Palmitate increases TLR4 content and stimulates expression of inflammatory genes in human myotubes.


Toll-like receptor 4 (TLR4) is the receptor of lipopolysaccharide (endotoxin) from Gram negative bacteria cell walls. Recent studies suggest that saturated free fatty acids (FFA) also can bind to TLR4 and impair insulin action by stimulating TLR4-driven pathways, including IKK/IkappaB/NF-kappaB. Previously, we reported that insulin-resistant (obese and type 2 diabetic) subjects have increased TLR4 gene expression and protein content in skeletal muscle. These subjects have elevated TLR4-driven (IKK/IkappaB/NF-kappaB) signaling, as evidenced by lower muscle IkappaB content and increased expression of the NF-kappaB regulated genes, IL-6 and SOD2. In this study, we hypothesized that: 1) increased expression of inflammatory genes (IL-6 and SOD2) in muscle are mediated by the elevated plasma FFA concentrations typically seen in insulin-resistant subjects; and 2) FFAs are involved in increased muscle TLR4 gene expression/protein content in insulin-resistant subjects. To test these hypotheses, we treated primary myotubes derived from normal-glucose-tolerant subjects with 200 μM palmitate for 6 h, which increased NF-kappaB activity by 2.5-fold (P<0.05), and induced IL-6 and SOD2 gene expression by 1.8-fold and 1.7-fold (P<0.05), respectively. Palmitate-induced IL-6 and SOD2 gene expression were completely abolished by blocking NF-kappaB activity through adenoviral-mediated expression of an IkappaB superrepressor. To examine whether FFAs play a role in increased muscle TLR4 expression/content in muscle from insulin-resistant subjects, human myotubes were treated with palmitate for 3 days. Chronic (3 day) treatment with 200 μM palmitate increased TLR4 gene expression (by 2.2-fold, P<0.05) and protein content (by 1.3-fold, P<0.05). In line with this finding, treatment with the TLR4 agonist monophosphoryl lipid A (10 μg/ml) for 6 h increased TLR4 gene expression by 1.8-fold (P<0.05). In contrast to FFA, 3-day treatment with insulin (100 nM), TNFα (20 ng/ml), and IL-6 (100 ng/ml), other systemic factors that are commonly elevated in insulin-resistant subjects, did not affect TLR4 content. In conclusion, elevation in plasma FFA concentration may contribute to increased gene expression of inflammatory proteins (IL-6 and SOD2) in muscle from insulin-resistant subjects, and this effect is likely
mediated by NF-kappaB. Increased TLR4 expression/signaling, possibly caused by elevated plasma FFAs, may contribute to the pathogenesis of insulin resistance in humans.

Key words: TLR4, NFkB, insulin resistance