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## **Investigation of the effect of caffeine on human brain metabolism by magnetic resonance spectroscopy and multimodal magnetic resonance imaging**

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Caffeine (Caf) is the most widely used psychostimulant worldwide. Yet the mechanisms of action of Caf on the central nervous system (CNS) are largely unknown. The purpose of this study was to longitudinally delineate the acute impact of Caf on cerebral metabolite changes in healthy subjects by proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) and multimodal magnetic resonance imaging subsequent to the ingestion of a Caf dose of 7.5 mg / kg body weight. This leads to tissue concentrations around 50  $\mu\text{M}$  and can be considered moderate as the toxic range starts  $> 200 \mu\text{M}$ . Multimodal magnetic resonance imaging included diffusion weighted imaging and  $\text{T2}'$  relaxometry. Each participant was examined before and over 65 minutes after per os Caf exposure. During the whole protocol, the subjects stayed within the scanner, enabling  $^1\text{H-MRS}$  data acquisitions based on the initial shim. Vegetative effects (heart rate) were registered continuously. At defined timepoints after Caf ingestion, blood samples were taken from the volunteers and analyzed. Based on information about habitual Caf consumption and on reported subjective alertness before and after Caf ingestion, volunteers were classified as either heavy or light Caf users and as either Caf sensitive or insensitive by two different non-hierarchical cluster analyses.

As absolute values of metabolite resonances from  $^1\text{H-MRS}$  are difficult to interpret, metabolite ratios have been used to quantify changes in cerebral metabolism. In this study, a significant effect of Caf on brain creatine (Cre), which is most often used as the reference metabolite, was observed. Using metabolite ratios for quantification without definite a priori knowledge about the kinetics of the reference metabolite may entail detrimental consequences. Using unsuppressed water as internal reference and referring to metabolite specific baseline rather than metabolite ratios seems to yield more unbiased concentrations. This approach is only feasible if all measurements are based on the initial shim.

Different aspects of the various effects of Caf on human brain were simultaneously characterized:

### 1. Water homeostasis

Neither the cerebral total water signal, nor cerebral osmolyte resonances, nor apparent diffusion coefficients (as a measure of water molecular diffusion) changed over time after Caf administration. It can be concluded that if Caf is to have an effect on fluid balance, these are compensated for in the CNS.

### 2. Creatine metabolism

Cre metabolite resonances significantly decreased after Caf ingestion. A trend towards increasing guanidinoacetate (GAA) levels was also observed. Increasing levels of the substrate (GAA) for the enzyme guanidinoacetate-methyl-transferase (GAMT) and simultaneously decreasing levels of its product (Cre) suggest that it might be inhibited by Caf. This was observed for the first time in the present study. A research cooperation with the *Metabolic Unit, VU University Medical Center, Amsterdam, NETHERLANDS* was established and the hypothesis that Caf inhibits GAMT was validated in cell culture experiments by these external cooperators.

### 3. Energy metabolism

As described elsewhere, rising lactate (Lac)/N-acetyl-aspartate (NAA) ratios after Caf ingestion had been observed in a Caf-intolerant group and within a group of regular users who had abstained for 1–2 months in a  $^1\text{H}$ -MRS experiment at 1.5 T. The present work measures similar effects in a cohort of healthy volunteers with no additional constraints on habitual Caf consumption or Caf sensitivity at higher field strength (3T) and with a measurement protocol that avoids ratios with a reference metabolite. In the present study cerebral Lac metabolite resonances increased and cerebral metabolite resonances of glucose (Glc), the principal energy substrate of the CNS, decreased over time after Caf ingestion. This reflects changes in cerebral energy metabolism. In blood an increasing trend of both Lac and Glc concentrations was observed. Cerebral metabolic rates of Glc and Lac were calculated and used to show that aerobic glycolysis and anaerobic glycolysis increase simultaneously. Through its action on adenosine receptors, Caf leads to simultaneous cerebral vasoconstriction and an increase in synaptic activity. To meet increasing cerebral energy demand and with the challenge of simultaneously decreasing cerebral blood flow due to vasoconstriction, brain tissue thus upregulates the two different energy sources of aerobic glycolysis and anaerobic glycolysis. The multimodal measurement protocol enabled the simultaneous recording of T2' relaxometry data. For the first time T2' relaxometric data was acquired in humans at 3T. Quantitative T2'-imaging (qT2') is a quantitative measurement of cerebral blood oxygen saturation. T2' decreased and R2' increased after Caf administration. These results are compatible with increasing oxygen extraction fraction and are in agreement with the data about energy metabolism from  $^1\text{H}$ -MRS.

### 4. Subgroup analysis

Habitual Caf consumption and Caf sensitivity did not correlate. No effect of the habitual Caf consumption on Caf sensitivity can be inferred. Caf sensitive individuals had a stronger increase in heart rate than Caf insensitive individuals, i.e. the subjective measure of Caf sensitivity correlated with measurable vegetative effects of Caf. Heavy users reached significantly higher blood peak Caf concentrations than light users. Caf sensitive users reached higher blood peak Caf concentrations than Caf insensitive individuals. No influence of the subgroup analysis of either habitual Caf consumption or Caf sensitivity was observed on water homeostasis or Cre metabolism. In contrast, the observed effects of Caf on energy metabolism showed a subtle modulation by habitual Caf consumption and Caf sensitivity. Caf insensitive volunteers had a greater increase in brain Lac than Caf sensitive volunteers and light Caf users had a greater increase in brain Lac than heavy users.

Future projects following the present work could include a  $^{31}\text{P}$ -MRS study. This could further elucidate the effect of Caf on Cre metabolism and on energy metabolism. The interplay between Cre and phosphocreatine (PCre) could be unraveled. Both  $^1\text{H}$ -MRS and  $^{31}\text{P}$ -MRS could also be performed on skeletal muscle to highlight the differences in the action of Caf on these different tissues. In a future study the methods used in the present work could be combined with perfusion measurements, e.g. with non-invasive arterial spin labeling (ASL). When incorporated into a multimodal MR experiment, the information from perfusion measurements would yield information about cerebral blood flow which could be directly correlated with the effects on energy metabolism observed in the present work: increasing oxygen extraction, increasing aerobic glycolysis and increasing anaerobic glycolysis.