Food in health and disease, scientific-professional journal of nutrition and dietetics (2014) 3 (2) 71-77

IS THERE CONNECTION BETWEEN BENZOATES FROM SOFT DRINKS AND HYPERACTIVITY AMONG OSIJEK PRESCHOOL CHILDREN?

Maja Miškulin[1]*, Nika Pavlović[2], Aida Mujkić[3], Jelena Vlahović[1]

[1]University of Osijek, Faculty of Medicine, Josipa Huttlera 4, 31 000 Osijek, Croatia
[2]Institute of Public Health for the Osijek-Baranja County, Franje Krežme 1, 31 000 Osijek, Croatia
[3]University of Zagreb, School of Medicine, Andrija Štampar School of Public Health, Rockefellerova 4, 10000 Zagreb, Croatia

Original scientific paper

Summary

Introduction: Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood-onset psychiatric disorders. The impact of certain food additives, preservatives and artificial colorings on the prevalence of this disorder is still controversial and not fully explored.

Aim: To explore the possible connection between benzoates from soft drinks and hyperactivity among Osijek preschool children.

Materials and methods: This cross-sectional study was conducted during April and May 2007 in Osijek, Eastern Croatia. A special questionnaire was administered to parents of 810 preschool children from kindergartens in Osijek (aged 5.9±0.7 years; 401/810, 49.5% boys and 409/810, 50.5% girls) during April and May 2007. The questionnaire contained questions on the preschool children age, gender, weight, average daily intake of soft drinks, type of soft drink consumed, food allergy and ten-item parents Conner's index for ADHD diagnose. The concentration of benzoates in 50 commercially available soft drinks was determined by the method of high-performance liquid chromatography (HPLC) with UV detector.

Results: Among all preschool children there were only 4.9% (40/810) of them who did not drink soft drinks. The mean concentration of benzoates in all samples was 97.7±26.7 mg/L. The questionnaire revealed that 95.1% (770/810) of study subjects consuming soft drinks were taking a mean of 0.6 L of soft drink per day, containing 58.6 mg of benzoates. Among those who did drink soft drinks there were 8.6% (66/770) of them positive for ADHD and none in group who did not.

Conclusion: ADHD is more common in the group of children who consume soft drinks and in that way intake larger amount of benzoates. It is not clear do these benzoates produce ADHD or just further facilitate ADHD that already exists. Further investigations are needed.

Key words: benzoates, soft drinks, hyperactivity, preschool children, Croatia.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common behavioral and neurodevelopmental disorder which is characterized by hyperactivity, impulsivity, and inattention in children and adolescents (Linnet et al., 2003). Prevalence of ADHD among school-aged children in different studies varies between 5 and 12% (Linnet et al., 2003; Grizenko et al., 2008) and the ADHD worldwide-pooled prevalence was estimated to be 5.29% (Polanczyk et al., 2007). It has also been stated that the prevalence of this disorder declines with increasing age (Golmirzaei et al., 2013). Concerning the preschool children there are only few studies dealing with the prevalence of this disorder in this age group and they showed prevalence of this disorder ranging from 4.3% to 31.1% (Ardalan et al., 2002; Hebrani et al., 2007; Soma et al., 2009; Meysamie et al., 2011). It is interesting that the results of majority of these studies indicate a marked difference in the prevalence of ADHD symptoms when the evaluation was performed by parents compared to teachers; the prevalence was consistently higher in the parent survey (Soma et al., 2009; Meysamie et al., 2011).

Although, the pathogenesis of ADHD is still un-

*Corresponding author: maja.miskulin@mefos.hr

known, primary and secondary factors are estimated to be implicated in ADHD pathogenesis. Primary roles are shaped in the cerebral cortex by catecholamine metabolism. Also, etiology of ADHD is attributed to genetic factors in about 80% (Golmirzaei et al., 2013). The secondary roles are created by various environmental factors (Pliszka, 2007; Millichap, 2008). Some of these factors, which are associated with ADHD, are pregnancy and birth related risk factors which are classified into three groups including prenatal, perinatal, and postnatal risk factors. Regarding prenatal risk factors, a large number of studies have shown that maternal exposure to alcohol, tobacco, and cocaine during pregnancy increases the risk of ADHD. On the other hand, some studies showed that prenatal viral infections are associated with increased risk of ADHD (Mann and McDermott, 2011; Golmirzaei et al., 2013). Various studies have demonstrated that preeclampsia, maternal anemia, lower serum level of iron and iodine, and trauma to abdomen during pregnancy are associated with increased risk of ADHD development (Mann and McDermott, 2011; Amiri et al., 2012). Regarding perinatal risk factors, a number of risk factors such as prematurity, low birth weight, and breech delivery are estimated to be associated with increased risk of ADHD (Millichap, 2008). Postnatal risk factors include postnatal viral infections such as measles, varicella, and rubella increasing the risk of developing ADHD (Millichap, 2008). Additionally, several other factors such as breast-feeding, head injury in early childhood and adolescence, encephalitis, convulsion and endocrine disorder are estimated to be risk factors for development of ADHD (Millichap, 2008; Golmirzaei et al., 2013). In addition to the mentioned factors, several sociodemographic factors such as maternal education, family income, male gender, and maternal age at pregnancy are known to be predictive factors for developing ADHD (Millichap, 2008; Amiri et al., 2012). Considering the socioeconomic status it has been concluded that although genetic and neurological determinants may be the primary predictors of the development of ADHD in a child, etiology appears to be influenced by socioeconomic situation of his/her family (Russell et al., 2014).

Under European regulation (EC) No. 1333/2008, food additives are defined as any substances 'not normally consumed as food itself' which are added to a food to perform a technological purpose e.g. preservation (European Commission, 2008). There are twenty-six categories of food additives outlined in this regulation, which fall broadly into two main categories depending on their purpose (1) safety and prevention of degradation of food by bacteria, oxidation or chemical reactions or (2) improvement of the taste, appearance or mouth-feel of the product (Martyn et al., 2013).

Benzoic acid and its salts (benzoate) may naturally be present in food, but with their antimicrobial properties they have a long history of use as food preservatives (Lazarević et al., 2011). Their use as food additives was limited by Joint FAO/WHO Expert Committee on Food Ad¬ditives (JECFA). According to JECFA acceptable daily intakes (ADI) of 0–5 mg/kg body weight for benzoic acid and benzoates have been established (WHO, 1997). Some studies suggested that very high intake of benzoic acid can cause adverse health effects such as metabolic acidosis, hyper¬pnoea and convulsions (WHO, 1997). Few epidemiologic studies reported allergic reactions to benzoic acid and benzoate (urticaria, rinhitis and pruritis) (Nettis et al., 2004; Asero, 2006; Lazarević et al., 2011). Among other products, as widely used food preservatives, benzoates are often used in soft drinks, such as carbonated drinks and various fruit juices. Soft drinks are likely to be the major contributing factor to the intake of benzoates for young children, due to the high levels of consumption of these products by this age group (Food Standards Agency, 2008).

In a recent literature, one of the most frequently investigated potential hazards associated with preschool children and food additives, is the influence of these substances on development of ADHD (Martyn et al., 2013). Until today, there were several studies that have dealt with the issue of the food additives intake and prevalence of ADHD in children but the impact of these substances on the prevalence of this disorder is still controversial and not fully explored because

the results of aforementioned studies are contradictory (Rowe and Rowe, 1994; Bateman et al., 2004; McCann et al., 2007; Connolly et al., 2010; Lok et al., 2013).
The aim of this study was to explore the possible connection between benzoates from soft drinks and hyperactivity among Osijek preschool children.

Materials and Methods

This cross-sectional study was conducted during April and May 2007, among parents of preschool children attending kindergartens in Osijek (Eastern Croatia). Participation in the study was voluntary, and the study was approved by the Ethics Committee of the Institute of Public Health for the Osijek-Baranja County. A total number of 1 378 specially designed anonymous questionnaires were delivered randomly to parents of preschool children attending kindergartens in Osijek. The overall response rate was 58.8% (810/1378), and all of the 810 completed questionnaires, were included in further statistical analyses. The final sample size consisted of 810 preschool children mean age 5.9±0.7 years, 49.5% (401/810) of boys and 50.5% (409/810) of girls.

Questionnaire

The anonymous questionnaire contained questions on the preschool children age, gender, weight, average daily intake of soft drinks, type of soft drink consumed, food allergy and ten-item Conner's abbreviated behavior rating scale for the ADHD diagnose. This ten-item is considered to be reliable instrument for screening purposes performed by parents in order to establish the existence of ADHD in a child (Gross-Tsur et al., 2006). The index consists of ten statements regarding the child's behavior and parents are supposed to express their agreement with the statements using the 4-point Likert scales (ranging from 0 for not at all true to 3 for very much true). The ADHD total score of 16 or above is considered indicative for existence of ADHD in the child.

Analytical methodology

The most popular and most widely available 50 soft drinks in Croatia were collected in grocery stores in the Osijek area. The concentration of benzoates (E210-E213) i.e. benzoic acid (E210), sodium benzoate (E211), potassium benzoate (E212) and calcium benzoate (E213), in 50 samples of commercially available soft drinks was determined by the method of high-performance liquid chromatography (HPLC) with UV detector on a Varian instrument (Walnut Creek, USA, 1993) (Varian Associates Inc, 1993) with a UV/VIS Star 9050 detector and Star 9012 pump. The method was standardized by AOAC; 979.08; Edition 18, Vol.2; Chapter 29. Standard solutions of benzoic acid (dr. Ehrenstorfer) were also prepared in accordance with the same norm.
Operating chromatography conditions were: room temperature; Zorbax C-18 column 5 μL, 150 mm x 4.6 mm, with Superguard LC-18 precolumn (20 mm x 4.6 mm); mobile phase: 20% acetic acid buffered to pH 3 with saturated solution of sodium acetate; flow rate 2 mL/min; wavelength 254 nm; injection volume 20 μL, and limit of detection 2.53 mg/L.
Soft drink samples were homogenized and degassed on „Branson 1210" ultrasonic bath, filtered through a 0.45-μm syringe filter and injected into the system.

Statistical analysis

Statistical analysis included data obtained by the laboratory analysis of soft drinks and data collected through the anonymous questionnaires. Normality of data distribution was tested by the use Kolmogorov-Smirnov test. All data were processed by the methods of descriptive statistics. The proportions were calculated and compared by the use of Fisher's exact test. Spearman's correlation coefficient was also calculated, to test the correlation between the amount of daily consumed soft drink and the presence of ADHD symptoms in a group of children who drank these drinks. $P<0.05$ was considered statistically significant. Statistical analysis was done by the SPSS Statistical Package for Windows, version

13.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The study sample consisted of 810 preschool children mean age 5.9±0.7 years (range 5.0-7.5 years), 49.5% (401/810) of boys and 50.5% (409/810) of girls attending the kindergartens in Osijek (Eastern Croatia). The mean weight of all children was 22.6±4.2 kg, ranging 13.0 to 60.0 kg.

Among all preschool children there were 95.1% (770/810) who drank soft drinks and 4.9% (40/810) of those who did not drink them at all. When looking the amount of daily consumed soft drinks among subjects who drank soft drinks it was discovered that this amount ranged from 0 L to 1.0 L daily, with 73.4% (565/770) of preschool children consuming the amount of 0.5 L or above (Table 1). The mean amount of daily consumed soft drink among children who drank soft drinks was 0.6 L.

Table 1. Preschool children from Osijek (Eastern Croatia) who drank soft drinks according to the daily consumed amount of soft drink and daily intake of benzoates via soft drink

Daily consumed amount of soft drink	Daily intake of benzoates via soft drinks	Preschool children from Osijek who consumed soft drinks N (%)
0 L	0 mg	20 (2.6)
0.20 L	19.5 mg	185 (24.0)
0.50 L	48.9 mg	208 (27.0)
0.75 L	73.3 mg	179 (23.3)
1.00 L	97.7 mg	178 (23.1)
Total		**770 (100.0)**

The results of the questionnaire showed that the preschool children from Osijek had most frequently consumed non-carbonated fruit juices (52.5%), ice teas (30.3%), coke (coca-cola) (12.1%), carbonated soft drink with orange flavor (3.4%) and at least other carbonated soft drinks (1.8%).

The mean concentration of benzoates in all samples of soft drinks was 97.7±26.7 mg/L. When calculating the amount of benzoates that children intake considering the amount of consumed soft drink it is evident that mean value of benzoates is 57.5±29.8 mg, ranging from 0 mg to 97.7 mg daily, depending on the amount of daily consumed soft drink. Considering that questionnaire revealed how 95.1% (770/810) of study subjects consuming soft drinks were taking a mean of 0.6 L of soft drink per day, it can be calculated that mean daily intake of benzoates via soft drinks among 95.1% of study population is 58.6 mg (Table 1).

According to the mean body weight of all children (22.6 kg) and mean value of benzoates in all soft drinks (57.5 mg) it is further evident that mean daily intake of benzoates in study population is 2.5 mg/kg body weight (50.0% of ADI), ranging from 0 to 4.3 mg/kg body weight (86.0% of ADI), also depending on the amount of daily consumed soft drink.

Among preschool children who drank soft drinks there were 8.6% (66/770) of them positive for ADHD and none (0%; 0/40) in group who did not drink them and this difference was statistically significant (Fisher's exact test; P=0.031).

When looking the children with ADHD symptoms according to the amount of daily consumed soft drink the study has showed that the number of children with the ADHD symptoms grew in parallel with the daily consumed amount of soft drink. Spearman rank correlation between the amount of daily consumed soft drink and the presence of ADHD symptoms in a group of children who drank these drinks was r=0.130; p=0.000 (Table 2).

Table 2. Preschool children from Osijek (Eastern Croatia) with ADHD symptoms according to the amount of daily consumed soft drink

The amount of daily consumed soft drink	Presence of the ADHD symptoms		Total
	No N (%)	Yes (%)	
0 L	20 (100.0)	0	20 (2.6)
0.20 L	178 (96.2)	7 (3.8)	185 (24.0)
0.50 L	192 (92.3)	16 (7.7)	208 (27.0)
0.75 L	159 (88.8)	20 (11.2)	179 (23.3)
1.00 L	155 (87.1)	23 (12.9)	178 (23.1)
Total	704 (91.4)	66 (8.6)	770 (100.0)

This study revealed that soft drinks are important source of benzoate intake among preschool children from Eastern Croatia, because 95.1% of children who drank those drinks in mean amount of 0.6 L daily through those drink intake around 2.6 mg/kg body weight of benzoates daily or 52.0% of ADI of benzoates. This finding confirms the fact that due to the high levels of consumption of these products by this age group, soft drinks are likely to be the major contributing factor to the intake of benzoates for young children, (Food Standards Agency, 2008). Also, this finding confirms results of some studies that have suggested that preschool children may have increased exposure and consumption of certain food additives, in comparison with adults and are therefore an important subgroup that should be addressed by exposure assessments (Goldman and Koduru, 2000; Huybrechts et al., 2011; Martyn et al., 2013). Generally speaking, considering the dietary habits, especially range of foodstuffs consumed during childhood it has been concluded that preschool children are likely to be the population group with the highest exposure to chemicals in the diet (Lawrie, 1998). When assessing the exposure of children to food additives, and other chemicals in diet, it is very important to bear in mind that because of some factors children are more vulnerable to their negative effects in relation to adults (Martyn et al., 2013). Young children may be more vulnerable than adults to chemical exposures as a result of their immature organ systems, rapid physical development and higher metabolic rates (Wilson et al., 2007). Furthermore, metabolic processes may differ between children and adults as, per kilogram body weight, smaller organs usually need more oxygen and nutrients than adult organs (Ginsberg et al., 2004). Finally, the distribution and absorption of chemicals throughout the body can differ, e.g. water-soluble substances are distributed over a relatively greater volume within the body of a child in comparison with that of an adult and can penetrate more easily into tissues and organs from the bloodstream (Martyn et al., 2013).

Considering the influence of benzoate intake on a children behavior this study points to the possible connection between the intake of benzoates through soft drinks and presence of the symptoms of ADHD among Osijek preschool children because ADHD was more common in the group of children who consume soft drinks and in that way intake larger amount of benzoates. This finding is in compliance with the results of studies conducted in the United Kingdom (Bateman et al., 2004; McCann et al., 2007), but is in contrast to the results of studies conducted in Ireland and Hong Kong, China (Connolly et al., 2010; Lok et al., 2013). This study also showed that the number of children with the ADHD symptoms grew in parallel with the daily consumed amount of soft drink, which further supports the existence of possible connection between the observed variables.

However, the definite conclusion cannot be drawn because of possible confounding factors that one should take into account when evaluating the results of this study. First of all, the evaluation of the existence and severity of ADHD symptoms among children was done by parents through

ten-item Conner's abbreviated behavior rating scale for the ADHD diagnose and studies dealing with the ADHD among preschool children showed a marked difference in the prevalence of ADHD symptoms when the evaluation was performed by parents compared to teachers with the prevalence's in the parent surveys being consistently higher (Soma et al., 2009; Meysamie et al., 2011). There are several possible explanations for this discrepancy. For example, parents may expect their children to be well-behaved and obedient, thus evaluating their behavior more strictly. Also, parents of preschool children may have difficulty determining whether a child's behavior is abnormal, thus resulting in more false positives in the parent survey (Soma et al., 2009; Meysamie et al., 2011). Second potential limitation of this study is an absence of questions regarding all possible risk factors for ADHD besides the intake of food additives such as genetic factors and other environmental factors, because it is possible that children who scored positive for ADHD were more prone to the development of ADHD symptoms due to some preexisting risk factors in their personal or family history that were not taken into account during this study. Finally, since the study is designed as a cross-sectional study, we cannot draw definitive conclusions about the established cause-effect relationships between the intake of benzoates through soft drinks and presence of the symptoms of ADHD among Osijek preschool children.

Conclusions

ADHD is more common in the group of children who consume soft drinks and in that way intake larger amount of benzoates. It is not clear do these benzoates produce ADHD or just further facilitate ADHD that already exists. Further investigations are needed.

Literature

1. Amiri S, Malek A, Sadegfard M et al (2012) Pregnancy-related maternal risk factors of attention-deficit hyperactivity disorder: a case-control study. ISRN Pediatr 2012:458064
2. Ardalan G, Farhud D, Shahmohammadi D (2002) Hyperactivity, attention and concentration deficit in preschool children. Iran J Pediatr 12:53–56
3. Asero R (2006) Sodium benzoate-induced pruritus. Allergy 61:1240–1241
4. Bateman B, Warner J, Hutchinson E et al (2004) The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch Dis Child 89:506–511
5. Connolly A, Hearty Á, Nugent A et al. (2010) Pattern of intake of food additives associated with hyperactivity in Irish children and teenagers. Food Add Contam 27:447–456
6. European Commission (2008) Regulation (EC) No. 1333/ 2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives. Off J Eur Union L 365.31.12.2008, p. 16 365/16
7. Food Standards Agency (2008) Survey of benzoates and sorbates in soft drinks. Food Survey Information Sheet No. 06/08. London, Food Standards Agency
8. Ginsberg G, Hattis D, Miller R et al (2004) Paediatric pharmacokinetic data: implications for environmental risk assessment for children. Pediatrics 113:973–983
9. Goldman LR, Koduru S (2000) Chemicals in the environment and developmental toxicity to children: a public health and policy perspective. Environ Health Perspect 108:443S
10. Golmirzaei J, Namazi S, Amiri S et al (2013) Evaluation of attention-deficit hyperactivity disorder risk factors. Int J Pediatr 2013:953103
11. Grizenko N, Shayan YR, Polotskaia A et al (2008) Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. J Psychiatry Neurosci 33:10–16
12. Gross-Tsur V, Goldzweig G, Landau YE et al (2006) The impact of sex and subtypes on cognitive and psychosocial aspects of ADHD. Dev Med Child Neurol 48:901–905
13. Hebrani P, Abdolahian E, Behdani F et al (2007) The prevalence of attention deficit hyperactivity disorder in preschool-age children in Mashhad, North-East of Iran. Arch Iran Med 10:147–151
14. Huybrechts I, Sioen I, Boon PE et al (2011) Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale, methods and design. Arch Public Health 69:1–12
15. Lawrie C (1998) Different dietary patterns in relation to age and the consequences for intake of

food chemicals. Food Add Contam 15:75–81
16. Lazarević K, Stojanović D, Rančić N (2011) Estimated daily intake of benzoic acid through food additives in adult population of South East Serbia. Cent Eur J Public Health 19:228–231
17. Linnet KM, Dalsgaard S, Obel C et al (2003) Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. Am J Psychiatry 160:1028 –1040
18. Lok KY, Chan RS, Lee VW et al (2013) Food additives and behavior in 8- to 9-year-old children in Hong Kong: a randomized, double-blind, placebo-controlled trial. J Dev Behav Pediatr 34:642–650
19. Mann JR, McDermott S (2011) Are maternal genitourinary infection and pre-eclampsia associated with ADHD in school-aged children? J Atten Disord 15:667–673
20. Martyn DM, McNulty BA, Nugent AP et al (2013) Food additives and preschool children. Proc Nutr Soc 72:109–116
21. McCann D, Barrett A, Cooper A et al (2007) Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet 370:1560–1567
22. Meysamie A, Fard MD, Mohammadi MR (2011) Prevalence of Attention-Deficit/Hyperactivity Disorder Symptoms in Preschool-aged Iranian Children. Iran J Pediatr 21:467–472
23. Millichap JG (2008) Etiologic classification of attention-deficit/hyperactivity disorder. Pediatrics 121:e358–e365
24. Nettis E, Colanardi MC, Ferrannini A et al (2004) Sodium benzoate-induced repeated episodes of acute urticaria/angio-oedema: randomized controlled trial. Br J Dermatol 151:898–902
25. Pliszka S (2007) Practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 46:894–921
26. Polanczyk G, de Lima MS, Horta BL et al (2007) The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 164:942–948
27. Rowe KS, Rowe KJ (1994) Synthetic food colouring and behaviour: a dose response effect in a double-blind, placebocontrolled, repeated-measures study. J Paediatr 125:691–698
28. Russell G, Ford T, Rosenberg R et al (2014) The association of attention deficit hyperactivity disorder with socioeconomic disadvantage: alternative explanations and evidence. J Child Psychol Psychiatry 55:436–445
29. Soma Y, Nakamura K, Oyama M et al (2009) Prevalence of attention-deficit/hyperactivity disorder (ADHD) symptoms in preschool children: discrepancy between parent and teacher evaluations. Environ Health Prev Med 14:150–154
30. Varian Associates Inc (1993) Varian chromatography systems: Operator's Manual. Walnut Creek, Varian Associates Inc
31. Wilson NK, Chuang JC, Morgan MK et al (2007) An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. Environ Res 103:9–20
32. World Health Organization (1997) Evaluation of certain food additives: forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 868. WHO, Geneva