



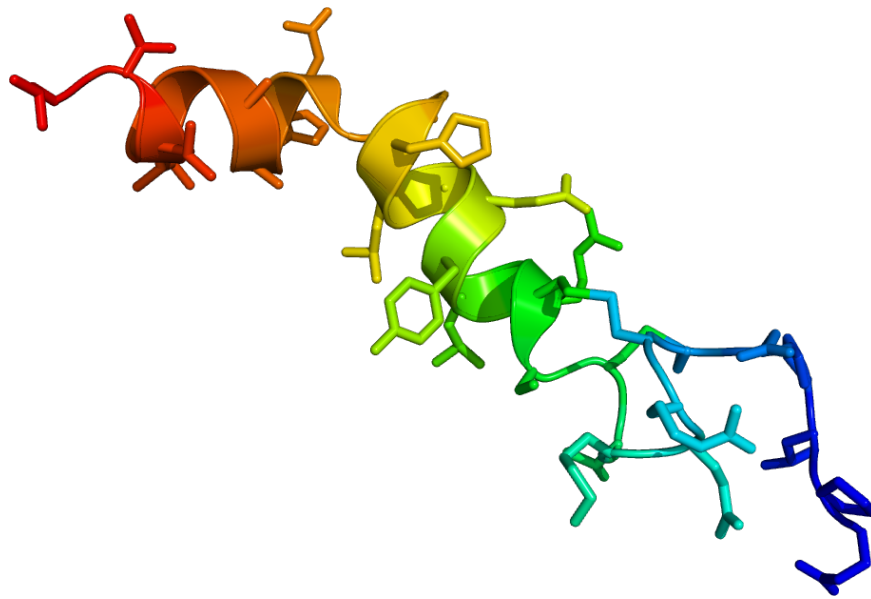
Human adipose tissue is a putative direct target of daytime orexin with favorable metabolic effects: a cross-sectional study

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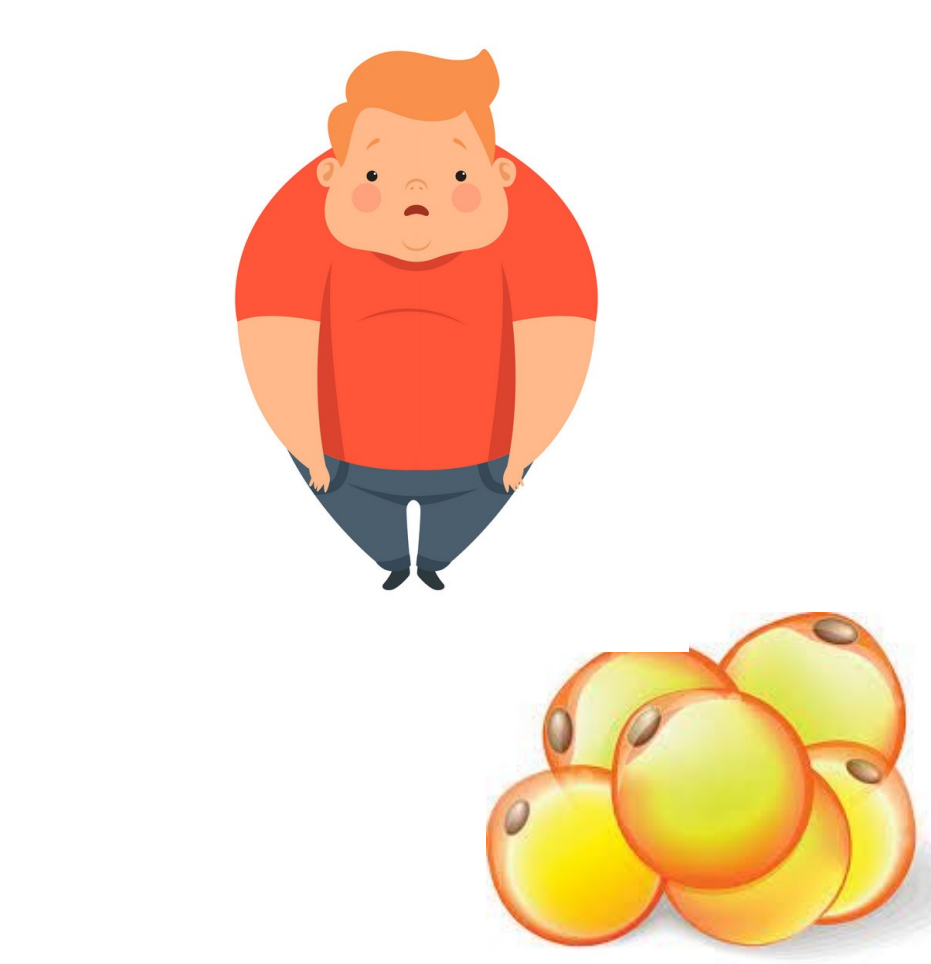
INTRODUCTION

Orexin (Ox) and its receptors constitute a family of two neuropeptides derived by proteolytic cleavage from the transcript of the *HCRT* gene (OxA and OxB) and two G-coupled receptors (Ox1R and Ox2R). The most characterized function of the system is the central regulation of the sleep–wake behavior. Ox rises during daytime in diurnal animals and consistently acts through central mechanisms to regulate metabolism and fuel homeostasis. Outside the CNS, Ox and/or its receptor(s) have been identified in the gastrointestinal tract, reproductive system, adrenal gland, heart, pancreas, and adipose tissue. Moreover, Ox is detectable in peripheral blood.



AIM of the STUDY

In human adipose tissue, only a few studies have attempted to assess the direct role of the Ox/OxR system. Given the scarcity of the information on the Ox/OxR system in human adipose tissue, we used samples from a human adipose tissue and plasma biobank to measure Ox and OxR expression in subcutaneous and visceral adipose tissues and measured circulating OxA levels. We hypothesized that greater daytime (when samples were collected) Ox system “input” would correlate with a healthier metabolic profile.



METHODS

- study population:** participants (n = 81) were recruited before undergoing elective abdominal surgery. Paired abdominal superficial subcutaneous and visceral adipose tissue biopsy specimens were obtained between 8 AM and 2 PM during surgery and immediately delivered to the laboratory. In addition, blood samples were collected between 7 AM and 8 AM for biochemical and lipid profile and for determining circulating OxA levels in the serum;
- cell culture:** the Chub-S7 cell line was derived from human subcutaneous adipose tissue. Cells were grown and differentiated into adipocytes;
- RNA extraction and quantitative real-time PCR:** total RNA was extracted from either biopsy specimens of human adipose tissue or from differentiated Chub-S7 adipocytes;
- Western blot analyses;**
- In vitro gene oscillation studies:** mature Chub-S7 adipocytes were synchronized using serum shock. Cells were harvested every 4 hours for 16 hours after washing with ice-cold phosphate-buffered saline (PBS);
- OxA measurement in the serum:** serum OxA levels were measured using a fluorescence kit.

RESULTS

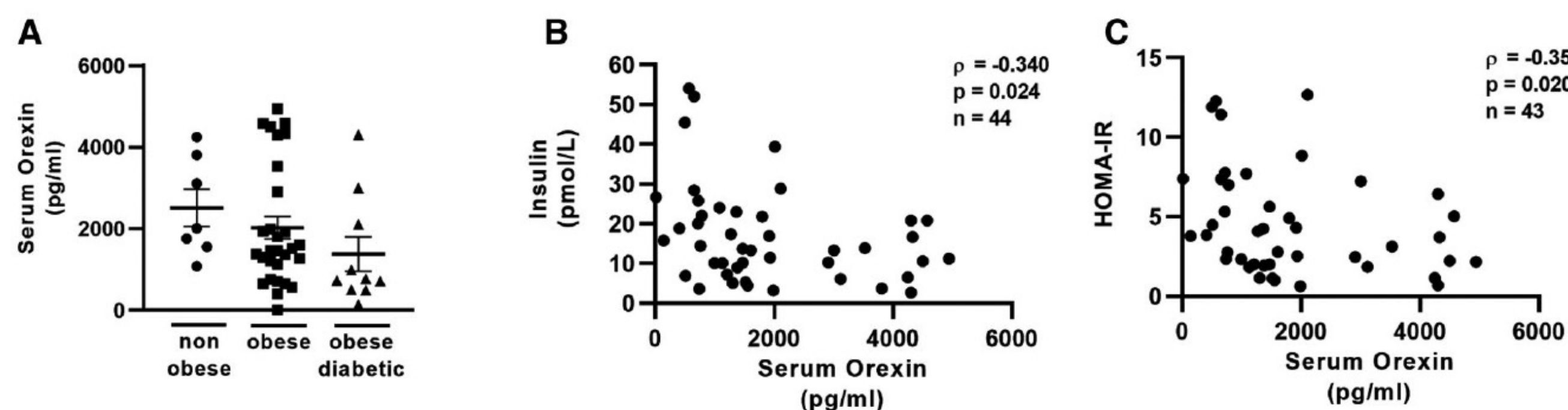


Figure 1 Morning peripheral blood OxA levels associate with an improved metabolic profile. (A) Serum OxA levels in nonobese participants, participants with obesity, and participants with obesity and diabetes (n = 46), (B) Spearman correlation with fasting insulin levels (n = 44), and (C) HOMA-IR (n = 43).

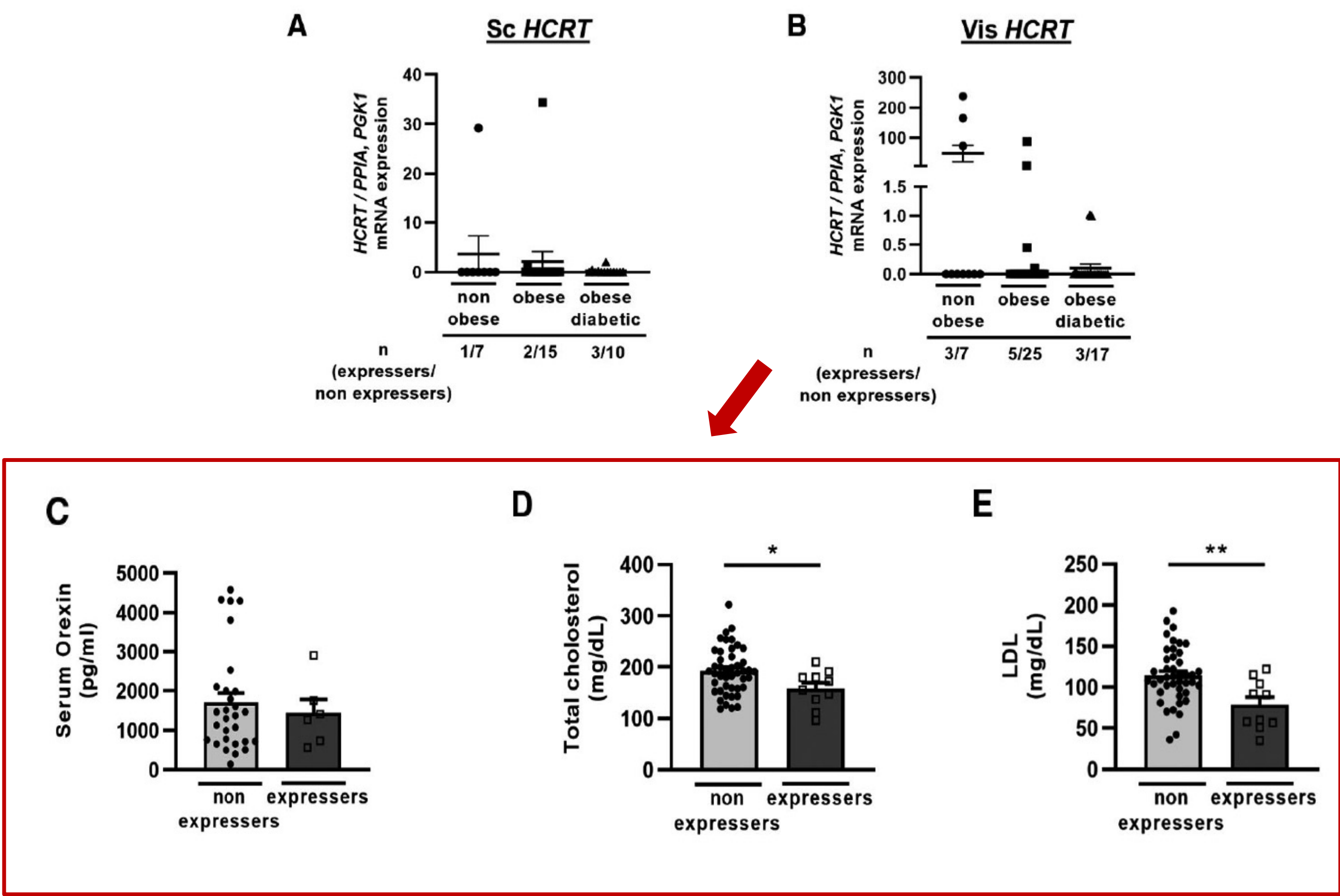


Figure 2 We next assessed whether local production of Ox can occur within human adipose tissue. In 15.4% of subcutaneous fat samples (A) and in 18.3% of visceral fat samples (B), *HCRT* mRNA was detectable. Circulating *HCRT* levels in expressers were similar to non-expressers of *HCRT* in visceral adipose tissue (C). Nevertheless, those who expressed *HCRT* mRNA in visceral adipose tissue exhibited significantly lower total and low-density lipoprotein (LDL) and cholesterol levels (D and E, respectively).

BIBLIOGRAPHY

- Perez-Leighton CE, Billington CJ, Kotz CM. Orexin modulation of adipose tissue. *Biochim Biophys Acta*. 2014;1842(3):440-445.
- Wang C, Wang Q, Ji B, et al. The orexin/receptor system: molecular mechanism and therapeutic potential for neurological diseases. *Front Mol Neurosci*. 2018;11:220.
- Kukkonen JP. Lipid signaling cascades of orexin/hypocretin receptors. *Biochimie*. 2014;96:158-165.

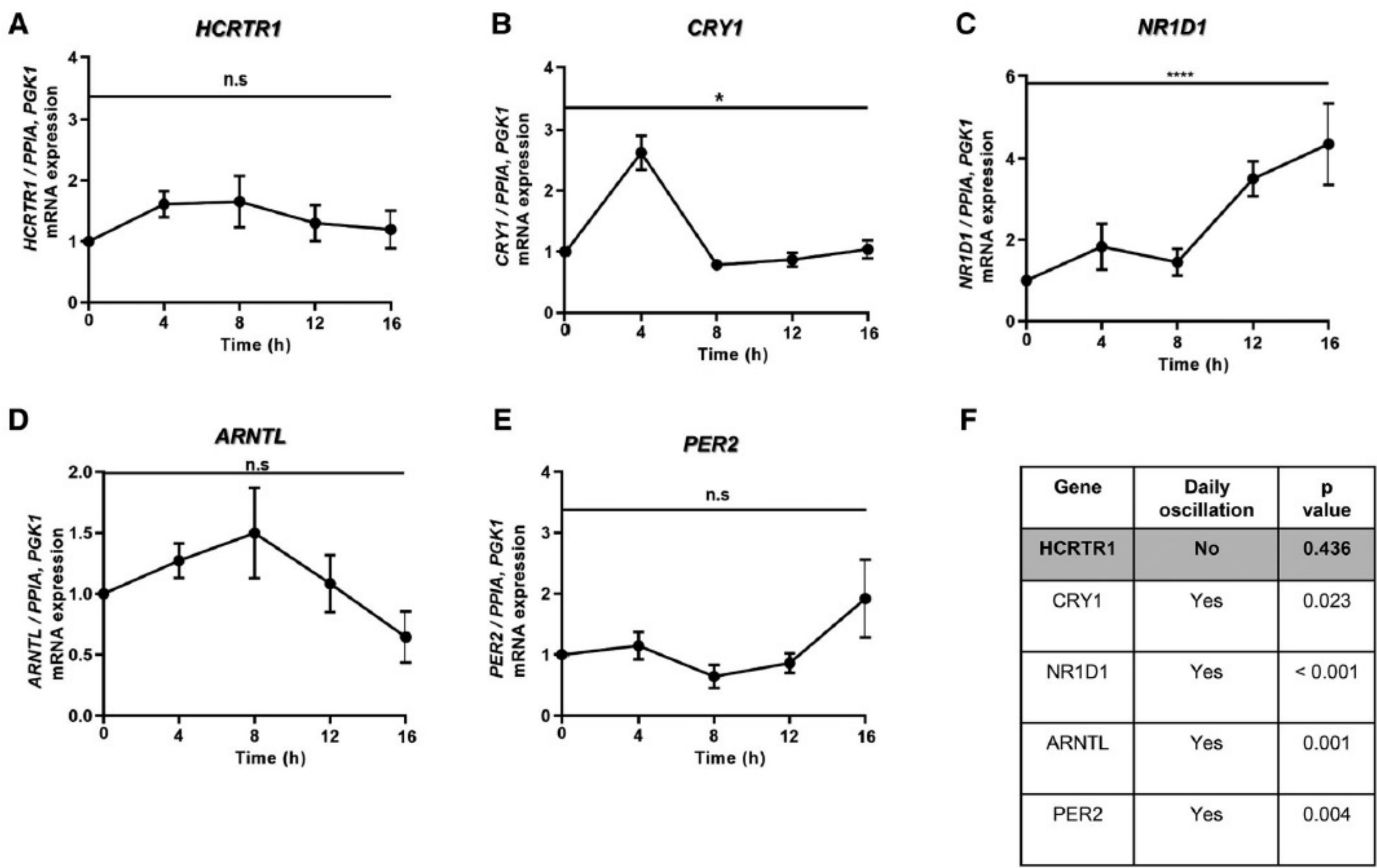


Figure 3 Ox is known to oscillate as part of its diurnal regulatory functions. To assess whether *HCRT* expression in adipocytes also oscillates, we used a human adipocyte cell line and subjected it to a classical paradigm of synchronization-oscillation. *HCRT* mRNA levels, readily detectable in these cells, did not significantly differ during the different time points compared with time 0 (A). These data suggest that the different times of day at which adipose tissue biopsy specimens were obtained are unlikely to significantly affect *HCRT* mRNA levels.

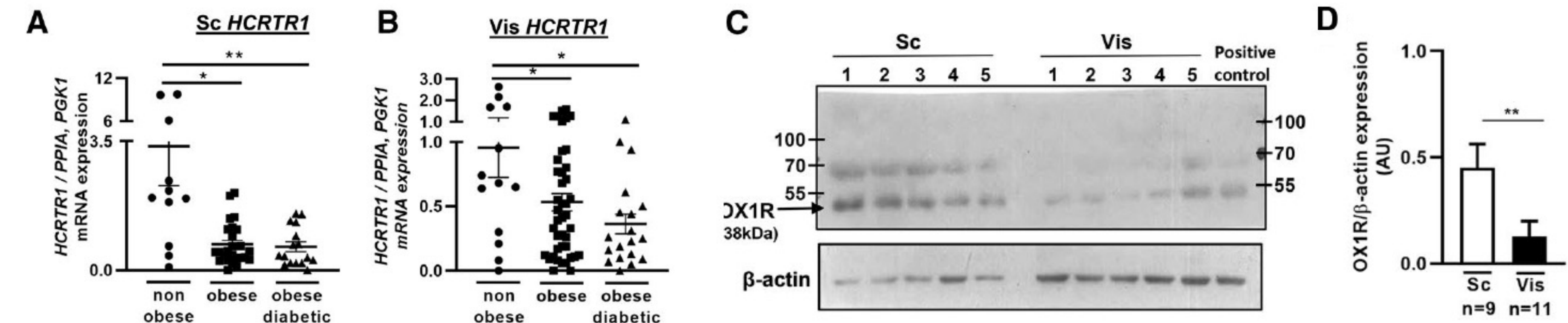


Figure 4 We measured the expression of *HCRT*s in human adipose tissue. *HCRT*2 was undetectable in both subcutaneous and visceral samples. In contrast, *HCRT*1 mRNA levels were readily detected in all samples. In both fat depots, the expression level tended to be lower in samples from persons with obesity and obesity and diabetes (A, B). Importantly, Ox1R was also detectable at the protein level (C,D).

CONCLUSIONS

Using a relatively large sample collection from a human adipose tissue and plasma biobank, we report the following main observations:

- Serum morning OxA levels correspond to improved metabolic lipid profile and insulin sensitivity.
- HCRT* mRNA is expressed in <20% of human adipose tissue samples. Yet, though an unlikely significant source of serum OxA, adipose tissue expression of *HCRT* associates with a better metabolic profile.
- Of the two *HCRT*s, we could not detect significant expression of *HCRT*2 mRNA, but *HCRT*1 is readily detectable in human adipose tissue samples. Expression level is lower in patients with obesity, especially if obesity is metabolically complicated by diabetes. In a human adipocyte cell model, *HCRT*1 mRNA does not exhibit diurnal oscillation.