



Alteration of daily urine 6-sulfatoxymelatonin, plasma corticosterone and hepatic clock gene expression in the folate deficient CBA mouse.

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Folate is a cofactor of CRY protein and is involved in methylation of many biological compounds including N-acetyl-tryptamine, the precursor of melatonin. These data prompted us to study in the mouse the influence of folate deficiency on daily melatonin production, plasma corticosterone—a marker of the circadian clock—and expression of clock genes in a peripheral tissue, the liver.

Eight week old CBA mouse submitted to a 12/12 light/dark cycle were fed an identical diet with or without folate (25 animals in each group). At the end of a 4 week treatment, 12h urine blocks (8h-20h, 20h-8h) were collected. After housing for 24h in the complete darkness to eliminate the possible masking effect of light on clock gene expression, the mice were hourly anaesthetised and killed under red light. Blood and organs were collected. Plasma folate and corticosterone and urine 6-sulfatoxymelatonin (aMT6s) were determined by radioimmunoassay. Hepatic RNAs were extracted with RNABLE (Eurobio[®]) and quantified using real time PCR (Light Cycler Roche[®])

Compared with controls, all animals fed the folate deficient diet showed a decreased erythrocyte folate concentration ($p < 0.0001$), a decrease in day and night urine aMT6s elimination ($p < 0.05$ for both), and a advanced phase for the 24h plasma corticosterone profile. A 2 way-ANOVA followed by multiple comparisons performed on the daily clock gene expression showed a phase-advance for *Cry1*, *Per1* and *Per3* expressions, a major increase of *Per2* expression during the overall subjective night, and an increase of *Cry1*, *Clock*, and *Bmal* expressions at the end of the subjective night.

The results obtained for aMT6s excretion in the CBA mice confirm those obtained in folate deficient rats. Taking into account the alterations of both folate metabolism and rhythms (decreased amplitude and advanced phase, fig. 1, 2) observed in old people, we suggest that the folate deficient CBA mice could be a model of aging.

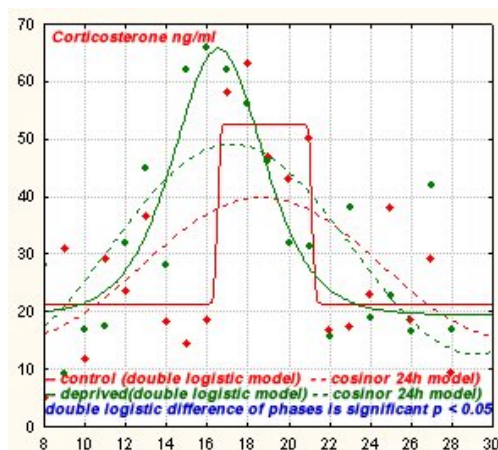


Figure 1: Plasma Corticosterone.

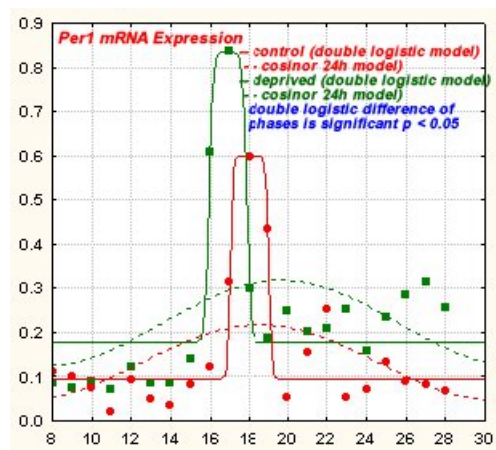


Figure 2: *Per1* mRNA Expression.

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