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RELATIONSHIPS BETWEEN DIET-RELATED CHANGES IN THE GUT MICROBIOME AND COGNITIVE FLEXIBILITY

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Abstract—Western diets are high in fat and sucrose and can influence behavior and gut microbiota. There is growing evidence that altering the microbiome can influence the brain and behavior. This study was designed to determine whether diet-induced changes in the gut microbiota could contribute to alterations in anxiety, memory or cognitive flexibility. Two-month-old, male C57BL/6 mice were randomly assigned high- fat (42% fat, 43% carbohydrate (CHO), high-sucrose (12% fat, 70% CHO (primarily sucrose) or normal chow (13% kcal fat, 62% CHO) diets. Fecal microbiome analysis, step-down latency, novel object and novel location tasks were performed prior to and 2 weeks after diet change. Water maze testing for long- and short-term memory and cognitive flexibility was conducted during weeks 5–6 post-diet change. Some similarities in alterations in the microbiome were seen in both the high-fat and high-sucrose diets (e.g., increased Clostridiales), as compared to the normal diet, but the percentage decreases in Bacteroidales were greater in the high-sucrose diet mice. Lactobacillales was only significantly increased in the high-sucrose diet group and Erysipelotrichales was only significantly affected by the high-fat diet. The high-sucrose diet group was significantly impaired in early development of a spatial bias for long-term memory, short-term memory and reversal training, compared to mice on normal diet. An increased focus on the former platform position was seen in both high-sucrose and high-fat groups during the reversal probe trials. There was no significant effect of diet on step-down, exploration or novel recognitions. Higher percentages of Clostridiales and lower expression of Bacteroidales in high-energy diets were related to the poorer cognitive flexibility in the reversal trials. These results suggest that changes in the microbiome may contribute to cognitive changes associated with eating a Western diet. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: executive function, intestinal microbiota, Western diet, Clostridiales, Bacteroidales, sucrose.

INTRODUCTION

The Western diet contributes to many chronic, diet-related illnesses in the United States, including the obesity epidemic (Cordain et al., 2005). Western diets are typically high in fat and simple carbohydrates (CHOs) (Cordain et al., 2005). Higher intake of fats and refined sugars are associated with deficits in cognitive flexibility and hippocampal-dependent memory in humans (Kalmijn, 2000; Francis and Stevenson, 2011) and an increase in the incidence of Alzheimer's disease (Pasinetti, 2002). There is evidence of a vicious cycle of hippocampal damage associated with a Western diet, followed by increased energy intake (Kanoski and Davidson, 2011; Kanoski, 2012). It is not clear whether these effects are due to direct or indirect influences of alternative energy sources on the brain.

Diets high in fat and/or sugar alter the microbiome of the gut (Li et al., 2009; Turnbaugh et al., 2009). Western diets in rodents are associated with increases in microbes in the phylum Firmicutes and decreases in Bacteroidetes (Turnbaugh et al., 2009; Ohland et al., 2013; Daulatzai, 2014; Patterson et al., 2014). Obese mice with a leptin mutation and obese people also show similar alterations in prevalence of these microbial phyla, compared to their lean counterparts (Ley et al., 2005, 2006b).

The gut microbiome in humans consists of approximately 100 trillion microorganisms (Ley et al., 2006a). Firmicutes and Bacteroidetes make up the majority of this population in the mouse and human gut (Ley et al., 2005, 2006a; Ley et al., 2008; Turnbaugh et al., 2009). Firmicutes are predominantly gram-positive bacteria, and include three classes: Bacilli, Clostridia and Erysipelotrichia (Ludwig et al., 2009). Bacteroidetes are gram-negative, anaerobic, rod-shaped bacteria, found in the environment and guts of animals (Eckburg et al.,

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CHO, carbohydrate; OTUs, operational taxanomic units.

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2005). In recent years it has become increasingly clear that the intestinal bacteria impact main functions in the body, including maturation of the immune system and metabolic processes. In fact, the effect of the composition of the microbiome in obesity is a good example of the importance of the bi-directional communication between the host and the resident microflora.

There is increasing evidence for an influence of the microbiome on the brain and behavior (Collins et al., 2012). Probiotics and/or antimicrobials can alter memory, anxiety and long-term potentiation and hippocampal and amydala levels of brain-derived neurotrophic factor (BDNF) in animals (Bercik et al., 2011; Collins et al., 2012; Davari et al., 2013; Hsiao et al., 2013). Fermented milk with a probiotic reduced activation of brain regions is associated with emotional responses in humans (Tillisch et al., 2013). The probiotic contained primarily bacteria from the Firmicutes; Bacilli; Lactobacillales order (Ludwig et al., 2009; Tillisch et al., 2013). A lean ground beef diet promoted greater bacterial diversity in the intestine and improved working and reference memory (Li et al., 2009). The current study was designed to determine whether specific changes within the microbiome could account for alterations in cognitive functions. High-energy diets were used to manipulate the gut microbiome (Li et al., 2009; Turnbaugh et al., 2009).

EXPERIMENTAL PROCEDURES

Animals

Eighteen male C57BL/6J mice (8 weeks of age) were purchased from JAX Laboratories (Bar Harbor, ME, USA). Mice were housed at the Oregon State University's animal facilities under a 12/12-h light/dark cycle with food and water available on an *ad libitum* basis. Three siblings were housed together in each cage for the first 2 weeks on a control chow diet (normal; Table 1). The mice were rotated between uncleansed cages for 2 weeks, until all the mice had been exposed similarly, in order to establish uniform microbiome baseline. Mice were then individually housed and, within each trio, were randomly assigned to a 42% fat diet (high fat), 70% CHO diet (66% sucrose; high- sucrose) or normal diet group (Table 1). Fecal collections for microbiome analysis and step-down latency and novel object and novel location tasks were performed prior to the diet change and were repeated 2 weeks after the diet change. Water maze testing for long- and short-term memory and cognitive flexibility was conducted during weeks 5 and 6 post-diet change.

Microbiome analysis

DNA extraction and 16S sequencing. Genomic DNA was extracted from approximately 100 mg of fecal sample using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions with the following changes: during the step-2 vortex step manual homogenization with a sterile pestle was performed to break up the sample, in step 3 the samples were heated to 94 degrees for 5 min, and step-6 vortexing was done for 4 min. All samples were diluted to 3 ng/μl and 5 μl of template went into each DNA amplification. Custom Lib-L Forward Primer A primers with unique MID barcodes, and Custom Lib-L Primer B primers with no MID, were designed to amplify the V3 and V4 regions of the 16S rRNA bacterial gene from each individual sample for sequencing.

Forward Primer Lib-L Primer A	**MID**	**Sequence Specific – LH 16SF**
CCATCTCATCCCTGCGTGTCTCCGAC***TCAG***	ACACGACGACT	GTGCCAGCMGCCGCGGTAA
Reverse Primer Lib-L Primer B		**Sequence Specific – LH 16S R**
CCTATCCCCTGTGTGCCTTGGCAGTC***TCAG***	**No MID**	GAGCTGACGACARCCATGCA

The barcoded 16S amplicons were sequenced using the Roche 454 GS Jr sequencing platform at the Oregon State University Center for Genome Research and

Table 1. Comparison of diets

	Normal (chow) PicoLab Rodent Diet 20 [a]	High-fat TD.88137 [b]	High-sucrose TD.98090 [b]
kcal/kg diet	4070	4500	4000
Percent of kcal provided by:			
Protein	24.7	17.3	17.7
Carbohydrate	62.1	42.7	70.4
Fat	13.2	42.0	11.8
Sucrose, g/kg diet	31.8	341.46	645.6

[a] purchased from LabDiet (St. Louis, MO).
[b] purchased from Harlan Laboratories (Madison, WI).

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