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Activation of Adipocyte mTORC1 Increases Milk Lipids in a Mouse Model of Lactation     
  
    
Reviewer 1: In this study, El Habbal and colleagues investigated the effects of constitutive mTORC1 activation in adipocytes induced by Tsc1 deletion via adiponectin Cre on mammary gland structure, function, milk composition, and offspring weights. Their main findings were that constitutive mTORC1 activation in adipocytes increased milk fat content mainly by increasing monounsaturated fatty acids and DHA and this leads to a higher milk caloric density and heavier offspring weight during lactation. The mechanisms by which constitutive mTORC1 activation in adipocytes promotes these actions still remain elusive. Please find below some suggestions that may strengthen manuscript message.  
  
1- It would be important to characterize mTORC1 physiology in mammary gland development and function in lactating dams. Is mTORC1 activated in mammary gland alveolar cells and adipocytes during lactation? What is the impact of prolactin on mTORC1 signaling in these cells?  
  
2- Tsc1fl/fl; Cre+/+. For clarity, please use the term Tsc1fl/fl for wild-type mice. The term Tsc1fl/fl; Cre+/+ may be confusing as it may be interpreted as the mice are positive for Cre, which is not the case. In addition, it would be important to measure mTORC1 activity in the adipocytes at mammary gland to show that the deletion of Tsc1 using the Cre-lox system was successful. These adipocytes are different from regular white adipose tissue residing adipocytes and there is no clear indication that adiponectin Cre works the same in these cells.  
  
3- Recent studies from Phil Scherer group have shown that mammary gland adipocytes de-differentiate upon lactation losing lipid droplets. So for some unknown reason constitutive mTORC1 activation in adipocytes is preventing this de-differentiation. Authors should discuss this.  
  
4- Please provide a brief explanation or a citation for the method for fatty acid extraction, semi-purification and derivatization used in the GC analysis. This is not a lipidomic analysis as mentioned in the Methods. This is only a simple analysis of milk fatty acid profile. In fact, a milk lipidomic analysis would be very informative to reveal the lipid species in milk.  
  
5- Please improve graphics presentation and readability. Gray lines are almost impossible to see in the gray background in Figure 1.  
  
6- Please provide the milk fatty acid content in absolute values, not in percentage. In my opinion, a Table containing the most predominant fatty acids in milk in both genotypes should be included in the manuscript. Please, it is important to detail the main fatty acids in each class that are being modified in milk between genotypes. The increase in DHA is unexpected and interesting. DHA is mainly oxidized at peroxisomes and/or serve as precursor for the synthesis of D-series resolvins. Any changes in the expression of enzymes involved in these processes?  
  
7- Changes in gene expression may not be the main mechanism by which mTORC1 regulates fatty acid and triacylglycerol synthesis. Other possible mechanisms should be explored. In this sense, authors should look at adiponectin, whose serum levels are markedly increased upon constitutive mTORC1 activation in adipocytes.  
  
  
  
Reviewer 2: In the current manuscript, the authors showed that adipocyte-specific hyper-activation of mTORC1 increase milk lipids and alters mammary gland adipocyte histology. The work described here is straightforward and the results in general support the conclusion.