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Journal of Nutritional Biochemistry xx (2015) xxx–xxx

Journal of Nutritional Biochemistry

High vitamin A intake during pregnancy modifies dopaminergic reward system and decreases preference for sucrose in Wistar rat offspring☆

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Received 9 November 2014; received in revised form 6 May 2015; accepted 18 August 2015

Abstract

High multivitamin (HV) content in gestational diets has long-term metabolic effects in rat offspring. These changes are associated with *in utero* modifications of gene expression in hypothalamic food intake regulation. However, the role of fat-soluble vitamins in mediating these effects has not been explored. Vitamin A is a plausible candidate due to its role in gene methylation. Vitamin A intake above requirements during pregnancy affects the development of neurocircuitries involved in food intake and reward regulation. Pregnant Wistar rats were fed AIN-93G diets with the following content: recommended multivitamins (1-fold multivitamins: RV), high vitamin A (10-fold vitamin A: HA) or HV with only recommended vitamin A (10-fold multivitamins, 1-fold vitamin A: HVRA). Body weight, food intake and preference, *mRNA* expression and DNA methylation of hippocampal dopamine-related genes were assessed in male offspring brains at different developmental windows: birth, weaning and 14 weeks postweaning. HA offspring had changes in dopamine-related gene expression at all developmental windows and DNA hypermethylation in the dopamine receptor 2 promoter region compared to RV offspring. Furthermore, HA diet lowered sucrose preference but had no effect on body weight and expression of hypothalamic genes. In contrast, HVRA offspring showed only at adulthood changes in expression of hippocampal genes and a modest effect on hypothalamic genes. High vitamin A intake alone in gestational diets has long-lasting programming effects on the dopaminergic system that are further translated into decreased sucrose preference but not food intake.

Keywords: Gestation; Vitamin A; Epigenetics; Reward system; Hippocampus; Dopamine

1. Introduction

Diets inadequate in nutrients and energy during pregnancy are well known to affect the *in utero* environment and subsequent brain development and function in offspring, but relatively little research has been directed at investigating the effect of intakes above requirements. High multivitamin (HV) in 10-fold the recommended amount of the complete vitamin mix in the AIN-93G diet fed during pregnancy predisposes male rat offspring to an obesogenic phenotype consisting of heavier body weight, greater food intake and impaired

Abbreviations: Comt, Catechol-*O*-methyl transferase; CpG, Cytosine-phosphate-guanine; *Darpp-32*, DA- and cyclic AMP-regulated phosphoprotein; *Dat*, Dopamine transporter; *Drd1*, Dopamine receptor 1; *Drd2*, Dopamine receptor 2; *Drd5*, Dopamine receptor 5; HA, AIN-93G diet with 10-fold the amount of vitamin A; HV, AIN-93G diet with 10-fold multivitamin levels; HVRA, AIN-93G diet with 10-fold multivitamins levels, 1-fold vitamin A; *Npy*, Neuropeptide Y; *Pomc*, Proopiomelanocortin; RARE, Retinoic acid response element; RV, AIN-93G diet with recommended amounts of multivitamins; *Th*, Tyrosine hydroxylase

☆ Financial Disclosure: Canadian Institute of Health Research, Institute of Nutrition, Metabolism and Diabetes, Reference MOP-93624.

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http://dx.doi.org/10.1016/j.jnutbio.2015.08.020
0955-2863/

blood glucose response [1,2]. Recently, methyl vitamins as a group (folate, vitamin B_6, vitamin B_{12}) and folate alone were shown to be components of the multivitamin diet that alter development of food intake circuits in the brain predisposing overeating [3,4]. The altered gene expression of offspring from rats fed HV diet [5] has been associated with epigenetic mechanisms, such as DNA methylation [3] and possibly histone modifications [6].

The role of fat-soluble vitamins in the altered phenotype of offspring from HV-fed dams has not been reported; however, vitamin A is a candidate for several reasons. First, it has a role in epigenetic mechanisms through enzymatic modifications of DNA (methylation) and histones (methylation and acetylation) [7]. One of vitamin A's metabolite, retinoic acid, regulates gene expression, thus affecting cellular function [8,9], *in utero* cellular proliferation, differentiation and apoptosis [8–10]. As well, *in vitro* studies have shown that vitamin A induced gene expression of the dopamine receptors *Drd1*, *Drd2* and *Drd5* in rat pituitary and human teratocarcinoma cell lines [11,12]. For this, vitamin A may also account for the obesogenic phenotype of offspring from dams fed the HV diet during pregnancy [1,2,5], suggesting that *in utero* vitamin A may be involved in the development of brain neural systems including those regulating reward and food intake. Reward processing is mediated by dopaminergic pathways in dopamine neurons in the ventral tegmental area and their projections to other forebrain structures, such as the hippocampus [13]. The hippocampus affects learned and motivational aspects of feeding behavior [14]. In the brain, vitamin A signaling occurs primarily in the hippocampus [15]. The dopaminergic system is critical in hedonically driven feeding behavior. In addition, hypothalamic regulation of food intake depends on many anorexigenic and orexigenic genes that regulate energy balance [16].

Therefore, the hypothesis of this report was that vitamin A intake above requirement during pregnancy affects gene expression and development of brain's neurocircuitry involved in the regulation of food intake and reward. Our objective was to compare with RV offspring the effect of a high vitamin A (HA) diet or the HV diet with recommended vitamin A levels (HVRA) on DNA methylation and expression of dopaminergic reward-related genes in hippocampus and food intake-related genes in the hypothalamus, food intake, and sucrose and oil preference in male Wistar rats.

2. Methods

2.1. Study design

To isolate the role of vitamin A in the AIN-93G vitamin mix when fed in the HV diet consumed during pregnancy, three groups of pregnant rats were fed one of the following diets: (1) a control diet with recommended vitamin mixture (RV), (2) a high (10-fold) vitamin A diet with all the other vitamins in recommended amounts in the AIN-93G diet (HA) and (3) a diet high in all vitamins (10-fold) with the exception of vitamin A, which was given at the recommended amount in the AIN-93G diet (HVRA). Because the HV diet fed during pregnancy alters development of neurocircuitry in the brain of the offspring [5], the latter diet (HVRA) was designed to further inform on the contribution of vitamin A to the effects of the HV diet. The effect of the maternal diet on the offspring was assessed at birth, weaning and 14 weeks postweaning. At birth, the dams were fed the RV diet, and after weaning, all offspring were fed a 60% high fat diet for 14 weeks to determine if the effects of gestational vitamin A on reward neurocircuitry modulate food preference and increase consumption of a diet known to cause excess food intake.

2.2. Animals and diets

First-time pregnant Wistar rats (2–3 days of pregnancy) were purchased from Charles River Inc., Quebec, Canada, and housed individually in ventilated plastic transparent cages with bedding in a 12:12 light–dark cycle at a temperature of 22±1°C. Animals had free access to water by an automated water system (Allentown Inc., Allentown, NJ, USA). The protocol was approved by the Institutional Animal Care and Use Committee of the University of Toronto.

Throughout gestation, three groups of dams (14–16 per group) were fed *ad libitum* the three commercially prepared diets (Dyets, Bethlehem, PA, USA) based on the AIN-93G diet [17] that was formulated by us. The three diets were identical in macronutrient (~20% as protein, ~64% as carbohydrate and ~16% as fat) and mineral content and differed only in vitamin content (Table S1). The HA diet contained 40,000 IU/kg of diet in the form of retinyl palmitate, an amount that is well below reported to cause toxicity in rats (50 mg/kg per day) [18] and that is achievable via quantities delivered in supplemental pills [19]. Based on our own research, a 10-fold compared to 1-fold dose of vitamin A achieves 1.40 μmol/L vs. 1.10 μmol/L of retinol in plasma and 4.0 μmol/g vs. 0.27 μmol/g wet tissue of retinyl palmitate in the liver of rats (n=9–13 per group, P<.05) [20]. The HVRA diet, the negative control, contained 10-fold amounts of the multivitamins in the AIN-93G diet, a level shown to induce an obesogenic phenotype in the offspring [1,2,5] but with the exception that it contained only the RV diet content of vitamin A. This diet was included to further isolate the effect of vitamin A alone from that of other vitamins known to be active in DNA methylation [4]. Vitamin composition is listed in Table S1. After birth, all dams received the RV diet. Litters were culled to 10 pups per dam on postnatal day 1, and at weaning, one male pup from each dam was randomly assigned to be fed the 60% high fat diet (with 20% as protein and 20% as carbohydrate) (Research Diets, New Brunswick, NJ, USA) with micronutrients adjusted to provide the same nutrient to energy ratio as in the AIN-93G diet. All pups (n=13–16 per group) were singly housed under the same conditions as dams from weaning until 14 weeks postweaning.

2.3. Tissue collection and gene expression analyses

Whole brains from offspring were removed upon sacrifice at birth, weaning and 14 weeks postweaning (1 pup/dam per time point). The hippocampus and hypothalamus were immediately dissected [21,22], frozen in liquid nitrogen and kept at −80°C until further analyses. Half of the hippocampus and hypothalamus were homogenized separately in Trizol (Invitrogen, Carlsbad, CA, USA), and RNA was isolated and quantified using Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, CA, USA). The other half of the hippocampus was used for DNA extraction. For each sample, 1 μg total RNA was reversed transcribed using the cDNA transcription kit (Applied Biosystems Inc., Foster City, CA, USA). All reactions were incubated at 25°C for 10 min, followed by 37°C for 120 min and 85°C for 5 min in an ABI Gene Amp PCR System 2700. Target gene expressions were determined by qRT-PCR using gene-specific TaqMan probes (Applied Biosystems Inc.) with TaqMan Gene Expression Master Mix (Applied Biosystems Inc.) on the ABI PRISM 7900 HT Sequence Detection System. The cycle conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The probes used were as follows: dopamine receptor 1 (*Drd1*; Cat#: Rn03062203_s1), dopamine receptor 2 (*Drd2*; Cat#: Rn00561126_m1), dopamine receptor 5 (*Drd5*; Cat#: Rn00562768_s1), DA- and cyclic AMP-regulated phosphoprotein DARPP-32 (*Ppp1r1b*; Cat#: Rn01452984_m1), dopamine transporter DAT (*Slc6a3*; Cat#: Rn00562224_m1), tyrosine hydroxylase (*Th*; Cat#: Rn00562500_m1), catechol-*O*-methyl transferase (*Comt*; Cat#: Rn01404927_g1), proopiomelanocortin (*Pomc*; Cat#: Rn00595020_m1) and neuropeptide Y (*Npy*; Cat#: Rn01410146_m1). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; Cat#: Rn99999916_s1) was used as an endogenous control after validating that it was not affected by our experimental treatments among other