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INTER-RELATIONSHIPS AMONG DIET, OBESITY AND HIPPOCAMPAL-DEPENDENT COGNITIVE FUNCTION

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Abstract—Intake of a Western diet (WD), which is high in saturated fat and sugar, is associated with deficits in hippocampal-dependent learning and memory processes as well as with markers of hippocampal pathology. In the present study, rats were trained to asymptote on hippocampal-dependent serial feature negative (FN) and hippocampal-independent simple discrimination problems. Performance was then assessed following 7 days on *ad libitum* chow and after 10, 24, 40, 60, and 90 days of maintenance on WD, on ketogenic (KETO) diet, which is high in saturated fat and low in sugar and other carbohydrates, or continued maintenance on chow (CHOW). Confirming and extending previous findings, diet-induced obese (DIO) rats fed WD showed impaired FN performance, increased blood–brain barrier (BBB) permeability, and increased fasting blood glucose levels compared to CHOW controls and to diet-resistant (DR) rats that did not become obese when maintained on WD. For rats fed the KETO diet, FN performance and BBB integrity were more closely associated with level of circulating ketone bodies than with obesity phenotype (DR or DIO), with higher levels of ketones appearing to provide a protective effect. The evidence also indicated that FN deficits preceded and predicted increased body weight and adiposity. This research (a) further substantiates previous findings of WD-induced deficits in hippocampal-dependent FN discriminations, (b) suggests that ketones may be protective against diet-induced cognitive impairment, and (c) provides evidence that diet-induced cognitive impairment precedes weight gain and obesity.

Key words: dementia, saturated fat, Pavlovian, memory, ketogenic diet, energy regulation.

INTRODUCTION

Energy dense diets that are high in saturated fat and sugar are often referred to as Western diets (WD) based on their widespread popularity in Western and Westernized societies. Previous studies using human and nonhuman animal models have shown that consuming WD is associated with not only weight gain and metabolic disease, but also with impaired hippocampal-dependent memory and the emergence of hippocampal pathologies (for details see reviews by Benoit et al., 2010; Kanoski and Davidson, 2011). In addition, some of these studies show that rats maintained on WD exhibit reductions in hippocampal brain-derived neurotrophic factor (Molteni et al., 2002; Kanoski et al., 2007) and hippocampal neurogenesis (Park et al., 2010), as well as increased hippocampal inflammation (Puig et al., 2012; Herculano et al., 2013) and blood–brain barrier (BBB) permeability (Kanoski and Davidson, 2011; Freeman and Granholm, 2012). Furthermore, under at least some test conditions, the effects of WD on hippocampal-dependent memory and BBB permeability depend on obesity phenotype (Davidson et al., 2012). Specifically, some rats (i.e., diet-induced obese (DIO)) maintained on a WD exhibit substantially more weight gain compared with rats fed standard low-fat, high carbohydrate chow, whereas other rats (i.e., diet-resistant (DR)) do not (Madsen et al., 2010). Davidson et al. (2012) reported that the obesity-prone DIO rats fed the WD showed impaired performance on a hippocampal-dependent discrimination problem and exhibited significantly higher BBB permeability compared to both DR rats on the same diet and chow-fed controls.

The present research attempts to expand what is known about the relationship between diet composition and the emergence of hippocampal-dependent cognitive dysfunction by addressing the following questions: (1) what types of diet-induced changes in physiological parameters (e.g., body weight, body adiposity, metabolic, hormonal, BBB permeability) are most predictive of diet-induced deficits in hippocampal-dependent memory? (2) What are the effects of a diet very high in fat and low in carbohydrate (i.e., ketogenic (KETO) diet) on hippocampal-dependent cognitive functioning? (3) What is the direction of the relationship between diet-induced changes in body weight, adiposity, and hippocampal-dependent cognitive performance?

These questions are of interest for several reasons: because KETO diets contain much higher levels of

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Abbreviations: ANOVA, analysis of variance; BBB, blood–brain barrier; BHB, beta-hydroxybutyrate; DIO, diet-induced obese; DR, diet resistant; EDTA, ethylenediaminetetraacetic acid; FN, feature negative; GLP-1, glucagon-like peptide-1; HE, high energy; KETO, ketogenic; PFC, prefrontal cortex; WD, Western diet.

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saturated fat and much lower levels of carbohydrate compared to the WD (Kinzig et al., 2005), if maintenance on the KETO diet produces disruptive effects that are similar to or of greater magnitude than those of the WD, this would suggest that dietary saturated fat may be a more important determinant of those effects than dietary carbohydrate. On the other hand, if the KETO diet has weaker or no adverse effects on hippocampal-dependent performance, this would suggest that dietary carbohydrate may be the more important factor. In addition, rats that consume KETO diets have been reported to show elevated levels of body fat without exhibiting significantly higher overall body weight compared to rats maintained on standard chow (Kinzig et al., 2005). Thus, the effects of a KETO diet on a behavioral task that depends on the hippocampus could help to evaluate the relative roles of body weight and body adiposity, per se, in the manifestation of those hippocampal-dependent cognitive effects. Finally, because the amount of carbohydrate in the diet is low, rats maintained on the KETO diet utilize ketone bodies that are converted from fat as the brain's primary energy (Bielohuby et al., 2011). This shift, which is indexed by elevated levels of circulating ketone bodies (i.e., ketosis), has been reported to have therapeutic effects on several brain disorders such as epilepsy (e.g., Lutas and Yellen, 2013) and mild cognitive dementia (Krikorian et al., 2012), and it may also have neuroprotective effects in the hippocampus (Samoilova et al., 2010). Thus, it is important to assess if the effects of consuming a KETO diet on cognitive functions that rely on the hippocampus depend on level of ketosis.

In addition to measuring circulating ketone bodies, we will also assess the relationship between diet-induced changes in blood glucose and triglyceride levels and in levels of the hormones, insulin, and glucagon-like peptide-1 (GLP-1). These measures are of special interest because the release of and sensitivity to each of these blood-borne factors have been associated with both hippocampal-dependent cognitive functioning and energy regulation (Farr et al., 2008; Malone et al., 2008; Isacson et al., 2011; Schioth et al., 2012).

While previous results have demonstrated that obesity and hippocampal-dependent cognitive dysfunction are related (e.g., Davidson et al., 2005, 2007; Kanoski and Davidson, 2011) the question of "What comes first – does obesity predict cognitive deficit or does cognitive deficit predict obesity?" remains open. To approach this question, we used a cross-lagged panel design (Kenny, 1975). The logic of this design is that if a given Factor A at early time point 1 predicts the value of another Factor B at later time point 2 better than Factor B at time point 1 predicts the value of Factor A at time 2, then based on the assumption that causes precede effects (i.e., temporal contiguity), the more likely direction of the relationship is that changes in Factor A precede and cause changes in Factor B. We performed several of these analyses using measures of obesity (e.g., body weight, body adiposity), ketosis, and cognitive performance as Factors A and B.

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 48 naïve male Sprague–Dawley rats, aged 60–75 days, and weighing between 275 and 300 g upon arrival from Harlan Inc. (Indianapolis, IN, USA). The animals were housed individually in a climate-controlled environment under a 12:12-h light:dark cycle with the light phase beginning at 07:00 h each day. The rats had free-access to water throughout the study, except during experimental sessions as described below. The rats were weighed daily during pre-training and immediately prior to each experimental session. The care and use of all animals in this study was reviewed and approved by the Purdue University Animal Care and Use Committee.

Apparatus

All behavioral training was conducted in eight conditioning chambers purchased from Med Associates (Georgia, VT, USA). The chambers were identical, each with aluminum end walls and clear plexiglass side walls, measuring $21.6 \times 21.6 \times 27.9$ cm and with floors consisting of stainless steel rods (0.48 cm in diameter) spaced 1.9 cm apart. A recessed food cup was located in the center of one end wall, and a 6-W jeweled panel light was located approximately 6 cm above and to the left of the opening for the food cup. Diffuse tone (1500 Hz), white noise, and clicker (3 Hz) stimuli (all approximately 78 db) were produced by auditory stimulus generators (Med Associates, ANL-926) located outside the conditioning chambers near the end wall opposite of the food magazine. A motorized pellet dispenser attached to each chamber was used to deliver 45-mg sucrose pellets to the food cup. A computerized infrared monitoring system with a photo transmitter and receptor was positioned in each chamber so that photobeam interruptions would index entries into the recessed area with the food cup. All experimental events (e.g., stimulus presentations, pellet delivery, recording of beam breaks) were controlled using MED PC IV (Med Associates) computer software. The body adiposity of each rat was measured using an EchoMRI-900 magnetic resonance body composition analyzer (Echo Medical Systems, LLC, Houston, TX, USA).

Procedures

Pretraining. The rats were fed standard rodent laboratory chow (LabDiets formula 5001, Purina, Framingham, MA, USA) *ad libitum* for 14 days after their arrival in the lab. Food rationing was then used to gradually reduce the body weight of each rat to 85% of the average weight obtained on the last 2 days of *ad libitum* feeding. Rats were weighed and handled daily during this period of food rationing. Magazine training