The assembly of lipid droplets and its effect on insulin sensitivity

Akademisk avhandling

Som för avläggande av medicine doktorsexamen
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Avhandlingen baseras på följande delarbeten:

I - PLD1 and ERK2 regulate cytosolic lipid droplet formation.
Andersson L*, **Bostrom P***, Ericson J, Rutberg M, Magnusson B, Marchesan D, Ruiz M, Asp , Huang P, Frohman MA, Boren J, Olofsson SO. *Journal of Cell Science.* 2006 Jun 1;119(Pt 11):2246-57.***** ***Contributed equally***

II - Cytosolic lipid droplets increase in size by microtubule-dependent complex formation.
Bostrom P, Rutberg M, Ericsson J, Holmdahl P, Andersson L, Frohman MA, Boren J, Olofsson SO. *Arteriosclerosis Thrombosis Vascular Biology*. 2005 Sep;25(9):1945-51. 2005.

III - SNAP23 is important for the fusion between lipid droplets: a novel role for the SNARE system with implications for the insulin sensitivity of muscle cells
Boström P, Andersson L, Rutberg M, Perman J, Lidberg U, Johansson BR, Fernandez-Rodriguez J, Ericsson J, Nilsson T, Borén J and Olofsson S-O. *Nature Cell Biology.* 2007 Nov;9(11):1286-93.

Abstract

Accumulation of neutral lipids, in particular triglycerides, in non-adipocytes is highly related to the development of insulin resistance and its consequences, type-2 diabetes and cardiovascular diseases. The accumulation of triglycerides occurs in so-called lipid droplets in the cytosol. The lipid droplet is a highly dynamic organelle consisting of a core of neutral lipids surrounded by a monolayer of amphipathic lipids and proteins. The mechanism of assembly of these droplets is poorly understood and the main aim of this thesis was to investigate this mechanism at the molecular level. Another aim was to determine the relationship between lipid storage and insulin sensitivity of the cell.

In paper I, gain- and loss-of-function experiments showed that phospholipase D1 promotes the formation of lipid droplets. In addition, a cytosolic protein required for assembly of the droplets was isolated and identified as the extracellular regulated kinase 2 (ERK2). The importance of ERK2 in the formation of lipid droplets was confirmed in intact cells using gain- and loss-of-function experiments. Both PLD1 and ERK2 were shown to be necessary for the effect of insulin on lipid droplet biosynthesis. Finally, ERK2 was shown to exert its effects through phosphorylation of the motor protein dynein.

Lipid droplets are formed as primordial structures with a diameter of 0.1–0.4 μm. In paper II, it was found that these primordial droplets grow in size by a fusion process that is independent of triglyceride biosynthesis. This conclusion was based on investigations in a cell-free system, on pulse-chase experiments in intact cells, and by 3D reconstructions of time-lapse studies of fluorescent droplets in intact cells. Intact microtubules and dynein were found to be essential for fusion between the droplets. The mechanism behind the fusion process was investigated further in paper III. The SNARE proteins SNAP23, VAMP4, and syntaxin5 were shown to be present on lipid droplets and to mediate their fusion. Previously described co-factors for SNARE-mediated fusion events (NSF and α-SNAP) were also found to be present on droplets.

It is well known that SNAP23 also mediates the insulin-stimulated fusion between transport vesicles containing the glucose transporter 4 (GLUT4) and the plasma membrane—a process that is essential for insulin-stimulated glucose uptake. Treatment of cells with oleic acid caused massive accumulation of lipid droplets, and also translocation of SNAP23 from the plasma membrane to sites within the cell, including lipid droplets. This was paralleled by an ablation of insulin-stimulated glucose uptake—an effect that was totally reversed by overexpression of SNAP23. Thus, SNAP23 may be a molecular link between insulin resistance and neutral lipid storage.

Key words: Diabetes, lipid metabolism, insulin resistance, lipid droplets, ADFP, SNARE
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