Inhibition of Inositol hexakisphosphate Kinase 1 (IP6K1) Does Not Increase Akt and mTOR Activity *in Vitro*

Richie D. Barclay, Neale Tillin, Chris Tyler & Richard W. A. Mackenzie

University of Roehampton London
Sport and Exercise Sciences

Background

Insulin like growth factor 1 (IGF - 1) is well characterised as a key stimulus of the Akt, an upstream target of the rapamycin (mTOR) pathway. Recent research has identified that insulin and IGF-1 share similar intracellular signalling roles[1]. Reduced Akt and mTOR activity are attenuating contributors in the development of skeletal muscle atrophy in ageing adults[2]. Limited data suggests that Inositol hexakisphosphate Kinase 1 (IP6K1) may inhibit Akt activity in hepatic cell lines [3] and diabetic rodent [4]. Our lab has recently shown that muscle contraction reduces muscle IP6K1 content while increasing pAkt in insulin resistant humans [5]. Given both glucose transport and protein synthesis require Akt activation, we investigated if IP6K1 inhibition reduces the IGF - 1 - Akt - mTOR signalling cascade.

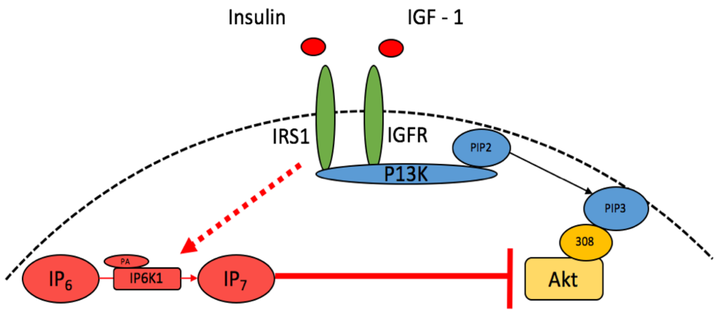

Figure 1. The insulin/IGF - 1 Signaling Cascade. The potential intracellular mechanism of IP6K1 on Akt Signaling. Abbreviations: IGF – 1, Insulin like growth factor 1; IRS1, Insulin receptor substrate 1; IGFR, Insulin like growth factor receptor; P13K, Phosphoinositide 3-kinase; IP6, myo-inositol hexakisphosphate; PA, phosphatidic acid; IP6K1, Inositol hexakisphosphate Kinase 1; IP7, 5-diphosphoinositol pentakisphosphate; PIP2, (4,5)-bisphosphate ; PIP3, phosphatidylinositol 3,4,5-trisphosphate; Akt, protein kinase B; Akt^{308}, Akt threonine 308.

Aims & Hypotheses

- IP6K1 will be downregulated in IGF - 1 + TNP treated C2C12 myotubes.
- To investigate Akt & mTOR activity in response to IP6K1 inhibition.
- Investigate what effect different concentrations of IGF - 1 has on IP6K1, Akt and mTOR signaling.

Methodology

C2C12 mouse skeletal myoblast cells were cultured in growth media (DMEM, 10% FBS, 1% anti - anti, 1% penicillin - streptomycin) under standard cell culture conditions (37 °C, 5% CO_2, 100 % humidity), changing media every 24 - 48 hours. Once confluence was reached, myoblasts were incubated in differentiation media (DMEM, 2% donor equine serum, 1% anti - anti, 1% penicillin - streptomycin) for 96 hours, changing media every 24 - 48 hours, to form mature myotubes.

Myotubes were treated with IGF - 1 (10ng/mL) +/- N2-(m-(trifluoromethyl)lbenzyl) N6-(p-nitrobenzyl)purine (TNP; 10µm) for 24 hours. Similarly, IGF - 1 was supplemented to mature myotubes in a serial concentration (10, 30, 50 ng/mL). Treated cells were lysed and total protein content determined using Lowry method. Western blot analysis was used to quantify t-Akt, p-Akt^{308}, p-Akt^{473}, t-mTOR, p-mTOR^{2448} and IP6K1. PathScan® Akt array kit was used to quantify relative intensity of signalling proteins surrounding the Akt – mTOR pathway in lysed homogenate.

Results

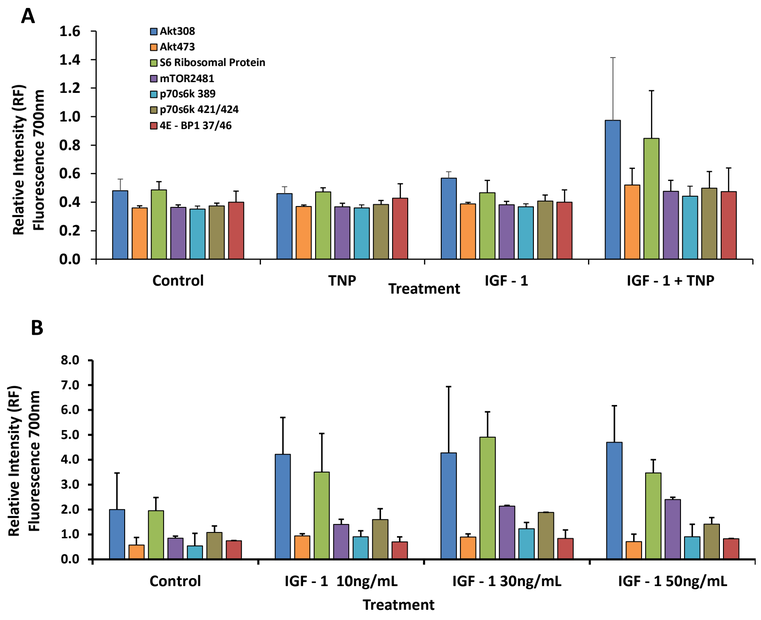

Figure 2. PathScan® Akt Array Fluorescently read (700nm) for A) IGF - 1 + TNP treated cells and B) serial concentration of IGF - 1. *P < 0.05 vs control. Values are mean ± SEM (n = 3).

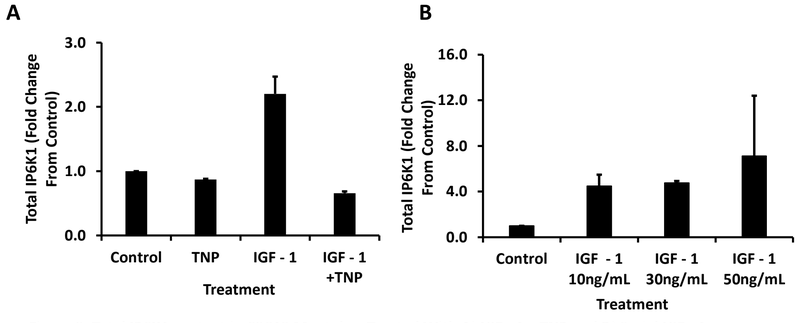

Figure 3. Total IP6K1 content in C2C12 Myotubes Treated With A) IGF - 1 + TNP and B) Serial IGF - 1 Concentration for 24hr. Analysed by Western blot. Values are mean ± SEM (n = 3). *P < 0.05 vs control.

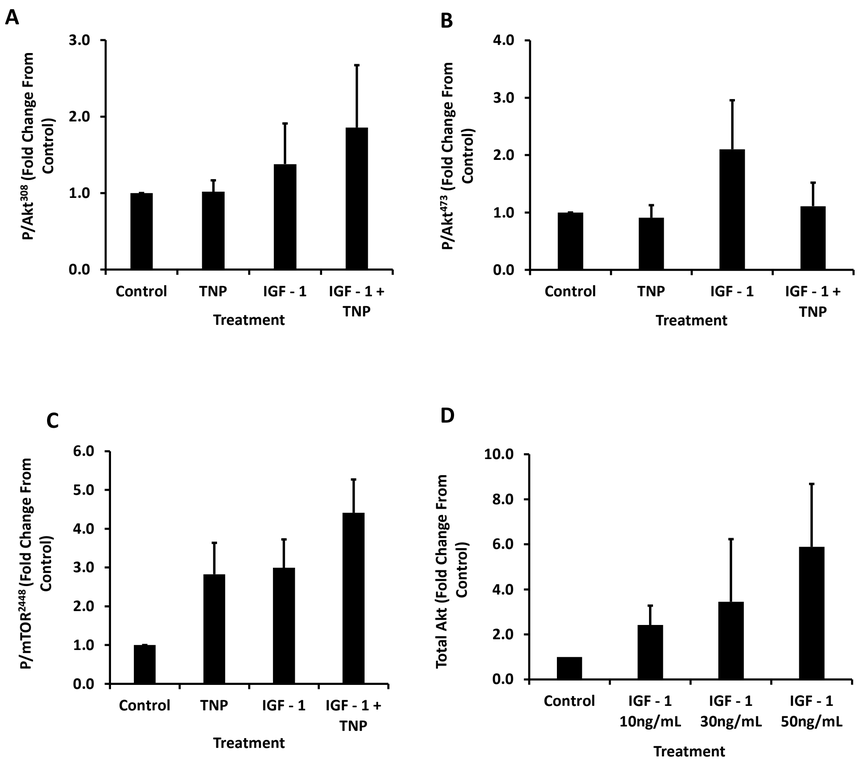

Figure 4. A) P/Akt^{308} Activity of IGF – 1 + TNP Treated Myotubes. B) P/Akt^{473} Activity of IGF - 1 + TNP Treated Myotubes. C) P/mTOR^{2448} Activity of IGF - 1 + TNP Treated Myotubes. D) Total Akt in a Serial Concentration of IGF – 1. Analysed by Western blot. Values are mean ± SEM (n = 3).

A significant condition x IP6K1 effect was noted (P < 0.05) between IGF - 1 treatment and control in C2C12 myotubes, whilst IGF – 1 + TNP decreased IP6K1 content compared to IGF – 1 alone (P < 0.05). Significant differences were observed between IGF - 1 + TNP and control in p-Akt^{308} and p-Akt^{473} (P < 0.05), however no significant differences were observed between IGF - 1 + TNP and control in p/Akt^{308}, p/Akt^{473} and p/mTOR^{2448} (P > 0.05). No significant differences between total IP6K1 and total Akt in varying concentrations of IGF - 1 were noted (P > 0.05).

Conclusions

This research, to the author's knowledge, was the first to characterise mTOR signalling in IP6K1 inhibited cultured muscle cells. Following inhibition by TNP treatment, IP6K1 inhibition in C2C12 myotubes did not effect downstream activity of Akt or mTOR. Given the small sample size, more research is required to confirm this mechanism. Lastly, increased concentrations had no additional effect on IP6K1 and Akt signalling but may increase downstream mTORC1 signalling.

References

1. O'Neill, B.T., Lauritzen, H.P., Hirshman, M.F., Smyth, G., Goodyear, L.J. & Kahn, C.R. (2015) Differential role of insulin/IGF-1 receptor signaling in muscle growth and glucose homeostasis. *Cell Reports.* 11(8) pp.1220-1235.
2. Haran, P. H., Rivas, D. A., & Fielding, R. A. (2012). Role and potential mechanisms of anabolic resistance in sarcopenia. *Journal of Cachexia, Sarcopenia and Muscle*, *3*(3), 157–162.
3. Chakraborty, A., Koldobskiy, M.A., Bello, N.T., Maxwell, M., Potter, J.J., Juluri, K.R., Maag, D., Kim, S., Huang, A.S. & Dailey, M.J. (2010) Inositol pyrophosphates inhibit akt signaling, thereby regulating insulin sensitivity and weight gain. *Cell.* 143(6) pp.897-910.
4. Ghoshal, S., Zhu, Q., Asteian, A., Lin, H., Xu, H., Ernst, G., Barrow, J.C., Xu, B., Cameron, M.D. & Kamenecka, T.M. (2016) TNP [N2-(m-trifluorobenzyl), N6-(p-nitrobenzyl) purine] ameliorates diet induced obesity and insulin resistance via inhibition of the IP6K1 pathway. *Molecular Metabolism.* 5(10) pp.903-917.
5. Naufahu, J., Elliott, B., Markiv, A., Dunning-Foreman, P., McGrady, M., Howard, D., ... & Mackenzie, R. W. (2017). High intensity exercise decreases IP6K1 muscle content & improves insulin sensitivity (SI2*) in glucose intolerant individuals. *The Journal of Clinical Endocrinology & Metabolism*.