METFORMIN IMPROVES EXTRACELLULAR MATRIX HOMEOSTASIS RELATED GENES AND TENOGENIC EXPRESSIONS ON INSULIN RESISTANCE INDUCED-HUMAN TENOCYTES IN VITRO

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Introduction: Type 2 diabetes mellitus (T2DM) and insulin resistance (IR) predispose patients to tendinopathy. Metformin, an anti-diabetic drug, has been reported to reverse cellular abnormalities in many tissues due to diabetes by targeting specific signaling pathways, i.e. AMPK pathway & inhibiting the activation of NF-kB proinflammatory pathway (Hattori, Kunihiro, Hattori, & Kasai, 2006; Lee et al., 2012). However, the effects of this drug on tendons have not been previously elucidated. To determine this, an in vitro study using IR induced human primary tenocytes (IR hTeno) model was conducted. Modulation of the expressed genes were examined following the treatment using Metformin.

Methodology: As a verification of hTeno IR model, cell lysates were harvested from hTeno treated with TNF-α for 24 h.To analyze the phosphorylation state of insulin receptor substrate-1 (IRS-1), Western Blot was performed. The hTeno were seeded for control (without any treatment); IR model (hTeno treated with TNF-α for 24, 48 & 72 h) and IR model treated with Metformin for 48 & 72h. Cells were harvested and examined for candidate tenogenic markers and ECM-related gene expression analysis.

Results: Western blot analysis demonstrated an increase in serine phosphorylation in hTeno treated with TNF-α (Figure 1). The mRNA gene expression levels of the candidate tenogenic markers, i.e. scleraxis (SCX), mohawk (MKX) and thrombospondin-4 (THBS-4), were upregulated ($p<0.05$) after treatment with Metformin for 72 h. ECM-related genes (COL1A1) were significantly upregulated after 48 h. Metformin also down-regulated ($p<0.05$) the ECM catabolic-related genes (MMP-9 & MMP-13) at all timepoints.

Conclusion: Metformin modulates the mRNA expression levels of candidate tenogenic markers and ECM-related genes in IR-induced hTeno. These observations suggest that Metformin may be suitable as a modulator in inflammation-induced cellular activities in IR hTeno; and thus may be potentially useful in treating diabetic tendinopathy.