protein synthesis. Cr uptake plateaued at 3 hours in response to saturating levels of extracellular Cr (5mM) and was 33% ($P<0.01$) higher in the presence of CHX, suggesting synthesis of a protein involved in regulating further Cr uptake. Global gene expression analysis using the Illumina Mouse WG6v2 Expression BeadChip system identified thioredoxin interactingprotein (Txnip) as the only significantly up-regulated gene under these conditions, elevated by 40% compared to non-treated cells ($P=0.036$). Quantitative RT-PCR verified up-regulation of Txnip mRNA (61% increase; $P=0.002$) and immunoblotting detected a 45% increase in Txnip protein after 3hr of Cr incubation ($P=0.01$). Treatment of cells with either 250 μM Cr or 5mM taurine, did not result in Txnip mRNA or protein up-regulation, confirming specificity for saturating Cr. Small interfering RNA against Txnip (siTxnip) reduced mRNA levels by 50% and attenuated the increase in Txnip gene expression in response to 5mM Cr (17% in siTxnip versus 45% in control cells). This was sufficient to maintain CrT activity at control levels during high [Cr], i.e. substrate inhibition was completely abolished. The *in vitro* observations were tested *in vivo* in relation to altered left ventricular [Cr]. Cr-deficient mice, due to deletion of the Cr biosynthetic enzyme guanidinoacetate methyltransferase ($GAMT^{-/-}$), had LV Txnip mRNA 35% lower than wild-types. Conversely, mice with cardiac overexpression of CrT had elevated LV [Cr] (mean 124 versus 74nmol/mg protein in WT) associated with a 58% increase in Txnip mRNA and 29% more Txnip protein when compared to wild-type littermates.

Conclusion Our work suggests a novel role for Txnip in the endogenous regulation of CrT activity in cardiomyocytes, thereby contributing to modulation of Cr homeostasis. The molecular mechanisms involved in the CrT-Txnip interaction, merit further elucidation.

214 REGULATION OF CELLULAR CREATINE HOMEOSTASIS: A ROLE FOR THIOREDOXIN INTERACTING PROTEIN (TXNIP)

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Background Creatine (Cr) uptake across the plasma membrane is particularly important in excitable cells that cannot synthesise Cr, such as cardiomyocytes, where creatine has a role in energy buffering and transport. Uptake is tightly controlled via a specific transmembrane Creatine Transporter (CrT), while cellular loss is slow, passive, and non-enzymatic. Earlier work has suggested that CrT activity is subject to substrate inhibition, but, that this requires *de novo* synthesis of unidentified protein(s). The purpose of this study was to identify this protein and to verify its role as a potential endogenous inhibitor of the CrT.

Methods and Results To achieve this we took a global gene array approach under varying conditions of cellular [Cr], and verified our findings using an *in vitro* assay of Cr-uptake using a radiolabelled ^{14}Cr method. 3T3 fibroblasts stably over-expressing CrT were incubated for 2-5 hours with either low (250 μM) or saturating (5 mM) Cr, with and without cycloheximide (CHX) to inhibit

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