Phosphoproteomics reveals the complexity of the human muscle exercise-regulated signalling network and a novel role for AKAP1 in regulating mitochondrial respiration via AMPK

N.J. Hoffman,[1,2] B.L. Parker,[1,2] R. Chaudhuri,[1,2] K.H. Fisher-Wellman,[2] M. Kleinert,[2,3] P. Yang,[2,4] S.J. Humphrey,[2] S. Trefely,[2] D.J. Fazakerley,[1,2] R. Jothi,[4] E.A. Richter[3] and D.E. James,[1,2] [1]School of Molecular Bioscience, Charles Perkins Centre, Education and Research Hub - D17, The University of Sydney, NSW 2006, Australia, [2]Garvan Institute of Medical Research, Diabetes and Metabolism Division, 384 Victoria Street, Darlinghurst, NSW 2010, Australia, [3]University of Copenhagen, Department of Exercise and Sport Sciences, August Krogh Building, 13 Universitetsparken, Copenhagen, DK-2100, Denmark and [4]Biostatistics Branch, National Institute of Environmental Health Sciences, National Institutes of Health, 111 T.W. Alexander Drive, MD A3-03, Research Triangle Park, NC 27709, USA.

Exercise plays an essential physiological role in whole body insulin sensitivity and metabolic homeostasis. A lack of physical activity causes insulin resistance in humans, and increased physical activity improves insulin sensitivity. However, only a few signalling pathways are known that are activated by exercise to trigger these beneficial effects in skeletal muscle, a major site of insulin resistance and exercise action. Therefore, it is crucial to map the complete repertoire of exercise activated pathways in order to identify the biology underlying exercise action in skeletal muscle and unleash exercise's full therapeutic potential. To fill the gap in our understanding of muscle exercise signalling, we performed a global, unbiased mass spectrometry (MS)-based phosphoproteomic analysis of human skeletal muscle biopsies obtained from four healthy male subjects before and after a single bout of high-intensity bicycle exercise.

The exercise signalling response between each subject was highly reproducible (average Pearson's correlation coefficient r=0.72). We identified 1,004 phosphosites that were significantly regulated with acute exercise (P<0.05, >1.5 fold change and false discovery rate <0.01). Many well characterized acute exercise-regulated signalling pathways were significantly regulated including the AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK), protein kinase A (PKA), protein kinase B (Akt), mammalian target of rapamycin (mTOR) and calcium pathways, as well as several pathways never previously known to be regulated by exercise. Of the 1,004 exercise-regulated phosphosites, more than 900 have not been previously quantified in exercised human muscle and the upstream kinases are unknown. Given the known therapeutic potential of the AMPK pathway, we performed two additional MS screens to specifically pinpoint novel AMPK substrates in the human muscle data including: (1) global MS-based phosphoproteomic analysis of rat L6 myotubes with and without AMPK agonist stimulation; and (2) a novel global AMPK in vitro kinase assay combined with targeted MS phosphopeptide quantification in human embryonic kidney (HEK-E) cells. Integration of these data sets revealed a number of potential novel AMPK substrates never previously described including A-kinase anchor protein 1 (AKAP1), an outer mitochondrial membrane scaffold protein that is known to bind PKA. We next performed detailed mechanistic studies to validate our MS findings and determine the biological significance of AMPK-mediated phosphorylation of AKAP1 Ser103. *In vitro* and *in vivo* validation experiments confirmed that AKAP1 is a *bona fide* muscle AMPK substrate. Furthermore, using complementary siRNA knockdown of AKAP1 and overexpression of an AKAP1 Ser103 phospho-dead mutant we have identified a novel role for Ser103 phosphorylation of AKAP1 in AMPK-mediated mitochondrial palmitate oxidation. These mechanistic studies highlight AKAP1 as a novel exercise- and AMPK-regulated phosphoprotein playing a critical role in muscle mitochondrial respiration. Taken together, the comprehensive human muscle exercise-regulated signalling network and targeted AMPK signalling screens contain a wealth of novel phosphoproteins for mechanistic dissection that will help reveal new biological mechanisms underlying exercise. These data will serve as an invaluable resource for future study by the exercise physiology research community.