Multi–omics in Metabolic Disease: the importance of circadian time

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Studies seeking to define the impact of genetic and dietary metabolic deficiencies in model rodents have often measured the metabolome or transcriptome from tissue samples harvested during the day, by convenience for the researchers. Mice and rats being nocturnal, means that these measurements were made during the resting/fasting phase of these animals. Thus, conclusions may have been affected with consequences of these deficiencies underestimated.

We have previously shown a bidirectional link between 1-carbon metabolism and the circadian clock in many species, such that 1-carbon metabolism regulates the circadian clock and vice-versa. It is therefore very likely that the consequences of 1-carbon metabolism deficiencies on metabolome or transcriptome will be dependent on the time of day at which they are measured.

Here, we show that mice fed a methionine/choline deficient (MCD) diet rapidly and dramatically lose normal circadian rhythms at the behavioural and molecular levels. The MCD diet is commonly used to induce steatohepatitis as a model for human NASH, but the weight loss and insulin hypersensitivity of mice under this diet are opposite to what is seen in NASH, questioning the validity of this model. Indeed, we demonstrate that, far from only affecting fatty acid metabolism in the liver, the MCD diet causes widespread changes in the liver and brain circadian transcriptomes and metabolomes, commensurate with changes in circadian locomotor activity rhythms, highlighting the systemic effects of this diet. Importantly, we show that conclusions on the impact of dietary deficiencies are highly influenced by the time at which measurements are made, calling for the inclusion of circadian time in metabolic disease study designs.

INTRODUCTION

The MCD diet is a classical method to induce hepatic steatosis in rodents as a model for human non-alcoholic steatohepatitis (NASH)[1]. The MCD causes a reduction in mitochondrial beta-oxidation, increased oxidative stress and changes in cytokines (notably TNFa) and adipokines production leading to steatohepatitis, inflammation and fibrosis[2-6]. While NASH in humans is usually associated with obesity and insulin resistance, rodents provided with an MCD diet display severe weight loss and improved insulin sensitivity, questioning the validity of the MCD model[7, 8].

We propose that the MCD diet, rather than solely a model for NASH, is rather a model for systemic dietary methyl metabolism deficiency. Indeed, methionine is required for the synthesis of the universal methyl donor S-adenosyl-L-methionine (SAM), and the MCD diet leads to a rapid decrease in SAM in the liver[2]. Donating its methyl, SAM becomes S-adenosyl-L-homocysteine (SAH), which is then hydrolysed to homocysteine and adenosine. Homocysteine can be remethylated back to methionine using either methyltetrahydrofolate in many tissues or betaine specifically in the liver. In the liver, choline is the precursor for betaine.

We have previously shown *in vitro* and *in vivo* that circadian rhythms are particularly sensitive to pharmacological or dietary interventions targeting the methyl cycle, and that many mRNAs coding for enzymes involved in 1-carbon metabolism have circadian rhythms of expression[9-13]. Together with the fact that NASH and liver fibrosis, both conditions driven by the MCD diet, are exacerbated by circadian misalignments[14], this highlights an intimate link between circadian rhythms and methyl metabolism.

Given the widespread rhythms in the expression of key rate-limiting enzymes and in the abundance of many metabolites, multi-omics analyses of metabolic diseases should take into account the time of day and the endogenous circadian time of the patients. Moreover, due to this cross-talk between metabolic and circadian rhythms, patients with dietary or genetic metabolic deficiencies may have unrecognised circadian disruption that may be detected by including circadian parameters in study designs. We illustrate this point by performing circadian phenotyping and multi-omics analyses in mice fed with the MCD diet.

RESULTS

Firstly, to define potential effects of the MCD diet on circadian rhythms, we fed control or MCD diet to C57-BL/6J mice kept in constant conditions of temperature and humidity with standard 12h light/12h dark cycles for 10 days before being transferred to constant darkness to allow expression of endogenous circadian rhythms. Mice had free access to food and water, and to a running wheel connected to a circadian locomotor activity recording system (ClockLab, Actimetrics) (Fig. 1).

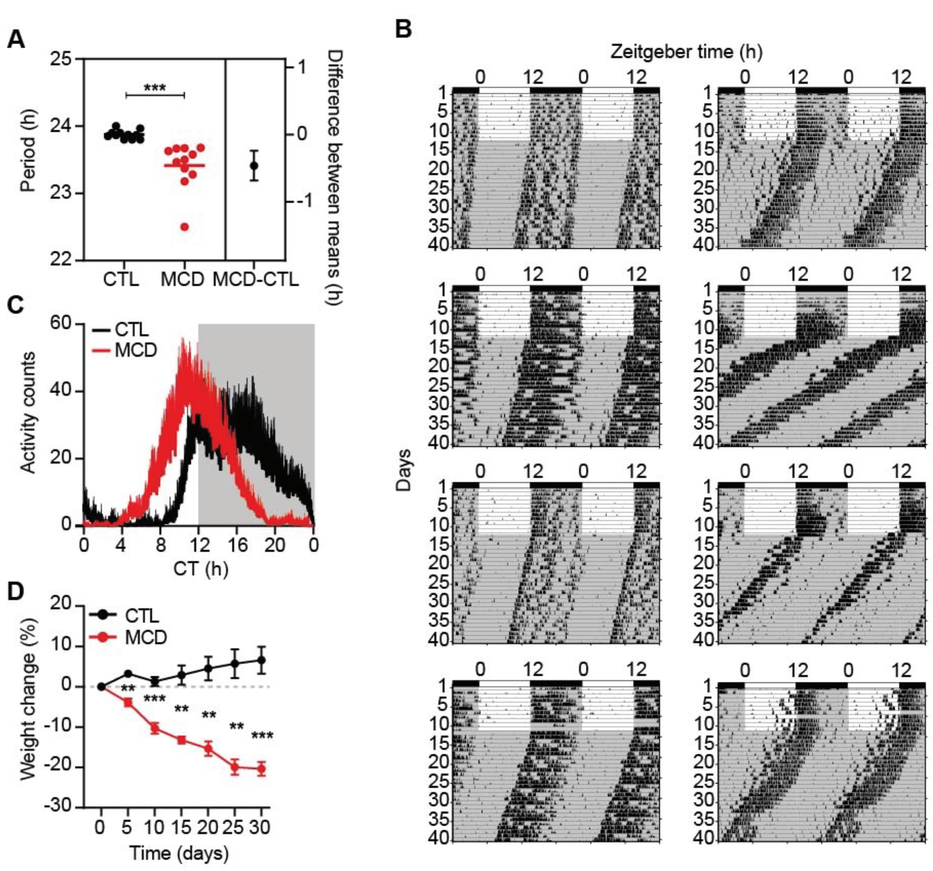

Fig. 1: Mice fed the MCD diet show dramatic changes in circadian organisation of activity. **A**, The MCD diet causes shortening of the endogenous circadian period. **B**, Mice fed a MCD diet (right pannels) show disrupted circadian organisation of wheel-running behaviour. Greyed area show when lights were turned off. **C**, Average activity profile of n = 4 mice. **D**, Mice (n =4) fed the MCD diet progressively lose weight. All data shown include equal number of male and female mice.

Secondly, to identify the mechanisms underlying these changes in circadian behaviour, we analysed the circadian transcriptome in the suprachiasmatic nucleus of the hypothalamus (SCN, where the master clock resides). In addition, to define whether the MCD diet also had an influence on circadian rhythms in the liver, its primary target, we also analyse the circadian transcriptome in this tissue (Fig. 2).

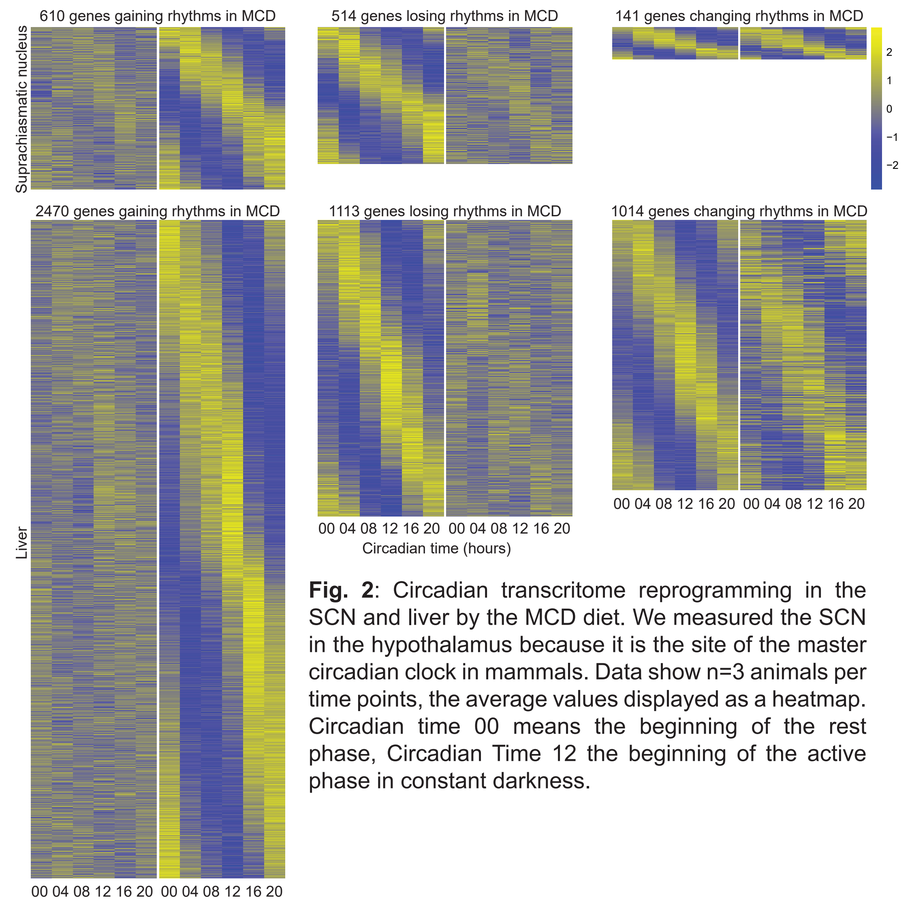

Fig. 2: Circadian transcritome reprogramming in the SCN and liver by the MCD diet. We measured the SCN in the hypothalamus because it is the site of the master circadian clock in mammals. Data show n=3 animals per time points, the average values displayed as a heatmap. Circadian time 00 means the beginning of the rest phase, Circadian Time 12 the beginning of the active phase in constant darkness.

Thirdly, to understand how 1-carbon metabolism in the SCN and the liver is affected by the MCD diet, we measured 1-carbon metabolites in these tissues at different time points during a circadian cycle (Fig. 3).

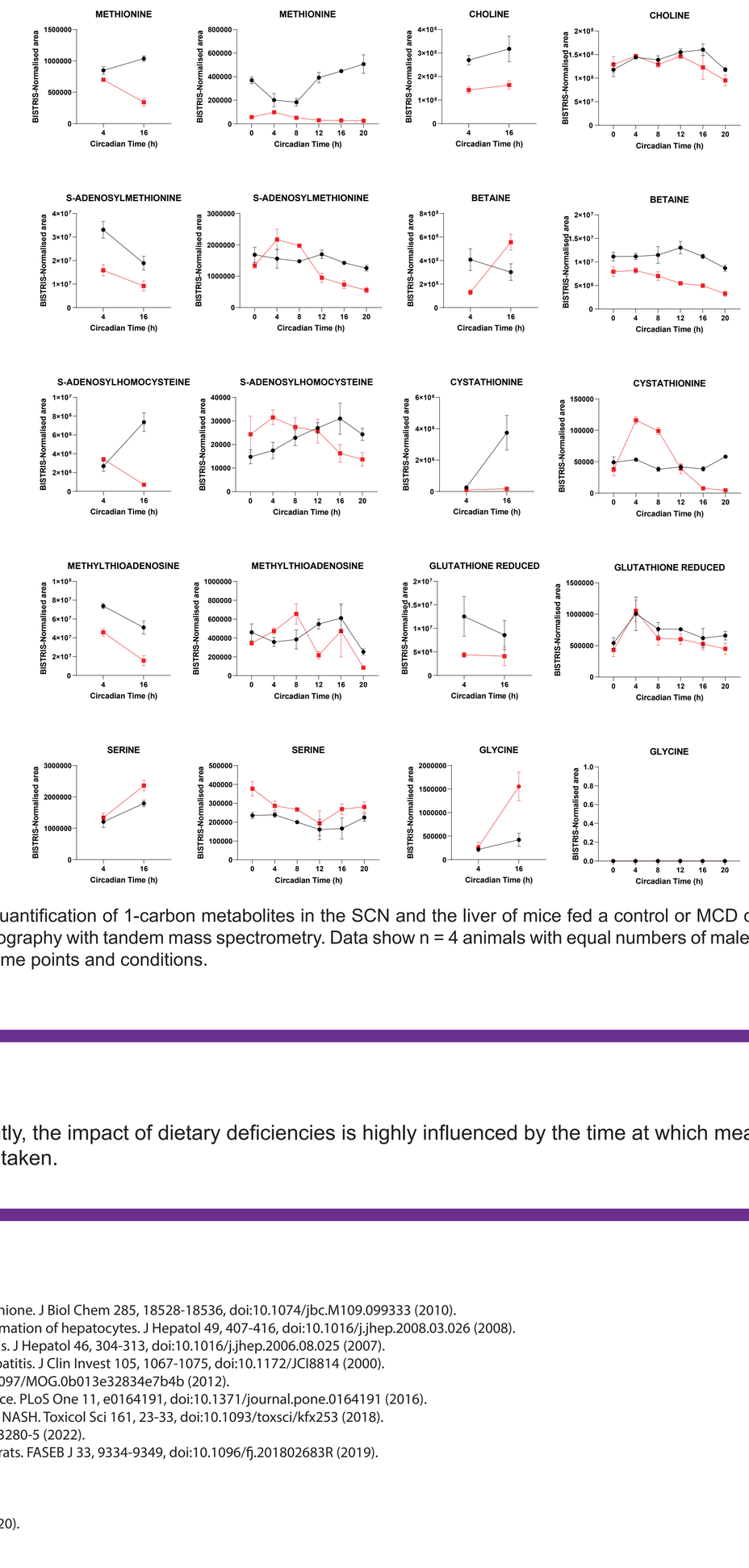

Fig. 3: Quantification of 1-carbon metabolites in the SCN and the liver of mice fed a control or MCD diet by Liquid Chromatography with tandem mass spectrometry. Data show n = 4 animals with equal numbers of male and females at each time points and conditions.

CONCLUSIONS

In conclusion, the MCD diet causes changes in the liver and brain physiology, likely affecting the whole body. Importantly, the impact of dietary deficiencies is highly influenced by the time at which measurements are made, calling for the inclusion of circadian time in metabolic disease study designs and when patient samples are taken.

REFERENCES

1 Conn, P. M. Animal models for the study of human disease. Second edition. edn, (Academic Press, an imprint of Elsevier, 2017).
2 Caballero, F., et al., Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J Biol Chem 285, 18528-18536, doi:10.1074/jbc.M109.099333 (2010).
3 Larter, C. Z., Yeh, M. M., Williams, J., Bell-Anderson, K. S. & Farrell, G. C. MCD-induced steatohepatitis is associated with hepatic adiponectin resistance and adipogenic transformation of hepatocytes. J Hepatol 49, 407-416, doi:10.1016/j.jhep.2008.03.026 (2008).
4 dela Pena, A., Leclercq, I. A., Williams, J. & Farrell, G. C. NADPH oxidase is not an essential mediator of oxidative stress or liver injury in murine MCD diet-induced steatohepatitis. J Hepatol 46, 304-313, doi:10.1016/j.jhep.2006.08.025 (2007).
5 Leclercq, I. A., Farrell, G. C., Field, J., Bell, D. R., Gonzalez, F. J. & Robertson, G. R. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. J Clin Invest 105, 1067-1075, doi:10.1172/JCI8814 (2000).
6 Corbin, K. D. & Zeisel, S. H. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Curr Opin Gastroenterol 28, 159-165, doi:10.1097/MOG.0b013e32834e7b4b (2012).
7 Chiba, T., et al., Evaluation of Methionine Content in a High-Fat and Choline-Deficient Diet on Body Weight Gain and the Development of Non-Alcoholic Steatohepatitis in Mice. PLoS One 11, e0164191, doi:10.1371/journal.pone.0164191 (2016).
8 Li, H., Toth, E. & Cherrington, N. J. Asking the Right Questions With Animal Models: Methionine- and Choline-Deficient Model in Predicting Adverse Drug Reactions in Human NASH. Toxicol Sci 161, 23-33, doi:10.1093/toxsci/kfx253 (2018).
9 Fukumoto, K., Ito, K., Saer, B., et al., Fustin, J. M., Excess S-adenosylmethionine inhibits methylation via catabolism to adenine. Commun Biol 5, 313, doi:10.1038/s42003-022-03280-5 (2022).
10 Abu Ahmad, N., et al., Betaine attenuates pathology by stimulating lipid oxidation in liver and regulating phospholipid metabolism in brain of methionine-choline-deficient rats. FASEB J 33, 9334-9349, doi:10.1096/fj.201802683R (2019).
11 Fustin, J. M., Methyl Metabolism and the Clock: An Ancient Story With New Perspectives. J Biol Rhythms, 7487304221083507, doi:10.1177/07487304221083507 (2022).
12 Fustin, J. M., et al., Methylation deficiency disrupts biological rhythms from bacteria to humans. Commun Biol 3, 211, doi:10.1038/s42003-020-0942-0 (2020).
13 Fustin, J. M., et al., RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell 155, 793-806, doi:10.1016/j.cell.2013.10.026 [doi] (2013).
14 Saran, A. R., et al., Circadian Rhythms in the Pathogenesis and Treatment of Fatty Liver Disease. Gastroenterology 158, 1948-1966 e1941, doi:10.1053/j.gastro.2020.01.050 (2020).