Hepatic autophagy dysfunction in mice following high fat feeding

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Autophagy is a highly conserved and dynamic housekeeping process that promotes cellular homeostasis and is acutely regulated by nutrient availability. Recently, dysfunctions in this process have been linked to insulin resistance and ectopic deposition of lipids in non-adipose tissue (Singh *et al.*, 2009; Cuervo & Wong, 2014). We investigated the effect of chronic high fat feeding on autophagy responses before and after acute glucose administration in the liver and skeletal muscle. C57BL/6 male mice were randomly divided into 2 groups (n=20 each) and were fed either a high-fat (HFD, 42% total energy from fat) or standard chow control (CON) diet for 10 weeks. After a 5h fast, mice underwent an oral glucose tolerance test administered *via* oral gavage. Prior to tissue sampling, mice were euthanased by cervical dislocation. Red *gastrocnemius* muscle and liver were collected at baseline and 15 min after glucose administration and the abundance of autophagy markers, lipid droplet-associated proteins and upstream signalling events were determined by immunoblotting. The study was approved by the Monash University Animal Ethics Committee (Code: MARP-2012-046). Data were analysed using a two-way ANOVA and statistical significance was set at $P<0.05$.

In the liver, the abundance of the lipidated form of LC3B and GABARAP were significantly higher in the HFD group compared to CON (main effect of diet, $P<0.05$), indicating a greater autophagosome content with HFD. The abundance of p62 was also elevated in HFD, compared to CON (main effect of diet, $P<0.05$), indicating a reduction in autophagic clearance by the lysosome, without any significant changes in the lysosome marker LAMP1. HFD also resulted in greater accumulation of the lipid droplet proteins PLIN2 and PLIN3 (main effect of diet, $P<0.05$) and inhibition of insulin signalling as demonstrated by reductions in pAktThr308 post glucose administration ($P<0.05$). In skeletal muscle, there was no main effects observed in autophagy markers (LC3B, GABARAP or p62), despite a tendency for a reduction in insulin signalling in HFD ($P=0.07$). Interestingly in muscle, there was a main effect of diet on LAMP1 levels ($P<0.05$), suggesting a higher lysosome content in the HFD group. There were no differences in markers of chaperone-mediated autophagy or mitophagy between HFD and CON in muscle or liver. Furthermore, there were no changes in any autophagy markers in liver or muscle following acute glucose administration. In conclusion, accumulation of autophagosomes in the liver following HFD is due to a reduction in autophagosome clearance by the lysosome. These autophagy impairments may contribute to hepatic lipid accumulation and metabolic dysfunction following high fat feeding.

Cuervo AM & Wong E. (2014) Chaperone-mediated autophagy: roles in disease and aging. *Cell Res* **24**: 92-104.

Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM & Czaja MJ. (2009) Autophagy regulates lipid metabolism. *Nature* **458**: 1131-1135.