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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