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Relationship between adipic acid concentration and the core symptoms of autism spectrum disorders

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ABSTRACT

Dicarboxylic acids are an important source of information about metabolism and potential physiopathological alterations in children with autism spectrum disorders (ASDs). We measured the concentration between dicarboxylic adipic and suberic acids in children with an ASD and typically-developing (TD) children and analyzed any relationships between the severity of the core symptoms of ASDs and other clinical features (drugs, supplements, drugs, or diet). The core symptoms of autism were evaluated using the DSM-IV criteria, and adipic acid and suberic acid were measured in urine samples. Overall, no increase in the concentration of adipic acid in children with ASDs compared to TD children, however when considering vitamin B supplementation in ASD there were significantly increased level of urinary adipic acid in children with an ASD not taking vitamin B supplementation compared to supplemented children or to TD children. No significant difference were observed in suberic acid. Interestingly, the increase in adipic acid concentration was significantly and indirectly correlated with the severity of the deficit in socialization and communication skills in children with an ASD. Therefore, therapeutic treatments aimed at decreasing adipic acid concentration might not be beneficial for treating the core symptoms of ASDs.

1. Introduction

Autism spectrum disorder (ASD) encompasses a group of a neurobehavioral disorders characterized by abnormalities in three behavioral domains: social interaction, communication, and repetitive stereotypic behaviors; it effects approximately 1% of children and is on the rise (MMWR, 2010). There are significant genetic mechanisms underlying these disorders and research studies have uncovered several metabolic abnormalities associated with the non-*syn*dromic forms of ASD (Adams et al., 2011; Frye and Rossignol, 2014; Volkmar and Pauls, 2003). Some of these alterations seem to be relevant in terms of clinical significance and are correlated with certain clinical features observed in ASDs depending on the metabolic alteration (Horder et al., 2013; Persico and Napolioni, 2013; Puig-Alcaraz et al., 2015; Ray et al., 2011) suggesting that, rather than being a 'global' neurobiological and biochemical abnormality, some of these changes are highly specific to particular brain areas or to a subgroup of patients with an ASD (Cholemkery et al., 2016; Kim et al., 2015).

The analysis of urinary dicarboxylic acids is a very important tool used in the diagnosis of several metabolic disorders (Liebich, 1986; Touma and Charpentier, 1992; Vianey-Liaud et al., 1987), some of them associated with the *syn*dromic forms of developmental disorders (Kurian et al., 2004; Triggs et al., 1992). Recently, it has been demonstrated that the concentration of some of these acids is increased in the urine of children with an ASD (Kałużna-Czaplińska et al., 2011) thus providing new evidence for metabolic disturbances as one potential root cause for this common disorder. In particular, among the dicarboxylic acids, two of them, namely adipic and suberic acid, are produced by the omega (ω)-oxidation pathway, a pathway that metabolizes fatty acids in some species of animals, including humans (Miura, 2013; Wanders et al., 2011, for review). This is an alternative to the beta (β)-oxidation pathway in which, instead of the β carbon, the ω carbon (the carbon most distant from the fatty acid carboxyl group) is oxidized, a process which occurs mainly in the endoplasmic reticulum. This process is normally a minor catabolic pathway for medium-chain fatty acids (10–12 carbon atoms), but becomes more important when β-oxidation is defective or impaired (Miura, 2013; Wanders et al., 2011). In certain pathophysiological states, such as diabetes, alcoholism, and starvation, ω-oxidation may provide an effective means for the elimination of toxic levels of free fatty acids (Miura, 2013).

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Beta-oxidation takes place in the mitochondria and when mitochondrial metabolism is impaired, β-oxidation can also be disturbed. Many reports have shown that a subgroup of patients with mitochondrial disorder also had an ASD (Brown and Rais, 2015; Guevara-Campos et al., 2013, 2015; Marin and Saneto, 2016) and that some pathways related to mitochondrial metabolism are altered in children with an ASD even though they do not fulfil the criteria for a classical mitochondrial disorder (Filipek et al., 2004; Oliveira et al., 2005; Rossignol and Frye, 2011; Weissman et al., 2008). Given this finding we postulate that altered mitochondrial metabolism can increase the activity of omega fatty acid oxidation, thus leading to increased production of adipic and suberic acid. However this logical link remains to be proven in children with an ASD.

In this study, we measured the concentration of adipic and suberic acids in urine samples from children with non-*syn*dromic ASDs and in typically developing children (TD) age- and sex-matched children. In addition, we investigated the relationship between the concentration of these metabolites and the core symptoms of ASDs. Study of the association between metabolic disturbances and symptomatology is required to shed some light on the role and significance of biochemical alterations in ASDs, and ultimately, to identify any possible links between specific neuropsychiatric alterations and the common pathophysiological changes present in this disease (Parellada et al., 2012). Finally, identifying differential biomarkers for a single core symptom of ASDs could be very helpful in pinpointing the specific mechanisms responsible for the phenotype and may help us to tailor new pharmacological treatments for children with an ASD that have these biochemical alterations.

Therefore in this study we assessed three main objectives:

1) First, we aimed to replicate the findings reported in recent literature related to the increase in adipic and suberic acid in children with an ASD. Because vitamin B2 is recommended for decreasing the levels of urinary dicarboxylic acid in metabolic disorders associated with increased production of these acids (Brivet et al., 1991; Triggs et al., 1992), and because one study showed that combined administration of vitamin B2, vitamin B6, and magnesium reduced the concentration of these acids in the urine of children with an ASD (Kałużna-Czaplińska et al., 2011), we investigated whether children supplemented with group B vitamins have a reduced concentration of adipic and suberic acids in their urine.
2) Next we evaluated the relationship between these alterations and each of the three core symptoms of ASDs according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DMS-IV).
3) Finally we studied the influence of several confounding factors that can modulate the strength of these associations such as age, gender, past or current intestinal problems, food allergies or intolerances, sleep problems (mainly difficulty in getting to sleep or early nocturnal awakening), vitamin supplement intake, docosahexaenoic acid (DHA) supplementation, mineral intake, prescription drug intake, antiepileptic drug intake.

2. Methods

2.1. Participants

Study participants were recruited from patients attending specialized and qualified centers for psychotherapeutic intervention in children with an ASD, all located in Valencia (Spain); data were collected between September 2012 and November 2013. All these children had previously been diagnosed with an ASD (including autistic disorder and pervasive developmental disorder not otherwise specified) by an experienced psychiatrist. An ASD was confirmed using the DMS-IV diagnostic criteria during a standard neurodevelopment examination and interview. In addition, all other relatively common genetic or neurological conditions responsible for syndromic ASDs (fragile X syndrome, Prader–Willi syndrome, MECP2, and karyotype alterations) were ruled out. For each child we performed the DMS-IV interview, a medical evaluation, took their medical history, did a physical examination, and assessed their metabolic test results, recording these findings in a clinical database. The parents' signed consent for their child's participation was obtained in all cases. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee at the University of Valencia (reference code H1397475950160).

2.2. Evaluation of the core autism spectrum disorder symptoms

All children had been previously diagnosed with an ASD using the ICD-10 (tenth revision of the international statistical classification of diseases and related health problems: i.e., the DSM-IV) criteria. We used the revised autism diagnostic interview™ (ADI-R) to evaluate the core symptoms of autism, based on the DMS-IV. The first section of the interview is used to assess the quality of the child's social interaction and includes questions about emotional sharing, offering and seeking comfort, social smiling, and responding to other children. The communication and language behavioral section investigates stereotyped utterances, pronoun reversal, and social usage of language. Stereotyped utterances are the few words or sounds that the individual uses and repeats most often. The restricted and repetitive behaviors section includes questions about unusual preoccupations, hand and finger mannerisms, and unusual sensory interests. Cut off scores are 10 for social interaction; 8 for communication and language, if verbal, and 7 if non-verbal; and 3 for restricted and repetitive behaviors. This diagnostic tool has a high correlation level with the diagnostic criteria reported in the DSM-IV and ICD-10 and has good psychometric properties (96% sensitivity and 92% specificity; Lord et al., 1995).

2.3. Medical interview and evaluation

Extensive medical histories of the ASD and control children were taken, i.e., a detailed history of pregnancy and labor, Apgar scores, vaccinations, any illnesses or traumatic events, diet, body mass index, vitamin, mineral or other supplementation intake, medication, diagnosis of epilepsy, gastrointestinal or sleep problems, regression, food allergies or intolerances, and laboratory results for *syn*dromic causes of ASD (fragile X, chromosomopathy, genetic diseases, etc.).

2.4. Determination of adipic and suberic acid concentration

On the first morning urine samples were collected from 26 ASD children (aged 4–13 years) and from 23 neurologically-healthy children (aged 4–12 years). The samples were centrifuged at 13,000 rpm for 10 min at 4 °C and the resulting supernatants were immediately stored at 80 °C until analysis. The two acids were extracted from the samples and derivatized before gas chromatography/mass spectroscopy (GC/MS) analysis, according the protocol previously published by Puig-Alcaraz et al. (2015): the acids were extracted from two samples of 500 μL of urine by mixing with 500 μL methanol/chloroform (3:1) and vortexing for 30 s The samples were maintained at −20 °C for 10 min and were then centrifuged at 12,000 rpm for 10 min The supernatant was evaporated with a vacuum-drier at room temperature. The residue