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9 **Increased day-to-day fluctuations in exhaled breath profiles**
 10 **after rhinovirus challenge in asthma**

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12 **Fluctuations in electronic nose-based breath profiles**

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Conflict of interest statement

Ariana Lammers has nothing to disclose; **Paul Brinkman** has nothing to disclose; **Louwrina H. te Nijenhuis** has nothing to disclose; **Rianne de Vries** reports personal fees and other from Breathomix BV, outside the submitted work; **Yennece W.F. Dagelet** has nothing to disclose; **Erik Duijvelaar** has nothing to disclose; **Binbin Xu** has nothing to disclose; **Mahmoud I. Abdel-Aziz** has nothing to disclose; **Susanne J. Vijverberg** has nothing to disclose; **Anne H. Neerincx** has nothing to disclose; **Urs Frey** has nothing to disclose; **Rene Lutter** reports grants from European Respiratory Society/Marie Curie (EU), during the conduct of the study; **Anke H. Maitland - van der Zee** reports grants from GSK, grants from Boehringer Ingelheim, grants from Chiesi, personal fees from Astra Zeneca, personal fees from Boehringer Ingelheim, grants from Vertex, outside the submitted work; **Peter J. Sterk** reports other from being Scientific Advisor and having an officially non-substantial interest in the start-up company Breathomix BV that produces eNoses and accompanying cloud algorithms, during the conduct of the study; **Anirban Sinha** has nothing to disclose.

Abstract

Background: Early detection/prediction of flare-ups in asthma, commonly triggered by viruses, would enable timely treatment. Previous studies on exhaled breath analysis by electronic nose (eNose) technology could discriminate between stable and unstable episodes of asthma, using single/few time-points. To investigate its monitoring properties during these episodes, we examined day-to-day fluctuations in exhaled breath profiles, before and after a rhinovirus-16 (RV16) challenge, in healthy and asthmatic adults.

Methods: In this proof-of-concept study, 12 atopic asthmatic and 12 non-atopic healthy adults were prospectively followed thrice weekly, 60 days before and 30 days after a RV16 challenge. Exhaled breath profiles were detected using an eNose, consisting of 7 different sensors. Per sensor, individual means were calculated using pre-challenge visits. Absolute deviations (|%) from this baseline were derived for all visits. Within-group comparisons were tested with *Mann-Whitney U* tests and receiver operating characteristics (ROC) analysis. Finally, Spearman's correlations between the total change in eNose deviations and fractional exhaled nitric oxide (FeNO), cold-like symptoms and pro-inflammatory cytokines, were examined.

Results: Both groups had significantly increased eNose fluctuations post-challenge, which in asthma started one day post-challenge, before the onset of symptoms. Discrimination between pre- and post-challenge reached an area under the ROC curve of 0.82 (95% CI=0.65-0.99) in healthy and 0.97 (95% CI=0.91-1.00) in asthmatic adults. The total change in eNose deviations moderately correlated with IL-8 and TNF α ($p \approx 0.50-0.60$) in asthmatics.

Conclusion: eNose fluctuations rapidly increase after a RV16 challenge, with distinct differences between healthy and asthmatic adults, suggesting that this technology could be useful in monitoring virus-driven unstable episodes in asthma.

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Keywords: asthma, biomarkers, "omics" and systems biology, virus.

Accepted Article

90

91 One of the major burdens in asthma, are episodes of loss of
92 control and exacerbations,^{1,2} characterized by acute flare-ups of respiratory symptoms, such as
93 shortness of breath, cough, wheezing and chest tightness. Commonly, these episodes are triggered
94 by respiratory viral infections³, especially rhinoviruses⁴⁻⁶, and can potentially lead to emergency
95 visits and hospitalisations, possibly requiring urgent medical interventions^{1,2} and incurring high
96 healthcare expenses.

97

98 Unfortunately, episodes of loss of control are hard to detect due to the poor correlation between
99 clinical symptoms and the underlying disease activity⁷. Furthermore, predictors of upcoming
100 exacerbations in an individual patient are currently lacking, except that recent (severe) asthma
101 exacerbations are predictive of future (severe) exacerbations^{8,9}. Moreover, treatment is modestly
102 effective during exacerbations¹⁰, which emphasizes the importance of strategies that limit the
103 development of exacerbations, preferably in the pre-symptomatic phase. Robust biomarkers
104 indicating disease severity and control over time, could help predict (the severity of) episodic flare-
105 ups in asthma. A metabolomic approach might be able to sufficiently capture subtle changes in
106 asthma control, possibly before symptoms occur.

107

108 Analysis of the exhaled breath's metabolic content (i.e. breathomics) is relatively new and of
109 interest in asthma, because of its non-invasive character and its potential to detect changes of
110 inflammatory profiles in asthmatic patients¹¹. Exhaled breath consists of volatile organic
111 compounds (VOCs), which are gaseous organic molecules that either originate from the body
112 itself (endogenous) or from the environment (exogenous). Endogenous VOCs can reflect the
113 metabolic processes occurring in the lungs and beyond¹². The studies by Brinkman *et al.*¹³ and
114 Fens *et al.*¹⁴ showed that exhaled breath could distinguish clinically stable from unstable episodes
115 in asthmatic patients, however only three time-points (i.e. baseline, loss of control and recovery)
116 were compared with weeks to months between visits. Moreover, the studies by Robroeks *et*
117 *al.*¹⁵ and van Vliet *et al.*¹⁶ showed that exacerbations could be predicted based on two-monthly
118 exhaled VOC measurements, using an offline breath analysis technique. Furthermore, our group

119 has previously shown that breath profiles change after an experimental rhinovirus-16
120 (RV16)infection in healthy and asthmatic adults¹⁷.However, only single time-point comparisons
121 and group averages were examined. Now, studies investigating the potential of real-time exhaled
122 breath analysis tomonitor and predict such episodes on a day-to-day basisshould follow.

123
124 It is believed that biological and therebymetabolic processes fluctuate over timeto maintain
125 homeokinesis and that external triggers can influence such fluctuations¹⁸. These metabolic
126 fluctuations may be reflected in exhaled breath metabolites and possibly be of value in detection
127 and prediction of loss of control/exacerbations in asthma. Since most exhaled breath studies are
128 cross-sectional or longitudinalwith low temporal resolution, knowledge about the day-to-day
129 fluctuations in exhaled breath profiles, with and without external triggers, in patients and healthy
130 controls, is lacking.

131
132 We hypothesized thatthe day-to-dayfluctuations of exhaled breathwould change after an external
133 trigger, and that this response would differ between healthy controlsandasthmatic patients, due
134 to differences in biological processes.Our first objective wasto compare the day-to-day
135 fluctuations from personal baselines in exhaled breath profiles between asthmatic and healthy
136 controls,before and after a rhinovirus (RV) challenge. Our second objective was to investigate
137 whether the magnitude of the altered eNose fluctuations were linked to pre-challenge
138 inflammatory markers and was reflected in post-challenge symptoms and inflammation, to
139 identify possible differences in biological processes between and within groups.

Methods

Study design

In this prospective, intervention study, asthmatic patients and healthy controls were followed for 60 days before and 30 days after a RV16 challenge (Figure 1). The study was conducted at the Amsterdam UMC, location AMC (Amsterdam, The Netherlands) from February 2016 till June 2017. Before inclusion, participants were screened and provided informed consent. Prior to the start of the study period, participants went through a run-in phase to familiarize themselves with all the different measurements performed in the study. Exhaled breath analysis was performed 3 times per week using an electronic nose (eNose).

This study, along with the safety of the virus, were approved by the medical ethical committee of the Amsterdam UMC, location AMC (Amsterdam, the Netherlands) and registered at the Netherlands Trial Register (NTR5426).

Study population

In this study, 12 intermittent or mild-to-moderate atopic asthmatics (based on the Global Initiative for Asthma criteria 2014, www.ginasthma.org) and 12 non-atopic healthy controls were included, aged 18 – 35 years, all non-smokers. Atopy was based on a skin prick test with common aeroallergens (See Supplementary Information). Asthmatics had to be clinically stable at inclusion, defined with no use of corticosteroids and no exacerbations, 6 weeks prior to inclusion.

Subjects were excluded when they had a cold (4 weeks prior to screening), a RV16 titre $\geq 1:8$ in serum (at the time of screening and before the RV16 challenge visit), a positive PCR for any respiratory virus in nasal lavage (at the day before the RV16 challenge) or when they were pregnant. Details about the inclusion and exclusion criteria can be found in the published work by Sinha *et al.*¹⁸.

All participants were recruited via advertisements at the outpatient clinics of various hospitals in Amsterdam and via social media. Furthermore, subjects from previous study cohorts of our hospital were contacted, only when they provided informed consent to be invited for future research.

170 **Rhinovirus challenge**

171 After the 60-day pre-challenge phase, participants were exposed to RV16 using a standardized and
172 validated challenge approach, as previously described^{18,19}. An experimental RV16 infection was
173 used with a nasal dose of inoculum of 100 TCID₅₀; tissue culture infective dose of the virus
174 required to cause cytopathy in 50% of the cells. This has been considered safe for *in vivo* testing
175 in human volunteers, during a scientific advice meeting at BfArM (Bonn, Germany, April 30, 2013).
176 The RV16 was prepared under good manufacturing practice (GMP) as part of the U-BIOPRED
177 study and tested in a dose-dependent manner in healthy individuals and mild asthma patients
178 (manuscript in preparation), which revealed that 100 TCID₅₀ was the lowest dose that effectively
179 infected those exposed and caused expected symptomology.

180

181 **Exhaled breath analysis**

182 *Measurement setup*

183 Real-time exhaled breath analysis was performed using an eNose, the SpiroNose, connected in
184 series with a spirometer (SpiroPerfect™, Welch Allyn) (Figure S1). The SpiroNose consists of seven
185 different cross-reactive metal-oxide sensors (Table S1), present in fourfold; twice on the inside
186 and twice on the outside of the device. The mixture of VOCs in exhaled breath is detected by the
187 inner sensors during the exhalation, while ambient VOCs are detected by the outer sensors.

188

189 *eNose measurement*

190 The eNose measurement was performed as described previously²⁰. In short, subjects were asked
191 to rinse their mouth three times thoroughly with water. Subsequently, exhaled breath analysis was
192 performed in duplicate with a 2-min interval. All participants were instructed to perform five tidal
193 breaths, followed by a single inspiratory capacity maneuver up to total lung capacity, a 5-second
194 breath-hold and slow (<0.4 L/s) maximal expiration towards residual volume, with their nose
195 clipped. A new mouthpiece, bacterial filter and nose clamp was used for each subject.

196

197 *Data processing*

198 Processing of the eNose sensor data was performed using MATLAB® as described in De Vries *et al.*²⁰
199 and included filtering, detrending, ambient correction and automated peak detection. The

highest sensor peak of each sensor signal was selected as the variable for further analysis. All sensor peaks were normalized to the most stable sensor, sensor 2, to minimize the inter-array differences. Therefore, data from sensor 2 is not included in the fluctuation analysis.

203

204 **Other outcomes**

205 *Home monitoring*

206 During the study, several clinical parameters were monitored at home: spirometry, the Wisconsin
207 Upper Respiratory Symptom Survey (WURSS-21) and Asthma Control Questionnaire (ACQ).
208 Spirometry and the ACQ-6 (six questions, with a score range of 0-6)²¹ were monitored daily in the
209 morning at home by the volunteers themselves, using a hand-held spirometry device (MicroDiary,
210 CareFusion). Lung function parameters of interest were the forced vital capacity (FVC), forced
211 expiratory volume in one second (FEV₁) and peak expiratory flow (PEF), expressed as percentages
212 of predicted. The WURSS-21 questionnaire (see Supplementary material) monitoring started at
213 the day of challenge.

214

215 *FeNO*

216 Doublefractional exhaled nitric oxide (FeNO) measurements were performed using the NIOX
217 MINO (Aerocrine AB, Sweden) during the study visits (thrice weekly) at the Amsterdam UMC
218 (Amsterdam, the Netherlands), according to American Thoracic Society recommendations²². We
219 used the average of the two measurements in our analysis.

220

221 *Nasal lavages*

222 Nasal lavages were collected once weekly pre-challenge and thrice weekly post-challenge during
223 the study visits, as described previously¹⁸. Standardized washings collected from the nose were
224 used for cytokine analyses by luminex: IFN- γ , IL-1 β , IL-6, IL-8, IL-10, IL-13, IL-17A, IL-33, IP-10 and
225 TNF- α (after the cells were removed by centrifugation).

226

227 **Sample size**

228 This proof of concept study had an explorative nature and was based on initial estimates of
229 fluctuating inflammatory biomarkers. Our sample size of 12 individuals per group was based on

previous studies by Turner *et al.*^{23,24}, in which detection of temporal variability in exhaled VOCs was possible with fewer data points (once weekly, for 6 months). Moreover, our sample size provides adequate power for multi-omics analysis according to the study by Li *et al.*²⁵.

233

234 **Statistical analysis**

235 *Baseline characteristics and clinical presentation*

236 Baseline characteristics are presented as mean and standard deviation (SD) for normally distributed variables, median and interquartile range (IQR) for skewed data and n (%) for categorical variables. The distribution of the data was visually examined using histograms and Q–Q (quantile-quantile) plots. Differences between groups were compared using the *Mann-Whitney U* or *Kruskal-Wallis* test for continuous variables and Chi-square test for categorical variables. Lung function, FeNO, WURSS-21 and ACQ scores were summarized by calculating means of the pre- and post-challenge and minima/maxima of the post-challenge at an individual level, followed by medians at group level, to compare baseline (pre-challenge) with the (maximal) response to the RV challenge. For the WURSS-21 score, data from the day of the RV16 challenge and the first day post-challenge were used for the “pre-challenge” phase, as daily home monitoring started on the day of challenge.

247

248 *eNose deviations*

249 Fluctuations in the eNose signals were examined for the pre- and post-challenge phase separately, in both asthmatic and healthy volunteers. For each sensor and subject, means were calculated based on all pre-challenge visits, serving as an individual baseline ($\bar{x}_{baseline}$) (Figure 2A, left graph). Per subject, deviations from this personal baseline were derived for all study visits, expressed as absolute percentages; $|(x - \bar{x}_{baseline}) / \bar{x}_{baseline}|$ with x being an observation and $\bar{x}$ the sample mean (Figure 2B, right graph). Next, the mean of these absolute deviation percentages was calculated for the pre- ($\bar{x}_{dev,pre}$) and post-challenge ($\bar{x}_{dev,post}$) phase, at individual and group level, consecutively. Within and between group comparisons were made using *Wilcoxon signed-rank* and *Mann-Whitney U* testing, respectively. Because of multiple testing, False Discovery Rate (FDR) adjusted p-values (q-values) were also calculated²⁶. Finally, receiver operating characteristic (ROC)

analysis was performed to calculate the discriminative power of the individual sensor fluctuations to distinguish between pre- and post-challenge within and between groups.

Linking change in eNose deviations to inflammatory markers and symptoms

First, we calculated the overall change in eNose fluctuations by summing up all the differences between pre- and post-challenge mean deviation percentages of all sensors (i), at an individual level ($\sum \bar{x}_{i,dev,post} - \bar{x}_{i,dev,pre}$) (Figure 2B, left graph). Next, we explored the link between the magnitude of the change in eNose fluctuations and post-challenge cold-like symptoms (WURSS-21), as well as pre- and post-challenge inflammatory marker levels (FeNO and cytokines). For this, the FeNO, WURSS-21 and cytokine data were log10-transformed and averaged over the last 10 days pre- ($\bar{x}_{marker,pre}$) and the first 10 days post-challenge ($\bar{x}_{marker,post}$), separately, for each subject (Figure 2B, right graph). Finally, Spearman's rank correlations between the total deviation difference and the mean FeNO, cytokine levels or WURSS-21 scores, pre- and post-challenge separately, were determined.

Statistics were performed in R (version 3.6.1) combined with R packages "pROC" and "RVAideMemoire". P-values and q-values < 0.05 were considered significant.

Results

Study cohort

In total, 24 participants were included in this study; 12 healthy and 12 asthmatic participants (inclusion chart, Figure S2). At baseline, there were no major differences between groups regarding sex, age, ethnicity, body-mass index (BMI), pack years and lung function (Table 1). Only FeNO was significantly different between groups ($p < 0.01$), with a median of 14 ppb (IQR: 12 – 21) in healthy controls and 45 ppb (IQR: 30 – 63) in asthmatics. The number of visits were similar between and within groups, with on average ~23 visits (range 20-29) before and ~11 visits (range 10-16) after the RV16 challenge.

Clinical presentation (pre- and post-challenge)

Either or a combination of serum antibody tests, along with clinical symptoms and RV Polymerase Chain Reaction (PCR) conducted on nasal lavage samples, confirmed that all the study participants were successfully inoculated with the RV16, as published previously¹⁸. During the whole study period, no exacerbations/loss of control, as defined by Reddel *et al.*¹, occurred in the participants. The strongest clinical effect was seen in the WURSS-21 score, which increased after the RV16 challenge in both groups. The maximal WURSS-21 score occurred three days post-challenge, with an average score of 35 ± 33 in asthmatics and 19 ± 9 in healthy participants (Figure S3). Using descriptive statistics, we did not find major changes in lung function, ACQ score or FeNO (Table S2). However, in-depth analysis on the development of the response to the challenge was carefully studied before using time series analysis, as previously published¹⁸.

eNose deviations

The following comparisons were made regarding the eNose deviations: 1) pre-viral challenge with post-viral challenge states and 2) diseased (asthma) cohort with healthy (control) cohort.

Pre- vs post-challenge

For both groups, the mean deviation of the eNose signals increased after the rhinovirus challenge in the majority of the sensors (Figure 3, Table S3). For healthy subjects, mean deviations increased

significantly in sensor 5 ($\Delta=2\% \pm 2$; $p<0.05$) and 7 ($\Delta=8\% \pm 8$; $p<0.01$) and for asthmatics in sensor 1 ($\Delta=6\% \pm 7$; $p<0.05$), 4 ($\Delta=3\% \pm 4$; $p<0.05$), 5 ($\Delta=9\% \pm 7$; $p<0.001$) and 6 ($\Delta=3\% \pm 3$; $p<0.001$) (Figure 3). The change in mean deviations per subject are shown in Figure 4 and S4. The highest area under the ROC curve (AUROCC) for discrimination between pre- and post-challenge was found in sensor 5, for both healthy 0.82 (95%CI: 0.65-0.99) and asthmatic subjects 0.97 (95%CI: 0.91-1.00) (Table 2). The eNose fluctuations of sensor 5 increased (on average) directly one day after the RV challenge in asthmatics (Figure S5). This was less evident in healthy subjects.

Healthy vs Asthma

When comparing the two groups, healthy controls had significantly larger deviations ($8\% \pm 6$) than asthmatics ($6\% \pm 5$) before the RV16 challenge in sensor 1 ($p<0.01$) (Table S3), with an AUROCC of 0.82 (95% CI: 0.65-0.99) (Table 2). Post-challenge, mean deviations were larger in asthmatics compared to healthy controls in sensor 5 ($15\% \pm 13$ and $6\% \pm 5$, respectively; $p<0.001$) and 6 ($6\% \pm 5$ and $3\% \pm 3$, respectively; $p<0.01$), and *vice versa* for sensor 7 ($17\% \pm 15$ and $32\% \pm 26$, respectively; $p<0.01$) (Table S3). The highest AUROCC for discrimination in post-challenge eNose fluctuations between asthma and healthy was 0.94 (95% CI: 0.83-1.00) based on sensor 5 (Table 2).

All differences remained (almost) statistically significant ($q\leq 0.051$) after FDR adjustment; all p -values and q -values are shown in Table S4. Individual absolute deviations for all visits and each sensor, are depicted in Figures S6-S8. Pearson correlations between eNose sensor peak values are listed in Table S5 and show a strong correlation between sensor 5 and 6 ($R=0.91$) due to the cross-reactive nature of the sensors.

Linking change in eNose deviations to inflammatory markers and symptoms

The total change in absolute eNose sensor deviations (i.e. the change in mean deviations summed up for all sensors, per subject) was slightly larger in asthma (20.4%, IQR 7.3 – 23.5) than in healthy (11.7%, IQR 7.3 – 21.7), although not statistically significant ($p=0.48$) (Figure S9). Only for asthma, this total change in deviations moderately correlated ($R\approx 0.50$) with four cytokines (Table 3); an increase in eNose fluctuations was inversely correlated with pre- and post-challenge IL-8 levels (pre: $\rho=-0.50$, $p=0.10$; post: $\rho=-0.60$, $p<0.05$) and post-challenge IL-1 β ($\rho=-0.49$, $p=0.11$),

336 IL-17A ($\rho=-0.49$, $p=0.11$) and TNF- α ($\rho=-0.55$; $p=0.07$) levels. For all outcomes (i.e. FeNO, WURSS-
337 21, cytokines) in healthy and asthma (except for the four previously mentioned
338 cytokines), the absolute correlation coefficients were <0.49 and p -values ≥ 0.14 (Table 3).

Discussion

In this prospective 90-day follow-up study, day-to-day fluctuations in exhaled breath signals rapidly increased after a RV16 challenge, in both non-atopic healthy and atopic asthmatic volunteers. We could distinguish between a stable and relatively unstable period with high accuracy, reaching a maximal AUROC of 0.97 in asthmatics and 0.82 in healthy controls. Asthmatics with a relatively larger increase in eNose fluctuations, had (towards) significantly lower IL-1 β and IL-17A levels (pre- and post-challenge), and a trend towards lower IL-8 and TNF- α levels (pre-challenge), possibly due to differences in biological processes between groups. In both groups, the change in eNose fluctuations due to RV16 challenge did not correlate with FeNO and cold-like symptoms (WURSS-21).

To our knowledge, we are the first to examine fluctuations in exhaled breath profiles in such an extensive follow-up study, additionally with a highly controlled exposure, in a well-defined study cohort with cases and controls. Our study complements the study of Brinkman *et al.*¹³, in which unstable (loss of control induced by steroid withdrawal) and stable (baseline and recovery) asthma periods were correctly classified with 86-95% accuracy, using breath analysis detected by an eNose platform. However, in our study we did not provoke loss of control, so a direct comparison cannot be made. Our study extends the study of Brinkman *et al.*, as we had a controlled exposure and included more (frequent) time-points (days vs weeks/months) designed exactly around the viral inoculation event. Moreover, we have improved the accuracy for discriminating pre- and post-challenge phases evidently, when compared to our previous study by Abdel-Aziz *et al.*¹⁷. In that study, although not the main focus, the discrimination between pre- and post-challenge only reached an AUROC of 0.79 in asthma and 0.76 in healthy, using single time point comparisons and "raw" sensor peak values. The improved discriminative accuracy of our current analysis, shows the added value of considering all time points and personal baseline sensor values.

As we had hypothesized, fluctuations in the eNose signals existed regardless the exposure²⁷⁻²⁹ and increased after the RV exposure. Fluctuations play an important role in the adaptive capacity of physiological systems to respond to a changing environment and can be too rigid or overly

369 unstable in asthma^{30,31}. Analysis of lung function, nasal eosinophils and neutrophils, and FeNO
370 from the same study as ours, showed that the adaptive capacity was lower in asthma¹⁸, possibly
371 explaining the larger increase in eNose fluctuations after the RV16 challenge in asthmatics
372 compared to healthy controls.

373 Secondly, the increase in eNose fluctuations after the RV16 challenge was detected by
374 different sensors between the two groups. One explanation could be the cross-reactivity of the
375 sensors, meaning a compound can be detected by several sensors and *vice versa*, which makes it
376 possible that the same or similar compounds were involved between groups. Another explanation
377 could be differences in (patho)physiological mechanisms between healthy and asthmatic
378 participants that become more prominent when local cells are activated (e.g. by viral
379 infection). There are several indications that metabolic activity in local cells asthma differs from
380 that in healthy individuals³². These metabolites are often of low molecular weight that can be
381 detected by eNose technology. It is intriguing that we did find moderate inverse correlations
382 between the increase in eNose fluctuations and the cytokine levels of IL-1 β , IL-17A, IL-8 and TNF- α ,
383 and not for any other mediators. As macrophages are abundantly present in the nasal
384 compartment³³, and M1-like macrophages are activated by RVs³⁴ at an early stage, it is likely that
385 M1-like macrophages gave rise to these cytokine levels, which can lead to neutrophilic
386 inflammation³⁵. Allergic asthmatics have reduced numbers of M1-like macrophages during RV-
387 induced exacerbations³⁶, which may explain the differences in eNose fluctuations. In addition,
388 airway epithelial cells are activated at an early stage by RV and give rise to mediators like IL-1 β
389 and IL-8^{37,38}. Together, this suggests that the eNose may detect RV-induced metabolic changes.

390 Finally, we showed that the change in eNose fluctuations was not correlated with FeNO or
391 WURSS-21 scores. It has been shown before that exhaled breath profiles or VOCs minimally
392 correlate with FeNO^{13,39,40}. This could possibly be explained by eNose signals representing a
393 composite interplay of multiple molecular constituents, making it multidimensional as compared
394 to FeNO. Regarding symptoms, a study by van der Schee *et al.*⁴¹ showed differences in VOC
395 profiles between wheezing and asymptomatic children, regardless of the presence (AUROCC 0.77)
396 or absence (AUROCC 0.81) of a RV. This distinction remained accurate even after symptoms
397 recovered in the RV positive group (AUROCC 0.84), but not as accurate in the RV negative group

398 (AUROCC 0.67), illustrating how breath profiles may reflect complex inflammatory processes
399 and/or (pre-existing) biological host-response differences regardless of symptoms.

400

401 The first strength of this carefully designed study was the controlled RV exposure and the long
402 follow-up period of three months, with multiple measurements per week, throughout the study. On
403 top of that, we included both healthy and asthmatic volunteers, allowing for investigation of
404 differences in response to the RV16 challenge between a diseased and control group. Secondly,
405 we examined fluctuations at individual level first, using personal baselines, before looking at
406 group averages. This reduced the issue of averaging out individual effects and enabled us to
407 compare normal day-to-day fluctuations with that when triggered with a perturbation. Finally, we
408 made steps towards clinical applicability by using a non-invasive and real-time method, namely
409 eNose technology, for detection of the exhaled breath profiles.

410

411 This study also has a few limitations. First of all, the RV16 exposure induced cold-like
412 symptoms, but no loss of control or exacerbation in asthma. This was likely due to our choice to
413 investigate mild asthmatics and a relatively mild RV strain, a choice driven by ethical
414 concerns. Nevertheless, the eNose was capable to detect differences in breath profile fluctuations
415 between the pre- and post-challenge phases, as well as between groups. Secondly, exhaled
416 breath profiles detected by eNose technology cannot be directly linked to (patho)physiological
417 pathways, as eNose sensors cannot detect and identify single VOCs, due to their cross-reactivity.
418 However, the eNose possibly did reflect macrophage activity or downstream interacting
419 processes. On top of that, it is not easy to delineate differences between groups that arise from
420 disease or atopy, as we included non-atopic healthy and atopic asthmatic
421 volunteers. Nevertheless, the majority of asthmatic subjects in reality also suffer from atopy and
422 hence our study resembled a real-life scenario⁴², in which unknown allergic exposures may have
423 influenced the eNose fluctuations. Even so, it would still be of interest to study eNose fluctuations
424 in atopic healthy and non-atopic asthmatics as well, also with respect to the generalizability of our
425 results. Our method of fluctuation analysis (i.e. summarizing all deviations per study phase, at
426 individual and group level) may have caused a loss of temporal and quantitative information on
427 day-to-day fluctuations. Furthermore, we did not perform internal or external validation due to the

428 limited sample size and the unavailability of comparable data matching the sampling frequency to
429 this intensive study. However, adding multi-omics' analyses offsets the requirement for a large
430 sample size²⁵, although we admit that it does not excludethe possible risk of overfitting (i.e.
431 unrepresentative AUROCCs)^{43,44}. Consequently, we believe thatour data merit a prospective
432 longitudinal study with a larger cohort, preferably in a real-life setting,including daily home
433 monitoring of exhaled breath during stable and unstable periods of asthma.Finally, a sample size
434 estimation was not possible due to the unknown effect sizes of the novel VOC markers, which was
435 compensated by unprecedented high sampling frequency in individuals.

436
437 Although these results cannot be directly applied in clinical practice in the current form, they
438 show that exhaled breath analysis could potentially be an useful and additional tool in monitoring
439 disease instability, as it captures a more comprehensive biomarker signal than that merely
440 captured by FeNO and clinical symptoms (WURSS-21). In addition, the increase in eNose
441 fluctuations appeared to start before the onset of cold-like symptoms in asthma, supporting its
442 potential for patient management at the point-of-care. A better understanding of normal and
443 protective homeokinesis versus diseased anddamaging fluctuationsis required, to discover how
444 treatment could be guided and the development of exacerbations could be limited, in asthmatic
445 patients. Non-invasive monitoring of exhaled markers using eNose technology can play an
446 important role in this.

447 448 **Conclusion**

449 Day-to-day fluctuations in exhaled breath profiles rapidly increased after a RV16 challenge with
450 distinct differences betweennon-atopic healthy and atopic asthmatic volunteers.The increase in
451 fluctuations did not seem to correlate with cold-like symptoms and FeNO, but slightly with some
452 of the investigated pro-inflammatory biomarkers, making it a complementary tool for
453 investigation of disease stability monitoring and possibly treatment adjustment purposes, in a
454 non-invasive manner.Our data justify the design of a longitudinal home monitoring study in a
455 real-life setting and larger population.

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Table 1. Baseline characteristics and the number of visits per group

	Healthy (n=12)	Asthma (n=12)
Sex (female)	7 (58%)	8 (67%)
Age (years)	21 ($\pm$ 1.5)	22.2 ($\pm$ 2.2)
Ethnicity (Caucasian)	11 (92%)	9 (75%)
BMI (kg/m ²)	22.2 ($\pm$ 1.6)	22.8 ($\pm$ 3.1)
Smoking (pack years)	1 ($\pm$ 0.17)	–
Baseline spirometry		
FEV ₁ % of predicted	106 ($\pm$ 12)	101 ($\pm$ 10)
FVC % of predicted	104 ($\pm$ 11)	104 ($\pm$ 10)
PEF % of predicted	108 ($\pm$ 14)	105 ($\pm$ 12)
FeNO (ppb)	14 (12 – 21)*	45 (30 – 63)*
Number of visits (<i>mean; range</i>)		
Total	34 (30 – 40)	35 (33 – 38)
Before challenge	23 (20 – 29)	23 (21 – 26)
After challenge	11 (10 – 12)	12 (10 – 16)

Data are presented as mean ($\pm$ SD), median (IQR), n (%) or else when stated, which have partly been published previously ¹⁸. BMI = body mass index; FEV₁ = forced expiratory volume in 1s; FVC = forced vital capacity; PEF = peak expiratory flow; FeNO = fractionalexhaled nitric oxide. Differences in the number of visits were due to personal reasons (i.e. missing visits) or when there was a delay in the RV16 challenge due to logistical reasons (i.e. extra visits).

* = significant difference (p<0.01)

Table 2.Area under the ROC curves for discrimination between study phase and groups

Sensor	Healthy pre vs. post	Asthma pre vs. post	Pre healthy vs. asthma	Post healthy vs. asthma
1	0.67 (CI: 0.44-0.90)	0.76 (CI: 0.55-0.96)	0.82 (CI: 0.65-0.99)	0.54 (CI: 0.27-0.81)
3	0.69 (CI: 0.46-0.92)	0.51 (CI: 0.26-0.76)	0.63 (CI: 0.39-0.87)	0.74 (CI: 0.52-0.95)
4	0.64 (CI: 0.40-0.88)	0.67 (CI: 0.43-0.91)	0.61 (CI: 0.37-0.85)	0.52 (CI: 0.27-0.77)
5	0.82 (CI: 0.65-0.99)	0.97 (CI: 0.91-1.00)	0.62 (CI: 0.39-0.86)	0.94 (CI: 0.83-1.00)
6	0.53 (CI: 0.28-0.78)	0.77 (CI: 0.57-0.97)	0.59 (CI: 0.34-0.84)	0.81 (CI: 0.64-0.99)
7	0.71 (CI: 0.49-0.92)	0.56 (CI: 0.32-0.81)	0.71 (CI: 0.49-0.93)	0.88 (CI: 0.74-1.00)

The area under receiver operating characteristic curve (AUROCC) for discrimination between pre- and post-challenge, as well as healthy and asthma, using mean sensor deviations. Numbers in bold are AUROCC>0.80; CI = 95% confidence interval.

Table 3. Correlation between total change in eNose fluctuations and symptoms or inflammation

	Healthy				Asthma			
	Pre		Post		Pre		Post	
	ρ (95%CI)	p-value	ρ (95%CI)	p-value	ρ (95%CI)	p-value	ρ (95%CI)	p-value
FeNO	-0.16 (-0.64 – 0.47)	0.62	0.20(-0.52 – 0.73)	0.53	0.00 (-0.73 – 0.61)	1.00	0.03 (-0.72 – 0.61)	0.94
WURSS-21	-	-	0.41(-0.23 – 0.79)	0.18	-	-	-0.14 (-0.72 – 0.55)	0.67
IFN-γ	-0.15(-0.71 – 0.52)	0.65	0.19(-0.45 – 0.76)	0.56	0.12(-0.59 – 0.68)	0.70	-0.03(-0.66 – 0.56)	0.92
IL-1β	0.17 (-0.54 – 0.75)	0.60	0.41 (-0.27 – 0.87)	0.18	-0.36 (-0.87 – 0.37)	0.26	-0.49 (-0.89 – 0.21)	0.11
IL-10	-0.13 (-0.77 – 0.63)	0.70	0.30 (-0.41 – 0.83)	0.34	-0.28 (-0.73 – 0.30)	0.38	-0.22 (-0.77 – 0.42)	0.48
IL-13	0.26 (-0.40 – 0.73)	0.42	0.34 (-0.41 – 0.87)	0.28	-0.20 (-0.77 – 0.48)	0.54	-0.49 (-0.82 – 0.12)	0.11
IL-17A	0.06 (-0.55 – 0.62)	0.85	0.14 (-0.53 – 0.72)	0.67	-0.37 (-0.89 – 0.33)	0.24	-0.45 (-0.87 – 0.18)	0.14
IL-33	-0.11 (-0.69 – 0.57)	0.73	-0.05 (-0.61 – 0.51)	0.89	0.00 (-0.70 – 0.66)	1.00	0.01 (-0.67 – 0.80)	0.99
IL-6	0.33 (-0.32 – 0.82)	0.30	0.05 (-0.57 – 0.68)	0.89	-0.26 (-0.81 – 0.44)	0.42	-0.41 (-0.76 – 0.24)	0.18
IL-8	0.29 (-0.40 – 0.80)	0.37	0.29(-0.33 – 0.81)	0.37	-0.50 (-0.98 – 0.10)	0.10	-0.60 (-0.89 – -0.03)	0.04
IP-10	-0.38 (-0.93 – 0.41)	0.22	-0.01 (-0.60 – 0.58)	0.97	-0.19 (-0.80 – 0.43)	0.56	-0.36 (-0.89 – 0.38)	0.26
TNF-α	0.22 (-0.45 – 0.73)	0.50	0.25(-0.41 – 0.83)	0.43	-0.45 (-0.90 – 0.23)	0.14	-0.55 (-0.92 – 0.01)	0.07

For FeNO, WURSS-21 and the cytokine levels, individual means were calculated based on 10 days pre- or post-challenge; WURSS-21 scores were only available

post-challenge. Next, Spearman's rank correlation coefficients (ρ) and bootstrapped ($n=1000$) 95% confidence intervals (CI) were calculated between the total change in eNose fluctuations and FeNO, WURSS-21 or cytokines in nasal lavage. Results with p-values <0.05 and/or $p \geq 0.50$ are marked in bold. FeNO = fractional exhaled nitric oxide; WURSS = Wisconsin Upper Respiratory Symptom Survey.

Figure legends

Figure 1: Study design. Overview of study design: all participants were screened (1 visit) to test eligibility and had a run-in phase (2 visits) to make participants familiarized with all measurements. During the study period of 90 days, participants had around 2-3 visits per week, with ~20 visits in the pre-challenge phase (60 days) and ~10 visits in the post-challenge phase (30 days).

Figure 2: Calculation of eNose deviations. Data from one subject with the vertical gray lines representing the RV challenge. **A)** The left graph shows the sensor values (dB) of one sensor, for all visits pre- and post-challenge. The mean of the sensor values pre-challenge (blue solid line, $\bar{x}_{baseline}$) served as the baseline, also for the post-challenge phase (blue dashed line). Next, deviations from this personal baseline were calculated (blue double arrows) and expressed as absolute percentages (|%|), as shown on the right. Finally, the average deviation pre- ($\bar{x}_{dev,pre}$) and post-challenge ($\bar{x}_{dev,post}$) were calculated and the difference (Δ_{sensor}) between these, per subject and sensor. **B)** The left graph shows the deviations from the personal baseline for all sensors (S1, S3-S7), expressed as absolute percentages (|%|). The difference in deviations (Δ_{sensor}) between the pre- and post-challenge phase was calculated for each sensor and summed to determine the total change in deviations ($\Sigma\Delta_{all\ sensors}$). On the right, data from one of the markers of interest (FeNO, cytokines and WURSS-21) are shown, from which 10-day averages were calculated for the pre- ($\bar{x}_{marker,pre}$) and post-challenge ($\bar{x}_{marker,post}$) phase (note that 10 days $\neq$ 10 visits). Finally, Spearman's rank correlations were calculated between the total change in deviation and the pre- and post-averages of the marker of interest, separately.

Figure 3: Total change in absolute eNose deviations (per sensor). The change (Δ = post - pre) in eNose fluctuations after the RV16 challenge, expressed as absolute mean deviation percentages (|%|). First, the difference between the personal mean deviations (pre- and post-challenge) was determined (Δ_{sensor}). Next, at group level, the average differences were calculated and are depicted in the graph for each sensor separately. In healthy controls sensor 5 ($p < 0.05$) and sensor 7 ($p < 0.01$) showed significant increases in fluctuations post-challenge, while in asthmatics sensor 1, 4 (both $p < 0.05$), 5 and 6 (both $p < 0.01$) were significantly different. * = significant difference ($p < 0.05$).

621 **Figure 4: Absolute eNose deviations pre- and post-challenge.** Absolute mean deviation percentages
622 (|%|) of all healthy (top) and asthmatic (bottom) participants. Only sensors with significant differences (also
623 after FDR adjustment) between the pre- (**blue**) and post-challenge (**orange**) phase, for either one or both
624 groups, are shown. Each dot represents the personal mean deviation pre- or post-challenge, connected by
625 a line, to visualize individual differences between the two study phases. Note that scales differ between
626 sensors, but not between groups.

Appendices

For additional information, tables and figures, we kindly refer to the supplementary material, which is supplied in a separate file.

Figures

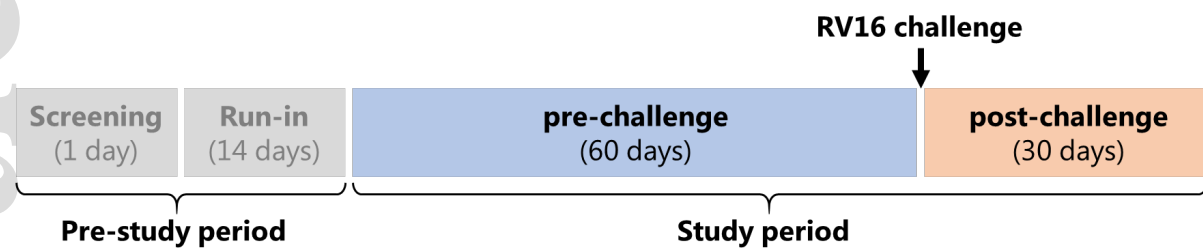


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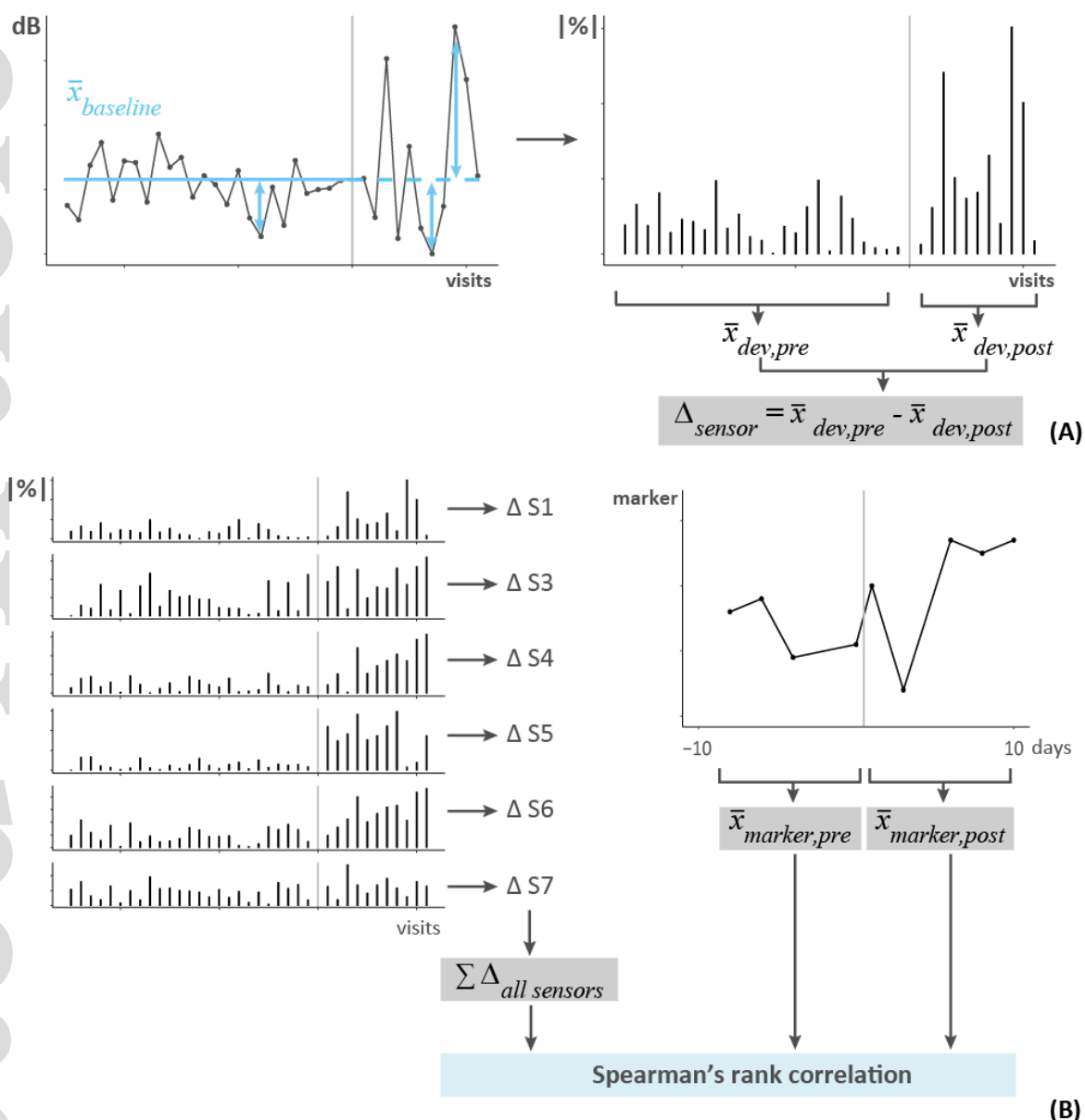


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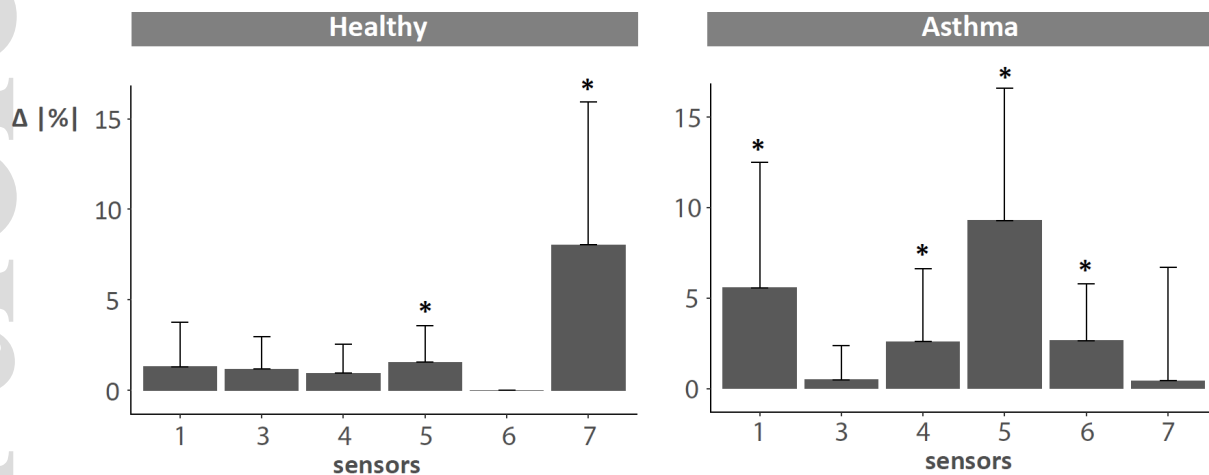


Figure 3: Total change in eNose fluctuations (per sensor). the change (Δ = post - pre) in eNose fluctuations after the RV16 challenge, expressed as absolute mean deviation percentages (|%|). First, the difference between the personal mean deviations (pre- and post-challenge) was determined (Δ_{sensor}). Next, at group level, the average differences were calculated and are depicted in the graph for each sensor separately. In healthy controls sensor 5 ($p < 0.05$) and sensor 7 ($p < 0.01$) showed significant increases in fluctuations post-challenge, while in asthmatics sensor 1, 4 (both $p < 0.05$), 5 and 6 (both $p < 0.01$) were significantly different. * = significant difference ($p < 0.05$).

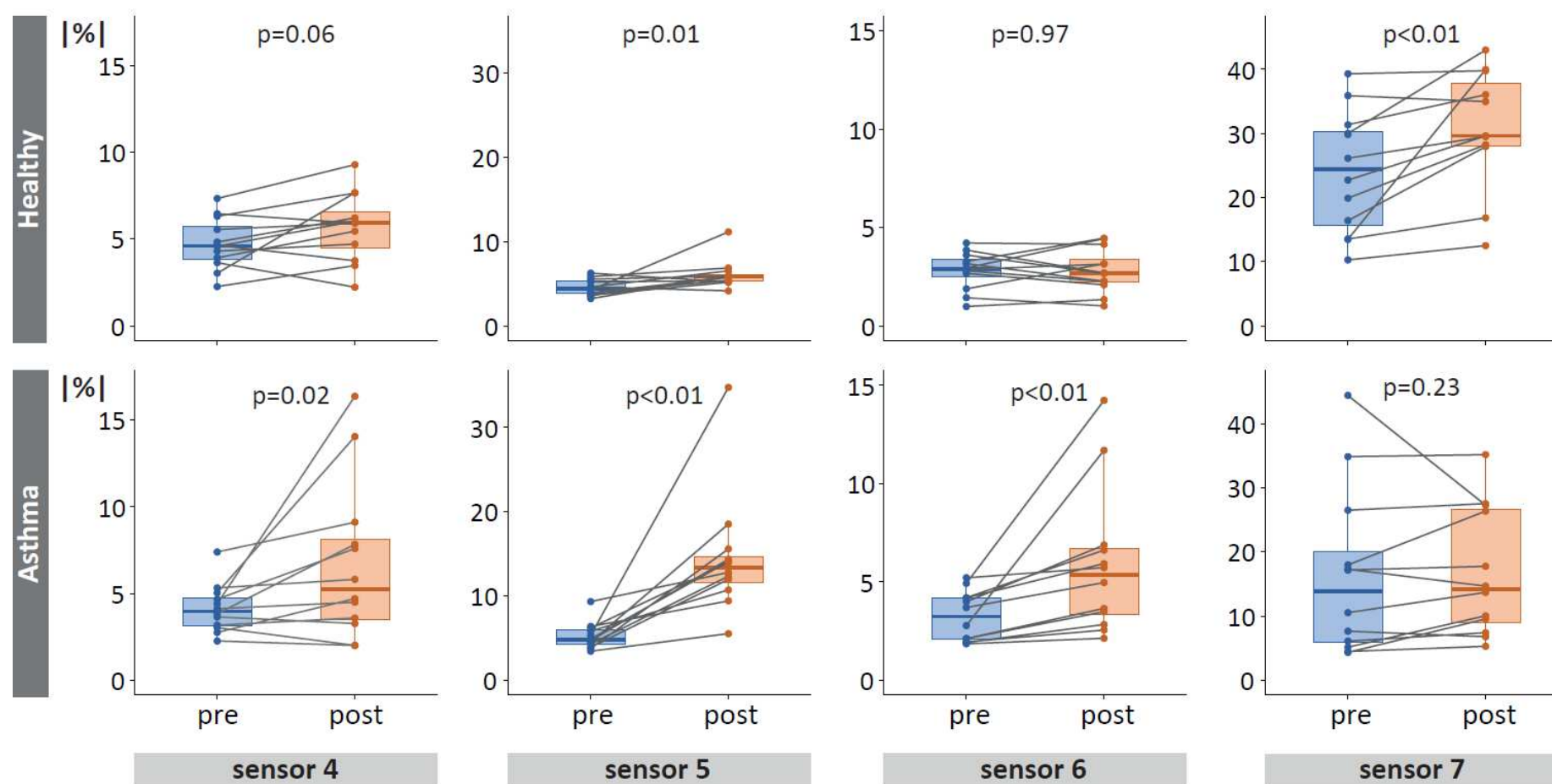


Figure 4: eNose fluctuations pre- and post-challenge. Absolute mean deviation percentages (%) of all healthy (top) and asthmatic (bottom) participants. Only sensors with significant differences (also after FDR adjustment) between the pre- (blue) and post-challenge (orange) phase, for either one or both groups, are shown. Each dot represents the personal mean deviation pre- or post-challenge, connected by a line, to visualize individual differences between the two study phases. Note that scales differ between sensors, but not between groups.

Supplementary material

Title: Increased day-to-day fluctuations in exhaled breath profiles after a rhinovirus challenge in asthma

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Methods

Skin prick test

Participants were considered atopic when allergic to one or more of 12 common aero allergens. The 12 common aero allergens tested were grass mix, tree mix, house dust mite, cat, dog, rabbit, guinea pig, cockroach, alternaria alternata, aspergillus fumigatus, cladosporium herbarum and latex. As a control, histamine and buffer control were tested.



Figure S1: eNose. The eNose (3) was attached to the rare end of a spirometer (1) and a bacterial filter (2) (left, side view). The eNose consists of four sensor arrays (two in duplicate) on the inner side of the eNose (blue circle), detecting the exhaled breath, and four on the outer side for detection of the environment (yellow circles) (right, front view).

Table S1. SpiroNose sensor type, sensitivity and detection range.

Sensor	Type	Highest sensitivity to	Range (ppm)
1	TGS 2602	VOCs (e.g. toluene) and odorous gases (e.g. ammonia and hydrogen sulphide)	1 – 30
2	TGS 2610	Butane and propane	500 – 10,000
3	TGS 2611-COO	Methane and natural gases	500 – 10,000
4	TGS 2600	Hydrogen, carbon monoxide and ethanol	1 – 30
5	TGS 2603	Trimethylamine and methyl mercaptan	1 – 30
6	TGS 2620	Alcohol and solvent vapors	50 – 5,000
7	TGS 2612	Methan, propane and iso-butane	500 – 10,000

ppm = parts per million, VOCs = volatile organic compounds. A similar table has been published previously (Ibrahim *et al.*, Allergy 2020, de Vries *et al.*, Ann Oncol 2019).

Wisconsin Upper Respiratory Symptom Survey – 21 --- *Daily Symptom Report*

Day:

Date:

Time:

ID:

Please fill in one circle for each of the following items:

	Not sick 0	Very mildly 1	2	Mildly 3	4	Moderately 5	6	Severely 7
How sick do you feel today ?	☐	☐	☐	☐	☐	☐	☐	☐

Please rate the average severity of your cold symptoms over the last 24 hours for each symptom:

	Do not have this symptom 0	Very mild 1	2	Mild 3	4	Moderate 5	6	Severe 7
Runny nose	☐	☐	☐	☐	☐	☐	☐	☐
Plugged nose	☐	☐	☐	☐	☐	☐	☐	☐
Sneezing	☐	☐	☐	☐	☐	☐	☐	☐
Sore throat	☐	☐	☐	☐	☐	☐	☐	☐
Scratchy throat	☐	☐	☐	☐	☐	☐	☐	☐
Cough	☐	☐	☐	☐	☐	☐	☐	☐
Hoarseness	☐	☐	☐	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐	☐	☐	☐
Feeling tired	☐	☐	☐	☐	☐	☐	☐	☐

Over the last 24 hours, how much has your cold interfered with your ability to:

	Not at all 0	Very mildly 1	2	Mildly 3	4	Moderately 5	6	Severely 7
Think clearly	☐	☐	☐	☐	☐	☐	☐	☐
Sleep well	☐	☐	☐	☐	☐	☐	☐	☐
Breathe easily	☐	☐	☐	☐	☐	☐	☐	☐
Walk, climb stairs, exercise	☐	☐	☐	☐	☐	☐	☐	☐
Accomplish daily activities	☐	☐	☐	☐	☐	☐	☐	☐
Work outside the home	☐	☐	☐	☐	☐	☐	☐	☐
Work inside the home	☐	☐	☐	☐	☐	☐	☐	☐
Interact with others	☐	☐	☐	☐	☐	☐	☐	☐
Live your personal life	☐	☐	☐	☐	☐	☐	☐	☐

Compared to yesterday, I feel that my cold is...

Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse
☐	☐	☐	☐	☐	☐	☐

WURSS -21® (Wisconsin Upper Respiratory Symptom Survey) 2004

Created by Bruce Barrett MD PhD et al., UW Department of Family Medicine, 777 S. Mills St. Madison, WI 53715, USA

Results

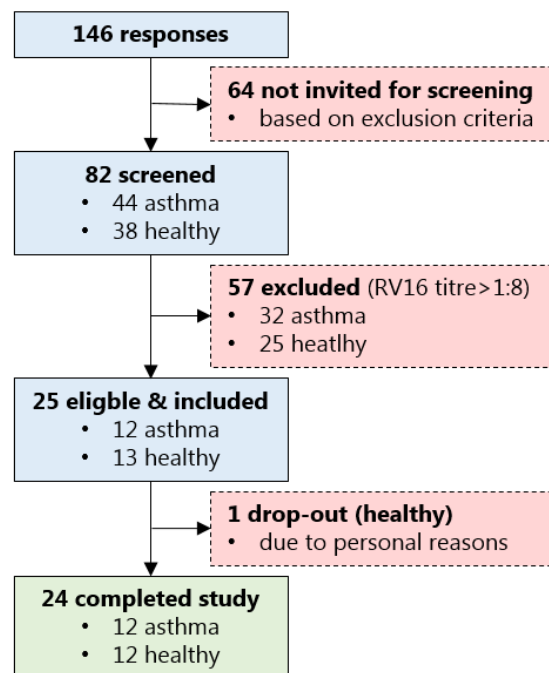


Figure S2: Inclusion flow chart. In total, 146 people were contacted of whom 64 met the exclusion criteria. From the 82 people who were screened, 57 had RV16 antibody levels >1:8 in serum. For the healthy group, we included 13 participants, as one subject had to drop-out because of personal reasons. Eventually 12 asthmatics and 12 healthy individuals completed the study.

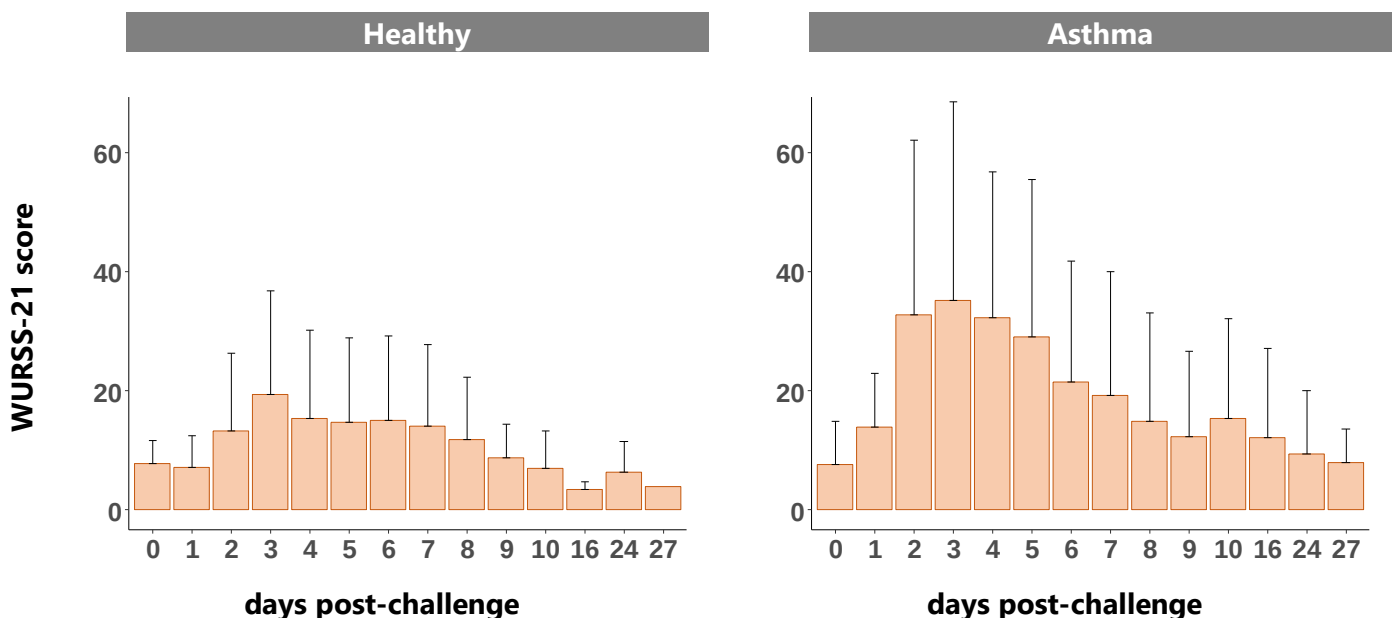


Figure S3: WURSS-21 scores post-challenge (per day). Wisconsin Upper Respiratory Symptom Survey (WURSS-21) scores per day for healthy (left) and asthmatic (right) volunteers expressed as means ($\pm$ SD). After visit 0, volunteers received the RV16 challenge. The study by Barrett *et al.* reported that the minimal clinical important difference (MCID) of the WURSS-21 score was 10.3 (Barrett, Health Qual Life Outcomes, 2009).

Table S2. Clinical presentation pre- and post-challenge

	Healthy (n=12)			Asthma (n=12)		
	Pre Median (IQR) of <u>mean</u>	Post Median (IQR) of <u>mean</u>	Median (IQR) of <u>min/max</u>	Pre Median (IQR) of <u>mean</u>	Post Median (IQR) of <u>mean</u>	Median (IQR) of <u>min/max</u>
Spirometry						
FVC (%) ^a	80 (78 – 89)	74 (67 – 87)	65 (57 – 81)	89 (77 – 91)	84 (77 – 91)	78 (69 – 85)
FEV ₁ (%) ^a	88 (82 – 91)	84 (76 – 90)	76 (68 – 85)	88 (74 – 90)	87 (74 – 90)	81 (68 – 85)
PEF (%) ^a	90 (84 – 100)	92 (77 – 104)	80 (60 – 98)	88 (81 – 93)	88 (78 – 95)	77 (58 – 86)
FeNO (ppb) ^b	14 (13 – 16)	16 (11 – 19)	26 (15 – 31)	46 (36 – 56)	43 (30 – 59)	78 (41 – 86)
WURSS-21 ^c	6 (4 – 10)	9 (6 – 17)	16 (14 – 28)	10 (6 – 18)	18 (8 – 35)	48 (18 – 70)
ACQ ^c	0.3 (0.3 – 0.3)	0.3 (0.3 – 0.3)	0.3 (0.3 – 0.3)	0.3 (0.3 – 0.4)	0.4 (0.4 – 0.4)	0.7 (0.5 – 0.9)

The results are expressed as medians and interquartile ranges (IQR) of the individual means (pre and post) or minima/maxima (post), to compare baseline with the (maximal) response to the RV challenge. First, the mean of all visits pre- and post-challenge and the minimum (spirometry data) or maximum (FeNO, WURSS-21 and ACQ) of the post-challenge were calculated at an individual. Next, the median (IQR) of the means pre- and post-challenge and minima/maxima post-challenge were calculated at group level. A more in depth analysis on this data has been previously published (Sinha *et al.*, eLife 2019).

a = based on daily home monitoring during the whole study period; b = based on clinical visits during the whole study period; c = based on daily home monitoring starting from the day of challenge, in which 'pre' was based on data from the day of challenge and 1 day post-challenge. FVC = forced vital capacity; FEV₁ = forced expiratory volume in 1s; PEF = peak expiratory flow; FeNO = fractional exhaled nitric oxide; WURSS = Wisconsin Upper Respiratory Symptom Survey; ACQ = asthma control questionnaire.

Table S3. Overview of eNose absolute deviation percentages per group and phase

Sensor	Healthy			Asthma	
	pre	post		pre	post
1	8.0 (±5.7)	9.3 (±6.9)		5.6 (±4.8)	11.1 (±12.9)
3	4.5 (±4.0)	5.8 (±4.0)		3.6 (±3.2)	4.0 (±3.5)
4	4.7 (±4.0)	5.7 (±4.9)		4.2 (±3.8)	6.7 (±7.0)
5	4.5 (±3.4)	6.1 (±4.5)		5.3 (±4.5)	14.5 (±13.3)
6	2.8 (±2.6)	2.8 (±2.6)		3.3 (±3.0)	5.9 (±5.4)
7	24.0 (±19.5)	32.1 (±26.2)		16.3 (±19.5)	16.8 (±14.7)

Group averages of absolute deviation percentages (%), expressed as mean (±SD), per study phase.

Table S4. P- and q-values for differences in eNose deviation percentages between study phases and groups

Sensor	Healthy		Asthma		Pre		Post	
	pre vs. post		pre vs. post		healthy vs. asthma		healthy vs. asthma	
	p-value	q-value	p-value	q-value	p-value	q-value	p-value	q-value
1	0.077	0.092	0.034	0.051	0.007	0.042	0.755	0.887
3	0.064	0.092	0.677	0.677	0.291	0.454	0.052	0.078
4	0.064	0.092	0.021	0.042	0.378	0.454	0.887	0.887
5	0.012	0.036	0.000	0.000	0.319	0.454	0.000	0.000
6	0.970	0.970	0.000	0.000	0.478	0.478	0.008	0.016
7	0.001	0.006	0.233	0.280	0.089	0.267	0.001	0.003

P-values and false discovery rate (FDR) adjusted p-values (q-values) of the differences between study phases (pre vs. post) per group and between groups (asthma vs. healthy) per study phase; using *Wilcoxon signed rank* test and *Mann-Whitney U* test, respectively. Numbers in bold are significantly different ($p < 0.05$).

Table S5. Correlations between eNose sensor peak values

	S1	S3	S4	S5	S6	S7
S1		0.23	0.16	0.00	0.08	-0.41
S3	0.23		-0.42	-0.15	-0.17	0.04
S4	0.16	-0.42		0.35	0.53	-0.34
S5	0.00	-0.15	0.35		0.91	-0.56
S6	0.08	-0.17	0.53	0.91		-0.63
S7	-0.41	0.04	-0.34	-0.56	-0.63	

Pearson correlations for all eNose measurements, with $R > 0.70$ marked in **bold**.

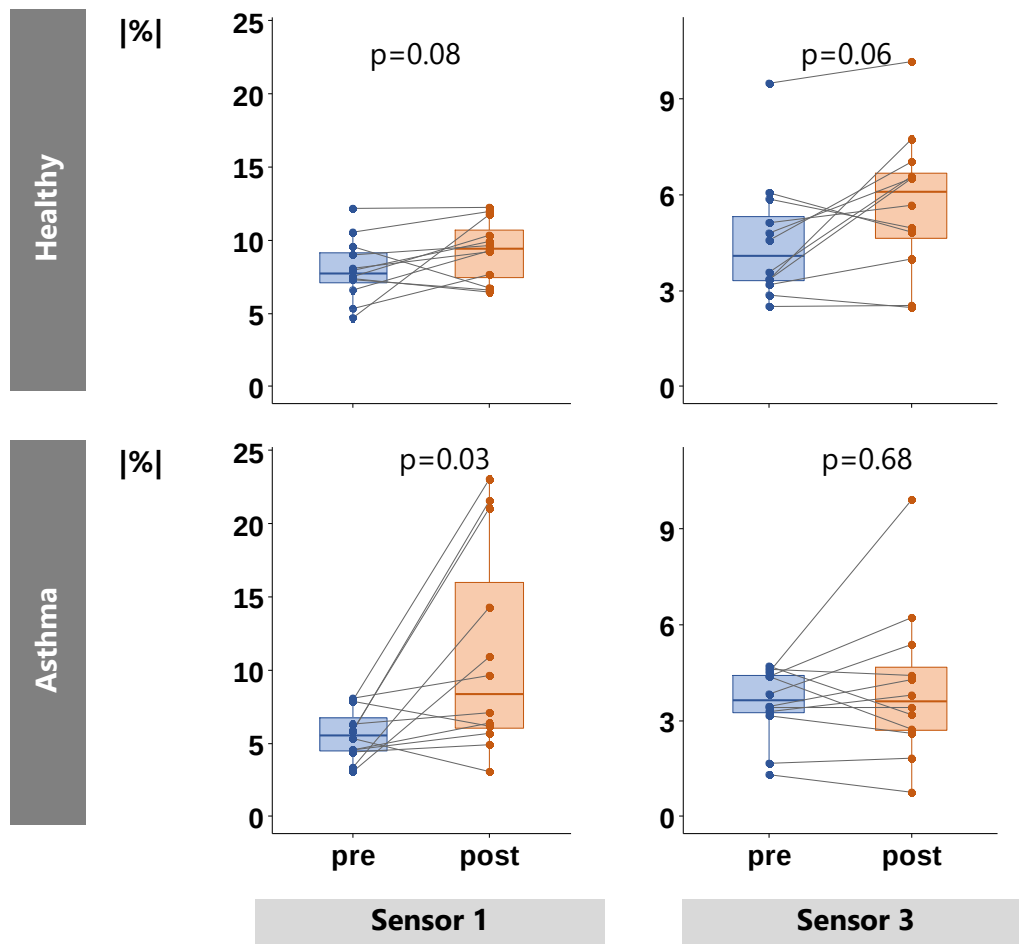


Figure S4: eNose deviations pre- and post-challenge. Absolute mean deviation percentages (|%|) for healthy (top) and asthmatic (bottom) participants. Only sensors that were not significantly different (after FDR adjustment) between the pre- (blue) and post-challenge (orange) phase for both groups, are shown. Each dot represents the personal mean deviation pre- or post-challenge, connected by a line, to visualize individual differences between the two phases.

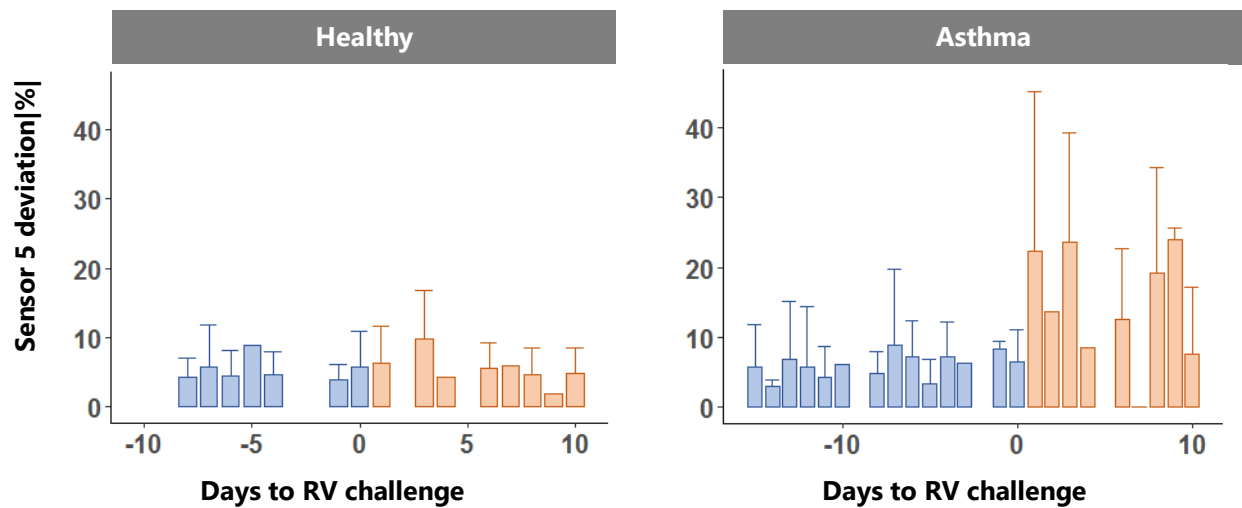


Figure S5: Absolute deviation percentages per day (sensor 5). Deviations in sensor 5 over time (days to or from the RV challenge) expressed as absolute deviation percentages (mean $\pm$ SD). The pre-challenge visit days are marked in **blue** and the post-challenge days in **orange**. For (some) asthma patients, a direct increase in eNose fluctuations is visible one day after the RV challenge. On certain days, eNose measurements were performed in one (no SD) or none of the participants (no bar).

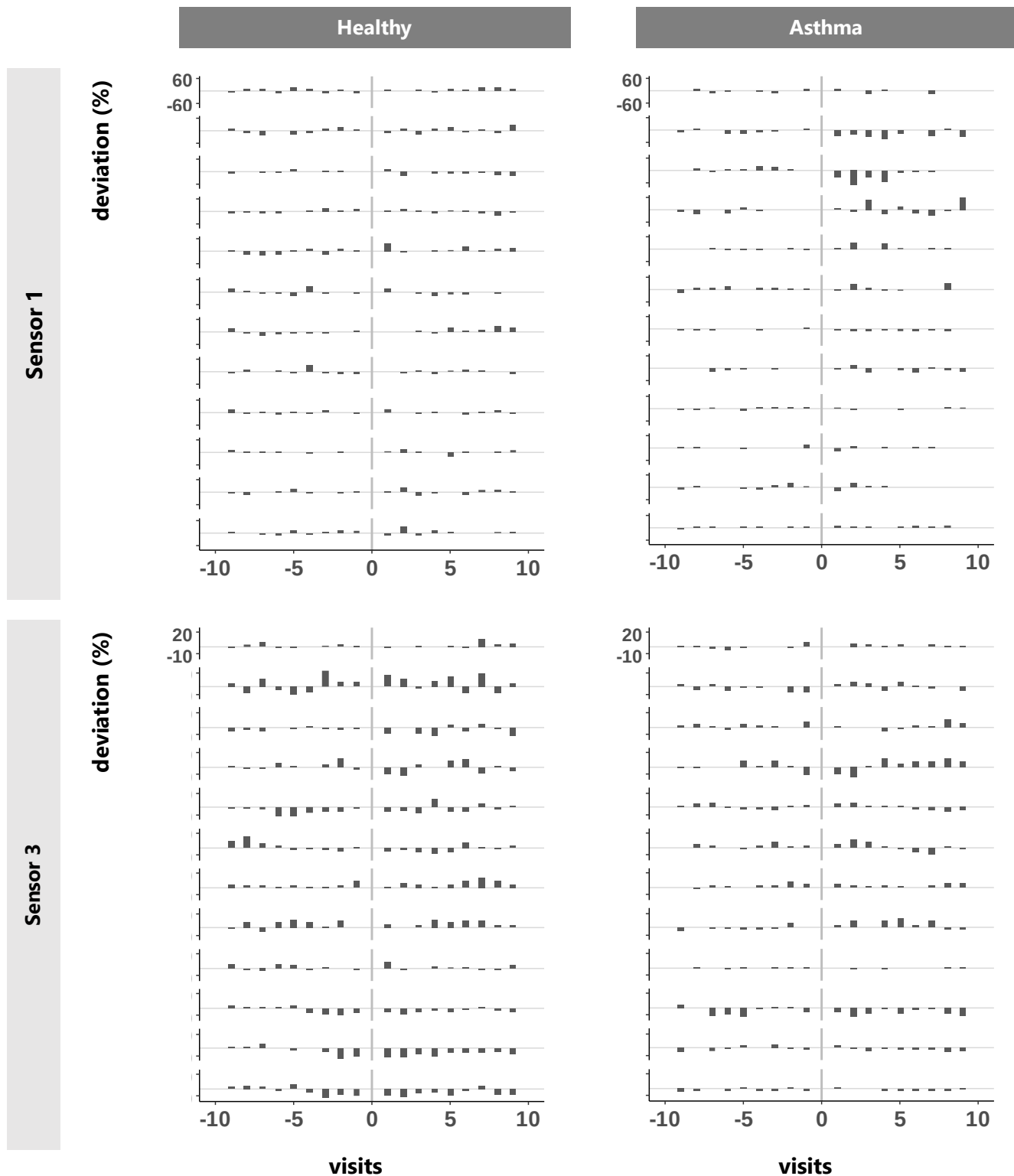


Figure S6: Time series eNose deviations per subject (sensor 1 & 3). Absolute deviation percentages from the individual baselines (horizontal grey lines = 0%) over time (visits) for sensor 1 and 3. Each row is an individual healthy (left) or asthmatic (right) participant. The light gray vertical line, marks the moment of the RV16 challenge. Note that visits are not always at the same day from the RV16 challenge.

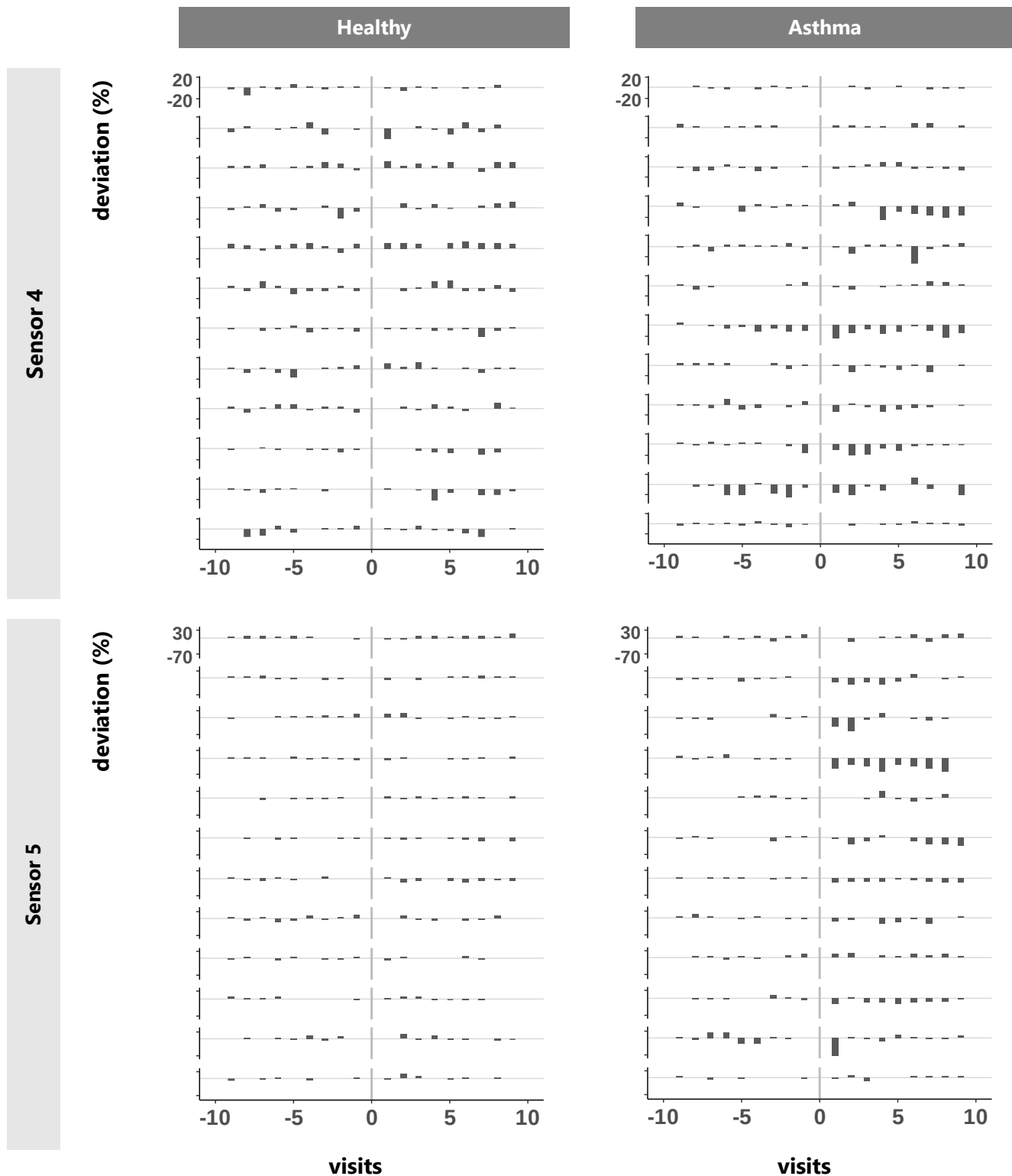


Figure S7: Time series eNose deviations per subject (sensor 4 & 5). Deviation percentages from the individual baselines (horizontal grey lines = 0%) over time (visits) for sensor 4 and 5. Each row is an individual healthy (left) or asthmatic (right) participant. The light gray vertical line, marks the moment of the RV16 challenge. Note that visits are not always at the same day from the RV16 challenge.

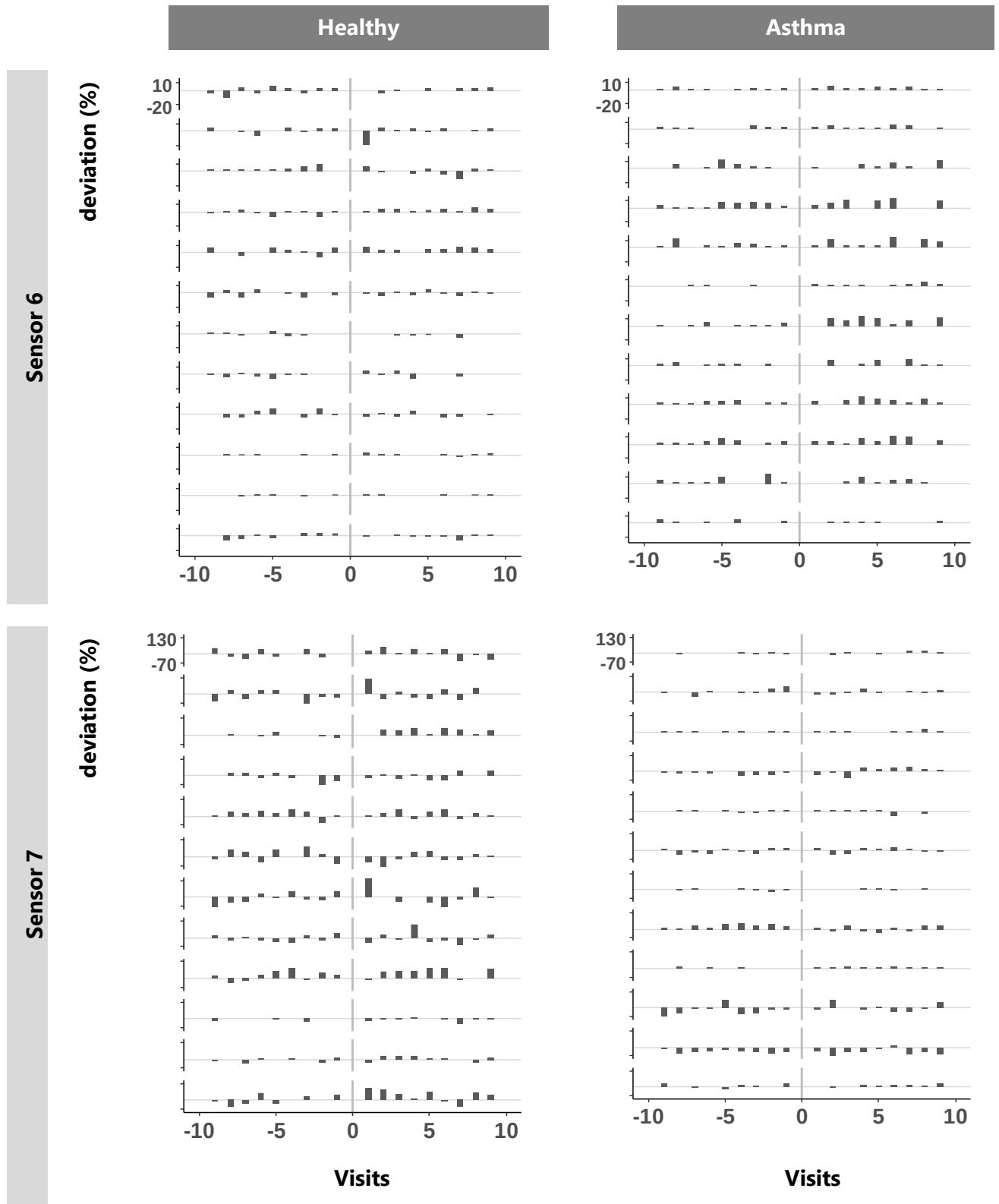


Figure S8: Time series eNose deviations per subject (sensor 6 & 7). Deviation percentages from the individual baselines (horizontal grey lines = 0%) over time (visits) for sensor 6 and 7. Each row is an individual healthy (left) or asthmatic (right) participant. The light gray vertical line, marks the moment of the RV16 challenge. Note that visits are not always at the same day from the RV16 challenge.

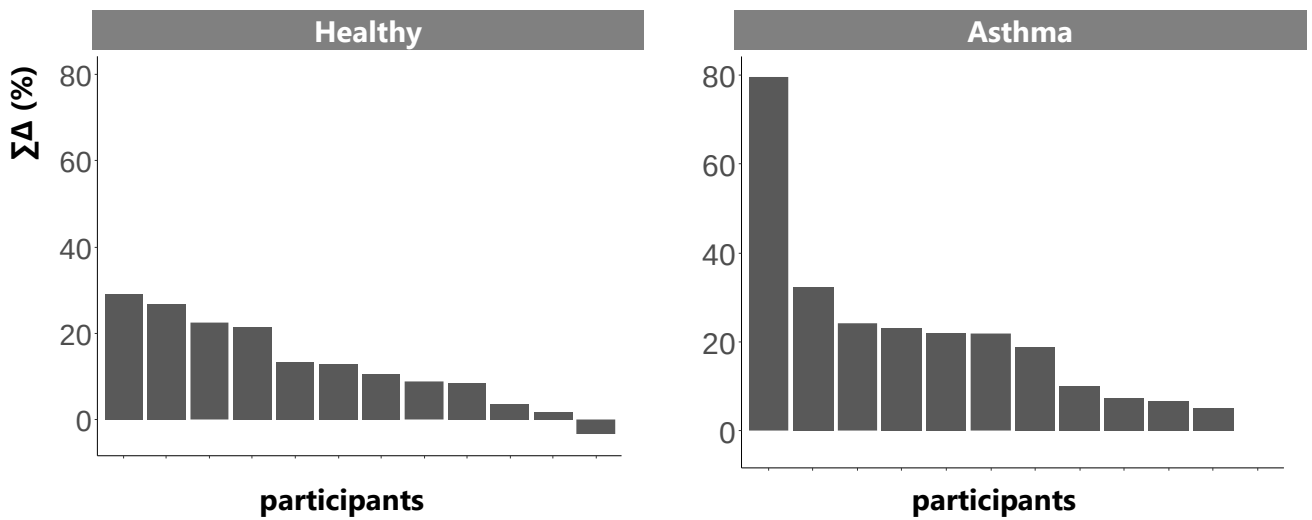


Figure S9: Total change in absolute eNose deviations (per participant). The total change (post – pre) in absolute eNose sensor deviations ($\Sigma\Delta_{all\ sensors}$). Each bar represents a healthy (left) or asthmatic (right) participant.