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Medium chain triglyceride diet reduces anxiety-like behaviors and enhances social competitiveness in rats

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ABSTRACT

Medium-chain triglycerides (MCT) are emerging as unique dietary supplements that are potentially relevant for the amelioration of brain dysfunctions. MCT are converted into ketones and free medium chain fatty acids that, in the brain, are highly effective energy sources to mitochondria and potentially less harmful than glucose metabolism to neurons. Given the recently established link between mitochondrial dysfunction and high anxiety and depression, we performed this study to investigate the effectiveness of an MCT-enriched diet to ameliorate anxiety- and depression-related behaviors in rats. Male rats were distributed into groups, according to their anxiety-like behaviors in the elevated plus maze. Each group was given either MCT-supplemented diet or an isocaloric control diet for fifteen days. Starting from the eighth day of diet, rats were exposed to different behavioral tests. MCT-fed rats exhibited reduced anxiety-like behaviors and enhanced social competitiveness, while their coping responses in the forced swim test were not affected by the treatment. When evaluated at the end of the two-week MCT diet, mitochondrial respiration was reduced in the medial prefrontal cortex (mPFC) while unchanged in the nucleus accumbens. In the mPFC, enzymes related to glycolysis and oxidative phosphorylation were also decreased by MCT diet, while proteins controlling glucose and glutamate transport were increased. Altogether, our findings strongly suggest the effectiveness of MCT diet to exert anxiolytic effects. In the brain, our results point to the mPFC as a brain region in which MCT supplementation improves transport and control of energy substrates.

1. Introduction

Medium-chain triglycerides (MCT) are saturated fats consisting of six to ten carbon atoms linked into chains predominantly found in limited foods such as coconut oil, palm kernel oil, and dairy fats. MCT provide fewer calories and are more rapidly absorbed and metabolized compared to long-chain triglycerides (LCT), whose longer length cause them to be stored as fat in the body. Consequently, MCT are increasingly being used in ketogenic diets as an alternative to the most frequently ketogenic diets composed of LCT. MCT can specifically affect brain function and are progressively used to improve neurobehavioral symptoms and cognitive function in clinical and preclinical studies of epilepsy (Gama et al., 2015; Packer et al., 2016), neurodegeneration (Costantini et al., 2008; Sharma et al., 2014; Zhao et al., 2012) and ageing (Balietti et al., 2010; Wang and Mitchell, 2016).

While both LCT and MCT are converted into ketones, beta hydroxybutyrate, acetone and acetoacetate in the liver, MCT is also converted into free medium chain fatty acids (MCFA). Ketones and MCFA are brain bioavailable via MCT and fatty acid transporters. In the brain, ketones serve primarily as fuel for neurons and astrocytes (Ebert et al., 2003). In contrast to glucose metabolism, ketones cause less oxidative stress when used as a mitochondrial energy source, and can bypass energy-consuming steps of the glycolytic and TCA cycle pathway (Prins and Matsumoto, 2014) Unlike LCT, MCFA rapidly cross the double mitochondrial membrane in the absence of carnitine (Williamson et al., 1968). Moreover, MCFA have been shown to affect both mitochondrial function and metabolism in cellular models (Hughes et al., 2014; Schönfeld and Wojtczak, 2016; Thevenet et al., 2016), and improve systemic energy metabolism *in vivo* (Zhao et al., 2012). Therefore, the emerging view is that ketones and MCFA may offer a means to improve energy metabolism

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efficiency without causing deleterious effects on neuronal function.

A potential application in which an MCT diet could be of benefit is anxiety. Emerging evidence points to the existence of mitochondrial dysfunction in individuals with high anxiety levels (Hovatta et al., 2010a; Tyrka et al., 2016; Wang et al., 2017), which is in line with data that individuals with mitochondrial disorders frequently display anxiety symptoms (Anglin et al., 2012; Morava and Kozicz, 2013). Mouse models of anxiety-like behavior display mitochondrial dysfunction at different stages of mitochondrial activity (Einat et al., 2005; Filiou et al., 2011). Importantly, high trait anxiety is a strong risk factor for developing stress-induced depression and anxiety disorders (Castro et al., 2012; Sandi et al., 2008; Sandi and Richter-Levin, 2009), and brain mitochondrial dysfunction is also emerging as an important hallmark of depression (Filipović et al., 2017; Gardner and Boles, 2011; Hovatta et al., 2010b; Marazziti et al., 2011; Wang et al., 2017). Several studies have evaluated the ability of LCT-based ketogenic diets to ameliorate anxiety and depression (Bostock et al., 2017; Kashiwaya et al., 2013; Sussman et al., 2015). However, to our knowledge, no study to date has examined the effects of a MCT-supplemented diet in the context of anxiety- and depression-related behaviors.

In humans, anxious subjects exhibit reduced competitiveness under stress (Goette et al., 2015), which can be predictive of a subordinate social status (Gilbert et al., 2009). Likewise, we have recently shown that high anxious rats show a propensity to become subordinate when paired against a low-anxious counterpart in a social dominance test (Hollis et al., 2015; van der Kooij et al., 2017). In the brain, this competitive disadvantage linked to high anxiety is associated with impaired mitochondrial function (i.e., decreased respiration, reduced protein content for the electron transfer chains I and II, and reduced ATP) in the nucleus accumbens (NAc), a brain region critically implicated in reward, motivation and exertion of effort (Hollis et al., 2015). Boosting mitochondrial function with nicotinamide in the NAc was able to increase social dominance (Hollis et al., 2015). Additionally, the medial prefrontal cortex (mPFC) has been implicated in social status and may also be subject to diet modulation (Wang et al., 2011). Mitochondrial function critically depends on substrate uptake and metabolism (Brand and Nicholls, 2011). Thus, the type of energy supply may have direct consequences for mitochondrial function by affecting the availability of substrates for oxidative phosphorylation, membrane potential, and proton motive force.

Therefore, here, we aimed to investigate the effectiveness of an MCT diet to decrease anxiety- and depression-related behaviors in adult, male Wistar rats. In addition, we aimed to assess the potential of the MCT diet to ameliorate social competitiveness and associated mitochondrial function in specific brain regions associated with social competition and anxiety.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, aged 2–3 months, were used for all experiments. Animals were individually housed in polypropylene cages (57 × 35 × 20 cm) with abundant pine bedding in a temperature- (23C) and light- (0700–1900 h) controlled room. All animals had ad libitum access to standard food and water. Upon arrival to the facility, animals were allowed to habituate to the vivarium for one week and were then handled for 2 min/d during 3 days prior to the start of all experiments. All experiments were performed with the approval of the Cantonal Veterinary Authorities (Vaud, Switzerland) and carried out in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU).

2.2. Behavioral tests

All behavioral manipulations were performed during the light phase by experimenters blind to treatment groups. All efforts were made to minimize the number of animals while maintaining statistical rigor.

2.2.1. Elevated plus maze

Prior to the dietary intervention, one hundred and two animals were tested for anxiety-related behavior in the EPM as previously described (Hollis et al., 2015). Lighting was maintained at 15–16 lux in the open arms and 5–7 lux in the closed. Depending on the amount of time spent on the open arm, animals were classified as high- (HA, ≤5% open arm duration), intermediate (IA, 6–20% open arm duration), or low-anxious (LA, ≥20% open arm duration). Before and in between testing, the apparatus was cleaned with a 5% EtOH solution. Three animals that fell off the maze during the procedure were omitted from the study. After EPM testing, rats were further subdivided into control (CON) and MCT feeding groups with similar overall average anxiety levels. In total, the group sizes were as follows: HA-CON = 14; IA-CON = 18; LA-CON = 17; HA-MCT = 15; IA-CON = 18; LA-CON = 17.

2.2.2. MCT dietary treatment and behavior testing schedule

Following group separation according to the EPM scores, one group (MCT) was given a diet of 5% medium chain triglycerides (40:60 of octanoic acid triglyceride and decanoic triglyceride) while the second group was given a control diet (5% sunflower oil) via specially formulated animal chow with traditional fat, carbohydrate and protein ratios (5% fat from sunflower oil, 35% starch, 3.5% crude fiber, 18% protein; Kliba Diet AIN93-G) similar to standard chow diets for 15 days. The duration was chosen based on previous evidence of rapid behavioral responses to short-term MCT supplementation (de Almeida Rabello Oliveira et al., 2008). Food was weighed daily to allow the calculation of food intake. Animals were left undisturbed for the first week of dietary exposure. On the 8th day, animals were evaluated for different behaviors using the following sequence of tests: light-dark box test, social dominance test, and social preference test. The experimental diet was continued throughout the behavioral testing, with two-day intervals of rest in between tests.

Finally, in order assess MCT effects on learned helplessness behavior, rats (10 IA rats from each treatment, matched for overall anxiety levels) were also assessed for forced swim test immobility for two consecutive days. After 15 days of dietary intervention, animals were euthanized and brain tissue collected for biochemical and metabolic analyses. See Fig. 1A for visual depiction of the schedule.

2.2.3. Social preference test

Animals were submitted to the social exploration test twice. The first test took place three days after phenotyping in the EPM to ensure that there were no baseline differences in social preference prior to the diet initiation (data not shown). Animals were tested again on the 12th day of dietary treatment to examine the effects of the MCT diet on social preference behavior.

The social preference test was performed in a rectangular, three-chambered box that included a central compartment where the rat was initially placed. Thereafter, retractable doors were removed and the rat could explore the left and right-compartment for 10 min. The left- and right-compartments were equipped with a floor-fixed transparent perforated Plexiglas cylinder that contained either an unfamiliar male juvenile rat or an unfamiliar object. The juveniles were between the ages of PND25-32 to avoid aggressive behavior from the experimental animal. Juveniles were habituated