Reviewer's report

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

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Reviewer: Ronald Evans

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In “Diurnal Variation in Human Adipose Tissue: Link to Metabolic Diseases and mTOR Signaling”, Loboda et. al. examine global gene expression patterns in adipose tissue samples collected from human patient biopsies at three different times of day under fed, fasted or sibutramine-treated conditions. They convincingly demonstrate that time of day has a greater effect on gene expression in human adipose tissue than does either fasting or the weight loss drug sibutramine. Indeed, approximately 25% of the expressed transcripts displayed significant diurnal expression, which is consistent with previous studies of circadian gene expression in liver, heart and adipose tissue in mice. Consistent with reports demonstrating the dependence of circadian clock timing in mouse peripheral tissues on feeding patterns, the authors found that fasting altered the timing of expression of circadian clock genes and other diurnally expressed genes in a manner suggestive of a fasting-induced delay of circadian rhythm in human adipose tissue. Sibutramine treatment mimicked or increased the effect of fasting on diurnal gene expression. Also consistent with previous reports from studies in rodents, transcripts central to cellular and organismal metabolism were significantly overrepresented among the diurnally expressed transcripts. Finally, the authors compared their diurnal gene signature to gene expression changes elicited by various pharmacological treatments in cultured cells and found that the most significantly associated gene signatures were those induced by inhibitors of the PI 3-kinase and mTOR signaling pathway. This association seems to be a weak point of the paper; therefore, the reference to mTOR signaling should be removed from the title.

The dominance of diurnal regulation is not entirely unexpected because previous studies have shown that a large fraction of the mouse transcriptome is rhythmically expressed in various peripheral organs; however, this seems to be the first report demonstrating the importance of diurnal regulation of gene expression in biopsies from a human metabolically important organ. The study design is well controlled and the results emphasize the importance of carefully controlling for the time of day in studies of tissue gene expression. Furthermore, this clear demonstration of diurnal transcription of many metabolically important enzymes in human adipose tissue underlines the probable role of circadian compartmentalization of metabolic processes in humans, which may have implications for the development and treatment of metabolic disease.

Major Compulsory Revisions:
None.

Minor Essential Revisions:

1. As explained above, “…and mTOR Signaling” should be deleted from the title.

2. In the abstract, “We demonstrate that the majority of genes in peripheral tissues are under diurnal regulation and demonstrating that the key processes…” should be changed to “We demonstrate that a large fraction of genes in peripheral tissues are under diurnal regulation and that the key processes…” because 25% is not a “majority” and to fix the grammar.

3. In the abstract, the claim that “Finally, we show that mTOR inhibitors significantly reversed the observed diurnal signature, consistent with the key role of mTOR in energy storage and the dyslipidemia observed in patients treated with mTOR inhibitors” seems overstated as it is my understanding that the authors did not themselves do any experiments using mTOR inhibitors but merely found an inverse correlation between their diurnal gene signature and the gene expression changes induced by mTOR inhibitors in cultured adipocytes in publicly available datasets. This sentence could be changed to “Finally, we find a significant association between transcripts that are diurnally regulated in our study and transcripts that are repressed by mTOR inhibitors, suggesting a possible link between mTOR signaling, diurnal gene expression and metabolic regulation.”

4. On page 6, the authors state: “We also report that the both the core clock genes and diurnal output genes were impacted with fasting and sibutramine. However, the effect was very subtle, hence very different from what had been observed in rodents, where the peripheral clock was profoundly affected by restricted feeding.” The comparison to the observations in rodents is not entirely fair because the experiment in rodents involved many days of restricted feeding while Loboda et. al. measured gene expression changes during a single day of altered feeding. The wording should be changed to reflect this. Also delete the unnecessary article “the” as indicated above by a strikethrough.

5. On page 13, the authors state that the correlation between their diurnal gene signature and the gene signatures elicited by PI 3-kinase and mTOR inhibitors in human cell lines “…indicates that the growth factor pathway (AKT/PI3K/mTOR) in the adipose is diurnally regulated and thus, growth inhibitors would be expected to reverse those changes” is inaccurate. The inverse relationship between genes that are induced by inhibitors of PI3K or mTOR and genes that are diurnally regulated in the Loboda et. al. study does not indicate that the AKT/PI3K/mTOR pathway is diurnally regulated. The last 2 sentences of the paragraph could be changed to “The results show that diurnal genes were significantly but negatively impacted by these compounds, suggesting that PI3K-mTOR inhibitors may alter diurnal patterns of gene expression, perhaps by regulating circadian clocks.”

Discretionary Revisions: None.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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

Declaration of competing interests:

I declare that I have no competing interests.