

CLINICAL STUDY

Plasma concentrations of selected antioxidants in autistic children and adolescents

Krajcovicova-Kudlackova M¹, Valachovicova M¹, Mislanova C¹, Hudecova Z¹, Sustrova M¹, Ostatnikova D²

Slovak Medical University, Bratislava, Slovakia. marica.kudlackova@szu.sk

Abstract: Few studies have demonstrated an increased vulnerability to oxidative stress in autism. The results of previous studies have shown that endogenous antioxidant defence is insufficient, indicating that exogenous antioxidant could play a crucial role for oxidative stress prevention in autism. Plasma concentrations of vitamins C, E, A, carotenoids β -carotene and lycopene were measured in 51 subjects with autistic spectrum disorders aged 5–18 years (27 children aged 5–10 years, 24 subjects aged 11–18 years). Older autistic group was compared with a group of healthy Slovak subjects aged 11–18 years. Older autistic subjects vs. healthy control showed significantly higher vitamin C and β -carotene plasma values with 92 % and 71 % vs 54 % and 13 % of optimal over-threshold values, respectively. This indicates a reduced risk of free radical disease. In younger vs. older autistic group the similarly high plasma vitamin concentrations were recorded. Favourable values of these vitamins suggested that consumption of fruit and vegetables in autistic subjects is optimal. Autistic average vitamin E and A plasma concentrations (non-significantly changed in comparison to control group) were below-threshold with low percentage of over-threshold values. Insufficient vitamin E and A plasma values indicate lower consumption of food rich in vitamins A and E (e.g. whole-grain products, plant oils, oil seeds, nuts, fat spreads and dairy products). Autistic average lycopene concentration is lower in comparison to published non-Slovak data. Conclusions of this pilot study suggest that plasma concentrations of exogenous antioxidants, vitamins E and A, and lycopene in autistic subjects are insufficient (*Tab. 1, Ref. 30*). Full Text (Free, PDF) www.bmj.sk.
Key words: autism, vitamins C, E, A, β -carotene, lycopene, children, adolescents.

Autism spectrum disorders (ASDs) are pervasive neurodevelopmental disorders influenced by genetic and environmental factors and characterized by three core symptom domains: speech and communication abnormalities, social functioning impairments and repetitive behaviours and restricted interests (Eigsti and Shapiro, 2003; James et al, 2004; Deth et al, 2008; Theoharides et al, 2008). Recent insights suggest that ASDs are disorders with genetic causes and a variety of genetic mechanisms may be involved, i.e. single gene disorders, copy number variations and polygenic mechanisms (Steyaert and De La Marche, 2008). Results of clinical research express that ASDs may be multi-system disorders affecting the gastrointestinal tract, the immune system and perhaps other systems (White, 2003; Sweeten et al, 2003). Oxidative stress, overactivation of the hypothalamic-pituitary-adrenal axis and increased gut-blood-brain-barrier permeability might be involved (White, 2003; Chauhan and Chauhan, 2006; Theoharides et al, 2008).

In children with autism, the vulnerability to oxidative stress was found to be increased and the capacity for methylation de-

creased (James et al, 2004; Kern and Jones, 2006; Geier and Geier, 2006; Deth et al, 2008). Relative to control children, the children with autism had a significantly lower baseline concentration of methionine, homocysteine, cystathionine, cysteine, total glutathione, reduced glutathione and significantly higher concentrations of S-adenosylmethionine, adenosine and oxidized glutathione. This metabolic profile is consistent with the impaired capacity for methylation and increased oxidative stress by significantly lower redox ratio of reduced glutathione to oxidized glutathione. Methionine synthase activity is required for dopamine-stimulated phospholipid methylation, a unique membrane-delimited signalling process mediated by the D4 dopamine receptor that promotes neuronal synchronization and attention, and synchrony is impaired in autism. Genetic polymorphisms adversely affecting sulphur metabolism, methylation, detoxification, dopamine signalling and the formation of neuronal networks occur more frequently in autistic subjects (Deth et al, 2008).

Several studies have suggested that increased oxidative stress testified by measuring the lipid peroxidation, detoxifying agents, antioxidant concentrations might have a pathological role in autism (McGinnis, 2004; Chauhan et al, 2004; Ming et al, 2005; Chauhan and Chauhan, 2006). Impaired antioxidant mechanisms are unable to inactivate free radicals that may induce a number of pathological processes. Thus, any abnormality in antioxidant defence systems could affect neurodevelopmental processes and

¹Slovak Medical University, Bratislava, and ²Faculty of Medicine of Comenius University, Bratislava, Slovakia

Address for correspondence: M. Kudlackova, PhD, DSc, Slovak Medical University, Limbova 12, SK-833 03 Bratislava 37, Slovakia.

Tab. 1. Group characteristics and plasma concentrations of antioxidants.

| | Autistic subjects | | Healthy subjects ^(A,B) |
|-----------------------------------|-------------------|----------------|-----------------------------------|
| | 5–10 y | 11–18 y | 11–18 y |
| n (boys+girls) | 27 (24±3) | 24 (22±3) | 77 (32±45) |
| average age (y) | 7.9±0.3 | 14.8±0.5 | 15.0±0.2 |
| vitamin C (µmol/l) | 70.9±3.9 | 75.3±2.9*** | 53.2±2.9 |
| >50 ^c | 89 % | 92 % | 54 % |
| β-carotene (µmol/l) | 0.771±0.096 | 0.725±0.081*** | 0.213±0.021 |
| >0.4 ^c | 78 % | 71 % | 13 % |
| vitamin E (µmol/l) | 18.4±1.2 | 20.6±1.2 | 22.2±0.7 |
| >30 ^c | 7 % | 4 % | 9 % |
| cholesterol (mmol/l) | 4.24±0.12 | 4.07±0.15*** | 4.86±0.16 |
| vitamin E/cholesterol (µmol/mmol) | 4.37±0.29 | 5.12±0.20* | 4.61±0.12 |
| >5.2 ^c | 26 % | 50 % | 23 % |
| vitamin A (µmol/l) | 1.37±0.05 | 1.54±0.08 | 1.58±0.04 |
| >2.2 ^c | 0 % | 4 % | 6 % |
| lycopene (µmol/l) | 0.347±0.048 | 0.324±0.042 | – |
| >0.45 ^d | 19 % | 25 % | – |

The results are expressed as mean±SEM, * – autistic vs healthy subjects aged 11–18 y, * p<0.05, *** p<0.001, ^A– Krajcovicova-Kudlackova et al (1997), ^B– Bederova et al (1997), ^c – limits for reduced risk of free radical disease by Gey (1995), ^d – average plasma value by Ford et al (2002)

could have an important role in the etiology of autistic disorder (Yorbik et al, 2002). A few autistic studies about the values of endogenous enzymatic or non-enzymatic antioxidants (superoxide dismutase - glutathione peroxidase – catalase activities, transferrin, ceruloplasmin) are published (Zoroglu et al, 2004; Chauhan et al, 2004; Chauhan and Chauhan, 2006). Evaluations of exogenous antioxidants or benefits from nutritional antioxidant interventions in autism are rare (McGinnis, 2004).

The main goal of this pilot study was to assess the plasma concentrations of antioxidative vitamins in autistic subjects as well as to compare these values with non-autistic subjects or with vitamin limits for reduced risk of free radical disease.

Subjects and methods

Randomly selected group of autistic subjects consisted of 27 children aged 5–10 years, 13 children aged 11–14 years and 11 adolescents aged 15–18 years. Diagnoses (by documentations of pediatricians): 36 autism, 6 atypical autism, 9 Asperger syndrome. Patients were divided into two groups with respect to recommended dietary allowances (RDAs) for antioxidative vitamins in dependence to age: 27 subjects aged 5–10 years, 24 subjects aged 11–18 years (RDAs, 1997). Intakes of vitamins, mineral and trace elements only in natural form were considered. Supplemented patients were excluded.

Blood was sampled after an overnight fasting using a standard procedure. EDTA was used as an anticoagulant. Concentrations of vitamins C, E, A and carotenoids (β-carotene, lycopene) were detected by HPLC methods (Hess et al, 1991, Cerhata et al, 1994). Concentrations of total cholesterol were measured by a standard laboratory method using Vitros 250 autoanalyzer (Johnson & Johnson, USA).

Results and discussion

In 33 children with autism vs. 29 healthy controls was measured significantly higher 8-isoprostane-F2α urinary excretion (Ming et al, 2005). Increased malondialdehyde plasma concentrations were detected in autistic children vs. non-autistic siblings (Chauhan et al, 2004). In 27 patients with autism vs. 26 normal controls increased thiobarbituric acid-reactive substance concentrations were found (Zoroglu et al, 2004). The lower redox ratio of reduced glutathione to oxidized glutathione was found in autistic children vs. non-autistic controls (James et al, 2004; Deth et al, 2008). Membrane phospholipids, the prime target of reactive oxygen species, are also altered in autism. The concentrations of phosphatidylethanolamine are decreased, and phosphatidylserine concentrations are increased in the erythrocyte membrane of children with autism vs. their healthy siblings (Chauhan and Chauhan, 2006). In group of 45 autistic children vs. 41 normal controls, activities of erythrocyte superoxide dismutase, erythrocyte and plasma glutathione peroxidase were significantly lower (Yorbik et al, 2002). The authors conclude: if similar abnormalities are present in brain, free radical accumulation could damage brain tissue. Concentrations of major antioxidant proteins namely, transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein) in serum were significantly reduced in autistic children vs. non-autistic siblings (Chauhan et al, 2004). There was a positive correlation between reduced concentrations of these proteins and loss of previously acquired language skills in children with autism.

An improved antioxidant status helps to minimize the oxidative damage and thus to delay or prevent pathological changes. Lines of defense include enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase), non-essential endogenous an-

tioxidants (glutathione, uric acid, proteins, ubiquinol 10 etc.) and essential radical scavengers (vitamins C, E, A and carotenoids – vitamin A precursor β -carotene and non-vitamin A precursor lycopene). In our previous studies the significantly reduced products of oxidative damage to DNA, lipids and proteins in healthy subjects were found at conditions of optimal over-threshold plasma antioxidant vitamin concentrations (over-threshold = reduced risk of free radical disease) (Gey, 1995) vs below-threshold insufficient plasma vitamin concentrations (Krajcovicova-Kudlackova et al, 2004, 2006, 2008).

Table 1 introduces plasma values of antioxidative vitamins C, β -carotene, E, E/cholesterol, A and carotenoid lycopene in 24 children with autism aged 5–10 years and in 27 autistic children and adolescents aged 11–18 years. Results of the second group are compared with healthy subjects at the same age, sole published results of antioxidant vitamin concentrations for Slovak subjects aged 11–18 year (Krajcovicova-Kudlackova et al, 1997; Bederova et al, 1997). In both, younger and older patients were measured high concentrations of vitamin C and β -carotene as vitamins derived from fruit and vegetables. Optimal concentrations for reduced free radical disease risk were found in 89 and 92 % of autistic subjects for vitamin C as well as in 78 and 71 % for β -carotene vs only 54 % and 13 % for vitamin C and β -carotene in controls. Epidemiologic data suggest that persons with diets rich in fruit and vegetables are at lower risk of several chronic diseases than are persons with diets poor in fruit and vegetables. The hazardous ratio for the highest vs. the lowest quartile of consumption was 0.74 for vitamin C, 0.68 for β -carotene and 0.65 for lycopene (Agudo et al, 2007). No autistic subject vs 10.4 % of control subjects has vitamin C plasma concentration $\leq 23 \mu\text{mol/l}$, which is indicated as an inadequate value (Kim et al, 2006).

Autistic E and A vitamins plasma concentrations are below the optimal threshold value of free radical disease prevention. These vitamin concentrations are similar or non-significantly lower to those in control group of healthy subjects. Over-threshold values for vitamin E were found only in 7 % and 4 % of autistic subjects in two groups vs 9 % in controls. Autistic vitamin A plasma values with a sufficient antioxidant effect were recorded in 0 % and 4 % of subjects of two groups vs in 6 % of controls. Inadequate values $\leq 12 \mu\text{mol/l}$ of vitamin E (Kim et al, 2006) were expressed in 4 patients vs 1 control subject.

Important antioxidant parameter indicating a protection of LDL against oxidation is molar ratio of vitamin E/cholesterol (Gey, 1995). Effective protection is recorded by vitamin E/cholesterol value $\geq 5.2 \mu\text{mol/mmol}$. In older autistic group vs control group vitamin E/cholesterol is significantly higher with 50 % of protective values vs 23 % in control subjects. In younger autistic group vs control group the calculated parameter is non-significantly lower. The favourable value of protective parameter for LDL in older autistic children and adolescents is a consequence of low cholesterol concentration (2.46–5.18 mmol/l). Tierney and co-authors (2006) published that 19 subjects with autism from 100 had their cholesterol concentrations lower than 2.58 mmol/l, which are below the 5th centile for children over 2

years. The findings suggest that in addition to Smith-Lemli-Opitz syndrome that can be associated with autism (Tierney et al, 2001) there may be other disorders of sterol metabolism or homeostasis associated with ASDs.

Distribution of plasma lycopene concentration in 4231 subjects aged 6–16 years was recorded in a wide range of 0.02–1.71 $\mu\text{mol/l}$ (Ford et al, 2002) or 0–1.51 $\mu\text{mol/l}$ in 129 subjects aged 1–18 years (Lenhartz et al, 1997). Lycopene, a more effective scavenger of free radicals than other carotenoids, is derived above all from tomatoes and tomato products. Autistic plasma lycopene concentrations are lower than the average plasma value from 4231 US children and adolescents (Ford et al, 2002). Lycopene concentration $> 0.45 \mu\text{mol/l}$ was found in 19 and 25 % of autistic subjects in two groups.

As is introduced above, a few studies demonstrated the presence of oxidative stress in autism. The results of other studies shown an insufficient endogenous antioxidant defence (lower serum proteins with antioxidative activity, lower antioxidant enzymes activities). Therefore, the crucial role of exogenous antioxidants can be indicated for prevention of oxidative stress in autism. The favourable plasma vitamin C and β -carotene concentrations (over-threshold limit for a reduced free-radical disease risk) suggest that the consumption of fruit and vegetables in autistic subjects is optimal. Vitamin E and A plasma concentrations below the threshold limit indicate lower consumption of food rich in vitamins (whole-grain products, plant oils, oil seeds, nuts, fat spreads, dairy products). Autistic average lycopene concentrations are lower in comparison to published non-Slovak data. These results suggest that plasma concentrations of exogenous antioxidants (vitamins E and A, and lycopene) in autistic subjects are insufficient and thus can impair the oxidative stress prevention.

References

1. Agudo A, Cabrera L, Amiano P, Ardanaz E, Barricarte A, Bereguer T, Chirlaque MD, Dorronsoro M, Jakszyn P, Larranaga N, Martinez C, Navarro C, Quirós JR, Sánchez MJ, Tormo MJ, González CA. Fruit and vegetable intake, dietary antioxidant nutrients, and total mortality in Spanish adults. *Amer J Clin Nutr* 2007; 85: 1634–1642.
2. Bederova A, Kudlackova M., Simoncic R, Grancicova E, Magalova T, Brtkova A. Total antioxidant status and antioxidative vitamins in selected group of children and adolescents. *Hygiena* 1997; 42: 92–98.
3. Cerhata D, Bauerova A, Ginter E. Ascorbic acid determination in serum by high performance liquid chromatography and its correlation with spectrophotometric determination. *Ces Slov Farm* 1994; 43: 166–168.
4. Chauhan A, Chauhan V, Brown WT, Cohen I. Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin – the antioxidant proteins. *Life Sci* 2004; 75: 2539–2549.
5. Chauhan A, Chauhan V. Oxidative stress in autism. *Pathophysiology* 2006; 13: 171–181.
6. Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. How environmental and genetic factors combine to cause autism: a redox/methylation hypothesis. *Neurotoxicology* 2008; 29: 190–201.

7. **Eigsti IM, Shapiro T.** A systems neuroscience approach to autism: biological, cognitive, and clinical perspectives. *Ment Retard Dev Disabil Res Rev* 2003; 9: 205–215.
8. **Ford ES, Gillespie C, Ballew C, Sowell A, Mannino DM.** Serum carotenoid concentrations in US children and adolescents. *Amer J Clin Nutr* 2002; 76: 818–827.
9. **Geier DA, Geier MR.** A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. *Horm Res* 2006; 66: 182–188.
10. **Gey KF.** Ten year retrospective on the antioxidant hypothesis of arteriosclerosis, threshold plasma levels of antioxidant nutrients related to minimum cardiovascular risk. *J Nutr Biochem* 1995; 6: 206–236.
11. **Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W.** Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int J Vitam Nutr Res* 1991; 61: 232–238.
12. **James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrandner JA.** Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Amer J Clin Nutr* 2004; 80: 1611–1617.
13. **Kern JK, Jones AM.** Evidence of toxicity, oxidative stress, and neuronal insult in autism. *J Toxicol Environ Health B Crit Rev* 2006; 9: 485–499.
14. **Kim YN, Lora KR, Giraud DW, Driskell JA.** Nonsupplemented children of Latino immigrants have low vitamin E intakes and plasma concentrations and normal vitamin C, selenium, and carotenoid intakes and plasma concentrations. *J Amer Diet Assoc* 2006; 106: 385–391.
15. **Krajcovicova-Kudlackova M, Simoncic R, Bederova A, Grancicova E, Magalova T.** Influence of vegetarian and mixed diet on selected haematological and biochemical parameters in children. *Nahrung* 1997; 41: 311–314.
16. **Krajcovicova-Kudlackova M, Dusinska M.** Oxidative DNA damage in relation to nutrition. *Neoplasma* 2004; 51: 30–33.
17. **Krajcovicova-Kudlackova M, Dusinska M, Valachovicova M, Blazicek P, Paukova V.** Products of DNA, protein and lipid oxidative damage in relation to vitamin C plasma concentration. *Physiol Res* 2006; 55: 227–231.
18. **Krajcovicova-Kudlackova M, Valachovicova M, Paukova V, Dusinska M.** Effects of diet and age on oxidative damage products in healthy subjects. *Physiol Res* 2008; 57: 647–651.
19. **Lenhartz H, Schweitzer R, Botticher D, Leichsenring M.** Plasma carotenoids in German children and adolescents. *Eur J Pediatr* 1997; 156: 71–73.
20. **McGinnis WR.** Oxidative stress in autism. *Altern Ther Health Med* 2004; 10: 22–36.
21. **Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC.** Increased excretion of a lipid peroxidation biomarker in autism. *Prostaglandins Leukot Essent Fatty Acids* 2005; 73: 379–384.
22. **RDAs – recommended dietary allowances in SR.** *J Min Health Slov Rep* 1997; 45: 58.
23. **Theoharides TC, Doyle R, Francis K, Conti P, Kalogeromitros D.** Novel therapeutic targets for autism. *Trends Pharmacol Sci* 2008; 29: 375–382.
24. **Steyaert JG, De La Marche W.** What's new in autism? *Eur J Pediatr* 2008; 167: 1091–1101.
25. **Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougale CJ.** Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Pediatrics* 2003; 112: 420–424.
26. **Tierney E, Nwokoro NA, Porter FD, Freund LS, Ghuman JK, Kelley RI.** Behavior phenotype in the RSH/Smith-Lemli-Opitz syndrome. *Amer J Med Genet* 2001; 98: 191–200.
27. **Tierney E, Bukelis I, Thompson RE, Ahmed K, Aneja A, Kratz L, Kelley RI.** Abnormalities of cholesterol metabolism in autism spectrum disorders. *Amer J Med Genet B Neuropsychiatr Genet* 2006; 141: 666–668.
28. **White JF.** Intestinal pathology in autism. *Exp Biol Med* 2003; 228: 639–649.
29. **Yorbik O, Saval A, Akay C, Akbiyik DI, Sohmen T.** Investigation of antioxidant enzymes in children with autistic disorder. *Prostaglandins Leukot Essent Fatty Acids* 2002; 67: 341–343.
30. **Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli E, Yetkin O, Meram I.** Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. *Eur Arch Psychiatry Clin Neurosci* 2004; 254: 143–147.

Received November 3, 2008.

Accepted January 23, 2009.