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Research report

Effect of diet on brain metabolites and behavior in spontaneously hypertensive rats

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HIGHLIGHTS

- Spontaneously hypertensive rats (SHR) are utilized as an animal model of ADHD.
- Studied omega 3 fatty acids in altering neural energy metabolism/behavior in SHR.
- The increase in omega 3 fatty acids associated with a reduction in Creatine levels.
- Omega 3 fatty acids possibly altering brain energy and phospholipid metabolism.
- Significant correlations were observed between brain metabolites and behavior.

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ABSTRACT

Attention-deficit hyperactivity disorder (ADHD) is a heterogeneous psychiatric disorder affecting 5–10% of children. One of the suggested mechanisms underlying the pathophysiology of ADHD is insufficient energy supply to neurons. Here, we investigated the role of omega 3 fatty acids in altering neural energy metabolism and behavior of spontaneously hypertensive rats (SHR), which is an animal model of ADHD. To this end, we employed Proton Magnetic Resonance Spectroscopy (^{1}H MRS) to evaluate changes in brain neurochemistry in the SHR following consumption of one of three experimental diets (starting PND 21): fish oil enriched (FOE), regular (RD) and animal fat enriched (AFE) diet. Behavioral tests were performed to evaluate differences in locomotor activity and risk-taking behavior (starting PND 44). Comparison of frontal lobe metabolites showed that increased amounts of omega 3 fatty acids decreased total Creatine levels (tCr), but did not change Glutamate (Glu), total N-Acetylaspartate (tNAA), Lactate (Lac), Choline (Cho) or Inositol (Ino) levels. Although behavior was not significantly affected by different diets, significant correlations were observed between brain metabolites and behavior in the open field and elevated plus maze. SHR with higher levels of brain tCr and Glu exhibited greater hyperactivity in a familiar environment. On the other hand, risk-taking exploration of the elevated plus maze's open arms correlated negatively with forebrain tNAA and Lac levels. These findings support the possible alteration in energy metabolites in ADHD, correlating with hyperactivity in the animal model. The data also suggest that omega 3 fatty acids alter brain energy and phospholipid metabolism.

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is a heterogeneous psychiatric disorder characterized by three core symptoms, namely inattention, hyperactivity and impulsivity. It affects approximately 5%–10% of children worldwide [1] with an onset in childhood and can persist into adulthood. ADHD can result in impairments in social relations, academic performance and execution of daily activities. In some cases it is accompanied by learning disabilities, anxiety, conduct disorder and mood disorders [1].

Although the pathophysiology of ADHD remains unclear [2], several factors have been implicated in its etiology. One hypothesis suggests that ADHD is the consequence of neural energy

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dysregulation, due to a malfunction in the astrocyte/neuron lactate shuttle leading to reduced availability of Lactate (Lac). The immediate effect of such a deficit is that during tasks that demand rapid, sustained neuronal firing, neurons may lack the energetic resources to maintain a rapid, precisely-timed, firing pace. The long-term consequence can be developmental impairments in myelination of axons due to decreases in Lac levels influencing oligodendrocyte synthesis of fatty acids and myelin [3,4].

Other studies have implicated Glutamate (Glu), an excitatory neurotransmitter, in the pathophysiology of ADHD, possibly due to its interaction with dopamine and norepinephrine [5] and/or its link to glycolysis and the astrocyte/neuron lactate shuttle [6]. In addition, several neuroimaging studies conducted in human populations with ADHD have utilized Proton Magnetic Resonance Spectroscopy (^{1}H MRS), which is a non-invasive method to quantify brain metabolites including Glu, and showed higher Glutamate + Glutamine (Glx) to total Creatine (tCr) ratio (Glx/tCr) and Glu/tCr ratio in the prefrontal cortex and striatum, and lower Glx to Inositol (Ino) ratio (Glx/Ino) in the anterior cingulate cortex (ACC) compared to healthy controls [7–9].

Yet another factor that has been implicated in the pathophysiology of ADHD is diet. Currently in western society, we consume a great quantity of highly processed foods, rich in sugar, sodium and saturated fat and low in omega 3 fatty acids, starting from a very early age. The "Western diet" has been implicated in increased hyperactivity, as well as increased odds of having ADHD [10,11]. Fatty acids that are often low in the Western diet are essential for brain development. Deficits in omega 3 fatty acids have been linked to different health and neurodevelopmental problems including ADHD, and a deficiency in omega 3 fatty acids produces symptoms such as a lack of attention or hyperactivity. Indeed, children with ADHD have been found to have reduced red blood cell omega 3 fatty acids compared to typically developing children [12–15]. Deficits in dietary omega 3 fatty acid have also been linked to alterations in glutamatergic and serotoninergic neurotransmission, as well as mesocortical and mesolimbic dopaminergic system dysfunction [16,17]. Furthermore, animal studies have shown that dopamine neurotransmission is also affected in animals with diets deficient in omega 3 fatty acids or offspring of animals deficient in omega 3 fatty acids, with an important decrease in dopaminergic transmission in the prefrontal cortex consistent with the findings in ADHD [17,18].

Since the pathophysiology of ADHD remains unknown and many families refuse classical psychopharmacological treatment with psychostimulants for their children, we set out to explore the role of omega 3 fatty acids in an animal model of ADHD to determine whether dietary management may hold potential treatment benefits [19]. In this study, we hypothesized that feeding a diet supplemented with omega 3 fatty acids, with an increased omega 3 to omega 6 fatty acid ratio, would improve energy metabolism and thereby impact on monoaminergic and glutamatergic systems and improve myelination.

To perform these studies, we utilized an animal model of ADHD, the spontaneously hypertensive rat (SHR), which presents with the behavioral characteristics of inattention, impulsivity and hyperactivity [20,21]. Although this model has some weaknesses, such as the incoherence of results in response to methylphenidate [22,23], no animal model can fully mimic a human psychiatric disorder. The SHR animal model meets many of the criteria necessary for consideration as an adequate animal model of ADHD [24,25]. Additionally, it has been shown that SHR have less polyunsaturated fatty acids (PUFA), specifically docosahexaenoic acid (DHA), in brain membrane, when compared to the control strain Wistar Kyoto rats (WKY) [26].

To test our hypothesis, we applied ^{1}H MRS techniques to assess the neurochemistry, through measurement of total Cr and Glu levels within the frontal cortex and behavioral alterations (hyperactivity and risk-taking behavior) in the SHR animal model fed a diet supplemented with fish oil and rich in omega 3 fatty acids (FOE). We focused on the frontal cortex due to its involvement in the pathophysiology of ADHD [27–29]. According to our hypothesis, we expected to see changes in total Cr as the main metabolite implicated in energy metabolism as well as in Glu due to its role in stimulating glycolysis and the astrocyte/neuron lactate shuttle [6]. We expected that these changes would be in agreement with those observed in human studies. To the best of our knowledge, there has been no MRS study investigating neurochemical changes in the brain of the SHR animal model related to diet. As secondary findings, we expected to uncover changes in N-acetylaspartate (NAA) and Choline compounds (Cho) levels as markers of improved myelination and in Lactate (Lac) levels as a result of increased availability and more efficient neurotransmission due to the FOE diet. Lastly, we expected to see an improvement in the behavior – that is decreased hyperactivity – in the group fed a diet supplemented with omega 3 fatty acids.

2. Methods

2.1. Animal preparation

Male SHR ($N = 35$), weighing 25–35 g (post natal day, PND, 21), were obtained from Harlan Laboratories. They were housed in pairs in Plexiglas cages in an environment controlled for temperature (22–24 °C) and humidity, with a reversed 12 h light/dark schedule (lights off at 09:00 and on at 21:00). Food and water were provided ad libitum. The rats were randomly divided into three groups upon arrival and each group was fed with one of three different diets from their day of arrival to the completion of the experiments: fish oil enriched diet (FOE, $N = 12$); regular diet (Prolab IsoPro RMH 3000) (RD, $N = 11$); animal fat enriched diet (AFE, $N = 12$). The three test diets were provided by TestDiet and LabDiet, FOE and AFE were custom-made diets whereas RD was a typical laboratory diet. Caloric distribution was calculated based on the diet formulation. All three diets were analyzed for fatty acids via gas chromatography (Table 1) at the Diagnostic Center for Population and Animal Health, Michigan State University (East Lansing, MI). All the experiments were done during the day, which was night time for these animals, i.e. their active time.

Rats were weighed on PND 58. All rats were acquired and cared for in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals. This study was approved by IACUC Committee of the University of Massachusetts Medical School.

2.2. Imaging data acquisition and analysis

All imaging experiments were performed using a 4.7 T/40 cm horizontal magnet (Oxford, UK) equipped with 450 mT/m magnetic field gradients and interfaced with a Biospec Bruker console (Bruker, Germany). A ^{1}H radiofrequency (RF) coil configuration (Insight NeuroImaging Systems, Worcester, MA) with 12 cm inner diameter was used.

Throughout the imaging experiment, rats were anesthetized with isoflurane (1–1.5%) delivered through a nose cone and custom-fitted with a head restrainer containing a built-in saddle coil. The body temperature of the animal was monitored and maintained at 37 °C with a feedback controlled heating pad. The imaging sessions took place at least 72 h after the completion of the final behavioral experiment (PND 63–65).

For each imaging session, anatomical images were acquired using a multi-slice fast spin-echo sequence (RARE)