A direct activator of AMPK provides strong glycemic and lipid control with potential to reduce body weight in type 2 diabetes patients.

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Type 2 Diabetes (T2DM) is characterized by the abnormal glucose and lipid metabolism due in part to resistance to the actions of insulin in different tissues. AMPK, a serine/threonine kinase, a cellular energy sensor that regulates the coordination of anabolic and catabolic processes and can be an attractive therapeutic target to treat T2DM. We report the anti-hyperglycemic effects of CNX-12-59 (a direct AMPK activator) in *in vitro* cell systems and diet-induced obese (DIO) mice.

CNX-012-59 has an EC50 of 80nM in the SAMS peptide phosphorylation assay. AMPK activation by CNX-012-59 is both dose and time dependent in liver and adipose. It significantly inhibits Isoproterenol mediated lipolysis in mature 3T3L1 adipocytes by ~40%. In the study in B6 DIO mice on high fat diet, CNX-12-59 (25mg/kg, BID, po for 8 weeks) reduced fasting blood glucose levels by ~15% (181.57±6.16 in HFD Vs 164±3.83 mg/dl in treatment) and body weight by ~7% (36.85±1.69 in HFD Vs 33±1.24 g in treatment). CNX-012-59 significantly reduced both liver (5.65±0.25 in HFD Vs 4.03±0.16 mg/dl in treatment) and plasma (207±10 in HFD Vs 152±13 mg/dl in treatment) triglycerides by ~30%. Moderate reduction in adipocytes size along with significant reduction in plasma Leptin levels (37.17±7.87 in HFD Vs 12.92±3.6 ng/ml in treatment) were observed along with reduced macrophage infiltration in adipocytes.

These findings indicate that direct activators of AMPK have potential to provide glycemic and lipid control and also reduce body weight and can be good therapeutic agents for the treatment of type 2 Diabetes.

CNX-012-59 is a *direct* highly potent AMPK Activator.

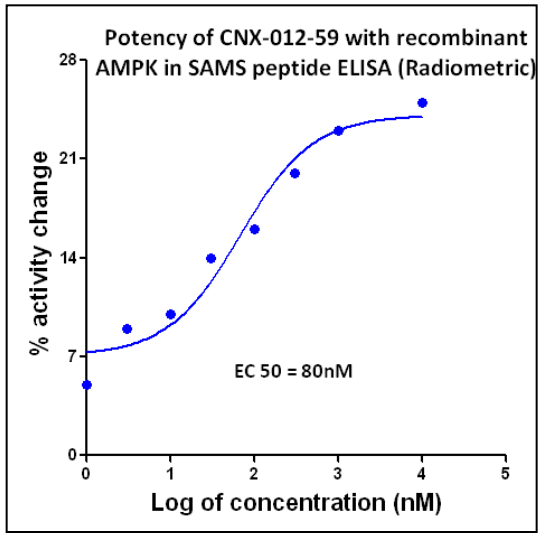
Selection and screening of compounds - our approach

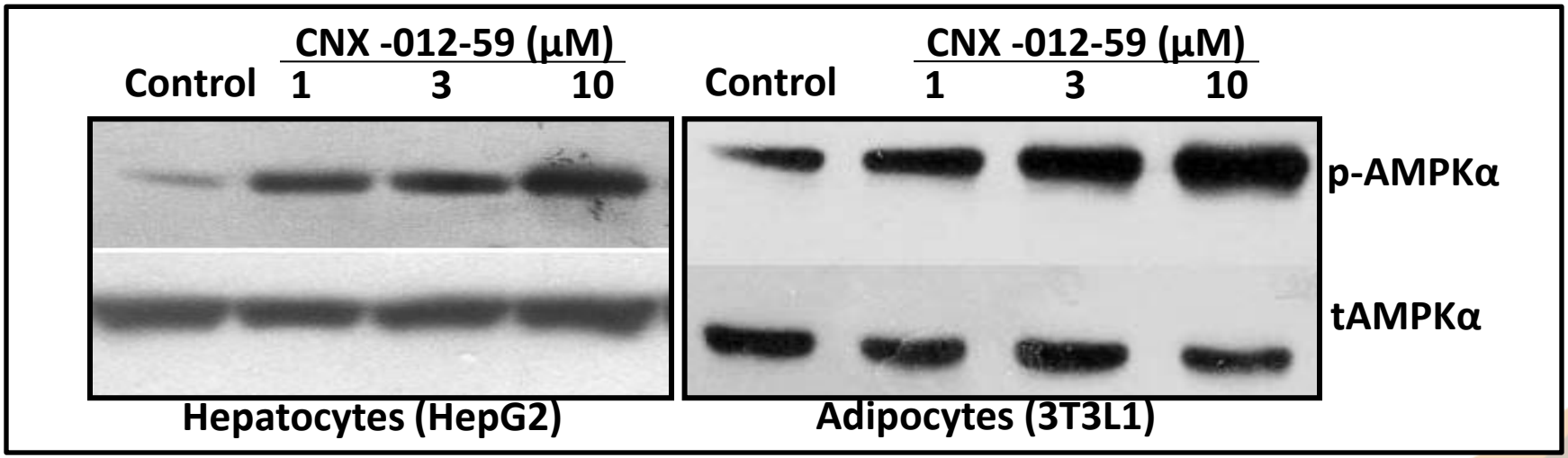
- Assays under disease mimicking conditions
- Rodent models of Disease
- Biomarkers

Focused library

- Direct B1 & B2 Activation
- Pan tissue activation
- Impact on isoform-specific downstream mechanisms and tissue end-points
- Minimal Blood brain barrier penetration

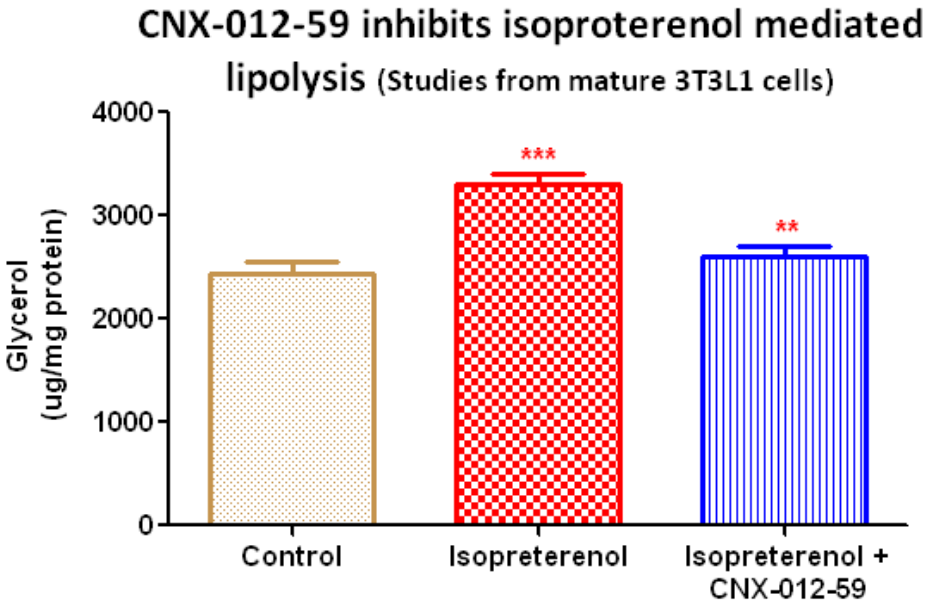
Direct AMPK activators with pan-tissue activity and impact on multiple metabolic parameters

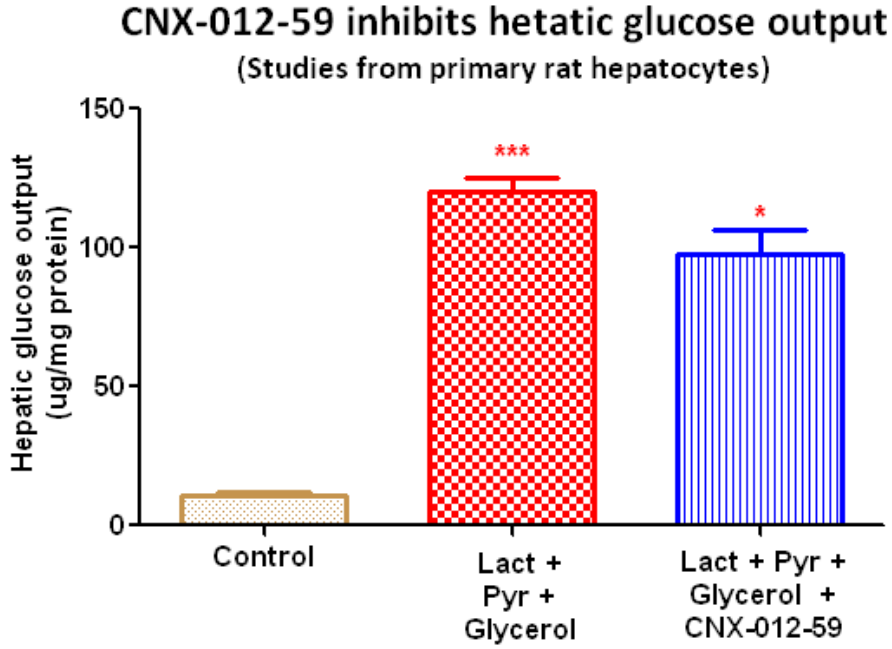




Detailed methodology will be available upon request

CNX-012-59 has a potential to control fasting glucose by inhibiting both hepatic glucose out put and adipose lipolysis





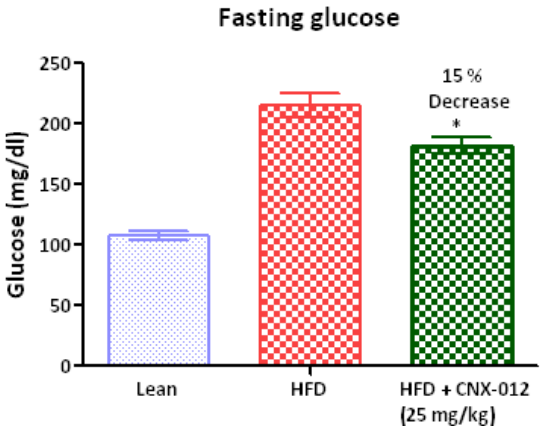
Statistical analyses were done using Dunnet's multiple comparison test with 95% confidence interval. * p<0.05, **p<0.01, p***<0.001.

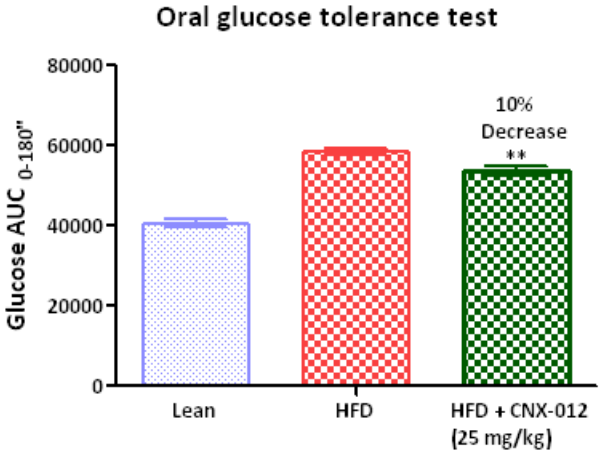
Rat primary hepatocytes were treated with serum-free, glucose-free media containing lactate and pyruvate (AA) either alone or with CNX-012 for 24h. Following this, glucose released in the media was estimated using the Glucose GOD FS assay kit (DiaSys, Germany) and normalized to total protein.

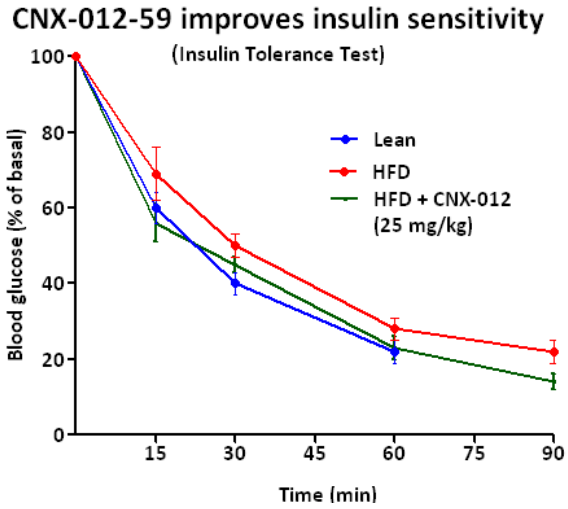
Mature adipocytes (3T3F442a) were treated with media containing Isoproterenol (IP) either alone or with CNX-012 for 24h. Subsequently, glycerol released in the media was measured using a free glycerol reagent (Sigma) and normalized to total protein.

Detailed methodology will be available upon request

CNX-012-59 controls both fasting, prandial glucose and improves insulin sensitivity – *Studies from C57 BL/6J DIO mice on HFD*



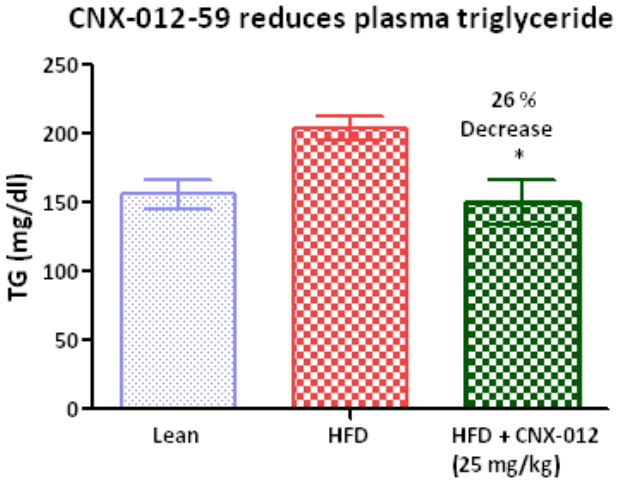


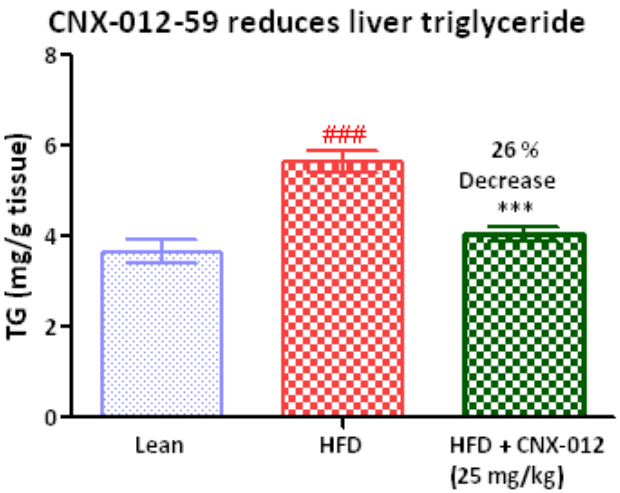


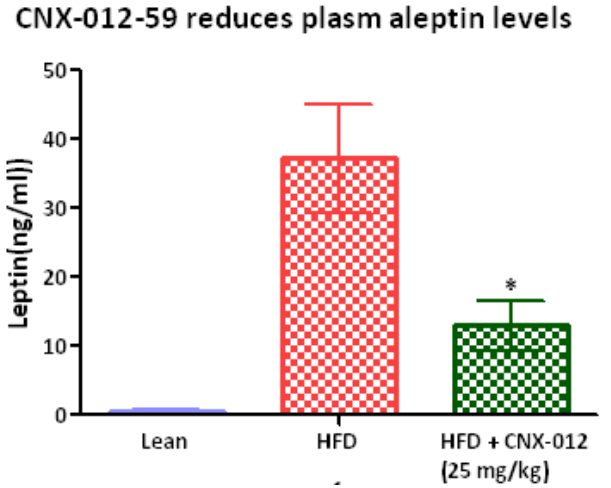
A.CNX_012_59 decrease hyperglycemia in obese C57 mice. Data in all panels are means ± S.E.M. (n = 8 group).
B. CNX_012_59 alter glucose disposal in glucose tolerance test. The blood glucose excursion profile from t = 0 to t = 180 min was used to integrate an area under the curve (AUC) for each treatment.
C. Effects of CNX_012_59 on insulin induced glucose clearance was evaluated by performing insulin tolerance test. Percentage changes in blood glucose levels were determined. (*)Significant difference from the HFD group as determined by student's *t*-test.

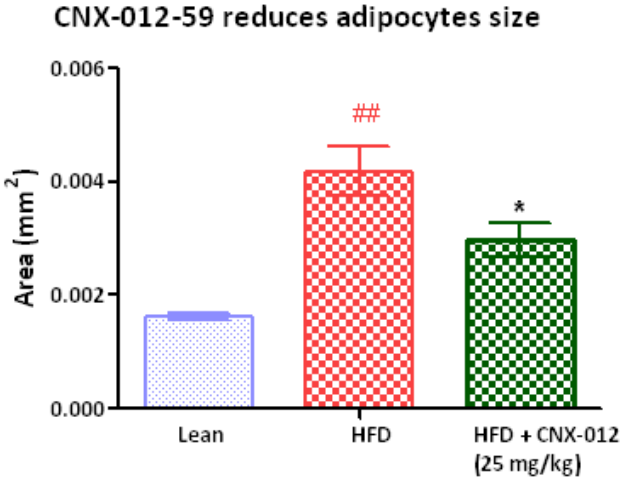
Detailed methodology will be available upon request

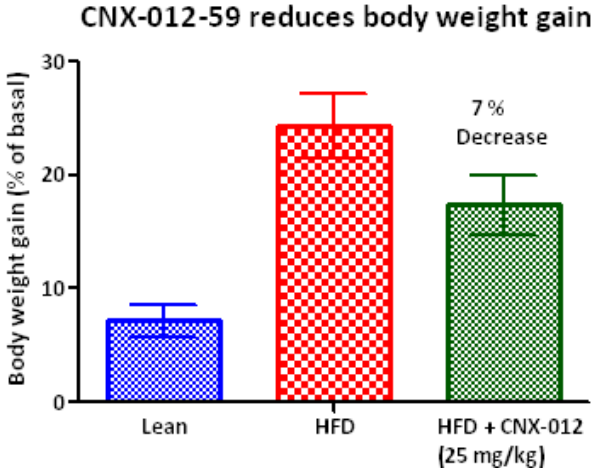
CNX-012-59 reduces plasma TG, liver TG, leptin, adipocytes size and controls body weight gain – *Studies from C57 BL/6J DIO mice on HFD*











All values presented as Mean±SEM,. n=8. Statistical significance was calculated using Student's t-test. P-value: (*)<0.05 and (***)<0.001, when compared to the HFD control

17 wk old C57 BL/6J DIO mice (HFD started at 6 wks of age) were treated with CNX-012 with a dose of 25 mg/kg body weight twice daily. At the end of the study, plasma TG, leptin and liver TG levels were estimated. Treatment showed a ~26% decrease in the TG levels and a significant reduction in plasma leptin levels along with redction in body weight gain and adipocyte size as compared to the HFD control.

Detailed methodology will be available upon request

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