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Palmitoylation of the adiponectin receptors, AdipoR1 and AdipoR2, is essential for function in vitro and in vivo

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Dysregulation of the adiponectin axis contributes to obesity-related cardiometabolic disorders making it an attractive therapeutic target. However, our understanding of the adiponectin receptors, AdipoR1 and AdipoR2, atypical seven transmembrane domain proteins, is rudimentary. We reasoned that elaboration of key properties of AdipoR1 and AdipoR2 would reveal therapeutic strategies. To address this we have employed *in silico*, molecular and cellular approaches.

First, using a series of complementary qualitative (microscopy) and quantitative (flow cytometry) assays we demonstrated that under steady-state conditions (no serum starvation) AdipoR1 exhibits robust (60%) cell-surface expression (CSE), whereas AdipoR2 is predominantly restricted to the ER [1]. Second, overexpression of AdipoR1 in HEK-293 cells resulted in acute activation of downstream signalling networks (AMPK, AKT, ERK and P38MAPK) whereas overexpression of AdipoR2 promoted more chronic activation (peaking at 15 min and 24 h) [2]. Third, characterisation of chimeric receptors (comprised of a series of AdipoR1/R2 and AdipoR2/R1 constructs) demonstrated that the differences in CSE and temporal signalling profiles of AdipoR1 and AdipoR2 are underpinned by the non-conserved regions (spanning $AdipoR1_{(1-70)}$ and $AdipoR2_{(1-81)}$) in the cytoplasmic 'trunks' of the receptors. Fourth, bioinformatics analysis (using CSS-Palm) revealed several putative palmitoylation sites including a conserved 'canonical' site (common to GPCRs) in the juxtamembrane region of both receptors as well as additional non-conserved sites. Palmitoylation of these sites was confirmed using Acyl-Biotinyl exchange chemistry and site-directed mutagenesis which also revealed rapid turnover of palmitoylation ($t_{1/2} < 60$ min). Moreover, palmitoylation of the canonical site in $AdipoR1_{(Cys124)}$ or $AdipoR2_{(Cys135)}$ was required for efficient CSE and coupling to downstream signalling networks (all $p < 0.05$).

Collectively these findings demonstrate fundamental differences between AdipoR1 and AdipoR2, highlight the importance of the cytoplasmic 'trunks' and post-translational regulation (palmitoylation) of the receptors. Studies are ongoing to elaborate whether changes in the latter contribute to the pathophysiology of cardiometabolic disease and afford novel therapeutic opportunities.

References

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The independent effects of dietary energy restriction and circuit exercise training on fat oxidation in patients with NAFLD

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Aim: To investigate the independent effects of 6-months of energy restriction or exercise training on whole-body and hepatic fat oxidation of patients with NAFLD.

Methods: Participants were randomised into either circuit exercise training (EX; $n = 13$; 3 h/week without changes in dietary habits), or dietary energy restriction without changes in structured physical activity (ER; $n = 8$). Respiratory quotient (RQ) and whole-body fat oxidation rates (Fat_{ox}) were determined by indirect calorimetry under basal, insulin-stimulated and exercise conditions. Severity of disease and steatosis was determined by liver histology; hepatic Fat_{ox} was estimated from plasma b-hydroxybutyrate concentrations; cardiorespiratory fitness (CRF) was expressed as VO2peak. Complete-case analysis was performed (EX: $n = 10$; ER: $n = 6$).

Results: Hepatic steatosis and NAFLD activity score decreased with ER but not with EX. β-Hydroxybutyrate concentrations increased significantly in response to ER (0.08 ± 0.02 vs. 0.12 ± 0.04, $P = 0.03$) but remained unchanged in response to EX (0.10 ± 0.03 vs. 0.11 ± 0.07, $P = 0.39$). Basal RQ decreased ($P = 0.05$) in response to EX, while this change was not significant after ER ($P = 0.38$). VO2peak ($P < 0.001$) and maximal Fat_{ox} during aerobic exercise ($P = 0.03$) improved with EX but not with ER ($P > 0.05$). The increase in β-hydroxybutyrate concentrations was correlated with the reduction in hepatic steatosis ($r = -0.56$, $P = 0.04$).

Conclusions: ER and EX lead to specific benefits on fat metabolism of patients with NAFLD. Increased hepatic Fat_{ox} in response to ER could be one mechanism through which the ER group achieved reduction in steatosis.

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Consumption of sugar sweetened beverages and type 2 diabetes incidence in Thai adults: Results from an eight year prospective study

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Introduction: The global prevalence of type 2 diabetes mellitus (T2DM) is high and increasing in countries undergoing rapid socio-economic development, such as Thailand. Sugar sweetened beverage (SSB) intake may contribute to the risk of developing T2DM. However, this has not been assessed in Thai adults. We aimed to assess the association between SSB intake and T2DM risk and whether this association was mediated by obesity in a prospective study of Thai adults.

Methods: Data were from Thai Cohort Study participants surveyed in 2005, 2009 and 2013. The sample included participants who were free of diabetes in 2005 and who were followed up in 2009 ($n = 59{,}314$) and/or in 2013 ($n = 39{,}175$). We used multivariable logistic regression to assess associations between SSB intake and four and eight year T2DM incidence. We used a counterfactual mediation analysis to explore potential mediation of the SSB intake and T2DM risk relationship.