



New Antidiabetes Agent Targeting Both Mitochondrial Uncoupling and Pyruvate Catabolism: Two Birds With One Stone

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Obesity and type 2 diabetes are emerging as global epidemics and impose huge burdens on patient families as well as society. They are leading causal factors for cardiovascular disease, neurological disease, cancer, and kidney disease and negatively impact health span and life span. However, treatment options are limited, and there is an unmet need for developing effective and safe medications. In this issue of *Diabetes*, Jiang et al. (1) report a new treatment strategy and a new antidiabetes agent.

Obesity arises from energy imbalance, and excessive energy is stored as triacylglycerol in fat (2). Mitochondria are an essential organelle responsible for cellular respiration and energy production. Mitochondria oxidize free fatty acids (FFAs) and glucose to produce ATP (referred to as oxidative phosphorylation). The inner mitochondrial membrane (IMM) contains an electron transport chain composed of the complexes I, II, III, IV, and V. Burning of FFAs and glucose produces high-energy electrons that are transported from complex I or II to complexes III and IV, where electron-carried energy is released to pump out protons, generating proton gradients across the IMM (Fig. 1). The IMM proton gradients drive ATP synthesis by complex V. The IMM also contains uncoupling proteins (UCPs) that mediate proton influx, thus suppressing ATP synthesis. UCPs release free energy as heat (thermogenesis) and are engaged in body temperature homeostasis. Brown adipose tissue UCP1 plays a pivotal role in the maintenance of body temperature homeostasis, particularly in rodents (3). UCP-mediated thermogenesis increases energy expenditure, thus protecting against obesity (3). In the 1930s, chemical uncoupler 2,4-dinitrophenol (DNP) was developed for obesity treatment; unfortunately, it had severe adverse effects (e.g., hyperthermia, hyperlactacidemia, death) and was stopped for clinical use (4,5). Nonetheless, the diabetes community continues to search for safe, therapeutic mitochondrial uncouplers for the treatments of obesity

and type 2 diabetes. Here, Jiang et al. identified a promising one called 6j.

DNP-induced uncoupling lowers ATP synthesis and ATP content, thereby increasing glycolysis to compensate for ATP deficiency. Increase in anaerobic glycolysis leads to pyruvate and lactate overproduction and hyperlactacidemia following DNP treatment (5). Of note, mitochondrial pyruvate dehydrogenase (PDH), which is tightly regulated through phosphorylation, catalyzes the first reaction of pyruvate catabolism (6). Pyruvate dehydrogenase kinases (PDKs) phosphorylate PDH at phospho-Ser293 and potentially inhibit PDH (6). Several PDK inhibitors, including dichloroacetic acid (DCA), have been developed to study the function of the PDK/PDH pathway. To combat hyperlactacidemia toxicity, Jiang et al. reasoned that DCA-stimulated PDH activation might reroute pyruvate from lactate production to PDH-mediated oxidation, thereby ameliorating DNP-associated hyperlactacidemia.

Jiang et al. first established cell culture systems and validated DNP and DCA actions in vitro. As expected, DNP potentially stimulates mitochondrial uncoupling, as revealed by marked increases in both oxygen consumption rate and ADP/ATP ratio. DCA inhibits PDK-induced phosphorylation of PDH at phospho-Ser293, leading to PDH activation and suppression of lactate synthesis (i.e., decrease in extracellular acidification rate) due to PDH-mediated pyruvate oxidation. Jiang et al. then tested DNP and DCA in mice. In line with previous reports, acute DNP administration induces hyperthermia, hyperlactatemia, and death at high doses. A chronic DCA treatment decreases both PDK-mediated phosphorylation of PDH (i.e., increases PDH activity) and lactate production. Notably, DCA also induces ectopic lipid accumulations in the liver and skeletal muscle. As an adaptive response to DCA-stimulated pyruvate catabolism and glucose oxidation, FFA oxidation is suppressed, contributing to muscle and liver steatosis.

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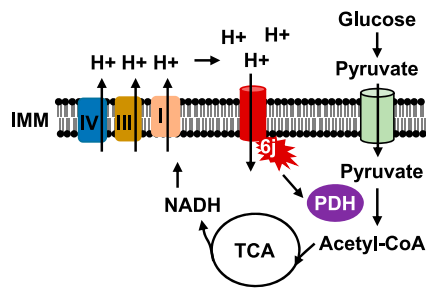


Figure 1—Protection against diabetes by dual mechanisms of mitochondrial uncoupling and pyruvate oxidation. 6j possesses dual properties of stimulating uncoupling and PDH-mediated pyruvate oxidation. Uncoupling increases mitochondrial respiration and burning of glucose and fatty acids. PDH activation reroutes glycolysis-derived pyruvate from lactate production to tricarboxylic acid cycle (TCA)-mediated catabolism, preventing hyperlactacidemia toxicity. I, II, and IV: complexes I, II, and IV.

Additionally, pyruvate-derived acetyl-CoA may serve as a lipogenic precursor to increase de novo lipogenesis. Intracellular lipid species are known to promote insulin resistance and type 2 diabetes. For instance, diacylglycerol activates protein kinase C- θ (PKC- θ) in skeletal muscle and PKC- ϵ in the liver, which in turn inhibit insulin signaling and induce insulin resistance (7,8). Accordingly, the authors observed that DCA treatment activates muscle PKC- θ and liver PKC- ϵ and impairs insulin signal transduction in these two tissues. Next, Jiang et al. tested an innovative idea that DNP and DCA dual treatments may preserve the antidiabetes effect while eliminating the adverse consequences. The authors elegantly demonstrated that in cell cultures, DCA-stimulated pyruvate oxidation abrogates DNP-induced lactate overproduction while DNP maintains its ability to stimulate mitochondrial uncoupling. In mice, DCA pretreatment (i.e., increased pyruvate oxidation) reverses DNP-induced hyperthermia, hyperlactacidemia, and death. In mice with dietary obesity, remarkably, DNP/DCA dual treatments considerably improve hyperglycemia, insulin resistance, and glucose intolerance. Furthermore, DNP decreases DCA-induced ectopic lipid accumulations and insulin resistance in the muscle and liver. These exciting results provide proof-of-concept evidence that concomitant stimulation of both mitochondrial uncoupling and pyruvate catabolism is a viable strategy for type 2 diabetes treatment. These observations also raise an intriguing possibility that mitochondrial uncoupling and pyruvate oxidation act coordinately to regulate mitochondrial selection of fuel substrates and metabolic flexibility. Next, Jiang et al. wondered, cleverly, whether they can design an agent that possesses dual properties of stimulating both mitochondrial uncoupling and PDH activation—two birds with one stone. They successfully engineered compound 6j (Fig. 1), using high-throughput screenings and structure/activity-based chemical modifications. Remarkably, chronic 6j administrations, like DNP/DCA dual treatments, considerably improve insulin resistance, glucose intolerance, and

liver steatosis in mice with either dietary or genetic (*db/db*) obesity. Thus, 6j and related agents hold great promise as new medications for the treatment of type 2 diabetes.

This work, like many other great studies, raises several interesting questions. ATP deficiency, due to mitochondrial uncoupling, potentially has adverse consequences on energy-demanding, ATP-sensitive cells (e.g., cardiomyocytes, neuronal subpopulations). Hence, cell type-specific 6j-related agents are expected to have additional safety properties. In line with this notion, recent studies highlight the translational significance of cell type-specific mitochondrial uncouplers (9,10). Aberrant mitochondrial uncoupling and depolarization may cause mitochondrial injury, mitochondria-originated cell death and inflammation, and/or mitophagy (11). Therefore, the potential effects of long-term 6j treatments on mitochondrial integrity, function, and mitochondrial diseases need to be further assessed. Given the paramount role of mitochondrial energy expenditure in body weight control, the impact of 6j and related agents on protection against obesity needs additional investigation. Interestingly, DCA suppresses DNP-induced hyperthermia, but the underlying mechanism is not fully understood. Finally, the direct molecular targets of 6j linking to uncoupling and PDH activation remain elusive, impeding structure/activity-based optimizations to further improve specificity and efficacy.

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Duality of Interest. No other potential conflicts of interest relevant to this article were reported.

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