Macrophage polarization induced by different toll-like receptor agonists mediate insulin responses in muscle cells

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Skeletal muscle is the main site of postprandial glucose disposal and defects in muscle glucose transport are critical in the development of type 2 diabetes. Diets inappropriately high in saturated fat are associated with a state of chronic inflammation and contribute to insulin resistance. Recent evidence had implicated toll-like receptors (TLRs), components of the innate immune system, in propagating fatty acid-induced inflammatory signals leading to insulin resistance. Direct exposure of muscle cells to palmitate, one of the most abundant dietary saturated fats, causes insulin resistance, an effect associated with signaling through TLRs. Bacterial infection is also associated with insulin resistance and direct administration of lipopolysaccharide (LPS), a wall component of Gram-negative bacteria and well-known TLR4 agonist, can cause insulin resistance. Macrophage-adipose cell cross-talk has been implicated in fatty-acid induced inflammation and insulin resistance, but the role of macrophage secreted factors on insulin responses in muscle cells is ill-defined. We hypothesized that conditioned medium from palmitate and LPS treated macrophages would cause insulin resistance in muscle cells. Treating RAW264.7 macrophages with 0.5 mM palmitate for 6 h increased the expression of inducible nitric oxide synthase (iNOS) by ~3-fold and did not alter the expression of arginase. Conditioned medium from palmitate-treated macrophages inhibited insulin-stimulated glucose uptake and GLUT4 translocation in L6-GLUT4*myc* muscle cells. This suggests that palmitate promotes macrophage polarization towards the M1 pro-inflammatory state, which can lead to the release of factors that cause insulin resistance in muscle cells. Contrary to our hypothesis, treating macrophages with 10 ng/mL of LPS for 24 hours increased the expression of arginase by ~4-fold and increased iNOS expression by ~3-fold, suggesting a polarization of these macrophages towards the M2 anti-inflammatory state. Moreover, conditioned medium from LPS-treated macrophages stimulated insulin-induced glucose uptake and GLUT4 translocation in L6-GLUT4*myc* muscle cells. Importantly, analysis of differences in macrophage secreted factors revealed that LPS induced a 16-fold increase in the anti-inflammatory cytokine, IL-10, whereas palmitate treatment only increased IL-10 by 2-fold. Exogenous IL-10 stimulated insulin action in muscle cells and attenuated insulin resistance caused by conditioned medium from palmitate-treated macrophages. These results indicate that macrophages may be an integral element linking inflammation and glucose homeostasis in skeletal muscle. Our data suggests that the polarization state of macrophages, which can be differentially regulated by innate immune responses to saturated fatty acids or LPS, can impinge or potentiate insulin responses in skeletal muscle cells.

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