TNFα impairs mitochondrial metabolism in 3T3-L1 adipocytes

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Background and Aims: The adipose tissue is now recognized as an endocrine/paracrine organ which can influence whole body metabolism not only through the release of non-esterified free fatty acids but also via many adipokines such as tumor necrosis factor α (TNFα). TNFα is a proinflammatory cytokine, which has been implicated as a contributing cause of insulin resistance. Although the effects of TNFα are well known, the mechanisms by which TNFα impairs insulin action are not well understood. A number of recent studies support the concept that insulin resistance is linked to mitochondrial dysfunction. The aim of this study was to investigate whether TNFα may affect pathways involved in mitochondrial biogenesis and fatty acid oxidation in 3T3-L1 adipocytes.

Materials and Methods: Differentiated 3T3-L1 adipocytes were treated with TNFα (3ng/ml, 72hrs) and in the final 24hrs of that incubation, cells were also treated with troglitazone (TGZ; 10μM), and aspirin (ASA; 5mM). Insulin sensitivity was determined by 2-deoxyglucose uptake and phosphorylation of key proteins in insulin signaling pathway. Following treatments, RNA was extracted and hybridised to custom-made cDNA microarrays containing ~ 12,000 elements. Real-Time PCR of mitochondrial-specific target genes was also performed to confirm microarray data.

Results: Insulin-stimulated glucose uptake was inhibited by 30-40% (p≤0.0002 compared to vehicle, n≥10) when 3T3-L1 adipocytes were exposed to TNFα for 72 hrs. Treatment with TGZ and/or ASA alleviated TNFα-induced inhibition of insulin-stimulated glucose uptake (p≤0.007 compared to TNFα alone, n≥6). Microarray analysis of 3T3-L1 adipocytes treated with TNFα ± TGZ and ASA (n=20 replicates per treatment) identified significant changes in the expression of several genes encoding key mitochondrial proteins such as citrate synthase (CS), CPT-1, PGC1α. CPT1, PGC1α and CS gene expression were all decreased following TNFα treatment and restored to basal levels with TGZ/ASA treatment. Real time PCR analysis confirmed the microarray results and showed that PGC1α mRNA levels were markedly decreased following TNFα treatment (p<10⁻⁵). Treatment with TGZ/ASA was reversed the suppressed PGC1α gene expression following TNFα treatment (p<10⁻⁴).

Conclusion: Our results showed that TNFα not only impairs insulin-stimulated glucose uptake but also genes encoding key mitochondrial proteins, particularly those involved in the regulation of fatty acid oxidation (PGC1α, CPT1, CS, PPARα) but not genes involved in mitochondrial biogenesis (mtTFA, NRF1, PPARγ).