# Response to Comment on: Tessari et al. Roles of Insulin, Age, and Asymmetric Dimethylarginine on Nitric Oxide Synthesis In Vivo. Diabetes 2013;62:2699–2708

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I have read with interest the comment letter by Kruszelnicka et al. (1) to our article recently published in *Diabetes* (2). Their letter contains useful observations and raises interesting questions. One is about the dissociation of the metabolic sensitivity to insulin from endothelial nitric oxide (NO) release and its vascular protective effects, and the other is on the specificity of the assay of blood nitrates (both $^{15}N$-labeled and unlabeled) as indices of NO generation.

In regards to the first issue, recent work by the Accili Laboratory, commented on by Kearney (3), report data on the dissociation between the metabolic and the vascular effects of insulin in the forkhead box class (FoxO)-deficient mouse model. The insulin signaling may diverge downstream of Akt, and this could explain tissue differences in the response to insulin in insulin-resistant states. These data provide a mechanistic view for the dissociation between the hormone effects also in vivo in humans. Caution is required however when translating data obtained in vitro to in vivo conditions, when multiple factors simultaneously operate. Even though the response(s) to insulin in individual tissues is uniformly affected, the presence of circulating inhibitors and/or regulators may alter the final responses to the hormone, as it could happen for asymmetric dimethylarginine in our study (2).

As regards the second issue, nitrate concentration was measured by the Griess reaction, i.e., following reduction of nitrates to nitrites, whereas $^{15}$N-nitrate enrichment is measured following nitration of benzene (2,4). Some substances may interfere with these reactions, among them peroxynitrite. Such interference is potentially relevant in data interpretation. However, an increase in peroxynitrite should not affect $^{15}$N-nitrate enrichment because NO (including the [$^{15}$N]-labeled species) is very quickly and efficiently converted to peroxynitrite. This suggests an extensive equilibration between the two compounds, provided that a selective intracellular compartmentalization does not significantly occur. Conversely, as concerns the measurements of nitrate concentration by the Griess reaction, although peroxynitrite can theoretically be decomposed into hydroxyl radical and nitrogen dioxide, such a backreaction is a thousand-times slower than the formation of peroxynitrite (5). As a matter of fact, both nitrates and peroxynitrite are fairly stable, and this would exclude a significant interconversion between these two compounds either in circulating blood, or after blood withdrawal, centrifugation, and storage, and even less in the frozen samples.

In conclusion, ours as well as other recently published articles challenge the somehow dogmatic concept about the existence of a uniform and comprehensive state of "insulin resistance," a condition that should be considered multifaceted, and not defined just on the basis of the reduced insulin-mediated glucose disposal. This more complex view may have relevant consequences not only from a mechanistic but also from a therapeutic standpoint.

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