

## Use of a GLUT4 translocation assay strategy to identify new insulin sensitisers with efficacy in vitro and in vivo

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**Background and Aims:** The aim of the current study was to identify new insulin sensitising agents from a small molecule library using a GLUT4 translocation assay screening strategy.

**Material and Methods:** We employed a fluorescence-based HA-GLUT4 translocation assay to determine the amount of GLUT4 present at the cell surface of differentiated rat L6 myotubes. The cells were rendered insulin-resistant using 0.3 mM palmitate for 16 h (68% decrease in GLUT4 content at the cell surface compared with myotubes incubated with 100 nM insulin for 20 min in the absence of palmitate). Insulin resistant L6 myotubes were then treated with small molecules from a library of ~1500 compounds at 10  $\mu$ M for 60 min. Positive hits were selected for their ability to increase GLUT4 content at the cell surface. Hits were further tested for their ability to reverse other models of insulin resistance in cultured 3T3-L1 adipocytes: chronic insulin stimulation (10 nM for 48 h), dexamethasone (500 nM for 8 days) or glucose oxidase (100 mU for 2 h). To test the in vivo efficacy of the lead compound, we examined its effect on glucose tolerance in insulin resistant high fat fed (HFF) mice (60% calories from saturated fat for 6 weeks). The lead compound was administered with the food for the last 4 weeks.

**Results:** Using this cell-based GLUT4 translocation assay, we identified 8 compounds that reversed the inhibitory effect of palmitate on insulin stimulated GLUT4 translocation in L6 myotubes. The magnitude of the protective effect was variable between the compounds: 30-40% rescue,  $p < 0.05$  (compounds designated as VVP086, VVP412 and VVP708); 60-70% rescue,  $p < 0.01$  (VVP326, VVP593, VVP600 and VVP912);  $> 80\%$  rescue,  $p < 0.005$  (VVP443). These compounds did not have a significant effect on GLUT4 translocation in the absence of insulin. We then tested the efficacy of these compounds on several models of insulin resistance in 3T3-L1 adipocytes. While several compounds were able to reverse insulin resistance induced by either chronic insulin stimulation, dexamethasone or glucose oxidase, VVP326, the berberine analog palmatine, achieved a significant effect in all models tested. We examined the effects of palmatine on glucose homeostasis in HFF mice. Palmatine (50mg/Kg) administered with the food for 4 weeks ameliorated glucose intolerance in HFF mice (23% decrease in the area under the curve compared with control HFF mice  $p < 0.05$ ,  $n = 8/\text{group}$ ). This effect was accompanied by a 55% reduction in body weight gain ( $p < 0.01$ ).

**Conclusion:** The GLUT4 translocation assay successfully identified a number of new compounds with insulin sensitising properties. Palmatine showed efficacy in all cellular insulin resistant models tested and ameliorated the glucose intolerance associated with high fat feeding in mice. The GLUT4 translocation assay is a useful screen for novel insulin sensitising agents and the hit compounds identified in this study represent potential targets for the development of new diabetes therapeutics.