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Western-style diet induces insulin insensitivity and hyperactivity in adolescent male rats

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HIGHLIGHTS

- Western-style diet induces insulin resistance and impairs episodic memory in rats.
- Western-style diet rats show hyperactivity and reduced anxiety compared to controls.
- Western-style diet did not affect performance on the attentional set shifting task.

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ABSTRACT

The prevalence of obesity in children and adolescents has increased rapidly over the past 30 years, as has the incidence of attention deficit hyperactivity disorder (ADHD). In 2012, it was found that overweight children have a twofold higher chance of developing ADHD than their normal weight counterparts. Previous work has documented learning and memory impairments linked to consumption of an energy-dense diet in rats, but the relationship between diet and ADHD-like behaviors has yet to be explored using animal models. Therefore, the purpose of this study was to explore the role of diet in the etiology of attention and hyperactivity disorders using a rat model of diet-induced obesity. Male Sprague–Dawley rats were fed either a control diet or a Western-style diet (WSD) for ten weeks, and specific physiological and behavioral effects were examined. Tail blood samples were collected to measure fasting blood glucose and insulin levels in order to assess insulin insensitivity. Rats also performed several behavioral tasks, including the open field task, novel object recognition test, and attentional set-shifting task. Rats exposed to a WSD had significantly higher fasting insulin levels than controls, but both groups had similar glucose levels. The quantitative insulin sensitivity check index (QUICKI) indicated the development of insulin resistance in WSD rats. Performance in the open field test indicated that WSD induced pronounced hyperactivity and impulsivity. Further, control diet animals were able to discriminate between old and novel objects, but the WSD animals were significantly impaired in object recognition. However, regardless of dietary condition, rats were able to perform the attentional set-shifting paradigm. While WSD impaired episodic memory and induced hyperactivity, attentional set-shifting capabilities are unaffected. With the increasing prevalence of both obesity and ADHD, understanding the potential links between the two conditions is of clinical relevance.

1. Introduction

Attention deficit hyperactivity disorder, ADHD, is the most common childhood psychiatric disorder in the US [1]. ADHD afflicts ~9% of school age children in the US [2] and reduces performance on standardized tests, grades earned in high school, and college graduation rate. It also increases the rate of delinquency and arrest [3]. Although the symptoms of ADHD subside in some individuals, as many as 30% of afflicted children have symptoms that persist into adulthood [4].

Meanwhile, the United States is also struggling with a childhood obesity epidemic. Over the past 30 years, obesity rates have risen from 5 to 17% in children and adolescents, exposing youth to comorbidities such as type 2 diabetes and nonalcoholic fatty liver disease [5,6]. In addition, obesity is a major contributor to metabolic syndrome as characterized by insulin resistance, high triglyceride levels, low levels of high density lipoprotein, and hypertension [7]. Moreover, there is a 50% chance that obese youths will struggle with obesity as adults [8].

There is mounting evidence that obesity and ADHD may be linked in children and adolescents [9]. In 2012, Erhart et al. reported that children with ADHD were 1.9 times more likely to be overweight and that overweight children had a 1.5-fold greater risk for developing ADHD [10]. Although such studies control for age, gender, and socio-economic

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status, the data is collected via parental questionnaires and may suffer from respondent error or bias. Further, restriction of Western-style foods has been recommended for children with ADHD that cannot be managed with pharmacotherapy [11]. To date, there is no animal model linking obesity to ADHD-like behaviors, but there are clear benefits of finding an appropriate animal model in which to explore the underlying physiological basis of this relationship.

Obesity and prolonged exposure to an energy-dense diet have been shown to produce a number of neurophysiological changes in rats, such as impaired glucoregulation, neuroinflammation, and alterations in the structure of the blood–brain barrier surrounding the hippocampus and hypothalamus [12–14]. Diets high in fat and/or sugar have also been shown to impair performance on a variety of behavioral tasks including the novel object recognition task, Morris Water Maze, stone T-maze, and radial arm maze, indicating compromised episodic and spatial memory (see [15] for review).

While the effects of diet and obesity on learning and memory have been well studied, their role in ADHD-associated behaviors (i.e. hyperactivity, impulsivity, and inattentiveness) is not well understood. It has been shown that diet-induced maternal obesity leads to impaired reversal learning, one measure of attention, in offspring even when they are maintained on a control diet [16]. Moreover, Kaczmarczyk et al. showed that methylphenidate, a commonly prescribed ADHD medication, reverses cognitive impairments induced by short-term exposure to high-fat diet [17]. However, the direct effect of consuming an energy-dense diet on specific attention tasks has yet to be examined.

Due to the multi-faceted nature of attention, many tasks have been developed to assess attention in rodents, including: the multiple-choice serial reaction time task [18], signal detection task [19], and attentional set-shifting task [20]. Impulsivity and hyperactivity have been measured with response-withholding tasks and the open field test [21–23]. The spontaneously hypertensive rat, an animal model of ADHD, has been validated using many of these behavioral tasks [1,24]. Recently, however, the homogeneity of spontaneously hypertensive rat behavior has been called into question [25].

The goal of the present study was to investigate the relationship between energy-dense diets and ADHD-like behaviors by exposing adolescent male Sprague–Dawley rats to either a Western-style or control diet for 12 weeks. The Western-style diet (WSD) is high in both saturated fat and sugars and is known to produce obesity [26]. We hypothesize that a WSD will induce metabolic issues such as obesity and insulin resistance, as well as memory impairments and ADHD-like behaviors in rats. Physiological parameters such as body weight, blood glucose, and insulin levels were monitored. Additionally, the rats' behavior was assessed with the open field test, novel object recognition task, and attentional set-shifting task.

2. Methods

2.1. Animals & dietary conditions

Male Sprague–Dawley rats (n = 18; p32; 120–124 g) were purchased from Harlan Laboratories (Virginia) and allowed five days to acclimate before the start of the study. The animals were housed individually in a climate-controlled environment under a 12:12 h light:dark cycle with the light phase beginning at 0900 h each day. All behavioral tests were conducted during the light phase between 1300 and 1800 h. Animals were weighed and handled daily. All procedures were approved by the Washington & Lee Institutional Animal Care and Use Committee.

Following the acclimation period, rats were randomly assigned to either a WSD (n = 9) or control diet (n = 9). The control group had ad libitum access to standard powdered chow containing 3.02 kcal/g (Lab Diet, #5001) and 13.4% of the calories were derived from fat. The WSD group had *ad libitum* access to powdered chow containing lard and dextrose (Teklad, #04489). The WSD supplied 4.4 kcal/g and 41.7% of the calories were derived from fat. Both diets were provided in glass cups, fastened to the front of the cages to prevent food spillage. Water was also provided *ad libitum*. Animals were fasted 12 h prior to glucose and insulin measurements. Further, food intake was restricted prior to performance of the attentional set-shifting task until body weight fell by 15%. During this period, 3 Honey Nut Cheerios were supplied along with a reduced amount of powered chow to expose animals to the rewarding variable in the attentional set-shifting task.

2.2. Fasting blood glucose & insulin levels

Fasting blood glucose and insulin levels were measured after 10 weeks of diet exposure. The animals were fasted overnight, and then blood samples were collected from a small incision made at the tip of the each rat's tail. Glucose measurements were obtained with an Accu-Chek Compact Plus meter. For detection of insulin levels, blood was also collected in heparinized glass capillaries at the same time, spun down, and plasma collected. Plasma samples were then stored at −20 °C until insulin levels were determined using a rat insulin ELISA kit as per the manufacturer's instructions (Crystal Chem, 90010).

2.3. Open field task

The open field apparatus was a 60 × 60 × 38 cm box made of laminate-covered wood. A rat was placed in the center of the open field and allowed to explore for 8 min. The apparatus was cleaned thoroughly with 70% ethanol solution between subjects to minimize conspecific cues. All trials were videorecorded for later analysis. The total distance traveled (cm), average velocity (cm/s) of each animal, and number of entries and time spent in the center of the box were analyzed using a customized Matlab script. The frequency and time (s) spent exploring and immobile were evaluated by student observers who were blind to the animal's dietary group.

2.4. Novel object recognition task

The novel object recognition (NOR) task is based on the tendency of rodents to preferentially explore novel objects over familiar ones [27]. In the first phase of NOR training, rats were habituated to the testing environment, the same box used in the open field test. On the day following habituation, two identical objects were placed in the center of the box, equidistant from each other and the sides of the box. Each rat was placed in the box and given 5 min to explore then returned to its home cage for 1 h. For the test phase, one of the original reference objects was replaced with a novel object that differed in color and shape but was approximately the same size. Objects included plastic duplo blocks and small plastic containers. The positions of the new and old objects were randomized between rats. The box and the objects were cleaned between individual trails with 70% ethanol. All trials were recorded for later analysis. Exploratory behavior was defined as coming within a centimeter or less of the object, sniffing the object, rearing on the object, or otherwise exploring the object. Following these guidelines, two observers who were blind to the animals' dietary condition analyzed video data separately. If total exploration time was less than 1 s, the trial was excluded from analysis. This resulted in 2 animals being dropped from analysis. Pearson correlations were performed in order to ensure a high degree of inter-rater reliability.

2.5. Attentional set-shifting task

The attentional set-shifting task (ASST) in rodents, sometimes called the sand-digging task, has parallels to human clinical assessments such as the Wisconsin card sorting task. The task requires minimal equipment and has a relatively short training time, as rats can be readily trained to dig in small pots for food rewards [28].