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The formation of lipid droplets: possible role in the development of insulin resistance/type 2 diabetes

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A B S T R A C T

Neutral lipids are stored in so-called lipid droplets, which are formed as small primordial droplets at microsomal membranes and increase in size by a fusion process. The fusion is catalyzed by the SNARE proteins SNAP23, syntaxin-5 and VAMP4. SNAP23 is involved in the insulin dependent translocation of GLUT4 to the plasma membrane, and has an important role in the development of insulin resistance. Thus fatty acids relocalize SNAP23 from the plasma membrane (and the translocation of GLUT 4) to the interior of the cell giving rise to insulin resistance. Moreover this relocalization is seen in skeletal muscles biopsies from patients with type 2 diabetes compared to matched control. Thus a missorting of SNAP23 is essential for the development of insulin resistance.

0. Introduction

Cytosolic lipid droplets consist of a core of neutral lipids (triglycerides and/or cholesterol esters) surrounded by a monolayer of amphipathic lipids, such as phospholipids and unesterified cholesterol. A multitude of proteins has been identified on the droplets (Fig. 1). The major proteins are the so-called PAT-domain proteins, which are named after their first identified members, perilipin, adipocyte differentiation-related protein (ADRP) and tail-interacting protein of 47 kD (TiP47). A number of other proteins have also recently been identified, some of which have been shown to be involved in lipid metabolism, sorting and trafficking processes, and processing of the droplets. Because of their complex structure and dynamic biology, lipid droplets are now regarded as dynamic organelles [1].

1. The lipid droplet and its biosynthesis

We have presented a model for the biosynthesis of lipid droplets (Fig. 2), which has been described in [2–4]. In brief, lipid droplets are formed as small (diameter < 0.5 μm) primordial droplet from microsomal membranes and the formation is highly dependent on triglyceride biosynthesis [5] but also on phospholipase D1 and the formation of phosphatidic acid [6]. Moreover, ERK2 is essential as it phosphorylates the motor protein dynein, which is then sorted on to lipid droplets allowing their transport on microtubules [6]. The lipid droplets can then increase in size by fusion [7], which requires transport on microtubules and is catalyzed by the SNARE proteins SNAP23, syntaxin-5, and VAMP4 [8].

The role of SNARE proteins in fusion processes has primarily been investigated in the fusion between transport vesicles and target membranes. In this fusion process, α-helical SNARE domains in the SNARE proteins forms a four-helix bundle (a stable superhelix), which forces the membranes together and promotes their fusion. A detailed molecular model for this process has been proposed [9,10]. The stable four-helix bundle present after the completed fusion is unwound by the ATPase *N*-ethylmaleimide-sensitive factor (NSF) and its adaptor protein α-soluble NSF adaptor protein (α-SNAP). (For reviews, see [10–13].)

The fusion process involved in lipid droplet growth most likely differs from the process that fuses a transport vesicle with a target membrane, particularly because the lipid droplet surface is an amphipathic monolayer in contrast to the bilayer that surrounds a transport vesicle. The fusion process between bilayers has been presented as an ordered sequence of events (stalk hypothesis) [12], and we postulate that fusion between lipid droplets is complete at an early stage in this sequence, equivalent to the creation of a 'fusion stalk' (i.e. when the two outer monolayers of the bilayers have fused, and there is a continuum between the hydrophobic portions of the two membranes). For lipid droplets, this would correspond to a fusion of the monolayers resulting in a connection between the two cores [8].

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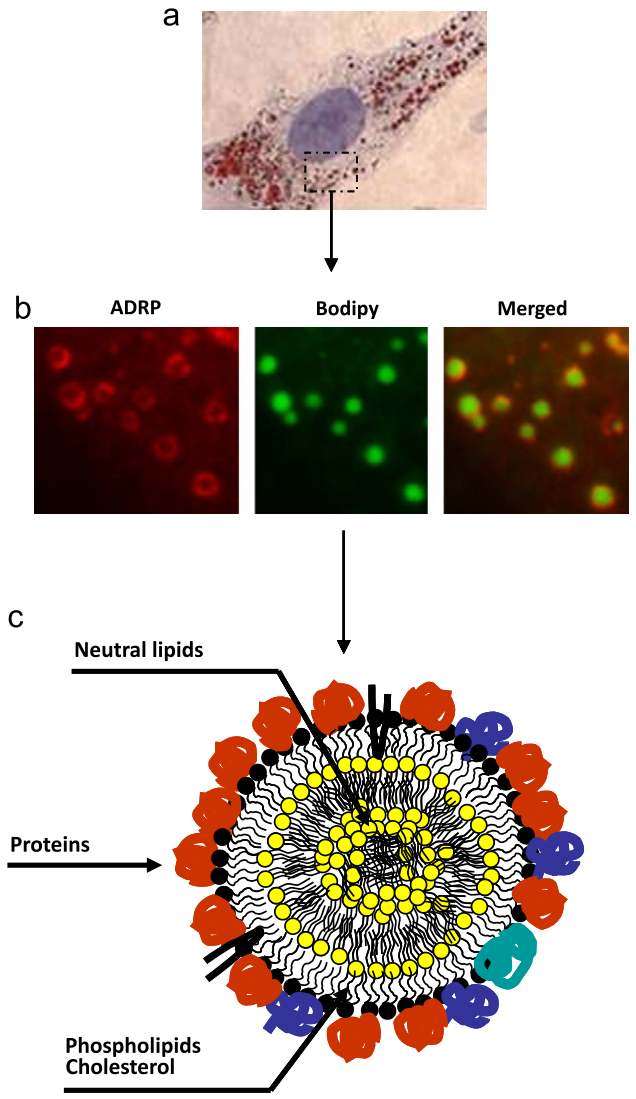

Fig. 1. The organization of the lipid droplet. (a) Lipid droplets in the cytosol stained with Oil Red. (b) Lipid droplets stained with antibodies to ADRP showing a ring like structure around the BODIPY stained core, most well seen in the merged figure. (c) A schematic organization of the lipid droplet.

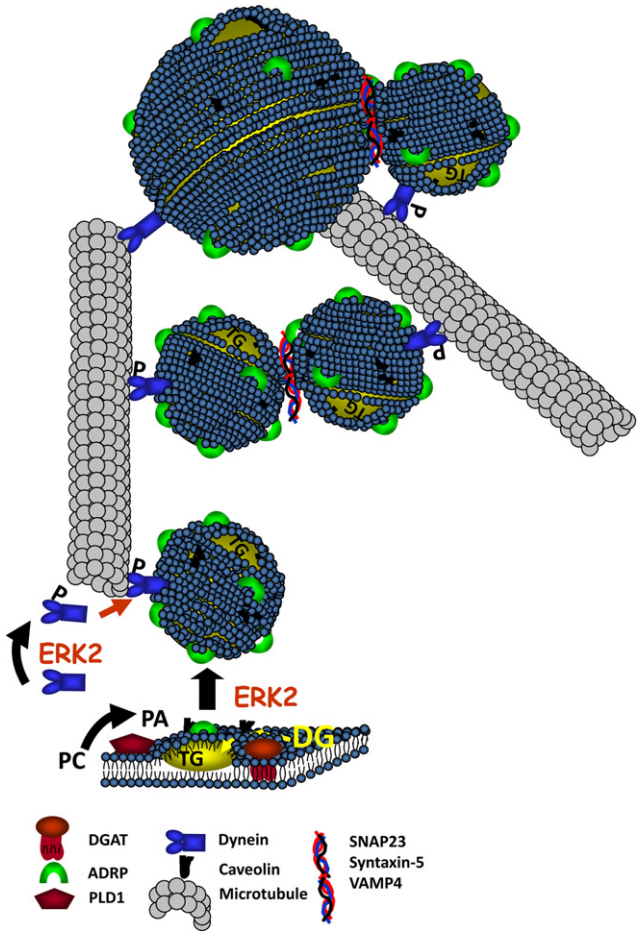

Fig. 2. Lipid droplets are formed from microsomes as primordial structures that are transported on microtubules and grow in size by fusion. The formation is driven by the biosynthesis of the neutral lipid (in this case triglycerides) but also requires phospholipase D1, and the formation of phosphatidic acid, as well as ERK2. ERK2 phosphorylated the motor protein dynein and sort this onto lipid droplets, allowing them to contact and travel along microtubules. This transport is of importance for their growth in size by a fusion process catalyzed by the SNAREs SNAP23, syntaxin-5, and VAMP4.

2. Implications of a fusion system based on SNARE proteins

We propose that the SNARE system acts to control the fusion of lipid droplets. Because oils in water fuse spontaneously, unprotected triglycerides would fuse to form large hydrophobic regions, which could influence the function of the cell. We hypothesize that this spontaneous fusion is reduced by protecting the triglycerides with amphipathic structures such as phospholipids and proteins, and that the function of the SNARE system is to restore the fusion capacity of intact droplets.

The SNARE catalyzed fusion between lipid droplets have implication for the distribution of the newly synthesized triglycerides which appear in the small newly formed droplets. If such droplets fuse with larger ones newly formed triglycerides will appear in such droplets. The mechanism is illustrated in Fig. 3.

3. Lipid droplets accumulation in skeletal muscle and the development of insulin resistance

The incidence of insulin resistance is rising to epidemic levels worldwide, with more than 350 million cases expected by 2030. Insulin resistance promotes the development of type 2 diabetes (T2D) and its life-threatening complication, cardiovascular disease.

The development of insulin resistance is highly related to the accumulation of ectopic triglycerides in lipid droplets in skeletal muscle. However, it is not thought that triglycerides *per se* promote insulin resistance, but rather factors associated with the lipid droplets or their biosynthesis and cellular processing.

Based on these results and the fact that SNAP23 is also involved in the insulin-dependent uptake of glucose, we described a unique mechanism that links the accumulation of lipid droplets with the development of insulin resistance ([8], reviewed in [2,14]; Fig. 4). SNAP23 combines with syntaxin-4 to form a t-SNARE complex in the plasma membrane (Fig. 4V), which is essential for the insulin-dependent translocation of the glucose transporter GLUT4 to the plasma membrane (Fig. 4I–IV). We tested and proved the hypothesis that lipid accumulation in the cell shifts SNAP23 from the glucose uptake mechanism in the plasma membrane to the interior of the cell (Fig. 4VII), resulting in insulin resistance [8]. Thus treatment of muscle cells with fatty acids decreased the levels of SNAP23 in the plasma membrane and increased the amount in the interior of the cell including on lipid droplets. This resulted in a cellular insulin resistance.

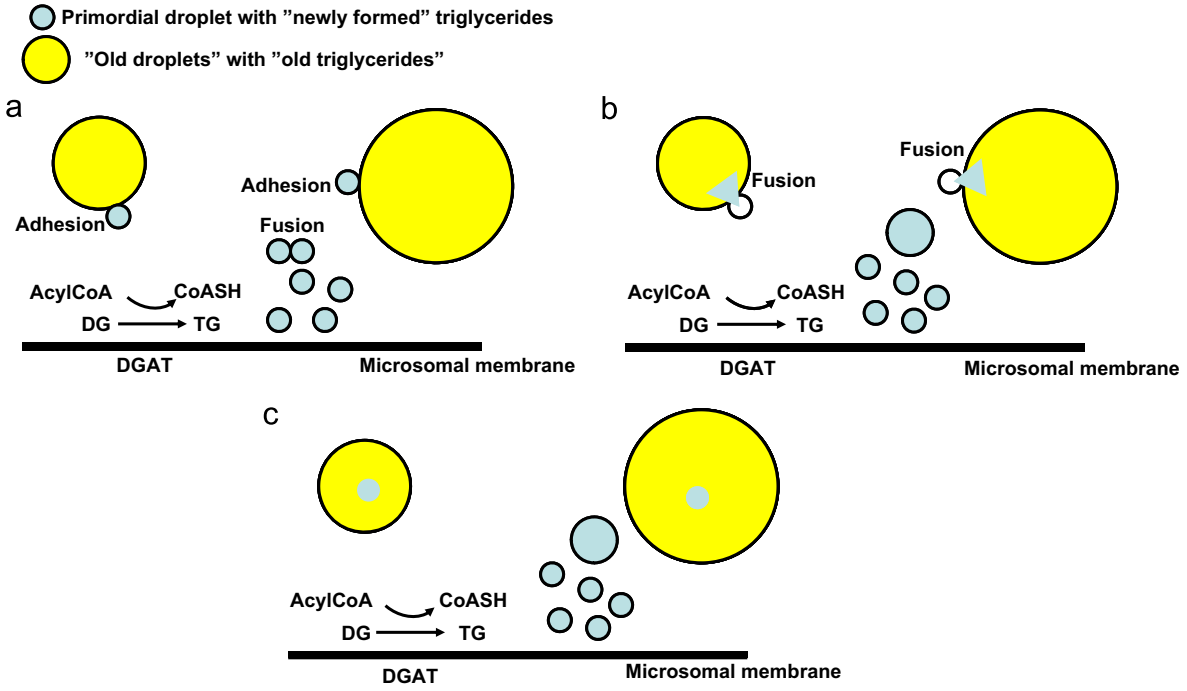

Fig. 3. The fusion between lipid droplets provides an explanation for the appearance of newly formed triglycerides in "old" droplets. The formation of lipid droplets is driven by the triglyceride biosynthesis presumable at the microsomal membranes. The small primordial droplets formed (blue) can fuse with each other forming larger droplets. But they can also fuse with the larger more mature droplets (yellow) which then acquire the newly formed triglycerides. Thus not only the size of the newly formed droplets will increase when the rate of triglyceride biosynthesis increases but also the "old" larger droplets that are not directly coupled to the triglyceride biosynthesis in the microsomal membranes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

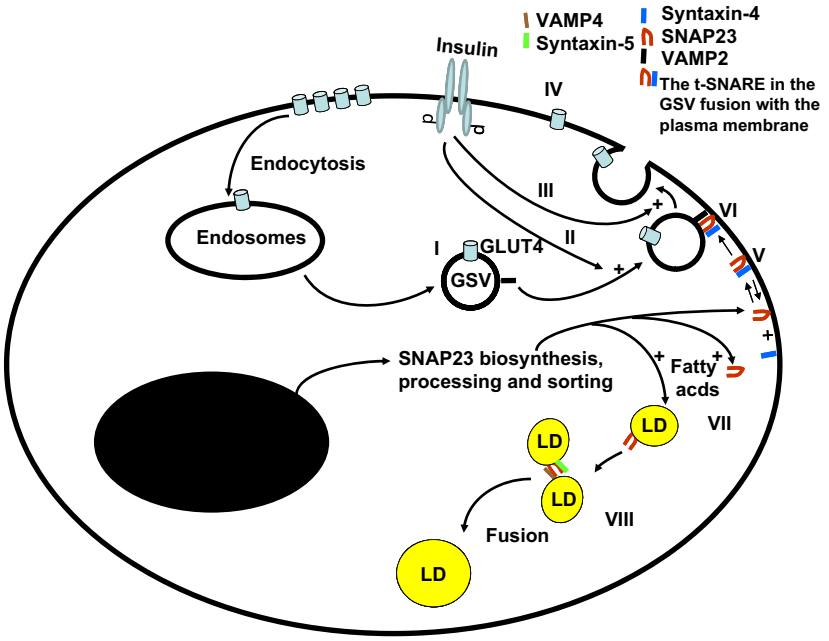

Fig. 4. Missorting of SNAP23 prevents the formation of the t-SNARE involved in the insulin-dependent GLUT4 translocation and induces insulin resistance. GLUT4 resides in GLUT4-specific vesicles (GSV) in the cytosol (I). The insulin signal transfers GSV to the plasma membrane (II) and promotes fusion of GSV with the membrane (III). The fusion process is driven by the interaction between a t-SNARE complex (syntaxin-4 and SNAP23) at the plasma membrane (V) and the v-SNARE VAMP2 on GSV (VI). This results in increased GLUT4 in the plasma membrane (IV) and increased glucose uptake. In insulin resistance/T2D, SNAP23 is missorted (VII) to the cytosol and lipid droplets, where it promotes fusion between the droplets (VIII). Fatty acids are involved in this missorting (VII).

We confirmed that this mechanism was present *in vivo* in humans by showing a redistribution of SNAP23 to the interior of the cell in skeletal muscle from patients with T2D compared with controls [15]. Thus this redistribution of SNAP23 was highly related to a increased systemic insulin resistance measured by a euglycemic hyperinsulinemic clamp.

Our studies in human skeletal muscle biopsies also demonstrated a role for the SNARE-regulating protein Munc18c in the development of insulin resistance [15]. Munc18c levels are increased in skeletal muscle (but not adipose tissue) from patients with T2D compared with controls [15]. We also observed that Munc18c forms a stable complex with syntaxin-4 that

completely excludes SNAP23 [15]. Thus Munc18c could have a role in the dissociation of SNAP23 from the t-SNARE complex with syntaxin. Interestingly the expression of Munc18c in human skeletal muscle cells increased when the cells were exposed to fatty acids. These results may point to Munc18c as a possible mediator between fatty acid treatment and the release of SNAP23 from the t-SNARE. However we could not demonstrate any redistribution of SNAP23 in skeletal muscle cells in which Munc18c had been overexpressed.

References

[1] S. Martin, R.G. Parton, Lipid droplets: a unified view of a dynamic organelle, Nat. Rev. Mol. Cell Biol. 7 (2006) 373–378.
[2] S.O. Olofsson, P. Bostrom, L. Andersson, M. Rutberg, M. Levin, J. Perman, J. Boren, Triglyceride containing lipid droplets and lipid droplet-associated proteins, Curr. Opin. Lipidol. 19 (2008) 441–447.
[3] S.O. Olofsson, P. Bostrom, L. Andersson, M. Rutberg, J. Perman, J. Boren, Lipid droplets as dynamic organelles connecting storage and efflux of lipids, Biochim. Biophys. Acta 1791 (2009) 448–458.
[4] P. Bostrom, L. Andersson, L. Li, R. Perkins, K. Hojlund, J. Boren, S.O. Olofsson, The assembly of lipid droplets and its relation to cellular insulin sensitivity, Biochem. Soc. Trans. 37 (2009) 981–985.
[5] D. Marchesan, M. Rutberg, L. Andersson, L. Asp, T. Larsson, J. Boren, B.R. Johansson, S.O. Olofsson, A phospholipase D-dependent process forms lipid droplets containing caveolin, adipocyte differentiation-related protein, and vimentin in a cell-free system, J. Biol. Chem. 278 (2003) 27293–27300.
[6] L. Andersson, P. Bostrom, J. Ericson, M. Rutberg, B. Magnusson, D. Marchesan, M. Ruiz, L. Asp, P. Huang, M.A. Frohman, et al., PLD1 and ERK2 regulate cytosolic lipid droplet formation, J. Cell Sci. 119 (2006) 2246–2257.
[7] P. Bostrom, M. Rutberg, J. Ericsson, P. Holmdahl, L. Andersson, M.A. Frohman, J. Boren, S.O. Olofsson, Cytosolic lipid droplets increase in size by microtubule-dependent complex formation, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1945–1951.
[8] P. Bostrom, L. Andersson, M. Rutberg, J. Perman, U. Lidberg, B.R. Johansson, J. Fernandez-Rodriguez, J. Ericson, T. Nilsson, J. Boren, et al., SNARE proteins mediate fusion between cytosolic lipid droplets and are implicated in insulin sensitivity, Nat. Cell Biol. 9 (2007) 1286–1293.
[9] C.G. Giraudo, A. Garcia-Diaz, W.S. Eng, Y. Chen, W.A. Hendrickson, T.J. Melia, J.E. Rothman, Alternative zippering as an on–off switch for SNARE-mediated fusion, Science 323 (2009) 512–516.
[10] T.C. Sudhof, J.E. Rothman, Membrane fusion: grappling with SNARE and SM proteins, Science 323 (2009) 474–477.
[11] W. Hong, SNAREs and traffic, Biochim. Biophys. Acta 1744 (2005) 493–517.
[12] R. Jahn, R.H. Scheller, SNAREs—engines for membrane fusion, Nat. Rev. Mol. Cell Biol. 7 (2006) 631–643.
[13] J. Malsam, S. Kreye, T.H. Sollner, Membrane fusion: SNAREs and regulation, Cell Mol. Life Sci. 65 (2008) 2814–2832.
[14] S.O. Olofsson, P. Bostrom, L. Andersson, M. Rutberg, J. Perman, J. Boren, Lipid droplets as dynamic organelles connecting storage and efflux of lipids, Biochim. Biophys. Acta 1791 (2009) 448–458.
[15] P. Bostrom, L. Andersson, B. Vind, L. Haversen, M. Rutberg, Y. Wickstrom, E. Larsson, P.A. Jansson, M.K. Svensson, R. Branemark, et al., The SNARE protein SNAP23 and the SNARE-interacting protein Munc18c in human skeletal muscle are implicated in insulin resistance/type 2 diabetes, Diabetes (2010).