

## Introduction

Insulin signaling is a result of interaction of many proteins, some of which are phosphorylated, others transported from within the cell into the plasma membrane. Proteins involved in insulin signalling can be regulated and incorrect regulation may lead to metabolic diseases.

One of the most rapidly emerging diseases involving insulin is diabetes melitus type 2. In order to fully understand the origins of the disease and proteins involved in regulation of insulin signaling experiment was designed to test whether two proteins - Mnk1 and Mnk2 - are regulating the proteins involved in the process such as protein kinase B, EIF4EBP1 and AS160.

## Materials & Methods

- Cells: Mouse Embryonic Fibroblasts (MEFs) - wild type and double knockout for Mnk1 and Mnk2; Nasopharyngeal carcinoma cells;
- Incubation Medium: DMEM GlutMAX, FPS, Antibiotic mix - Streptomycin+Penicillin;
- Additives to medium: Optimen + fugene 6 + siRNA; Optimen + fugene 6 + cDNA, Dharmafect + siRNA, Dharmafect + cDNA;
- Procedure for transfection: add additives to medium 72 hours before insulin treatment;
- Procedure for insulin treatment: growth until 90% confluency, starvation for 2 h (GLUT4 translocation) or 12 h (PKB, AS160, 4EBP1 phosphorylation); insulin treatment for 20 minutes (translocation) or 5, 15 and 30 minutes (phosphorylation), SDS PAGE electrophoresis and Western Blotting;
- Sample preparation: cell lysing (phosphorylation) or homogenation and lysing (translocation); spectroscopic measurements of concentration of proteins, standarisation of samples and boiling for 5 minutes in 95°C.

## Acknowledgments

I would like to thank Professor Chris Proud for his support and to Rebecca Stead for her guidance.

## Results

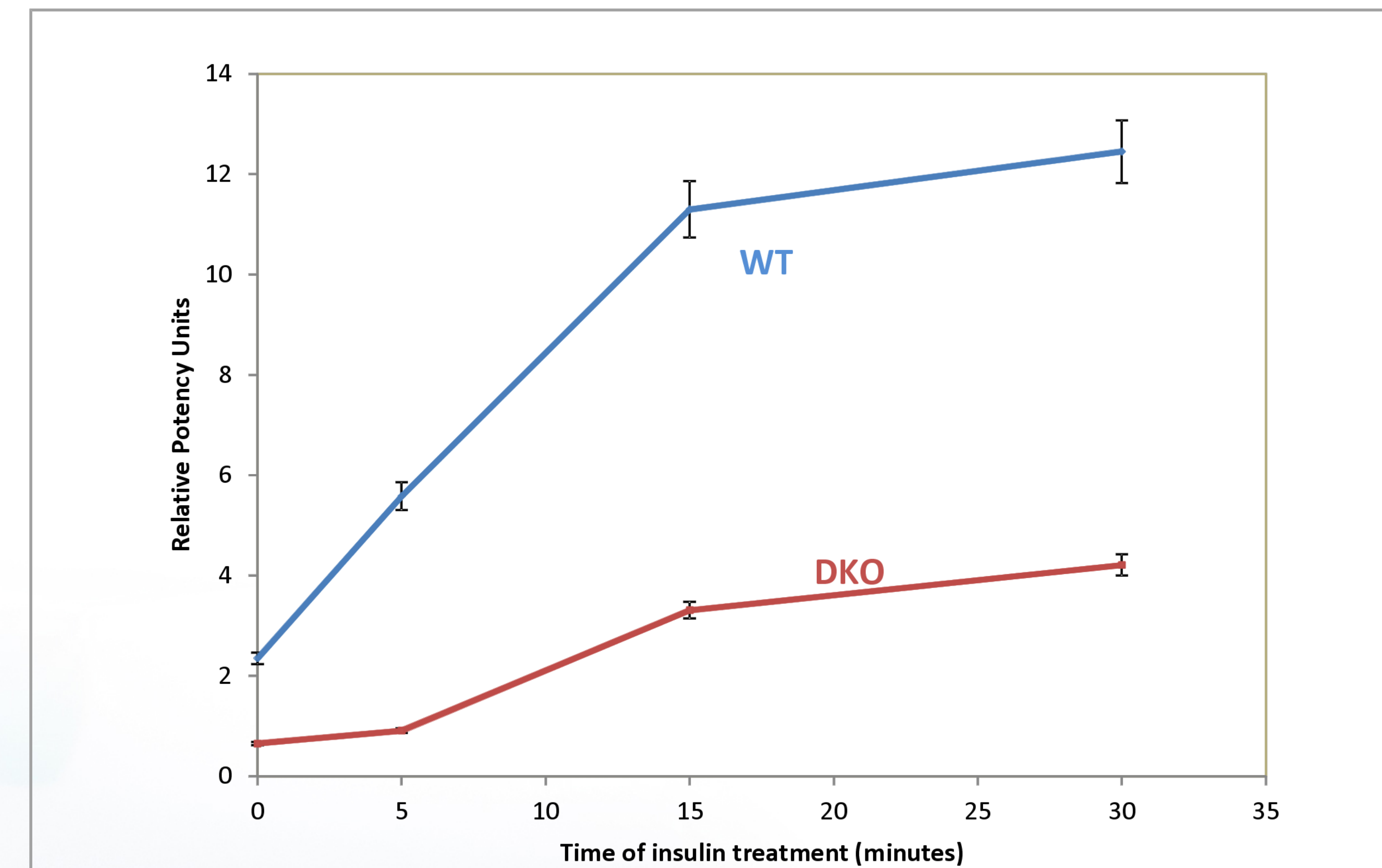


Figure 1 - PKB Phosphorylation in wild type and double knockout MEFs

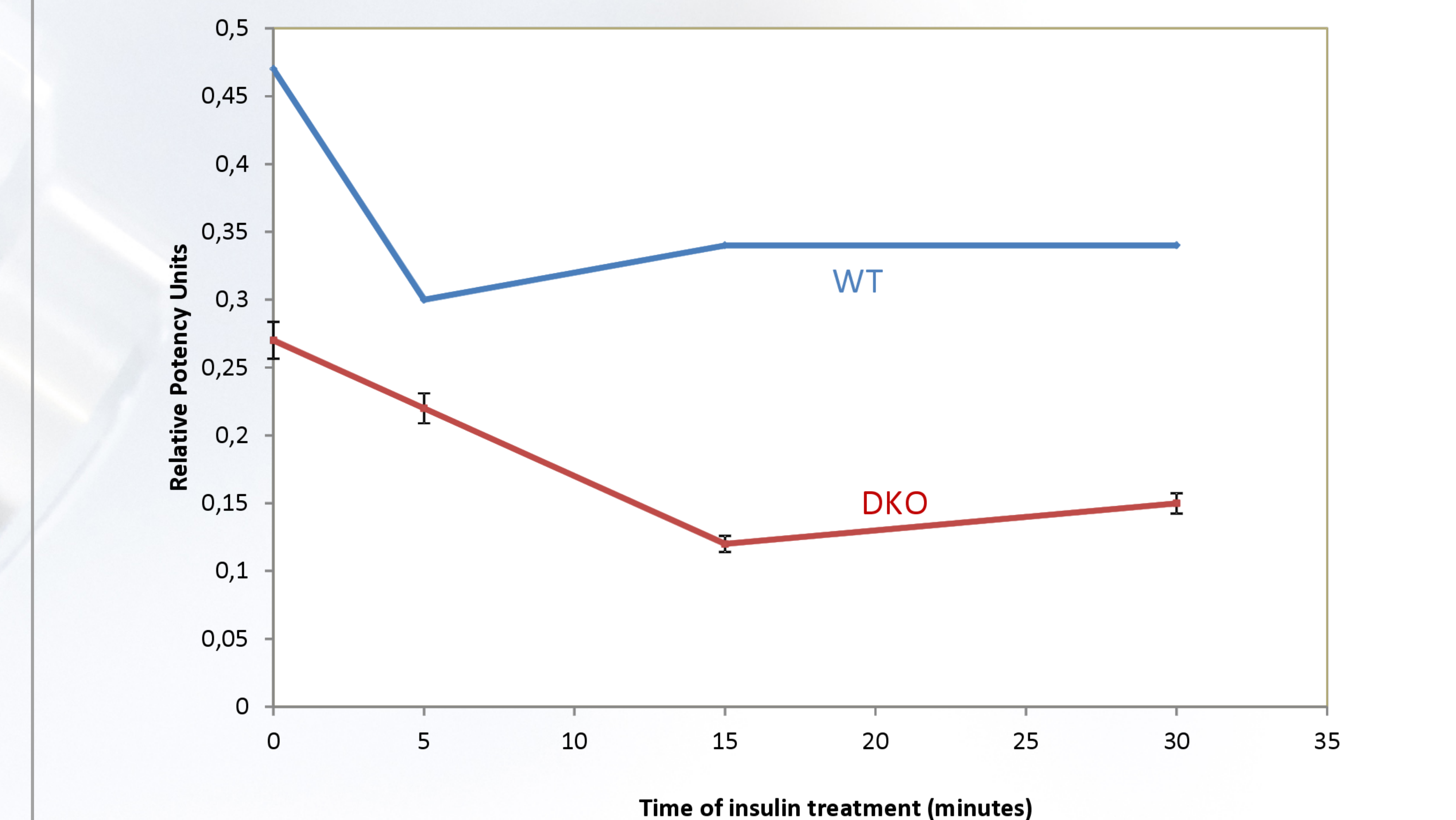


Figure 2 - AS160 Phosphorylation in wild type and double knockout MEFs

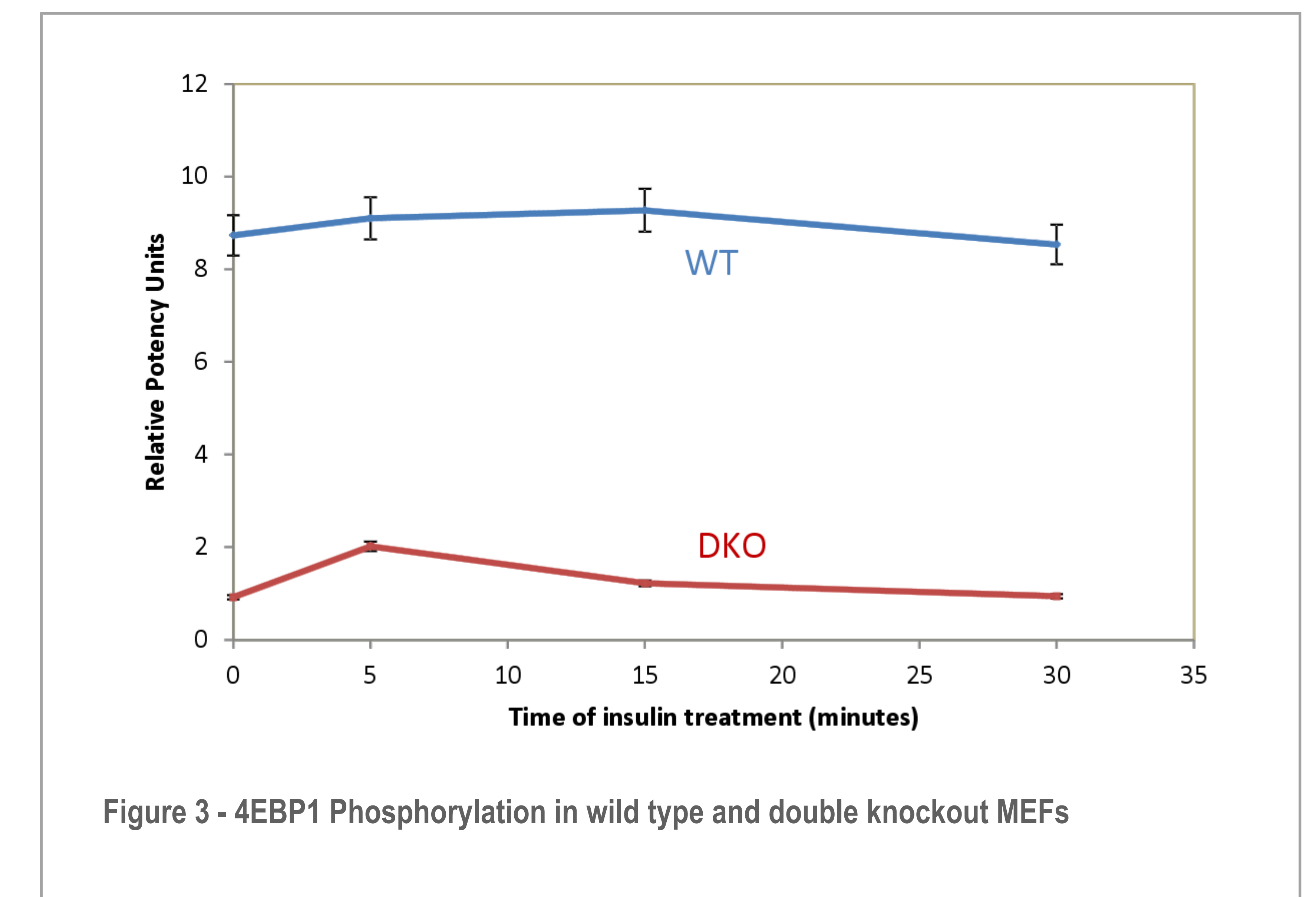


Figure 3 - 4EBP1 Phosphorylation in wild type and double knockout MEFs

## Conclusions

- Mnk1 and Mnk2 proteins significantly increase phosphorylation of PKB, AS160 and 4EBP1. There is a clear correlation between the presence of Mnk1 and Mnk2 and phosphorylation of those three proteins (Figures 1-3).
- Mnk1 and Mnk2 increase translocation of GLUT4 from internal storage to plasma membrane. Presence of both Mnk1 and Mnk2 caused increase in translocation of GLUT4 from GLUT4 storage vesicles to plasma membrane.
- Introduction of siRNA for Mnk1 and Mnk2 leads to decrease in phosphorylation of proteins and lack of translocation of GLUT4. Phosphorylation of PKB is inhibited in cells with introduced siRNA. A similar effect can be noticed in DKO.

Understanding the process of Mnk1 and Mnk2 involvement in insulin signaling would allow:

- development of new diagnostic tool
- emergence for possible drugs targetting Mnk1/2 pathway in insulin signaling
- improved knowledge of insulin signaling and proteins involved