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WORD COUNT	9179	TIME SUBMITTED	01-DEC-2023 08:07PM
		PAPER ID	104844806

Name of Journal: *World Journal of Psychiatry*
Manuscript NO: 88545
Manuscript Type: ORIGINAL ARTICLE

Basic Study

Nutritional epigenetics education improves diet and attitude of parents of children with autism or attention deficit/hyperactivity disorder

Dufault RJ *et al*. Nutritional epigenetics education improves diet

Abstract

BACKGROUND

Unhealthy maternal diet leads to heavy metal exposures from the consumption of ultra-processed foods that may impact gene behavior across generations, creating conditions for the neurodevelopmental disorders known as autism and attention deficit/hyperactivity disorder (ADHD). Children with these disorders have difficulty metabolizing and excreting heavy metals from their bloodstream, and the severity of their symptoms correlates with the heavy metal levels measured in their blood. Psychiatrists may play a key role in helping parents reduce their ultra-processed food and dietary heavy metal intake by providing access to effective nutritional epigenetics education.

AIM

To test the efficacy of nutritional epigenetics instruction in reducing parental ultra-processed food intake.

METHODS

The study utilized a semi-randomized test and control group pretest-posttest pilot study design with participants recruited from parents having a learning-disabled child with autism or ADHD. Twenty-two parents who met the inclusion criteria were randomly selected to serve in the test (n = 11) or control (n = 11) group. The test group participated in the six-week online nutritional epigenetics tutorial, while the control group did not. The efficacy of the nutritional epigenetics instruction was determined by measuring changes in parent diet and attitude using data derived from an online diet survey administered to the participants during the pre and post intervention periods. Diet intake scores were derived for both ultra-processed and whole/organic foods. Paired sample t-tests were conducted to determine any differences in mean diet scores within each group.

RESULTS

There was a significant difference in the diet scores of the test group between the pre- and post-intervention periods. The parents in the test group significantly reduced their intake of ultra-processed foods with a pre-intervention diet score of 70 (mean = 5.385, SD = 2.534) and a post-intervention diet score of 113 (mean = 8.692, SD = 1.750) and the paired *t*-test analysis showing a significance of $P < 0.001$. The test group also significantly increased their consumption of whole and/or organic foods with a pre-intervention diet score of 100 (mean = 5.882, SD = 2.472) and post-intervention diet score of 121 (mean = 7.118, SD = 2.390) and the paired *t*-test analysis showing a significance of $P < 0.05$.

CONCLUSION

Here we show nutritional epigenetics education can be used to reduce ultra-processed food intake and improve attitude among parents having learning-disabled children with autism or ADHD.

Key Words: Epigenomics; Parenteral nutrition; Autism; Attention deficit/hyperactivity disorder; Ultra-processed foods; Heavy metals

Dufault RJ, Adler KM, Carpenter DO, Gilbert SG, Crider RA. Nutritional epigenetics education improves diet and attitude of parents of children with autism or attention deficit/hyperactivity disorder. *World J Psychiatry* 2023; In press

Core Tip: Ultra-processed foods remain a source of heavy metal exposure in American families. The bioaccumulation of inorganic mercury and lead in the blood increases the severity of symptoms in children with autism and attention deficit/hyperactivity disorder *via* paraoxonase-1 gene modulation. Providing parents with nutritional epigenetics instruction may reduce their intake of ultra-processed foods and empower them to influence their child's behavior through dietary changes.

INTRODUCTION

Excessive consumption of ultra-processed foods is a characteristic of unhealthy diet due to the increased intake of saturated fats and added sugars and decreased intake of essential micronutrients zinc (Zn), calcium (Ca), magnesium, vitamins A, C, D, E, B12, and niacin[1,2]. Increasing evidence links consumption of ultra-processed foods to the development of various disease conditions including obesity, type-2 diabetes, cardiovascular disease[3], and adverse child neurodevelopment[4,5]. In a recent diet study involving 2377 pairs of pregnant women and their offspring, Puig-Vallverdú *et al*[4] found an adverse association between maternal prenatal consumption of ultra-processed foods and verbal functioning in the offspring during early childhood. Zupo *et al*[5] performed a literature review of the evidence showing an association between maternal pre-natal diet and adverse neurodevelopmental outcomes in offspring and found a positive association between maternal prenatal diet high in ultra-processed foods and adverse verbal intelligence and executive functioning in the offspring during middle childhood. While the health problems associated with the consumption of ultra-processed foods are indisputable, the precise factors (*e.g.,* food ingredients, contaminants, additives, nutrient profiles) that cause the problems are not yet fully understood[3].

A few food ingredients have been studied by researchers and their role in the development of disease is becoming clearer. Ward was the first to report the health problems of hyperactive children affected by the consumption of yellow food colors including the development of eczema and/or asthma, respiratory and/or ear infections, poor speech, and poor coordination[6]. In a behavioral response study of hyperactive children and an age and gender matched control group, Ward determined only hyperactive children showed significant losses in serum Zn levels along with increased levels of overactivity and aggression following the consumption of yellow food colors #5 and #6[7]. Milne and Neilsen[8] reported significant losses in Ca from the consumption of high fructose corn syrup (HFCS) in a small study of men and cautioned

that a high fructose containing diet could lead to bone loss and the development of osteoporosis over time. Food colors yellow #5 and #6 and HFCS are a few examples of food ingredients commonly found in ultra-processed foods that are suspected of causing health problems by creating nutritional deficit and/or mineral imbalance. Numerous studies now indicate that reducing ultra-processed food intake will help eliminate the nutritional deficits that lead to poor health outcomes[2,3]. In a recent review, Touvier *et al*[3] stated "public health policies to reduce the consumption of ultra-processed food cannot wait". This statement especially applies to birth outcomes. In a recent scoping review, Cortés-Albornoz *et al*[9] found nutritional deficits during pregnancy are the recognized cause of some adverse birth outcomes and contribute to increased risk of several neuropsychiatric disorders, including those found in the autism spectrum, and attention deficit/hyperactivity disorder (ADHD).

In addition to the problem of creating nutritional deficits, excessive consumption of ultra-processed foods results in dietary heavy metal exposures[10,11]. Heavy metal exposures *via* the consumption of ultra-processed foods are allowed by food safety laws that regulate contaminant concentrations in various food ingredients. For example, in the case of synthetic petroleum-based food coloring, the United States Food and Drug Administration requires manufacturers to test and certify each batch of coloring to ensure the concentration of heavy metals does not exceed the levels permitted by law (≤ 1 ppm mercury (Hg), ≤ 10 ppm lead (Pb), and ≤ 3 ppm arsenic (As)[12]. These "certified" food colors (*e.g.*, yellow #5, yellow #6, red #40) are found in many ultra-processed food products consumed by both children[13] and adults in the United States and remain a significant health concern[14,15].

In addition to petroleum-based food coloring, heavy metal contaminants may be found in other highly consumed food ingredients such as high fructose corn syrup which is used by beverage companies as an added sweetener in soft drinks. Dufault *et al*[16] was the first to publish findings of Hg in HFCS. In subsequent studies, Rideout *et al*[17] and Wallinga *et al*[18] also reported finding low levels of (Hg in food products containing corn syrup or HFCS. Corn sweeteners, including HFCS, are at risk of

inorganic-Hg (I-Hg) contamination due to their manufacturing process which involves the application of mercuric chloride to the corn starch at the beginning of the process to prevent starch degrading enzymes[19] and the use of Hg-grade chlor-alkali chemicals throughout the manufacturing process to adjust the pH of the various corn products[16].

Heavy metal contaminants are found throughout the food supply. Emerging evidence suggests prenatal exposure to heavy metals can influence the development of the child's immune system and contribute to the development of various disease conditions[20]. Prenatal exposures to cadmium (Cd), Pb, and/or Hg have been linked to the development of asthma, eczema, food allergy, and adverse respiratory outcomes in children[20-22]. Heavy metals may be transmitted to the fetus during pregnancy *via* cord blood. In a birth cohort study of 1751 pregnant women, Jeong *et al*[23] found maternal blood Pb and Hg concentrations correlated with the levels found in cord blood and in children up to age 5 years. In a more recent birth cohort study of children diagnosed with autism and/or ADHD in Norway, Skogheim *et al*[24] determined prenatal exposures to different heavy metals during gestation could adversely impact child neurodevelopment[24]. Hg, Pb, Cd, and As were among the notable heavy metals found to be involved in the development of autism and ADHD.

Heavy metals are detoxified in the body by metallothionein (MT) metal carrier proteins that must bind with Zn and copper (Cu) which are the elements required to regulate MT gene expression[25,26]. MT proteins play a vital role in metal trace element homeostasis within cells and tissues as heavy metals are detoxified and eliminated from the body[25]. In this capacity, MTs serve the immune system as potent antioxidant proteins because they protect DNA against oxidative stress that may occur if heavy metals accumulate in the body[25]. Dietary elements that reduce Zn availability or deplete Zn stores may disable MT functioning creating conditions for the bioaccumulation of heavy metals in cells and the buildup of oxidative stress which destroys cell membranes and tissues[27]. Dietary elements or contaminants known to reduce or deplete Zn include excessive exposures to heavy metals Hg[27,28], Pb, Cu, Cd, silver (Ag), Bismuth (Bi)[28], HFCS[27,29], yellow #5 and yellow#6[7], alcohol and some

drugs[29]. Recent studies suggest the synaptic pathway for the development of autism may be the disruption of glutamatergic synapses which can be influenced by prenatal Zn deficiency and MT dysfunction both of which affect brain tissue metal-ion homeostasis[30].

Prenatal Zn deficiency arising from the maternal consumption of ultra-processed foods is one example of how reproductive risk occurs and can lead to behavioral abnormalities and impaired immunocompetence in children[31]. Uriu-Adams and Keen[31] found poor diet leading to Zn deficiency, for even a short period of time, can present a risk to the developing fetus. More recent literature reviews indicate prenatal and offspring Zn deficiency are now strongly associated with the development of autism and ADHD[32,33]. Heavy metal exposures from consumption of ultra-processed foods compromise maternal and child Zn status and cause MT gene malfunction which leads to the bioaccumulation of heavy metals in blood[27]. Multiple studies indicate the heavy metal levels found in the blood of children with autism or ADHD correlate with the severity of their symptoms[34-39]. Recent rat studies demonstrate the toxic and synergistic effects of low dose combined exposures to Hg, Pb, and Cd on hippocampal neurons[40,41]. These studies show dietary co-exposures to Hg, Pb, and Cd correlate to Hg, Pb, and Cd levels in blood and the intensity of oxidative stress in brain tissue with impacts on rat learning and memory impairment[40,41].

Evidence suggests nutritional deficits and heavy metal exposures associated with poor diet (*e.g.*, excessive consumption of ultra-processed foods) are the primary epigenetic factors responsible for the development of autism and ADHD *via* MT gene dysfunction[27] and paraoxonase-1 (PON1) gene suppression[11]. The field of science that studies the effects of heavy metal exposures and diet on gene behavior is nutritional epigenetics. Figure 1 shows the constructs of the nutritional epigenetics model for autism and ADHD that explains how dietary factors (*e.g.*, HFCS, yellow #5, yellow #6, Hg and Pb exposures) from the consumption of ultra-processed foods lead to the development of nutritional deficits and mineral imbalances that disrupt MT gene function. MT gene disruption leads to the bioaccumulation of heavy metals in the blood

and problem behaviors and impaired learning in offspring. Maternal exposures to Pb and Hg *via* unhealthy or poor diet, lifestyle, and environment are transmitted to the child *via* cord blood[23] and are likely responsible for the development of autism and ADHD in children[24,42], especially in the case of PON1 gene suppression[10,11,43] which can result in oxidative stress[44] and changes in DNA methylation patterns[45]. Expression of the PON1 gene results in the body's production of the Ca dependent paraoxonase enzyme that detoxifies organophosphate (OP) pesticide residues[46] found widely in the United States food supply[47]. Prenatal OP pesticide exposures are neurotoxic, producing lasting effects on the behavioral health of children[48] and may impact their ability to learn when PON1 gene activity levels are low[11]. Inhibitors of PON1 gene activity include dietary exposures to fructose (*e.g.*, HFCS), Hg and Pb, and nutritional deficits in fatty acids, Ca, and selenium (Se)[10]. Diet interventions aimed at educating parents on each of the constructs that form this model may lead to the adoption of healthier diets.

MATERIALS AND METHODS

The study utilized a semi-randomized test and control group pretest-posttest pilot study design with participants recruited from parents having a learning-disabled child with autism or ADHD. Prior to participant recruitment, the curriculum for the tutorial intervention was developed and built online at the non-profit Food Ingredient and Health Research Institute[49]. The online curriculum was modeled after a previous online nutritional epigenetics course that was focused on teaching college students how to reduce their intake of ultra-processed foods and improve their intake of whole and organic foods while reducing their blood I-Hg and glucose levels[50]. For this diet intervention study, we focused the curriculum on the known factors of the western diet that contribute to heavy metal exposures and nutritional deficits that may impact gene behaviors in children with autism and ADHD. In learning how specific food ingredients contribute to heavy metal exposures, impact nutrient status and/or gene behavior

(Figure 1), and child health and learning outcomes, parents may then have the knowledge they need to feed themselves and their children a healthy diet.

Curriculum and tutorial development

The curriculum consisted of a textbook titled, "The Toxic Western Diet: What Parents Must Know to Protect Their Family," that was written by the principal investigator for use in conjunction with six online modules of instruction[51]. There were learning objectives posted online for each module of instruction along with four assigned activities. Every week each participant was expected to complete four activities which included a reading assignment from the textbook, three discussion questions to be answered in a discussion group setting, and two other activities which varied depending on the topic (*e.g.,* video assignment, kitchen cupboard survey, online scavenger hunt for facts, meal preparation assignment). The hands-on activity and video assignments for each chapter are provided in the textbook which accompanies this article as supplemental material in PDF file format for the purpose of study duplication.

The textbook chapters and associated modules of instruction are titled as follows: (1) Chapter 1: How food regulates and supports gene function; (2) Chapter 2: What we eat or don't eat leads to disease; (3) Chapter 3: Ingredients that add heavy metals to your body; (4) Chapter 4: What we know about corn sweeteners; (5) Chapter 5: What we know about pesticides; and (6) Chapter 6: How we can create a safe food environment. Each textbook chapter provides at least 42 science-based references to support the content. During the study, all references were accessible online at the tutorial web page. Each chapter presented several lessons that helped improve parent understanding of the role diet plays on their child's behavior and health. Table 1 provides detailed information on the content covered in each module of instruction.

The textbook has been updated[52] and the tutorial is now available in a study guide hardcopy format[53]. Supplementary material provides an example of one module of instruction in the tutorial/study guide[53]. It may be used in conjunction with Chapter 4

of the current textbook which is titled, "Ingredients that add heavy metals to your body"[52]. In this chapter, parents read the results of previous studies that show consumption of food colors yellow #5 and yellow #6 by hyperactive children leads to Zn losses and increased hyperactivity[6,7]. Parents learn that products with these food colors sold in the European Union must carry the following mandatory warning on their labels: "May have an adverse effect on activity and attention in children[54,55]." A hands-on activity in the study guide involves the parent conducting a survey while using the tables in this chapter to find and list all the food products in their kitchen that contain yellow #5, yellow #6, and other ingredients with allowable heavy metal residues. Another hands-on activity involves the parent finding a recipe to prepare a Zn rich meal for the family. Parents learn that Zn is needed by the body to build the MT transporter protein that supports heavy metal excretion. The textbook is geared to teach parents how to avoid consuming ultra-processed food products known or likely to contain ingredients with heavy metal or pesticide residues while increasing consumption of whole foods that contain the essential nutrients required by the body to support gene function so that it can build the proteins needed to improve child health, behavior, and learning outcomes.

Study design for evaluating tutorial

The study was a semi-randomized test and control group pretest-posttest pilot study design. The tutorial intervention lasted six weeks, and participants were surveyed online at baseline and post intervention. In accordance with the protocol, all the participants provided written informed consent to participate in the study. The protocol for the study was approved and found to be exempt from further review by the A.T. Still University Institutional Review Board (IRB).

Sample size calculation

The required sample size was calculated before the start of the study. A prior study using the same survey instrument[50] produced mean diet scores at pre and post

intervention of 16.0 and 23.2 with the standard deviations of 1.72 and 2.83, with n = 10. Based on calculation procedures provided by Kirkwood and Stein[56], a sample size of less than 10 was determined to be adequate for this study. The variables for the sample size analysis are shown in Table 2. The formula used to determine the sample size for each group (test and control) is provided by Kirkwood and Sterne[56] as follows:

$$\frac{(u + v)^2 (\sigma 1^2 + \sigma 0^2)}{(\mu 1 - \mu 0)^2} > n$$

n (number required for each group), assuming power = 90%, u = 1.28; assuming significance level = 5%, v = 1.96.

Substituting estimated values for the means and standard deviations outlined in Table 2 results in the following:

$$\frac{(1.28 + 1.96)^2 (1.72^2 + 2.83)^2}{(23.2 - 16.0)^2} > 2.2 \text{ (number required for each group)}$$

According to the calculation, at least three participants were needed in each group (test and control) for this study. If 20 participants were recruited to enroll in the study with 10 participants serving in the test group and 10 participants serving in the control group, there would be enough data to include in the final analysis even with a few dropouts.

Participant recruitment and eligibility

Participants were chosen from a population of parents who have learning-disabled children with behavior problems commonly associated with autism, developmental delay, and ADHD. Recruitment was primarily conducted using a Facebook[57] web page announcement hosted by the non-profit Food Ingredient and Health Research Institute[49]. The recruitment web page was "shared" with various organizations across the United States with a mission to serve parents with learning-disabled children. Approximately 250 parents responded to the recruitment web page by sending inquiry emails with most being excluded due to their child's existing diet. Parents with children on special diets (*e.g.,* Feingold, gluten free, or casein free) were excluded from the study.

Eligibility for participation in the tutorial project was determined using the protocol approved by the A.T. Still University IRB, which included the use of a screening questionnaire. Supplementary material provides a copy of the screening questionnaire. Parents of learning-disabled children demonstrating the most severe behavior problems were given priority for enrollment in the tutorial. Severity was determined by the number of child behaviors observed by the parents within the last 24 h as recorded on the screening questionnaire checklist. Observed child behaviors included any of the following: Frequent tantrums, hyperactivity, self-injury (head banging), pica (chewing on objects), running away, and aggression (hitting, name-calling). Of the 93 parents who filled out and submitted the screening questionnaire, 23 met the inclusion criteria for the study and were invited to participate in the tutorial project. Prior to their enrollment in the study, all parents interviewed face to face *via* Skype or by phone with the investigator and were provided with the opportunity to ask questions about their participation in the study before signing the informed consent form. Participants were alternately assigned to the test or control group when eligibility was confirmed and after receipt of the signed informed consent form.

Eligible parents had a learning-disabled child between the age of 34 mo and 8 years, daily access to the internet, and documentation of their child's disability. All participants reported having a minimum educational level equivalent to a high school diploma. For this basic study, only the test group received nutritional epigenetics instruction *via* the online tutorial intervention. The participants in the test group began the tutorial on the same date and had the flexibility of any other online course. They were able to access the tutorial at any time anywhere in the world if they had internet access. One parent dropped out of the study before the tutorial began, which left 11 parents serving in the test group and 11 parents serving in the control group. Pre and post intervention, all participants completed the online diet survey questionnaire comprised of 30 dietary habit questions and 10 participant characteristic questions.

Survey instrument design and use

A survey questionnaire was developed using the Survey Monkey[58] website. The survey consisted of one item to determine parental belief about the role of diet in child behavior, several items to collect qualitative demographic data to characterize the family units (*e.g.,* highest level of education, race or ethnicity, age of learning-disabled child, parent observation of child behavior in past 24 h), and food frequency questions to characterize parental dietary intake. The food frequency questions were modeled after those used by the National Cancer Institute[59] to measure dietary intake in the past month and designed to determine the proportion of time specific whole foods or ultra-processed foods were eaten by the parent. There were seventeen questions for measuring whole and/or organic food intake and thirteen questions for measuring ultra-processed food intake. Food products were considered ultra-processed if they contained more than one ingredient along with at least one ingredient known, or likely, to contain heavy metal residues. Products made from conventional flour with pesticide residues or bleached flour were considered ultra-processed foods. Whole foods, originating from a farm (one ingredient) were considered less processed and healthier to consume.

All food frequency questions used a similar structure. For example, an item used to measure the intake of dark green vegetables, a whole food, was written as follows: During the past month, how many times did you eat dark green vegetables (romaine lettuce, spinach, broccoli, kale, Swiss chard, collard, or other greens)? The possible responses included: Never, rarely (once or twice a month), once a week, several times a week, daily (1-2 servings), several times a day (3 or more servings). An example of a question used to measure the intake of cereal, an ultra-processed food, was written as follows: During the past month, how many times did you eat ready to eat cereal (corn flakes, rice crisp, corn squares, fruity o's, oat circles, *etc.*)? The same set of responses were used for each question. A question used to measure the intake of swordfish or tuna was included in the ultra-processed food category because their consumption can lead to Hg exposure, and one purpose of the tutorial was to decrease consumption of foods that may contain heavy metal or pesticide residues.

The food frequency questions did not follow any specified order in the survey (whole food *vs* ultra-processed food), so parents were not aware of their significance. Item design was intentional and tracked back to lessons learned about the importance of certain nutrients in modulating gene function and child behavior. For example, some items used to measure intake of ultra-processed foods were associated with food ingredient labels containing corn sweeteners and food colors that may lead to heavy metal exposures and Zn losses. All the food frequency questions are provided in Tables 3 and 4.

The food frequency questions had been pilot tested successfully and validated in a previous clinical trial that involved the delivery of an online nutritional epigenetics course to students at a tribal college located on an Indian reservation[50]. The diet survey utilized during that trial was comprised of the same food frequency questions used for the diet survey administered during this study. The survey was validated to correctly measure intake of ultra-processed and whole foods through biomarker collection (*e.g.*, blood) and analyses[50]. College students who significantly reduced their intake of ultra-processed foods and increased their intake of whole and/or organic foods had lower inorganic blood Hg levels compared to students who did not participate in the online nutritional epigenetics course[50]. During this study, parents in the test and control groups were administered the survey online pre and post intervention during a specified period. Each parent received a link to the confidential survey *via* email.

Data analysis

The qualitative demographic data was analyzed to determine the percent of family units with or without college, percent of family units in each ethnic group, percent of participants observing specific problem behaviors in the last 24 h, and percent of families having learning-disabled children in different age groups.

Quantitative data in ratio form (proportion) was analyzed to determine how many times in the past month different foods were eaten by the parent. The data was coded and each response in the healthier range was awarded a score of 1. Less healthy

responses received a score of 0. For example, more frequent consumption of whole and/or organic foods achieved a higher score, as did less frequent consumption of ultra-processed foods. Each item was scored positively with a 1 if the participant reported a diet habit consistent with the instruction. A diet behavior score was calculated for each participant as the sum of the 1's across all questions. The mean diet score for each group (test and control) was also calculated for each period (pre and post intervention). The scoring procedure for this study was the same procedure used for the tribal college study[50]. A two-sample paired *t*-test was conducted to determine any significant difference in parental diet behavior between the pre and post periods.

Comparisons of pre- and post-intervention diet scores for test and control groups were performed using a two-sample paired *t*-test, with two tails and alpha = 0.05. All *t*-tests were conducted in R. Version 4.3.0 (2023-04-21ucrt). The following four assumptions of the *t*-test are met: (1) No outliers - outliers were tested in two stages using box plots and Grubbs test. Stage 1: Each of the distributions were examined for outliers using box plots in R. Outliers were noted for the distribution of process - post-intervention test group and the distribution of process - post-intervention control group. Both these outliers were for the item, "eat grain products made of wheat such as macaroni, bread, hamburger or hot dog buns, or spaghetti". Another outlier was found for the distribution of process - pre-intervention control group for the item, "eat foods prepared with organic flour". Stage 2: The outliers found in stage 1 were not found to be outliers when tested using the Grubbs test with alpha = 0.01. The Grubbs test is known as the maximum normed residual test or the extreme studentized deviate test[60,61]; (2) Normality: The Shapiro-Wilk normality test in R showed no distributions with a *P*-value < 0.01. All distributions were found to be normally distributed; (3) Random: Subjects were assigned randomly to test and control groups as described in the participant recruitment and eligibility section above; and (4) Equal variance: R does not assume equal variance between the samples for the *t*-test. The default status in R for the *t*-test is unequal variance. The Welch's *t*-test was performed for all *t*-tests in R. The

statistical methods of this study were reviewed and verified by Dr. Raquel Crider of the Food Ingredient and Health Research Institute.

RESULTS

Participant demographic characteristics

Table 5 shows the demographic characteristics of the parents who participated in the nutritional epigenetics tutorial study. All (100%) of the participants reported having some college. Participant race or ethnic group only varied slightly between test and control groups. The age distribution for the learning-disabled children in the family units was the same for both groups. Although the gender of the parents was not determined by the questionnaire, pre-intervention interviews confirmed all participants were female. Problem child behaviors observed by parents in the last 24 h varied only slightly between groups.

Efficacy of tutorial on parent diet

Table 3 shows the changes in dietary intake of ultra-processed foods among the parents between the pre and post intervention period. The unit of analysis is the item of the online diet survey. The processed food section of the diet survey had 13 items. Each participant that responded with a healthy response to a single item was awarded a score of 1. For example, during the pre-intervention period, one person in the test group gave a healthy response (*e.g.*, "never, rarely, once a week") to the question "during the past month, how many times did you eat sweet snacks such as candy, cookies, ice cream…...?" After undergoing the intervention, 8 participants in the test group gave a healthy response to the same question so the score for this item on sweet snack intake increased from 1 to 8. After participating in the intervention, parents in the test group adopted a healthier eating pattern for sweet snacks. The only items in this part of the survey for which scores did not change for the test group were the questions asking about grain products made of wheat and white rice.

Table 4 shows the dietary intake of whole and/or organic food among the parents between the pre and post intervention period. The unit of analysis is the item of the online diet survey. The whole and/or organic food section of the diet survey had 17 items. Each participant that responded with a healthy response to a single item was awarded a score of 1. For example, during the pre-intervention period, four participants in the test group each received a score of 1 because each gave a healthy response (*e.g.*, "rarely, once a week, several times a week, every day") to the question "during the past month, how many times did you eat foods prepared with organic flour…...?" After undergoing the intervention, 9 participants in the test group gave a healthy response to the same question so the score for this item on "foods prepared with the organic flour" increased from 4 to 9. After participating in the intervention, parents in the test group adopted a healthier eating pattern by eating more organic foods thus reducing their pesticide exposure. Their intake of organic flour, organic produce and organic bread showed impressive post-intervention scores, with each item increasing 5 to 6 points. The participants likely also reduced their I-Hg exposure because organic flour is unbleached and does not contain the allowable Hg residue that may be found in the bleached flour products[62].

Table 6 presents an analysis of changes in parent dietary intake within both the test and control groups pre- and post-intervention. Paired sample *t*-tests were conducted to determine any differences in mean diet scores within each group, test, and control. While there were no significant differences in the consumption of ultra-processed or whole foods by control group participants between the pre- and post-intervention periods, there were significant differences in the dietary habits of the test group participants. The test group diet score for avoiding ultra-processed food increased from 70 to 113 between the pre- and post-intervention periods. The paired *t*-test analysis was significant with $P < 0.001$. For the whole and/or organic food category, the total pre-intervention diet score is 100, and the post-intervention score is 121 for the test group. The paired *t*-test analysis was significant with $P < 0.05$. The findings presented in Table 6 indicate the parents in the test group who completed the tutorial significantly

increased their consumption of whole and/or organic foods and significantly decreased their consumption of ultra-processed foods.

Table 7 presents a summary with the key findings of the *t*-test results showing changes in parental dietary intake within groups pre- *vs* post-intervention. The summary shows all the important statistical information along with the tested hypotheses and the effect size for each of the *t*-tests.

Table 8 presents the data collected to measure the changes in the parents' attitude about their ability to control their child's behavior through diet. The survey item was worded "To what extent do you agree with the following statement: I have the ability to control the behavior of my family's learning-disabled child through diet". The data was coded "1" if a parent responded with "strongly agree" or "agree" and "0" for all other responses. The total and mean score for each group was calculated along with the standard deviation. During the pre-intervention period, three parents in the test group and four parents in the control group responded in agreement to the item statement. During the post-intervention period, ten parents in the test group and three parents in the control group responded in agreement to the item indicating a change in attitude had occurred among the parents who participated in the tutorial.

Feedback was collected from the parents who completed the online nutritional epigenetics tutorial. Table 9 shows the feedback provided by the test group. The feedback indicates the hands-on activities helped parents learn to read food ingredient labels. The kitchen cupboard survey activities helped parents differentiate between ultra-processed and minimally processed foods. Parents indicated they changed their shopping habits by reducing or eliminating toxic food ingredients from their family's diet.

The study results showed the use of an online nutritional epigenetics tutorial could facilitate and influence healthy dietary changes in parents of children with autism and ADHD. The parents in the test group significantly reduced their consumption of ultra-processed foods and increased their consumption of whole and organic food after participating in the tutorial. Parents who participated in the tutorial became more

confident in their ability to control their child's problem behaviors through dietary changes. The feedback from the parents in the test group indicates they became better able to manage their family's dietary environment and eating behavior at home. During the online discussions, some parents reported seeing improvements in their child's behavior with the changes in family diet.

DISCUSSION

Parents play a crucial role in shaping a child's food preferences because they choose what foods to feed their children[63]. Food preferences begin developing in the fetus in utero and continue after birth during breast feeding and/or formula feeding[64-66]. There is evidence to suggest children eat, or learn to eat, what their parents eat. Dolwick and Persky[67] found an unhealthy dietary pattern of excessive ultra-processed food intake by parents is associated with unhealthy child feeding behaviors, including the excessive intake of ultra-processed foods. An *et al*[68] conducted a cross-sectional study of caregivers of children (n = 408) in China to determine whether caregiver feeding behaviors were associated with child ultra-processed food intake. Caregiver feeding habits were positively associated with children's consumption of ultra-processed foods including a higher frequency, and larger amount, of ultra-processed food intake[68]. Nutrition interventions aimed at parent education are recommended because such instruction may create a healthier home food environment and improve child feeding and health outcomes[67,68].

Few studies have been conducted to test the efficacy of parent education programs aimed at improving the diet of parents of learning-disabled children. In a sample of 356 parents of children with autism, spina bifida or Down syndrome, Polfuss *et al*[69] found parent feeding behaviors are significantly related to the child's weight but there was no attempt to provide a nutrition intervention. To address the feeding problems of food aversion and selectivity in children with autism, Sharp *et al*[70] conducted a pilot study to determine the feasibility of two separate parent education programs: one focused on the management of eating aversions *via* behavioral interventions and the other focused

on providing nutrition education with strategies for meal structure and diet expansion. The nutrition parent education program proved to be more promising at alleviating the problematic mealtime behaviors among the children but required further study[70]. Thorsteinsdottir *et al*[71] conducted a seven-week intervention trial to determine the effect of taste education on problematic mealtime behaviors in children with autism or ADHD and their parents. Children and parents received separate nutrition instruction and then combined family instruction[71]. The combined family instruction involved six kitchen sessions, each lasting 90 min, and focused on building food preparation skills using games as a base for delivering sensory and taste education[71]. The results of the study showed superior outcomes with stable effects after six months in the family groups that underwent the taste education[71].

The aim of this basic study was to test the efficacy of a six-week nutrition intervention aimed at educating parents of learning-disabled children on the role ultra-processed foods play in creating conditions for the development of autism and ADHD. The nutrition intervention was delivered online *via* tutorial and focused on teaching parents the constructs of the nutritional epigenetics model for autism and ADHD (Figure 1). Parents learned which food ingredients in ultra-processed foods contribute to heavy metal exposures, impact nutrient status, and/or gene behavior. Through the instruction, parents became aware of the fact that they could choose to create a healthy and safe home food environment for their children (Chapter 6 of textbook, Module 6 of tutorial). The outcome measure we chose to assess the efficacy of the nutrition intervention was change in parent diet, including ultra-processed food intake and organic/whole food intake. We demonstrated that in six weeks, with nutritional epigenetics instruction, you could significantly change parent diet. The expected outcome (H1) was that parents would reduce their intake of ultra-processed foods and they did.

One limitation of this study is that we did not collect pre- and post-intervention food frequency data for the learning-disabled children. We did not ask the parents if they changed their children's diet because of the instruction they received during the nutrition intervention, so we do not know if the parental changes in diet affected the

diets of the children. We do have the parent feedback data in Table 8 that indicates parents did change the way they shop for food and plan their family's meals. There is little evidence to suggest that learning disabled children will mirror their parents' dietary pattern aside from the observations made by Thorsteinsdottir *et al*[71]. For follow up studies, we recommend that hands-on parent-child cooking and meal preparation activities be incorporated into the existing nutritional epigenetics curriculum along with the collection of pre- and post-intervention child food frequency data so that researchers can measure any changes in child dietary intake patterns.

Another limitation of this study may be the relatively small sample size. However, we did use the calculation procedures provided by Kirkwood and Sterne[56] to determine the minimum sample size for each group (test and control). A prior study using the same survey instrument[50] produced mean diet scores at pre and post intervention of 16.0 and 23.2 with the standard deviations of 1.72 and 2.83, with $n = 10$. Based on calculation procedures provided by Kirkwood and Stein[56], a sample size less than 10 was determined to be adequate for this study. According to our calculations, at least three participants would be needed for each group; we ended up with eleven participants in each group (test and control) for this study. Each of the H0 and H1 hypotheses in this study were supported using the classical *t*-test.

Another limitation of this study could be the introduction of selection bias from the participant recruitment process. We used social media (Facebook) to recruit the participants. The underlying assumption of using social media is parents who use social media have access to the internet. Because we required participants to have at least a high school education and internet access, lower income parents may not have been able to participate. There is a digital divide in the United States where lower income and less educated parents are less likely to own a computer, or use social media and the internet[72,73]. Minorities are underrepresented in our study as 68% of our participants were predominantly white and non-Hispanic. Out of the twenty-two participants in this study, only 32% were members of a minority group. This type of selection bias is known as demographic bias. Because a higher proportion of black children in the

United States are classified as having intellectual disability[74] and lower income is an important factor associated with receiving a diagnosis of autism/ADHD[75], follow up studies will need to be adequately funded to ensure the nutritional epigenetics curriculum is designed to be effective at teaching less educated and lower income parents from minority backgrounds. Future studies involving low-income families will also need to ensure that parents have access to whole and/or organic foods especially if they live in food deserts.

The need to implement nutrition education interventions focused on teaching parents the constructs of the nutritional epigenetics model for autism and ADHD is now critical in the United States. At a minimum, parents must learn to avoid feeding their children the ingredients in ultra-processed foods that contribute to heavy metal exposures, impact nutrient status, and/or gene behavior. Trends in ultra-processed food consumption among youth in the United States are increasing at an alarming rate. Wang *et al*[76] found ultra-processed food consumption among United States youth aged 2-19 increased from 61.4% to 67% while the total caloric intake of whole or minimally processed foods decreased from 28.8% to 23.5% between 1999 and 2018. The increasing trends in ultra-processed food consumption among youth are alarming because strong evidence now suggests the consumption of ultra-processed foods is associated with heavy metal and pesticide exposures and the development of autism, developmental delay, and/or ADHD[42]. With the increasing ultra-processed food intake, we see increasing trends in the number of children struggling with these neurodevelopmental disorders. Dufault *et al*[42] reported a 242% increase in the number of children ages 6-21 in the United States receiving special education services under the autism category while student enrollment remained flat between 2006 and 2021. The need for special education services for children under the other health impaired (including ADHD) and developmental delay categories increased 83% and 184%, respectively, during the same period[42]. Parent nutrition education programs focused on facilitating reductions in ultra-processed food intake are key to preventing the learning disabilities that are

increasing in prevalence with each passing year: autism, developmental delay, and ADHD.

Children with autism and ADHD are routinely diagnosed by healthcare providers using criteria specified by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders[77]. The current diagnostic criteria do not include recommendations for biomarker analyses, even though children with autism and ADHD have elevated levels of Pb and/or I-Hg in their blood compared to their neurotypical peers[10]. Baj *et al*[78] concluded in a recent review that the analysis of trace elements as biomarkers could be relevant tools in the diagnosis, prevention, and treatment of children with autism and neurological disorders associated with heavy metal exposures. Guidelines for selecting analytical methods for the measurement of blood Pb and Hg in children with autism or ADHD are already available[10]. Because the severity of symptoms in autism and ADHD correlate directly to the heavy metal levels in their blood[10,38,78,79], it may be beneficial to utilize these biomarker tools to monitor treatment strategies[78], especially in the case of dietary interventions which may decrease heavy metal exposures. One final limitation of this study is that we did not have funding to collect biomarker data. In addition to educating parents on the role ultra-processed foods play in the development of autism and ADHD, future research efforts could include the collection of blood samples from afflicted children pre- and post-intervention (parent education) to determine any changes in the heavy metal levels.

Due to their inability to excrete heavy metals[10], a common co-morbid condition of autism and ADHD is gut dysbiosis[80,81]. It is not surprising that one of the toxic effects of heavy metals, in single or multi-metallic combination, is the alteration of metabolic profiles that lead to gut dysbiosis[82]. In a recent review, Zafar and Habib[83] found the following bacteria to be greatly decreased in the gastrointestinal tract of children with autism: Bacteroides, bifodobacterium, lactobacillus, and prevotella. In a separate review, Boonchooduang *et al*[81] found bifodobacterium and bacteroides to be the best candidates for analyses in stool samples of children and adolescents with ADHD.

Dietary co-exposure to heavy metals is the least studied area of gut health; however, Mangalam *et al*[84] found a decline in Prevotella in response to heavy metal mixtures in the gut. Studying the impact of dietary heavy metal exposures on gut microbiota may also be of value when children and parents consume a western diet comprised mostly of ultra-processed foods. Future research efforts could be focused on developing guidelines for collecting and analyzing stool samples to determine the makeup of the gut biome in children with autism and ADHD.

With respect to the prevention of these neurodevelopment disorders, to date, only one study has attempted to determine a relationship between prenatal dietary heavy metal exposure and the offspring's gut microbiome: Midya *et al*[85] found prenatal Pb exposure is associated with reductions in Bifidobacterium within the gut microbiota of children at 9-11 years of age. In their conclusion, Midya *et al*[85] stated that the current Pb exposure guidelines in the United States and Mexico are not sufficient to protect the human gut microbiome from the deleterious effect of dietary Pb exposure. More research needs to be conducted to determine how prenatal exposures to the heavy metal mixtures found in ultra-processed foods may impact the child's gut microbiome.

Future research could also focus on determining whether diet can be modulated to reduce heavy metal exposures associated with gut dysbiosis in children with autism or ADHD. Emerging evidence suggests that diet does have a moderating influence on the gut microbiome and the pathophysiology of neurodevelopmental disorders such as those found in the autism spectrum[86]. The role of diet as a modulator of neuroinflammation is presented clearly in a recent review conducted by Kurowska *et al*[87]. Several studies show that consuming a diet rich in whole foods (*e.g.,* fruits, nuts, vegetables, herbs, legumes) while avoiding foods that promote inflammation (*e.g.,* ultra processed foods) results in reduced risk of neurological disease associated with gut dsybiosis[87]. In designing our nutrition education intervention for parents of children with ADHD or autism, we thought it foremost to provide instruction on how to avoid consuming ultra-processed foods that contain ingredients with allowable heavy metal residues or increase the risk of heavy metal exposure. In conducting future studies, it

may also be useful to measure any changes that may occur in the gut microbiota in children with autism and ADHD in response to changes in diet that reduce heavy metal exposures.

The risk of heavy metal exposure from eating ultra-processed foods has been clearly demonstrated in studies conducted by Khan *et al*[88], Wells *et al*[89], and Raehsler *et al*[90]. Khan *et al*[88] found heavy metal concentrations in food products significantly correlated with the heavy metal levels detected in human blood samples. Wells *et al*[89] verified Hg exposure from non-fish food occurs through the consumption of vegetable oil, an ingredient commonly found in ultra-processed foodstuffs. Raehsler *et al*[90] determined excessive intake of ultra-processed "gluten-free" food may lead to significantly higher levels of Cd, Pb, and Hg in the blood. The heavy metal exposures from drinking contaminated water, or eating ultra-processed food, will destroy the metabolic processes in the human body *via* oxidative stress[91]. The nutritional epigenetics model for autism and ADHD shows how this oxidative stress occurs (Figure 1) and may thus be a useful tool for understanding other pathologies associated with heavy metal exposures.

In addition to autism and ADHD, heavy metal exposures, especially Cd, Pb, and Hg, are positively associated with the development of atherosclerotic cardiovascular disease[92] and non-alcoholic fatty liver disease[93]. From a toxicological perspective, it is interesting to note that non-alcoholic fatty liver disease is associated with ultra-processed food intake in a dose-response manner similar to the dose-response relationship showing heavy metal toxicity[94]. Not surprisingly, numerous pathologies are associated with ultra-processed food intake[95]. In a recent review, Elizabeth *et al*[95] examined forty-three articles to determine any associations between ultra-processed food intake and adverse health outcomes. Of the forty-three articles, thirty-seven found excessive ultra-processed food intake was associated with at least one of the following pathologies: Obesity, overweight, cancer, type-2 diabetes, depression, irritable bowel syndrome, cardiovascular disease, and all-cause mortality[95]. Any nutrition education program that helps individuals significantly reduce their intake of ultra-processed

foods will be useful because evidence suggests that switching to a healthy diet will prevent disease and/or improve health outcomes[96].

The pre- and post-intervention outcomes presented in Table 6 show the nutrition education program used in this study was an effective tool because parents who received the nutritional epigenetics instruction significantly decreased their ultra-processed food intake ($P < 0.001$) and significantly increased their whole and/organic food intake ($P < 0.05$). Table 7 provides details on the reliability of our outcome measurements from a statistical perspective. Parent nutritional epigenetics instruction is a novel nutrition education intervention because evidence suggests that if parents reduce their consumption of ultra-processed foods, then their children will also reduce their consumption of ultra-processed foods[67,68]. Using the nutritional epigenetics model as a teaching tool for helping parents reduce their consumption of ultra-processed foods may lead to healthier home food environments and subsequent improvements in child diet by reducing the heavy metal exposures associated with autism and ADHD. More research is needed to verify the reductions in heavy metal exposures that may be associated with reducing ultra-processed food intake.

Meanwhile heavy metal residues continue to be a problem in the American food supply. The United States Congress recently released two reports on the problem of heavy metals, including I-Hg and Pb, in baby foods sold in America[97,98]. Dietary heavy metal exposures, I-Hg and Pb, are an important construct in the nutritional epigenetic model for autism and ADHD (Figure 1). In addition to collecting and analyzing blood samples for Hg and Pb, changes in MT and PON1 gene activity levels could also be measured in children with autism and ADHD pre- and post-parental nutritional epigenetics instruction. Meguid *et al*[99] successfully measured changes in the genetic expression of MT-1 in children with autism after Zn supplementation. Numerous studies have already been conducted successfully to measure PON1 gene activity in response to changes in diet[100]. Further studies that use nutritional epigenetics instruction to modulate diet could shed light on the role ultra-processed foods (and

heavy metal exposures) play in the development of autism and ADHD *via* MT gene disruption or PON1 gene suppression[101].

CONCLUSION

The aim of this basic study was to test the efficacy of a six-week nutritional epigenetics tutorial in improving dietary behavior patterns and attitudes of parents having a learning-disabled child with autism or ADHD. Evaluation of the tutorial showed it was an effective tool because it provided parents with the instruction and information needed to reduce poor dietary habits and facilitate healthy dietary changes over a 6-wk period. The parents who completed the tutorial significantly reduced their intake of highly processed foods, increased their intake of whole and/or organic foods, and changed their attitude about their ability to influence their child's behavior through diet. Nutritional epigenetics instruction can be used to facilitate healthy changes in diet and attitude among parents of learning-disabled children within a six-week period.

ARTICLE HIGHLIGHTS

Research background

Ultra-processed foods contain heavy metal and pesticide residues. Specific food ingredients and heavy metal contaminants found in ultra-processed foods may result in mineral imbalances that impact or disrupt gene expression. Evidence suggests prenatal nutritional deficits and heavy metal exposures associated with poor diet are the primary epigenetic factors responsible for the development of autism and attention deficit/hyperactivity disorder (ADHD) *via* metallothionein gene dysfunction and paraoxonase-1 gene suppression. The excess consumption of ultra-processed foods by parents is associated with the development of these neurodevelopmental disorders.

Research motivation

The prevalence of autism and ADHD is increasing in the United States. The key problem to be solved is the excess consumption of ultra-processed foods by parents.

Parents must be encouraged to reduce their consumption of ultra-processed foods. In educating parents on the role ultra-processed foods play in the development of autism and ADHD, they may become empowered to change their diets.

Research objectives

The aim of this basic study was to test the efficacy of a six-week nutritional epigenetics tutorial in reducing parental ultra-processed food intake.

Research methods

The parent education intervention we created was novel as it is the first ever to provide instruction focused on the constructs of the nutritional epigenetics model for autism and ADHD. In learning how what they eat determines how their genes behave, parents in the test group chose to change their diets. We measured their dietary changes pre- and post-intervention using a novel, pre-tested and validated, diet survey with questions designed to measure ultra-processed or whole/organic food intake. Ultra-processed foods are characterized as having more than one ingredient along with at least one ingredient known, or likely, to contain heavy metal residues.

Research results

The literature review conducted for this basic study revealed maternal ultra-processed food consumption is associated with adverse child neurodevelopment. This new finding further strengthens the nutritional epigenetics model for autism and ADHD that was initially published in 2009 as the Mercury Toxicity Model. The literature review also revealed the role of dietary heavy metals in creating the co-morbid condition of gut dysbiosis that is found in children with autism and ADHD. Dietary heavy metal exposures continue to be a problem in the United States ultra-processed food supply.

Research conclusions

The nutritional epigenetics model for autism and ADHD that this study uses as the basis for the nutritional epigenetics instruction is not new but is further refined by the results of this study. The success of the nutritional epigenetics tutorial in helping parents reduce their intake of ultra-processed foods may be attributed to the parents' acceptance of the model constructs. They know the ultra-processed food supply is contaminated with heavy metal and pesticide residues. Two reports issued by the United States Congress in 2021 confirm there is a heavy metal problem in the ultra-processed food supply.

Research perspectives

In conducting future research, it may be useful to collect blood samples from the children and parents for heavy metal analyses pre- and post-six-week intervention that includes nutritional epigenetics instruction. If the children present with symptoms associated with autism and/or ADHD, then blood mercury and lead levels are important to measure. Pre- and post-intervention stool samples could also be collected to measure any changes in the gut biome, especially if a child presents with symptoms of gut dysbiosis.

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