# Medium chain fatty acids are metabolised by the hypothalamus and regulate energy balance in healthy mice

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Nutrient sensing by hypothalamic neurons is vital for the control of food intake and energy expenditure. The arcuate, the ventromedial and the lateral nuclei of the hypothalamus contain specific populations of neurons that are sensitive to long chain fatty acids (LCFA). Intracellular metabolism is hypothesized as a key component of LCFA neuronal signaling. In addition to a signaling role, fatty acids (FA) may be an important source of energy within the brain. Whilst glucose is its primary energy source, the brain also has the capacity to oxidise LCFAs such as palmitic acid and oleic acid, however, the metabolic fates of FA within the hypothalamus are not yet understood.

Rat blood and CSF collection experiments were carried out in the UK according to the Animals (Scientific Procedures) Act, 1986 (U.K. Government Home Office) including the recent revision incorporating the European Directive 2010/63/EU on the protection of animals used for scientific purposes. Experiments involving CSF and blood sampling received ethical approval from the University of Warwick ethical review committee and were covered by the Home Office Project license PPL. Mouse experimental protocols were approved by the Monash Animal Research Platform Animal Ethics Committee, Monash University, Australia, and conformed to the National Health & Medical Research Council (NHMRC) code of practice. For collection of CSF and blood samples and for *in vivo* radioactive tracer experiments, rats and mice were anaesthetized with isoflurane inhalation, with a 4% dosage for induction and 2% for maintenance.

To first determine the availability of FA to the hypothalamus, the free fatty acid (FFA) composition of rat blood and cerebrospinal fluid (CSF) was assessed by mass spectrometry. The FFA profiles of whole blood and CSF differed significantly in saturation. In blood, 41% of total FFA were saturated, 16% were monounsaturated and 42% were polyunsaturated, while the CSF FFA were almost exclusively saturated (89%), with low concentrations of mono- and polyunsaturated FFA (4% and 7%, respectively). Blood and CSF FFA profiles were both predominantly composed of LCFA (96% and 94%, respectively), but medium chain fatty acids (MCFA) constituted a larger portion of the CSF FFA pool (4%) than was seen in the blood (1%).

In subsequent experiments in mice using radiolabelled FA, we demonstrated that the hypothalamic uptake of arterial-administered LCFAs is negligible, but MCFAs are readily taken up and oxidised. To elucidate a potential role of MCFA in appetite and energy homeostasis, lean mice were gavaged with water, trioleic acid (long-chain triglyceride (LCT)) or tri-octanoic acid (medium-chain triglyceride (MCT)) prior to the main feeding time for mice (1600 h). In the first 8 hours after gavage, MCT reduced voluntary food intake by 53% and 41% compared with water and LCT, respectively. LCT did not reduce food intake beyond the caloric load of the treatment. No ‘rebound’ feeding effect was seen in any treatment group after 8 hours. The reduction in food intake following MCT gavage was accompanied by an increase in energy expenditure, but not in physical activity. These data demonstrate that lean mice respond to MCT by reducing their food intake and increasing their energy expenditure. Understanding the mechanisms by which MCT can regulate energy balance may provide avenues for the development of novel therapeutics for treating obesity.