**Title:** Decreased oral glucose tolerance and insulin response during biological evening versus morning among adults under free-living conditions

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*The abstract body (including title, introduction, methods, results, conclusion and support) is limited to 350 words.*

**Introduction:** Melatonin secretion during the biological night impairs glucose tolerance *in vivo* and suppresses insulin secretion from beta-cells *in vitro*, yet its implication in adults under free-living conditions and its relevance for time-of-day assessment of glucose tolerance remain undetermined.

**Methods:** In the Shift work, Heredity, Insulin, and Food Time (SHIFT) Study (*#NCT02997319*), we tested the hypothesis that compared to the biological morning, an oral glucose tolerance test (OGTT) performed during the biological evening will reveal relatively impaired glucose tolerance and reduced insulin secretion. In this large randomized crossover study, participants completed two 2-hour 75-g OGTTs: morning (3 hours after self-reported habitual free day wake time) and evening (1 hour before self-reported habitual free day bedtime). Blood glucose, insulin, and melatonin were determined. Incremental 2-hour area under the curve (iAUC) for increases in glucose and insulin using the trapezoidal method, insulin sensitivity index (ISI), corrected insulin response (CIR), and oral disposition index (DIo) were calculated. Morning and evening values were compared using paired t-tests.

**Results:** In 83 healthy, non-night workers (73% female; 33.3±11.8 years; 25.46±5.0 kg/m$^2$), we confirmed 10-fold higher endogenous melatonin concentrations at biological evening compared to biological morning assessments (morning, 2.35±3.93 pg/ml *vs.* evening, 26.16±24.72 pg/ml; $P$<0.001). Compared to the morning OGTT, evening OGTT postprandial glucose was 91% higher (glucose iAUC: morning, 4,523±2587 mg.min/dl *vs*. night, 8,652±2773 mg.min/dl; $P$<2.2x10$^{-16}$), while no difference in postprandial insulin iAUC ($P$=0.82) was noticeable. Whereas time-of-day had no

influence on ISI (*P*=0.87), the evening OGTT had significantly lower CIR (*P*=0.022) and DIo (*P*=0.003) compared to the morning OGTT.

**Conclusion:** These observations from adults under free-living conditions extend earlier findings from *in vitro* and controlled human experiments by confirming impaired glucose tolerance likely from reduced insulin response to glucose during the biological evening relative to the biological morning. These findings provide first insights into how chronic exposure to nighttime eating, such as among night shift workers, may result in increased diabetes risk.

**Support:** NIH-R01DK105072