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Regulation of lipid metabolism in skeletal muscle by IDOL

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Inducible Degrader Of the Low-density lipoprotein Receptor (LDLR), IDOL, is an E3 ligase that targets LDLR, very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (apoeR2) for degradation. We have previously demonstrated that IDOL is an evolutionarily conserved regulator for lipid uptake. Furthermore, single nucleotide polymorphisms in IDOL are associated with altered plasma cholesterol levels in humans. Studies have demonstrated a role for IDOL in the brain and liver; however, the importance of IDOL in the regulation of skeletal muscle lipid metabolism remains to be determined.

Using a range of *in vitro, ex vivo* and *in vivo* approaches, we aimed to determine the role of IDOL in the regulation of lipid metabolism in skeletal muscle. Ectopic expression of IDOL in C2C12 myotubes was associated with an increased extracellular acidification rate. Interestingly, it was also associated with a 10-fold increase in fibroblasts growth factor (FGF)-21. Similarly, administration of IDOL adeno-associated virus (AAV; 1E10) to the tibialis anterior (TA) was associated with a marked increase in FGF-21 expression compared to the contralateral control-treated TA muscle in mice. A significant reduction in a range of lipid species including several ceramide species was also observed in muscle of IDOL-AAV treated mice. In contrast, comparison of IDOL fl/fl and IDOL fl/fl $MCK^{Cre/-}$ mice demonstrated that skeletal muscle specific deletion of IDOL was associated with poorer glucose handling as indicated by a glucose tolerance test following either chow or high fat diet, in the absence of a phenotype in body weight or percent fat.

Together, these studies establish a role for IDOL in the regulation of skeletal muscle lipid metabolism. Further studies are warranted to understand the underlying mechanisms by which IDOL mediates these effects.

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Combining intermittent fasting with high intensity interval training reduces fat mass by increasing fat oxidation and mobilization

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Combining lifestyle interventions such as intermittent fasting (IF) and high intensity interval training (HIIT) result in higher fat loss than IF and HIIT alone. However, the molecular pathways that underpin these changes are unclear. The present study investigated the effects of IF and/or HIIT on molecular markers of epididymal adipose tissue function. Eight-week-old male mice (C57BL/6) were initially fed a high fat (HF, 60%) and sugar (S, 30% w/v) diet for 12 weeks to induce obesity. They were then randomly allocated to four groups: IF (fasting for 2 alternate days/week), HIIT (3 days/week), combined IF + HIIT (2 alternate fasting days and 3 days HIIT) and CON (*ad libitum* HF/S diet) for 12 weeks. Body weight, fat mass (Echo MRI) and epididymal adipose tissue gene expression (qRT-PCR) were measured at the end of intervention period. At the end of intervention period, IF and IF + HIIT groups displayed significantly lower body weights ($p < 0.05$), fat mass ($p < 0.05$) and expression of the leptin gene ($p < 0.05$) compared to both CON and HIIT groups. The IF + HIIT group displayed increased expression of genes related to fatty acid oxidation (Hydroxyacyl-coenzyme A dehydrogenase; (HADH), $p < 0.05$) and intracellular trafficking (Fatty acid binding protein 4; FABP4), $p < 0.01$)) compared to IF group only. A significant positive correlation ($R = 0.66$, $p < 0.05$) between HADH and FABP4 was also observed in IF + HIIT group. Simultaneous higher expression of both HADH and FABP4 suggests that combination of IF and HIIT has a synergistic effect on reduction in fat mass possibly due to increased fatty acid oxidation and mobilization. In summary, IF with or without HIIT are effective strategies for reducing fat mass accumulation potentially via different molecular signaling pathways within adipose tissue.

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Does activation of TRPM8 with menthol or icilin reduce blood glucose and body weight?

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Brown adipose tissue (BAT) is a potential target against obesity and T2D, because activation of BAT induces oxidation of fatty acids and glucose and increases energy expenditure. Theoretically, BAT is activated by beta 3 adrenergic receptors (β3AR). However, β3AR agonists have been unsuccessful in reducing weight and blood glucose in humans. Cold temperatures can also activate BAT and increase the metabolism of fatty acids and glucose. Transient receptor potential cation channel subfamily M member 8 (TRPM8) is a cold-sensing cation channel expressed on BAT and is activated by menthol and icilin, with icilin being more potent.

Diet-induced C57Bl/6J mice ($n = 17$) were divided into four treatment groups: vehicle (100 L s.c); menthol (40 mg/kg s.c, 100 L.); low dose of icilin (2 mg/kg s.c, 100 L) and high dose of icilin (5 mg/kg s.c, 100 L) and treated daily for 1 week. Mice were implanted with biotelemetry devices (G2 E-mitter, STARR Life Sciences Corp, PA, USA). Food intake and body weight were recorded daily. BAT temperature and activity were recorded every minute. Glucose tolerance was assessed by ipGTT before and after 7-days of drug treatment. Body composition was assessed by DEXA (Lunar PIXI densitometer, PIXImus, WI USA) immediately after GTT.

Food Intake

Mice treated with low dose of icilin had significantly reduced food intake on D5-7 ($p < 0.05$), menthol did not change food intake.

Body Weight and Composition

Low dose icilin reduced body weight on D6-7 ($p < 0.05$). Menthol treatment did not change body weight.

BAT Temperature and Activity

Still being analysed.

Glucose Tolerance

Low dose icilin treatment reduced glucose throughout the GTT ($p < 0.05$) but did not change baseline or resting blood glucose. Menthol did cause any significant changes to blood glucose.

These results demonstrate that icilin, a TRPM8 agonist, is more potent than menthol at reducing body weight, body fat and glucose tolerance.

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Muscle-specific NOX4 deficiency impairs antioxidant defence, mitochondrial biogenesis and exercise capacity

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Skeletal muscle constantly produces reactive oxygen species (ROS) and ROS generation is increased during exercise. Both mitochondria and NADPH oxidases (NOXs) have been implicated as sources of ROS in muscle, but there is evidence for NOXs being key drivers of exercise-induced ROS. However, definitive evidence for this and the precise NOX involved remain unknown. Contraction-induced ROS generation is important in driving antioxidant defence and mitochondrial adaptive responses that are key to the health-promoting effects of exercise. The mechanisms by which ROS coordinate antioxidant defence, mitochondrial biogenesis and insulin sensitivity remain incompletely understood. The focus of the current study is on skeletal-muscle NOX4 and its role on skeletal muscle exercise metabolism, antioxidant defence and mitochondrial biogenesis. Muscle-specific NOX4 knockout mice [*Mck*-Cre; $Nox4^{(fl/fl)}$] were fed either a standard chow diet or a high-fat diet and were then subjected to exercise capacity and endurance tests. Furthermore, primary myoblasts were isolated to delineate the cell intrinsic mechanisms by which NOX4 elicits its effects on mitochondrial biogenesis and antioxidant defence. NOX4-deficiency resulted in reduced muscle mass, energy expenditure and impaired exercise and endurance capacity. In addition, both NOX4-deficient mice and primary myoblasts showed impaired mitochondrial biogenesis and reduced expression of antioxidant defence genes. Our results highlight NOX4 *as a key regulator of* antioxidant defence, exercise capacity and mitochondrial metabolism.

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Amino acid restriction through B^0ATI (Slc6a19) Inhibition: A potential target for treating diabetes

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B^0ATI (Slc6a19) is a sodium dependent neutral amino acid transporter catalyzing the secondary active transport of neutral amino acids across the brush border membrane of kidney and intestine. The surface expression of B^0ATI requires either collectrin or angiotensin converting enzyme 2 (ACE2) in the kidney and intestine, respectively. A Slc6a19 KO mouse showed neutral aminoaciduria in urine as observed in Hartnup disorder, a benign medical condition which is caused by mutations in the Slc6a19 gene. Further characterization of these mice revealed that lack of B^0ATI improves glucose tolerance and enhances fat metabolism. This would suggest that pharmacological inhibition of B^0ATI using chemical compounds could lead to new drugs to treat type 2 diabetes (T2DM).

An initial screen of 20,000 compounds was carried out using a high throughput screening (HTS) assay based on membrane depolarization. This generated a group of 64 inhibitory compounds. Based on the strongest inhibition of B^0ATI-mediated transport, 33 compounds were selected for further characterization. Radiolabelled amino acid uptake assays were used to determine the potency (IC50) and mechanism (competitive or non-competitive) of inhibition, as well as the specificity of B0AT1 inhibitors.

Five novel B^0AT1 inhibitors with IC50 values below 10 μM were identified from the HTS of a small molecule compound library. These compounds will be further tested using *in-vivo* pharmacological studies.

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NOX4 deficiency impairs insulin sensitivity

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Reactive oxygen species, produced by all living organisms as natural by-products of oxygen metabolism and by specialised enzymes known as NAPDH oxidases (NOXs), have been shown to elicit both deleterious and protective effects in various human diseases, including obesity and type 2 diabetes. In particular, ROS such as H_2O_2, produced by NOXs has far postulated to act as a secondary messenger, facilitating insulin signalling by inactivating protein tyrosine phosphatases. The focus of the current study is on the role of skeletal-muscle and liver NOX4 in glucose metabolism, insulin sensitivity and insulin signalling. Muscle-specific NOX4 knockout mice [*Mck*-Cre; $Nox4^{(fl/fl)}$] and liver-specific NOX4 knockout mice [*Alb*-Cre;$Nox4^{(fl/fl)}$] were fed either a standard chow diet or a high-fat diet and subjected to insulin and glucose tolerance tests as well as hyperinsulinaemic–euglycaemic clamps. Furthermore, we isolated primary myoblasts and hepatocytes from $Nox4^{(fl/fl)}$, *Mck*-Cre; $Nox4^{(fl/fl)}$ *and Alb*-Cre;$Nox4^{(fl/fl)}$ mice to delineate the mechanisms involved. Skeletal muscle NOX4-deficiency resulted in glucose intolerance and insulin resistance in both chow and high fat fed mice. Moreover, NOX4 deficiency in liver exacerbated the development of obesity, hepatic steatosis and insulin resistance in mice fed a high fat diet. NOX4 deficiency in myoblasts or hepatocytes also attenuated insulin signalling as assessed by monitoring the PI3K/AKT signalling. Our results highlight the importance of muscle and liver NOX4-derived ROS in the promotion of insulin signalling and the prevention of insulin resistance. Our findings point towards NOX4-derived ROS being required for glucose metabolism.

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