The effects of acute exercise and creatine supplementation on Akt signalling in human skeletal muscle

R.J. Snow, C.R. Wright, M.J. Quick, A.P. Garnham, K.K. Watt and A.P. Russell, The Centre for Physical Activity and Nutrition Research (C-PAN), School of Exercise and Nutrition Sciences, Deakin University, VIC 3125, Australia.

Introduction. The protein kinase Akt has recently been described as a major signalling pathway regulating skeletal muscle remodelling. Stimulation of the Akt signalling pathway through IGF/IRS/PI3K activates a series of downstream anabolic targets including Glycogen Synthase Kinase 3β (GSK-3β) and the mammalian target of rapamycin (mTOR) (Bodine *et al.,* 2001) and can also inhibit catabolic pathways involving Forkhead Transcription Factors (FOXO) and their atrophy gene targets, atrogin-1 and MuRF1 (Stitt *et al.,* 2004). It has recently been shown that Akt and GSK-3β phosphorylation is increased within a few hours following moderate intensity endurance exercise (Mascher *et al.,* 2007). However, whether endurance exercise regulates other Akt targets such as mTOR or FOXO was not determined. Creatine supplementation is also associated with increases in IGF-1 mRNA (Louis *et al.,* 2004) and by analogy, could also increase the phosphorylation and activation of Akt. It is therefore possible that both endurance exercise and creatine supplementation could stimulate Akt and its downstream pathways. At present the effect of endurance exercise and creatine supplementation on the phosphorylation of Akt and its downstream signalling targets has not been investigated in humans. The aim of this study was to determine the effects of acute endurance exercise, with/and without creatine supplementation, on the phosphorylation status of proteins involved in the Akt signalling pathways; Akt/GSK-3β, Akt/mTOR, Akt/FOXO.

Methods. Sixteen healthy male subjects performed single leg cycling at 65% VO2 peak until exhaustion. Subjects were randomly assigned to a creatine or placebo group and were administered either 0.4 g/kg of creatine monohydrate with 0.4 g/kg of glucose or 0.8 g/kg of glucose, respectively, for five days post exercise. Muscle biopsies were taken before the exercise bout and at 3 hours, 1 day and 5 days post exercise from both the exercising and non-exercising legs. Biopsies were taken for the non-exercising leg to control for the potential confounding effects of inflammation induced by multiple muscle biopsies. Cytosolic and nuclear proteins were separated and phosphorylation levels of Akt, GSK-3β, mTOR, 4EBP1, Foxo1 and Foxo3a were analyzed by western blotting. IGF-1 mRNA expression was measured using quantitative PCR.

Results. A 3-way ANOVA revealed a strong trend ($p = 0.067$) and a significant interaction ($p = 0.023$) between treatment (creatine *vs* placebo) x exercise x time for Akt and GSK3β phosphorylation levels, respectively. For Akt, further analyses demonstrated a trend ($p = 0.076$) for an interaction between treatment and time suggesting an increase in Akt phosphorylation levels at days 1 and 5 in the creatine group. Along similar lines, GSK3β phosphorylation levels were increased at days 1 and 5 in the creatine group. There was no interaction or main effects for mTOR, 4EBP1, Foxo1 and Foxo3a phosphorylation levels or IGF-1 mRNA.

Conclusion. These observations suggest that creatine supplementation for 1 to 5 days may increase the activity of the Akt/ GSK-3β signalling pathway. Acute single leg endurance exercise does not appear to affect the phosphorylation levels of Akt or its downstream signalling proteins, when measured 3 hours, 1 day and 5 days post exercise.

Bodine SC. Stitt TN, Gonzalez M, Kline WO, Stover, GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ & Yancopoulos GD. (2001) *Nature Cell Biology*, **3:** 1014-9.

Louis M, Van Beneden R, Dehoux M, Thissen JP & Francaux M. (2004) *FEBS Letters*, **557:** 243-7.

Mascher H, Andersson H, Nilsson PA, Ekblom B & Blomstrand E. (2007) *Acta Physiologica (Oxford)*, **191:** 67-75.

Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, Gonzalez M, Yancopoulos, GD & Glass DJ. (2004) *Molecular Cell*, **14:** 395-403.