RHYTHM OF MICE METABOLIC TESTS – WHEN TO MEASURE?

AQUINO, Carlos Alberto Queiroz de[1]*; PERES, Laura da Silva White[2]*; MARINHO, Letícia de Oliveira[3]*; CASTRUCCI, Ana Maria[3]; SUA-CESPEDES, Cristhian David[3]

[1] Veterinary medicine, Department of Animal Sciences, Federal University of the Semi-Arid Region, Mossoró, RN - Brazil.

[2] Veterinary medicine, Department of Agricultural Sciences, University Anhembi Morumbi, SP - Brazil.

[3] Department of Physiology, Institute of Biosciences, University of São Paulo, SP - Brazil

*These authors have the same contribution as the first authors

Corresponding author: cristhian.sua@usp.br

Introduction. In organisms, processes such as gene expression, hormone secretion, as well as metabolic pathways are regulated by biological rhythms, under the influence of environmental and social cues. The measurement of metabolic products of glucose and lipid pathways in humans and animals follows a protocol to avoid pre-analytical interferences, but little is discussed about the influence of the time of day at which they are measured. **Objective.** Our aim was to determine the most appropriate time of day for the application of glucose and lipid metabolism tests in C57Bl/6J (WT) and *Trpa1*$^{-/-}$ (KO- knockout) mice kept in thermoneutrality. **Methods.** Ten males of each genotype, ± 5-month-old, maintained in thermoneutrality (30 ± 1°C) for at least 2 weeks with a 12:12 light-dark photoperiod was used (CEUA-IBUSP 373/2021). The animals were separated into three groups according to the time of measurement: 07:00 h (beginning of the resting phase), 13:00 h (mid-resting phase) and 19:00 h (beginning of the activity phase). All animals were fasted for 10 h before the metabolic test. Fasting blood glucose levels were measured using strips and glucometer. Later, 2g/kg glucose was intraperitoneally injected and a GTT curve was calculated at 15, 30, 60, 90, and 120 min after injection. After 3 days the animals were euthanized, and blood was collected to obtain serum. This sample was utilized to determine total cholesterol and triglycerides by colorimetric kit. The mean values were compared by Two-way ANOVA followed by Bonferroni post-test for $p<0.05$. **Results.** The fasting glucose values showed no relevant statistical differences among the groups and times evaluated. The WT group exhibited an increase tendency at 7:00 h (start of activity). Regarding GTT, both genotypes showed peaks 15 and 30 minutes after glucose administration at all time points evaluated. At 13:00 h the WT mice still displayed elevated levels 60 minutes after glucose injection, suggesting an increased time to metabolize glucose. That was evidenced in the knockout animals at 19:00 h. The area under the curve (AUC) demonstrated differences between the genotypes only at 13:00

h suggesting a better metabolization of the KO animals at this time point. The AUC also showed no significant difference for this metabolic test among the temporal points analyzed or between genotypes at each time point. Cholesterol levels showed no significant differences between the genotypes and the times, suggesting the possibility of measuring this lipid at any time of day. On the other hand, triglyceride levels were higher in WT animals compared to KO group at 07:00 h. Interestingly, in the WT mice, cholesterol levels decreased throughout the day, suggesting 07:00 h as the peak of this metabolite. The reduction in the KO animals was only evident at 19:00 h, suggesting that the triglyceride measurements in this genotype must occur in any temporal point of the light phase. **Conclusion.** We conclude that for these genotypes, on thermoneutrality, there is no best time for blood glucose measurement while the cholesterol and triglyceride determination is recommended during the light phase.

Keywords: Metabolic tests; Rhythm; Mouse