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COMMENTARY

Burn after feeding. An old uncoupler of oxidative phosphorylation is redesigned for the treatment of nonalcoholic fatty liver disease

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Summary Uncoupling of oxidative phosphorylation (OXPHOS) in brown adipose tissue can be used by hibernating animals to produce heat at the expense of their fat mass. In a recent work, Dr Shulman et al. generated a liver-targeted derivative of the prototypical OXPHOS uncoupler 2,4-dinitrophenol that alleviated steatosis, hypertriglyceridemia and insulin resistance in several models of nonalcoholic fatty liver disease and type 2 diabetes.

Oxidative phosphorylation (OXPHOS) is a fundamental biological process allowing the synthesis of mitochondrial ATP in most cells. During OXPHOS, energy production is coupled to the oxidation of endogenous substrates such as fatty acids and pyruvate. In some physiological situations, oxidation of these substrates can be uncoupled from ATP synthesis. This is for instance the case in brown adipose tissue (BAT) where OXPHOS uncoupling is a key mechanism whereby hibernators are generating heat to maintain a body temperature compatible with life [1,2]. OXPHOS uncoupling in BAT is mediated by the uncoupling protein-1 (UCP1), which is embedded within the inner mitochondrial membrane [1,2]. UCP1 facilitates proton entry into the mitochondrial matrix, and thus the energy of the proton-driven membrane potential ($\Delta\psi$) is dissipated into heat instead of being converted in ATP by the ATP synthase (Fig. 1A). Importantly, OXPHOS uncoupling is leading to a stimulation of substrate oxidation since this biochemical process is no longer controlled by ATP synthesis. In BAT, the preferred oxidative substrates are fatty acids that have been stored as triacylglycerol (TAG) molecules prior to hibernation [1,2] (Fig. 1A). At the end of winter, hibernating animals can lose as much as 40% of their body weight, mainly as fat mass, which has been burnt for thermogenesis [2—4].

Abbreviations: BAT, brown adipose tissue; CYP, cytochrome P450; DAG, diacylglycerol; DNP, 2,4-dinitrophenol; FAO, fatty acid oxidation; DNPME, 2,4-dinitrophenol-methyl ether; NAFLD, nonalcoholic fatty liver disease; OXPHOS, oxidative phosphorylation; PKCε, protein kinase C-ε; ROS, reactive oxygen species; TAG, triacylglycerol; T2D, type 2 diabetes; UCP1, uncoupling protein-1; WAT, white adipose tissue.

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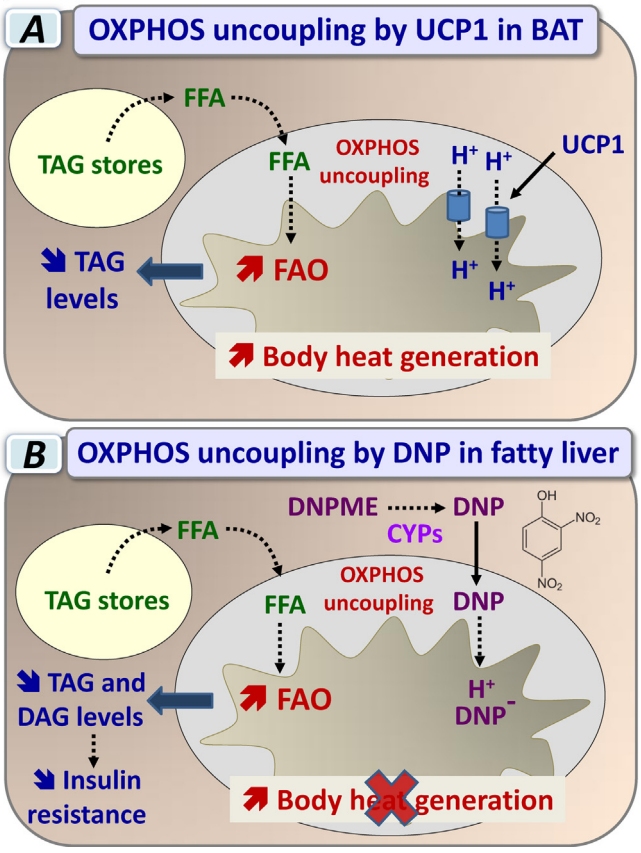

Figure 1 Consequences of oxidative phosphorylation uncoupling on fat metabolism and heat production. Panel A. In the brown adipose tissue (BAT), uncoupling of oxidative phosphorylation (OXPHOS) is mediated by the uncoupling protein-1 (UCP1), which is inserted within the inner mitochondrial membrane. UCP1 transfers protons (H^+) across the inner mitochondrial membrane, thus stimulating the mitochondrial fatty acid oxidation (FAO) and generating heat. Indeed, during OXPHOS uncoupling, mitochondrial FAO is uncoupled from ATP production and thus the energy of the proton-driven membrane potential $\Delta\psi$ is dissipated into heat instead of being used for ATP synthesis. This system is utilized by the hibernating animals to maintain a body temperature compatible with life. This is associated with a large decrease in the triacylglycerol (TAG) levels stored in adipose tissue because TAG are hydrolyzed into free fatty acids (FFA), which are subsequently used as fuel for the mitochondrial FAO pathway. Panel B. In liver, 2,4-dinitrophenol (DNP) is able to uncouple OXPHOS and stimulate mitochondrial FAO because this protonophoric uncoupler is able to transport protons across the inner mitochondrial membrane. In a recent work, Dr Shulman et al. generated a derivative of DNP, DNP-methyl ether (DNPME) that can be metabolized to DNP by cytochromes P450 (CYPs), thus allowing DNP formation principally in the liver [19]. This local OXPHOS uncoupling avoids an increase in body temperature but leads to a reduction of TAG and diacylglycerol (DAG) levels in fatty liver. Alleviation of steatosis is also associated to a reduction of plasma hypertriglyceridemia (not shown) and insulin resistance in liver and skeletal muscle.

Many researchers working in the field of mitochondria are using for different purposes synthetic OXPHOS uncouplers such as 2,4-dinitrophenol (DNP) or carbonyl cyanide m-chlorophenylhydrazone (CCCP). These compounds are also referred to as protonophoric uncouplers because their chemical structures allow the transport of protons across the inner mitochondrial membrane. Hence, DNP and CCCP can induce maximal mitochondrial respiration with different substrates including fatty acids, loss of the membrane potential $\Delta\psi$ and abolition of ATP synthesis in isolated mitochondria and intact cells [5–7]. It is also noteworthy that different pharmaceuticals currently on the market can uncouple OXPHOS. Indeed, this has been shown with the local anesthetic bupivacaine, the antiarrhythmic agent amiodarone, the nonsteroidal antiestrogen tamoxifen and different nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen and salicylic acid [7–10]. Importantly, OXPHOS uncoupling can be an important mechanism whereby drugs can induce liver injury in some patients [10–12].

The treatment of obesity and related metabolic disorders such as type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD) is a matter of extensive investigations because these diseases are a major burden for public health and medical care systems. Regarding NAFLD, reduction of de novo lipogenesis and stimulation of fat oxidation are two major pharmacological strategies that are currently tested [13,14]. In particular, drug-induced increased fat utilization can be achieved by way of activation of peroxisome proliferator-activated receptor-α (PPARα), PPARδ, and AMP-activated protein kinase AMPK [13–17]. Another strategy under investigation is the stimulation of mitochondrial fatty acid oxidation (FAO) by the OXPHOS uncoupler DNP and its derivatives DNP-methyl ether (DNPME), as proposed by Dr Shulman et al. [18,19]. Indeed, this group already showed in 2004 that the treatment of obese rats for 3 days with low doses (16 mg/kg/day) of DNP alleviated fatty liver and hepatic insulin resistance [18]. Furthermore, amelioration of the latter metabolic disturbance was accompanied with a normalization of the activity of protein kinase C-ε (PKCε) and c-Jun NH2-terminal kinase-1 (JNK1), two kinases impairing insulin signaling [18].

Although these early results were encouraging and demonstrated the effectiveness of this pharmacological strategy, the authors wished to develop a safer compound for further investigations [19]. Indeed, DNP was used in the 1930's in different countries as a treatment of obesity but this remedy was rapidly withdrawn from the market due to significant toxicity [20]. For instance, severe hyperthermia, cardiovascular collapse and death were sometimes observed [21]. In addition, skin rashes, cataracts, agranulocytosis and neutropenia, deafness and liver injury with jaundice occurred in some patients [20,21]. Hence, although DNP-induced OXPHOS uncoupling and stimulation of mitochondrial FAO can alleviate obesity and fatty liver [18,20,21], its low therapeutic index and adverse effects preclude any registration to national drug agencies.

In order to develop a safer OXPHOS uncoupler, Dr Shulman et al. thus generated 5 derivatives of DNP that could be metabolized to DNP by cytochromes P450 (CYPs) [19]. Indeed, the working hypothesis was that hepatic biotransformation of the DNP derivatives to the parent compound would