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Title: A noninvasive method for detecting the metabolic stress response in rodents: characterization and disruption of the circadian corticosterone rhythm

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Taking blood samples to assess certain stress hormones, such as corticosterone (CORT), is a commonly used procedure with rodents. Blood sampling is invasive and poses a serious limitation in the detection and quantification of the stress response. Factors such as anesthesia, the physiologic response to blood loss, restraint, pain and the anticipation of a stressful stimulus, have been shown to disrupt basal CORT stress levels (Tuli *et al.* 1995, Haemisch *et al.* 1999). Tail veil sampling by either tail vein nick or tail snip induces a stress response if the sampling takes longer than 3 minutes (Vahl et al 2005). Metabolites of CORT have been previously extracted and measured in feces (Cavigelli et al. 2005) providing us with a non-invasive procedure for measuring stress hormones. We sought to determine the effect of a stressor (i.e. blood sampling through a tail veil catheter) on fecal excrement pattern and circadian rhythm of fecal CORT metabolites. Fecal and blood samples were collected from 24 male (4 week old) SD rats individually housed in metabolic cages for 1 week prior to data collection. Rats were fed daily at 13:30 (5g food / 100g body weight). Fecal samples were collected hourly for 24 hours pre- and post-blood draw and were immediately stored at -80° C. Blood samples were obtained from 3 rats every 3 hours, with each animal being sampled only once by tail vein catheterization. We determined fecal and plasma CORT levels with a radioimmunoassay (RIA) kit. We measured fecal sample output, weight, and circadian rhythm in locomotor activity. Using fecal samples to assess CORT levels in any rodent behavioral study will: a) provide insight into stress levels of study animals, b) reduce the likelihood of introducing an unwanted stressor by blood sampling, and c) allow for frequent serial sample collections and CORT measures to assess long-term changes or changes in the circadian rhythm of these stress hormones.

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