RESPONSE TO COMMENT ON ADAM ET AL.

Metformin Effect on Nontargeted Metabolite Profiles in Patients With Type 2 Diabetes and in Multiple Murine Tissues. Diabetes 2016;65:3776–3785

Jonathan Adam,[1,2,3] Stefan Brandmaier,[1,2,3] Martina Troll,[1,2,3] Markus Rotter,[1,2,3] Robert P. Mohney,[4] Margit Heier,[2] Jerzy Adamski,[3,5,6,7] Yixue Li,[8] Susanne Neschen,[3,6] Gabi Kastenmüller,[3,9] Karsten Suhre,[10] Donna Ankerst,[11] Thomas Meitinger,[12,13] and Rui Wang-Sattler[1,2,3]

Diabetes 2017;66:e3–e4 | DOI: 10.2337/dbi17-0010

We appreciate the comments of Jegatheesan et al. (1) on our article (2), as well as the remarks of Irving et al. (3). Jegatheesan et al. (1) also summarize citrulline's beneficial effects, specifically in nonalcoholic fatty liver disease, and characterize this amino acid as a coin with two sides. They suggest citrulline as a marker of insulin resistance as well as an insulin-sensitizing amino acid (1). Although this is an interesting question, it was not the subject of our study and therefore could not be addressed (2).

We would like to emphasize that we did not speculate about a direct association between high citrulline and insulin resistance. In fact, we did not observe significantly different relative concentrations of citrulline in participants with normal glucose tolerance and with impaired glucose tolerance or type 2 diabetes (T2D) not treated with antidiabetes drugs (ndt-T2D) in the KORA (Cooperative Health Research in the Region of Augsburg) cohort (2). However, we observed significantly lowered values of citrulline when comparing the relative serum concentrations (nontargeted approach) of metformin-treated T2D (mt-T2D) patients with those of more than 1,000 participants with normal glucose tolerance, impaired glucose tolerance, and ndt-T2D, as Fig. 1*A* in our article showed (2). Given the assumption that these individuals represent normal levels, the observed values in mt-T2D patients are lower and not just a normalization of a supposed T2D-induced "citrullinemia" (1). All this is corroborated in murine plasma, skeletal muscle, and adipose tissue but not in the liver (2). We fully agree that understanding of the underlying mechanisms would benefit from further investigations including kidney and gut tissues. Unfortunately, these data were not present in our previous study (2).

We advise caution with the statement by Jegatheesan et al. (1) that "the liver does not play any significant role in these interorgan exchanges because liver citrulline is almost exclusively synthesized locally and channeled into the urea cycle." It has been shown that citrulline can be taken up by the liver from the blood (4). Irrespective of the fact that the

[1]Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany
[2]Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, Germany
[3]German Center for Diabetes Research (DZD), Neuherberg, Germany
[4]Metabolon, Inc., Durham, NC
[5]Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, Neuherberg, Germany
[6]Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany
[7]Institute of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising, Germany
[8]Key Lab of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
[9]Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany
[10]Department of Physiology and Biophysics, Weill Cornell Medical College in Qatar (WCMC-Q), Education City—Qatar Foundation, Doha, Qatar
[11]Lehrstuhl für Mathematische Modelle Biologischer Systeme, Technische Universität München, Garching, Germany
[12]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany
[13]Institute of Human Genetics, Technische Universität München, Munich, Germany

Corresponding author: Rui Wang-Sattler, rui.wang-sattler@helmholtz-muenchen.de.

S.N. is currently affiliated with Sanofi Deutschland GmbH, R&D Diabetes Research & Translational Medicine, Frankfurt am Main, Germany.

liver citrulline values we presented in Fig. 1*B* of our article (2) are not significantly influenced by metformin intake, it is known that the liver is the main target of metformin (5).

In summary, we would like to expand on the question raised about the two sides of the coin to the potential coherence. Is metformin's beneficial effect on insulin sensitivity mediated not only by inhibiting gluconeogenesis (5) but also by its effect on the nitric oxide cycle (3) and the consequent alteration of citrulline concentrations? We therefore support the suggestion by Jegatheesan et al. (1) to investigate the interaction of citrulline and metformin. Clinical studies to investigate the specific role of citrulline in T2D and its potential treatment benefits as a supplement to metformin are highly promising.

Funding. Part of this project was supported by European Union Seventh Framework Programme grant HEALTH-2013-2.4.2-1/602936 (Project CarTarDis).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Jegatheesan P, De Bandt J-P, Cynober L. Comment on Adam et al. Metformin effect on nontargeted metabolite profiles in patients with type 2 diabetes and in multiple murine tissues. Diabetes 2016;65:3776–3785 (Letter). Diabetes 2017; 66:e1–e2. DOI: 10.2337/db16-1592

2. Adam J, Brandmaier S, Leonhardt J, et al. Metformin effect on nontargeted metabolite profiles in patients with type 2 diabetes and in multiple murine tissues. Diabetes 2016;65:3776–3785

3. Irving BA, Spielmann G. Does citrulline sit at the nexus of metformin's pleotropic effects on metabolism and mediate its salutatory effects in individuals with type 2 diabetes? Diabetes 2016;65:3537–3540

4. van de Poll MCG, Siroen MPC, van Leeuwen PAM, et al. Interorgan amino acid exchange in humans: consequences for arginine and citrulline metabolism. Am J Clin Nutr 2007;85:167–172

5. Madiraju AK, Erion DM, Rahimi Y, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 2014;510:542–546