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Predictive value of exhaled nitric oxide in healthy infants for asthma at school age

To the Editor:

Exhaled nitric oxide fraction (F_{ENO}) is a noninvasive biomarker that is elevated in subjects with asthma and allergic diseases [1]. Nitric oxide (NO) is produced by three NO synthase (NOS) enzymes: neuronal, endothelial and inducible (iNOS), all present in the human lung [2]. The increase of F_{ENO} in asthmatic patients has mainly been attributed to an activation of iNOS, mediated through proinflammatory cytokines in the airways [3].

Early monitoring of airway inflammation assessed by elevated F_{ENO} provides information on asthma evolvement and helps to identify subjects at risk. Few studies have investigated the association between F_{ENO} during early childhood and asthma at school age. In pre-school children with recurrent respiratory symptoms, those with higher F_{ENO} were at an increased risk of asthma at 6 years [4]. In a selected cohort of infants with eczema, increased F_{ENO} prior to any wheezing episodes was associated with an increased risk of asthma at 5 years [5]. Notably, in these studies, F_{ENO} was measured in high-risk children who were already exposed to environmental factors known to modify F_{ENO} levels [6–8].

The value of F_{ENO} after birth to predict later symptoms before relevant exposure to environmental factors has been prospectively investigated in only two studies [9, 10]. Latzin *et al.* [9] reported that infants born to atopic mothers had increased F_{ENO} prior to respiratory symptoms and that this association was enhanced in mothers who smoked. In infants born to asthmatic mothers, Chawes *et al.* [10] showed that increased F_{ENO} was associated with recurrent wheezing episodes during the first year of life but not thereafter.

Taken together, while there is cumulative evidence that elevated F_{ENO} in high-risk children after a possible impact by environmental exposures is associated with later asthma [4, 5], it is unknown if F_{ENO} after birth, prior to a possible influence by post-natal environmental exposures and first respiratory symptoms, is associated with asthma. Given that environmental factors are known to induce NOS activity and modify F_{ENO} [6, 7, 11, 12], we hypothesised that F_{ENO} measured after birth, and before relevant exposure to these factors, is not associated with asthma at school age.

The aim of this prospective cohort study was to investigate if F_{ENO} levels after birth in unselected newborns are associated with asthma or atopy at school age. This prospective birth cohort study comprised of unselected, healthy, term-born infants recruited in the region of Bern, Switzerland [13]. At 5 weeks of age, F_{ENO} was measured from multiple breaths during natural sleep, as previously described [9, 14], with a rapid response chemiluminescence analyser (CLD 77; EcoMedics, Duernten, Switzerland) (analysis software: WBreath version 3.28.0.0; ndd, Zurich, Switzerland).

At 6 years of age, asthma was assessed by study physicians with questions adapted from the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire [15], defined as a history of wheezing within the 12 months prior to follow-up. Study physicians assessed atopy, defined as allergic asthma, allergic rhinitis, atopic dermatitis or positive skin-prick test (SPT). An SPT including seven common allergens was determined to be positive in the case of hives bigger than positive control, histamine, with any of the tested allergens [13]. We assessed risk factors for asthma or atopy of the child: parental asthma was defined as self-reported or doctor-diagnosed asthma; parental atopic disease was defined as allergic asthma, hay fever or eczema by history.

The Governmental Ethics Committee of the Canton of Bern, Switzerland approved the study and informed, written consent was obtained at enrolment.

Logistic regression was used for analysis of an association between F_{ENO} at birth and asthma, atopy and positive SPT at school age. Adjustment for confounders (*e.g.* parental asthma and atopy) and nonconfounding factors (minute ventilation, which is known to modify F_{ENO} [16]) was done. Linear regression was used for the analysis of an association between parental variables and F_{ENO} at birth. Data are presented as odds ratios or difference in F_{ENO} (in parts per billion) with 95% confidence intervals. Data were analysed with STATA 13 (STATA Corporation, College Station, TX, USA). Patient characteristics for those with and without follow-up were compared using Mann–Whitney U-test and Chi-squared test.

We measured F_{ENO} in 278 infants with 44 (16%) being excluded for technical reasons, resulting in 234 subjects. Of those 26 (12%) were lost to follow-up, resulting in 208 final study participants. Demographics,

exposure to risk factors and F_{ENO} levels did not differ between subjects followed up and those lost to follow-up (data not shown). For the entire group, neither maternal atopy nor maternal smoking was associated with postnatal F_{ENO}. Smoking during pregnancy was only associated with decreased F_{ENO} levels in infants of nonatopic mothers (−2.91 ppb, 95% CI −5.76– −0.048 ppb). These findings were similar to previously published data [16], although the fraction of smoking mothers was lower in this study (9% *versus* 13% [16]). At 5 weeks of age, mean F_{ENO} was 13.9 ppb (range 1.8–32.9 ppb). Parents of 43 (20%) children had asthma and those of 126 (61%) were atopic; 19 (10%) mothers smoked during pregnancy. Among 6-year-olds (age range 5–7 years), 31 (15%) had asthma with 13 (6%) allergic asthma cases, and none of the children used corticosteroids. There were 62 (30%) atopics and in 164, an SPT was completed with 26 (19%) being positive.

F_{ENO} at birth was not associated with asthma, atopy or positive SPT at school age. Per 1-ppb increase in F_{ENO}, the simple and adjusted odds ratios for asthma were 0.99 (95% CI 0.92–1.07) and 0.97 (95% CI 0.89–1.06); for atopy, 0.99 (95% CI 0.94–1.05) and 0.99 (95% CI 0.94–1.06); and for positive SPT, 0.95 (95% CI 0.88–1.03) and 0.95 (95% CI 0.87–1.03), respectively (table 1).

Our study is the first to investigate the association between F_{ENO} at birth and asthma at school age in unselected infants. It was previously shown that the association of elevated F_{ENO} after birth with respiratory symptoms is restricted to infancy [10]. In this study, we found supporting evidence for this finding, since F_{ENO} after birth was not associated with diagnosis of school age asthma.

Based on this finding, and on previous studies in selected high-risk populations, we propose two different models of F_{ENO} metabolism in early infancy (figure 1). In model 1, elevated F_{ENO} is an expression of an intrinsic mechanism, determined by pre- and early postnatal risk factors. In this model, F_{ENO} levels during infancy would then not be altered by environmental factors, and F_{ENO} measured at birth could serve as a predictor for later asthma and atopy. In model 2, environmental factors (*e.g.* infections or air pollution) are needed to induce iNOS [6, 7, 11], which then results in elevated F_{ENO}. In this hypothesis, we would expect no association between F_{ENO} at birth (measured before environmental exposures) and school-age outcomes. F_{ENO} may then only serve as a phenotype-specific biomarker in infants after the first activation of the environmentally or genetically induced iNOS. We speculate that the second, rather than the first, model better explains NO metabolism after birth, since studies measuring F_{ENO} after environmental exposures found an association between F_{ENO} and asthma development, while our study, measuring F_{ENO} before environmental exposures, did not find any association.

Measurements were performed using a face mask, which is the only available technique for measuring F_{ENO} in infants at this time. This introduces the possibility that NO from the upper airways contributes to the overall F_{ENO} measured. However, we believe this potential contribution is unlikely due to the fact that the nasal sinuses of infants are not developed.

Asthma prediction with F_{ENO} might further be hampered by the physiological variability of F_{ENO} *per se*, by an intersubject variability of up to 50% [18], and influenced by different measurement techniques. In school-aged children, comparison of F_{ENO} measured from single *versus* multiple breaths resulted in higher F_{ENO} values using the latter technique [19]. Multiple-breath F_{ENO} measurement technique is, at this time, the only one available for infants. The present study is limited by the low number of asthmatics (n=31) and the questionnaire-based assessment for diagnosis, which could lead to possible misclassification. In general, the cohort reflects the epidemiological situation in Switzerland, with a low prevalence of mild-to-moderate

TABLE 1 Association between exhaled nitric oxide fraction (F_{ENO}) in newborns and subsequent diagnoses of asthma, atopy and positive skin-prick test (SPT) in school-aged children

Outcome	Exposure F_{ENO}			
	Simple[#] model		Adjusted[¶] model	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Asthma[+]	0.99 (0.92–1.07)	0.970	0.97 (0.89–1.06)	0.586
Atopy[§]	0.99 (0.94–1.05)	0.976	0.99 (0.94–1.06)	0.971
SPT positive[f]	0.95 (0.88–1.03)	0.285	0.95 (0.87–1.03)	0.256

Odds ratios and 95% confidence intervals per 1-ppb increase in F_{ENO} were determined by logistic regression. [#]: F_{ENO} was adjusted for minute ventilation [16]; [¶]: additionally adjusted for sex, parental asthma, parental atopy and smoking during pregnancy; [+]: defined as a history of wheezing within the 12 months prior to follow-up; [§]: defined as the presence of asthma, allergic rhinitis, atopic eczema or positive prick test; [f]: positive in case of hives bigger than positive control, histamine, with any of the tested allergens.

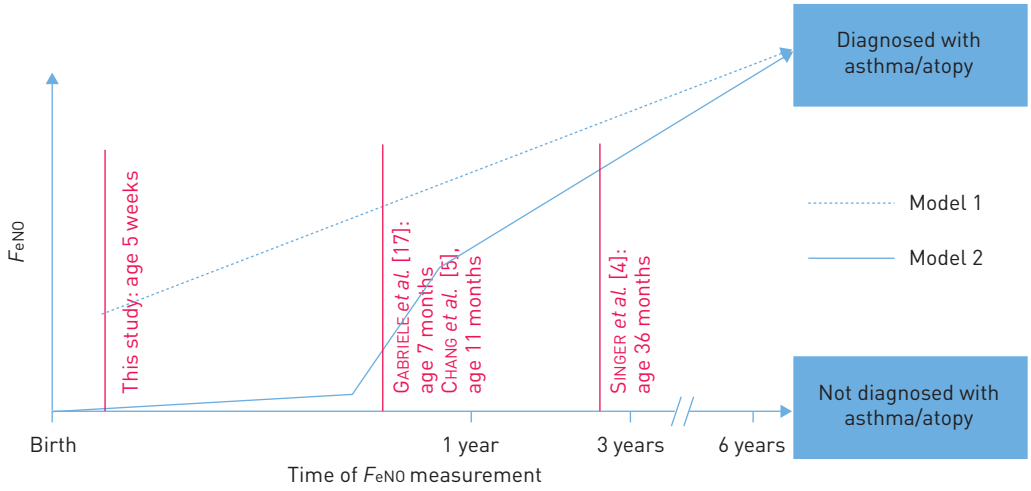

FIGURE 1 We measured exhaled nitric oxide fraction (F_{ENO}) at 5 weeks of age, while previous studies measured F_{ENO} in older children during time ranges. Based on this and previous studies, we propose two hypotheses for F_{ENO} metabolism during infancy. In model 1, elevated F_{ENO} is an expression of an intrinsic mechanism determined by pre- and early postnatal risk factors. In this model, F_{ENO} levels during the first year of life would then not be altered by environmental factors and F_{ENO} measured at birth could serve as a predictor for asthma/atopy at school age. In model 2, environmental factors (*e.g.* infections or air pollution) are needed to induce nitric oxide synthases, which then results in elevated F_{ENO}. In this model, we would expect no association between F_{ENO} at birth (measured before environmental exposures) and asthma/atopy at school age. F_{ENO} may then only serve as a phenotype-specific biomarker in infants after the first activation of environmentally induced nitric oxide synthesis.

asthmatics. Coincidentally, however, in our study sample, only mild intermittent asthmatics (without corticosteroid use in the last 12 months) were included, but this study was conducted in a prospective, unselected cohort, representing the general population. In contrast to previous studies in high-risk populations, we measured F_{ENO} at a single point in time after birth, excluding possible age- or time-dependent effects on F_{ENO}.

The interpretation of F_{ENO}, its predictive value and its modifiers are age dependent [4, 5, 7, 9–11]. Postnatal F_{ENO} metabolism seems to be modified by various environmental factors. On a cellular level, maternal tobacco smoke modified NOS activity in the fetal vascular bed in newborns [12]. Consistent with this observation, postnatal F_{ENO} is modified by prenatal tobacco smoke exposure in offspring [16, 17], interestingly enough, in an interaction with maternal atopy [16]. In contrast to the pre- and early postnatal situation, infancy and preschool age seems to be critical for further gene–environment interactions through exposures other than smoking and maternal atopy and their impact upon NO metabolism [4, 5].

In summary, we show that postnatal F_{ENO} measured in unselected healthy newborns is not associated with asthma diagnosis at school age. We speculate that NO metabolism may play a role in the pathophysiology of childhood asthma and atopy only after exposure to environmental factors at preschool age. To confirm that environmental exposures indeed modify NOS expression during infancy, frequent longitudinal assessment of F_{ENO} levels and NOS expression would be necessary. Our findings should encourage further research on factors impacting upon NO metabolism during infancy that can serve as targets for new preventive strategies on childhood asthma development.

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F_{ENO} in newborns before exposure to environmental factors is not associated with school-age asthma development http://ow.ly/ShQQ300xiN2

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