ORIGINAL RESEARCH

Intestinal Permeability and Nutritional Status in Developmental Disorders

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ABSTRACT

Context • Autism is a developmental disorder with a possible connection between dietary components and triggering or worsening of symptoms. An altered intestinal permeability might allow absorption of incompletely digested peptides (gluten and casein) that could produce opioid-like activity on the brain, causing significant changes in behavior.

Objective • To assess the intestinal permeability and nutritional status of participants with developmental disorders to determine if changes in the intestinal mucosal barrier and/or injury to the intercellular junctions have occurred that might justify application of further dietary modifications.

Design • To assess intestinal permeability, the research team analyzed participants' urine under fasting conditions, using gas chromatography to determine chromatographic peaks. To assess nutritional status, the team determined participants' heights and weights and performed a bioelectric bioimpedance examination at least 4 hours after their most recent meal. In addition, the team determined food intake using three diet diaries. They asked participants and caregivers to register each food consumed during 2 nonconsecutive weekdays and 1 weekend day.

Setting • The study occurred at the Ribeirao Preto School of Medicine, Sao Paulo University.

Participants • Seven participants aged 9 to 23 years with developmental disorders (the developmental group, DG) completed the study. The research team recruited them through the Association of Friends of the Autistic Persons of Ribeirao Preto in Ribeirao Preto, Brazil. The control group (CG) consisted of nonsmoking healthy volunteers in the general population who were similar in age to the experimental group and did not suffer from diseases that potentially could influence nutritional status and intestinal function.

Intervention • To assess intestinal permeability, participants ingested 150 mL of an isosmolar solution of the sugars mannitol (2 g) and lactulose (7.5 g) under fasting conditions and the researchers collected all voided urine over a period of 5 hours.

Outcome Measures • Using chromatographic peaks, the research team quantified the mannitol and lactulose in participants' urine by calculating the percentage excreted in relation to the ingested amounts of sugar. This calculation gave them the lactulose-to-mannitol ratio (L/M). To evaluate nutritional status, they used data regarding bioimpedance resistance, heights, and weights to estimate lean mass and body water (in liters). They classified adults and adolescents using the body mass index (BMI). For children (2-10 y), they classified participants' height-to-age and weight-to-height ratios. The research team used food intake to examine the macronutrient interval, the mean added sugar consumption, and the quantity of protein, in g/kg weight.

Results • Participants with developmental disorders (n = 7) were more likely to be overweight. Their usual diet revealed a high intake of lipids (%) and proteins (g/kg) (compared to reference values) and a high intake of calories (kcal) and carbohydrates (%) (compared to CG) as well as a high intake of food sources that are important contributors of casein and gluten. The DG's (n = 7) mean mannitol excretion was lower, and their L/M higher than the CG's (n = 7) ($P < .05$). Their increased L/M may indicate atrophy of the intestinal-mucosa surface and/or injury to the intercellular junctions or the effect of some other abnormality. The small number of participants, however, prevented more complex statistical analysis.

Conclusions • Researchers need to complete additional studies to confirm the existence of abnormalities in autistic individuals' intestines and to justify the use of dietary restrictions on gluten and casein to improve the symptoms of autism. (*Altern Ther Health Med.* 2012;18(2):19-24.)

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Autism is a complex spectrum of clinically heterogeneous, neurodevelopmental disorders, now commonly known as autism spectrum disorders (ASD).[1] The etiology of autism is still unknown, and the possible causes proposed, separately or in combination, are viral infections, genetic predisposition, abnormalities of the limbic and cerebellar systems, and dysfunction of the immune, hepatic, and gastrointestinal systems.[2] In view of the increase in the number of cases diagnosed over the last few years,[3,4] the need is growing to determine the causes and the physiopathological mechanism of autism.

For more than 2 decades, investigators have been trying to clarify a possible connection between dietary components and the triggering or worsening of symptoms of ASD.[5] A more recent review emphasized that the causes of ASD are still undefined, but the study's authors discussed the recent onset of theories concerning gastrointestinal abnormalities in children with ASD.[6] Altered intestinal permeability is the basis for the theory that an intestine that allows leakage of incompletely digested peptides might produce opioid-like activity on the brain, causing significant changes in behavior.[2]

In one of the few studies found in the literature, D' Eufemia et al (1996) reported that approximately half of a small cohort of children with ASD had abnormally high intestinal permeability.[7] Another study, however, did not detect differences in this measure in a group of autistic children.[8] More recently, researchers found a high percentage of abnormal intestinal-permeability values among patients with autism and their relatives compared with normal subjects. Additionally, patients on a gluten– and casein–free diet reported significantly lower intestinal-permeability values compared with those who were on an unrestricted diet and with healthy children.[9]

The available literature has not confirmed the presence of abnormal gastrointestinal permeability in individuals with ASD, and the research has not correlated the presence of increased intestinal permeability with the various clinical and behavioral aspects of ASD.[10]

To collect evidence of the participation of dietary components in the triggering of autism or in the exacerbation of symptoms, researchers have conducted some studies to assess the effects of dietary restrictions on the improvement of the behavioral signs and symptoms of autistic groups. Although most of the results obtained were controversial,[10,11] a growing number of institutions have proposed the use of gluten-poor and casein-poor diets to improve the symptoms of autism, even if this practice means removing food groups important to the maintenance of the affected patients' nutritional status. Complicating these proposed changes, obtaining compliance with any dietary plan is difficult with autistic individuals because they often show an eating routine with a repetitive feeding pattern and selectivity in choice of food groups, textures, and even certain food-packaging types.[12,13]

As with autism,[9] some researchers have assessed the impact of restricted diets on intestinal permeability in individuals with intestinal inflammatory diseases.[14,15] Few studies, however, have evaluated the nutritional status and intake of subjects with ASD.[16-18]

METHODS

Hypothesis

An assessment of intestinal permeability and nutritional status in individuals with developmental disorders will show changes in the intestinal mucosal barrier and/or injury to the intercellular junctions that might justify application of further dietary modifications.

Participants

Seven participants aged 9 to 23 years with developmental disorders (DG) completed the study. The research team recruited them through the Association of Friends of the Autistic Persons of Ribeirao Preto (AMA) in Ribeirao Preto, Brazil. Medical practioners had diagnosed five of the participants with autism spectrum disorders (ASDs), four with autism, one with a pervasive developmental disorder-not otherwise specified (PDD-NOS), and two with mental retardation (MR). The practitioners made these designations based on the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV).[19]

The current study's inclusion criterion included full urinary control without the use of diapers. Exclusion criteria included clinical evidence of gastrointestinal diseases such as celiac disease, Crohn's disease, ulcerative colitis, or tropical sprue; an individual or familial history of food allergies; gastrointestinal infections; and the presence of diseases associated with changes in intestinal physiology such as diabetes and human immunodeficiency virus.[20]

The CG consisted of nonsmoking healthy volunteers in the general population who were similar in age to the experimental group and did not suffer from diseases with potential influence on nutritional status and intestinal function. Participants were alcohol-free for at least 10 days prior to the study and did not use any medicine that could interfere with intestinal physiology during the intestinal-permeability test.[7]

The ethics committee at the University Hospital of the Ribeirao Preto School of Medicine approved the study, and all participants and persons responsible for them gave written informed consent to participate.

Intestinal-permeability Assessment

Permeability refers to the property of the intestinal epithelium or of a membrane that enables passage of a solute by nonmediated diffusion down a concentration gradient or a pressure gradient without the mediation of specific biochemical carrier system.[21]

The research team began the test of intestinal permeability in the morning with the collection of basal urine. After participants ingested 150 mL of an isosmolar solution of the sugars mannitol (2 g) and lactulose (7.5 g) the team collected all voided urine over a 5-hour period.[22]

The syrup solution was prepared by the research team, packaged in a 200 mL amber bottles, and stored under refrigeration at 8.0˚C until used. After ingesting the syrup, participants were not permitted to consume food until 1 hour had elapsed to prevent interference with syrup metabolization in the intestine.

Basal urine was tested using the same methodology as the 5-hour urine collection, described below:

> *The sugar permeation test began at 8:00 AM, following an 8-hour fast. Each subject emptied his bladder completely and the collection from voiding was used as a baseline sample (blank). The subject drank 150 mL of water that contained 7.5 g lactulose and 2.0 g mannitol. Thereafter, all of the urine over the next 5 hours was collected into one container. During the collection period, the baseline sample and the 5-hour collection were preserved in a graduated bottle by the addition*

Table 1. Comparisons of Excreted Mannitol and Lactulose Fractions and Lactulose/mannitol Ratio Between Groups

Variable	Control Group (n = 7)			Developmental Disorders Group (n = 7)			P-Value
	Mean (SD)	Minimum and Maximum Values	95% Confidence Interval	Mean (SD)	Minimum and Maximum Values	95% Confidence Interval	
Mannitol, %	17.9 (3.2)	14.2-23.8	14.9-20.9	10.8 (6.3)	0.7-17.9	4.9-16.6	.03
Lactulose, %	0.12 (0.08)	0.02-0.28	0.04-0.19	0.3 (0.3)	0.04-0.89	0.02-0.58	.16
Lactulose/ mannitol Ratio	0.007 (0.006)	0.001-0.02	0.0005-0.01	0.039 (0.038)	0.003-0.11	0.003-0.07	.02

Abbreviations: SD, standard deviation.

of sodium flouride, 10 or 100 mg, respectively. After collection, urine volumes were recorded. They measured the total urine output in a graduated cylinder and recorded the data. They homogenized the samples and aliquots of 15 mL were taken from the baseline and 5-hour urine collections and stored at –30˚C for subsequent analysis by gas chromatography, approximately 1 month after the samples were collected.

If a participant's basal urine contained excreted mannitol and lactulose, the research team subtracted its value from the amount obtained in the relevant stored sample.[23] According to the chromatograms obtained on participants' stored samples, the amounts excreted in the basal urine were negligible, measuring below the limit of quantification (0.002 mg/mL mannitol and 0.0002 mg/mL for lactulose)[24] and therefore, were values that did not influence the final results.

The research team analyzed the urine samples according to a previously standardized derivatization technique.[23] The gas chromatography used a capillary column according to the conditions that Dumas et al has described.[25] The gas chromatograph, a Shimadzu model GC-17A (Shimadzu Corp, Kyoto, Japan), was equipped with an autoinjector in split mode; the AT-1 capillary column was 10 m long, 0.53 mm in diameter, and 1.2 mm thick with a flame ionization detector. The team used helium as the carrier gas with a flow of 2.4 mL/min and linear velocity of 30 cm/s, which provided a pressure of 10 kPa in the capillary column.

Based on the areas of the chromatographic peaks, the research team quantified mannitol and lactulose by calculating the percentage excreted in relation to the ingested amounts of sugar and obtained the lactulose-to-mannitol ratio (L/M). The team calculated the cut-off range of normal values by adding twice the value of the standard deviation (SD) to the CG's obtained mean, namely L/M > 0.020.

Nutritional Status

Anthropometric Assessment. The research team determined participants' weights using a Plenna digital scale (Plenna®, Sao Paulo, Brazil) with a capacity of 150 kg and with 100 g graduations. They used a stadiometer with 1-cm graduations to measure height and performed a bioelectric bioimpedance examination at least 4 hours after the most recent meal (a minimum 4-hour delay was necessary for participants that consumed breakfast before participating in the study) using a Biodynamics® instrument (Biodynamics Corporation, Seattle, Washington).

The research team used data regarding resistance, height, and weight to estimate lean mass and body water (in liters) based on the equation that Sun et al[26] recommended for adolescents and adults. They selected the equation that Suprasongsin et al[27] recommended for the determination of lean mass in children, with fat mass being calculated as the difference.

Table 2. Comparisons of Age, Anthropometric, and Body-composition Data Between Groups

Variable	Control Group (n = 8) Mean (SD)	Developmental Disorders Group (n = 7) Mean (SD)	*P*-value
Age, y	17.7 (4.9)	15.8 (6.5)	.77
Weight, kg	52.4 (8.9)	66.9 (25.2)	.61
Height, m	1.61 (0.1)	1.59 (0.2)	.86
Body mass index, kg/m^2	20.0 (1.6)	25.6 (5.5)	.04
Fat mass, %	22.4 (5.3)	20.9 (7.7)	.53
Lean mass, %	77.5 (5.3)	79.0 (7.7)	.53

Abbreviations: SD, standard deviation.

Table 3. Comparisons of Calorie and Macronutrient Intake Data Between Groups

Variable	Reference Values	Control Group (n = 7) Mean (SD)	Developmental Disorders' Group (n = 7) Mean (SD)	*P*-value
Intake, Kcal	–	1787.3 (348.1)	2191.5 (179.4)	.03
Intake, Kcal/kg	–	35.0 (7.9)	37.2 (14.7)	.86
Proteins, g/kg	0.80-0.95	1.29 (0.28)	1.15 (0.4)	.69
Proteins, %	10-15	14.9 (0.6)	12.2 (1.3)	.001
Carbohydrates, g/kg	–	4.3 (1.0)	5.3 (2.4)	.53
Carbohydrates %	55-75	49.1 (2.6)	56.7 (4.7)	.007
Lipids, g/kg	–	1.4 (0.3)	1.2 (0,4)	.45
Lipids, %	15-30	35.9 (2.5)	31 (3.9)	.01
Added sugar, g	–	7.6 (12.8)	5.4 (7.8)	>.99
Added sugar, %	<10	2.0 (3.7)	0.9 (1.3)	.79

The research team assessed nutritional status according to the corresponding gender and age range. For children (2-10 y), they classified the height-to-age and weight-to-height ratios according to the parameters set by Jelliffe[28] and Waterlow.[29] For adolescents (11-18 y), they used the body mass index (BMI) curve that the National Center for Health Statistics[30] has proposed, with the cut-off points that Frisancho defined.[31] For adults, they used the BMI values that the World Health Organization (WHO)[32] has stipulated.

The research team considered percentages of body fat above 25% for men and above 32% for women as cut-off values defining overweight condition.[33] For children and adolescents, the team determined the predicted cut-off values of body fat for age range and gender, according to Taylor et al.[34]

Determination of Food Intake. The research team determined food intake using 3 diet diaries. They asked participants and caregivers to register each food consumed during 2 nonconsecutive weekdays and 1 weekend day. They instructed participants and their relatives about portion size, home measurements, and types of foods and preparations consumed. The team used the NutWin software Version 1.5 (NutWin Support Program on Nutrition, Paulista School of Medicine, Federal University of Sao Paulo, Sao Paulo) for the nutritional calculations (energy and macronutrients).

The research team assessed the macronutrient interval and the mean added-sugar consumption according to the values recommended by the WHO,[35] and the quantity of protein, in g/kg weight, according to the values that the US Institute of Medicine's Dietary Reference Intakes stipulated.[36]

Statistical Analysis

The research team reported data as means ± SD and determined the differences between groups by the nonparametric Mann-Whitney test with the level of significance set at $P < .05$.

RESULTS

A total of 7 participants in the DG completed the study: 3 boys (mean age: 9.19 ± 0.78 y), one with MR and the others with autism; 1 adolescent female (age: 17 y) with PDD NOS; and 3 adult males (mean age: 22 ± 1 y), two with autism and one with MR.

Eight volunteers participated in CG: 2 boys (mean age: 10 ± 1, 4 y), 2 adolescent females (mean age: 17, 5 ± 0, 7 y) and 4 adult males (mean age: 20, 7 ± 2, 2 y).

Intestinal-permeability Data

The DG showed a low mannitol excretion compared to the CG (Table 1). Although lactulose excretion did not differ between the groups (Table 1), 2 participants presented discrepant values (0.51% and 0.89%).

The L/M was about 5 times higher in the DG vs the CG (Table 1). Furthermore, 4 participants in the DG (57.0%) had an L/M above 0.020 (0.028-0.11), the cut-off point for normal values: three of them were autistic subjects, and one had PDD NOS. Of these four, one autistic subject had a higher L/M due to increased lactulose excretion (L/M = 0.028; 0.51% of lactulose; 17.9% of mannitol); two had an increased ratio due to reduced mannitol excretion (L/M = 0.064 and 0.038; % mannitol = 7.6% and 5.2%; % lactulose = 0.05% and 0.2%, in this order); and one had a higher L/M due both to an increase in lactulose and a reduced mannitol excretion (L/M = 0.11; 0.89% of lactulose; 7.9% of mannitol). No participant reported any side effects with the syrup intake.

Nutritional-status Data

Anthropometric Data. The DG had a higher BMI compared to the CG ($P < .05$) (Table 2). Six of the 7 participants from the DG had a higher weight than recommended, with 4 participants being classified as obese and two as overweight according to the corresponding gender and age range.

All participants of the CG were in normal weight, according to the corresponding gender and age range.

Food Intake Data. For the DG, Table 3 shows a high average intake of calories (absolute value) and carbohydrates (in percentage of total energy intake) vs the CG. Although the consumption of lipids

and proteins (in percentage of total energy intake) of the DG is smaller compared to the CG, lipid values (in percentage of total energy intake) and mean protein consumption per Kg of current body weight of the DG are above stipulated values.[35, 36] The average consumption did not exceed the value stipulated for simple sugars (10% or less of the total energy intake).[35] However, this type of food was very present in food records of the group.

DISCUSSION

Intestinal Permeability

The sensitivity and specificity of the L/M is high.[24] Furthermore, the L/M is highly sensitive when compared to other methods,[37] and researchers consider it to be a reference for the assessment of intestinal permeability in various situations and diseases.[38]

In the current study, the L/M was higher than normal in 57.0% of the DG, with a significantly higher mean for the DG vs the CG, indicating changes in intestinal permeability in the DG.

The values obtained for the DG were compatible with those reported in other studies. A similar permeability study that Horvath et al[39] did using the lactulose-to-mannitol recovery ratio, found that 76% of children tested had an elevated lactulose-to-mannitol recovery ratio; however, the work did not include a control group.

The values of mannitol and lactulose excretion for the CG were similar to those reported in the literature. Farhadi et al[23] detected 14.2% mannitol excretion, 0.16% lactulose excretion, and an L/M of 0.013 in healthy individuals. Dumas et al[25] detected 14.5% mannitol excretion, 0.27% lactulose excretion, and an L/M of 0.018.

In the current study, the low number of participants due to low enrollment and to difficulties in urine collection prevented more complex statistical analysis. The confounding impact of probiotic or intestinal flora on intestinal permeability, the lack of matched controls, and the inconsistent profile of autistic subjects with elevated L/M are some limitations of the study.

Atrophy of Intestinal-mucosa Surface. In the current study, the research team detected a reduced mannitol excretion in the DG, of which three with a L/M above the cut-off point had a decreased mannitol excretion. Johnston et al[40] mentioned atrophy of the intestinal mucosa, which is present in individuals with celiac disease, as a possible cause of lower mannitol uptake since mannitol is absorbed through the aqueous pores of the enterocytes, reflecting absorptive capacity.

Horvath et al (1999)[41] evaluated the structure and function of the upper gastrointestinal tract in a group of patients with autism who had gastrointestinal symptoms. Histologic examination in 36 children revealed grade I or II reflux esophagitis in 25 (69.0%), chronic gastritis in 15, and chronic duodenitis in 24. The study reported low digestive-enzyme activity relating to intestinal carbohydrates in 21 children (58.3%). The most frequent finding was low lactase activity in 14 of the 21 children, with pathologic disaccharidase results.[41] Lactase is expressed only in the small intestine and is confined to absorptive enterocytes on the villi.[42] Therefore, the decrease in lactase activity might be justified by the possible dysfunction in intestinal villi as evidenced by a decrease in mannitol uptake.

Injury to Intercellular Junctions. D'Eufemia et al[7] detected an increased L/M (0.036-0.298) in nine of 21 (43%) children with autism and in none of the healthy age-matched controls. The excreted mannitol fraction of the study's autistics was similar to that of its control group, whereas lactulose excretion was greater than for the control group. In another study, the increase in L/M values both in patients with ASD (36.7%) and their relatives (21.2%) was mainly due to the 2- to 3-fold increase in lactulose compared with mannitol recovery.[9] In this study, despite the fact that excretion of lactulose for the patients with ASD did not differ from that of the control group, two individuals had increased values.

Lactulose, a disaccharide, is absorbed via a paracellular route (reflecting barrier function). According to White and to Wakefield et al, the greater lactulose levels indicate a possible injury to the intercellular junctions (tight junctions) of the intestinal mucosa, with a consequent increased passage of molecules of higher molecular weight (>180 Da). This alteration might be the mechanism for increased passage of food-derived peptides through a damaged gut mucosa that may interfere with the central nervous system.[2,11]

In this context, some recent studies have tried to determine if a close relationship exists between dietary change and the onset of symptoms in patients with ASD. Available research data do not support the use of a casein-free diet, a gluten-free diet, or combined gluten-free, casein-free diet as a primary treatment for individuals with ASD.[10]

In summary, the rates of excretion of the 2 sugars suggest the possibility of changes of molecular permeation through the intercellular space (paracellular route) and of the presence of absorptive dysfunction (transcellular route) among autistics.

Nutritional Status

The research team also assessed participants' usual diets, which revealed reasonable consumption of carbohydrates and dairy products. These foods are sources of casein and gluten, substances that caregivers would usually remove from dietary schemes to relieve the symptoms of autism.

Children with ASD have the potential to be obese. In a retrospective review of charts from 1992 to 2003 in children aged 3 to 18 years with ASD, Curtin et al found that the overall prevalence rates of "at risk for overweight" and "overweight" were 35.7% and 19.0%, respectively.[43] These prevalence data are similar to rates in children aged 6 to 19 years in the general population at NHANES study from 2001-2002.[44] When stratified by age categories, the researchers report that children with ASD in the 12- to 18-year age range present the highest prevalence rates of 80% and 50% for being "at risk for overweight" and "overweight," respectively,[43] compared with 30.9% and 16.1%, respectively, in the general population.[44]

In the current study, participants were more likely to be overweight. The higher caloric intake associated with a higher percentage of fat may have contributed to weight gain in the DG. Few studies have investigated the nutritional conditions in autism. Recent investigations have demonstrated an association between weight gain and the use of antipsychotic medications such as resperidone, commonly used for the treatment of these individuals.[45] Of the 7 participants in this study, 5 were using resperidone, and 1 was using carbamazepine, a drug that also has weight gain as a side effect.

Characterization of food intake revealed the constant presence of foods that are gluten and casein sources in the habitual diet, with milk and dairy products corresponding to 16% of the total protein ingested and bakery items corresponding to 11%.

CONCLUSION

In addition to assessment of intestinal permeability, a fundamental need exists to determine the nutritional status and especially, the diet of autistic individuals in view of the possible application of dietary gluten and casein restrictions that might reduce their behavioral disorders. When researchers confirm the existence of intestinal changes and the sensitivity to gluten and casein, caregivers can implement a restricted diet for autistic individuals, adapting their diets to ensure adequate food choices without exposing them to nutritional risk.

Researchers, however, have not fully characterized the type of injury present in the mucosa of autistic individuals. The small sample investigated in the current study and the small number of studies in this field indicate the need for further research. The research needs to confirm the existence of abnormalities in intestinal functioning and to define the costs and benefits of a test for screening and/or managing patients with ASD before practitioners adopt such a practice for widespread clinical and nutritional use.

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