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Urinary nitric oxide excretion in infants with eczema

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Short title: Urinary nitric oxide and eczema

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Abstract

Eczema is characterized by inflammation of the skin and is commonly associated with food allergy. It has been suggested that nitric oxide (NO) is an important player in eczema, food allergy and intestinal inflammation. The aim of this study was to assess the levels of urinary NO breakdown products in infants with eczema and the effect of eczema treatment on NO levels. Ninety-four infants with eczema, 58 boys and 36 girls, with a mean age of 7.5 ± 5.2 months (mean \pm SD) at inclusion were examined twice with an interval of 6 weeks. The sum of nitrite and nitrate was measured colorimetrically in urinary samples from both visits and compared with clinical data concerning eczema severity, nutrition, gastrointestinal symptoms, asthma, and skin prick positivity. The levels of NO products increased significantly from the first to the second visit: 289; 374 μ M (median; IQR) versus 457; 678 μ M (median; IQR) ($p < .001$) in parallel with a significant improvement of the eczema. After eczema treatment consisting of skin care and elimination diet during the 6-week interval between evaluations, the NO levels approached the values previously found in healthy children. The results support previous studies indicating that the homeostasis of nitrogen radicals is disturbed in childhood eczema.

Key words: pediatric, eczema, clinical immunology, nitric oxide

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Introduction

Eczema affects as much as 10-20% of children worldwide (1, 2). Characterized by inflammation of the skin, it is associated with food allergy in approximately 35% of the affected children (3). Altered intestinal permeability, with increased absorption of large molecules but normal uptake of low-molecular weight species, has been reported in eczema with and without food allergy, indicating an intestinal mucosal defect in eczema (4).

It has been implied that nitric oxide (NO) and reactive nitrogen products play a key role in the regulation of inflammatory responses (5). NO formation is catalyzed by NO synthases (NOS): two constitutive isoforms, cNOS, and one inducible, iNOS. The constitutive forms produce low amounts of NO and have generally been associated with the regulation of homeostatic functions. iNOS, which produces large amounts of NO and is induced in various cells by inflammatory stimuli, has been linked to severe tissue damage, but has also been shown to induce healing of the skin and intestinal mucosa (6). NO reacts rapidly with oxygen, yielding nitrite and nitrate, which are excreted into the urine.

In asthma, the level of NO in exhaled air is frequently used to monitor the inflammatory status of the bronchial epithelium (7). Traditionally, NO has been considered to act mainly as a pro-inflammatory agent (8). This concept has recently been challenged, however, in a study on human bronchial epithelium that suggests the involvement of NO in an anti-inflammatory feedback loop (9).

Less is known about the role of NO in eczema. In an animal model using NC/Nga mice to simulate human eczema, NO breakdown products were increased in serum, but decreased in skin lesions, compared with control animals (10).

Two previous studies reporting NO products in children with eczema have shown diverging results, but also have major differences in design and sampling compartments (11, 12). Taniuchi et al followed up 88 children with eczema, examined twice, and their nitrite/nitrate

levels in serum were also compared with the levels of 12 non-atopic children. They detected higher serum levels of nitrite/nitrate in patients than in controls and found that nitrite/nitrate levels decreased when the eczema was treated (11). However, in a cross-sectional study Omata et al (12) found lower levels in 27 eczematous children than in 25 healthy controls when measuring urinary nitrite/nitrate levels. Further, they found no correlation between nitrite/nitrate levels and eczema severity. They did, however, find increased levels of 8-OH-guanosine, a marker of oxidative stress in the children with eczema (12).

We have previously investigated urinary NO production in children with celiac disease (CD) (13, 14). Children with untreated CD, i.e. with small intestinal mucosa characterized by severe inflammation, displayed significantly higher levels of nitric oxide products in urine compared with children on a gluten-free diet with normalized mucosa. Moreover, CD severity correlated well with the level of NO urinary products (13, 14). These studies provide a comparison for the levels of NO in children with a different disease.

We hypothesized that children with severe eczema would show a high level of urinary NO, which should decrease after eczema treatment.

The aim of this study was to assess the levels of urinary NO breakdown products in children with eczema and to investigate a possible correlation between eczema improvement and NO formation.

Materials and Methods

Patients

The study group comprised 94 children, 58 boys and 36 girls, participating in a prospective study of clinical and immunological development in small children with eczema and suspected food allergy (15). They were examined twice with a 6-8 week interval. Morning urinary samples were collected on both occasions, and skin prick tests (SPTs) to cow's milk and egg were performed at the first visit. The age of the children at the first and second visits was 7.5 ± 5.2 months (mean \pm SD) and 10 ± 5.4 months (mean \pm SD), respectively. The infants were referred from primary care physicians from June 1999 to September 2001 to the pediatric units in the cities of Linköping, Jönköping, Norrköping and Hudiksvall in Sweden. Inclusion criteria were age below two years, eczema at referral and suspected food allergy. The entire study group comprised 123 infants. For the present study, we chose those with complete data, including urinary samples from both visits. The subset of children described in this paper did not differ significantly from the entire study cohort with respect to eczema severity. The Hanifin – Rajka criteria were used to diagnose eczema (16). Eczema severity was assessed with the Severity Scoring of Atopic Dermatitis (SCORAD) method (17), taking into account the extent and severity of eczema as well as the consequences of the skin disorder (degree of pruritus and sleeping disorder assessed by the parents). Using this classification, children were judged to have mild (SCORAD ≤ 25 points; n=58), moderate (SCORAD 26 – ≤ 50 points; n= 31), or severe (SCORAD >50 points; n= 5) eczema. At referral, all children were judged as having eczema by the referring physician. The majority of children, 84/94, had already been prescribed topical steroid treatment on referral, viz. steroid class I (mild) for 63 children, class II for 18 children, and class III for three children. Therefore, 9 children had SCORAD 0 at the first visit.

The parents received detailed instructions and practical demonstrations on how to treat eczema and dry skin at the first visit in the study. When there was eczema and a significant positive skin prick test to milk and/or egg, parents were advised to put the child on a temporary elimination diet. On referral, no child or breastfeeding mother was on an elimination diet.

According to Swedish recommendations for infants, baby food should not contain any food items with high levels of nitrite/nitrate, such as certain vegetables (spinach, beetroot, rhubarb, fennel and celery) or smoked and salted meat products. These recommendations were given to the mothers at the well-baby clinics both orally and in a pamphlet about good baby feeding.

The criteria used for asthma were an episode of wheezing if related to exposure to allergens or combined with atopic eczema, or at least three episodes of wheezing in the absence of atopy and exposure to allergens (n= 20) Gastrointestinal allergy was defined as vomiting and/or diarrhea at least twice after intake of a specific food (n=17).

A comprehensive description of the clinical data has been given previously (15).

Methods

In the urinary sample, the sum of nitrite and nitrate was measured and taken as an indirect indicator of NO production (18). In short, the nitrite content was assessed with a colorimetric method based on the Griess reaction for nitrite. In a PBS-diluted sample, nitrate was converted using nitrate reductase from *Aspergillus* (19). Next, 50 μ l of the diluted urine was mixed with 10 μ l NADPH (1 μ M) followed by 40 μ l 80 U/l nitrate reductase (Roche, Basel, Switzerland), glucose-6-phosphate (500 μ M) and glucose-6-phosphate dehydrogenase (160 U/l). The reaction mixture was incubated at room temperature for 45 min. The mixture was then used for the Griess assay of nitrite by adding 100 μ l sulfanilamide (1% in 5% phosphoric

acid) and 100 μ l naphthylethylenediamine (0.1 %). The resultant color was read at 540 nm with a Vmax spectrophotometer (Molecular Devices, Sunnyvale, CA).

SPT

SPT was performed as a prick-prick test as described previously (17). In accordance with the EAACI position paper, the results were considered positive when the mean diameter (half of the sum of the largest diameter and its perpendicular) of the wheal was ≥ 3 mm greater than the negative control (20).

Statistics

The data were not normally distributed, so the non-parametric Mann-Whitney U-test was used to compare groups. Differences associated with p values of less than .05 (2-tailed) were considered significant. Spearman's correlation test was used to evaluate correlations.

Ethics

The study was approved by the Human Research Ethics Committee at the Faculty of Health Science, Linköping University. Informed consent was obtained from the parents.

Results

Skin prick test positivity and eczema assessments

In 62 children, SPTs to egg and/or milk were positive, whereas in 32 children SPTs were negative.

The SCORAD value for the whole group decreased significantly from the first to the second visit, viz. from 17.4; 24.9 (median; IQR) to 9.1; 13.9 (median; IQR) ($p < .001$). For the SPT-positive children the corresponding values were 20; 22.9 (median; IQR) at the first visit, and 9.1; 14.2 (median; IQR) at the second ($p < .001$). For the SPT-negative children, the values were 15.5; 15.7 (median; IQR) at the first visit and 9.0; 13.2 (median; IQR) at the second (n.s).

Nitrite/nitrate in urine at the first and second measurements

For the whole group, the urinary excretion of NO breakdown products was 289; 374 μM (median; IQR) on the first occasion and 457; 678 μM (median; IQR) on the second ($p < .001$). Similarly, in SPT-positive children, the levels were 240; 360 μM (median; IQR) on the first occasion and 408; 565 μM (median; IQR) on the second ($p < .005$; Fig.1). For SPT-negative children, the corresponding values were 324; 354 μM (median; IQR), and 530; 847 μM (median; IQR) ($p < .05$; Fig 1). There was no significant difference between the SPT-positive and SPT-negative groups at the first or second visit. For the majority of children, 60/94, the values at the second measurement were higher than at the first. Ten children in the present study displayed a value above 1406 μM , which was the ± 2 SEM level we found in a previous study comprising healthy reference children (13). The individual change between the assessments in these 10 infants is depicted in Fig. 2.

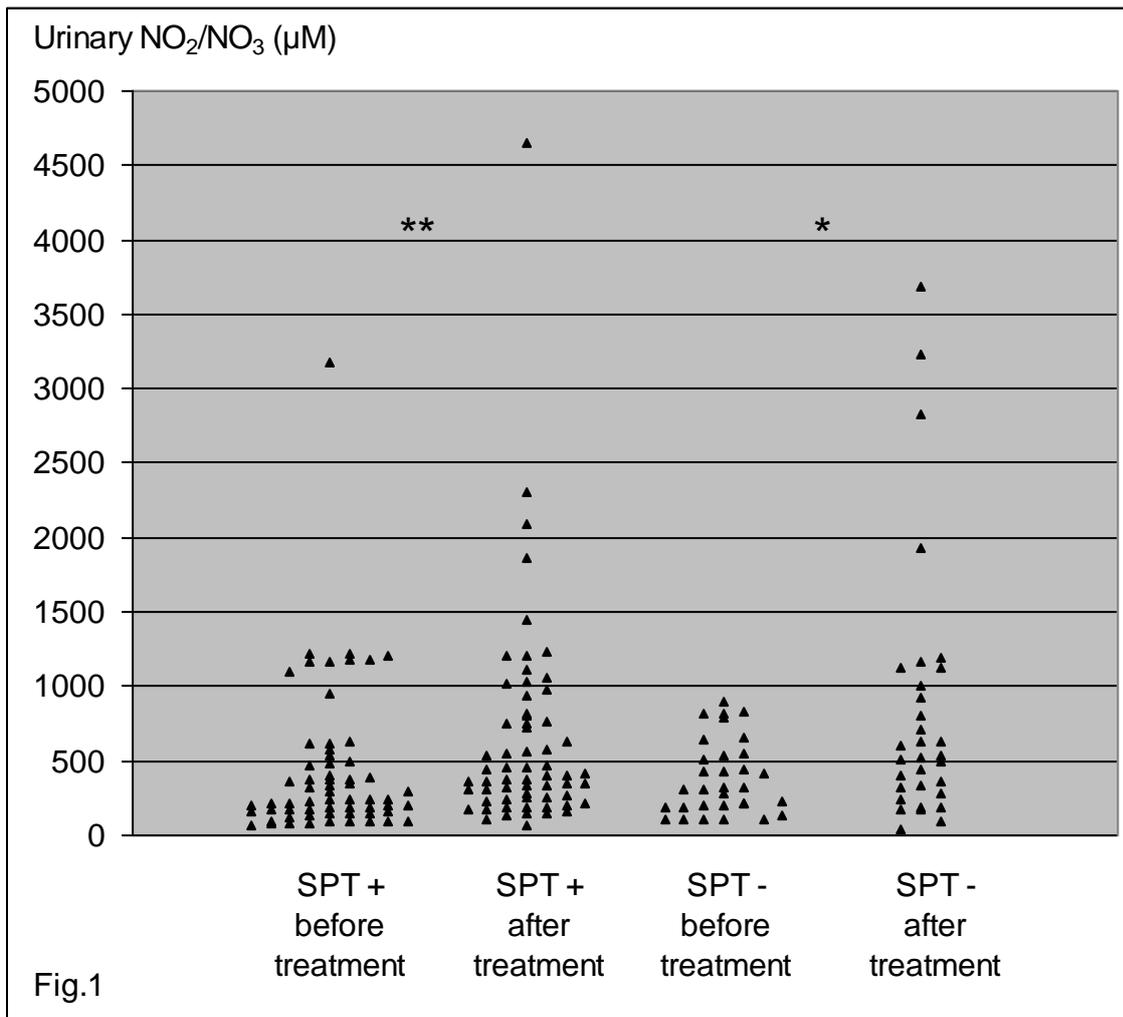


Fig. 1. Urinary nitric oxide breakdown products in children with eczema. The values of nitrite/nitrate were significantly higher after treatment, 457; 678 μM (median; IQR), than before treatment, 289; IQR 374 μM ($p < .001$). In the SPT+ group, the median value was 240 before and 408 after treatment ($p < .005$), and in the SPT – group the median values were 324 before, and 530 after treatment ($p < .05$). SPT = skin prick test.

Effects of sex, age, breastfeeding, atopic symptoms and eczema severity on urinary nitrite/nitrate levels

The values were similar in boys and girls, 290; 393 μM (median; IQR) versus 289; 329 (median; IQR) (n.s), respectively, and there was no correlation between nitrite/nitrate levels and age. Ninety-one of the 94 children had been breastfed at least 2 months, 52/94 were still being breastfed at the first visit, and 38/94 at the second. Nitrite/nitrate levels were similar: 237; 310 μM (median; IQR) in the breastfed and 326; 450 μM (median; IQR) in the non-breastfed group (n.s.). In addition, 20 children with asthma displayed similar levels to those

without, 387; 425 μM (median; IQR) and 237; 313, μM (median; IQR) respectively (n.s). Infants with symptoms of gastrointestinal allergy had similar levels of nitrite/nitrate to those without; i.e. 308; 383 μM (median; IQR) compared with 262; 357 μM (median; IQR) (n.s.). There was no correlation between nitrite/nitrate levels and eczema severity at either the first or second measurement. Finally, there was no significant difference in nitrite/nitrate levels between children with mild (n=58), moderate (n=31) or severe eczema (n=5).

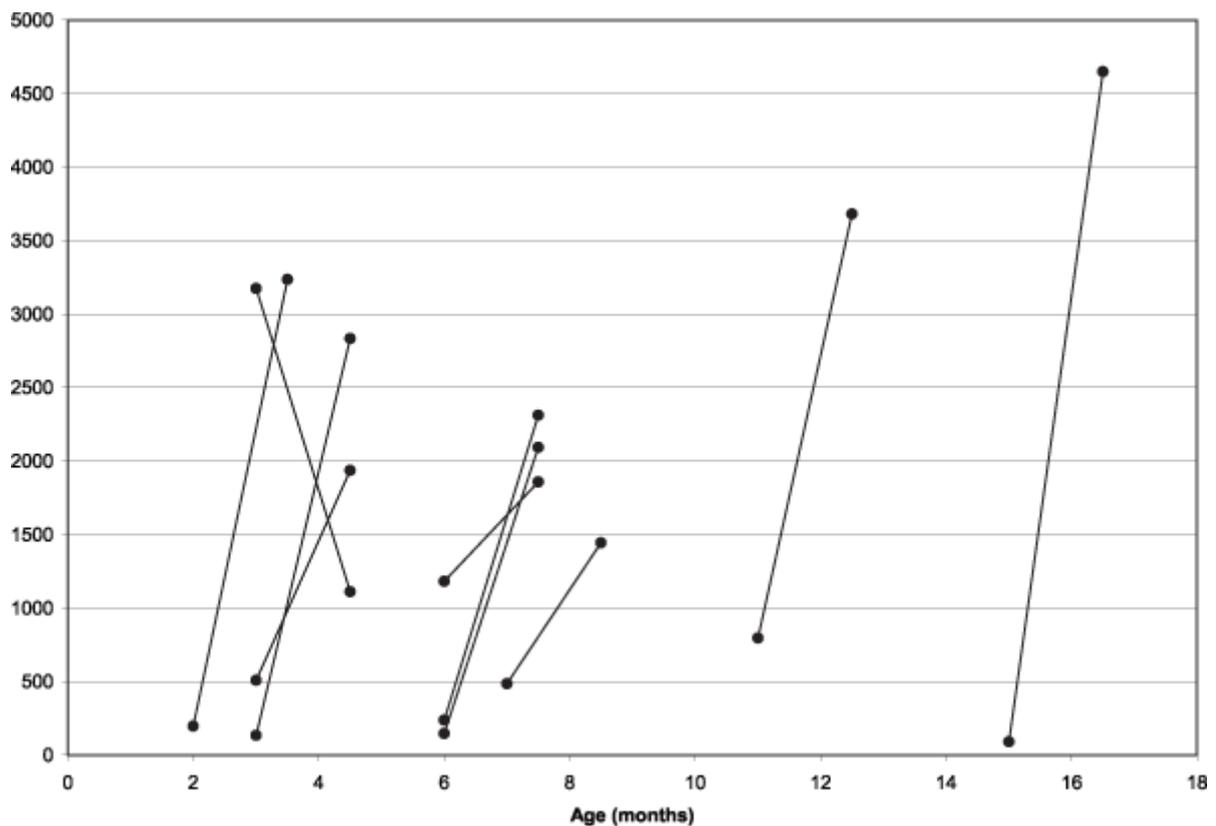


Fig. 2. Individual changes in nitrite/nitrate levels in urine, before and after eczema treatment, in the 10 children displaying a value above 1406 μM representing the ± 2 SEM in a previous study of healthy reference children.

Discussion

Urinary NO levels at the first visit were 289; 374 μM (median; IQR) which was much lower than those observed in a previous study with healthy children (13). As expected, the eczema improved after treatment with skin care and/or food interventions, as evidenced by lower eczema scores. However, contrary to our expectations, urinary NO breakdown products increased significantly as eczema improved. This is a novel finding and contrasts with a previous study of children with eczema and the effect of treatment (11). In that study, the children showed significantly increased levels of serum nitrate compared with healthy children, and lower nitrate levels after eczema treatment. Moreover, the serum nitrate levels correlated with the severity of the eczema (11). There are, however, several differences between the studies. Taniuchi et al used serum samples, and the children in that study were older (mean age 2.2 years) than the infants in the present study. This makes it likely that their eczema was more chronic. No information was provided with respect to diet, i.e. intake of food rich in nitrite/nitrate, or sensitization to foods requiring elimination diets (11). Moreover, the findings reported by Taniuchi et al were not confirmed in a study on adults with eczema performed by Kuzik et al (21), who reported no correlation between plasma NO levels and eczema severity. In fact, in a subgroup they found significantly higher NO plasma levels during eczema remission, i.e. their results corroborated ours.

The compartment chosen for analysis, i.e. serum or urine, may also be of importance. A recent study using a mouse model mimicking eczema-like conditions found higher NO levels in serum, but lower levels in the skin lesions (10). Our results support those of Omata et al (12), who also used urinary measurements. They reported lower levels of urinary nitrite/nitrate in children with eczema and no correlation with eczema severity.

In the present study, urinary excretion of NO products significantly increased after treatment, from 289; 374 μM (median; IQR) on the first occasion to 457; 678 μM (median; IQR) at the second measurement, i.e. after treatment. To explain the higher levels of nitrite/nitrate, substantial consumption of food products containing nitrite/nitrate could be considered a confounding factor at the second assessment. This is, however, a most unlikely explanation because parents in Sweden are discouraged from serving these food items to their infants. A second source of NO production in the body might be untreated asthma, as several studies have verified the usefulness of analyzing exhaled NO products as a sign of airway inflammation (7). Among the 94 children in this study, 20 were diagnosed as having asthma, but their urinary nitrite/nitrate levels did not differ from those of the children without airway problems. A further explanation of the change following treatment could be a general anti-inflammatory effect caused by the local steroids used to treat the eczema. This was probably not the case because most children were already being treated with local steroids at the first assessment. Considering that the children were a few months older at the second measurement, increased age might be thought to cause the increase. This, however, is not a plausible explanation as there was no correlation between age and NO breakdown levels. Moreover, it has previously been shown that urinary nitrite/nitrate levels in healthy children decrease with age (22).

We have previously assessed the content of NO breakdown products in children with celiac disease (CD). With untreated CD, the children displayed very high nitrite/nitrate values, $4147 \pm 1102 \mu\text{M}$ (mean \pm SEM, n=20) (13). Moreover, during gluten exposure, increased expression of iNOS was demonstrated in small intestinal biopsy samples (23), which made increased urinary excretion of nitrite/nitrate in this condition a reasonable finding. After treatment with a gluten-free diet, i.e. when healing of the intestinal mucosa had been demonstrated by a small intestinal biopsy, the nitrite/nitrate levels were $1078 \pm 1084 \mu\text{M}$

(mean \pm SD, n=25) (14). The levels in healthy children of the same age were $1174 \pm 116 \mu\text{M}$ (mean \pm SEM, n=53) (14). In comparison, urinary nitrite/nitrate in the children with eczema in the present study was clearly lower, especially before treatment for eczema, 289; 374 μM (median; IQR), but also at the second sampling after treatment, 457; 678 μM (median, IQR). At this point, they were increasing toward the higher levels previously observed in healthy children and children with CD on a gluten-free diet.

The inflammatory reaction in eczema is thought to result in an activation of the stress system of the organism, e.g. acute exacerbation of atopic eczema correlated with high levels of urinary pentosidine, a marker of oxidative stress (24). It has been suggested that oxidative stress and impaired homeostasis of oxygen/nitrogen radicals are involved in childhood eczema (12). On the other hand, NO has been attributed with a physiological role in the dermis, protecting the skin surface and facilitating wound healing (25).

We hypothesize that our findings of low urinary levels of NO in children with active eczema and increased levels after treatment might be explained by an upregulation of iNOS, as observed in human bronchial epithelial cells in asthma (9). Moreover, most, but not all, of the children in our study had elevated NO levels after treatment, which might suggest individual variations in the feedback system (9).

A recent study by Linkasalo et al (26) reported elevated levels of bronchial NO output in children and adolescents with severe eczema, even without established asthma. Patients with severe eczema had enhanced NO output compared with patients with mild eczema. Compared with this study, their patients were older (7-22 years), and all had increased wheat-specific IgE and signs of eosinophilic inflammation.

In conclusion, contrary to our expectations we found that the levels of urinary NO breakdown products increased significantly after treating the eczema. In parallel, the skin improved. After

treatment, the NO values approached those found in healthy children, an increase that might be due to an upregulation of iNOS. The results support previous studies indicating that the homeostasis of nitrogen radicals is disturbed in childhood eczema.

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References

1. JOHANSSON SGO, BIEBER T, DAHL R et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, Oct 2003. *J Allergy Clin Immunol* 2004; 113: 832-6.
2. ASHER MI, MONTEFORT S, BJÖRKSTEN B et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet* 2006; 368:733-43.
3. SAMPSON HA. Update on food allergy. *J Allergy Clin Immunology* 2004; 113(5): 805-819.
4. JACKSON PG, LESSOF MH, BAKER RW, FERRETT J, MACDONALD DM. Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981; I: 1285-6.
5. GUZIK TJ, KORBUT R, ADAMEK-GUZIK T. Nitric oxide and superoxide in inflammation and immune regulation. *J Physiol Pharmacol* 2003; 54: 469-487.
6. KUBES P. Inducible nitric oxide synthase: a little bit of good in all of us. *Gut* 2000; 47(1):6- 9.
7. PINJENBURG MWH, DE JONGSTE JC. Exhaled nitric oxide in childhood asthma: a review. *Clin Exp Allergy* 2008; 38:246-59.
8. KOLB H, KOLB-BACHOFEN V. Nitric oxide in autoimmune disease– cytotoxic or regulatory mediator? *Immunol Today* 1998;19: 556-61.
9. ERIKSSON U, EGERMANN U, BIHL MP et al. Human bronchial epithelium controls Th2 responses by Th1-induced, nitric oxide-mediated STAT5 dephosphorylation: implications for the pathogenesis of asthma. *J Immunol* 2005; 175: 2715-2720.

10. KUBO M, KAMBAYASHI Y, TAKEMOTO K, OKUDA J, MUTO M, OGINO K.
Reactive nitrogen species formation in eosinophils and imbalance in nitric oxide metabolism are involved in atopic dermatitis-like skin lesions in NC/Nga mice. *Free Radic Res* 2005; 39: 719-27.
11. TANIUCHI S, KOJIMA T, HARA MT K et al. Increased serum nitrate levels in infants with atopic dermatitis. *Allergy* 2001; 56: 693-5.
12. OMATA N, TSUKAHARA H, ITO S et al. Increased oxidative stress in childhood atopic dermatitis. *Life Sci* 2001; 69: 223-8.
13. SUNDQVIST T, LAURIN P, FÄLTH-MAGNUSSON K, MAGNUSSON KE, STENHAMMAR L. Significantly increased levels of nitric oxide products in urine of children with celiac disease. *J Pediatr Gastroenterol Nutr* 1998; 27: 196-8.
14. LAURIN P, FÄLTH-MAGNUSSON K, SUNDQVIST T. Increase of nitric oxide urinary products during gluten challenge in children with coeliac disease. *Scand J Gastroenterol* 2003; 38: 55-60.
15. NORRMAN G, TOMICIC S, FAGERÅS BÖTTCHER M, OLDAEUS G, STRÖMBERG L, FÄLTH-MAGNUSSON K. Significant improvement of eczema with skin care and food elimination in small children. *Acta Paed* 2005; 94:1384-88.
16. HANIFIN JM, RAJKA G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; Suppl 92: 44-7.
17. Consensus report of the European Task Force on Atopic dermatitis. Severity scoring of Atopic dermatitis: the SCORAD index. *Dermatology* 1993; 186: 23-31.
18. VERDON CP, BURTON BA, PRIOR RL. Sample pretreatment with nitrate reductase and glucose-6-phosphate dehydrogenase quantitatively reduces nitrate while avoiding interference by NADP⁺ when the Griess reaction is used to assay for nitrate. *Anal Biochem* 1995; 224: 502-508.

19. GILLIAM MB, SHERMAN MP, GRISCAVAGE JM, IGNARRO LJ. A spectrophotometric assay for nitrate using oxidation by *Aspergillus* nitrate reductase. *Anal Biochem* 1993; 212(2): 359-65.
20. Position Paper: allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. *Allergy* 1993; 48 (suppl 14): 48-82.
21. GUZIK TJ, ADAMEK-GUZIK T, CZERNIAWSKA-MYSTIK G, DEMBINSKA-KIEC A. Nitric oxide metabolites in children and adult patients with atopic eczema/dermatitis syndrome. *Allergy* 2002; 57:856.
22. TSUKAHARA H, HIRAOKA M, HORI C, MIYANOMAET T, KIKUCHI K, SUDO M. Age-related changes of urinary nitrite/nitrate excretion in normal children. *Nephron* 1997; 76: 307-9.
23. HOLMGREN PETERSON K, FÄLTH-MAGNUSSON K, MAGNUSSON KE, STENHAMMAR L, SUNDQVIST T. Children with celiac disease express inducible nitric oxide synthase in the small intestine during gluten challenge. *Scand J Gastroenterol* 1998; 33: 939-43.
24. TSUKAHARA H, SHIBATA R, OHTA N et al. High levels of urinary pentosidine, an advanced glycation end product, in children with acute exacerbation of atopic dermatitis: relationship with oxidative stress. *Metabolism* 2003; 52:1601-5.
25. CALS-GRIESON MM, ORMEROD AD. Nitric oxide function in the skin. *Nitric Oxide* 2004; 10: 179-93.
26. LINKOSALO L, LEHTIMÄKI L, LAITINEN J, KAILA M, HOLM K; MOILANEN E. Increased bronchial NO output in severe atopic eczema in children and adolescents. *Pediatr Allergy Immunol* 2008; 19: 426-32.