

外国語要旨

Title: The biological significance of fatty acid composition in the phospholipid monolayer on the mature lipid droplets

Name: Kotoko Arisawa

Introduction

Mature adipocytes store excess lipids in intracellular lipid droplets (LDs). LDs are composed of a hydrophobic core of triacylglycerol (TAG) and cholesterol ester surrounded by a monolayer of phospholipids studded with a variety of proteins. During adipocyte differentiation, LDs can expand and LD-associate protein localization pattern might be mediated. Recent studies have shown that the size and surface protein properties of individual LDs are influenced by the amount and the type of head groups of phospholipids on the surface of LDs. However, the significance of fatty acid composition in the monolayers of LDs has not been elucidated.

I previously reported that the fatty acid composition of the phospholipid monolayer changes significantly during the differentiation of 3T3-L1 adipocytes (Arisawa *et al.*, J. Biochem., 2013). However, it is still uncertain whether this change could play a role in LD maturation. The degree of saturation of fatty acid chains in the bilayer membrane structure is known to control membrane physical properties such as fluidity, curvature, and packing density. Therefore, the fatty acid compositions in monolayer phospholipids of LDs might be also considerably important for physical properties of LDs. It is possible that these physical properties affect the size of LDs or the protein targeting to LDs, thus I focused on the relationship between the fatty acid composition of phospholipid monolayer and the LD growth.

Study 1 Saturated fatty acid in the phospholipid monolayer influenced the size of lipid droplets

First, I examined the differences in lipid composition between small and large LDs, and confirmed the significance of these lipid properties for size determination of LDs. To obtain large LDs, I generated NIH3T3 cells overexpressing fat-specific protein 27 (FSP27 cells), which induces the fusion of LDs. Although FSP27-overexpressing cells had larger LDs compared with control (mock) cells, the levels of core TAG and surface monolayer phospholipids such as PC and PE in the LDs were similar between FSP27 cells and mock cells. However, FSP27-overexpressing cells had more saturated fatty acids in the phospholipid monolayer of the LDs compared with mock cells.

Next, I examined whether the lipid extract from larger LDs possessed the biophysical properties for constructing large-droplet emulsions. LD-like oil-in-water emulsions were prepared from the lipid extract of LDs from each cell. The droplet size of emulsions reconstituted from lipid extracts of FSP27 cells was larger than that of emulsions prepared from mock cells. This experiment showed that the lipids from large LDs by themselves had the physical properties required to form large LDs.

To further investigate the effects of the degree of phospholipid unsaturation on the size of LDs, I synthesized artificial emulsions of a lipid mixed with distearoylphosphatidylcholine (DSPC, diC18:0-PC) or with dioleoylphosphatidylcholine (DOPC, diC18:1n-9-PC) and compared the particle size of LD-like emulsions. The droplet size of emulsions with saturated PC (DSPC) was larger than that with unsaturated (DOPC), suggesting

that saturated fatty acids in the phospholipid monolayer acted on the form or stability of large LDs.

Finally, I measured the packing density of surface phospholipid monolayers in emulsions. The results showed that the monolayer based on saturated fatty acids had less fluidity than the layer based on unsaturated fatty acid. It is possible that lipid packing defects occur more frequently on saturated phospholipid monolayers compared to monolayers formed from unsaturated phospholipids due to the restricted lateral diffusion of saturated PC. Therefore, the core TAG of emulsions might be exposed to the aqueous phase by lipid packing defects. To eliminate this unstable condition, it is possible that saturated phospholipid emulsions attempt to coalesce and stabilize by providing sufficient phospholipids to the monolayer surface.

Study 2 The fatty acid compositions of phospholipid monolayer mediate Perilipin1 localization

Perilipin1 is located on the surface layer of LDs and prevents lipase association with the surface of larger LDs in white adipocytes. On the other hand, Perilipin2 and Perilipin3, which show sequence similarity to the N-terminal region of Perilipin1, localize on nascent LDs but not large LDs. Thus I hypothesized that Perilipin family proteins localization might be mediated by the phospholipid fatty acid composition in the LDs that change during adipocyte differentiation.

As I generated NIH3T3 cells overexpressing Perilipin1, these cells were treated with saturated or unsaturated free fatty acids, respectively. Immunofluorescence analysis showed that Perilipin1 localization to LDs was promoted by the addition of unsaturated fatty acids, but not that of saturated fatty acids. Next, artificial emulsions were used to verify the association between the localization of Perilipin1 and the degree of unsaturation of fatty acid in LD monolayer. I synthesized artificial emulsions of a lipid mixed with DSPC and with DOPC, and then compared the binding capacity of Perilipin1 to each emulsions. The results showed that Perilipin1 could easily bind to the DOPC emulsion surface, suggesting that binding of Perilipin1 to LDs is induced by a loose packing of phospholipid according to the content of unsaturated fatty acids in the monolayer. Therefore, I hypothesize that amphiphilic alpha helical domain of Perilipin1 interact with hydrophobic regions of surface lipids. When Perilipin1 binding to emulsions were disrupted by octyl β -D-thioglucopyranoside detergent, which is hydrophobic interaction inhibitor, the isolated emulsions lose their coating of Perilipin1. In the experiments of the inhibition of disrupting electrostatic interactions using with sodium carbonate, the amount of Perilipin1 on isolated emulsions also reduced.

These results suggest that Perilipin1 localizes to the lipid droplet surface via both hydrophobic and electrostatic interactions. Moreover, Perilipin1 on the DOPC emulsions were more influenced by these inhibition effect compared with on the DSPC emulsions.

Conclusion

In this study, I confirmed the significance of these lipid properties to form and maintain large LDs. Our results suggest that saturated fatty acid chains in phospholipid monolayers might establish the form and stability of large LDs. On the other hand, unsaturated fatty acid chains as seen in matured LDs in 3T3-L1 adipocyte might contribute to the localization of LD-associated proteins such as Perilipin1. These results show that the significance of the fatty acid chains of phospholipid monolayer for the establishment of mature LDs.