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Equally elevated concentrations of exhaled nitric oxide in nonatopic and low-sensitized atopic asthmatics

Heikki Ekroos^{a,*}, Annamari Rouhos^b, Paula Pallasaho^c, Jouko Karjalainen^d, Seppo Sarna^e, Anssi R.A. Sovijärvi^a

^a Division of Clinical Physiology and Nuclear Medicine, Laboratory Department, Helsinki University Hospital, Helsinki, Finland

^b Department of Respiratory Medicine, Helsinki University Hospital, Helsinki, Finland

^c Department of Allergology, Skin and Allergy Hospital, Helsinki University Hospital, Helsinki, Finland

^d Department of Internal Medicine, Central Military Hospital, Helsinki, Finland

^e Department of Public Health, Helsinki University, Helsinki, Finland

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KEYWORDS

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Summary

Background: Some studies show concentrations of exhaled nitric oxide (FENO) in nonatopic asthma and in healthy subjects to be similar, but include asthmatics on inhaled steroids, which is likely to interfere with the results.

Aim: Comparison of FENO between nonatopic asthmatics, low-sensitized and high-sensitized atopic asthmatics, and healthy controls.

Methods: We studied 85 non-smoking, steroid-naïve young men with recently diagnosed symptomatic asthma and 10 healthy controls. FENO was measured according to European Respiratory Society Guidelines. In skin prick tests of 13 common aeroallergens, subjects with a total sum of prick wheals 3–10 mm were regarded as low-sensitized and those with >10 mm, as high-sensitized. Flow–volume spirometry, standardized histamine challenge, and an exercise test were also carried out.

Results: Prick tests revealed 14 subjects to be nonatopic and 71 atopic. In high-sensitized subjects with atopic asthma, the FENO median (25–75 quartiles) was significantly higher, 34.9 (21.3–53.8) parts per billion (ppb), than in subjects with nonatopic asthma, 15.2 (9.7–24.7) ppb ($p < 0.001$), both being significantly higher than in healthy controls, 6.6 (5.2–8.5) ppb ($p < 0.001$). FENO levels were similar in nonatopic and in low-sensitized atopic asthmatics, with no difference between them in bronchial responsiveness to histamine and exercise.

* Corresponding author. Porvoo Hospital, Sairaalanatie 1, PL 500, 06151 Porvoo, Finland. Tel.: +358 50 5743949; fax: +358 19 548 2257.
E-mail address: heikki.ekroos@kolumbus.fi (H. Ekroos).

Conclusion: Among steroid-naïve young male asthmatics, FENO was equally elevated in non-atopic asthma and in low-sensitized atopic asthma but lower than in those with high-sensitized atopic asthma. These differences in FENO between asthma groups parallel the differences in airway function disturbance in terms of responsiveness to histamine or exercise.

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Introduction

Both similarities and differences in the mechanisms of airway inflammation and in its clinical features occur between atopic and nonatopic asthma,¹ which is characterized by airway inflammation, bronchial hyperresponsiveness (BHR) and variable airway obstruction.² Features of nonatopic asthma are usually greater severity, female predominance, later onset, and greater steroid dependence than in atopic asthma.³

Exhaled nitric oxide (FENO) is a useful diagnostic tool, as it is frequently elevated in asthma, especially in atopic subjects.^{4–6} The level of FENO in nonatopic asthmatics has been similar or slightly higher than in healthy controls.^{4,5} Some studies have also included asthmatics who are using inhaled steroids, which can interfere with comparisons.⁴ The level and type of allergic sensitization affect FENO values in atopic asthma, with perennial allergens having the greatest effect.⁷

BHR to histamine or methacholine is more prominent in atopic asthma than in nonatopic asthma of similar severity.⁸ Associations between FENO and BHR to histamine or to exercise depend on the atopic status.⁹ Furthermore, the number of positive skin prick test reactions seems to correlate positively with exercise-induced asthma in young athletes,¹⁰ and the severity of exercise-induced bronchoconstriction (EIB) associated with cumulative atopy score.¹¹ We, therefore, grouped the atopic asthmatics according to their level of sensitization.

Our main aim was to compare the FENO of nonatopic asthmatics with that of both low- and high-sensitized atopic asthmatics and that of healthy control subjects in a population of steroid-naïve, non-smoking male military conscripts. The second aim was to compare the characteristics of airway responsiveness to histamine and exercise with the type of allergic sensitization.

Methods

Subjects

The 85 study subjects had been referred to the Central Military Hospital by military physicians from various areas in Finland because of respiratory symptoms including dyspnoea, wheezing, cough, sputum production, or symptoms related to exercise during the 1 month prior to the study. They were aged 18–27 years, with no history of childhood asthma, who were recently diagnosed as having asthma according to American Thoracic Society (ATS) criteria: 12% reversibility in FEV₁ in spirometry, 15% reversibility in PEF after bronchodilator or 20% daily variability in PEF¹ (Table 1). In Finland, all conscripts are exposed to much outdoor exercise in the forest and thus have a similar exposure to allergens (same kind of accommodation, no animal exposure). Conscripts were consecutively included in the study if they were lifelong non-smokers, had used no inhaled or oral steroids or chromones during the preceding 2 months, and had no evidence of respiratory infections within the

Table 1 Characteristics of study subjects.

		Healthy subjects (n = 10)	Nonatopic asthma (n = 14)	Atopic asthma low-sensitization ^a (n = 13)	Atopic asthma high-sensitization ^a (n = 58)
Age	mean (range)	19.1 (18–20)	20.4 (18–26)	20.3 (18–26)	19.9 (18–27)
Height	mean (range)	179 (170–188)	177 (170–183)	176 (162–191)	175 (160–191)
Weight	mean (range)	74.8 (60–91)	76.0 (49–101)	69.7 (56–82)	76.5 (56–129)
Body mass index	mean (range)	23.3 (18–29)	24.2 (17–31)	22.3 (21–23)	23.8 (19–31)
FVC % of pred ^b	mean (SD)	104.2 (13.8)	97.5 (7.6)	102.7 (10.1)	102.1 (12.2)
FEV ₁ % of pred ^b	mean (SD)	102.4 (12.2)	88.5 (6.7)	94.7 (8.6) ^c	93.7 (9.6)
B-eos	mean (SD)	110.0 (37.3)	135.1 (91.7)	255.1 (274.1)	234.5 (146.3)
Number of positive reactions	mean (SD)	—	—	1.5 (0.5)	5.8 (2.0)
Mean wheal size	mm (SD)	—	—	4.3 (1.9)	6.0 (1.6)
Total atopy score	mean (SD)	—	—	6.23 (2.7)	35.0 (17.0)

FVC: forced expiratory vital capacity; FEV₁: forced expiratory volume in 1 s; and B-eos: blood eosinophils.

^a Total prick wheal sum of 3–10 mm, were regarded as low-sensitized, and those with >10 mm as high-sensitized.

^b Baseline values (Viljanen et al.).

^c Significant difference between nonatopic and low-sensitization atopic asthma; *p* < 0.05.

preceding 3 weeks. Inclusion criteria for forced expiratory volume in 1 s (FEV₁) was >70% of predicted. Use of inhaled short-acting beta-agonists was not allowed later than 12 h before the FENO and lung function measurements. In addition, 10 non-smoking, nonatopic healthy conscripts, who were hospitalized for non-respiratory reasons, volunteered to become our control group (Table 1).

Study design

On the first study day, flow–volume spirometry, a broncho-dilatation test, and skin prick tests were carried out, and on the second day, an exercise challenge was performed. FENO was measured at 7–8 a.m. on the third study day. The histamine challenge test was performed immediately after the FENO determination. No study measurements were performed during pollen season. This study was approved by the Ethics Committees of the Department of Medicine at Helsinki University Hospital and the Central Military Hospital. Each participant gave his written informed consent.

Spirometry

FEV₁, forced vital capacity (FVC), and the FEV₁/FVC ratio were determined with a flow–volume spirometer (Medikro M904, Kuopio, Finland) according to the European Respiratory Society guidelines,¹² with Finnish reference values for spirometry.¹³

Skin prick tests

Skin prick tests for assessment of atopy were performed with 13 common aeroallergens: alder, birch, cocksfoot, timothy, mugwort, horse, dog, cat, sheep's wool, house dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), *Alternaria*, and *Cladosporium*, with a solvent (glycerin) as the negative and histamine as the positive control. A wheal with a diameter of at least 3 mm in the presence of expected reactions to the control solutions was regarded as positive, and subjects with at least one positive reaction were regarded as atopic. Total prick wheal sum was calculated by adding the diameters of the wheals of each positive reaction excluding histamine.¹⁴ Subjects with a total prick wheal sum of 3–10 mm, were regarded as low-sensitized, and those with >10 mm were regarded as high-sensitized. Mean wheal size, number of positive responses, and type of sensitization were also analyzed.

Histamine challenge

BHR was assessed by histamine challenge with a dosimetric method and controlled tidal breathing.¹⁵ A provocative dose of histamine inducing a 15% decrease in FEV₁ (PD₁₅FEV₁) was calculated from the logarithmically transformed histamine doses by linear interpolation. The degree of bronchial hyperresponsiveness was classified with PD₁₅FEV₁ values. PD₁₅FEV₁ less than 0.1 mg was considered high, PD₁₅FEV₁ between 0.1 and 0.4 mg as moderate and PD₁₅FEV₁ between 0.4 and 1.6 mg as mild, respectively.

Exercise challenge

A standardized 8-minute test of running was performed by a previously described method¹⁶ outdoors on a 150-meter circular track between 9 and 11 a.m. Running speed was adjusted by monitoring each subject's heart rate with a Sport Tester™ PE 3000 heart rate meter (Polar Electro KY, Kempele, Finland). Subjects raised their heart rate to 85% of their predicted maximal rate, during a 2-minute warm-up, and maintained this rate (165–175 beats/min) for the remaining 6 min of the exercise. PEF values were measured just before the exercise and immediately and 5, 10, 20, and 30 min afterwards of three PEF measurements on each occasion, the highest value was recorded for analysis. The response to the exercise challenge was the maximum percentage fall in PEF (Δ PEF%) after exercise.

Measurement of FENO

FENO was measured as previously described¹⁷ with a chemiluminescence analyzer (Sievers 270B, Boulder, CO, USA). Expiratory airflow and exhaled volume were measured with pneumotachograph simultaneously with FENO in real time, an exhalation procedure performed according to European Guidelines on exhaled NO measurement.¹⁸ Before the measurement, patients rinsed their mouths with sodium bicarbonate solution to eliminate any nitric oxide (NO) eventually produced in the mouth. For measurements of end-expiratory NO, subjects inhaled 100% oxygen (NO-free) and then, without nose-clips, exhaled slowly from total lung capacity over a period of 15 s. They exhaled against a flow resistor (Hans Rudolph, Model #7100R, 100 cmH₂O/L/s, flow range 0–0.5 L/s) to close the soft palate, thus avoiding any nasal NO contamination. Exhalation flow in the flow window was between 0.08 and 0.15 L/s, and the mean individual exhalation flow ranged between 0.09 and 0.12 L/s. The mean value was recorded for a 3-second period from the end-exhaled NO plateau. For at least three successive measurements, the mean value was recorded for analysis. The acceptable coefficient of variation of a single measurement was less than 0.15.

Statistical methods

FENO and PD₁₅FEV₁ were skewed and log transformed before analysis to achieve a near normal distribution. FENO and PD₁₅FEV₁ levels were reported as medians and 25–75% quartiles, and the other parameters as means (SD). Differences between anthropometric data, FENO, PD₁₅FEV₁, and severity of EIB in the high-sensitized and low-sensitized atopic and nonatopic patients and healthy controls were analyzed with ANOVA. ANOVA tests were continued with Dunnett's test based on two hypotheses: In the first, healthy subjects served as the reference group. In the second hypothesis, the high-sensitized atopic asthma group served as the reference group. A separate comparison was between those with nonatopic asthma and low-sensitized atopic asthma.

Multiple linear regression analysis was undertaken using log (FENO) value as the outcome variable. The model

evaluated the severity of EIB (Δ PEF%), log (PD15FEV₁), B-eosinophils, atopy score, seasonal vs perennial sensitization, number of positive prick reactions, and size of each prick reaction with a stepwise method for atopic patients. Variables in the model were excluded in a stepwise fashion if they did not reach significance at the 5% level.

All tests were 2-tailed, with a *p*-value of less than 0.05 considered significant. All analyses were performed with SPSS version 15.0 (SPSS, Chicago, IL, USA).

Results

Of the 85 subjects, the vast majority, 71 were atopic, and of these 58 (82%) were high-sensitized (total prick wheal sum >10 mm) and 13 (18%) low-sensitized (total prick wheal sum 3–10 mm). Sensitization to perennial allergens was present in 89% of the high-sensitized and 62% of the low-sensitized subjects. For these four groups, see Table 1 with patient characteristics, basic pulmonary function, and differences in atopic status, and Table 2 and Fig. 1 with FENO levels and degree of BHR. The median for FENO in the high-sensitized atopic patients was more than double than that in the nonatopic asthmatics. Low-sensitized and nonatopic asthmatics had similar FENO levels. Elevated levels (FENO > 12 ppb)^{18,17} appeared in 57% of subjects with nonatopic asthma, whereas this level was exceeded by 71% of subjects with low- and 91% with high-sensitized atopic asthma.

FEV₁ of predicted value was slightly higher in low-sensitized atopic asthma subjects than in those with nonatopic asthma (Table 1). BHR to histamine and to exercise between subjects with nonatopic asthma and with low-sensitized atopic asthma were similar, but high-sensitized atopic asthmatics had significantly more severe BHR to both histamine and exercise than did those in nonatopic asthmatics (*p* < 0.05, both comparisons) (Table 2).

Linear regression analysis showed that perennial sensitization to cat, severity of EIB and blood eosinophils were the only significant determinants of FENO in atopic asthmatics (Table 3).

Discussion

Higher levels of FENO appeared in our nonatopic asthmatics than in healthy controls. Levels of FENO and BHR to histamine and to exercise in nonatopic asthmatics and low-sensitized atopic asthmatics were similar, but high-sensitized atopic asthmatics showed more severe BHR to both exercise and histamine and showed higher levels of FENO than did those in nonatopic and low-sensitized atopic asthmatics. In atopic asthmatics, the most important determinants of FENO were perennial sensitization to cat, severity of EIB and blood eosinophils.

Our study subjects were ideal for evaluating the association of atopy with FENO and BHR in asthma: symptomatic and steroid-naïve young non-smoking men with a recent diagnosis of asthma but without any interfering diseases. The skin prick tests included the most important allergens in Finland.¹⁹ The elevated FENO in 57% of our nonatopic asthmatics is consistent with earlier findings on the

Table 2 Exhaled nitric oxide and bronchial responsiveness to histamine and to exercise in study groups.

	Healthy subjects (n = 10)	Nonatopic asthma (n = 14)	Atopic asthma (n = 13)	Atopic asthma low-sensitization (n = 13)	Atopic asthma high-sensitization (n = 58)
FENO, ppb	6.5 (5.1–8.0) ^{§§§}	15.2 (9.7–24.7) ^{***§§§}	16.7 (10.8–25.6) ^{***§§§}	16.7 (10.8–25.6) ^{***§§§}	34.9 (21.3–53.8) ^{***}
PD15FEV ₁ , mg	– ^a	0.82 (0.38–3.2) [§]	1.1 (0.59–1.1) ^{§§}	0.37 (0.16–0.96)	0.37 (0.16–0.96)
PD15FEV ₁ < 1.6 mg	0	57	43	86	86
PD15FEV ₁ < 0.4 mg	0	29	14	53	53
EIB, % fall in PEF	ND	10.3 (7.7) ^{§§}	12.3 (6.8)	18.6 (14.1)	18.6 (14.1)
EIB, % fall in PEF > 10%	ND	36	69	78	78

FENO: exhaled nitric oxide; PD15FEV₁: provocative dose of histamine causing a 15% fall in FEV₁; EIB: exercise-induced bronchoconstriction; and ND: not done.

p* < 0.05, *p* < 0.01, ****p* < 0.001 compared to healthy subjects.

§*p* < 0.05, §§*p* < 0.01, §§§*p* < 0.001 compared to high-sensitized atopic asthma.

^a All values over 1.6 mg (no bronchial hyperreactivity).

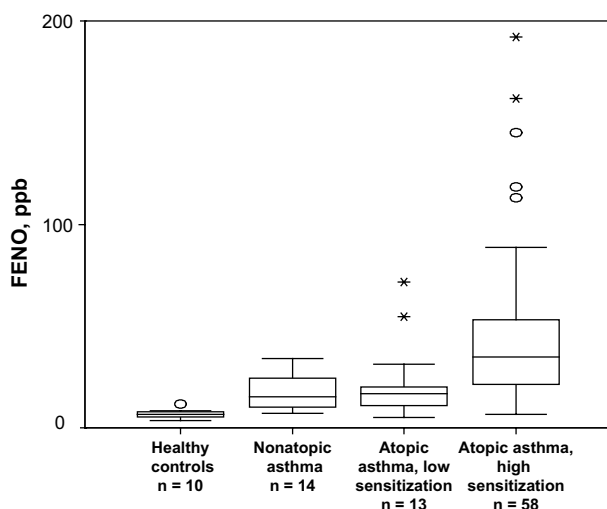


Figure 1 Boxplot presentation of the distribution of exhaled nitric oxide (FENO) in the groups (outliers are circles and extreme values are asterisks).

association of FENO level with asthma, irrespective of atopy,²⁰ and with a decrease in FENO in nonatopic asthmatics after treatment with inhaled steroids.²¹ In contrast, Ludviksdottir and coworkers found similar FENO levels in nonatopic and atopic asthma, but the majority of their patients were on inhaled steroids.⁴ In contrast with our results FENO levels have been similar in steroid-naïve nonatopic asthma, nonatopic rhinitis, and in nonatopic healthy subjects, which can in part explain the very stable or mild asthma during study.⁵

Our study confirms earlier findings that the FENO level is higher in atopic asthma than in nonatopic asthma.⁷ The mechanisms for this are still obscure. One study of bronchial biopsies has shown that the number of T-lymphocytes and interleukin-4 (IL-4) positive cells in bronchial biopsies of atopic asthmatics are higher than in nonatopic asthmatics,² furthermore, IL-4 is one of the most important cytokines inducing FENO production in asthmatic airways. Moreover, a study by Chistodoupoulos and coworkers has shown an increased number of α -IL-4R mRNA-positive cells in atopic asthmatic subjects compared to nonatopic asthmatics.²² T-lymphocytes and eosinophils thus may probably be more highly activated and may produce more cytokines in atopic asthma than in nonatopic asthma, which may account for the differing levels of FENO in nonatopic and atopic asthma. Our finding showed blood eosinophils to be one determinant of FENO both in atopic and nonatopic asthmatics. The similar FENO in nonatopic and low-sensitized atopic asthma probably reflects a similar low level of eosinophilic airway inflammation, even though the level of blood eosinophils is not similar, whereas the high eosinophilic bronchial inflammation may be more marked in the high-sensitized group.²³

Linear regression analysis showed, moreover, that in atopic asthmatic patients, the severity of EIB and number of blood eosinophils, and also sensitization to cats were the most important determinants of FENO. Our results are consistent with those of Olin and coworkers showing that being sensitized to perennial allergens in asthma raised the

Table 3 Multiple linear regression model with log (FENO) as dependent variable and severity of EIB (Δ PEF%), log (PD15FEV₁), B-eosinophils, atopy score, perennial sensitization, number of positive prick reactions, and each prick reaction with stepwise method in atopic patients ($n = 71$).

Variable	B	p-value	Partial correlation
Severity of EIB (Δ PEF%)	0.023	0.044	0.342
Log (PD15FEV ₁)	-0.0251	0.05	-0.254
B-eos	0.001	0.049	0.231
Atopy score	0.156	0.202	0.167
Perennial sensitization	0.239	0.049	0.255
Number of positive prick reactions	0.188	0.123	0.201
Birch	0.096	0.434	0.103
Alder	-0.043	0.725	-0.1504
Cocksfoot	0.142	0.253	0.15
Timothy	0.150	0.22	0.161
Mugwort	-0.022	0.856	-0.024
Horse	0.097	0.446	0.1
Dog	0.124	0.31	0.133
Cat	0.250	0.039	0.267
Sheep's wool	0.234	0.053	0.251
<i>Dermatophagoides farinae</i>	-0.078	0.488	-0.083
<i>Dermatophagoides pteronyssinus</i>	-0.191	0.092	-0.2
<i>Alternaria alternata</i>	-0.038	0.732	-0.041
<i>Cladosporium herbarum</i>	0.107	0.34	0.114

EIB: exercise-induced bronchoconstriction; Δ PEF%: maximum percentage fall in PEF after exercise challenge; PD15FEV₁: provocative dose of histamine causing a 15% fall in FEV₁; B: regression coefficient, and Partial correlation: correlation between dependent and each independent variable taking into account the other variables in the model.

level of FENO compared to asthmatic patients' sensitization to seasonal allergens and to that of nonatopic asthmatics.⁷ Moreover, atopic subjects sensitized only to house dust mite (HDM), independently of having asthma or not, had an increased level of FENO.²⁴ Furthermore, levels of FENO have been significantly higher in patients with asthma who were both sensitized and exposed to relevant allergen than in those who were sensitized but not exposed. FENO thus may be a marker of the airway inflammation induced by domestic exposure to allergens in sensitized asthmatics.²⁵ Sensitization to perennial allergens (e.g., cat and HDM) has also led to increase levels of FENO in asthma.⁸ In atopic children, elevated FENO was associated with sensitization to perennial allergens, but not to seasonal allergens such as grass pollen. Furthermore, an increase in FENO was associated with BHR and current wheezing, suggesting that FENO was more than just a marker for atopy.²⁶ In Finland HDM is not a major allergen because of central heating and dry indoor air, while cats produce the most important perennial allergen, especially for younger adults.¹⁹

To the best of our knowledge, this is the first study showing that steroid-naïve nonatopic asthmatics and low-sensitized atopic asthmatics have similar levels of FENO as well as BHR both to histamine and to exercise. Similar

exposure to relevant allergens during military service (same kind of accommodation, no animal exposure) may contribute. All study subjects had asthmatic symptoms during the previous month, which in atopic patients was found to be associated with FENO on population study.²⁷ In atopic patients sensitization to cats was strongly associated with FENO. Few ($n = 2$) subjects were sensitized to cats among the low-sensitized asthmatics, which also explain their FENO being similar to that of the nonatopics. The mean number of positive responses in skin prick tests was 5.7 in those high-sensitized and 1.4 in those low-sensitized, making contact with relevant allergens more likely for those high-sensitized. Furthermore, the mean size of the skin prick test response was clearly smaller in low-sensitized than in high-sensitized subjects, which also reflects the higher atopic tendency of the latter. The size of the skin prick test wheal correlates with the likelihood of clinical symptoms from that allergen.²⁸

Our finding that the level of BHR to histamine and exercise is lower in nonatopic asthma than in high-sensitized atopic asthma is consistent with previous findings.²⁹ Furthermore, asthmatics sensitized and then exposed to high levels of sensitizing allergens had lower baseline FEV₁ and more severe BHR than did asthmatics not sensitized and exposed to relevant allergens.⁸ Schwartz and coworkers showed in a population study that BHR to methacholine increased with number of positive skin prick tests.³⁰ In one retrospective study in asthmatics, skin prick reactivity to aeroallergens was also associated with BHR to methacholine.³¹ Furthermore, Rouhos and coworkers showed that among steroid-naïve patients with suspected asthma, only atopics showed a marked association between FENO and severity of exercise- or histamine-induced bronchoconstriction.⁹ In our study, the degree of atopy was significant where BHR to an indirect stimulus was concerned: The magnitude of EIB was higher in high-sensitized atopic asthmatics than in nonatopic asthmatics. Similarly, the reactivity to AMP has been higher in atopic than in nonatopic asthmatics.³² Differences in BHR between these groups may be explained by the recent finding that accumulation of mast cells in the smooth muscle compartment is more prominent in atopic than in nonatopic asthma.³³

Our study indicates that FENO is equally elevated in nonatopic asthma and in low-sensitized atopic asthma but lower than in high-sensitized atopic asthma among young adult conscripts. These differences in FENO between the asthma groups parallel the differences in airway function disturbance in terms of responsiveness to histamine or exercise in steroid-naïve non-smoking young men.

Conflict of interest statement

The authors have no conflict of interests.

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