Reviewer's report

Title: Diurnal Variation in Human Adipose Tissue: Link to Metabolic Diseases and mTOR Signaling

Version: 1 Date: 14 September 2008

Reviewer: Jeffrey Gimble

Reviewer's report:

This study extends prior circadian studies on murine adipose tissue to human subjects. It represents the first clinical study analyzing human adipose tissue diurnal biology at the transcriptomic level. The findings are correlated relative to a robust circadian gene marker (Per) in the context of the fed, fasting, and sibutramine treated conditions.

Major Compulsory Revisions:

1. In the context of references to papers by Ptitsyn or Zvonic, it would be appropriate to include the work by Ando H et al Endocrinology 146:5631, 2005. This study was the first to report the oscillatory expression of the circadian genes in murine adipose tissue by RT-PCR.

2. Pg16. The authors introduce the concept of HDAC drugs in their discussion as a future direction predicted by their analyses. The authors need to cite the following papers:


   These and related publications have documented that deacetylases act on the components of the core or common circadian regulatory apparatus and impact oscillatory expression profiles. This prior work needs to be acknowledged and incorporated into the discussion.

3. Pg 16. The authors point to a relationship of diurnal patterns in adipose tissue and the mTOR/PI3K/AKT pathways. This association is one that is logical, and to a degree, predictable since the next step in the progression is glycogen synthase kinase 3 beta. Most biochemical pathways describing the core or common circadian oscillatory apparatus will include GSK3B and/or casein kinase 1 epsilon as components. Their role is to phosphorylate the Per protein, which is then targeted to the proteasomal pathway for degradation. Again, this concept with appropriate references should be acknowledged in the Discussion.

4. Pg 17, end of first paragraph. In their comments associating glucose metabolism to the diurnal gene expression profile, the authors are encouraged to acknowledge the publication by Rudic RD et al PLOS Biology 2:e377, 2005 "Bmal1 and clock, two essential components of the circadian clock, are involved in glucose homeostasis".
Minor Essential Revisions:
1. Table 1. The sixth most enriched pathway identified is the PPAR signal pathway. In light of its important role in adipogenesis and lipogenesis, it would be appropriate to include it for comment in the Results and/or Discussion.
2. Figure 4. Identify the X axis in the figure itself. Identify the Y axis directly as "fold change".
3. Figure 5. Specify the identity of the Y axis. As presented, it could be easily understood as the "fold change" or the "number of genes".

Discretionary Revisions:
1. Consider:
Reporting the mean BMI and age of the study subjects on pg 7.
Including any physiological measures of circadian biology (body temperature, blood pressure measures, serum corticosterone levels) that might have been evaluated in the study in addition to the transcriptome itself.
Identifying the biopsy site on pg 20 rather than waiting solely to doing so in the methods.

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

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
Yes. I have applied for a patent relating to circadian mechanisms regulating adipogenesis and obesity.