High fat diet remodels the intestinal lymphatic vasculature to promote obesity and glucose intolerance

E. Cao,[1] M.J. Watt,[2] C. Nowell,[1] G. Gracia,[1] H. Chu,[1] L. Hu,[1] C.J.H. Porter[1] and N.L. Trevaskis,[1] [1]Monash Institute of Pharmaceutical Sciences, Monash University, Parkville 3010, Australia and [2]Biology of Lipid Metabolism Laboratory, Department of Physiology, Monash University, Clayton, VIC 3165, Australia .

Insulin resistance (IR) underpins a spectrum of prevalent and inadequately treated cardiometabolic diseases, including type 2 diabetes (T2D). Excess adipose, particularly visceral adipose tissue (VAT) in the abdomen, increases the risk of IR. The expanded VAT releases pro-inflammatory and metabolic mediators that promote IR. In diabetic *db/db* mice the permeability of intestinal collecting lymphatic vessels is significantly increased and in transgenic mice with hyperpermeable lymphatics the adipose surrounding lymphatics is expanded. The content of inflammatory and metabolic mediators in intestinal lymph is also increased in response to high fat diet (HFD. Together these findings suggest that increased intestinal lymph extravasation to surrounding VAT promotes VAT expansion. Our aim is to determine whether intestinal lymph access to VAT is increased in HFD thereby promoting VAT expansion and pathogenic changes that promote whole body IR.

Firstly, to confirm that intestinal lymph access to surrounding VAT is increased in response to HFD, the structure and permeability of intestinal lymphatic vessels within VAT was assessed in mice fed standard chow fat diet (CFD) or HFD using immunofluorescence analysis of mesenteric lymph vessels and evans blue lymphangiography, respectively. In mice fed the HFD *vs* the CFD there was a progressive increase in mesenteric collecting lymph vessel complexity and permeability within the VAT over time. The remodelling and permeability increased with time from the early stage (week 6-15 HFD feeding) to the chronic stage (week 32 HFD feeding) of diet induced obesity. Secondly, metabolomics and FACS analysis revealed increases in the majority of lipid species, several pro-inflammatory mediators including prostaglandins, and CD4+ and CD8+ T cells in the HFD *vs* CFD fed mesenteric lymph. Prostaglandins promote lymphangiogenesis and COX-2 inhibition has been shown to reduce lymphangiogenesis and lymph vessel hyperpermeability. Treatment with a COX-2 inhibitor, celecoxib, successfully prevented and reversed the changes in lymph composition, lymphatic vessel mispatterning and hyperpermeability resulting in improved glucose tolerance. *In vivo* experiments were conducted under isoflurane inhalation. *In vitro* studies further confirmed that HFD conditioned lymph promotes pathogenic changes in adipocytes with the potential to induce whole body IR. Treatment of 3T3-L1 adipocytes with lymph, particularly from HFD fed mice, enhanced adipocyte adipogenesis (PPARγ, leptin, C/EBPα), inflammation (IL-6), lipogenesis (Ap2, triglyceride), and lipolysis (ATGL, HSL, FFA).

In conclusion, intestinal lymph extravasation to surrounding VAT is enhanced in response to HFD and increased lymph exposure to adipocytes, particularly pro-inflammatory HFD fed lymph, promotes functional changes in adipocytes consistent with the changes that promote adipose expansion and IR *in vivo*. Further, reversing the lymphatic changes and hyperpermeability in HFD fed mice *via* treatment with celecoxib was able to restore glucose tolerance. These studies provide evidence that intestinal lymph extravasation to VAT is increased with HFD feeding thereby promoting VAT expansion and pathogenic changes that promote whole body IR. These studies advance the fundamental understanding of the pathogenic drivers of IR. Subsequent studies will probe the potential to attenuate pathogenic changes in VAT and thereby treat whole-body IR by modulating lymph content and/or lymph access to VAT.