Adipocyte Protein S3-12 Coats Nascent Lipid Droplets*

Most animals store lipid intracellularly in protein-coated droplets. The protein coat usually contains at least one member of the PAT (perilipin, adipose differentiation-related protein, and TIP47) family. Evidence suggests that PAT proteins control access to the lipid they enclose. The protein S3-12, which has sequence similarity to the PAT proteins, was found in a screen for adipocyte-specific proteins. The adipocyte expression of S3-12 and its similarity to the PAT proteins suggest that S3-12 is involved in adipocyte lipid storage. To test this hypothesis, we supplemented 3T3-L1 adipocytes with fatty acids and assessed the distribution of S3-12 by immunofluorescence microscopy. Prior to fatty acid incubation, S3-12 was distributed diffusely throughout the cytoplasm on punctate structures of heterogeneous size. After 10 min of lipid loading, S3-12 localized to 500-nm structures concentrated at the adipocyte periphery. After longer incubations, S3-12 coated the surface of lipid droplets up to several micrometers in diameter. Initially, these droplets were distinct from those droplets surrounded by perilipin; but by 240 min, most perilipin-coated droplets had some S3-12 on the surface as well. We additionally report that the formation of S3-12-coated droplets 1) required glucose and fatty acids that can be incorporated into triacylglycerol, 2) was blocked by an inhibitor of triacylglycerol synthesis, and 3) was insulin-dependent. This study reports for the first time the early morphological events in the genesis and maturation of adipocyte lipid droplets.

To survive periods of nutrient scarcity, animals have evolved the ability to store energy as neutral lipid during periods of nutrient excess. Most animals store neutral lipid in droplets surrounded by one or more proteins of the PAT (perilipin, adipose differentiation-related protein (ADRP), and TIP47) family (1). Until now, S3-12 has not been reported to associate with lipid droplets. Thus, it has been unclear whether or not S3-12 is a PAT protein.

The precise functions of PAT proteins are not known. The best characterized members are the perilipins, which facilitate lipid storage and play an important role in remodeling the adipocyte lipid droplet during lipolysis. A current model proposes that, under non-lipolytic conditions, perilipin surrounds lipid droplets, thereby blocking access of intracellular lipases to the underlying neutral lipids (6–9). Lipolytic activation of adipocytes through a cAMP-dependent pathway leads to hyperphosphorylation of perilipin and presumably to a change in its conformation that permits access of lipases to the neutral lipid core (10, 11). One of these lipases, hormone-sensitive lipase, is phosphorylated following lipolytic activation and translocates to the lipid droplet surface (12, 13). This recruitment of hormone-sensitive lipase requires the presence of perilipin that has intact amino-terminal phosphorylation sites (14). Finally, lipolytic activation of 3T3-L1 adipocytes fragments the lipid droplets (6, 15). These remodeling events change the morphology of the adipocyte to an extent that is observable by light microscopy. The importance of perilipin to lipid metabolism has been demonstrated in vivo. Consistent with the role of perilipin as a barrier to lipolysis, perilipin knockout mice have reduced triacylglycerol (TAG) in their adipose tissue and are resistant to both diet-induced and genetic obesity (7, 9). ADRP also may act as a lipolytic barrier around lipid droplets since overexpression of ADRP in cultured cells leads to increased fatty acid uptake (16) and to increased lipid droplet size and number (17).

Most cells import, esterify, and package fatty acid as TAG in lipid droplets. The events required to synthesize and package neutral lipid into PAT protein-coated droplets are poorly understood. The intracellular location of neutral lipid synthesis remains uncertain (18, 19). Furthermore, how neutral lipid gets from the site of synthesis to the core of the lipid droplets is not known. Given the major remodeling events observed when adipocytes are stimulated to release fatty acids, it is likely that remodeling also occurs when adipocytes are stimulated to store TAG. Accommodating and transporting hydrophobic TAGs

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‡The abbreviations used are: ADRP, adipose differentiation-related protein; TAG, triacylglycerol; BSA, bovine serum albumin; MES, 4-morpholineethanesulfonic acid; PBS, phosphate-buffered saline; WAT, white adipose tissue; BAT, brown adipose tissue.
likely require major changes in the arrangement of adipocyte membranes.

Intracellular trafficking of PAT proteins may be important for neutral lipid packaging. For example, cultured cells grown in serum-containing medium without supplemental lipids have few lipid droplets and express little ADRP protein, but such cells express abundant TIP47 as a soluble cytoplasmic protein. Supplementation of the medium with fatty acid induces lipid droplet formation, increases the amount of ADRP protein (2), and causes TIP47 to move onto nascent lipid droplets (4). Given the sequence similarity of S3-12 to the PAT proteins, we hypothesized that S3-12 participates in lipid droplet biogenesis. We report that S3-12 associates with the surfaces of nascent lipid droplets under conditions that promote TAG synthesis in a time-, substrate-, and insulin-dependent manner.

EXPERIMENTAL PROCEDURES

Reagents—Unless otherwise indicated, reagents were obtained from Sigma. Essentially fatty acid-free bovine serum albumin (BSA) was purchased from Intergen Co. (catalogue no. 3320; Purchase, NY). Protein A-Sepharose 4 Fast Flow was purchased from Amersham Biosciences. Octyl glucoside was purchased from Roche Applied Science. Antibodies—The antibody to perilipin was purchased from Research Diagnostics Inc. (catalogue no. prog29; Flanders, NJ), that to flotillin-2/epidermal surface antigen (ESA) from BD Biosciences (catalogue no. 610383), and that to UCPI (ungrouping protein-1) from Alpha Diagnostic International, Inc. (San Antonio, TX). The antibodies to the carboxy termini of flotillin-1 and of S3-12 (S3-12C) were raised in rabbits and affinity-purified against peptides SISQVNHNKPLRTA and GPFTSMPCGQL, respectively, by Research Genetics (Huntsville, AL). The antibody that recognizes the S3-12 amino terminus (S3-12N) was raised in rabbits and affinity-purified against S3-12 peptide MSASG-DGTRVPPKSKG by BIOSOURCE (Camarillo, CA).

Fatty Acids—Free fatty acids were solubilized with sodium hydroxide and bound to BSA at a molar ratio of 5:1.

Immunoprecipitations—Proteins were captured with antibodies bound to protein A-Sepharose beads as described previously (20).

TAG Measurements—Adipocytes were differentiated and trypsinized as described under “Indirect Immunofluorescence Microscopy,” replated at their original density, treated as described in the figure legends, washed five times, and harvested in TNET (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, and 1% Triton X-100) with 0.5% cholate. To normalize TAG for protein, the protein content of the lysate was measured using the BCA assay (Pierce) with BSA as the standard. The antibody to perilipin was purchased from Research Diagnostics Inc. (catalogue no. progp29; Flanders, NJ) and affinity-purified against peptides SISQVNHNKPLRTA and GPFTSMPCGQL, respectively, by Research Genetics (Huntsville, AL). The antibody that recognizes the S3-12 amino terminus (S3-12N) was raised in rabbits and affinity-purified against S3-12 peptide MSASG-DGTRVPPKSKG by BIOSOURCE (Camarillo, CA).

Statistical Analysis of TAG Measurements—The amount of TAG measured in adipocytes treated with oleate clearly differed from that measured in the untreated adipocytes and in adipocytes treated with oleate + tricinarin C (see Fig. 5A). However, it is not clear from inspection that the (oleate + tricinarin C)-treated adipocytes had more TAG than the untreated adipocytes. Because the mean amounts of TAG and S.D. values in the amount of TAG have a large and positive correlation, statistical analysis was performed on log-transformed data. The null hypothesis was tested as follows. For each day’s experiment, the measurements from the untreated adipocytes (n = 6) and from the (oleate + tricinarin C)-treated adipocytes (n = 6) were subtracted from the mean of the oleate-treated adipocytes (n = 6). These difference scores were used in an analysis of variance using two factors, treatments with two levels, days with three levels, and six observations within each of these six groups (see Fig. 5 legend).

Cell Culture—3T3-L1 mouse fibroblasts (American Type Culture Collection, Manassas, VA) were propagated and differentiated into adipocytes as described (21). Adipocytes were maintained in 10% FBS in “complete medium,” consisting of Dulbecco’s modified Eagle’s medium (catalogue no. 11065-084; Invitrogen) supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin.

Immunoblotting—Proteins were resolved on 4–12% NuPAGE gels run in the MES buffer system (Invitrogen) and transferred to nitrocellulose. Immuno-reactive proteins were revealed by enhanced chemiluminescence with ECL™ (Amersham Biosciences). Molecular mass standards were Precision Plus Dual Color Markers from Bio-Rad.

Protein Extraction from Tissue—C57BL/6 mice were asphyxiated with carbon dioxide in a protocol approved by the Animal Care Committee of Washington University. Tissues were harvested and immediately frozen in liquid nitrogen. The tissues were ground in liquid nitrogen and transferred to a tube that contained TNET with 1% octyl glucoside (TNETO) or TNET with 1% cholate (TNETC) supplemented with protease inhibitors (Complete™ EDTA-free tablet, Roche Applied Science). The ground tissue/detergent homogenate was vortexed for 30 s, incubated on ice for 10 min, vortexed for 30 s, and centrifuged at 21,000 × g for 20 min at 4 °C. Protein concentrations of the supernatants were determined by the BCA assay as described above under “TAG Measurements.”

Immunohistochemistry—Interscapular fat from a C57BL/6 mouse was fixed in 4% paraformaldehyde, paraffin-embedded, and cut into 4-μm sections for immunostaining. Antigens were unmasked with Nuclear Declarer (Biocare Medical, Walnut Creek, CA) in the case of S3-12 staining and with citrate buffer (pH 6) in the case of UCPI staining. Affinity-purified anti-S3-12N antibody was used at 5.5 μg/ml. Primary antibody to UCPI was diluted 1:400. Secondary antibody was biotinylated goat anti-rabbit IgG (PerkinElmer Life Sciences). Antigen-antibody complexes were detected with streptavidin-horseradish peroxidase (catalogue no. P0397; Dako Corp., Carpinteria, CA; followed by 3,3′-diamino-benzidine (Sigma). Slides were counterstained with hematoxylin. Tyramide amplification was used for S3-12 (but not UCPI) staining.

Indirect Immunofluorescence Microscopy—Day 6 3T3-L1 adipocytes were trypsinized, replated at 30–50% of their original density, and allowed to recover for 2 days prior to being used in experiments. Adipocytes were treated as described in the figure legends and then fixed with 2% formaldehyde in phosphate-buffered saline (PBS) for 10 min. The coverslips were washed with PBS and then incubated for 60 min with both anti-S3-12N (1.4 μg/ml) and anti-perilipin (diluted 1:10,000) antisera in microscopy buffer (1% BSA and 0.1% saponin in PBS). The coverslips were washed again with PBS and incubated for 30 min with Alexa 594-conjugated donkey anti-rabbit IgG (catalogue no. A-21207; Molecular Probes, Inc., Eugene, OR) and Alexa 488-conjugated goat anti-guinea pig IgG (catalogue no. A-11073), both diluted 1:1000 in microscopy buffer. Lipids were stained by the addition of 1 μg/ml BODIPY® 493/503 (catalogue no. D-3922; Molecular Probes, Inc.) added from a 1 mg/ml stock in ethanol. The coverslips were mounted and viewed with a Zeiss Axioplan-2 microscope. Images were captured with a Hamamatsu Orca CCD camera. Image acquisition and post-processing were performed with Northern Eclipse software (Empix, Mississauga, Ontario, Canada).

Fig. 1. Anti-S3-12 antibodies recognize a single protein from mouse adipose tissue. Proteins were extracted from 500 mg of mouse WAT with TNETC. Proteins were captured with 1 μg of the indicated antibodies. The immunoprecipitates were divided into three aliquots each, and each aliquot was loaded into a single lane of a SDS-polyacrylamide gel. After transfer, the membranes were probed with anti-S3-12N antiserum (1:3000 dilution) or with anti-S3-12C antibody (600 ng/ml). Flo1, flotilin-1.
Scoring Adipocytes for S3-12-coated Lipid Droplets—After adipocytes were treated as described in the figure legends, they were stained for perilipin and S3-12 as described above. Adipocytes in each field were identified by the presence of large perilipin-coated lipid droplets. After adipocytes were identified, the light filters were changed to reveal S3-12 staining. Adipocytes were counted as having S3-12-coated lipid droplets if they had either a well defined ring of peripheral S3-12-staining puncta or S3-12-staining lipid droplets with discernible cores. For each coverslip, 10 fields were counted with an average of 18 adipocytes/field.

RESULTS
Preparation of Antisera Specific for S3-12—To generate specific reagents for the subcellular localization of S3-12, we raised antisera against the amino-terminal (S3-12N) and carboxyl-terminal (S3-12C) peptides of murine S3-12. Each of the resulting antibodies immunoprecipitated from murine white adipose tissue (WAT) a single polypeptide of the predicted size of S3-12 (~150 kDa) that was recognized upon immunoblotting by both antibodies. Neither antibody recognized proteins precipitated by a control rabbit polyclonal antibody to an unrelated protein (flotillin-1). Thus, the anti-S3-12N and anti-S3-12C antibodies specifically recognize the amino and carboxyl termini of murine S3-12, respectively (Fig. 1).

S3-12 Protein Expression Is Largely Limited to WAT—The original report of S3-12 showed that S3-12 RNA was highly expressed in WAT, with little or no expression in other tissues (5). Expression in brown adipose tissue (BAT) was not reported. We immunobotted various tissue lysates for comparison with 3T3-L1 adipocyte differentiation. Cellular proteins were extracted with TNETO during the differentiation protocol on the days indicated. 20 μg of extracted protein were resolved by SDS-PAGE, transferred, and probed with the antibody indicated. Flot2, the antibody that recognizes flotillin-2/ESA, which is expressed at constant levels during 3T3-L1 adipocyte differentiation (36) and confirms equal loading of protein.
tion, the same membrane was reprobed with an antibody to perilipin, the expression of which is limited to adipocytes (brown and white) and to steroidogenic cells. Heart and skeletal muscle showed a clear S3-12 signal, but no detectable perilipin. This suggests that there is a small amount of non-adipocyte S3-12 expressed in heart and skeletal muscle (Fig. 2A), but we cannot rule out that the S3-12 detected in these tissues was from muscle adipocytes. BAT expressed much less S3-12 compared with WAT, despite similar perilipin expression in samples equally loaded for protein. Thus, it is likely that BAT expresses little or no S3-12 protein. This conclusion is consistent with the immunohistochemistry (Fig. 2B), in which

![Fig. 3. Redistribution of S3-12 during oleate supplementation. 3T3-L1 adipocytes were treated for the times indicated with 1.8 mM albumin-bound oleate in complete medium and then fixed and stained for S3-12 (A, D, G, and J) and perilipin (B, E, H, and K). The merged images (C, F, I, and L) show staining for S3-12 protein in red and perilipin protein in green. Overlapping signals for S3-12 and perilipin are revealed as yellow. Bars = 10 μm.](image-url)
the anti-S3-12N antibody strongly stained WAT, but not BAT. As preadipocytes differentiate into mature adipocytes, they undergo structural and biochemical remodeling. Like the PAT protein perilipin, S3-12 increased dramatically from days 2 to 4, and levels continued to increase through the conclusion of the experiment on day 10 (Fig. 2).

**S3-12 Is Not Present on the Large, Perilipin-positive Lipid Droplets of Untreated Adipocytes**—We used the anti-S3-12N antibody to probe the subcellular localization of S3-12 in 3T3-L1 adipocytes by indirect immunofluorescence microscopy. When these adipocytes were cultured in complete medium as described under “Experimental Procedures,” S3-12 was present most abundantly on cytoplasmic punctate structures that ranged in size from barely resolvable to −1-μm diameter (Fig. 3A). The staining intensity of these structures increased with their size. This staining was excluded from the perilipin-positive lipid droplets (Fig. 3, A–C).

**Lipid Loading of Adipocytes Leads to the Formation of a Discrete Pool of S3-12-coated Lipid Droplets**—TAG accumulation can be driven by the addition of esterifiable fatty acids to the media of cells in culture (22). During lipid loading of non-adipocytes such as HeLa cells and MA-10 Leydig cells, the PAT protein ADRP accumulates, and TIP47 moves from the cytosol onto lipid droplets (4). To assess if S3-12 is also affected by lipid loading, we incubated adipocytes in complete medium supplemented with albumin-bound oleate. Oleate supplementation caused time-dependent remodeling events that changed the intracellular distribution of S3-12 as well as lipid droplet size and number. After 10 min of lipid loading, the diffuse punctate staining of S3-12 observed before treatment (Fig. 3A) was replaced by a pattern of discrete, much larger puncta of more uniform size (~500 nm) and staining intensity (Fig. 3D). These discrete uniform puncta were arranged in a concentric ring around the perilipin-coated droplets, which surrounded the nucleus. A separate pool of S3-12 was present on puncta within the perilipin ring of droplets (Fig. 3, D–F). At 60 min of lipid loading, the S3-12 structures had increased in size, and many had developed discernible hollow cores. The S3-12 structures and perilipin-coated droplets remained in separate concentric rings at 60 min (Fig. 3, D–F).
By 240 min, the S3-12 structures had increased further to several micrometers in diameter (Fig. 3 J). Also by this time point, S3-12 staining was clearly apparent for the central pool of perilipin-positive droplets (Fig. 3, J–L). In the 60- and 240-min treatments, we observed that for both pools of lipid droplets, those lipid droplets most proximal to the nucleus were the largest and most intensely stained. The hollow cores seen in the S3-12-positive structures at 60 and 240 min were consistent with these structures being lipid droplets. To investigate this possibility, we incubated 3T3-L1 adipocytes with oleate or palmitate and then co-stained for S3-12 protein and for lipid with the hydrophobic fluorochrome BODIPY® 493/503 (Fig. 4 A). In this experiment, immunodetectable S3-12 surrounded cores that contained lipid, thereby confirming that these structures were lipid droplets.

To obtain a quantitative assessment of the oleate effect, we counted 3T3-L1 adipocytes with S3-12-coated lipid droplets in the presence or absence of 1.8 mM oleate in complete medium after 60 min of incubation. In independent experiments (six replicates for each condition) performed on 3 different days, we examined a total of 697 adipocytes. In the absence of oleate, only 1 of 697 adipocytes had S3-12-coated lipid droplets. With oleate present, 92% of the adipocytes contained S3-12-coated droplets at 60 min.

**Formation of S3-12-coated Lipid Droplets Requires Long Chain Fatty Acids** — Fatty acids differ in the rate and extent to which they can be incorporated into TAG (23). We tested whether fatty acids that are poorly incorporated into TAG would also lead to the redistribution of S3-12 to lipid droplets. Loading of adipocytes with the medium and short chain saturated fatty acids butyrate (4:0), octanoate (8:0), and decanoate (10:0), which are poorly incorporated into TAG, did not induce the formation of S3-12-coated lipid droplets (Fig. 4 B). As observed with oleate (18:1), incubations with the long chain fatty acids linolenate (18:3) and palmitate (16:0) drove the formation of S3-12-coated lipid droplets (Fig. 4B). However, at the 60-min time point, induction of S3-12-coated lipid droplets by the saturated fatty acid palmitate was less extensive than that induced by the monounsaturated oleate or the polyunsaturated linolenate (Fig. 4B). Incubations with palmitate required 180 min to achieve a similar degree of S3-12 redistribution as that seen with oleate at 60 min (Fig. 4A). Given that palmitate is not...
as easily incorporated into TAG as oleate (24), these data, together with the data above relating to fatty acyl chain length (Fig. 4B), suggest that S3-12 coating of lipid droplets may require incorporation of fatty acids into TAG.

The coating of lipid droplets by S3-12 is fully reversible. Removal of oleate-supplemented medium and its replacement with lipid-poor medium result in the return of S3-12 to its basal distribution within 3 h (Fig. 4C).

Inhibiting TAG Synthesis Blocks the Formation of Oleate-induced S3-12-coated Lipid Droplets—That formation of S3-12-coated droplets was dependent upon fatty acid substrates that can be incorporated into TAG suggested that the genesis of these structures requires new TAG synthesis. To test this hypothesis, we treated adipocytes with triacsin C. Triacsin C inhibits acyl-CoA synthetase-1 and -4. This inhibition largely blocks TAG synthesis, but has less effect on phospholipid metabolism (25, 26). At 10 μM, triacsin C greatly reduced oleate-induced TAG accumulation during a 180-min incubation (Fig. 5A). At this concentration, triacsin C prevented oleate-induced S3-12-coated lipid droplet formation (Fig. 5B, compares panels I and II). Triacsin C did not block the nucleation of S3-12 onto discrete uniform puncta (Fig. 5B, panel II) similar to those observed at 10 min of oleate loading in the absence of triacsin C (Fig. 3D). Surprisingly, treatment of adipocytes with triacsin C but without oleate supplementation also showed significant nucleation of S3-12 onto similar puncta (Fig. 5B, panel III).

Formation of Oleate-induced S3-12-coated Lipid Droplets Requires Insulin and Substrates for TAG Synthesis—Insulin is the primary anabolic hormone that promotes TAG synthesis in adipocytes. Because of the dependence of S3-12-coated droplet formation on TAG synthesis and on the presence of esterifiable long chain fatty acids, we reasoned that the absence of insulin or of glucose, the other substrate required for TAG synthesis,
would prevent the development of these droplets. To examine the role of insulin and glucose, we identified a set of serum-free conditions that produced S3-12-coated droplets in a similar percentage of adipocytes as we had observed in complete medium with oleate supplementation. Specifically, PBS (pH 7.4) supplemented with 2% BSA, 1.8 mM albumin-bound oleate, 25 mM glucose, and 10 nM insulin was sufficient to cause the formation of S3-12-coated lipid droplets in 82% of adipocytes after 60 min (Fig. 6A). In most adipocytes, the combination of oleate and glucose without insulin or of oleate and insulin without glucose was insufficient to drive the formation of S3-12-coated lipid droplets (Fig. 6A). Similar to the results of the experiment performed in the presence of serum (Fig. 5B), triacsin C inhibited S3-12-coated droplet formation under serum-free conditions (Fig. 6A). 1 nM insulin was sufficient to drive significant S3-12-coated droplet formation in the presence of oleate and glucose substrates by 60 min (Fig. 6B).

**DISCUSSION**

The data in this work reveal a previously unrecognized process of adipocyte lipid droplet biogenesis and maturation. Our major finding is that promotion of TAG synthesis in adipocytes results in the formation of S3-12-coated lipid droplets that initially are distinct from the pool of perilipin-coated lipid droplets. The pool of S3-12-coated droplets was not observed when adipocytes were cultured in lipid-poor complete medium. By 60 min of oleate treatment, there were two distinct populations of lipid droplets: the small peripheral droplets coated by S3-12 but not perilipin and the large perinuclear droplets coated by perilipin but not S3-12. With prolonged incubations, co-staining of S3-12 and perilipin on the more centrally located lipid droplets suggests an additional step in the maturation of S3-12-positive droplets. Our data do not indicate whether the co-staining of S3-12 and perilipin corresponds to fusion of these distinct pools of droplets or to the exchange of coat components. These data extend our understanding of how newly synthesized TAG is packaged and transferred to the mature lipid droplets. Return of S3-12 to its basal diffuse distribution upon removal of oleate from the medium suggests that S3-12 may cycle to and from the lipid droplet depending upon the nutritional status of the adipocyte.

The lipid droplets that S3-12 coats in response to lipid loading have properties that suggest they are filled with newly synthesized TAG. First, substrates for TAG synthesis, oleate and glucose, are required for the formation of S3-12-coated lipid droplets. Furthermore, the extent of this formation positively correlates with the ease with which the fatty acid substrate is incorporated into TAG. Second, the anabolic hormone insulin is required for a robust response to these substrates. Third, if TAG synthesis is inhibited with triacsin C, the formation of S3-12-coated lipid droplets is blocked. Fourth, there is a clear time-dependent increase in the size of the structures identified by S3-12. Finally, during the time period when a large number of S3-12-coated lipid droplets form, a large amount of TAG accumulates. These properties of the S3-12-coated lipid droplets suggest that they may correspond to the esterification compartment for TAG synthesis, but this hypothesis remains for future experimental investigation.

The dependence of S3-12 lipid droplet formation on insulin is not surprising given the role of insulin as an anabolic hormone that promotes TAG storage in adipocytes at multiple levels. Insulin increases the activity of lipoprotein lipase in adipose tissue (27), which increases the amount of fatty acid substrate available for TAG synthesis. Insulin also increases the transport of both glucose and fatty acids into adipocytes by promoting the trafficking of specific glucose (28, 29) and fatty acid (30) transporters to the plasma membrane. Insulin also positively regulates the expression of genes involved in TAG synthesis, including acyl-CoA:diacylglycerol acyltransferase (31), acyl-CoA synthetases, and steroyl-CoA desaturase-1 (32). The overexpression of peroxisome proliferator-activated receptorγ1, a target of the thiazolidinedione insulin sensitizers, in transgenic mouse liver has been associated with hepatic lipid accumulation (steatosis) and increased adipocyte-specific gene expression, including that of S3-12 (33). Our data extend these previous findings of the importance of insulin for TAG synthesis and storage by revealing a novel insulin action in the assembly of nascent lipid droplets in adipocytes.

It is instructive to place S3-12 in the context of the PAT family. We have now demonstrated that S3-12 functions as a regulated PAT protein in that it associates with lipid droplets in a hormone- and substrate-dependent manner. The PAT family members reported to date can be thought of as consisting of proteins of ubiquitous expression (ADRP and TIP47) or largely adipocyte-restricted expression (perilipin and S3-12). Viewed in another light, PAT family members are either constitutively present on lipid droplets (perilipin and ADRP) or present on droplets only under certain metabolic conditions (TIP47 and S3-12). Future studies will be needed to determine the precise functions of each of the family members in the biogenesis, maturation, and regulation of lipid droplets.

Over the past decade, the adipocyte has received considerable attention as an endocrine cell that regulates energy metabolism via secretion of “adipokines” such as leptin, tumor necrosis factor-α, adiponectin, and resistin (34). However, it is important not to ignore the lipid storage function of the adipocyte. The ability of the adipocyte to efficiently package and store TAG in lipid droplets is critical not only for energy storage, but also for partitioning lipid away from other tissues such as liver, muscle, and pancreas, where lipids may have adverse or “toxic” effects on cellular function with resulting insulin resistance and/or insulin secretory dysfunction (35). In this context, elucidation of the mechanisms by which the adipocyte efficiently packages and stores TAG in different adipose depots may reveal underlying causes of obesity- or lipodystrophy-associated lipotoxicity.

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