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Subcellular localization of skeletal muscle lipid droplets and PLIN family proteins OXPAT and ADRP at rest and following contraction in rat soleus muscle


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GROWING EVIDENCE INDICATES that skeletal muscle lipolysis is regulated by protein-protein interactions occurring on the surface of triacylglycerol (TAG)-storing lipid droplets (22, 31). The most abundant proteins found on the lipid droplet surface are the PLIN family of proteins, of which there are five members. Each member of the family is named after the founding protein of the PAT family (perilipin, PLIN), with the different proteins numbered sequentially (PLIN1–5) (19). The PLIN family consists of five members: perilipin (PLIN1), ADRP (ADR), adipophilin (PLIN2), tail-interacting protein of 47 kDa (TIP47), PLIN3, S3–12 (PLIN4), and OXPAT (MLDP, LSD5, PLN5) (28). In general, lipid droplets are coated with one or more members of this protein family, each serving important roles in regulating lipolysis. It is thought that each PLIN protein plays a unique role in regulating lipid metabolism due to distinct tissue distributions; however, the mechanisms regulating lipid droplet dynamics in skeletal muscle are largely unknown.

To date PLIN1 is the only PAT protein for which a distinct role has been established in regulating both TAG storage and lipolysis in adipocytes. At rest in adipocytes, PLIN1 coats the lipid droplet regulating the activities of the lipases [adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) to the triglycerides (TAGs)] (5, 29). ATGL activity requires activation by comparative gene identification-58 (CGI-58) (34). At rest, CGI-58 is prevented from interacting with ATGL because it is colocalized with PLIN1 on the surface of lipid droplets. HSL is found in the cytoplasm and has several serine residues that when phosphorylated result in activation and translocation to the lipid droplet (4, 6). In adipocytes, activation of the lipases occurs via a hormonal route. During lipolytic stimulation catecholamines bind to β-adrenergic receptors on the cell surface. Adenylate cyclase is then activated through the action of a stimulatory G protein, the intracellular level of cAMP is elevated, and cAMP-dependent PKA is activated. PKA phosphorylates both perilipin and HSL (5, 15, 29). The phosphorylated perilipin releases CGI-58, which is now free to interact and activate ATGL, promoting degradation of TAGs to diacylglycerols (11). Perilipin phosphorylation also permits the phosphorylated HSL to access the lipid droplet (29, 30, 38). This may be due to phosphorylated perilipin leaving the lipid droplet or causing the large droplets to fragment into smaller ones, thereby increasing the accessible droplet surface area, which may enhance the activity of ATGL and HSL at the lipid droplets (25).

However, information on lipid droplet dynamics in skeletal muscle is scarce, and researchers have extrapolated
current knowledge from other tissues to make conclusions/hypotheses about skeletal muscle (26). Given that PLIN1 is not present in skeletal muscle and the tissue distributions of both PLIN2 and PLIN5, these two PAT proteins are the most likely candidates to be involved in regulating muscular lipid storage and use (27). PLIN2 is ubiquitously expressed and is one of the predominant PAT proteins found in skeletal muscle (3, 32). The exact role of PLIN2 is currently unknown; however, because of its position on the lipid droplet membrane, it may regulate lipolysis through interactions with lipolytic enzymes at the lipid droplet surface (21, 32). Studies in cell culture (Chinese hamster ovary cells and human embryonic kidney 293 cells) have shown that PLIN2 coats the lipid droplet membrane and is stable in the presence of neutral lipids but is otherwise targeted for degradation by proteasomes (21, 45, 46). Information in skeletal muscle regarding PLIN2 distribution and its association with lipid droplets is limited and contradictory. One study determined that PLIN2 is highly colocalized (80%) to the lipid droplet surface, making it an ideal marker of lipid droplets (32), while a recent study has found that this is not the case, with only 64% of PLIN2 associated with lipid droplets (36).

PLIN5 is unique in that its distribution is restricted to tissues that undergo high rates of lipolysis, such as skeletal muscle (specifically type 1 fibers), cardiac muscle, liver, and brown adipose tissue (8, 44, 47). Because of this unique distribution, it has been hypothesized that PLIN5 facilitates the oxidation of intracellular lipids in these tissues. Further, PLIN5 expression is induced by physiological, pharmacological, and genetic perturbations that increase utilization of fatty acids for oxidative phosphorylation (8, 44, 47). For example, PLIN5 mRNA and protein content is increased in skeletal muscle (3, 32). The exact role of PLIN2 is currently unknown; however, because of its position on the lipid droplet membrane, it may regulate lipolysis through interactions with lipolytic enzymes at the lipid droplet surface (21, 32). Studies in cell culture (Chinese hamster ovary cells and human embryonic kidney 293 cells) have shown that PLIN2 coats the lipid droplet membrane and is stable in the presence of neutral lipids but is otherwise targeted for degradation by proteasomes (21, 45, 46). Information in skeletal muscle regarding PLIN2 distribution and its association with lipid droplets is limited and contradictory. One study determined that PLIN2 is highly colocalized (80%) to the lipid droplet surface, making it an ideal marker of lipid droplets (32), while a recent study has found that this is not the case, with only 64% of PLIN2 associated with lipid droplets (36).

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Given that the location of a protein is generally related to its physiological function, the myocellular locations of both PLIN2 and PLIN5 are crucial in understanding their roles. Therefore, to fully understand how PLIN5 may regulate skeletal muscle lipolysis, it is important to first determine the location of this protein at rest and following contraction. The purpose of this study is twofold; we plan first to investigate the myocellular location and colocalization of lipid droplets, PLIN2, and PLIN5 and second to determine whether PLIN5 is recruited to lipid droplets with contraction. It was hypothesized that contraction would recruit a cytosolic pool of PLIN5 to the lipid droplet surface, allowing fatty acids to be oxidized and used for energy.

MATERIALS AND METHODS

Animals. A total of 10 male Long-Evans rats (~4–6 wk old, body mass 163 ± 13 g) were used in this study. Animals were housed in groups within the Brock University Animal Facility, where they were maintained on a 12:12-h light-dark cycle at ~22°C. The rats were fed a standard rodent diet and had ad libitum access to food and water. All experimental procedures and protocols were approved by the Brock University Animal Care and Utilization Committee and conform to all Canadian Council on Animal Care guidelines.

Muscle preparation. Animals were anesthetized via intraperitoneal injection of pentobarbital sodium (6 mg/100g body wt), and then the left and right soleus muscles were removed and placed in an organ bath, where they were assigned to one of two experimental groups: (1) rest or (2) stimulated. To briefly summarize the preparation, each soleus muscle was dissected from tendon-to-tendon, sutures were tied in-situ, the muscle was removed and immediately placed in an organ bath (Radnoti Glass Technology, Monrovia, CA), which contained 15 ml of fully oxygenated liquid Sigma medium 199 and suspended at a resting tension of 1 g force. The incubation medium was continuously gassed with 95% O_2, 5% CO_2, and temperature was maintained at 25°C by circulating distilled water from a bath through an outer water jacket of the incubation reservoir (1). Muscles were incubated at rest for 30 min to equilibrate. After the initial incubation, the muscles were assigned to either the rest or stimulated group.

Stimulation protocol. Following the equilibration period the muscles remained at rest or were stimulated to contract for 30 min. Initially, optimal stimulus voltage was determined by assessing force responses (Grass Telefactor force transducer, West Warwick, RI) to single electrical pulses (Grass Model FT03 with P11T amplifier). Stimulus intensity was increased from 10 V in 10-V increments, until a plateau in twitch force was reached, after which stimulus voltage was increased to ~1.25 of this level. During the 30-min stimulus protocol, muscles received repeated volleys of brief (150 ms) but high-frequency (60 Hz) trains at a train rate of 20 tetani/min (muscles were suspended at 1 g of resting tension throughout). This protocol has previously proven to elicit maximal rates of triglyceride pool turnover and rates of TAG oxidation without the development of fatigue (9). Throughout this period, muscle force production was recorded using Grass Polyview Data Acquisition and Analysis System (West-Warwick, RI) and analyzed using the Polyview Reviewer (Grass Polyview Data Acquisition and Analysis System; Astro-Med, West-Warwick, RI).

Metabolite analysis. Following the 60-min incubations, soleus muscles were removed from the bath and cut into two pieces, with one piece being snap frozen in liquid nitrogen for metabolite analysis and the other piece being mounted for histochemical analysis (see below). The extraction process was completed after the frozen soleus sections were freeze dried (Labconco; Kansas City, MO). Muscles were powdered and any visible blood and connective tissue were removed, and then acid was extracted for measurement of muscle metabolites (ATP, PCr, and lactate). Muscle metabolite content was determined by fluorometric techniques according to the procedures described by Harris et al. (16) and modified by Green et al. (14). Each sample was analyzed in triplicate during the same analytical session for each of the measured metabolites.

Table 1. Muscle metabolite concentrations

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>ATP (mmol/kg dry wt)</th>
<th>PCr (mmol/kg dry wt)</th>
<th>Lactate (mmol/kg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>21.27 ± 1.4</td>
<td>56.49 ± 4.2</td>
<td>12.57 ± 1.8</td>
</tr>
<tr>
<td>Stimulated</td>
<td>20.09 ± 0.9</td>
<td>45.18 ± 3.1†</td>
<td>25.05 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (mmol/kg dry wt). PCr, phosphocreatine. *Significant difference between experimental groups (P < 0.05). †Trend (P = 0.053).
**Immunohistochemical analysis.** The muscle section used for histochemical analysis was oriented for transverse sections and mounted, in embedding medium (Cryomatrix, Pittsburgh, PA), 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. Sections were (10 μm thick) thaw mounted onto slides and stored at −80°C until immunohistochemical staining.

To permit the examination of lipid droplets stained by oil red O (ORO; O0625; Sigma-Aldrich, St. Louis, MO) together with immunolabeled PLIN5 or PLIN2, the same protocol developed by Koopman et al. (20) was utilized (20, 37, 41, 42). PBS was used for dilution of antibodies and reagents, and for use in the washing steps of the protocol. 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 treated with 0.5% Triton X-100 in PBS for 5 min and washed three times with PBS for 5 min. Sections were then incubated for 1 h at room temperature with a primary antibody against PLIN5 (no. GP31; Guinea pig polyclonal; Progen, Heidelberg, Germany) or PLIN2 (no. GP40; Guinea pig polyclonal; Progen) in the appropriate dilution (1:50 and 1:100, respectively). Incubation was followed by three

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**Fig. 1.** Digitally captured images of one single field of view (×40 magnification) taken from a soleus muscle cross section. Oil red O (ORO), PLIN5, and PLIN2 immunofluorescent staining. A: PLIN2 (green; left), ORO (red; middle), and combined staining (right), showing higher levels of PLIN2 and lipid droplets closer to the sarcolemmal region. B: PLIN5 (green; left), ORO (red; middle), and combined staining (right), showing higher levels of PLIN5 and lipid droplets closer to the sarcolemmal region. C: PLIN5 (green; left), PLIN2 (red; middle), and combined staining (right).
5-min washes in PBS. Then the appropriate fluorescent secondary antibody Alexa Fluor 488 (1:200 in PBS; A-11073; Goat anti-guinea pig; Molecular Probes, Invitrogen, Breda, The Netherlands) was applied for 60 min at room temperature. This was followed by a 15-min wash with three exchanges of PBS. The slides were then immersed in the working solution of ORO for 30 min. Slides were rinsed three times in deionized water. Cover slips were mounted with a prolonging agent (no. P36930; Prolong Gold anti-fade reagent; Invitrogen).

Double staining of PLIN5 and PLIN2 together was performed by simultaneous incubation with primary antibodies against PLIN5 (no. GP31; Guinea pig polyclonal; Progen, Heidelberg, Germany) and PLIN2 (ab37516; chicken polyclonal; Abcam, Cambridge, MA). Visualization of primary antibody binding was achieved with the following secondary antibodies: Alexa Fluor 488 (1:200 in PBS; A-11073; Goat anti-guinea pig; Molecular Probes, Invitrogen), and Alexa Fluor 555 (1:200 in PBS; A-21437; goat anti-chicken; Molecular Probes). Control steps were performed to eliminate the chance of cross-reaction between the ORO and one of the antibodies, or between the pair of antibodies, where either ORO or one of the antibodies were omitted; resulting in no signal for all cases (data not shown).

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 and the 488 and 550 fluorophores, the FITC UV (450–490 nm) and TRITC (510–560 nm) excitation filters were used. Digitally captured images (×40 magnification), four fields of view/muscle cross section (17.9 ± 1.1 fibers/field of view), were processed and analyzed using imaging software (NIS-Elements AR 3.00; Nikon Instruments, Melville, NY). An intensity threshold representing minimal values corresponding to lipid droplets, PLIN2, and PLIN5 was set manually and applied uniformly in all images.

The lipid droplets, PLIN5, and PLIN2 fluorescent signals were quantified for each muscle fiber, resulting in a total of 4,315 muscle fibers analyzed for each muscle cross section. Fiber area, as well as the number and area of objects emitting a fluorescent signal, were recorded. Muscle fiber lipid droplet, PLIN2, and PLIN5 content was expressed as the fraction of the measured area that was stained (41).

Within each separate muscle cross section, 67 ± 5 muscle fibers were selected to further investigate the lipid droplet, PLIN2, and PLIN5 distribution patterns. To determine distribution, the area of the objects within eight successive bands of 2 μm in width from the sarcolemma toward the central region (16 μm from sarcolemma) of each muscle fiber was recorded. Lipid and protein content of these bands was recorded as area fraction (area stained divided by total area measured in each band/central region) (41).

Colocalization of the fluorescent signals was determined in four fields of view per muscle cross section. Pearson’s linear regression analyses were performed for each field of view, and coefficients of determination (r²) were averaged for each cell.

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 intraobserver and interobserver reliability were evaluated by two investigators. The intraobserver reliability involved the two investigators performing analysis of one image three times, at least 1 wk apart. The interobserver reliability involved two independent investigators performing analysis for three separate images. These tests proved to be reliable with a coefficient of variation <5% for both intraobserver and interobserver reliability.

Statistics. Differences in total content and colocalization coefficients were evaluated by t-tests. Comparisons of lipid droplet and protein content in successive 2-μm bands from the sarcolemma were performed using a two-way ANOVA (rest or stimulated, level of band) and a best-fit multiple regression analysis. Tukey post hoc tests were performed when significance was detected. Statistical significance was set at P < 0.05. All data are expressed as means ± SE.

RESULTS

Muscle viability and force. The viability of incubating muscles at 25°C was verified by the maintenance of muscle ATP, phosphocreatine, and lactate concentrations (Table 1). Further, adequate oxygenation and muscle viability were assessed by the ability to maintain force production over the duration of
contractions (30 min), as previously demonstrated (9, 33). The initial isometric force normalized to soleus mass was 106.8 ± 11.4 g/g wet wt (n = 10). During stimulation, force output was recorded at 5-min intervals, and this was not noted to vary by more than 5% of initial isometric force at any point during the protocol. Accordingly, little or no fatigue or muscle degradation was evident in our results, indicating that muscle remained stable and viable during the entire protocol.

Lipid droplet, PLIN2, and PLIN5 content. The average muscle fiber area was 2,631 ± 87 μm². Figure 1 shows representative images of rat skeletal muscle cross sections viewed with an immunofluorescence microscope following incubation with lipid droplet protein antibodies (PLIN2 and PLIN5) combined with ORO. The antibodies directed against PLIN2 (Fig. 1A: green; 1C: red) and PLIN5 (Fig. 1B: green) successfully stained the proteins with visually distinct dots in the central area of the muscles fibers. In addition, areas of intense fluorescence signal can be seen in the subsarcolemma region most likely reflecting accumulations of densely packed lipid droplets. Fiber lipid droplet content was greater in the resting muscle group compared with the stimulated group (0.030 ± 0.009% rest vs. 0.012 ± 0.006% area lipid stained contraction, P = 0.029; Fig. 2A). Fiber PLIN2 content did not differ between resting and stimulated muscles (0.22 ± 0.05% rest and 0.14 ± 0.04% area PLIN2-stained contraction, P = 0.23; Fig. 2B). Fiber PLIN5 content did not differ between resting and stimulated muscle groups (0.035 ± 0.004% rest and 0.038 ± 0.009% are PLIN5 stained stimulated, P = 0.081; Fig. 2C).

Lipid droplet, PLIN2, and PLIN5 distribution. Lipid droplet distribution is presented as lipid content in 2-μm-wide bands from the sarcolemma toward the central region (16 μm) of the fiber and is expressed as the area fraction lipid stained (Fig. 3A). There was a greater lipid content in resting muscles compared with stimulated muscles at all band levels (P = 0.001). The concentration of the lipid droplets appeared to be greater closer to the sarcolemma; however, no significant difference was found for lipid droplet content between the different band levels (P = 0.23). Regression analysis demonstrates that there was an exponential decline in lipid content from the sarcolemma to the center of the fiber in the resting muscles (P = 0.001, r² = 0.99). Regression analysis of the stimulated muscles revealed that there was a linear decline in lipid droplet content starting from the sarcolemma (slope = -0.0023 ± 0.0006, P < 0.001, r² = 0.93).

Both PLIN5 and PLIN2 distribution mirror the lipid droplet distribution showing distinct intracellular dots and a higher concentration closer to the sarcolemma (Fig. 1, A and B). There was a main effect for PLIN2 content between rest and stimulated muscles regardless of band level (P = 0.004, Fig. 3B). PLIN2 content was significantly greater in the 2-μm region compared with the 6-, 8-, 10-, 12-, 14-μm, and center regions (P < 0.05). Regression analysis demonstrated that in both resting and stimulated muscles, there was an exponential decline in PLIN2 content from the sarcolemma to the central region of the fiber (P < 0.0001, r² = 0.99 rest; P = 0.0004, r² = 0.98 stimulated). PLIN5 content did not differ between resting and stimulated muscles when analyzed for content in 2-μm-wide bands from the sarcolemma toward the onset of the central region of the fiber (P = 0.34, Fig. 3C). PLIN5 content was significantly greater in the 2- and 4-μm regions compared with the 8-, 10-, 12-, 14-μm, and central regions (P < 0.05).
PLIN5 content in the 6-μm region was significantly greater compared with the 14-μm and center region \((P < 0.05)\). Regression analysis demonstrated that in both resting and stimulated muscles, there was a linear decline in PLIN5 content from the sarcolemma to the central region of the fiber \((\text{slope} = -0.0085 \pm 0.0009, P < 0.0001, r^2 = 0.94 \text{ rest}; \text{slope} = -0.0078 \pm 0.0010, P = 0.0003, r^2 = 0.91 \text{ stimulated})\).

Colocalization of lipid droplets and PLIN2, and lipid droplet and PLIN5. Combined ORO staining of lipid droplets with PLIN2 or PLIN5 allowed the position of the lipid droplets to be observed in relation to each of these PAT proteins. All images were quantified by Pearson’s correlation coefficient \((r^2)\). Immunostaining of PLIN2 was combined with ORO staining on muscle sections and viewed with wide field fluorescence microscopy (Fig. 4A). The Pearson’s correlation coefficient demonstrated that 66.4 ± 0.02% (rest) and 65.5 ± 0.02% (stimulated) of PLIN2 colocalized with lipid droplets with no significant difference between experimental groups \((P = 0.48)\). Colocalization of PLIN5 and lipid droplets demonstrated that 55.0 ± 0.02% (rest) and 57.7 ± 0.03% (stimulated) of PLIN5 colocalized with lipid droplets with no significant difference between experimental groups \((P = 0.47, \text{Fig. 4B})\). Combined staining of PLIN5 and PLIN2 reveals that 67.2 ± 0.02% (rest) and 66.5 ± 0.02% (stimulated) of PLIN5 and PLIN2 are colocalized with no significant difference between rest and stimulated muscles \((P = 0.792, \text{Fig. 4C})\).

**DISCUSSION**

This is the first study to investigate the content, distribution, and colocalization of lipid droplets, PLIN2, and PLIN5 in contracted skeletal muscle. The main findings from this study are that PLIN5 is not recruited to lipid droplets with muscle contraction and that both PLIN2 and PLIN5 are partially colocalized to lipid droplets at any given time, indicating that skeletal muscle lipid droplets differentially express PAT proteins.

This stimulation protocol was chosen to maximize intramuscular TG lipolysis and was effective as evidenced by the significant reduction in lipid droplet content in the stimulated group compared with the rested group (Fig. 2A) \((9)\). This decline in lipid content in the stimulated group was significant regardless of subcellular location, as demonstrated when fibers were analyzed using successive 2-μm bands from the sarcolemma to the fiber center. Further, regression analysis reveals that with stimulation there is a greater decline in lipid content in the subsarcolemmal region as the relationship changes from an exponential decline to a linear decline with stimulation (Fig. 3A). Overall, total PLIN2 content was not significantly different between experimental groups; however, there was a significantly lower PLIN2 content as a main effect when analyzed by regional band content (Figs. 2B and 3B). The absence of a significant difference in overall PLIN2 content may have been due to the large variance in muscle fiber content from the subsarcolemmal region to the center of the muscle fibers. Nonetheless, the significant drop in PLIN2 content with contraction in the present study is in agreement with previous data in cultured human embryonic kidney 293 and hamster ovary fibroblastic cells, demonstrating that PLIN2 is targeted for degradation if it is not bound to the lipid droplet membrane \((21, 46)\). Consequently, as the lipid droplet volume decreased during the contraction protocol, the PLIN2 bound to those lipid droplets may have been degraded in the cytosol. However, in the present study, only ~66% of PLIN2 content was found colocalized to lipid droplets, suggesting that 34% of the PLIN2...
protein is located in the cytosol. There are only two previous studies in skeletal muscle with varying results describing the colocalization of PLIN2 and lipid droplets. One study in rat soleus demonstrated a strong ~80% association between PLIN2 and lipid droplets, with no difference in this association in response to epinephrine or contraction (32). Although the other study in rested human vastus lateralis muscle demonstrated a partial, ~64%, association between PLIN2 and lipid droplets (36). Together, these results suggest that there is a consistent proportion of PLIN2 associated with lipid droplets, with some found free in the cytosol. Further research is needed to determine the physiological role that PLIN2 plays both at the lipid droplet surface and in the cytosol in skeletal muscle. Total PLIN5 content did not change with stimulation, and analyses of PLIN5 distribution further reveals no difference poststimulation (Figs. 2C and 3C).

A novel finding of this study is that both PLIN2 and PLIN5 mirror the myocellular distribution of the lipid droplets. Lipid content, as well as PLIN2 and PLIN5 content, is found in high density at the subsarcolemmal region with a progressive decline toward the central regions of the muscle fiber (Fig. 3, A–C). This lipid droplet distribution is similar to previous studies, which interestingly, the amount of lipid was higher in the periphery of the myofibers compared with the fibers’ central region (7, 35, 41). Interestingly, the lipid droplet and PAT protein distribution pattern observed in the present study is similar to the distribution pattern that has also been reported for mitochondria (7, 10, 35, 39). Shaw et al. (35) were the first to show that intramuscular TAG content and mitochondria have a similar pattern of distribution in human muscle with the highest density in subsarcolemmal regions with a progressive decline in the deeper regions of the muscle cell. This is in accord with previous reports in human skeletal muscle showing 2- to 3-fold greater mitochondrial density in the subsarcolemmal region compared with the intermyofibrillar area (7, 10, 24, 35, 40). It is possible that the similar distribution and close proximity of lipid droplets, PLIN2, PLIN5, and mitochondria may indicate a functional relationship, allowing the lipids to be hydrolyzed and oxidized efficiently during exercise (17, 35, 36, 39).

Lipid droplets appear to be differentially coated with PLIN2 and PLIN5. Both PLIN2 and PLIN5 were found to be partially colocalized with lipid droplets (~66% and 55%, respectively), and no significant changes in colocalization to lipid droplets in response to contraction were observed. It is not possible to determine the physiological significance of the partial colocalization of PLIN2 or PLIN5 with lipid droplets; however, it is hypothesized that subpopulations of lipid droplets presenting different PAT proteins have specific functions within skeletal muscle (36, 43, 44). Cell culture studies (murine fibroblasts, human embryonic kidney cells, MEA-RH7777 cells, and primary rat hepatocytes) have demonstrated that overexpression of PLIN2 encourages lipid accumulation (18, 21, 23), whereas downregulation of PLIN2 results in elevated rates of basal lipolysis (2). Thus, PLIN2 is thought to form a protective coat restricting lipolysis, and it may be possible that ~66% of the lipid droplets are protected from lipolysis. We believe that PLIN5 may play a role in regulating the interaction of this lipase and the lipase coactivator. Very recently, it was demonstrated that PLIN5 facilitates lipolysis by promoting the colocalization and functional interaction of CGI-58 and ATGL in the basal state (12, 13). Interestingly, while PLIN5 binds both CGI-58 and ATGL, the same PLIN5 molecule does not bind both at the same time (13). Moreover, ATGL interacts with PLIN5 in the cytoplasm and on intracellular structures lacking neutral lipid, indicating that the interaction does not require lipid droplets (13). Perhaps the portion of PLIN5 that is not colocalized to lipid droplets regulates how ATGL interacts with CGI-58 on the lipid droplet surface. Further study is needed to determine how PLIN5 regulates the interaction of ATGL and CGI-58 on the lipid droplet surface promoting lipolysis.

**Perspectives and Significance**

In summary, this study directly analyzed the spatial organization of lipid droplets, PLIN5, and PLIN2 in skeletal muscle in terms of content, distribution, and colocalization in response to contraction. The primary finding of this study is that PLIN5 is not recruited to lipid droplets with muscle contraction. Lipid droplets were found to be heterogeneous in nature in terms of their PAT protein content, and both PLIN2 and PLIN5 mirror the distribution of lipid droplets. Further investigation is needed to establish the exact roles that these proteins play in skeletal muscle lipid metabolism and should focus on the interactions that these proteins may have with lipases and lipase coactivators. Understanding the intricate mechanisms by which PAT proteins contribute to lipid storage and use will help to clarify both the physiology of healthy cells and tissues, as well as the basis of some important metabolic diseases.

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**DISCLOSURES**

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

**REFERENCES**


SKELETAL MUSCLE PLIN PROTEIN SUBCELLULAR LOCALIZATION


