Psychoneuroendocrinology 62 (2015) 252–264

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

High-fructose diet during periadolescent development increases depressive-like behavior and remodels the hypothalamic transcriptome in male rats

Constance S. Harrell[a], Jillybeth Burgado[b], Sean D. Kelly[a], Zachary P. Johnson[c], Gretchen N. Neigh[a,d,*]

[a] Department of Physiology, Emory University, Atlanta, GA 30322, USA
[b] Neuroscience and Behavioral Biology Program, Emory University, Atlanta, GA 30322, USA
[c] Division of Developmental & Cognitive Neuroscience, Yerkes National Primate Research Center, USA
[d] Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 21 August 2015
Accepted 26 August 2015

Keywords:
High-fructose
Diet
Stress
Adolescence
HPA axis
Depressive-like behavior

ABSTRACT

Fructose consumption, which promotes insulin resistance, hypertension, and dyslipidemia, has increased by over 25% since the 1970s. In addition to metabolic dysregulation, fructose ingestion stimulates the hypothalamic-pituitary-adrenal (HPA) axis leading to elevations in glucocorticoids. Adolescents are the greatest consumers of fructose, and adolescence is a critical period for maturation of the HPA axis. Repeated consumption of high levels of fructose during adolescence has the potential to promote long-term dysregulation of the stress response. Therefore, we determined the extent to which consumption of a diet high in fructose affected behavior, serum corticosterone, and hypothalamic gene expression using a whole-transcriptomics approach. In addition, we examined the potential of a high-fructose diet to interact with exposure to chronic adolescent stress. Male Wistar rats fed the periadolescent high-fructose diet showed increased anxiety-like behavior in the elevated plus maze and depressive-like behavior in the forced swim test in adulthood, irrespective of stress history. Periadolescent fructose-fed rats also exhibited elevated basal corticosterone concentrations relative to their chow-fed peers. These behavioral and hormonal responses to the high-fructose diet did not occur in rats fed fructose during adulthood only. Finally, rats fed the high-fructose diet throughout development underwent marked hypothalamic transcript expression remodeling, with 966 genes (5.6%) significantly altered and a pronounced enrichment of significantly altered transcripts in several pathways relating to regulation of the HPA axis. Collectively, the data presented herein indicate that diet, specifically one high in fructose, has the potential to alter behavior, HPA axis function, and the hypothalamic transcriptome in male rats.

1. Introduction

Fructose consumption has increased by at least 25% in the past 30 years (Havel, 2005) due to increases in added sweeteners such as sucrose and high-fructose corn syrup. Adolescents are the highest consumers of fructose at 72.8 g/day, with a quarter of adolescents consuming at least 15% of their daily caloric intake from fructose alone (Vos et al., 2008). This is part of a global energy imbalance, resulting in a growing epidemic of metabolic syndrome (Rutledge and Adeli, 2007). The epidemic is not restricted to adults, as today over 20% of American adolescents are obese (Elliott et al., 2002) and Type II diabetes' rates are increasing among youth (Nadeau and Dabelea, 2008).

Diets high in fructose have implications beyond an excess caloric consumption. Such diets alter insulin, blood pressure, and lipid profiles in animal models (Hwang et al., 1987; Catena et al., 2003) and humans (Stanhope et al., 2009; Teff et al., 2009). Fructose consumption also raises corticosterone levels in rats (Brindley et al., 1981; Brindley et al., 1985) and elevations in corticosterone may be responsible for fructose-induced hepatic gluconeogenesis (Kinote et al., 2012). The role of glucocorticoids in fructose metabolism is particularly relevant given the clinical data indicating an increased prevalence of depression among diabetic patients (Anderson et al., 2001). Altered hypothalamic-pituitary-adrenal (HPA) axis signal-

* Corresponding author at: Department of Physiology, Department of Psychiatry and Behavioral Sciences, Emory University, 615 Michael Street, Atlanta, GA 30322. Fax: +1 404 727 2648.
E-mail address: gretchen.neigh@emory.edu (G.N. Neigh).

http://dx.doi.org/10.1016/j.psyneuen.2015.08.025
0306-4530/

ing is a classic feature of, and risk factor for, depression (Heim et al., 2008b).

Chronic stress, which disrupts HPA axis signaling (Bourke et al., 2013) and is associated with increased incidence of depression (Neigh et al., 2009) or depressive-like behavior (Bourke and Neigh, 2011), can exacerbate the effects of diet by promoting palatable food consumption (Pecoraro et al., 2004) and by inducing insulin resistance (Kaufman et al., 2007). A history of early life stress not only increases the risk of depression in adulthood (Neigh et al., 2009; Bale et al., 2010) but also increases the risk of metabolic dysfunction (Williamson et al., 2002). Adolescence is a "critical period" of development that shapes both stress responses (Romeo, 2010) and adult metabolism (Dietz, 1994). For these reasons, we designed our study to examine the interaction of adolescent stress and high-fructose diet on behavior, the HPA axis, and the hypothalamic transcriptome.

We hypothesized that fructose consumption beginning at weaning would induce metabolic disruption paralleling increases in anxiety-like and depressive-like behavior, and that fructose consumption would create a susceptibility to behavioral alterations in response to a subthreshold chronic adolescent stress. Further, we hypothesized that these behavioral and metabolic changes would correspond to alterations in HPA axis output both at baseline and in response to an acute stressor. Finally, we used whole-transcriptome RNA sequencing of the hypothalamus to determine the scope of changes in gene expression induced by a high-fructose diet during periadolescent development.

2. Materials and methods

2.1. Animal husbandry

Timed pregnant Wistar rats ($n = 22$) were obtained on gestational day 12 to produce the periadolescent cohort from Charles River (Wilmington, MA), while male Wistar rats ($n = 16$, PND 56) were obtained from Charles River (Wilmington, MA) to produce the adult cohort. Shipping stress during puberty can alter behavioral outcomes (Laroche et al., 2009) but shipping of pregnant dams has not been shown to alter developmental outcomes without a pharmacologic challenge (Ogawa et al., 2007); thus, shipping was conducted during in utero development to produce the periadolescent cohort. Animals for the adult-only diet exposure were obtained from Charles River as adults and acclimated to colony conditions for seven days prior to introduction of the high fructose diet (Capdevila et al., 2007). Rats were housed on a 14:10 reverse light:dark cycle in a facility controlled for humidity (60%) and temperature (20–23 °C). For the periadolescent cohort, litters were culled on postnatal day (PND) 3 to eight pups per litter and weaned on PND 23 ($n = 134$). Culled litters contained both male and female pups, but only male offspring were used in the current study. All experiments were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of Emory University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Diet and metabolic measurement

Either two days post-weaning (Periadolescent Cohort; PND 25; Chow-Non-Stress, $n = 44$; Chow-Stress, $n = 23$; Fructose-Non-Stress, $n = 43$; Fructose-Stress, $n = 24$) or at PND 64 (Adult Cohort, Chow, $n = 8$; Fructose, $n = 8$, all Non-Stress), male rats were pair-housed and assigned to either the Lab Rodent Diet 5001 or a high-fructose diet. The numbers and endpoints for each cohort are further clarified in Supplemental Table 1. In addition, the experimental timelines for each cohort are visualized in Supplemental Fig. S1.

The primary goal of using the high-fructose diet was to elicit physiologic changes typically associated with fructose consumption in humans, including altered lipid storage and hyperglycemia (Havel, 2005; Tappy and Le, 2010) and to examine concomitant effects on the brain and behavior but not to mimic common human consumption. This 55% high-fructose diet has previously been used to elicit such physiological changes in rodents, most notably increased adiposity, hyperglycemia, and hypertension. It is estimated that about 10% of total caloric intake for the United States population is from fructose with higher consumption among adolescents. One fourth of adolescents have been reported to consume at least 15% of daily calories from fructose (Vos et al., 2008). However, these percentages do not reflect the increase in mass of fructose consumed, as increased fructose consumption in humans has coincided with substantial increases in caloric consumption, primarily due to increases in carbohydrate consumption (Marriott et al., 2009). In addition, these estimates in humans are based on self-reported dietary recall, which typically underestimates consumption, particularly in obese subjects and adolescents. Obese adults underreport energy intake by an average of 47% and adolescents underreport by an average of 20% (Schoeller, 1995). Nonetheless, the diet's effects should be understood in the context of an animal model useful for exploring potential effects of a given macronutrient (fructose) on energy homeostasis and stress response and not as a replica of the human condition.

The "periadolescent" cohorts were so named as their diet intake spanned the beginning of adolescence through adulthood, while the "adult" cohort consumed the high-fructose diet during adulthood only. All major outcomes were tested in adulthood for all cohorts. While adolescence is difficult to define precisely in rats as in humans, it is accepted that infancy and "childhood" end at weaning (PND21-23) and that adulthood begins at PND60 (Spear, 2000, McCormick and Mathews, 2007). The diet timelines were thus selected based on the aim to fully cover the adolescent period in the periadolescent cohorts and not in the adult cohorts; and additionally based on evidence from the literature that 8-10 weeks on a similar high-fructose diet will induce metabolic changes (Huang et al., 2004, Nakagawa et al., 2006).

Non-stressed rats were pair-housed throughout the study, while stressed rats remained pair-housed until the initiation of stress, and single-housed thereafter. The fructose diet used (Research diets D05111802) is 55% fructose while the standard chow (Lab Diet 5001) normally used is 0.30% fructose. Both diets were supplemented with comparable levels of vitamins and minerals deemed necessary for rodent health, and were reviewed by veterinary staff and approved by IACUC. The details of the macronutrients of each diet have been listed in Supplemental Table 2.

Metabolic measures were taken from a subset of the periadolescent cohort and the adult cohort. Blood glucose was tested near weekly after an overnight fast by tail prick using a *Freestyle* glucometer. Animal weights were also taken concurrently with glucose readings. Research assistants, carefully accounting for any spilled food, measured food consumption daily and caloric consumption was determined thereof. To determine caloric efficiency, the body mass gained per week per animal was divided by the mean weekly caloric consumption calculated per cage (of pair-housed animals) divided by two. While imprecise, this type of approximation should only serve to increase variability in caloric efficiency and thus increase probability of returning a false negative result as opposed to producing a false positive.

Fat pads were collected from only a subset of periadolescent animals after weeks on the diet (described below). Weight and fasting blood glucose were assessed in the stress cohorts prior to and after the mixed modality stress, and a subset of the stress & non-stressed animals in each diet cohort were submitted to a glucose tolerance