Abstract: 167P

Autophagy Flux in Human Peripheral Blood Mononuclear Cells in the 24 h Fasted and Fed State

Vicky Kuriel, Giselle Allsopp, Tim Connor, Sanna Barrand, Greg Kowalski, David L Hamilton, Kirsten F Howlett, Christopher S Shaw

Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC 3216, Australia.

Autophagy is a cellular recycling process that plays a central role in optimal cellular function. Dysfunctional autophagy has been linked to accelerated ageing and a broad range of chronic diseases yet the dynamic measurement of autophagy flux in humans has been a significant limitation to the translational of preclinical data. Peripheral blood mononuclear cells (PBMCs) offer a practical and minimally invasive method to quantify autophagy flux in humans which is responsive to leucine and insulin *ex vivo* (1). However, this method has not been applied to PBMCs in response to physiological nutrient and hormonal variations that occur *in vivo*. This study compared autophagy flux and activation of upstream signalling pathways in human peripheral blood mononuclear cells (PBMCs) following a 24 h fast and in the fed state. Blood samples from twelve healthy young individuals (7 females, 5 males, age: 30 ± 6 years, BMI: 24.3 ±1.8 kg/m2) were collected after a 24 h fast and following ingestion of a mixed meal. Whole blood following the 24 h fast and 1 h and 2 h post-feeding were incubated at 37oC with and without the lysosomal inhibitor chloroquine for 1 h. PBMCs isolated through centrifugation were assessed for markers of autophagy flux (LC3-I, LC3-II and p62) and the activation of upstream signalling proteins through immunoblotting. Plasma insulin, branched chain amino acids and triacylglycerol concentrations were significantly elevated (P<0.001), and β- hydroxybutyrate, glycerol and free fatty acids were significantly reduced (P<0.001) in the fed-state as compared to the fasted state. Chloroquine incubation resulted in ~4-fold increase in LC3-II, indicative of successful inhibition of lysosomal degradation (P<0.001). However, there was no significant difference in autophagy flux when comparing the 24 h fasted and fed state (ΔLC3-II of 2.8 ± 1.25 AU versus 2.11 ± 1.07 AU for fasted and 1 h post-meal, respectively, P=0.40). Compared to the fasted state, activation of the mTOR signalling pathway was apparent in the fed state via an ~3-fold increase in p-S6Ser235/236 (P=0.02) but this did not translate to downstream autophagy signalling, as p- ULK1Ser757was unaffected (P>0.05). These preliminary findings suggest that while human PBMCs are amenable to assessment of autophagy flux, it is unclear whether they are responsive to the physiological metabolite and hormonal changes that occur following a 24 h fast and subsequent refeeding. Further work is required to demonstrate the utility of using PBMCs to assess changes in autophagy flux and whether they reflect other human tissues.

1. Bensalem J, Hattersley KJ, Hein LK, Teong XT, Carosi JM, Hassiotis S, et al (2021). Measurement of autophagic flux in humans: an optimized method for blood samples. *Autophagy*. **17**, 3238-3255.