Abstract:

Many processes in mammalian body exhibit circadian rhythms. These rhythms are driven by an intricate system composed of the central pacemaker, suprachiasmatic nuclei (SCN) in the brain, which entrains the peripheral oscillators in various organs, such as pancreas, liver, colon and lungs. Circadian clocks are autonomously driven in each cell based on molecular circuits involving so-called clock genes, such as BMAL, CLOCK, PER and CRY. Age-dependent impairment of physiological functions of mammalian body, such as behaviour and metabolic functions, has been well documented. However, it has not been fully elucidated whether the impairment is linked with worsening of the circadian clock function.

The aim of our study was to find out whether i) aging affects basic properties of the circadian clock in SCN and peripheral organs, such as pancreas, colon, liver and lungs, ii) aging-induced changes in glucose homeostasis affect the properties of the circadian clock in the pancreas, and iii) the sensitivity of circadian clock in SCN and peripheral organs to disturbances in environmental lighting conditions is altered during aging.

We used groups of adult (9 months) and aged (25 months) animals which were subjected to 3 different light regimes, namely to light/dark regime (LD 12:12), constant light (LL) and constant darkness (DD) in order to study the synchronization of the circadian system during aging. We assessed properties of their peripheral circadian clocks in vivo by creating expression profiles of clock genes by RT qPCR. Metabolic wellbeing of experimental animals was assessed by IPGTT tests and by determining the levels of expression of genes linking circadian clock with metabolism. Furthermore, we studied the aging clock in vitro using organotypic explants from SCN, pancreas and lungs of genetically modified mice expressing clock gene PER2 fused with gene for luciferase enabling us to detect bioluminescence rhythms of the clock. Additionally, synchronization capacity of peripheral clocks was tested in vitro by repeated 8-h treatments.

We revealed that the exposure to LL worsened age-dependent decline in behavioural rhythms. The ability to produce robust Per2\textsuperscript{LUC} bioluminescence rhythms in vitro in SCN explants was not compromised under any of applied light regimes. The results also revealed tissue-specific effects of aging on the rhythmic production of Per2\textsuperscript{LUC} protein. Aged pancreas showed robust oscillation of circadian clock; however, pancreas exhibited high sensitivity to in vivo lighting conditions, which did not improve after providing rhythmic signal in vitro. On the other hand, aging significantly affected the period of the clock in the lungs under all lighting conditions and the LL exposure did not have effect on the clock in the lungs.

Our aged mice developed hyperinsulinemic hypoglycaemia which was likely due to the Pclo-mediated insulin hyper-secretion together with Slc2a2-mediated glucose transport impairment in pancreas. Pp1r3c-related glycogen storage and Sgk1-related glucose transport in the liver were also impaired. Expression of clock genes in pancreas was only marginally affected by aging, it upregulated the expression of BMAL1 and downregulated the expression of CLOCK.

Altogether, our results demonstrate that since the SCN-driven locomotor activity was clearly affected by aging and the molecular circadian core clock mechanism was relatively resilient to aging, possibly the pathways responsible for this phenomenon are downstream of the core clock mechanism of SCN. We also demonstrated, that aging does not compromise
pancreatic clock, but significantly affects formal properties of the \textit{in vitro} circadian clock in the lungs. Our findings provide a possible explanation for previously demonstrated relationship between disturbance of the circadian system and disordered glucose homeostasis. This includes diabetes mellitus type 2 in subjects exposed to long-term shift work.

**Key words:** circadian clock, aging, pancreatic clock, SCN, Per$^{2\text{LUC}}$, T2DM