Role of insulin receptor substrate-2 serine phosphorylation in the regulation of liver metabolism

K.F. Howlett,[1] C. Swinton,[2] B.J. van Denderen,[3] K. Sakamoto[4] and S.L. McGee,[2] [1]Center for Physical Activity and Nutrition, School of Exercise and Nutrition, Deakin University, VIC 3217, Australia, [2]Metabolic Research Unit, School of Medicine, Deakin University, VIC 3217, Australia, [3]Department of Medicine, St. Vincent's Institute of Medical Research, University of Melbourne, VIC 3065, Australia and [4]Nestlé Institute of Health Sciences, Lausanne 1015, Switzerland.

Introduction: Insulin mediates diverse metabolic responses in the liver, including both glucose production and storage, through the canonical insulin signalling pathway. Insulin receptor substrate (IRS) proteins are key mediators of the insulin signalling pathway, where they act as docking proteins between cell surface receptors and a complex network of intracellular signalling molecules. Whole body (Kubota *et al.*, 2000; Previs *et al.*, 2000; Withers *et al.*, 1998) and liver specific (Valverde *et al.*, 2003) knockout animal models implicate IRS-2 as the principle mediator of glucose homeostasis in the liver, and demonstrate that impaired IRS-2 signalling contributes to the development of hepatic insulin resistance. Phosphorylation of IRS proteins on serine sites is a key control process under both physiological and pathological conditions (Copps & White, 2013). However, very few sites on IRS-2 have been identified and examined in detail as to their effects on insulin mediated signalling pathways and subsequent cellular processes.

Methods: IRS-2 serine (Ser, S) phosphorylation sites, S587 and S604, that are located close to or within a unique IRS-2 kinase loop binding domain that interacts with the insulin receptor, were identified from results using high resolution mass spectrometry-based proteomics designed to predict physiological substrates of insulin regulated kinases (Humphrey *et al.* 2013). To examine the role of these specific IRS-2 serine phosphorylation sites on insulin signalling and glucose metabolism in the liver, S587 and S604 of mouse IRS-2 were substituted to alanine (A) using site-directed mutagenesis. FAO hepatoma cells were transfected with wild type (WT) or S587/604A IRS2 plasmids and insulin-stimulated intracellular signalling, gene expression and measures of glucose metabolism were performed.

Results: The insulin-stimulated phosphorylation of forkhead box class O (FoxO1 pSer256), a transcription factor that coordinates glucose utilisation and storage, was significantly attenuated in mutant S587/604A IRS-2 cells compared to WT controls. However, the expression of the FoxO1-dependent gluconeogenic genes, glucose-6-phosphatase catalytic subunit (G6Pc) and phosphonenolpyruvate carboxykinase (PEPCK), and glucose production were reduced ($P<0.05$) to a similar extent in both WT and mutant cells in response to insulin. In contrast, the expression of the FoxO1-dependent pyruvate dehydrogenase kinase-2 (PDK2) gene, which regulates pyruvate flux through the pyruvate dehydrogenase complex, was significantly increased in mutant IRS2 cells and was associated with a greater ($P<0.05$) glycolytic to oxidative flux (ECAR/OCR) ratio in S587/604A IRS-2 cells compared to WT cells in glucose media.

Conclusion: These data suggest that these unique serine phosphorylation sites on the insulin signalling protein, IRS-2, control specific aspects of hepatocyte glucose metabolism that could play an important role in hepatic energy substrate selection.

Copps KD, White MF. (2012). *Diabetologia* **55**, 2565-2582.

Humphrey SJ, Yang G, Yang P, Fazakerley DJ, Stöckli J, Yang JY, James DE. (2013). *Cell Metab* **17**, 1009-1020.

Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T. (2000). *Diabetes* **49**, 1880-1889.

Previs SF, Withers DJ, Ren JM, White MF, Shulman GI. (2000). *J Biol Chem* **50**, 38990-38994.

Valverde AM, Burks DJ, Fabregat I, Fisher TL, Carretero J, White MF, Benito M. (2003). *Diabetes* **52**, 2239-2248.

Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF. (1998). *Nature* **391**, 900-904.