Autophagy modulation in the liver and skeletal muscle of high-fat fed mice

G. Morales-Scholz,[1,2] K.F. Howlett,[1] R.M. Murphy,[3] G.M. Kowalski,[1] C.R. Bruce[1] and C.S. Shaw,[1] [1]Institute of Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC 3126, Australia, [2]Research Institute of Human Movement Sciences, School of Physical Education, University of Costa Rica, San José, 11501, Costa Rica and [3]Department of Biochemistry and Genetics La Trobe Institute for Molecular Science La Trobe University Melbourne, VIC 3086, Australia.

Autophagy is a highly conserved and dynamic process that is acutely regulated by nutrient availability and contributes to the maintenance of cellular homeostasis. Insulin resistance has been linked to defects in both macroautophagy (Singh *et al.*, 2009) and chaperone-mediated autophagy (CMA) (Cuervo & Wong, 2014). We aimed to investigate the influence of chronic high fat feeding on autophagy responses in the liver and skeletal muscle.

C57BL/6 male mice were randomly divided into 2 groups and were fed either a high-fat (HFD, n=20) or standard chow (Chow, n=20) 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 samples were collected before and 15 min after glucose administration and the abundance of macroautophagy (LC3-I, LC3-II, p62, TP53INP2) and CMA (LAMP2A) markers were determined by immunoblotting. Data were analysed using a two-way ANOVA and statistical significance was set at $P<0.05$.

In the liver, the abundance of LC3-I was significantly lower in the HFD group compared to Chow ($P<0.05$), but LC3-II was unchanged. This resulted in an increase in the LC3-II/LC3-I ratio which bordered on statistical significance ($P=0.07$), indicating greater lipidation of LC3 following HFD. Moreover, p62 levels in the liver tended to increase in the HFD group ($P=0.08$) indicative of reduced autophagosome degradation. Additionally, the abundance of TP53INP2, another autophagosome degradation marker, decreased following HFD ($P<0.05$). In skeletal muscle, there were no significant changes observed in LC3 isoforms, LC3-II/I ratio, p62 nor TP53INP2 levels with HFD. However, there was a significantly lower abundance of LAMP2A in skeletal muscle following HFD ($P<0.05$). There were no changes in any autophagy marker in muscle or liver in response to glucose administration.

These results indicate that a greater proportion of LC3 lipidation in the liver following HFD may be due to reduced autophagosome clearance by the lysosome. In skeletal muscle, the reduction in LAMP2A with HFD could indicate a reduced capacity for CMA. The importance of CMA to skeletal muscle homeostasis and quality control requires further investigation.

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.