not continue into adulthood, as there are no significant differences at 3 months of age. This higher adipose tissue mass may be attributed to the milk composition during suckling. Others have shown that milk obtained from dams fed a HFD reflects the diet consumed, exposing offspring to a similar diet as the mother while suckling [20, 43, 54]. Interestingly, skeletal muscle neutral lipid content of offspring from high fat fed dams remained higher than control offspring at 3 months of age. Lipid analysis of plantaris muscle from these offspring revealed that there was a higher triacylglycerol content in pups from the HFD dams in spite of the fact that they had been maintained on control diet since weaning. Further analysis demonstrated that this increase was due to higher content of SFA, MUFA, PUFA, *n*3 PUFA, and *n*6 PUFA in the muscle triacylglycerols. In addition to changes in lipid content, PLIN5 protein content in offspring from high fat fed dams was higher than control offspring at weaning as well as at 3 months of age. This indicates that maternal diet may have long lasting effects on the ability of skeletal muscle to store and utilize triacylglycerols. Interestingly, Hsieh et al. [24] demonstrated that PLIN5 specifically targets lipid droplets composed of mainly triacylglycerols [24]. This may be an adaptive response in an attempt to buffer excess dietary lipids into muscle lipid


A
HFD DAM
HFD M21
HFD F21
HFD M3
HFD F3
CON DAM
CON M21
CON F21
CON M3
CON F3
~82 Kda
ACTIN
ACTIN
Dams
Offspring
HSL (A.U.)
0.20
0.15
0.10
0.05
0.00
Control
HFD
Weaning
3 Months
B
~50 Kda
ACTIN
ATGL (A.U.)
0.4
0.3
0.2
0.1
0.0
0.6
C
~42 Kda
ACTIN
CGI-58 (A.U.)
2.0
1.5
1.0
0.5
0.0
Pups

◀ **Fig. 3** **a** HSL protein content (arbitrary units) in dams (*left panel*) and offspring (*right panel*) with representative Western blot. **b** ATGL protein content (arbitrary units) in dams (*left panel*) and offspring (*right panel*) with representative Western blot. **c** CGI-58 protein content (arbitrary units) in dams (*left panel*) and offspring (*right panel*) with representative Western blot. Representative blots are shown from left as high fat (HF) dam, 21 day old high fat male (HF M21), 21 day old high fat female (HF F21), 3 month old high fat male (HF M3), 3 month old high fat female (HF F3), control dam (CON), 21 day old control male (CON M21), 21 day old control female (CON F21), 3 month old control male (CON M3), and 3 month old control female (CON F3)

droplets. Recently, PLIN5 has been shown to localize to mitochondria in smooth and skeletal muscle cells [8, 44] as well as link lipid droplets to mitochondria [62]. Further, overexpression of PLIN5 results in a more intimate interaction between the lipid droplets and mitochondria [8]. It is possible that the offspring from high fat fed dams have an increased interaction between lipid droplets and mitochondria due to the increased PLIN5 content, potentially to regulate oxidation during muscle lipolysis.

In offspring, regardless of maternal diet, there was a significant decline in PLIN2 and PLIN5 protein content from weaning to 3 months of age, although, offspring exposed to the maternal HFD rather than CON diet had higher PLIN5 at both time points. This finding is of particular interest, as these changes in PLIN protein content do not reflect the skeletal muscle lipid content, particularly in the offspring from the control fed dams. This is the first study to demonstrate a potential developmental role for PLIN proteins in skeletal muscle. Due to the proposed roles of PLIN2 and PLIN5 in lipid droplet growth and synthesis it is possible that the higher PLIN2 and PLIN5 content demonstrated at weaning in comparison to 3 months of age may be due to differences in growth rates at these time points. For example, at weaning through 1 month of age, pups gained weight more rapidly than between 2 and 3 months of age [37]. To support this rapid growth, there is likely a larger influx of lipids into skeletal muscle, providing the necessary fuel for proper growth and development.

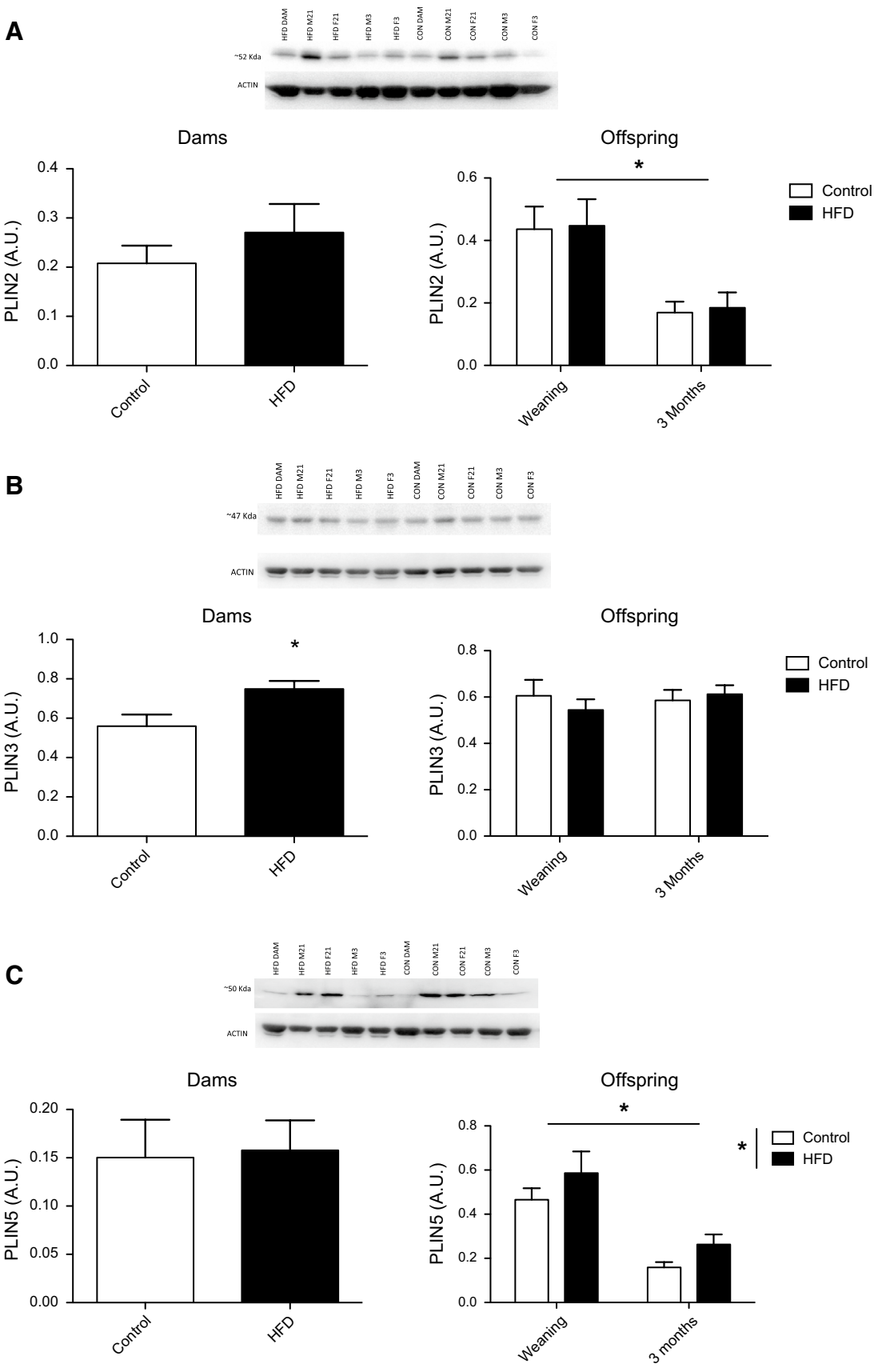
A secondary purpose to this study was to investigate the effect of a 16-week HFD on muscle lipid, lipase, and PLIN protein content of dams. As expected, the HFD resulted in a significant accumulation of intramuscular lipids as well as an accompanying increase in total fat pad weight. These results are consistent with the theory of adipose tissue expandability, where there is a delay in adipocyte hyperplasia and/or the adipose tissue expansion limit is reached and lipids accumulate in other tissues [57, 60]. It is also possible that the accumulation of intramuscular lipids is the result of increased skeletal muscle free fatty acid uptake and/or reduced lipolysis [6, 38, 39, 45]. Examination of the protein content of the rate limiting lipase ATGL, its co-activator CGI-58, and HSL revealed no significant difference in HSL and CGI-58 content between high fat fed and control dams, and a 38 % decline in ATGL protein content in high fat fed dams, although, this change was insignificant ($p = 0.07$) as shown previously in HF fed mice [3].

Interestingly, higher skeletal muscle lipid content of high fat fed dams was accompanied by higher PLIN3 protein content. A functional role in skeletal muscle has not been confirmed for PLIN3, but studies in other cell types indicate that PLIN3 is involved in nascent lipid droplet expansion as well as new lipid droplet formation [14, 64, 66]. Specifically, in response to lipid loading in both HeLa cells and adipocytes, PLIN3 appears to move from the cytosol and onto lipid droplets indicating a role in lipid droplet growth [64, 66]. The present study is the first to demonstrate increased PLIN3 content within skeletal muscle in response to a HFD. Previous work by Badin et al. [3] show no significant change in PLIN3 content following 4 weeks of a HFD initiated at 5 weeks of age. These apparently disparate findings may be due to differences in rodent species studied or to the duration of the diet.

This study did not detect changes in PLIN2 or PLIN5 protein content in dams fed a HFD. This is interesting as it is thought that both PLIN2 and PLIN5 are involved with lipid droplet growth and that protein content shadows intramuscular lipid content in situations when lipid content is higher, PLIN2 and PLIN5 contents are also higher [41, 48–51, 65]. Further, previous work has demonstrated that both PLIN2 and PLIN5 are increased in response to high fat feeding [3, 7, 16]. Previously, de Wilde et al. [16] demonstrated using both a C2C12 cell line as well as gastrocnemius of C57BL/6J mice fed a HFD (45 % kcal of palm, olive or safflower oil) that the expression of PLIN2 increased more in response to unsaturated versus saturated fats. Therefore, it is possible that the type of dietary fat differentially regulates the expression of each PLIN protein.

The lack of change in PLIN5 content in the present study in comparison to the study by Badin et al. [3] may again be due to the duration of the diet and potentially the time course of increased PLIN5 expression. HFD of ~4 weeks are known to initially increase mitochondrial content and oxidation [23], however HFD >4 weeks result in a decrement of mitochondrial content and function [2]. It is possible that PLIN5 protein content follows a similar pattern as the mitochondrial content as recent findings indicate that PLIN5 may have a function in mitochondrial fatty acid oxidation [8, 9]. Future work is needed to elucidate this time course and the role that these two PLIN proteins may have in response to different fatty acids in skeletal muscle lipid storage and use.

This study is the first to examine the effects of a HFD on skeletal muscle PLIN protein content in offspring as well as dams. Our findings show that a maternal HFD may have


A
HFD DAM
HFD M21
HFD F21
HFD M3
HFD F3
CON DAM
CON M21
CON F21
CON M3
CON F3
~52 Kda
ACTIN
Dams
Offspring
PLIN2 (A.U.)
0.4
0.3
0.2
0.1
0.0
0.6
0.4
0.2
0.0
*
Control
HFD
Weaning
3 Months
B
~47 Kda
ACTIN
PLIN3 (A.U.)
1.0
0.8
0.6
0.4
0.2
0.0
C
~50 Kda
ACTIN
PLIN5 (A.U.)
0.20
0.15
0.10
0.05
0.00
3 months

◀ **Fig. 4** **a** Plin2 protein content (arbitrary units) in dams (*left panel*) and offspring (*right panel*) with representative Western blot. *Significantly lower than weaning ($p < 0.05$). **b** Plin3 protein content (arbitrary units) in dams (*left panel*) and offspring (*right panel*) with representative Western blot. *Significantly higher than control dams ($p < 0.05$). **c** Plin5 protein content (arbitrary units) in dams (*left panel*) and offspring (*right panel*) with representative Western blot. *Significantly lower than weaning and significantly higher from control ($p < 0.05$) at weaning and 3 months. Representative blots are shown from left as high fat (HF) dam, 21 day old high fat male (HF M21), 21 day old high fat female (HF F21), 3 month old high fat male (HF M3), 3 month old high fat female (HF F3), control dam (CON), 21 day old control male (CON 21 M), 21 day old control female (CON F21), 3 month old control male (CON M3), and 3 month old control female (CON F3)

long lasting effects on skeletal muscle metabolism in offspring, as demonstrated by higher muscle triacylglycerol and PLIN5 protein content in young adult offspring of high fat fed dams. Dams were also shown to be responsive to a HFD. PLIN3 protein content increased in response to a 16-week HFD, thus indicating that PLIN3 may be important in longer-term diet-induced lipid storage in skeletal muscle.

Acknowledgments The in vivo portion of this study was funded through start-up funding from Brock University to W. Ward. Analyses were funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to S. J. Peters. Lipid analyses were funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to P.J. LeBlanc. Laboratory infrastructure support was provided by the Canadian Foundation for Innovation (B. Roy), the Ontario Innovation Trust (B. Roy), and NSERC. R. MacPherson is the recipient of an Ontario Graduate Scholarship in Science and Technology, as well as a NSERC Doctoral Postgraduate Scholarship. L. Castelli received graduate student support through an Ontario Graduate Scholarship (OGS). P. Miotto received graduate student support through a Canadian Institutes of Health Research (CIHR) Frederick Banting and Charles Best Canada Graduate Scholarship. W. Ward holds a Canada Research Chair in Bone and Muscle Development.

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the authors.

References

1. Alsted TJ, Nybo L, Schweiger M, Fledelius C, Jacobsen P, Zimmermann R, Zechner R, Kiens B (2009) Adipose triglyceride lipase in human skeletal muscle is upregulated by exercise training. Am J Physiol Endocrinol Metab 296:E445–E453
2. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW 3rd, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, Cortright RN, Wasserman DH, Neufer PD (2009) Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Investig 119:573–581
3. Badin PM, Vila IK, Louche K, Mairal A, Marques MA, Bourlier V, Tavernier G, Langin D, Moro C (2013) High-fat diet-mediated lipotoxicity and insulin resistance is related to impaired lipase expression in mouse skeletal muscle. Endocrinology 154:1444–1453
4. Bayol SA, Macharia R, Farrington SJ, Simbi BH, Stickland NC (2009) Evidence that a maternal "junk food" diet during pregnancy and lactation can reduce muscle force in offspring. Eur J Nutr 48:62–65
5. Bayol SA, Simbi BH, Stickland NC (2005) A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. J Physiol 567:951–961
6. Bonen A, Parolin ML, Steinberg GR, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Heigenhauser GJ, Dyck DJ (2004) Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. FASEB J 18:1144–1146
7. Bosma M, Hesselink MK, Sparks LM, Timmers S, Ferraz MJ, Mattijssen F, van Beurden D, Schaart G, de Baets MH, Verheyen FK, Kersten S, Schrauwen P (2012) Perilipin 2 improves insulin sensitivity in skeletal muscle despite elevated intramuscular lipid levels. Diabetes 61:2679–2690
8. Bosma M, Minnaard R, Sparks LM, Schaart G, Losen M, de Baets MH, Duimel H, Kersten S, Bickel PE, Schrauwen P, Hesselink MK (2012) The lipid droplet coat protein perilipin 5 also localizes to muscle mitochondria. Histochem Cell Biol 137:205–216
9. Bosma M, Sparks LM, Hooiveld GJ, Jorgensen JA, Houten SM, Schrauwen P, Kersten S, Hesselink MK (1831) Overexpression of PLIN5 in skeletal muscle promotes oxidative gene expression and intramyocellular lipid content without compromising insulin sensitivity. Biochim Biophys Acta 844–852:2013
10. Bradley NS, Heigenhauser GJ, Roy BD, Staples EM, Inglis JG, LeBlanc PJ, Peters SJ (1985) The acute effects of differential dietary fatty acids on human skeletal muscle pyruvate dehydrogenase activity. J Appl Physiol 104(1–9):2008
11. Brasaemle DL (2007) Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. J Lipid Res 48:2547–2559
12. Brasaemle DL, Barber T, Wolins NE, Serrero G, Blanchette-Mackie EJ, Londos C (1997) Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. J Lipid Res 38:2249–2263
13. Brown DA (2001) Lipid droplets: proteins floating on a pool of fat. Curr Biol 11:R446–R449
14. Bulankina AV, Deggerich A, Wenzel D, Mutenda K, Wittmann JG, Rudolph MG, Burger KN, Honing S (2009) TIP47 functions in the biogenesis of lipid droplets. J Cell Biol 185:641–655
15. Dalen KT, Dahl T, Holter E, Arntsen B, Londos C, Sztalryd C, Nebb HI (2007) LSDP5 is a PAT protein specifically expressed in fatty acid oxidizing tissues. Biochim Biophys Acta 1771:210–227
16. de Wilde J, Smit E, Snepvangers FJ, de Wit NW, Mohren R, Hulshof MF, Mariman EC (2010) Adipophilin protein expression in muscle–a possible protective role against insulin resistance. FEBS J 277:761–773
17. Ducharme NA, Bickel PE (2008) Lipid droplets in lipogenesis and lipolysis. Endocrinology 149:942–949
18. ED O (1993) Guide to the care and use of experimental animals, 2nd ed. In: Canadian Council on Animal Care 1
19. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
20. Franco JG, Fernandes TP, Rocha CP, Calvino C, Pazos-Moura CC, Lisboa PC, Moura EG, Trevenzoli IH (2012) Maternal high-fat diet induces obesity and adrenal and thyroid dysfunction in male rat offspring at weaning. J Physiol 590:5503–5518

21. Fukushima M, Enjoji M, Kohjima M, Sugimoto R, Ohta S, Kotoh K, Kuniyoshi M, Kobayashi K, Imamura M, Inoguchi T, Nakamuta M, Nawata H (2005) Adipose differentiation related protein induces lipid accumulation and lipid droplet formation in hepatic stellate cells. In Vitro Cell Dev Biol Anim 41:321–324
22. Gao J, Serrero G (1999) Adipose differentiation related protein (ADRP) expressed in transfected COS-7 cells selectively stimulates long chain fatty acid uptake. J Biol Chem 274:16825–16830
23. Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, Holloszy JO (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci USA 105:7815–7820
24. Hsieh K, Lee YK, Londos C, Raaka BM, Dalen KT, Kimmel AR (2012) Perilipin family members preferentially sequester to either triacylglycerol-specific or cholesteryl-ester-specific intracellular lipid storage droplets. J Cell Sci 125:4067–4076
25. Imamura M, Inoguchi T, Ikuyama S, Taniguchi S, Kobayashi K, Nakashima N, Nawata H (2002) ADRP stimulates lipid accumulation and lipid droplet formation in murine fibroblasts. Am J Physiol Endocrinol Metab 283:E775–E783
26. Kimmel AR, Brasaemle DL, McAndrews-Hill M, Sztalryd C, Londos C (2010) Adoption of PERILIPIN as a unifying nomenclature for the mammalian PAT-family of intracellular lipid storage droplet proteins. J Lipid Res 51:468–471
27. Kishimoto K, Urade R, Ogawa T, Moriyama T (2001) Nondestructive quantification of neutral lipids by thin-layer chromatography and laser-fluorescent scanning: suitable methods for "lipidome" analysis. Biochem Biophys Res Commun 281:657–662
28. Koopman R, Schaart G, Hesselink MK (2001) Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. Histochem Cell Biol 116:63–68
29. Listenberger LL, Ostermeyer-Fay AG, Goldberg EB, Brown WJ, Brown DA (2007) Adipocyte differentiation-related protein reduces the lipid droplet association of adipose triglyceride lipase and slows triacylglycerol turnover. J Lipid Res 48:2751–2761
30. Liu P, Ying Y, Zhao Y, Mundy DI, Zhu M, Anderson RG (2004) Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic. J Biol Chem 279:3787–3792
31. Londos C, Sztalryd C, Tansey JT, Kimmel AR (2005) Role of PAT proteins in lipid metabolism. Biochimie 87:45–49
32. Macpherson RE, Herbst EA, Reynolds EJ, Vandenboom R, Roy BD, Peters SJ (2012) Subcellular localization of skeletal muscle lipid droplets and PLIN family proteins OXPAT and ADRP at rest and following contraction in rat soleus muscle. Am J Physiol Regul Integr Comp Physiol 302:R29–R36
33. Macpherson RE, Ramos S, Vandenboom R, Roy BD, Peters SJ (2013) Skeletal muscle PLIN proteins, ATGL and CGI-58, interactions at rest and following stimulated contraction. Am J Physiol Regul Integr Comp Physiol 304:R644
34. Macpherson RE, Vandenboom R, Roy BD, Peters SJ (2013) Skeletal muscle PLIN3 and PLIN5 are serine phosphorylated at rest and following lipolysis during adrenergic or contractile stimulation. Physiol Rep 1:e00084
35. Mahadevappa VG, Holub BJ (1987) Quantitative loss of individual eicosapentaenoyl-relative to arachidonoyl-containing phospholipids in thrombin-stimulated human platelets. J Lipid Res 28:1275–1280
36. Minnaard R, Schrauwen P, Schaart G, Jorgensen JA, Lenaers E, Mensink M, Hesselink MK (2009) Adipocyte differentiation-related protein and OXPAT in rat and human skeletal muscle: involvement in lipid accumulation and type 2 diabetes mellitus. J Clin Endocrinol Metab 94:4077–4085
37. Miotto PM, Castelli LM, Amoye F, Leblanc PJ, Peters SJ, Roy BD, Ward WE (2013) Maternal high fat feeding does not have long-lasting effects on body composition and bone health in female and male wistar rat offspring at young adulthood. Molecules 18:15094–15109
38. Moore HP, Silver RB, Mottillo EP, Bernlohr DA, Granneman JG (2005) Perilipin targets a novel pool of lipid droplets for lipolytic attack by hormone-sensitive lipase. J Biol Chem 280:43109–43120
39. Moro C, Bajpeyi S, Smith SR (2007) Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. Am J Physiol Endocrinol Metab 294:E203–E213
40. Murphy DJ (2001) The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog Lipid Res 40:325–438
41. Peters SJ, Samjoo IA, Devries MC, Stevic I, Robertshaw HA, Tarnopolsky MA (2012) Perilipin family (PLIN) proteins in human skeletal muscle: the effect of sex, obesity, and endurance training. Appl Physiol Nutr Metab 37:724–735
42. Prats C, Donsmark M, Qvortrup K, Londos C, Sztalryd C, Holm C, Galbo H, Ploug T (2006) Decrease in intramuscular lipid droplets and translocation of HSL in response to muscle contraction and epinephrine. J Lipid Res 47:2392–2399
43. Purcell RH, Sun B, Pass LL, Power ML, Moran TH, Tamashiro KL (2011) Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. Physiol Behav 104:474–479
44. Ramos SV, MacPherson RE, Turnbull PC, Bott KN, LeBlanc P, Ward WE, Peters SJ (2014) Higher PLIN5 but not PLIN3 content in isolated skeletal muscle mitochondria following acute in vivo contraction in rat hindlimb. Physiol Rep 2
45. Russell AP (2004) Lipotoxicity: the obese and endurance-trained paradox. Int J Obes Relat Metab Disord 28(suppl 4):S66–S71
46. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WH, Nicolay K, Schrauwen P (2005) Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. Obes Res 13:2088–2094
47. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM (2008) Maternal obesity at conception programs obesity in the offspring. Am J Physiol Regul Integr Comp Physiol 294:R528–R538
48. Shaw CS, Shepherd SO, Wagenmakers AJ, Hansen D, Dendale P, van Loon LJ (2012) Prolonged exercise training increases intramuscular lipid content and perilipin 2 expression in type I muscle fibres of patients with type 2 diabetes. Am J Physiol Endocr Metab
49. Shaw CS, Sherlock M, Stewart PM, Wagenmakers AJ (2009) Adipophilin distribution and colocalization with lipid droplets in skeletal muscle. Histochem Cell Biol 131:575–581
50. Shepherd SO, Cocks M, Tipton KD, Ranasinghe AM, Barker TA, Burniston JG, Wagenmakers AJ, Shaw CS (2012) Preferential utilization of perilipin 2-associated intramuscular triglycerides during 1 h of moderate-intensity endurance-type exercise. Exp Physiol 97:970–980
51. Shepherd SO, Cocks M, Tipton KD, Ranasinghe AM, Barker TA, Burniston JG, Wagenmakers AJ, Shaw CS (2013) Sprint interval and traditional endurance training increase net intramuscular triglyceride breakdown and expression of perilipin 2 and 5. J Physiol 591:657–675
52. Simar D, Chen H, Lambert K, Mercier J, Morris MJ (2012) Interaction between maternal obesity and post-natal over-nutrition on skeletal muscle metabolism. Nutr Metab Cardiovasc Dis 22:269–276
53. Stellingwerff T, Boon H, Jonkers RA, Senden JM, Spriet LL, Koopman R, van Loon LJ (2007) Significant intramyocellular lipid use during prolonged cycling in endurance-trained males as assessed by three different methodologies. Am J Physiol Endocr Metab 292:E1715–E1723

54. Sun B, Purcell RH, Terrillion CE, Yan J, Moran TH, Tamashiro KL (2012) Maternal high-fat diet during gestation or suckling differentially affects offspring leptin sensitivity and obesity. Diabetes 61:2833–2841
55. Tansey JT, Sztalryd C, Hlavin EM, Kimmel AR, Londos C (2004) The central role of perilipin a in lipid metabolism and adipocyte lipolysis. IUBMB Life 56:379–385
56. Timmers S, de Vogel-van den Bosch J, Hesselink MK, van Beurden D, Schaart G, Ferraz MJ, Losen M, Martinez-Martinez P, De Baets MH, Aerts JM, Schrauwen P (2011) Paradoxical increase in TAG and DAG content parallel the insulin sensitizing effect of unilateral DGAT1 overexpression in rat skeletal muscle. PloS One 6:e14503
57. Unger RH, Scherer PE (2010) Gluttony, sloth and the metabolic syndrome: a roadmap to lipotoxicity. Trends Endocr Metab 21:345–352
58. van Loon LJ, Koopman R, Manders R, van der Weegen W, van Kranenburg GP, Keizer HA (2004) Intramyocellular lipid content in type 2 diabetes patients compared with overweight sedentary men and highly trained endurance athletes. Am J Physiol Endocr Metab 287:E558–E565
59. van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA, Saris WH (2003) Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. J Physiol 553:611–625
60. Virtue S, Vidal-Puig A (1801) Adipose tissue expandability, lipotoxicity and the metabolic syndrome—an allostatic perspective. Biochim Biophys Acta 338–349:2010
61. Wainwright PE (1998) Issues of design and analysis relating to the use of multiparous species in developmental nutritional studies. J Nutr 128:661–663
62. Wang H, Sreenevasan U, Hu H, Saladino A, Polster BM, Lund LM, Gong DW, Stanley WC, Sztalryd C (2011) Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria. J Lipid Res 52:2159–2168
63. White CL, Purpera MN, Morrison CD (2009) Maternal obesity is necessary for programming effect of high-fat diet on offspring. Am J Physiol Regul Integr Comp Physiol 296:R1464–R1472
64. Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A, Bickel PE (2005) S3-12, Adipophilin, and TIP47 package lipid in adipocytes. J Biol Chem 280:19146–19155
65. Wolins NE, Quaynor BK, Skinner JR, Tzekov A, Croce MA, Gropler MC, Varma V, Yao-Borengasser A, Rasouli N, Kern PA, Finck BN, Bickel PE (2006) OXPAT/PAT-1 is a PPAR-induced lipid droplet protein that promotes fatty acid utilization. Diabetes 55:3418–3428
66. Wolins NE, Rubin B, Brasaemle DL (2001) TIP47 associates with lipid droplets. J Biol Chem 276:5101–5108