*These authors contributed equally to this work as joint first authors.
‡Dr Swoboda is currently affiliated with the Molecular Biotechnology Section, University of Applied Sciences, Campus Vienna Biocenter, Vienna, Austria.
§Dr Papadopoulos is currently affiliated with the Center for Paediatrics and Child Health, Institute of Human Development, University of Manchester, Manchester, United Kingdom.
Supported by the FAST project 201871 of the FP7 of the European Union and by the Austrian Science Fund (FWF) projects P23350-B11 and F4605.
Disclosure of potential conflict of interest: B. Linhart has received research support from the FAST Project (EU grant) and Austrian Science Fund (FWF) project P23350-B11. R. Valenta has received research support from the Austrian Science Fund (FWF) and the FPF Program "FAST" and serves as a consultant for Biomay AG, Vienna, Austria. N. G. Papadopoulos has received consultancy fees from AbbVie, Novartis, Menarini, Meda, ALK-Abelló, and GlaxoSmithKline; has received research support from Nestlé, Merck, and GlaxoSmithKline; has received lecture fees from Novartis, Uriach, GlaxoSmithKline, Allergopharma, Stallergenes, and MSD; and has received payment for development of educational presentations from AbbVie, Sanofi, Menarini, and Meda. The rest of the authors declare that they have no relevant conflicts of interest.

## REFERENCES

1. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: food allergy. J Allergy Clin Immunol 2012;129:906-20.
2. Tsabouri S, Triga M, Makris M, Kalogeromitros D, Church MK, Priftis KN. Fish and shellfish allergy in children: review of a persistent food allergy. Pediatr Allergy Immunol 2012;23:608-15.
3. Bugajska-Schretter A, Elfman L, Fuchs T, Kapiotis S, Rumpold H, Valenta R, et al. Parvalbumin, a cross-reactive fish allergen, contains IgE-binding epitopes sensitive to periodate treatment and Ca2+ depletion. J Allergy Clin Immunol 1998;101:67-74.
4. Swoboda I, Bugajska-Schretter A, Verdino P, Keller W, Sperr WR, Valent P, et al. Recombinant carp parvalbumin, the major cross-reactive fish allergen: a tool for diagnosis and therapy of fish allergy. J Immunol 2002;168:4576-84.
5. Swoboda I, Bugajska-Schretter A, Linhart B, Verdino P, Keller W, Schulmeister U, et al. A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. J Immunol 2007;178:6290-6.
6. Zuidmeer-Jongejan L, Swoboda I, Valenta R, Linhart B, Rigby R, Versteeg SA, et al. Development of a hypo-allergenic recombinant parvalbumin for first-in-man SCIT of fish allergy. Int Arch Allergy Immunol 2015;166:41-51.
7. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics 2003;111:1601-8.
8. Zuidmeer-Jongejan L, Fernandez-Rivas M, Poulsen LK, Neubauer A, Asturias J, Blom L, et al. FAST: towards safe and effective subcutaneous immunotherapy of persistent life-threatening food allergies. Clin Transl Allergy 2012;2:5.

Available online March 5, 2015.
http://dx.doi.org/10.1016/j.jaci.2015.01.015

## Endogenous metabolites and inflammasome activity in early childhood and links to respiratory diseases

*To the Editor:*

The NLR family, pyrin domain containing 3 (NLRP3) inflammasome is a pivotal host platform for sensing of endogenous and exogenous danger signals and for the subsequent orchestration of inflammatory responses. Beside pathogenic agents, endogenous dietary metabolites such as fatty acids can trigger the assembly of NLRP3 inflammasomes, cytosolic protein complexes that activate caspase-1 (CASP1), leading to the cleavage of pro–IL-1β and pro–IL-18.[1] NLRP3 and the expression of pro forms of the IL-1β cytokine family are transcriptionally induced by Toll like receptor (TLR) activation. Although the link between glucose or fatty acids and the activation of NLRP3 inflammasome is well established in mice,[2] comparable data for humans were reported only for adipose tissue.[3] Studies for children are completely missing so far. Therefore, in the present study, our aim was to investigate whether the metabolic state is associated with the immature immune system already in early childhood. By performing a broad metabolic profiling, we explored the involvement of endogenous metabolites in the expression of TLR and NLRP3 inflammasome components in children at birth and at age 1 year using samples from the prospective birth cohort LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk).[4]

In the LINA study, 629 mother-child pairs (622 mothers and 7 twin pairs) were recruited from March 2006 until December 2008 in Leipzig, Germany.[4] Annually, around the birthday of the child, follow-up investigations were performed with questionnaire evaluations for respiratory (wheezing, airway infections, bronchitis, asthma) and allergic (atopic dermatitis, hay fever, allergic sensitization) outcomes and clinical visits including blood collection. In children's blood samples at birth (cord blood, n = 471) and at age 1 year (n = 513), mRNA expression for NLRP3, CASP1, MyD88, TLR1, TLR2, TLR5, TLR6, TLR7, TLR9, and TLR10 as well as the concentration of the effector cytokines IL-1β (protein and mRNA), IL-18 (mRNA), and specific IgE was measured. Metabolomic analysis was performed in sera of 495 newborns (cord blood) and 449 1-year-old children by using the Absolute*IDQ* p180 Kit (Biocrates Life Science AG, Innsbruck, Austria). Method details, characteristics of the study population, median values, and interquartile ranges for all metabolites and immune parameters are given in Tables E2-E4 in this article's Online Repository at www.jacionline.org.

At birth as well as in the first year of life, high hexose levels correlated with increased expression of TLRs and CASP1 and in part with NLRP3. Contrarily, a reduced expression of TLRs and inflammasome components was measured in the presence of high concentrations of amino acids and lysophosphatidylcholines (lysoPCs). To control for multitesting, we applied a Bonferroni correction (significance level $P < 4.2 \times 10^{-4}$) and could show that the observed associations between metabolites and immune/inflammasome parameters remained significant (data are presented as heat maps of Spearmen correlation coefficients) (Fig 1). Furthermore, by calculating mean ratios adjusted for the possible confounding factors season of birth, mode of delivery, cat keeping, parental history of atopy, sex, and parental education, the entirely detected relationships remained significant (see Figs E2 and E3 in this article's Online Repository at www.jacionline.org). Although the concentration of metabolites and inflammatory parameters at birth did not correlate with concentrations in the first year of life (see Tables E3 and E4), the relationship between metabolites and inflammatory parameters at each time point was nearly identical (Fig 1). These findings indicate that there is a valid relationship between sugars, lysoPCs, amino acids, and TLRs/inflammasome components that is dynamically modulated by the metabolic state.

It is obvious from *in vitro* and *in vivo* animal studies that high glucose levels provide a priming signal for IL-1β by activation of thioredoxin-interactin protein, which subsequently binds to NLRP3, inducing IL-1β secretion in islet cells.[2] In addition, high concentrations of glucose induce the generation of reactive oxygen species, which is a sufficient second signal for promoting

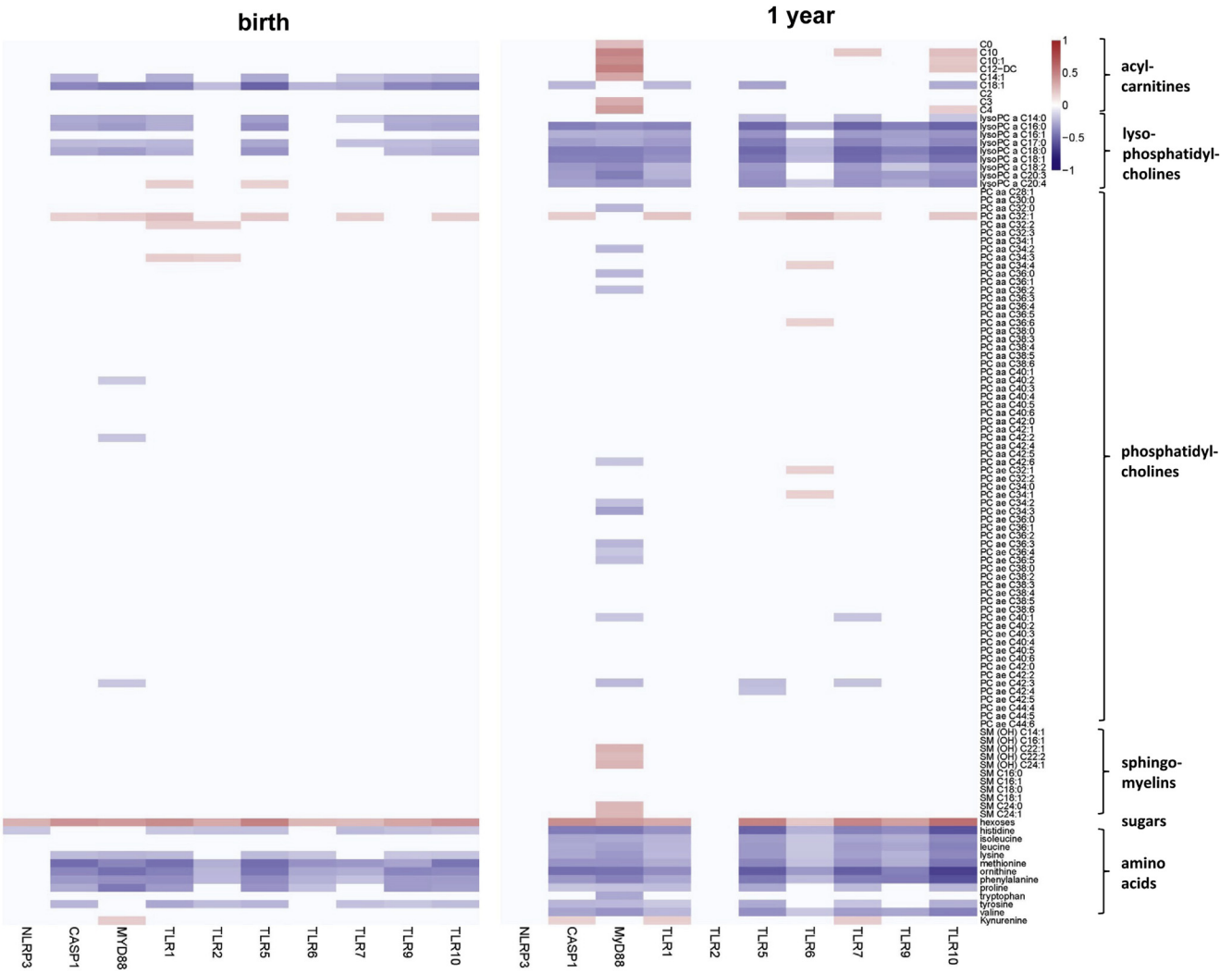


**FIG 1.** Heat maps present the correlation between blood metabolite concentrations and expression of TLRs/MyD88 and inflammasome components (NLRP3/CASP1) at birth (cord blood) and in the first year of life (1 year). The color of each cell in the heat map represents the correlation coefficient (*R*) from Spearman correlation. Only significant values after Bonferroni correction ($P < 4.2 \times 10^{-4}$) are presented.

**TABLE I.** Relationship between IL-1β protein and IL-1β mRNA concentrations at birth (cord blood), 1-year blood, and the development of childhood diseases in the first (1 y) and second year (2 y) of life, respectively*

| | IL-1β protein blood concentrations | | IL-1β mRNA blood expression | |
|---|---|---|---|---|
| | Birth | 1 y | Birth | 1 y |
| | aOR (95% CI)† | | | |
| Outcome at age | 1 y | 2 y | 1 y | 2 y |
| Atopic dermatitis | 0.97 (0.74-1.28) | 1.26 (0.97-1.63) | 0.98 (0.74-1.31) | 0.81 (0.58-1.15) |
| Sensitization: | | | | |
| to food allergens‡ | 1.13 (0.88-1.46) | 1.04 (0.80-1.35) | 0.99 (0.76-1.28) | 0.86 (0.62-1.19) |
| to inhalant allergens‡ | 1.33 (0.75-2.38) | 0.92 (0.63-1.34) | 1.02 (0.56-1.87) | 0.84 (0.52-1.36) |
| Bronchitis | **1.25 (1.01-1.54)**§ | **1.23 (1.05-1.44)**§ | 0.91 (0.73-1.13) | **1.23 (1.00-1.50)**§ |
| Wheezing | 1.15 (0.93-1.42) | 1.11 (0.93-1.32) | **1.26 (1.00-1.54)**§ | **1.27 (1.01-1.59)**§ |
| Airway infections | 1.62 (0.88-2.99) | 1.09 (0.90-1.32) | 1.02 (0.83-1.24) | 1.07 (0.84-1.36) |

*aOR*, Adjusted odds ratio.

*Analysis was performed using a logistic regression model with IL-1β values categorized in quartiles.

†aOR for parental educational level, month of birth, mode of delivery, cat keeping during pregnancy, siblings, sex, and smoking during pregnancy. Atopic dermatitis and allergic sensitization were additionally adjusted for parental history of atopy.

‡According to the Phadia CAP System, concentrations of more than 0.35 kU/L were regarded as positive.

§Significant numbers are in boldface ($P < .05$).

inflammasome activation.[2] Our epidemiological data show that at least in cord blood, the NLRP3 expression is associated with high hexoses levels. Furthermore, the expression of the NLRP3 downstream target CASP1 was strongly correlated with high hexose levels at birth as well as in the first year. We validated these findings by performing *in vitro* assays using PBMCs from healthy adult donors and demonstrated that glucose induces a higher expression of NLRP3, CASP1, as well as IL1B (see Fig E7 in this article's Online Repository at www.jacionline.org). *In vitro* experiments with human monocytes already demonstrated that high glucose induces TLR mRNA expression,[5] which is in line with our results showing that elevated blood concentration of hexoses is related to increased mRNA expression of TLRs and the adaptor molecule MyD88 at both analyzed time points. Our *in vitro* data again support this result by showing a glucose-induced activation of MYD88 (Fig E7). Earlier experimental studies show that the *Nlrp3* promotor is activated by TLR/MyD88-mediated signaling.[6] Our data support these results; we found that increased expression of TLRs/MyD88 correlated with increased expression of NLRP3 and CASP1 (see Figs E4 and E5 in this article's Online Repository at www.jacionline.org). We could furthermore show that both NLRP3 and CASP1 were related to the expression of the effector cytokines IL-1β (mRNA and protein) and IL-18 (mRNA) (see Fig E6 in this article's Online Repository at www.jacionline.org). Thus, our results may provide the first evidence that the association between hyperglycemia, inflammasome activation, and IL-1β production reported so far only in animal models[2] is also present in humans.

As further metabolites linked to inflammasome activity we have identified amino acids and lysoPCs. Our results may point to an anti-inflammatory effect of both groups showing a strong negative association with TLR and inflammasome component expression. These data are in line with previous reports showing that lysoPCs may act as attenuators of sepsis and as immune suppressors[7,8] whereas dietary supplementation with certain amino acids (eg, valine, histidine, and arginine) was reported to alleviate tissue and systemic inflammation.[9,10] Our *in vitro* data sustain these findings at least for amino acid (valine) treatment where we observed a diminished expression of CASP1, IL1B, and MyD88 but not of NLRP3 and the analyzed TLRs.

On the basis of our data and findings from other groups, it is reasonable that an imbalance between endogenous metabolites (eg, elevated glucose levels and reduced amino acid levels) may lead to an increased inflammatory status. Indeed, in our study, molecules capable of sensing metabolites (TLRs and NLRP3/CASP1) were associated with increased concentrations of the effector cytokines IL-1β and IL-18. The role of these cytokines for the development of childhood diseases is poorly investigated. Here, we found that cord blood IL-1β (Table I) but not IL-18 (Table E5) concentrations were predictive for the development of bronchitis and wheezing by age 2 years. Thus, the balance between endogenous metabolites seems to be important for the regulation of an inflammatory immune response independently of invading pathogens. Because we could show that the inflammasome effector cytokine IL-1β was linked to a higher risk of respiratory diseases during the first 2 years of life, our results may give evidence that the metabolome is of importance for respiratory diseases in early childhood.

We thank all LINA participants as well as Anne Hain, Beate Fink, and Gabriele Heimpold for their excellent technical assistance and Melanie Nowak for her study organization assistance.

*Gunda Herberth, PhD*[a]*
*Kirsten Offenberg, MSc*[a]*
*Ulrike Rolle-Kampczyk, PhD*[b]
*Mario Bauer, PhD*[a]
*Wolfgang Otto, PhD*[c]
*Stefan Röder, PhD*[d]
*Konrad Grützmann, Dipl.Bioinf*[a]
*Ulrich Sack, PhD*[e]
*Jan-Christoph Simon, PhD*[f]
*Michael Borte, PhD*[g]
*Martin von Bergen, PhD*[c,h]
*Irina Lehmann, PhD*[a]
*for the LINA Study Group*

From the Departments of [a]Environmental Immunology, [b]Metabolomics, and [c]Proteomics, UFZ-Helmholtz Centre for Environmental Research Leipzig, [d]UFZ-Helmholtz Centre for Environmental Research Leipzig, Core Facility Studies, [e]the Institute for Clinical Immunology, University of Leipzig, [f]Universitätsklinikum, Klinik für Dermatologie, Venerologie und Allergologie, and [g]Children's Hospital, Municipal Hospital "St. Georg," Academic Teaching Hospital of the University of Leipzig, Leipzig, Germany; and [h]the Department of Biotechnology, Chemistry, and Environmental Engineering, Aalborg University, Sohngaardsholmsvej, Aalborg, Denmark. E-mail: gunda.herberth@ufz.de. Or: irina.lehmann@ufz.de.

*These authors contributed equally to this work.

The LINA study was supported by Helmholtz institutional funding (Helmholtz Centre for Environmental Research—UFZ).

Disclosure of potential conflict of interest: U. Sack has consultant arrangements with Indago GmbH and has received travel support from Beckman Coulter. The rest of the authors declare that they have no relevant conflicts of interest.

## REFERENCES

1. Gross O, Thomas CJ, Guarda G, Tschopp J. The inflammasome: an integrated view. Immunol Rev 2011;243:136-51.
2. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010;11:136-40.
3. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011;17:179-88.
4. Herberth G, Hinz D, Roder S, Schlink U, Sack U, Diez U, et al. Maternal immune status in pregnancy is related to offspring's immune responses and atopy risk. Allergy 2011;66:1065-74.
5. Dasu MR, Devaraj S, Zhao L, Hwang DH, Jialal I. High glucose induces Toll-like receptor expression in human monocytes: mechanism of activation. Diabetes 2008;57:3090-8.
6. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, et al. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol 2009;183:787-91.
7. Murch O, Collin M, Sepodes B, Foster SJ, Mota-Filipe H, Thiemermann C. Lysophosphatidylcholine reduces the organ injury and dysfunction in rodent models of gram-negative and gram-positive shock. Br J Pharmacol 2006;148:769-77.
8. Yan JJ, Jung JS, Lee JE, Lee J, Huh SO, Kim HS, et al. Therapeutic effects of lysophosphatidylcholine in experimental sepsis. Nat Med 2004;10:161-7.
9. Aihara K, Osaka M, Yoshida M. Oral administration of the milk casein-derived tripeptide Val-Pro-Pro attenuates high-fat diet-induced adipose tissue inflammation in mice. Br J Nutr 2014;112:513-9.
10. Niu YC, Feng RN, Hou Y, Li K, Kang Z, Wang J, et al. Histidine and arginine are associated with inflammation and oxidative stress in obese women. Br J Nutr 2012;108:57-61.

Available online March 7, 2015.
http://dx.doi.org/10.1016/j.jaci.2015.01.022

## The influence of asthma control on the severity of virus-induced asthma exacerbations

*To the Editor:*

Our understanding of asthma control has evolved in recent years to include both the idea of current control (gauged mostly