Glucose and insulin modulate the expression and activity of the thioredoxin antioxidant system in cultured human skeletal muscle fibres

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The thioredoxin antioxidant system is composed of thioredoxin-1 and -2, the endogenous inhibitor thioredoxin binding protein-2 (TPB-2), and thioredoxin reductase. It is ubiquitously expressed and regulates redox-sensitive signalling pathways and cellular redox balance. Reactive oxygen species (ROS), glucose and insulin modulate the thioredoxin antioxidant system. Glucose increases TBP-2 expression in a dose dependent manner and TBP-2 mRNA transcripts are elevated in *vastus lateralis* muscles of prediabetics and diabetics. *In vivo* and *in vitro*, TPB-2 expression is inversely correlated to glucose uptake in peripheral tissues. Stressed cells secrete thioredoxin and thioredoxin plasma levels are increased in patients with glucose intolerance. Regulation of the thioredoxin antioxidant system is complex and has not been well studied in skeletal muscle. A time course study was completed to investigate how glucose and insulin affect the gene and protein expression and cellular localisation of the thioredoxin-1 and -2, TBP-2 and thioredoxin reductase in cultured skeletal muscle fibres. The effects of glucose and insulin treatment on muscle ROS production and deoxy-glucose uptake were also evaluated.

Primary skeletal muscle cell cultures were established from *vastus lateralis* skeletal muscle biopsies from healthy elderly donors (N = 6, 69 ± 3 years) and grown in 5% oxygen (normoxia for muscle tissue) and 5% carbon dioxide. At passage 4, muscle fibres at 4 days post-differentiation were treated with 5.5 mM (normal-glycaemia) or 15.5 mM (hyperglycaemia) glucose in the presence of 100 pM insulin for 2 h, 6 h, and 48 h for experimental analysis. Gene expression of thioredoxin-1 and TBP-2, but not thioredoxin-2, was increased following 48 h of treatment with 5.5 mM or 15.5 mM glucose plus insulin compared with 2 h and 6 h ($p < 0.05$). At 48 h, the increase in TBP-2 mRNA transcripts tended to be greater in cells exposed to hyperglycemia, whereas the increase in thioredoxin-1 gene expression was unaffected by glucose concentration. Similar to gene expression, at 48 h of treatment with 5.5 mM or 15.5 mM glucose plus insulin TBP-2 protein levels were significantly increased in the nucleus and cytoplasm compared to 2 h and 6 h ($p < 0.05$). At 48 h the increase in TBP-2 protein expression in the nucleus was independent of glucose concentration, whereas in the cytoplasm TBP-2 protein levels tended to be 2.5-fold higher in response to hyperglycemia. Cytoplasmic and nuclear thioredoxin-1 protein levels were similar following 2 h, 6 h, and 48 h of treatment. Neither glucose nor insulin affected cytoplasmic thioredoxin reductase protein levels following up to 48 h of treatment. Since hyperglycemia is associated with increased thioredoxin secretion, an ELISA assay was used to measure secreted thioredoxin in the media following 24 h and 48 h of treatment with 5.5 mM or 15.5 mM glucose plus 100 pM insulin. Secreted thioredoxin was higher following 48 h than 24 h of treatment ($p = 0.05$), which helps explain the possible discrepancy between thioredoxin-1 gene and protein expression. Media thioredoxin content was increased by ~10% in muscle fibres treated with 5.5 mM glucose plus insulin and by ~40% in muscle fibres treated with 15.5 mM glucose plus insulin. Thus, up to 48 h of chronic exposure to 100 pM insulin and 5.5 mM or 15.5 mM of glucose are needed to affect thioredoxin-1 or TBP-2 gene and/or protein expression and localisation in cultured skeletal muscle fibres. The increase in TBP-2 and thioredoxin-1 gene expression in response to chronic insulin exposure may be mediated by increased ROS levels. Muscle fibres were treated for 48 h with 5.5 mM or 15.5 mM glucose with or without 100 pM insulin and ROS production was assessed by monitoring the oxidation of 2′,7′-dichlorodihydrofluorescein-diacetate using a scanning fluorometer. In muscle fibres treated with 5.5 mM glucose, insulin increased ROS levels by ~30% ($p = 0.06$) and in cells treated with 15.5 mM glucose, insulin increased ROS levels by ~45% ($p < 0.05$). Given the effects of glucose and insulin on ROS levels and thioredoxin antioxidant system components, basal and insulin stimulated deoxy-glucose uptake was assessed following in 48 h of treatment with 5.5 mM or 15.5 mM glucose plus 100 pM insulin. Hyperglycemia reduced basal ($p < 0.05$) and insulin stimulated (100 nM; $p = 0.06$) glucose uptake. In conclusion, chronic rather than acute exposure to insulin and hyperglycaemia increased ROS levels in cultured muscle fibres and this was associated with altered thioredoxin signalling and reduced glucose uptake.