Response to Comment on: Reyna et al. (2008) Elevated Toll-Like Receptor 4 Expression and Signaling in Muscle From Insulin-Resistant Subjects. *Diabetes* 57:2595–2602

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We thank Shapiro et al. (1) for their interest in our study (2) and their comment about the role of low-grade endotoxemia in the pathogenesis of insulin resistance in type 2 diabetic subjects. The notion that elevated levels of lipopolysaccharide (LPS) in the circulation derived from a gastrointestinal source could be responsible for the inflammatory state present in insulin-resistant subjects (3,4) is provocative and merits further investigation. Although caution is warranted when extrapolating from a small number of studies (3,4), in the future it would be useful to measure circulating LPS levels in insulin-resistant subjects and determine whether a correlation exists between the plasma LPS concentration and toll-like receptor (TLR)4 expression/signaling in tissue.

As pointed out by Shapiro et al. (1), there is some evidence (obtained mostly in inflammatory cells) indicating that pre-exposure to LPS decreases the response to subsequent challenges with this molecule. The mechanism responsible for this phenomenon is not clear, but it is thought to involve an increase in nuclear factor κB (NFκB) p50 inhibitory homodimers, presence of endotoxin-neutralizing molecules in plasma, and downregulation of TLR4 expression/signaling. Because insulin-resistant subjects are chronically exposed to TLR4 agonists, such as saturated fatty acids (SFAs) and possibly LPS, one might expect a downregulation of TLR4. However, this does not seem to occur in vivo. For example, we observed upregulation of TLR4 in muscle of obese and type 2 diabetic subjects (2), while a similar phenomenon has been reported in adipose tissue of insulin-resistant mice (5,6). It is unclear why insulin-resistant animals and humans seem to "escape" from the chronic TLR4 stimulation. Instead of TLR4 downregulation, we actually found that acute exposure to monophosphoryl lipid A, a specific TLR4 agonist, increased TLR4 gene expression in primary human myotubes (2). Consistent with this observation, others have reported increased TLR4 expression upon LPS stimulation in endothelial (7) and dendritic cells (8). Therefore, the effect of LPS on TLR4 expression seems to vary depending on the experimental conditions, particularly the cell type examined.

In contrast to SFAs, n-3 polyunsaturated fatty acids (PUFAs) appear to blunt TLR4-mediated inflammatory responses in macrophages (9) and adipocytes (5) and prevent high-fat diet–induced insulin resistance in rodents (10). These results suggest that n-3 PUFAs could be used to treat insulin resistance in humans. Indeed, epidemiological studies suggest that diets rich in unsaturated fatty acids and low in SFAs are associated with enhanced insulin sensitivity (11,12). The Kuopio, Aarhus, Naples, Wollongong, Uppsala (KANWU) study, one of the largest (162 subjects) controlled dietary studies, showed that substituting SFAs with monounsaturated fatty acids improved insulin sensitivity in healthy individuals (13). Nonetheless, in that study, addition of n-3 PUFAs (3.6 g/day of fish oil) had no effect on insulin sensitivity. Moreover, another study reported worsening of insulin sensitivity after administration of high-dose fish oil (5.9 g/day) in type 2 diabetic subjects (14). In contrast to these studies, another large (324 subjects) trial found that fish oil (3.0 g/day) improved insulin sensitivity in obese individuals (15). It is not clear why the beneficial effect of n-3 PUFAs on insulin action in cell culture and rodent studies has not been confirmed in humans. Type 2 diabetes is a multifactorial disease with complex genetic and environmental variables, and short-term nutritional interventions might be insufficient to improve insulin sensitivity. Taking into account that the anti-inflammatory activity may vary among different sources of fish oils (16), the discrepant results could also be due to variations in the purity and ratio of n-3 PUFAs (eicosapentaenoic and docosahexaenoic acids) contained in the fish oil preparations used in different clinical trials. Studies using recently available (and Food and Drug Administration–approved) highly enriched re-esterified n-3 PUFA concentrates might help to clarify the role of n-3 PUFAs in the treatment of insulin-resistant disorders in humans.

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