# Targeted mitochondrial uncoupling beyond UCP1 – the fine line between death and metabolic health

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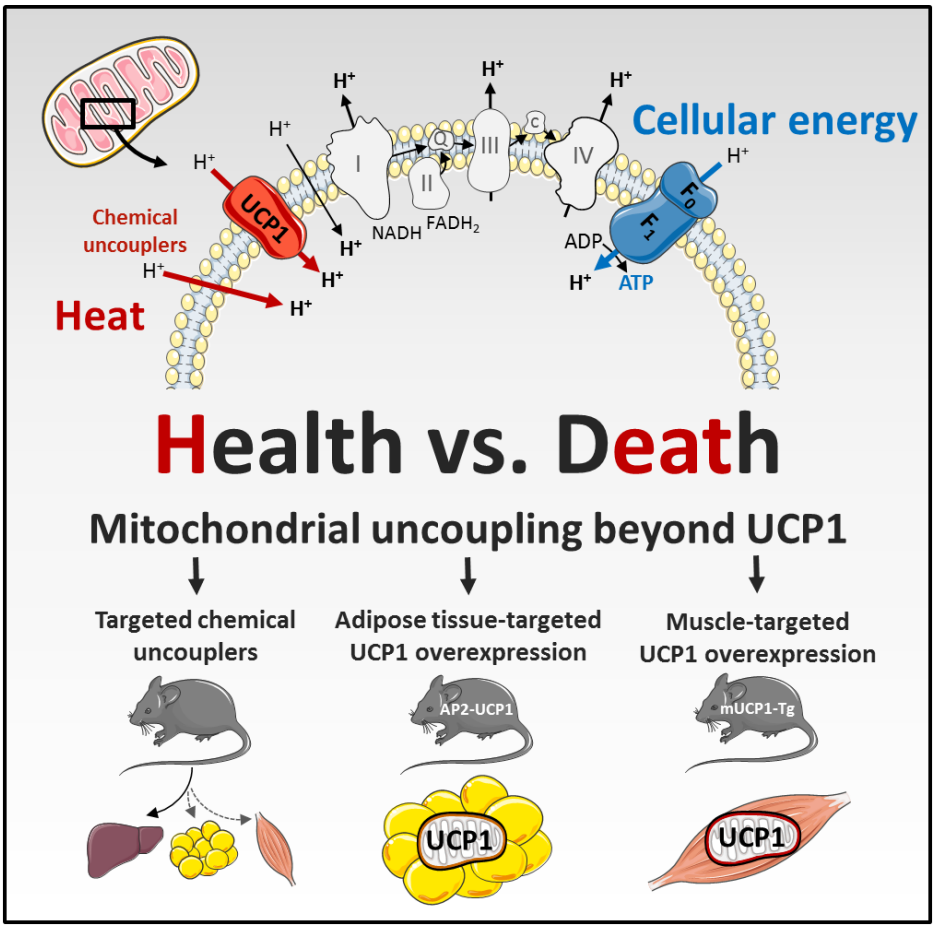
**Highlights**:

- Ectopic mitochondrial uncoupling increases substrate oxidation in target tissues.
- Novel chemical uncouplers tackle obesity, diabetes and fatty liver disease.
- Targeted UCP1 overexpression ameliorates obesity, hypertriglyceridemia and insulin resistance.
- Muscle-targeted UCP1 overexpression promotes adaptive metabolic remodeling, endocrine crosstalk and survival.

20 **Graphical abstract**

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**ABSTRACT**

In the early 1930s, the chemical uncoupling agent 2,4-dinitrophenol (DNP) was promoted for the very first time as a powerful and effective weight loss pill but quickly withdrawn from the market due to its lack of tissue-selectivity with resulting dangerous side effects, including hyperthermia and death. Today, novel mitochondria- or tissue-targeted chemical uncouplers with higher safety and therapeutic values are under investigation in order to tackle obesity, diabetes and fatty liver disease. Moreover, in the past 20 years, transgenic mouse models were generated to understand the molecular and metabolic consequences of targeted uncoupling, expressing functional uncoupling protein 1 (UCP1) ectopically in white adipose tissue or skeletal muscle. Similar to the action of chemical mitochondrial uncouplers, UCP1 protein dissipates the proton gradient across the inner mitochondrial membrane, thus allowing maximum activity of the respiratory chain and compensatory increase in oxygen consumption, uncoupled from ATP synthesis. Consequently, targeted mitochondrial uncoupling in adipose tissue and skeletal muscle of UCP1-transgenic mice increased substrate metabolism and ameliorates obesity, hypertriglyceridemia and insulin resistance. Further, muscle-specific decrease in mitochondrial efficiency promotes a cell-autonomous and cell-non-autonomous adaptive metabolic remodeling with increased oxidative stress tolerance. This review provides an overview of novel chemical uncouplers as well as the metabolic consequences and adaptive processes of targeted mitochondrial uncoupling on metabolic health and survival.

**Keywords:**

Energy metabolism, Longevity, Mitochondria, Obesity, Protonophore, Uncoupling protein 1

**Abbreviations:**

AMPK, AMP-activated protein kinase; aP2, adipocyte protein 2; ATP, adenosine triphosphate; BAT, brown adipose tissue; CRMP, controlled-released mitochondrial protonophore; DNP, 2,4-dinitrophenol; DNPME, DNP-methylethyl; FGF21, fibroblast growth factor 21; NEN, niclosamide ethanolamine salt; OXPHOS, mitochondrial oxidative phosphorylation; RMR, resting metabolic rate; ROS, reactive oxidant species; T2D, type 2 diabetes; TPP, triphenylphosphonium; UCP1, uncoupling protein 1, WAT, white adipose tissue

# Content

## 1 Introduction

Metabolic diseases such as obesity, hypertriglyceridemia and type 2 diabetes (T2D) have reached an epidemic level globally [1, 2]. Metabolic health is closely associated with body weight and whole-body energy balance which can be regulated by the amount of energy intake or energy expenditure. Targeting processes that lead to a reduction in mitochondrial coupling/efficiency could be a promising therapeutic strategy to combat obesity and its co-morbidities. The uncoupling protein 1 (UCP1) is the first identified and most studied uncoupling protein, discovered almost 40 years ago [3, 4]. Despite the existence of other UCPs such as UCP2 [5] and UCP3 [6, 7], only UCP1 seems to mediate energy dissipation as heat for adaptive thermogenesis via functional mitochondrial uncoupling *in vivo* [8, 9]. UCP1 is predominantly expressed in brown adipose tissue (BAT) mitochondria and dissipates upon activation the proton gradient across the inner mitochondrial membrane [10], thus uncoupling electron transfer system from ATP synthesis and accelerating mitochondrial oxidative phosphorylation (OXPHOS) in order to maintain ATP homeostasis [11]. Basically, mitochondrial uncoupling refers to a loss of coupling between the mitochondrial inner membrane electrochemical proton gradient and the synthesis of ATP (Fig. 1), thereby releasing energy as heat [12]. Within BAT depots, this metabolic process is called non-shivering thermogenesis which is UCP1-dependent and strongly increased by cold exposure [13, 14]. The manipulation of UCP1 activity is an excellent approach to influence energy expenditure and a natural defense against obesity. However, it is localized in BAT, a tissue which, when not activated by cold induction, represents only a small part of the human body [15-18].

Pharmacological agents that increase metabolic rate by increasing uncoupling of mitochondrial OXPHOS were intensively studied in the past. However, systemic chemical mitochondrial uncoupling agents, such as 2,4-dinitrophenol (DNP) or carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) lack selectivity and have a narrow therapeutic window, largely due to their severe side effects and toxic doses [19]. Instead, tissue-specific and thereby targeted mitochondrial uncoupling has been investigated during the last decades as a powerful strategy to regulate whole-body energy homeostasis and metabolic health for the treatment of obesity and associated metabolic disorders. This review provides an overview of the metabolic consequences and adaptive processes in response to a targeted treatment with chemical uncoupling agents or ectopic overexpression of functional UCP1, as a model of tissue-targeted mitochondrial uncoupling.

## 2 Metabolic impact of chemical mitochondrial uncoupling agents

Apart from dietary and pharmacological interventions affecting satiety or intestinal absorption efficiency to decrease energy intake, increasing energy output through pharmacological uncoupling has been proposed as a weight-loss therapy [20]. Below we review well-known and novel chemical uncoupling agents and discuss their relevance for metabolic health and treatment of human metabolic disease.

### 2.1 DNP (2,4-Dinitrophenol)

The first and best-studied example is the artificial uncoupler DNP, a lipid-soluble weak acid which acts as a chemical protonophore and allows protons to leak across the inner mitochondrial membrane [21], mimicking the uncoupling effect of activated UCPs. In the 1930s, DNP was widely used to treat obesity [22]. Nevertheless, because at high doses nonspecific uncoupling in all tissues causes dangerous side effects including hyperthermia and death [23], DNP was withdrawn from the market by the US Food and Drug Administration (FDA) in 1938. Case reports demonstrated that an acute administration of 20–50 mg per kilogram of body weight in humans can be lethal [24].

Nevertheless, in 2015 in the UK, a substantial increase was reported in clinical presentations with toxicity and associated high mortality caused by exposure to DNP [25]. In contrast, recent studies demonstrate that long-term treatment with low doses of DNP protects against diet-induced obesity, improves insulin sensitivity and increases lifespan in mice [26, 27]. Because of the strong effects on body weight in humans and survival in mice, the mechanism of action of DNP remains under investigation as a potential approach for the treatment of obesity and associated metabolic disorders (Fig. 2). In 2006, Murphy and colleagues developed a mitochondrial-targeted form of DNP, by coupling it to the lipophilic triphenylphosphonium (TPP) cation, which accumulates within mitochondria driven by the membrane potential [28]. They found that MitoDNP was extensively taken up by mitochondria, however no increase in uncoupling could be observed. Six years later Chalmers et al. developed a compound called MitoPhotoDNP, a mitochondria-targeted photoactivated protonophore [29]. Comparable with MitoDNP, it is targeted to mitochondrion by TTP, but releases DNP only in response to directed irradiation with UV light. Indeed, MitoPhotoDNP led to the selective uncoupling of individual and/or several mitochondria within a cell when used in conjunction with fluorescence imaging. Thus, MitoPhotoDNP represents a promising tool to elucidate the effects of mitochondrial uncoupling on cellular metabolism in vitro which is of great importance in the development of less toxic protonophore.

## 2.2 Next generation chemical uncouplers

Several novel mitochondrial- or tissue-targeted chemical uncouplers with a higher safety and therapeutic value were developed and investigated in the last couple of years (Fig. 2). In 2010, the group of Vladimir P. Skulachev synthesized penetrating cation/fatty acid anion pairs as mitochondria-targeted protonophore. While initially searching for mitochondria-targeted antioxidants, they discovered that the synthesized plastoquinone derivates SkQ1 (10-(6´-plastoquinonyl) decyltriphenylphosphonium) and $C_{12}$TPP (dodecyltriphenylphosphonium) potentiated the fatty acid-induced uncoupling of respiration and OXPHOS in isolated rat-liver mitochondria [30]. SkQ1 was further investigated as mitochondria-targeted antioxidant for potential treatment of various age-related diseases, such as Alzheimer's disease [31] or retinopathy [32, 33]. Furthermore, a recent study demonstrated that the mitochondrial-targeted $C_{12}$TPP effectively increased oxygen consumption in isolated brown-fat mitochondria, independent of UCP1, and abolished diet-induced obesity in mice by reducing food intake, increasing the resting metabolic rate and overall fatty acid oxidation [34].

In addition, Rhodamine 19 butyl ester ($C_4$R1), a short-chain alkyl derivative of Rhodamine 19, was found to decrease the membrane potential and stimulate respiration of isolated liver mitochondria as well as reduce oxidative stress induced by brain ischemia and reperfusion in rats [35]. In addition, similar to the *in vivo* effects of $C_{12}$TPP, the penetrating cation $C_4$R1 effectively reduced body weight and fat mass of obese mice fed a high-fat diet [36]. Thus, both $C_{12}$TPP and $C_4$R1 are considered as novel promising candidates for mild mitochondrial uncoupling anti-obesity drugs.

With the overall aim to identify mitochondrial-targeted uncouplers that lack off-target activity at the plasma membrane, the lipophilic weak acid named BAM15 was recently identified by a small molecule library and bioenergetics screening approach [37]. Interestingly, the authors could show that although BAM15 is less cytotoxic because it does not depolarize the plasma membrane, it still effectively uncouples OXPHOS in L6 myoblast mitochondria *in vitro*. Furthermore, first *in vivo* experiments demonstrated that BAM15 protects mice from acute renal ischemic-reperfusion injury, whereas effects on metabolic diseases have not be been addressed so far.

However, the above-mentioned uncoupling agents are not selective for particular mitochondria and the challenge still is to find a way to deliver those molecules to mitochondria within individual tissues or cells. Two additional novel mitochondrial uncouplers, named C1 and CZ5, were recently uncovered performing a high-throughput screening assay for modulators of mitochondrial membrane potential [38, 39]. The small-molecule compound C1 increased fat oxidation and activity of key cellular energy sensor AMP-activated protein kinase (AMPK) [40] acutely 2 hours after intraperitoneal injection specifically in liver of lean mice as well as reduced hyperglycemia and plasma fatty acids in diabetic *db/db* mice after long-term oral administration for 4 weeks [38]. Remarkably, the compound CZ5 was described to act as a cell type-specific uncoupler that only targets skeletal muscle and adipose tissue, but not liver [39]. Chronic orally administrated CZ5 for 25 days ameliorated diet-induced obesity via both increased energy expenditure and suppressed food intake. Besides, CZ5 treatment improved glucose and lipid metabolism *in vivo*, accompanied by an activated AMPK phosphorylation in targeted white fat depot and skeletal muscle.

Finally, a recent study by Tao et al. could show that treatment with niclosamide ethanolamine salt (NEN) uncouples mammalian mitochondria at upper nanomolar concentrations and increases energy expenditure and lipid metabolism in mice [41]. Interestingly, NEN represents a salt form of niclosamide (5-chloro-salicyl-(2-chloro-4-nitro) anilide), an anthelmintic drug approved by the FDA for treating intestinal infections of tapeworms [42]. Remarkably, oral NEN efficaciously prevented and treated hepatic steatosis and insulin resistance in high-fat-fed mice and improved glycemic control accompanied by delayed disease progression in *db*/*db* mice. Moreover, NEN activated AMPK in a dose- and time-dependent manner in liver, but not in muscle and adipose tissue of treated mice. Thus, the authors concluded that liver is a direct target of NEN treatment and AMPK-activation as key mechanism on promoting lipid oxidation.

### 2.3 Liver-targeted chemical uncouplers

A study has recently demonstrated that a functionally liver-targeted derivative of DNP, DNP-methyl ether (DNPME), reversed a high-fat diet-induced hypertriglyceridemia, hepatic steatosis, and whole-body insulin resistance in rats without inducing hyperthermia or associated hepatic or systemic toxicities [43]. Here, the authors hypothesized that derivatives of DNP, such as DNPME, would be preferentially metabolized by the hepatic cytochrome P450 system to yield the active protonophore DNP in hepatocytes as primary target cells. Interestingly, they reported that the 50% lethal dose ($LD_{50}$) of DNPME is almost 10-fold higher than that of classic DNP. In a follow-up study, the same group developed a controlled-release oral formulation of DNP, called CRMP (controlled-release mitochondrial protonophore) in order to further improve the safety and efficacy of DNP, consequently increasing the therapeutic window of this agent [44]. Using a polymer hydroxypropylcellulose/ethylcellulose coating system they generated a novel DNP version with lower peak plasma levels and sustained-release pharmacokinetics. In rat models of diet-induced or genetic obesity, daily CRMP administration reversed hepatic steatosis, insulin resistance, T2D, steatohepatitis, and liver fibrosis without detectable toxicity [44].

Altogether, tissue-targeted chemical mitochondrial uncoupling agents provide an elegant strategy to combat obesity and associated disorders, although the issue of dose-dependence and self-limitation remains an important open question (Fig. 3A). Thus, regulation and activation of endogenous proteins with uncoupling action, such as UCP1 or the adenine nucleotide translocase (ANT) [45], may provide an alternative strategy for dose-independent and self-limiting tissue-restricted mitochondrial uncoupling.

## 3 Targeted expression of UCP1 to white fat depots

White adipose tissue (WAT) plays an important role as an endocrine regulator and is involved in whole body glucose as well as energy homeostasis. However, its major role is the control of systemic fatty acids levels and the storage of metabolic energy, thus the opposite of energy burning thermogenic brown adipose tissue.

### 3.1 Metabolic consequences of white adipose tissue-targeted mitochondrial uncoupling

In 1995, Kopecky and coworkers established a transgenic mouse model (AP2-UCP1 mice) expressing UCP1 in WAT [46-48]. In these mice, the fat-specific AP2 promoter was used to drive expression of UCP1, resulting in enhanced protein expression of UCP1 in both WAT and BAT. Notably, whereas the total protein amount of transgenic UCP1 in WAT of adult mice did not exceed 2% of native UCP1 found in bona fide BAT depots, this was still sufficient to uncouple OXPHOS in WAT adipocytes [49]. Moreover, AP2-UCP1 mice showed resistance to diet induced obesity, which is consistent with the hypothesis, that thermogenesis from elevated expression of UCP1 reduced adiposity (Fig. 3B). In contrast, the transgene particular led to BAT atrophy which impaired thermogenic mechanisms for protecting body temperature [50]. In fact, it was shown that ectopic WAT-targeted UCP1 overexpression particularly activated AMPK and mitochondrial biogenesis in unilocular white adipocytes [51, 52]. Transgenic UCP1 also reduced lipogenesis and modulated lipolysis and hormonal control of lipid metabolism [53, 54] but resulted in only a marginal stimulation of RMR [50]. Moreover, UCP1 expression in WAT of AP2-UCP1 mice decreases with age [52], suggesting a posttranscriptional control of the ectopic UCP1 expression or an elimination of UCP1-containing adipocytes with time. Finally, Yamada et al. could demonstrate that ectopic expression of very low levels of UCP1 in epididymal WAT through injection of a UCP1 adenovirus vector reversed both insulin and leptin resistance, improved glucose tolerance and decreased food intake in both diet-induced and genetically obese mouse models [55].

### 3.2 Induction of endogenous UCP1 in white fat depots

Nowadays research focus intensively on the induction of endogenous UCP1 in WAT depots, also called the “browning” of white fat, which is characterized by the infiltration or transdifferentiation of so called beige/brite fat cells within white adipose tissue depots [56]. It was already reported 30 years ago that cold stimulus induces multilocular, UCP1-positive fat cells within certain WAT depots [57, 58]. Today we know that endogenous UCP1 can be induced in WAT not only by cold exposure, but also in response to different pharmacological and nutritional stimuli [59] as well as by secreted endogenous factors [60, 61]. In line with the results of ectopic expression of UCP1 in WAT, the induction of WAT browning has been associated with improved metabolic health [62]. However, while it was demonstrated that UCP1 in brite/beige adipose tissue mitochondria is indeed thermogenically functional [63], we could recently demonstrate a clear dissociation of WAT browning from obesity resistance and improved glycemic control and insulin sensitivity [61]. Thus, the precise physiological relevance of WAT remodeling, including UCP1 expression, still remains hotly debated [64].

## 4 Targeted expression of UCP1 to skeletal muscle

Recent studies implicated that BAT and muscle cells, but not WAT cells, differentiate from a common precursor [65], suggesting that BAT cells are more similar to muscle cells than to white adipocytes. Moreover, skeletal muscle represents a plastic and highly metabolic active organ that constitutes up to 40% of total body mass in mammals and is the major contributor to RMR and total energy expenditure [66-68]. Therefore, skeletal muscle represents a predominant site of glucose

disposal and plays a crucial role in glycemic control [69] as well as uptake and utilization of plasma lipoprotein-derived and free fatty acid [70, 71]. In contrast, chronic metabolic disorders such as obesity and T2D are closely associated to impaired muscle mitochondrial function [72, 73] and an involvement of skeletal muscle in mitochondrial dysfunction-associated diseases is frequent [74]. Thus, proper muscle mitochondrial performance is tightly connected to metabolic health and the question came up whether skeletal muscle represents another suitable tissue for targeted mitochondrial uncoupling?

### 4.1 Metabolic consequences of muscle-targeted mitochondrial uncoupling

Almost 16 years ago, the first transgenic mouse model with ectopic muscle-targeted UCP1 overexpression (mUCP1-Tg) driven by the rat myosin light chain 2 (MYL2) promoter was described by Li et al. demonstrating that low muscle UCP1 expression doubled muscle oxygen consumption without affecting thermoregulation [75]. Moreover, transgenic mice with muscle-targeted UCP1 overexpression under control of the mouse muscle creatine kinase (MCK) [76] or the human skeletal actin (HSA) [77] promoter were generated. Again, while mUCP1-Tg mice indeed show an increased mitochondrial uncoupling and a reduced muscle mitochondrial OXPHOS capacity [78], body temperature was normal or rather decreased with declining ambient temperature [77]. mUCP1-Tg mice display increased energy expenditure [77] and a whole range of metabolic improvements such as an increased metabolic flexibility [79], muscle glucose uptake and fatty acid oxidation [80, 81], as well as an increased insulin sensitivity accompanied by decreased insulin levels, especially under high-fat diet-feeding. Interestingly, this increased insulin sensitivity is independent of diet and body fat accumulation suggesting a dissociation of obesity and insulin resistance in mUCP1-Tg mice [79, 82, 83]. Surprisingly, not only muscle is affected in mUCP1-TG mice. Furthermore they show an increased glucose uptake [84], an augmented lipid metabolism [79] as well as an induction of beige/brite adipocytes in WAT depots [85], suggesting an endocrine role of skeletal muscle uncoupling. Overall, improved glucose tolerance accompanied by an increased insulin sensitivity and muscle glucose uptake seems to be the most robust metabolic phenotype of mUCP1-Tg models (Fig 3C).

### 4.2 Muscle-targeted mitochondrial uncoupling promotes adaptive metabolic remodeling

Glycolytic and oxidative metabolic processes are rapidly activated to maintain cellular energy homeostasis in skeletal muscle. In line with the above-mentioned liver- and WAT-targeted mitochondrial uncoupling approach it was shown that muscle-restricted mitochondrial uncoupling also led to an increased AMPK activity [84, 86]. Notably, AMPKα2 activity, but not AMPKα1 was highly induced in muscle of mUCP1-Tg mice, and loss of active AMPKα2 promoted a severe reduction of overall muscle integrity together with a highly diminished physical activity tolerance and impaired mitochondrial biogenesis [80]. This revives the significance of AMPK for regulating cellular plasticity in response to chronic decreased mitochondrial energy efficiency. Interestingly, enhancing AMPK activity in brown adipocytes also increased BAT activity [87]. Thus, targeting AMPK as a key mediator of a cell-autonomous adaptive response holds therapeutic potential for the treatment of obesity and associated metabolic disorders.

Furthermore, muscle-targeted mitochondrial uncoupling promotes cell-non-autonomous effects and endocrine crosstalk via secretion of myokines. Treatment of mouse C2C12 muscle cells *in vitro* with the chemical uncoupler FCCP resulted in a strong induction of integrated stress response (ISR) as well as fibroblast growth factor 21 (FGF21) as myokine [85, 88]. Notably, muscle mitochondrial uncoupling induces FGF21, which was previously described as enhancer of WAT browning [89], in skeletal muscle of mUCP1-Tg mice *in vivo* [85]. Indeed, using mUCP1-Tg/FGF21-knockout mice,

we recently demonstrated that cell-non-autonomous WAT browning and metabolic remodeling is fully FGF21 dependent [61]. Remarkably, the cell-autonomous muscle metabolic adaptation and obesity resistance was independent of FGF21 action as a myokine. With regard to metabolic health and aging it was shown that transgenic overexpression of FGF21 in liver markedly extends lifespan in mice probably in an auto-/paracrine manner by blunting the growth hormone/insulin-like growth factor-1 signaling pathway [90]. Furthermore, a recent study could demonstrate that serum FGF21 levels in humans are related to BAT activity [91]. However, to date, the ultimate contribution of FGF21 to a cell-autonomous and cell-non-autonomous response on effects of targeted mitochondrial uncoupling and survival remains to be elucidated.

### 4.3 Muscle-targeted mitochondrial uncoupling delay age-related disease

Remarkably, two independent studies found that skeletal muscle-targeted respiratory uncoupling promotes survival [82, 86] and diminishes age-related diseases, such as atherosclerosis, vascular disease and blood pressure as well as diabetes [86, 92]. Interestingly, markers of lipid-oxidative stress levels were highly induced in muscle of mUCP1-Tg mice independent of the diet-feeding regime [93]. In addition, increased activity of endogenous antioxidant defense enzymes such as catalase or superoxide dismutase (total SOD) provides evidence for an elevated rather than reduced formation of reactive oxidant species (ROS) in muscle of mUCP1-Tg mice. Mitochondria are a major site of cellular ROS production [94] which by itself represents an important mediator of mitochondrial stress signaling to promote cellular adaptation [95]. In line with that, glutathione metabolism was induced followed muscle-targeted ectopic mitochondrial uncoupling [96, 97] which fits with the induced serine and glycine biosynthetic pathway [96], as both amino acids are important precursors for glutathione biosynthesis [98].

Initially, a “rate of living” hypothesis has been proposed by Pearl in the late 1920s, predicting that increased energy metabolism would increase the ROS production and thus reduce life span [99]. While 40 years later Peter Mitchell´s chemi-osmotic hypothesis provided the basis for understanding the actual process of OXPHOS and energy/ATP synthesis [100], it took another 40 years to reveal that an inefficiency of the respiratory chain through mitochondrial uncoupling might increase longevity by reducing ROS generation, despite increased energy expenditure [101]. This so called “uncoupling to survive” hypothesis was supported by the study of Speakman et al., showing that mice with highest metabolic intensity and mitochondrial uncoupling live longer than littermates with lower metabolic rate [102]. However, whether mild mitochondrial uncoupling under physiological conditions indeed plays a role as alleviator of oxidative damage remains unclear [103]. Although mitochondrial function and increased oxidative stress are usually associated with aging [94], a ROS-induced cellular stress adaption through an increased endogenous antioxidant defense system is in line with the concept of “mitohormesis”, suggesting a link between mild oxidative stress and enhanced cellular function [104]. To date, mitohormesis has been best studied in *Caenorhabditis elegans* (*C. elegans)* as a model for neurologic and metabolic diseases [105, 106]. In addition, a *Drosophila* model of mild muscle mitochondrial distress showed preserved mitochondrial and muscle function during aging and a prolonged lifespan [107]. Thus, the significance of the “uncoupling to survive” hypothesis related to longevity by uncoupling-mediated reduced ROS formation [101] should be re-evaluated taking into account the concept of “mitohormesis” based on the *in vivo* mitochondrial adaptation in response to muscle-targeted ectopic mitochondrial uncoupling.

Altogether, despite using different promoters, all mUCP1-Tg mice display an overall improved metabolic phenotype of increased insulin sensitivity, reduced obesity and increased survival (Fig.

3C). However, the molecular mechanisms behind the phenotype are quite complex and not fully understood so far.

## 5 Conclusion and therapeutic perspectives

While discussing potential therapeutic targets for obesity, T2D and fatty liver disease strategies of life style change such as dietary restriction and regular exercise programs should always be kept in mind for improvement of metabolic health status. Nevertheless, if proofed to be safe and effective, tissue-targeted chemical mitochondrial uncoupling agents still provide an additional therapeutic strategy to combat metabolic syndrome and associated disorders.

Studies using transgenic mice with targeted UCP1 overexpression uncovered key molecular mechanisms how mitochondrial uncoupling affects energy metabolism and metabolic health *in vivo*. Thereby, it was proven that AMPK plays a crucial role as a housekeeper for mitochondrial function to maintain energy homeostasis and cellular integrity. Together with mitochondrial uncoupling-induced endocrine crosstalk via secretion of cytokines, such as FGF21, this potentially could open up new avenues of investigations that may help to understand how a specific target tissue is sufficient to reprogram and tune the metabolic health of the whole organism. It is worth mentioning that the first human mitochondrial disease discovered around 50 years ago, Luft´s disease, leads to a muscle atrophy due to increased uncoupled mitochondrial oxidative phosphorylation and energy depletion within skeletal muscle which is also affecting whole-body energy metabolism [108]. Thereby, Luft and colleagues described the first example how a single dysfunctional organelle within one specific tissue can affect the whole organism. However, our knowledge regarding the integrated signaling network of cell plasticity remains rudimentary and further studies are required to enlarge our understanding.

Overall, there is a still increasing interest in mitochondrial uncoupling during the past 20 years [109], and we are just half-way on our journay to discover a safe “polypill” that treats metabolic disorders such as obesity and T2D. Whether the light at the end of the tunnel will be a targeted chemical mitochondrial uncoupler or a train remains to be unknown.

## 6 Conflict of interests

The authors declare no conflict of interest.

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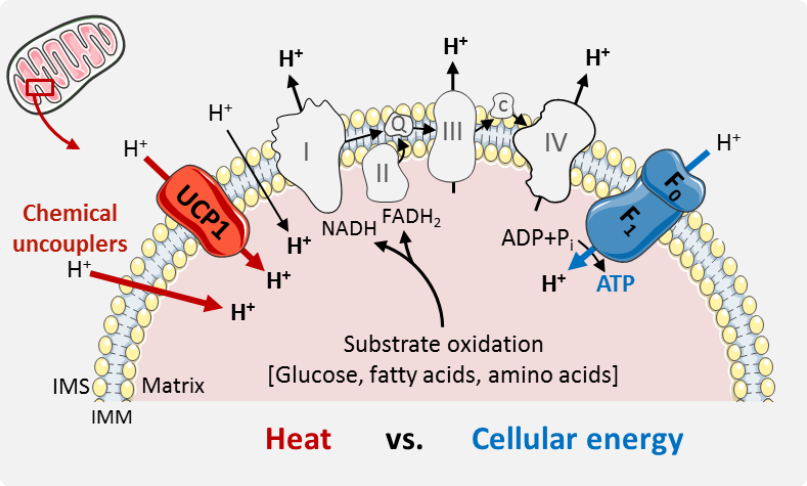


**Fig. 1. Mitochondrial oxidative phosphorylation and uncoupling.** Cellular energetics is efficiently controlled by rate of the electron transfer system (ETS) and oxidative phosphorylation, referred to as the mitochondrial respiratory chain (RC). Substrate muscle metabolism of glucose (glycolysis), fatty acids (β-Oxidation) and amino acids is closely coupled with ATP formation through mitochondrial RC. The primary reducing equivalents of the ETS are nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide ($FADH_2$) which are mainly generated by the tricarboxylic acid cycle or during β-Oxidation of fatty acids/acyl-CoA. Electrons received from NADH or $FADH_2$ are passed through the series of OXPHOS complexes in the RC, ultimately reducing oxygen to water. This electron flow particular through complex I, III and IV results in pumping of protons from the matrix into the intermembrane space (IMS), generating a membrane potential ($\Delta\psi m$) and proton motive force that in turn is used to generate ATP from ADP and inorganic phosphate ($P_i$) via the ATP synthase ($F_1/F_0$). Predominantly expressed in brown adipose tissue, uncoupling protein 1 (UCP1) dissipates the proton gradient across the inner mitochondrial membrane (IMM), thus uncoupling ETS from ATP synthesis and accelerating mitochondrial RC activity in order to maintain energy homeostasis [11]. During mitochondrial uncoupling the energy amount stored in the proton gradient is released as heat. See text for details on chemical uncouplers. This figure was created using Servier Medical Art (http://www.servier.com).

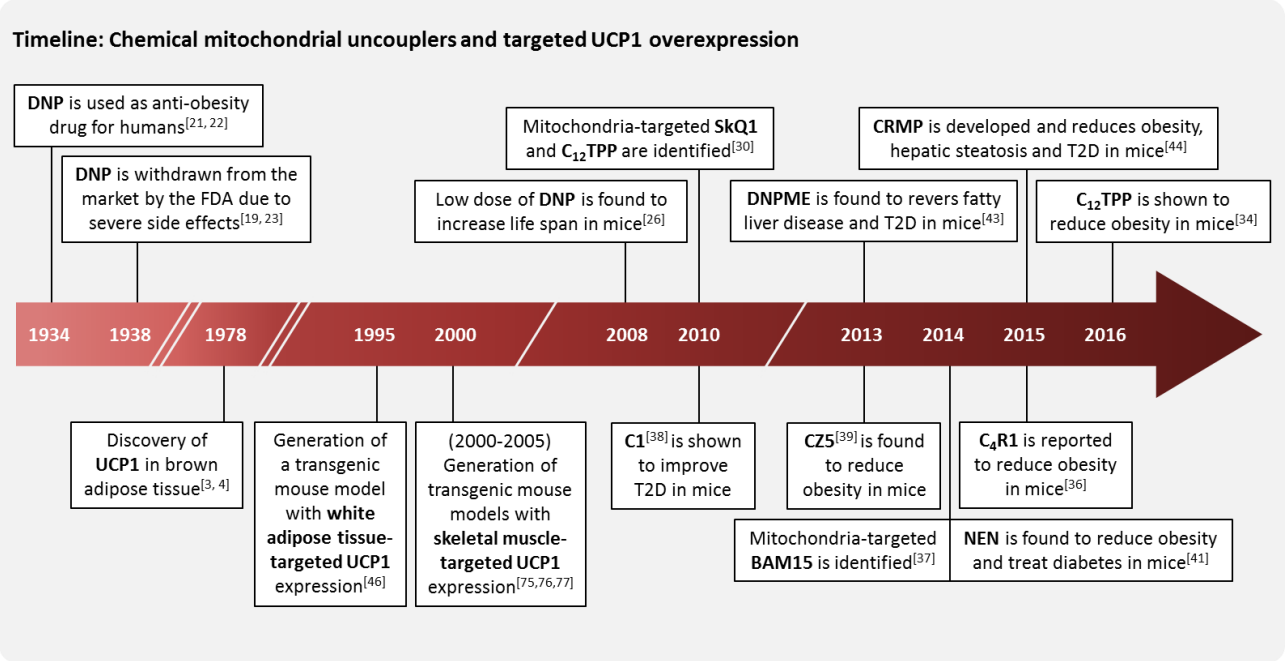


**Fig. 2. Timeline of chemical uncoupling agents and targeted UCP1 overexpression.** This illustrates the increasing interest in mitochondrial uncoupling for the treatment of obesity and associated metabolic disorders during the past 20 years. Of note, in parallel the re-evaluation of the role of brown adipose tissue (BAT) for the treatment of obesity took place, supported by observations using positron emission tomography–computed tomography (PET/CT) scanning that revealed the presence of BAT in adult humans [15-18]. Moreover, in the last decade research focused intensively on the induction of endogenous UCP1 in white adipose tissue depots, also called the "browning" of white fat, and the effects on metabolic health [56, 62]. Abbreviations: BAM15, $N^5$,$N^6$-bis(2-Fluorophenyl)-oxadiazolo-pyrazine-5,6-diamine [37]; CRMP, controlled-released mitochondrial protonophore [44]; $C_4$R1, Rhodamine 19 butyl ester [36]; C1, nomenclature not defined [38], CZ5, nomenclature not defined [39]; $C_{12}$TPP, dodecyltriphenylphosphonium [34]; FDA, US Food and Drug Administration; DNP, 2,4-dinitrophenol; DNPME, DNP-methylethyl [43]; NEN, niclosamide ethanolamine salt [41]; SkQ1, 10-(6´-plastoquinonyl) decyltriphenyl-phosphonium) [30]; UCP1, uncoupling protein 1. See text for further details.

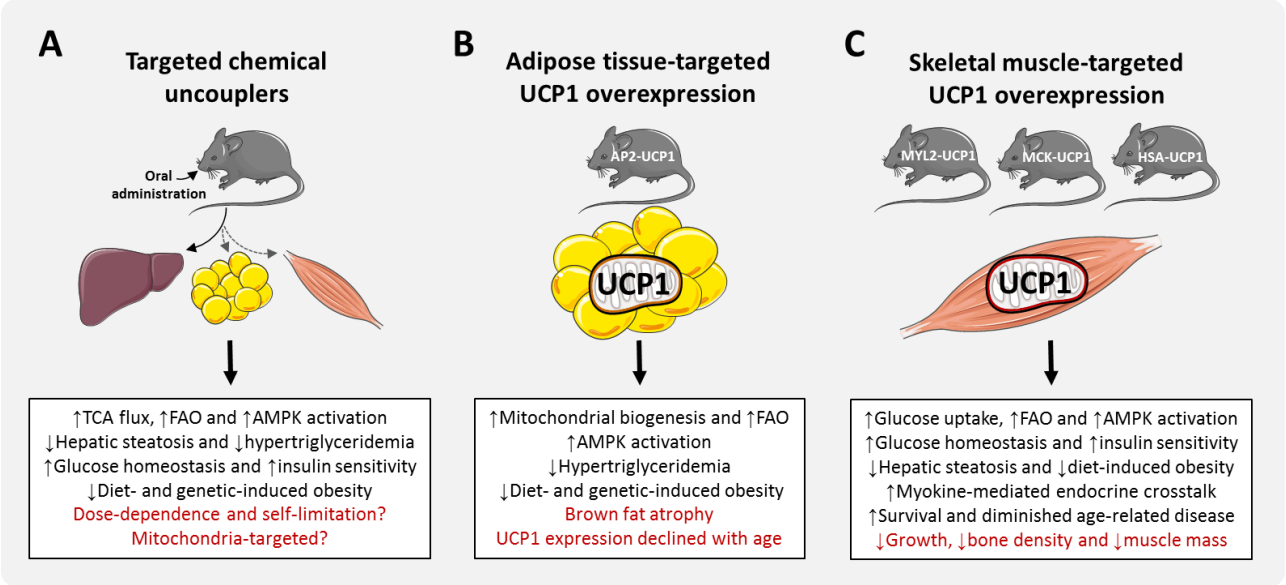


**Fig. 3. Overview on metabolic effects of chemical mitochondrial uncouplers and targeted UCP1 overexpression. (**A) Tissue-targeted chemical mitochondrial uncoupling agents provide an additional elegant strategy to combat metabolic syndrome and associated disorders. However, the issues of target tissue, dose-dependence and self-limitation remain important open questions. (B)+(C) Studies using transgenic mice with targeted UCP1 overexpression uncovered key molecular mechanisms how mitochondrial uncoupling affects energy metabolism and metabolic health *in vivo*. Abbreviations: AMPK, AMP-activated protein kinase; AP2, adipocyte protein 2; FAO, fatty acid oxidation; HSA, human skeletal actin; MCK, muscle creatine kinase; MYL2, myosin light chain 2, TCA, tricarboxylic acid cycle; UCP1, uncoupling protein 1. See text for further details. This figure was created using Servier Medical Art (http://www.servier.com).