6. Fitzpatrick AM, Teague WG, Burwell L, Brown MS, Brown LA. NIH/NHLBI Severe Asthma Research Program. Glutathione oxidation is associated with airway macrophage functional impairment in children with severe asthma. Pediatr Res 2011;69:154-9.
7. Bossley CJ, Saglani S, Kavanagh C, Payne DN, Wilson N, Tsartsali L, et al. Corticosteroid responsiveness and clinical characteristics in childhood difficult asthma. Eur Respir J 2009;34:1052-9.
8. Ueda N, Tsuboi K, Uyama T. Enzymological studies on the biosynthesis of N-acylethanolamines. Biochim Biophys Acta 2010;1801:1274-85.
9. Berdyshev EV, Schmid PC, Krebsbach RJ, Hillard CJ, Huang C, Chen N, et al. Cannabinoid-receptor-independent cell signalling by N-acylethanolamines. Biochem J 2001;360:67-75.
10. Duncan RS, Chapman KD, Koulen P. The neuroprotective properties of palmitoylethanolamine against oxidative stress in a neuronal cell line. Mol Neurodegener 2009;4:50.

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Urinary metabolomic changes as a predictive biomarker of asthma exacerbation

To the Editor:

Exacerbations requiring hospitalization represent a high risk in patients with severe asthma. Predictive biomarkers of exacerbations are needed but are not available. Oxidative stress resulting from pulmonary reactive oxygen species formation is involved in asthma, leading to physiologic damage.[1] Asthma exacerbation is associated with increased oxidative stress,[2] with increased levels of carbon monoxide, nitric oxide, nitrotyrosine, and H_2O_2 found in breath condensate. Aldehydes and alkanes, the end products of lipid peroxidation, are also known to be involved in asthma-related oxidative stress.[3]

Urine is a stable, noninvasively collectable body fluid with a complex metabolic composition, the profiling of which might have a role in asthma diagnosis and monitoring. The 2 techniques used in this study, comprehensive bidimensional gas chromatography coupled to mass spectrometry (GC × GC-ToFMS) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, allow the analysis of volatile and nonvolatile compounds, thus providing complementary information on the urinary metabolome.

The aim of this pilot study was to assess the urinary metabolic changes linked to asthma exacerbation. Aldehydes and alkanes were studied by using GC × GC-ToFMS analysis, and NMR was used to provide an overview of changes in major metabolites involved in central metabolic pathways.

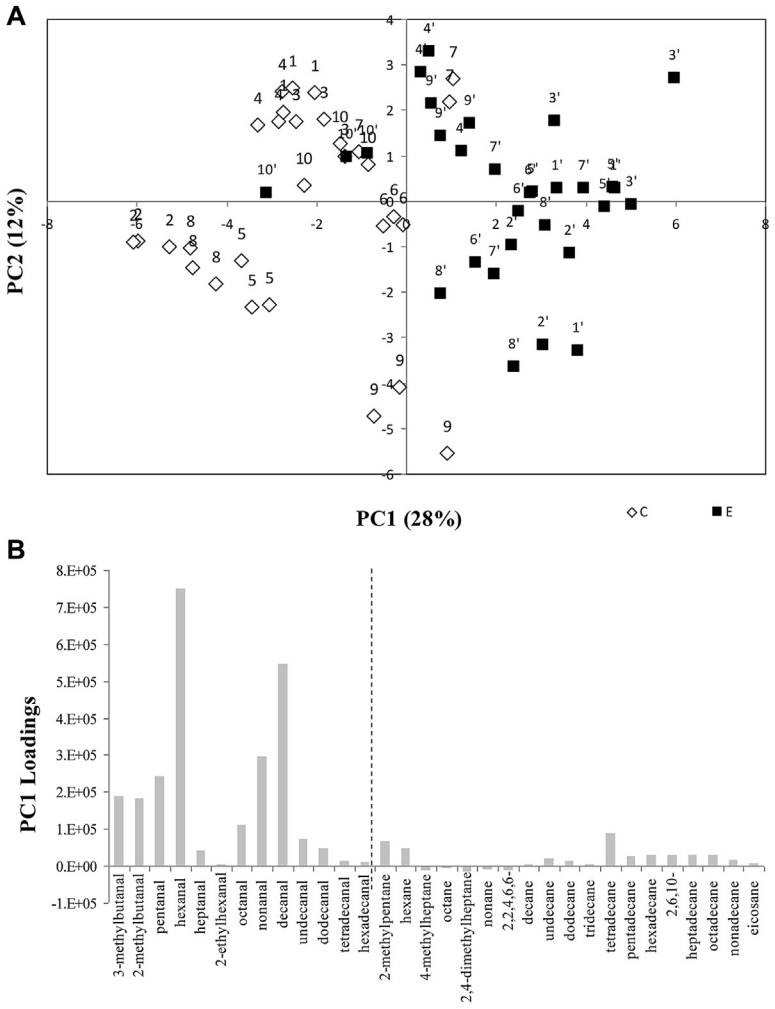

FIG 1. PCA applied to GC × GC peak areas of aliphatic aldehydes and alkanes detected in the urine of subjects in the stable state and under exacerbation conditions. **A,** PC1 versus PC2 score scatter plot in which *c* corresponds to the control condition *(open symbols)* and *e* corresponds to exacerbation *(solid symbols)*. The numbers included close to the symbols correspond to the sample ID. **B,** PC1 loadings plot explaining the separation observed in the score map (positive loadings = compounds increased in exacerbated condition).

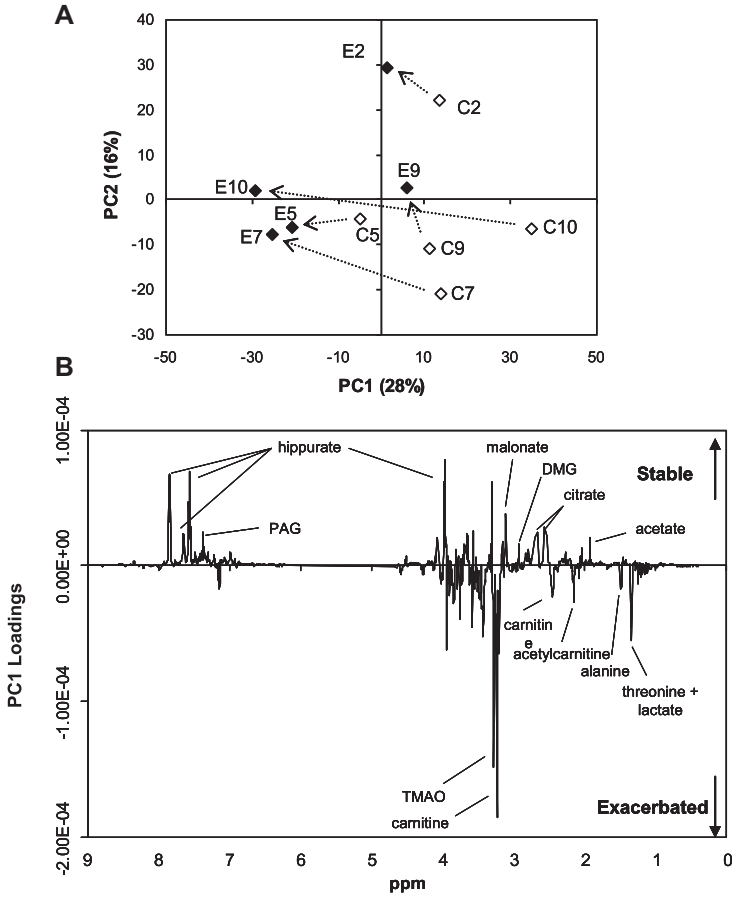

FIG 2. PCA applied to ^{1}H-NMR urine spectra of subjects in the stable state and under exacerbation conditions. **A,** PC1 versus PC2 score scatter plot. *Arrows* highlight the direction of change from stable *(open symbols)* to exacerbation *(solid symbols)* conditions for each subject. The numbers included close to the symbols correspond to the sample ID. **B,** PC1 loadings plot explaining the separation observed in the score map (positive loadings = compounds increased in stable conditions; negative loadings - compounds increased in exacerbated conditions).

A prospective cohort included 10 adult asthmatic patients. Written informed consent was provided, as was approval from the ethics board of the Hospitais da Universidade de Coimbra. All of the patients had a definite diagnosis of asthma (see the Methods section in this article's Online Repository at www.jacionline.org). All patients were studied at 2 different stages: during exacerbation (see the Methods section in this article's Online Repository at www.jacionline.org) and during a control state, as defined by results of the Asthma Control Test.

Urine was collected during exacerbation and in the stable state (see the Methods section in this article's Online Repository). All samples were analyzed by using solid-phase microextraction (SPME)/GC × GC-ToFMS,[4] and samples from 5 of 10 subjects were also analyzed by using ^{1}H-NMR spectroscopy (see the Methods section in this article's Online Repository).[5]

Exploratory principal component analysis (PCA) was applied to GC × GC peak areas, as well as to ^{1}H-NMR spectra, to extract the metabolic features of the exacerbation (see the Methods section in this article's Online Repository).

All patients (5 male and 5 female patients; mean age, 50 ± 18.8 years) were treated before exacerbation with inhaled corticosteroids and long-acting β-agonists. Seven patients had an allergic phenotype (see Table E1 in this article's Online Repository at www.jacionline.org).

PCA was applied to data matrices comprising aliphatic aldehydes and alkanes (Fig 1; and see Table E2 in this article's Online Repository at www.jacionline.org). The score scatter plot of the first and second principal components (PC1 and PC2; Fig 1, *A*), explaining 40% of the total variability, show the separation of exacerbation-related samples from control samples along PC1. The corresponding PC1 loadings (Fig 1, *B*) show that subjects under the exacerbated conditions were characterized by higher levels of alkanes and aldehydes. For 9 of the subjects, the aldehyde and alkane content consistently increased in the exacerbation state compared with the stable condition (see Fig E1 in this article's Online Repository at www.jacionline.org).

The ^{1}H-NMR spectrum shows signals from several amino acids and derivatives, organic acids, amines, and other metabolites, such as glucose and creatinine (see Fig E2 in this article's Online Repository at www.jacionline.org). The score scatter plot of the 2 main PCs (explaining almost 50% of the total variability) shows, for each subject, a clear trend for sample scores to shift from the positive to the negative PC1 axis between stable and exacerbated conditions (Fig 2, *A*). The PC1 loadings plot unveiled the signals