Divergent autophagy responses in the liver and skeletal muscle of diabetic (db/db) mice

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Autophagy is a conserved catabolic process sensitive to nutrient availability, where intracellular dysfunctional material is recycled. Macroautophagy and chaperone mediated autophagy (CMA) are the most well studied types of autophagy and both involve the delivery of cellular components to the lysosome for degradation. Macroautophagy involves the recognition and engulfment of organelles or cytosolic components by the autophagosome before delivery to the lysosome. Alternatively, CMA involves targeting of specific proteins by the chaperone Hsc-70 and subsequent entry into the lysosome *via* the receptor LAMP-2A. Insulin resistant (IR) tissues typically display accumulation of autophagy targets such as lipids, aggregated proteins and dysfunctional mitochondria. Recently, hepatic autophagy dysfunction has been observed in IR conditions. However, the impact of IR on the acute regulation of autophagy by nutrient availability, as well as its influence on skeletal muscle remains elusive. We investigated the effect of overt diabetes on autophagy markers in mouse liver and skeletal muscle in both the fasted and the fed state.

C57BL6 db/db and control heterozygous (db/+; n=18 per group) mice were fed a standard chow diet for 8 weeks. Mice were killed by cervical dislocation either in the fed state (ad libitum food access) or after a 4 h fast and liver and *quadriceps* muscle were collected. The abundance of markers for macroautophagy, CMA and lysosomes were determined by immunoblotting. Data were analysed using a two-way ANOVA and statistical significance was set at $P<0.05$.

In the liver, markers of autophagosome content (lipidated LC3B) and autophagosome degradation (p62) remained unchanged between all groups. However, the amount of non-lipidated LC3B decreased ($P<0.05$) in db/db compared to control mice indicating a lower capacity for macroautophagy. Moreover, the substrate targeting chaperone (Hsc-70) was downregulated ($P<0.05$) in db/db compared to control mice, while the abundance of the CMA receptor (LAMP2A) remained unchanged, suggesting a potential decrease in CMA capacity. In skeletal muscle, both LC3B isoforms were higher ($P<0.05$) in db/db mice, whereas p62 abundance was unchanged, suggesting increased macroautophagy capacity and autophagosome content in db/db mice. Furthermore, the abundance of LAMP2A was decreased ($P<0.05$) in the db/db mice, compared to the control, and an analogous trend was found for Hsc-70 ($P=0.06$), suggesting a lower CMA capacity. There was no difference in any macroautophagy or CMA markers between the fed and 4 h fasted state in either skeletal muscle or liver.

Taken together, these findings demonstrate a tissue specific influence on autophagy pathways in response to diabetes, where a reduction in macroautophagy capacity in the liver occurs alongside an upregulation of macroautophagy in skeletal muscle. Work is ongoing to further clarify the observed responses and their impact in both tissues. These findings will contribute to the existing body of evidence on the pathophysiology of type 2 diabetes.