The Anti-obesity Effects of Xanthohumol are Mediated Partly by the Adenosine Monophosphate-Protein Kinase Signaling Pathway

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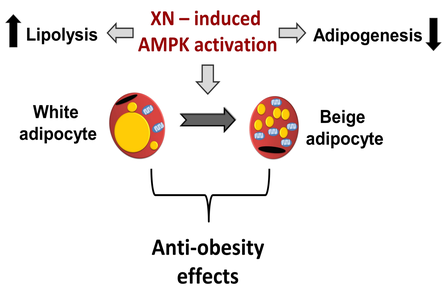
Abstract

Xanthohumol (XN), a flavonoid compound extracted from the hop plant Humulus lupus, has been studied for its anti-cancer and anti-adipogenic effects. In this study, we have investigated the effects of XN on the inhibition of adipogenesis and the induction of browning in 3T3-L1 adipocytes. Furthermore, we provide evidence for the first time on the role of adenosine monophosphate-activated protein kinase (AMPK) signaling pathway in XN-induced anti-obesity effects. Browning of white adipose tissue, WAT, is emerging as a novel approach to address obesity. AMPK is activated in response to stress-like exposure to cold and has been shown to induce browning of WAT. 3T3-L1 preadipocytes were differentiated using a cocktail comprised of insulin, dexamethasone, isobutyl methyl xanthine, and rosiglitazone in DMEM supplemented with 10% FBS following an 8-10 adipogeneic differentiation protocol. Mature adipocytes were treated with either 0.01% DMSO or varying doses of XN for 24-48hrs. Treatment of mature 3T3-L1 adipocytes with XN 6.25μM and 25μM decreased lipid content during adipogenesis and increased the expression of uncoupling protein 1 (UCP1), in a dose-dependent manner in mature adipocytes. XN further increased mitochondrial activity in mature adipocytes after 24 hours, suggesting browning of adipocytes. To demonstrate the role of AMPK pathway in XN-induced anti-obesity effects, mature adipocytes were treated with either 0.01% DMSO or XN 25μM in the presence or absence of dorsomorphin, an established inhibitor of the AMPK pathway, and 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMPK stimulator. XN increased the expression of phospho-AMPK and this XN-induced increase in AMPK activation was diminished in the presence of dorsomorphin. On the other hand, XN+AICAR demonstrated an additive effect on the activation of AMPK. Likewise, dorsomorphin reversed XN-induced inhibition of adipogenesis while XN+AICAR demonstrated an additive effect on the inhibition of lipid content during adipogenesis. These results provide evidence for the potential role of AMPK pathway in XN-induced anti-obesity effects in 3T3-L1 adipocytes.

Hypothesis



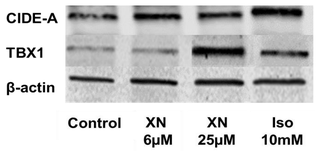
Research Goals:

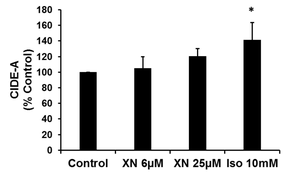
Demonstrate multifaceted anti-obesity effects of XN in 3T3-L1 adipocytes and to determine the effects of XN on the induction of beiging.

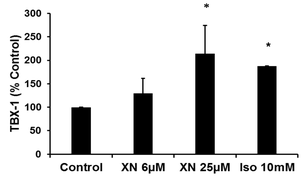
Investigate the possible role of AMPK signaling pathway in XN-induced beiging.

XN Induces Beiging in Mature 3T3-L1 Adipocytes

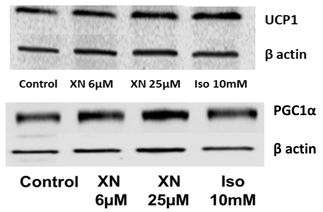
XN upregulates beige markers Cidea and Tbx1 in mature 3T3-L1 adipocytes.

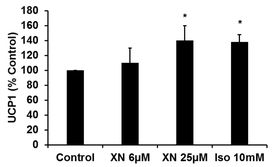


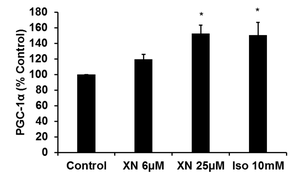




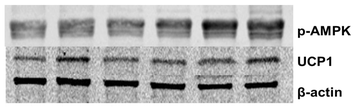
XN increases thermogenesis and induces mitochondrial biogenesis.



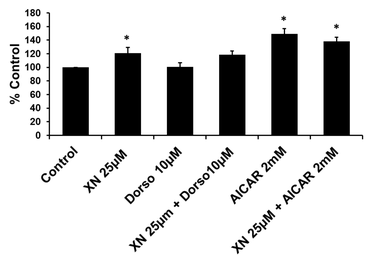




Role of AMPK in XN – induced Beiging in 3T3-L1 Adipocytes

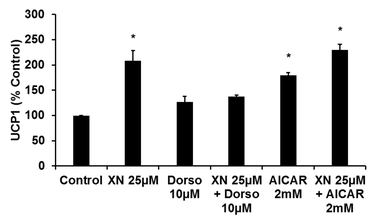


p-AMPK expression in mature adipocytes

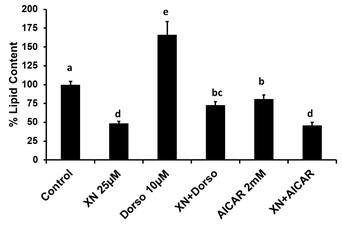


XN – induced upregulation of UCP-1 is mediated partly through AMPK signaling pathway

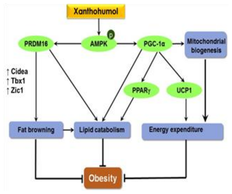
UCPI expression in mature adipocytes



XN – induced inhibition of adipogenesis is reversed by AMPK inhibitor, dorsomorphin



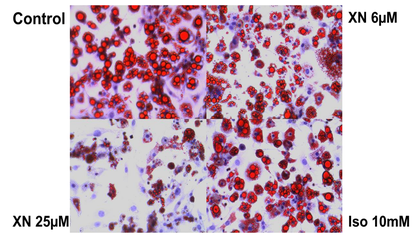
Role of AMPK in XN-induced anti-obesity effects: a working model



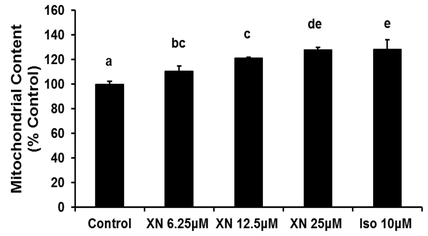
*p <0.05
Means that are not denoted with a common letter are different; a, b, c, d and e: p < 0.05

XN Inhibits Adipogenesis

XN inhibits lipid accumulation in 3T3-L1 adipocytes in a dose-dependent manner: Oil Red O Staining



XN increases mitochondrial content in 3T3-L1 mature adipocytes.



Conclusions

- XN drives the beiging of mature 3T3-L1 adipocytes as demonstrated by increased beige marker expression.
- XN inhibits adipogenesis and stimulates lipolysis.
- XN increases thermogenesis as witnessed by the up-regulation of UCP1 and mitochondrial biogenesis, indicative of further beiging.
- XN activates AMPK signaling pathway as seen with the upregulation of p-AMPK expression.
- XN-induced upregulation of UCP1 expression is dependent on AMPK signaling pathway.
- Further studies will be conducted to demonstrate that XN-induced anti-obesity effects are partly mediated through the activation of AMPK pathway.

Financial Support

Funding was provided by the Philadelphia College of Osteopathic Medicine Biomedical Research Program.