

concentration was measured by GC/MS, and turnover by GC/C/IRMS, from muscle biopsies taken immediately before and after exercise. Muscle biopsies were dissected free of visible fat and lyophilized, after which lipid fractions were isolated using solid phase extraction and derivatized to fatty acid methyl esters prior to analysis.

Results: Fasting and 2-hour glucose concentrations were higher in PD vs. NGT (fasting [glucose] 106 ± 1.8 vs. 92 ± 1.2 mg/dl, 2h [glucose] 137 ± 10.3 vs. 101 ± 4.5 mg/dl, $p < 0.05$ for both), by study design, otherwise age (57 ± 1.4 vs. 58 ± 1.6 years), body mass index (32 ± 1.0 vs. 31 ± 1.0 kg/m²), body fat (35 ± 1.8 vs. 37 ± 1.8 %), and VO₂max (25 ± 1.3 vs. 22 ± 1.3 ml O₂/kg/min) were not different. Furthermore, whole-body substrate use and circulating concentrations of insulin, glucagon, free fatty acids, glycerol, lactate and catecholamines were not different between groups at rest or during exercise. Insulin action (Si) at baseline was similar between the groups, but insulin secretion (AIR & DI) was significantly lower in PD (AIR 146 ± 24 vs. 428 ± 67 μ U/ml, DI 375 ± 60 vs. 1231 ± 145 $\times 10^{-4}$ /min, PD vs. NGT, $p < 0.01$ for both). Basal IMTG concentration was higher (43.1 ± 5.2 vs. 23.5 ± 6.3 μ g/mg dry weight, $p = 0.04$) and turnover lower (0.24 ± 0.04 vs. 0.37 ± 0.5 %/hr, $p = 0.05$) in PD vs. NGT, whereas no such differences were seen for diacylglycerol (DAG) at baseline. No differences were observed in IMTG or DAG concentration or turnover during exercise, or in IMTG and DAG saturation at any time point. IMTG turnover during exercise correlated with DI, but not Si, in both groups ($R^2 = -0.438$, $p = 0.03$). Analyses were performed using repeated measures ANOVA.

Conclusion: The current study demonstrates that in groups matched for obesity, higher IMTG concentration and lower IMTG turnover, distinguish pre-diabetes from NGT. Diacylglycerol and fatty acid saturation of intramuscular lipids do not appear a major point of differentiation. Insulin secretion, rather than insulin action, was lower in pre-diabetes and correlated with IMTG turnover. How, or if, IMTG turnover influences insulin secretion, or vice versa, remains to be determined.

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Intravenous AICAR administration reduces hepatic glucose output and inhibits whole-body lipolysis in type 2 diabetes patients

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Background and aims: The AMP-activated protein kinase (AMPK) pathway is intact in type 2 diabetes patients and is seen as a target for the treatment of this disease. Here, for the first time, we assessed the effect of the AMPK activator 5-aminoimidazole-4-carboxamide riboside (AICAR) on both glucose and fatty acid metabolism *in vivo* in type 2 diabetes patients.

Material and methods: Stable isotope methodology with blood and muscle biopsy sampling were combined to assess glucose and fatty acid kinetics following continuous intravenous infusion of AICAR (0.75 mg.kg⁻¹.min⁻¹) and/or saline in 10 male, type 2 diabetes patients (age: 64 ± 2 y; BMI: 28 ± 1 kg/m²).

Results: Plasma glucose rate of appearance (Ra) was reduced following AICAR administration, while plasma glucose rates of disappearance (Rd) were equal in the AICAR and control test. Consequently, blood glucose disposal (Rd expressed as % of Ra) was increased following AICAR infusion ($P < 0.001$). Accordingly, a greater decline in plasma glucose concentration was observed following AICAR infusion ($P < 0.001$). Plasma FFA Ra and Rd were both significantly reduced in response to AICAR infusion, which was accompanied by a significant net decline in plasma FFA concentration. Although the phosphorylation state of AMPK in skeletal muscle was not increased, we observed a significant rise in the phosphorylation of acetyl-CoA carboxylase (ACC; $P < 0.001$).

Conclusion: We conclude that intravenous AICAR administration reduces hepatic glucose output and augments glucose disposal, thereby lowering blood glucose concentrations *in vivo* in type 2 diabetes patients. Furthermore, AICAR administration stimulates hepatic fatty acid oxidation and/or inhibits whole-body lipolysis, thereby reducing plasma FFA concentrations.

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Use of a GLUT4 translocation assay strategy to identify new insulin sensitizers 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 insulin sensitising agents from a small molecule library using a GLUT4 translocation assay screening strategy.

Materials 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 μ 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 (50 mg/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 $p < 0.05$, $n = 8$ /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.

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PPAR-sparing insulin sensitizers; path for development and clinical evaluation

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Background and aims: We have proposed that development of insulin sensitizer therapies has been delayed because of focus on nuclear receptor activation. Further, superior pharmacological profiles, [e.g., less plasma volume expansion (and edema) as well as greater reductions in blood pressure and corrections of dyslipidemia] might be obtained by minimizing direct activation of nuclear receptors. These studies seek to establish whether a series of