Reviewer’s report

Title: Microarray gene expression profiling of subcutaneous adipose tissue in obesity: Distinct expression of cell-cycle- and differentiation-related genes

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Reviewer: Lawrence Chan

Reviewer’s report:

The authors used customized microarray analysis to study the gene expression pattern of human subcutaneous adipose tissues focusing on 3 biological areas, namely, cell cycle, adipocyte differentiation and lipid metabolism with a total of 319 cDNA examined, and from this concentrated on data on 17 genes whose expression was significantly different in the obese subjects. The material used consists of RNA isolated from subcutaneous fat of 15 morbidly obese and 10 normal women. Validation was done by extending the analysis to additional human samples using qRT-PCR, and also included ob/ob and normal mice. Since the study adopted a targeted approach, by necessity, it was biased. On the other hand, with all the controls and validation, it provides highly usable information on the 3 groups of genes examined.

I have one recommendation for major compulsory revision - adding pref1 analysis. I also suggest below some histological data, not essential or compulsory, but would add to the paper if such data can be obtained. I recommend a few minor essential revisions, which deal more with expanding or revising the discussion.

Major compulsory revision:

The most interesting finding was perhaps that the gene expression profile suggested an increase in the amount of undifferentiated adipocytes in the obese subjects compared to normal subjects. This appeared to be in conflict with the increased adiposity in obesity. However, it is likely that the obese subjects are using more preadipocytes reserves for differentiation (if the limited preadipocyte reserve hypothesis is correct), or that other cell types or stem cells are driven toward preadipocyte lineages in respond to the body’s need for lipid storage in obesity.

1. Even though distinguishing between these possibilities is not easy, the authors should further examine whether (1) there are mixed populations of adipocytes/preadipocytes (by histological examination; c.f. Yourka D. Tchoukalova, Michael G. Sarr, and Michael D. Jensen 2004, AJP) or (2) other preadipocyte markers (such as pref1) are indeed increased in the adipose tissue of obese subjects.

Minor Essential Revisions:
1. Although the authors cite Ref 43 [Spalding et al.] as indicating that the no. of
adipocytes is set during childhood and adolescence and stays constant in adulthood, they also pointed out that Spalding et al. found that obese individuals generate more adipocytes per year than lean individuals. The situation is complex, and the discussion should be expanded slightly to include the additional work from the same lab that published Ref#43 (Diabetes 59: 105, 2010) taking into account the role of hyperplasia vs hypertrophy in these samples from obese subjects.

2. The finding of PCK1 downregulation in obese subjects is at odds with the finding in mice as pointed out by the authors on p. 13. But it should be noted that PCK1 is not merely a gluconeogenic gene; it is also important in glyceroneogenesis (c.f., Richard Hanson’s many studies) using the same glycolytic intermediates to produce G3P for glycerol synthesis. One possible reason that PCK1 is reduced in obesity may be that too much TG is being stored and the adipose tissue may have devised a way to release some of its store through lipolysis (perhaps mediated by glucocorticoid which downregulates PCK1 in adipose tissue). This possibility should be further explored in their discussion.

3. The results of genes involved in lipid metabolism such as LPL, NPC2, FACL1 and 4 were not discussed in the paper. The names of the latter two genes had been suggested to use the unified nomenclature system as Acsl 1 and 4 (cf. Douglas G. Mashek, Karin E. Bornfeldt et al. 2004 JLR). Perhaps a little more speculative discussion of these findings is in order.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.