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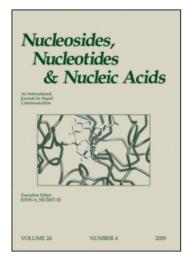
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## Urinary Methylxanthine and Autistic Disorder: Absence of Previously Reported Correlation

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## NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS Vol. 23, Nos. 8 & 9, pp. 1249–1251, 2004

# **Urinary Methylxanthine and Autistic Disorder: Absence of Previously Reported Correlation**

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#### **ABSTRACT**

We were unable to reveal significant difference in the levels of xanthine and methylxanthines in the urine samples from 59 patients diagnosed with autistic symptoms and 64 age- and sex-matched normal volunteers. Our data suggest that abnormalities in xanthine and methylxanthine excretion (US Patent 20020019406 A1, Feb. 12, 2002) represent distincly uncommon symptoms in autism.

Key Words: Theobromine; Methylxanthine; Autism; HPLC chromatography.

#### INTRODUCTION

Inherited disorders on purine biosynthesis and interconversion have been often associated with autistic symptoms, focusing the attention on the metabolic fate of endogenous and exogenous purines in patients with behaviour abnormalities. Great expectations in patient's families have arisen from recent reports<sup>[1,2]</sup> indicating that urinary methylxanthine levels are below the normal range in a significant fraction (47%) of autistic children, while xanthine levels are above the control values. Urinary

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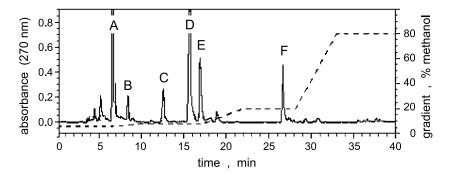


Figure 1. HPLC chromatogram of urine sample 6 hours after chocolate ingestion. Gradient elution was performed as shown (broken line). Aqueous phase: 0.05% acetic acid; organic phase: methanol. A: uric acid; B: xanthine; C: 7-methyluric acid; D: 7-methylxanthine; E: 3-methylxanthine; F: theobromine.

7-methylxanthine concentrations below the threshold level of 3.4 µg/mL have been regarded diagnostic of autistic disorder. Methylxanthine themselves or compound that are involved in the generation of xanthine have been administered to the patients to improve the symptoms of autistic disorder. The aim of this work is to assess the prevalence of abnormalities in methylxanthine excretion among Italian patients.

#### MATERIALS AND METHODS

Urine samples were collected from 59 patients diagnosed with symptoms of autistic disorder and 64 age- and sex-matched normal volunteers. The samples were diluted in equal volume of water, brought to pH 3.5, filtered through 0.2  $\mu$ m filter units (Lida Manufacturing Corp.), and injected onto a  $\mu$ -Bondapak phenyl column (3.9  $\times$  150 mm; Waters Corp.) in tandem with an Ultrasphere octyldecylsilane column (4.6  $\times$  250 mm; Beckman Instruments). Separation was performed by using the gradient profile shown in Fig. 1. Uric acid peak on the chromatograms was used as internal standard. Identification of metabolites was based on migration times and characteristic diode-array spectra.

#### RESULTS AND DISCUSSION

We found that the levels of xanthine and 7-methylxanthine in the urine samples vary greatly day by day as a result of dietary changes. If autistic patients or healthy controls were restricted to an isocaloric methylxanthine-free diet for few weeks, 7-methylxanthine content in urine became undetectable (<  $0.2 \mu g/mL$ ), suggesting that the endogenous synthesis of 7-methylxanthine was negligible in both groups of subjects. On the other hand, ingestion of 100 g chocolate (about 740 mg theobromine) resulted in a sustained increase of excretion of theobromine, 3-methylxanthine, 7-methylxanthine, and 7-methyluric acid, that reached a plateau 6-10 hours after the

meal. About 6% of the ingested theobromine was excreted within 12 hours, mainly as 7-methylxanthine. The range of 7-methylxanthine concentrations, which we found in the urine 6 hours after the ingestion of chocolate, was very similar in the autistic patients (median: 0.21 mg/mL; range: 0-0.89 mg/mL) and in the healthy controls (median: 0.20 mg/mL; range: 0-0.46 mg/mL). Urinary xanthine levels in autistic patients was comparable to those in normal controls. On the whole, our study indicate that abnormalities in methylxanthine excretion (if any) should be a noticeable rare event among Italian patients with autistic symptoms.

#### REFERENCES

- 1. Zhang, J.; Rivers, G.; Peyser, J.; Zack, B.; Horvath, K.; Rushe, J. In *Analysis of Compounds in the Urine of Autistic Children with HPLC and Mass Spectrometry*, World Congress of Pediatric Gastroenterology, Boston, MA, August 5–9, 2000.
- 2. Rusche, J.R.; Zhang, J. Methylxanthines in the Diagnosis and Treatment of Autistic Disorder. US Patent 20020019406 A1, February 12, 2002.