The multiple facets of mitochondrial regulations controlling cellular thermogenesis.

Florian Beignon (florian.beignon@univ-angers.fr)
Université Angers: Universite d'Angers
Naig Gueguen
Université Angers: Universite d'Angers
Hélène Tricoire-Leignel
Angers University: Universite d'Angers
César Mattei
Angers University: Universite d'Angers
Guy Lenaers
Université Angers: Universite d'Angers

Research Article

Keywords: mitochondria, temperature, energy balance, thermogenesis, uncoupling

Posted Date: June 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1665727/v1

Abstract

Understanding temperature production and regulation in endotherm organisms becomes a crucial challenge facing the increased frequency and intensity of heat strokes related to global warming. Mitochondria, located at the crossroad of metabolism, respiration, Ca^{2+} homeostasis and apoptosis, were recently proposed to further act as cellular radiators, with an estimated inner temperature reaching 50°C in common cell lines. This inner thermogenesis might be further exacerbated in organs devoted to produce consistent efforts as muscles, or heat as brown adipose tissue, in response to acute solicitations. Consequently, pathways promoting respiratory chain uncoupling and mitochondrial activity, such as Ca^{2+} fluxes, uncoupling proteins, futile cycling and substrate supplies, provide the main processes controlling heat production and cell temperature. The mitochondrial thermogenesis might be further amplified by cytoplasmic mechanisms promoting the over-consumption of ATP pools.

Considering these new thermic paradigms, we discuss here all conventional wisdoms linking mitochondrial functions to cellular thermogenesis in different physiological conditions.

Highlights

Beyond its role as the principal source of ATP production, mitochondria can be considered as cellular radiators, with a possible local temperature of 50°C. This physiological contribution to heat production is regulated by many mechanisms targeting mitochondrial activity levels, and can be further stimulated by uncoupling the respiratory chain and membrane potential from ATP synthesis, to respond to drastic cold stresses. In addition, exergonic reactions using large ATP amounts in cell functions, like muscle shivering or Ca^{2+} leak from the SR, can contribute to generate heat by inducing an over-stimulation of mitochondrial activity to replenish ATP pools. Cell variations in thermal flows might thus promote heterogeneous subcellular temperature gradients that might contribute to novel regulatory pathways controlling cell physiology.

Internal Heat Production In Endotherms

Body temperature in endotherms, such as mammals, is crucial for the management of most biological functions. The ability to produce internal heat and maintain a constant body temperature was acquired during animal evolution, and is closely linked to original processes controlling energy metabolism. Indeed, an increased metabolic rate in endotherm species produces sufficient heat to raise body temperature, even at rest **(Legendre et Davesne 2020).**

Thermodynamics laws help to understand the link between heat production and energy metabolism. Energetic metabolism combines catabolic reactions, which break down molecules into smaller units, and anabolic reactions, which promote the synthesis of complex molecules from smaller units. Catabolic reactions are spontaneous and exergonic, i.e. more energy is released than consumed, whereas anabolic reactions are non-spontaneous endergonic reactions, requiring energy input from exergonic reactions.

However, the coupling between exergonic and endergonic reactions is imperfect, and a significant fraction of the energy dissipates as heat **(Ricquier 2006; Chouchani, Kazak, and Spiegelman 2018).**

In eukaryotic cells, mitochondria have a crucial role in ATP and heat productions, as the conjunction of most exergonic and endergonic reactions lies in this organelle especially through the oxidative phosphorylation (OXPHOS) **(Lowell et Spiegelman 2000; Ricquier 2006; Chouchani, Kazak, et Spiegelman 2018)**.

I. Mitochondria: The Center Of Cell Energy Or Heat Production?

As the center of ATP production, mitochondria are well-recognize as the powerhouses of eukaryotic cells**.** Indeed, the proton (H^+) motive force (Dp) generated by the electron transfer chain (ETC) system drives the ATP synthesis by the mitochondrial F0F1-ATP synthase, thus converting a significant part of the energy released from substrate oxidation to ADP phosphorylation (OXPHOS, see box 1).

****Box 1****

While mitochondrial ATP synthesis is widely quoted, the thermogenic role of mitochondria is much more neglected. Yet the maximal overall thermodynamic efficiency of mitochondrial ATP synthesis is in the range of 40% **(Nath, 2016)**, meaning that about 60% of the input energy is dissipated as heat (Figure 1, Box 2).

****Box 2****

Furthermore, this value is calculated with respect to the ideal OXPHOS coupling efficiency, while the effective one can be very different *in vivo.* Indeed, the degree of OXPHOS coupling is variable and modulated **(M D Brand, Harper, et Taylor 1993)** according to tissues and cells**(Cairns et al. 1998),** their energetic need and metabolic states. Thus, in mammalian cells, mitochondrial thermogenesis is the predominant form of energy production by respiration, far ahead of ATP synthesis. **(J. A. N. Nedergaard, Cannon, et Lindberg 1977).**

Two main mechanisms can further increase mitochondrial heat production rate: 1) the reduction of the thermodynamic efficiency of OXPHOS, at a constant or increased rate of substrate oxidation, thus increasing heat production at the expense of ATP production; 2) the increase in ATP turnover and consequently the substrate oxidation rate, with an inherent loss of energy as heat. Both mechanisms are not mutually exclusive, and will be discussed in the following sections.

Ii. Mitochondrial Heat Production Through Reduced Oxphos Efficiency

The coupling of redox reactions at the ETC to ATP synthesis by the F0F1-ATP synthase through H^+ flux is not perfect and any alteration of OXPHOS efficiency will favor mitochondrial heat production. Two main

mechanisms are known:

(A) the dissipation of the proton electrochemical gradient through the inner mitochondrial membrane (IMM), by uncoupling the use of Δp from ATP synthesis (Figure 2B).

(B) the modification of the stoichiometry between substrate oxidation, electron transfer reactions and proton translocation across the IMM (Figure 2A).

(A) Proton leak, the most powerful mechanism to support heat production.

a) Proton leak mechanisms

The most efficient way to increase thermogenesis consists in uncoupling. Mitochondrial uncoupling is a general term referring to any pathway that enables H^+ reentry into the matrix independently of ATP production. Thus, uncoupling induces futile cycle, linking H^+ leak through the inner mitochondrial membrane (IMM), via endogenous H^+ conductance pathways, and subsequent backward H^+ pumping by ETC in order to maintain the Δp across the IMM. This H^+ leak leads to a decreased in ATP production, favoring heat production, consistent with the energy conservation law **(Ricquier 2006)** (Figure 3). This energy-dissipating cycling accounts for a varying proportion of cellular metabolism, depending on the tissue type, but involves remarkable energetic costs in endotherms, as up to 25% of rat basal metabolic rate can be related to H^+ leak **(D. F. Rolfe et Brown 1997; D. F. Rolfe et al. 1999).** H^+ leak may be a constitutive process related to IMM biochemical features, termed basal H^+ leaks, or induced in specific conditions requiring thermogenesis.

Basal H^+ leak is an unregulated process, which depends on the H^+ driving force (Δp), being negligible at low potential at maximal phosphorylating rates, while increasing exponentially as Δp rises **(Nicholls 1974; Martin D. Brand et al. 2005).** Although poorly understood, the mechanisms of basal H^+ leak depend on IMM physicochemical properties, such as its fatty acid composition. For instance, the amount and saturation of cardiolipin **(Hoch 1992),** together with the ω6/ω3 fatty acid ratio increase basal H+ leak intensity **(Bobyleva et al. 1997; Pehowich 1999) (Fontaine et al. 1996; Martin D Brand et al. 2003).** However, this concept remains debated **(Hulbert et Else 2004)**, since no difference was found in H^+ conductance in liposomes prepared from liver mitochondria from different species, despite a three-fold difference in the unsaturation index of the phospholipid fatty acyl groups **(Martin D. Brand et al. 2005).** Alternatively, the magnitude of H^+ current across the IMM also correlates with the abundance of the adenine nucleotide translocase (ANT), a transmembrane IMM channel protein shuttling ADP for ATP **(Shabalina et al. 2006).** ANT may account for 50–70% of basal H^+ leak in muscle mitochondria (**Martin D. Brand et al. 2005**). The related molecular mechanism still remains elusive, but is independent of ANT ATP/ADP exchange activity. Thus, the basal H^+ leak may be related to IMM lipid composition and to the amount of restricted transmembrane carriers, such as ANT **(Shabalina et al. 2006; Jastroch et al. 2010)**.

Besides this basal H^+ leak, the most important uncoupling process corresponds to an induced and regulated Δp decrease, termed inducible H^+ leaks, and related to the expression of specific mitochondrial carrier proteins, like the uncoupling proteins (UCPs). The best characterized process of thermogenesis arises from UCP1, which is required for the inducible H^+ leak in brown and beige adipocytes. Conversely to heat shock proteins whose expression is induced by a hot stress, UCP1 expression is driven by a cold stress **(A. D. Nguyen et al. 2018).** UCP1 mediates H^+ transport from the intermembrane space back to the matrix, consistently decreasing the Δp. This process induces a tremendous activation of oxidations to restore the Δp. Thus, once activated, UCP1 uncouples substrate oxidation from ATP production, converting most energy in heat **(Ricquier 2017)**. Since UCP1 discovery **(Lin et Klingenberg 1980)**, its key role in heat production in the brown adipose tissue (BAT) was evidenced **(Ricquier 2017).** As of today, UCP1 is considered a major thermogenesis player in BAT adaptive thermogenesis.

While mitochondria from BAT specifically produce heat through uncoupling, H^+ leak is not a distinctive property of the mitochondria of the specialized thermogenic tissues. For instance, hepatocyte coupling efficiency in a broad range of species was found in the range of 75-80% of the ideal value **(M. D. Brand 2005)**, with H^+ leak accounting for up to 26 and 22% of the oxygen consumption in resting and active rat hepatocytes, respectively **(M. D. Brand et al. 1994; D. F. Rolfe et al. 1999)**. Similar coupling efficiencies were observed in other cell types **(Buttgereit, Brand, et Müller 1992; Jekabsons et Nicholls 2004),** while in perfused rat muscle, H^+ leak could account for up to 55% of the mitochondrial oxygen consumption in resting condition **(D. F. S. Rolfe et al. 1999)** and up to 38% in contracting fibers **(D. F. Rolfe et Brand 1996)**. These data indicate that H^+ leak in tissues different from primary thermogenic ones can have a consistent contribution to heat production. The molecular identity of the uncoupling protein in non-thermogenic tissues has been a long matter of debates. On the basis of their sequence similarities, four additional UCP isoforms UCP 2 to 5 were discovered, with restricted expression patterns in tissues such as heart, kidney, pancreas, neurons, smooth muscles and skeletal muscles **(Mao et al. 1999; Boss et al. 1997; Yu et al. 2000; Fleury et al. 1997)**. However, the functions of these UCPs are imperfectly known **(Ježek et al. 2018)**. Although UCP3 has a possible *in vivo* effect on thermal homeostasis **(Riley et al. 2016)**, UCP2 to 5 could regulate superoxide production **(Ježek et al. 2018)**. Notably, the low contents of UCP2 to 5 in tissues do not allow a strong uncoupling, as does UCP1 in BAT. Indeed, the range of UCP1 uncoupling in BAT mitochondria is ≈55 mV, while UCP2 uncoupling in lung is lower than 12.5 mV **(Jezek et al. 2004)**. Moreover, respiration analyses first pointed ANT as the main actor of the induced H^+ leak in skeletal muscle and liver **(Andreyev AYu et al. 1989; M. D. Brand 2005).** These results were further confirmed by direct patch-clamp measurements on IMM from several tissues such as skeletal muscle, heart, liver and kidney, using both pharmacological and genetic (ANT1/ANT2-deficient mice) approaches **(Bertholet et al. 2019)**. The ANT-mediated uncoupling requires protonatable free fatty acids, which would act as cofactors in H^+ translocation. The ubiquitous and high expression of ANT in many cell types may promote increased energy expenditure, i.e. in diet-induced thermogenesis. Thus, two proteins are nowadays considered as thermogenic uncoupling proteins: the ubiquitously expressed ANT and the fat-specific UCP1.

Finally, proton re-entry also accompanies metabolite and ion transports, therefore increasing conductance of any ion through the IMM could dissipate the Δp and uncouple the substrate oxidation by ETC from ATP synthesis. The most significant example is the mitochondrial Ca^{2+} cycling, which requires electrogenic ion-exchange and consequently induces mitochondrial uncoupling **(Filadi et Greotti 2021).** Furthermore, activities of K^+ channels and electrogenic transporters embedded in the IMM **(O'Rourke 2007; Urbani et al. 2021)**, can also induce uncoupling and reduce the OXPHOS efficiency, while supporting heat production However, induced-H^+ leak appears to be the only regulated mechanism specifically dedicated to thermogenesis.

b) Proton leak regulation

Regulations of H^+ leak have been extensively studied in BAT, where UCP1-dependent thermogenesis prevails especially during adaptive non-shivering thermogenesis **(Chouchani, Kazak, et Spiegelman 2018; Cohen et Kajimura 2021; Bertholet et Kirichok 2021)**. The regulation of UCP1 activity is not detailed in this review, but lipid and hormonal signaling as well as redox control are relevant pathways involved in UCP1-related inducible uncoupling.

Mitochondrial ROS production was described as a main regulator of BAT adaptive thermogenesis **(Mills et al. 2018; Schneider et al. 2016; Chouchani et al. 2016; Lettieri-Barbato 2019)** (reviewed by **Chouchani, Kazak, et Spiegelman 2017).** Indeed, thermogenesis activation in mouse BAT by cold temperature (4°C) or β-adrenergic stimulation results in elevated production of mitochondrial superoxide, hydrogen peroxide and lipid hydroperoxides **(Stier et al. 2014)**. Furthermore, genetic and pharmacological elevations of adipocyte ROS levels promote thermogenesis, while *in vivo* pharmacological depletion of mitochondrial lipid peroxides and superoxides impairs BAT thermogenic respiration **(Chouchani, Kazak, et Spiegelman 2018).** Mechanistically, thermogenic activation by mitochondrial ROS is mediated through reversible cysteine modifications of target proteins **(Chouchani et al. 2016).** For example, UCP1 Cys253 is targeted by oxidation, which increases UCP1 response to fatty acid (FA) during thermogenesis **(Chouchani et al. 2016).** In this respect, free long chain FAs are widely described as essential components of H^+ leak activation by UCP1 and ANT **(reviewed by Bertholet et Kirichok 2021).** Indeed, free cytosolic FAs can reach millimolar concentrations during active lipolysis in BAT **(J. Nedergaard et Lindberg 1979)**, while micromolar concentrations are sufficient to activate H^+ leak via UCP1 or ANT *in vitro* **(Garlid et al. 1996; Brustovetsky et Klingenberg 1994).** Conversely, free purine nucleotides, such as ATP and ADP, are described as the main physiological inhibitors of H^+ leak (for a detailed review, see **(Bertholet et Kirichok 2021)**). Note that there is a competition between nucleotide exchange and FA-dependent H^+ leak in the ANT translocation pathway. Thereby, in non-thermogenic tissues, ANT activity could switch from H^+ leak, promoting heat production, to ATP/ADP translocation, promoting ATP production, and consequently act as a modulator of heat in energy balance **(Bertholet et al. 2019)**.

Finally, T3 and T4 thyroid hormones (TH) are also involved in mitochondrial uncoupling **(Yehuda-Shnaidman, Kalderon, et Bar-Tana 2014).** Indeed, TH treatment is associated to decreased IMM potential

in human lymphocyte **(Mihara et al. 1999)** and to an excessive increased TCA cycle flux, compared to the level of ATP synthesis **(Johannsen et al. 2012).** Even if TH mode of action in promoting mitochondrial uncoupling remains elusive, TH may favor Ca^{2+} release by IP_3 receptors on the endoplasmic reticulum membrane **(Yehuda-Shnaidman, Kalderon, et Bar-Tana 2014; Teixeira, dos Santos, et Pazos-Moura 2020).**

Finally, H^+ leak increases the temperature which could itself enhance H^+ leak process and decreases OXPHOS efficiency. Indeed, in liver mitochondria *of Bufo bufo*, the increase of temperature promotes H^+ leak **(Monternier et al. 2014; Roussel et Voituron 2020).**

(B). Heat production through modulation of H^+ pump stoichiometry

a) Electron and $H^{\pm}$ slip

In theory, a decrease of the proton pumps efficiency would also result in a reduced P/O ratio (Figure 3). "Electron slip" refers to electron transfer without H^+ pumping (decrease of the H^+/e^- stoichiometry) through the cytochrome c oxidase (CIV) **(Papa et al. 1991; Babcock et Wikström 1992; Ferguson-Miller 1996; Kadenbach 2003; Wikström et Springett 2020)**. Electron slip may represent a protection against ROS production at high ΔYm, but currently, little information is available on the use of this mechanism in a physiological context. However, evidences indicate that its contribution in the regulation of mitochondrial efficiency and heat production is negligible **(M. D. Brand, Chien, et Diolez 1994; M. P. Murphy 1989; Porter et Brand 1995; Kadenbach 2003).**

"H^+ slip", or F0F1-ATP synthase uncoupling, refers to reduced coupling between the F1 catalytic activities and the H^+ translocation by F0. The H^+/ATP stoichiometry can be predicted from the ratio of catalytic β subunits number and that of H^+ binding c subunits, c/β **(Steigmiller, Turina, et Gräber 2008)**. However, the actual H^+/ATP stoichiometry can vary, leading to energy dissipation by F0F1-ATPsynthase as heat **(Giovanna Lippe et al. 2019).** The dissipative pathways and their modulation are still not well understood. 17β-estradiol, at micromolar concentrations, can also induces an "intrinsically slipping state" of F0-ATP synthase, resulting in a decreased ATP/O ratio **(Moreno et al. 2013).** Oxidative posttranslational modifications occur at a selective cysteine in ATP synthase subunits, which may act as a redox sensor modulating ATP synthase function **(S.-B. Wang et al. 2011)**. Oxidation of thiol residues located in F0 **(Zanotti et al. 1992; Yagi et Hatefi 1987)** and the formation of a disulphide bond between subunits b of two adjacent F-ATP synthase monomers **(G Lippe, Dabbeni Sala, et Sorgato 1988)** induce uncoupling of ATP synthase.

Thus, if slipping does not seem to be directly involved in the regulation of thermogenesis, these mechanisms could induce some heat production under cellular stress conditions.

b) Electron leak

A decreased coupling efficiency can also occur through electron leaks, when electrons “escape” the ETC pathway prior to the oxygen reduction by the cytochrome c oxidase, to generate superoxide ($O_2^{\bullet -}$) **(Chouchani, Kazak, et Spiegelman 2018; Jastroch et al. 2010)**. Within ETC, the sites of superoxide production are mainly associated to complex I (CI) and secondly to complex III (CIII). Mitochondrial enzymatic complexes such as the dihydrolipoamide dehydrogenase, the flavoenzymes α-glycerophosphate dehydrogenase, or the electron-transferring flavoprotein:Q oxidoreductase (ETFQOR) of fatty acid β-oxidation were also reported to produce ROS. **(St-Pierre et al. 2002; Tretter et Adam-Vizi 2007)**. Briefly, electron leaks in CI can occur at high NADH/NAD^+ ratio eliciting an increased level of reduced FMN cofactor and $O_2^{\bullet -}$ production, or during reverse electron transport **(Jastroch et al. 2010).** Electron leaks in CIII can occur when Q_i site inhibitors prevent electron removal from the complex during the Q-cycle, leading to $QH^{\bullet}$ formation in Q_0 site and $O_2^{\bullet -}$ production **(Jastroch et al. 2010).**

The in vitro superoxide production was estimated at 0.12-2% of O_2 consumption in isolated mitochondria **(Kudin et al. 2004).** However, experimental conditions may lead to overestimated values, leading to lower *in vivo* superoxide production **(Michael P. Murphy 2009).** Therefore, it is unlikely that this process significantly contributes to substantial heat production. Nevertheless, electron and H^+ leaks are intricately related, as ROS production is highly sensitive to H^+ leak-related Δp decrease **(Michael P. Murphy 2009),** while superoxide production can indirectly impact mitochondrial heat production by regulating H^+ leak (cf. proton leak regulation) (Figure 3). Moreover, mitochondrial superoxide production is increased by heat stress **(Belhadj Slimen et al. 2014)**.

c) Substrate utilization

In addition to the H+ leak amplitude, the OXPHOS efficiency also differs according to the catabolic routes feeding the ETC. Besides complex I which catalyzed NADH oxidation, several ETC oxidoreductases are unable to pump protons, particularly the complex II, the glycerol phosphate dehydrogenase and the ETFQOR linked to fatty acid oxidation **(Adeva-Andany et al. 2019)**. Depending on whether the ETC is fed on NADH or a FAD prosthetic group, the H^+/O_2 stoichiometry will differ :$10H^+/2e^-$ or $6H^+/2e^-$, respectively **(M. D. Brand 2005)**, ultimately modifying the ATP/O stoichiometry and thus, mitochondrial heat production. One main example could be the two NADH shuttles.

Indeed, 2 NADH per glucose molecule are formed in the cytosol during glycolysis which cannot cross the IMM, but have to be transported by two major shuttles into the mitochondrial matrix: (i) the malate-aspartate shuttle, which exchanges cytosolic NADH for mitochondrial NADH to supply the CI **(Borst 2020),** and (ii) the glycerol-3-phosphate (G3P) shuttle, which catalyzes an apparent exchange of cytosolic NADH for mitochondrial FADH through the cross talk between the NADH-dependent cytosolic G3P dehydrogenase (G3PDH) and the FADH-dependent mitochondrial G3PDH (mG3PDH). mG3PDH, located on the outer surface of the inner mitochondrial membrane directly transfers electrons to CIII via the ubiquinone pool **(Mráček, Drahota, et Houštěk 2013)**. This last shuttle is less efficient, since only 2 ATPs are generated per oxygen reduced, the remaining energy being dissipated as heat **(Dawson et Cooney**

1978). Thus, the use of the G3P shuttle may promote heat production (Figure 3). The relative activity of these two shuttles is tissue dependent, G3P shuttle being highly active in muscle, BAT and brain **(Mráček, Drahota, et Houštěk 2013)**. In this respect, using calorimetry and high resolution respirometry, **Masson et al. 2017** demonstrated that mGPDH substrate oxidation increases heat production compared to NADH substrate oxidation in permeabilized flight muscles of bumblebees. The authors hypothesized that these insects use the mitochondrial G3P pathway to facilitate heat production in flight muscles **(Masson et al. 2017).** Transgenic mice lacking the mG3PDH showed a slight reduction in obligatory thermogenesis, compensated by increased BAT facultative thermogenesis **(DosSantos et al. 2003)**.

Iii. Increasing Mitochondrial Heat Production By Increasing Respiration Rates

As mentioned earlier, the OXPHOS efficiency, which mainly depends on the H^+ leak, is a major element in the regulation of mitochondrial heat production. However, energy can also be dissipated through mechanisms that do not involve mitochondrial uncoupling but stimulate cellular ATP hydrolysis. Increasing H^+ flux through the ATP synthase to sustain ATP synthesis thereby stimulates both the substrate oxidation rate and inherent energy loss as heat (Figure 4).

a) Futile cycling

Mechanisms that increase ATP turnover include ATP-consuming futile cycles, such as the creatine/phosphocreatine (Cr/PCr) cycle and the calcium import/export cycle, which can be regulated by adjusting these cycle rates to cellular ATP requirements. Their dependence on ATP production underlines that mitochondrial oxidations coupled to ATP synthesis, not just uncoupling, may play a role in heat production (Figure 4).

Futile Cr/PCr cycle

Creatine Kinase (CK) catalyzes the reversible reaction: $PCr2- + MgADP- + H^+$ « $MgATP2- + Cr$ and can either utilize PCr to regenerate ATP or synthesize PCr to generate ADP. The CK/PCr system displays tissue- and cell-specific CK isoforms with defined subcellular locations, connecting sites of ATP utilization (ATPases) with sites of ATP production, i.e. mitochondria, through PCr/Cr shuttling (Figure 4). The mitochondrial mtCK is located in the intermembrane space and is tightly coupled to ATP-synthesis and respiratory chain activity via the ANT, consuming ATP and releasing PCr and ADP. Thus mtCK maintains a high local ADP/ATP ratio and high phosphorylating rates in mitochondria (For review, see **Wallimann, Tokarska-Schlattner, et Schlattner 2011)**. This process is crucial in cells with high energy and/or fluctuating requirements such as striated muscles, brain and neuronal cells **(T Wallimann et al. 1992; Theo Wallimann et al. 2007).** However, this energy transfer mechanism has recently been involved in the stimulation of heat production through substrate oxidation and futile cycle of creatine dephosphorylation in thermogenic fat cells **(Chouchani, Kazak, et Spiegelman 2018).** This futile cycle, associated with low creatine and ADP concentrations, drives ATP hydrolysis, resulting in the stimulation of mitochondrial

respiration **(Kazak et al. 2015; Bertholet et al. 2017; Chouchani, Kazak, et Spiegelman 2018)**. In addition, low creatine level itself is linked to thermogenesis deregulation **(Yamashita et al. 1995; Wakatsuki et al. 1996)**. This process initially described in mitochondria isolated from beige fat of cold-exposed animals, and later in all adipose tissues, is considered as a key effector of non-shivering thermogenesis **(Chouchani, Kazak, et Spiegelman 2018)**. In this respect, the deletion of the glycine aminotransferase prevents creatine biosynthesis, (Adipo-Gatm KO mice) and results in cold intolerance, independently of UCP1 protein abundance **(Kazak et al. 2017)**. This creatine phosphorylation-dependent heat production process seems to be regulated by the tissue nonspecific alkaline phosphatase (TNAP) which hydrolyses phosphocreatine to initiate a futile cycle of creatine dephosphorylation and phosphorylation in mitochondria **(Sun et al. 2021).** Today, even if several *in vivo* studies demonstrated the implication of the creatine futile cycle in thermogenesis, further studies are required to understand its molecular process and regulations.

Futile calcium cycling

Ca^{2+} plays a central role in cell signaling and is involved in the regulation of multiple cellular functions related to the metabolism **(Glancy et Balaban 2012; Rizzuto et al. 2012).** Ca^{2+} homeostasis is driven by the ER and mitochondria. The latter one is a “sink” accumulating large amounts of Ca^{2+} in its matrix, by direct ER-mitochondria Ca^{2+} transfer **(Filadi et al. 2018)** through the Mitochondrial Calcium Uniporter (MCU) **(Rossi, Pizzo, et Filadi 2019).** An increase in the matrix Ca^{2+} concentration results in activation of three rate-limiting enzymes dehydrogenases in feeding electrons at complex I (CI): pyruvate (PDH), α-ketoglutarate (αKGDH), and isocitrate (ICDH) dehydrogenases **(McCormack, Halestrap, et Denton 1990)**. PDH activation occurs through the dephosphorylation of the catalytic subunit by a Ca^{2+}-dependent phosphatase **(R M Denton, Randle, et Martin 1972)**, which leads to an increase of pyruvate oxidation generating NADH for CI and acetyl-CoA for the TCA cycle. αKGDH and ICDH dehydrogenases are directly activated by Ca^{2+} binding, thereby enhancing TCA cycle flux **(Rutter et Denton 1988; Richard M. Denton 2009).** Finally, the two redox shuttles are activated by Ca^{2+}, the G3P (see section II.B.c) **(Hansford et Zorov 1998)** and by two mitochondrial metabolite transporters, aralar1 and citrin, which are isoforms of the malate-aspartate shuttle **(Contreras et al. 2007)**. Thereby, Ca^{2+} also stimulates the mitochondrial NADH re-oxidation generated by glycolysis. Whether the activity of complex V (ATP synthase) rises directly in response to elevated mCa^{2+} is a matter of debate **(Wescott et al. 2019).** Thus, Ca^{2+} plays an integrative role, increasing reduced equivalent (NADH and FADH2) availability and enhancing the electron flow through the ETC, which supports mitochondrial energy metabolism in parallel to the activation of ATP consuming processes in the cytosol **(Jouaville et al. 1999; Balaban 2009)** with downstream consequences on heat production. Ca^{2+} mainly couples activation of oxidative metabolism with increased ATP use for cellular works, such as muscle contraction. Alternatively, another important process of non-shivering thermogenesis (NST) was recently discovered in skeletal muscle and implies futile ATP hydrolysis **(Blondin et Haman 2018).** This mechanism is based on futile Ca^{2+} cycling and ATP hydrolysis in the sarcoplasmic-reticulum (SR) or endoplasmic-reticulum (ER), and involves the

transmembrane Ca^{2+}-ATPase (SERCA) and the sarcolipin (Sln), a small peptide controlling SERCA-mediated ATP turnover in muscle **(Gamu et al. 2014).** In physiological conditions, SERCA couples ATP-hydrolysis to Ca^{2+} sequestration in the SR/ER **(L. de Meis 2002).** However, the direct binding of Sln to SERCA decreases the coupling efficiency between ATP hydrolysis and Ca^{2+} pumping back, thereby promoting both ATP hydrolysis and Ca^{2+} accumulation in the cytosol **(Smith et al. 2002; Chouchani, Kazak, et Spiegelman 2018).** This leads to heat production by the SERCA ATPase activity and the activation of the Ca^{2+}-dependent pathways regulating muscle metabolism and mitochondrial activity (see below) **(Sahoo et al. 2013)**. In this respect, overexpression of Sln in mice fed with high fat diet increases the metabolic rate: their muscle show enhanced oxidative capacity along with a rise of mitochondrial biogenesis and fatty acid transport protein expression **(Pant, Bal, et Periasamy 2016)**. Clarke et al. (2012), further hypothesized that changes in calcium cycling and mitochondrial function may be involved in post-prandial heat production **(Clarke et al. 2012)**. ATP-consuming Ca^{2+} futile cycling was also shown to contribute to beige fat energy expenditure and systemic glucose homeostasis in response to cold exposure through activation of the ryanodine receptor 2 (RyR2), promoting the extrusion of ER-calcium. In addition of the futile ATP consumption responsible for energy dissipation, lower efficiency of SERCA2b leads to higher Ca^{2+} import into mitochondria, enhanced tricarboxylic acid and pyruvate dehydrogenase activity, and finally ATP synthesis for ATP-dependent thermogenesis **(Ikeda et al. 2017)**. Interestingly, in BAT, SERCA 1 was localized at the fusion sites between the ER and mitochondrial outer membranes, and where it induces SERCA/RyR mediated Ca^{2+} futile cycling. This Ca^{2+} increases stimulates the rate of respiration and heat production both in coupled and uncoupled mitochondria, which can be inhibited by rotenone, KCN, and CI and CIV inhibitors, demonstrating the key role of ETC activation by Ca^{2+} in this thermogenesis **(Leopoldo de Meis et al. 2010)**.

Consequently, dysregulations of mitochondrial Ca^{2+} signaling are featuring pathologies, such as neuronal, cardiac and muscle disorders, as well as diseases related to excessive body temperature. In this respect pathologic variants affecting Ca^{2+} channels, are causing malignant hyperthermia (MHT) and exertional heat stroke (EHS) **(Rosenberg et al. 2015; Rossi, Pizzo, et Filadi 2019)** (Figure 4, and box 3).

****Box 3****

b) Metabolic sensor

When ATP is used to drive cellular mechanical or metabolic pathways, the consecutive decrease in the mitochondrial ATP/ADP ratio rises ATP synthesis rate by F0F1-ATP synthase, which elevates mitochondrial oxidative metabolism and inherent heat production. Thus, mitochondrial thermogenesis is likely to vary depending on chronic or acute ATP global needs. For example, in muscles exposed to challenging efforts requiring tremendous high energetic input, myocytes mitochondria will exhibit higher OXPHOS rates than in resting conditions **(McLaughlin et al. 2020),** and consequently will produce more heat **(Yamada 2017).**

The equilibrium between ATP production and consumption represents a major cellular challenge, in order to maintain a physiological energy balance, whatever the internal or external solicitations. ATP hydrolysis results in ADP and inorganic phosphate, ADP being recycled by the adenylate kinase to ATP + AMP, according to the reaction 2ADP ↔ ATP + AMP. Thus, following ATP consumption, both ADP/ATP and AMP/ATP ratios increase and are sensed by the AMP-activated protein kinase (AMPK). Once activated, the AMPK activates enzymes involved in catabolism, glucose uptake and mitochondrial biogenesis **(Hardie, Ross, et Hawley 2012)**. In this respect, AMPK activation stimulates metabolism favoring heat production, as suggested by its crucial involvement in BAT thermogenesis (Q. Wang et al. 2021)**.**

In parallel, sirtuin 3 (SIRT3), which is a mitochondrial NAD^+-dependent deacetylase, acts as a cellular energetic sensor of the NADH/NAD^+ ratio **(Nogueiras et al. 2012).** During the OXPHOS process, $FADH_2$ and NADH are oxidized in FAD and NAD^+, which can lead to SIRT3 activation. Once activated, SIRT3 regulates many mitochondrial protein activity involved in metabolism, through post translational modifications **(Nogueiras et al. 2012)**. For example, SIRT3 deacetylates and activates TCA cycle enzymes, the glutamate dehydrogenase (GDH), SDHA subunit of the CII and NDUFA9 from the CI **(Schlicker et al. 2008; Cimen et al. 2010; Ahn et al. 2008; Shi et al. 2005)**. Furthermore, it has been suggested that SIRT3 mediated deacetylation in mitochondria is essential for UCP1-dependant BAT thermogenesis though the regulation of many substrate uptake and oxidation upstream of UCP1 **(Sebaa et al. 2019)**. However, the links between SIRT3 and heat production in other tissues are not yet clarified.

V. Questions Raised By Considering Mitochondria As Cell Radiators

Recent data disclosed that mitochondria could form "hot spots", namely micro-domains where the temperature greatly exceeds the body temperature set point of 37°C. Thanks to a variety of fluorochromes such as inorganic dyes, synthetic polymers or genetically–encoded fluorescent proteins, different studies have reported a temperature gradient between mitochondria and the cytoplasm (reviewed by **(Macherel et al. 2021).** In HeLa cells, mitochondrial uncoupling with FCCP protonophore (carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone), leads to an increase of 6-9°C of the mitochondrial temperature **(Nakano et al. 2017)**, while oleic acid as an alternative nutrient source or ionomycin, a calcium ionophore, increase temperature by ~2°C **(Di et al. 2021).**

Moreover, in HEK293 and human fibroblast cell lines grown in standard conditions, mitochondrial temperature surprisingly reaches a maximal temperature of 50°C, without any exogenous stimulation to promote uncoupling **(Chrétien et al. 2018).** Although the precise measurement of mitochondrial temperature differs depending on the nature of the method and the experimental conditions used, the fact that mitochondria is the most important source of heat production, acting as a cellular radiator, raises many conceptual questions.

First, a high intra-mitochondrial temperature challenges the activity and stability of proteins and DNA, together with the IMM fluidity **(Slimen et al. 2016)**. In this respect, the presence of mitochondrial Heat Shock Protein 70 (HSP70), the high GC composition of the mitochondrial genome and the abundance of

cardiolipins in the IMM might represent evolutionary adaptive mechanisms, coping with high local temperatures **(Nasr et al. 2019).**

Second, a mitochondrial temperature of 45-50°C must generate an important intracellular temperature gradient, which is predicted to be physically unsustainable across cells, according to theoretical rates of heat transfer through aqueous media **(Baffou et al. 2014; 2015).** However, the previous theoretical models assumed that mitochondria are spherical organites producing heat at their surface, a concept which contrasts with the existence of the outer and inner mitochondrial membranes, the latter forming dynamic cristae appearing as radiator lamellae, whose structure evolves according to the energetic state **(Lane 2018; Joubert et Puff 2021).** In addition, the IMM is an impermeable lipid bilayer composed of densely packed proteins and a peculiar phospholipid composition, including cardiolipins **(Claypool et Koehler 2012)**, which might define a heat retention compartment **(Lane 2018).** Since the emergence of the concept of hot mitochondria, several studies have strengthened this concept with new experimental approaches to assess their temperature. For example, using a nanohybrid heater-thermometer combining fluorescent nanodiamond and polydopamine, variations in thermal conductivity were disclosed within a single cell **(Sotoma et al. 2021)**, allowing the characterization of transient temperature spikes. A further reconciliation between theory and practice was suggested by the observation of picosecond temperature-difference spikes, compatible with the 50°C temperature of mitochondria. Indeed, when these picosecond spikes were averaged over time, a 10°C difference of temperature was observed between mitochondria and cytoplasm, consistent with the maintenance of a chronic steady-state temperature gradient, rather than acute picosecond heat spikes **(Fahimi et Matta 2021).** Moreover, the concept of transient heat release was also observed experimentally, as a transient heat shock of ≈7.5°C was observed during proton uncoupling using the chemical uncoupler BAM15 **(Rajagopal et al. 2019).** Nevertheless, more convincing physical explanations remain to be identified to support the concept of “hot” mitochondria” **(Macherel et al. 2021).**

Finally, the optimal range of temperature for the CIV and possibly the ATPase activities could be 50°C, suggesting that the optimal condition for the respiratory chain is close to the high local temperature, and parallels the thermogenic function of mitochondria **(Chrétien et al. 2018)**. However, this result is surprising and does not agree with previous observations. For example, incubating the CIV of beef heart at 43-45°C lead to a decrease the H^+/e^- stoichiometry close to 0 **(Sone et Nicholls 1984).** The positive influence of high temperature on respiratory chain complexes and ATP synthase remains to be determined but if this result was confirmed, links between heat and energy productions might represent a virtuous cycle: ATP production promotes heat production, and heat production could in turn favor ATP production. Moreover, this concept also reflects our questioning about the origin of mitochondrial endosymbiosis. Indeed, phylogenetic elements suggest that mitochondria ancestors were thermophile α-probacteria **(Nasr et al. 2019).** Otherwise, it was suggested that proto-mitochondria endosymbiont permitted an internal heat production into the archaeal ancestor host, who lived in high temperature environment, allowing it to colonize cooler biotopes **(Dunn 2017)**. Thus, the understanding of mechanisms of mitochondrial temperature regulations is a research field to explore and some answer

track begin to emerge. Indeed, mitochondrial heat production was recently associated with Δ9-fatty acid desaturase DESAT1 activity which introduces a double bond at the Δ9 position of the acyl moiety of acyl-CoA, leading to the enhancement of F1Fo-ATPase-dependent mitochondrial respiration and consistent heat production **(Murakami et al. 2022).**

Concluding Remarks And Future Perspectives

Recent advances highlighted the large thermal heterogeneity at subcellular scales, exemplified by a possible physiological temperature of 45-50°C into mitochondria, promoting mitochondria as the cell radiators. Understanding how these radiators regulate and is regulated by heat production is intimately related to the generation and dissipation of the H^+ gradient through the IMM and its connection or not to the ATP synthesis and consumption. At the crossroads between physics and cell biology, emerging studies are now required to determine thermogenesis levels in the different cellular compartments, and heat flows throughout the cell. Ultimately, mechanisms regulating mitochondrial thermogenesis could result in identifying novel heat-related messengers regulating crucial cellular processes.

Outstanding Questions Box

- Although robust experiments led to the conclusion that mitochondria temperature reaches 50°C *in vitro* in HEK293 and human fibroblast cell lines, there is a crucial need to confirm this observation in alternative cell models, and *in vivo*.
- In this respect, generating novel thermosensitive probes highly sensitive to faint temperature changes located in the different cellular components are mandatory to better explore dynamic changes and distribution of cellular temperature, and heat flows.
- If the different mechanisms involved in cellular thermogenesis are now well identified, future important challenges will consist in identifying local thermostats or thermal sensors, and how they control the ON-OFF switch to modulate or not mitochondrial heat production.
- Are there any physiological pathways that are controlled by temperature gradients and heat flows within or in-between different cellular compartments?
- How enzymatic activities from mitochondrial matrix proteins can cope with a local temperature of 50°C remains an open avenue?
- One might questions the existence of all mechanisms described in this review, in endotherm or ectotherm animal species, or in other eukaryote phyla, and in mammals that escaped endothermy, like the naked mole-rat (*Heterocephalus glaber*) from Ethiopia.
- In this respect, there is an important challenge to understand heat over-production in live-threatening pathological conditions as malignant hyperthermia, exertional heat strokes, as well as local heat production in processes like inflammation and tumorigenesis.

Glossary

- **Adenine nucleotide translocase (ANT):** mitochondrial ADP/ATP carrier that exchanges ATP with ADP across the inner mitochondrial membrane.
- **Adenosine triphosphate (ATP) / Adenosine diphosphate (ADP):** key molecules in the management of cellular energy. The hydrolysis of ATP to ADP provides energy to drive most chemical reactions involved in all cellular processes.
- **Brown adipose tissue (BAT):** adipose tissue subtype which main function is to ensure thermogenesis, through lipolysis of adipocytes, in mammals.
- **Calcium (Ca^{2+}):** ions that participate in many signaling pathways, as a second messenger regulating biological functions such as muscle contraction, nerve conduction and metabolism.
- **Exertional Heat Stroke (EHS):** severe pathological life threatening reaction characterized by a drastic increase in body temperature during a physical exertion, high external temperatures or both.
- **Endoplasmic reticulum (ER)/Sarcoplasmic reticulum (SR):** organelles involved in protein synthesis and folding, and lipid synthesis, which also constitute the main intracellular Ca^{2+} store, essential for muscular cell contraction.
- **Electron-transferring flavoprotein (ETF):** flavoprotein that function as an electron acceptor for dehydrogenases.
- **Heat shock protein (HSP):** family of proteins involved in cellular stress response such as un-physiological heat, cold, UV light or tissue damages.
- **Inositol triphosphate receptor 3 (IP_3R):** membrane glycoprotein complex localized in ER/SR that acts as a Ca^{2+} channel activated by inositol trisphosphate (IP_3).
- **Malignant Hyperthermia (MHT):** severe reaction in response to volatile anesthetic agents whose symptoms result in pathological muscle rigidity, fever and heart rate.
- **Mitochondrial calcium uniporter (MCU):** mitochondrial transmembrane protein described as the main actor in mitochondrial calcium uptake.
- **Nicotinamide adenine dinucleotide ($NADH/NAD^+$):** reduced and oxidized form of a coenzyme involved in redox reactions that carry electrons from one reaction to another.
- **Non-shivering thermogenesis (NST):** Process related to an increase in metabolic heat production that is not associated with muscle activity.
- **Oxidative phosphorylation (OXPHOS):** an aerobic metabolic pathway where NADH and $FADH_2$ are oxidized by a series of protein complexes within the mitochondria to produce ATP.
- **Proton gradient (Δp):** gradient associated to the higher H^+ concentration in the intra-membrane space than in the matrix, which results from the respiratory chain activity and serving as the driving force for ATP synthesis by the mitochondrial ATP synthase.
- **Reactive oxygen species (ROS):** reactive chemicals formed from O_2, such as $O_2^{\bullet -}$, that play a role in cell signaling and homeostasis, in addition to causing cellular damages by altering DNA, proteins or lipids, when over-produced.

- **Sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA):** a Ca^{2+} ATPase that transports Ca^{2+} from the cytosol into the ER/SR lumen by hydrolyzing ATP.
- **Sarcolipin (Sln):** small peptide regulating SERCA activity.
- **Sirtuin 3 (SIRT3):** a NAD-dependent deacetylase localized in mitochondria regulating many metabolic functions.
- **Tricarboxylic acid cycle (TCA cycle):** a series of chemical reactions occurring in the mitochondrial matrix where the oxidation of Acetyl-CoA, derived from carbohydrates, lipids, and proteins, provides reduced cofactors to feed the OXPHOS.
- **Uncoupling proteins (UCPs):** mitochondrial inner membrane proteins acting as transporters to dissipate H^+ gradient and generate heat, leading to mitochondrial stimulation.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: All authors approved the final version of the manuscript.

Availability of data and material: Not applicable.

Competing interests: The authors declare that there are no relevant financial or non-financial competing interests to report.

Funding: This study was funded by ANR (ANR-ASTRID TEMPO-MITO Project N° ANR-21-ASTR-0010) and DGA/MinDef. FB is supported by a PhD. fellowship from DGA/Région Pays de la Loire.

Authors' contributions: All authors were involved in the redaction and edition of the manuscript.

Acknowledgements: We wish to warmly thank the french Délégation Générale pour l'Armement and the Région Pays de la Loire for supporting this project.

References

1. Abeele F, Vanden S, Lotteau S, Ducreux C, Dubois N, Monnier A, Hanna D, Gkika et al (2018) « TRPV1 Variants Impair Intracellular Ca2 + Signaling and May Confer Susceptibility to Malignant Hyperthermia ». *Genetics in Medicine*, juin. https://doi.org/10.1038/s41436-018-0066-9
2. Adeva-Andany MM, Carneiro-Freire N Mónica Seco-Filgueira, Carlos Fernández-Fernández, et David Mouriño-Bayolo. 2019. « Mitochondrial β-Oxidation of Saturated Fatty Acids in Humans ».*Mitochondrion*46 (mai):73–90. https://doi.org/10.1016/j.mito.2018.02.009
3. Ahn B-H, Kim H-S, Song S, Lee IH, Liu J, Vassilopoulos A, Deng C-X, et Toren Finkel (2008). « A Role for the Mitochondrial Deacetylase Sirt3 in Regulating Energy Homeostasis ». *Proceedings of the National Academy of Sciences* 105 (38): 14447–52. https://doi.org/10.1073/pnas.0803790105

4. Andreyev AYu TO, Bondareva VI, Dedukhova EN, Mokhova VP, Skulachev LM, Tsofina NI, Volkov TV, Vygodina (1989) « The ATP/ADP-Antiporter Is Involved in the Uncoupling Effect of Fatty Acids on Mitochondria ». *European Journal of Biochemistry* 182 (3): 585–92. https://doi.org/10.1111/j.1432-1033.1989.tb14867.x
5. Babcock GT, Wikström etM (1992) « Oxygen Activation and the Conservation of Energy in Cell Respiration ». Nature 356(6367):301–309. https://doi.org/10.1038/356301a0
6. Baffou G, Rigneault H, Marguet D, Ludovic Jullien (2014) « A critique of methods for temperature imaging in single cells ». Nat Methods 11(9):899
7. ———. « Reply to:" Validating subcellular thermal changes revealed by fluorescent thermosensors" and" The 10 5 gap issue between calculation and measurement in single-cell thermometry" ». *Nature methods* 12 (9): 803
8. Balaban RS (2009) « The Role of Ca(2+) Signaling in the Coordination of Mitochondrial ATP Production with Cardiac Work ». Biochim Biophys Acta 1787(11):1334–1341. https://doi.org/10.1016/j.bbabio.2009.05.011
9. Beam TA, Emily F, Loudermilk DF, Kisor (2017) « Pharmacogenetics and Pathophysiology of CACNA1S Mutations in Malignant Hyperthermia ». Physiol Genom 49(2):81–87. https://doi.org/10.1152/physiolgenomics.00126.2016
10. Belhadj Slimen, Imen T, Najar A, Ghram H, Dabbebi MB, Mrad, Manef Abdrabbah (2014) « Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review ». Int J Hyperth 30(7):513–523. https://doi.org/10.3109/02656736.2014.971446
11. Bertholet AM, Edward T, Chouchani L, Kazak A, Angelin A, Fedorenko JZ, Long S et al (2019) « H + Transport Is an Integral Function of the Mitochondrial ADP/ATP Carrier ». Nature 571(7766):515–520. https://doi.org/10.1038/s41586-019-1400-3
12. Bertholet AM, Kazak L, Chouchani ET, Bogaczyńska MG, Paranjpe I, Wainwright GL, Bétourné A, Kajimura S, Spiegelman BM, Yuriy Kirichok (2017) « Mitochondrial patch clamp of beige adipocytes reveals UCP1-positive and UCP1-negative cells both exhibiting futile creatine cycling ». Cell Metabol 25(4):811–822
13. Bertholet AM, Yuriy Kirichok (2021) « Mitochondrial H + Leak and Thermogenesis ». *Annual Review of Physiology*, novembre. https://doi.org/10.1146/annurev-physiol-021119-034405
14. Blondin DP, et François H (2018) « Shivering and Nonshivering Thermogenesis in Skeletal Muscles ». Handb Clin Neurol 156:153–173. https://doi.org/10.1016/B978-0-444-63912-7.00010-2
15. Bobyleva V, Bellei M, Pazienza TL, Umberto Muscatello (1997) « Effect of Cardiolipin on Functional Properties of Isolated Rat Liver Mitochondria ». IUBMB Life 41(3):469–480. https://doi.org/10.1080/15216549700201491
16. Borst P (2020) « The Malate-Aspartate Shuttle (Borst Cycle): How It Started and Developed into a Major Metabolic Pathway ». IUBMB Life 72(11):2241–2259. https://doi.org/10.1002/iub.2367
17. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Jean-Paul G (1997) « Uncoupling Protein-3: A New Member of the Mitochondrial Carrier Family with Tissue-Specific

Expression ». FEBS Lett 408(1):39–42. https://doi.org/10.1016/S0014-5793(97)00384-0

18. Bosson C, Rendu J, Pelletier L, Abriat A, Chatagnon A, Brocard J, Brocard J et al (2020) « Variations in the TRPV1 Gene Are Associated to Exertional Heat Stroke ». *Journal of Science and Medicine in Sport*, mai. https://doi.org/10.1016/j.jsams.2020.04.018
19. Brand MD (2005) « The Efficiency and Plasticity of Mitochondrial Energy Transduction ». Biochem Soc Trans 33(5):897–904. https://doi.org/10.1042/BST0330897
20. Brand MD, Chien LF, Ainscow EK, Rolfe DF, Porter RK (1994) « The Causes and Functions of Mitochondrial Proton Leak ». Biochim Biophys Acta 1187(2):132–139. https://doi.org/10.1016/0005-2728(94)90099-x
21. Brand MD, Chien LF, Diolez etP (1994) « Experimental Discrimination between Proton Leak and Redox Slip during Mitochondrial Electron Transport ». Biochem J 297(janvier):27–29. https://doi.org/10.1042/bj2970027
22. Brand MD, Harper ME, Taylor HC (1993) « Control of the effective P/O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes ». Biochem J 291(3):739–748. https://doi.org/10.1042/bj2910739
23. Brand MD, Julian L, Pakay A, Ocloo J, Kokoszka DC, Wallace PS, Brookes J, Cornwall (2005) « The Basal Proton Conductance of Mitochondria Depends on Adenine Nucleotide Translocase Content ». Biochem J 392(2):353–362. https://doi.org/10.1042/BJ20050890
24. Brand MD, Nigel Turner A, Ocloo PL, Else AJ, Hulbert (2003) « Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds. » Biochem J 376(Pt 3):741–748. https://doi.org/10.1042/BJ20030984
25. Brustovetsky N, Klingenberg etM (1994) « The Reconstituted ADP/ATP Carrier Can Mediate H + Transport by Free Fatty Acids, Which Is Further Stimulated by Mersalyl ». J Biol Chem 269(44):27329–27336
26. Buttgereit F, Brand MD, Müller etM (1992) « ConA Induced Changes in Energy Metabolism of Rat Thymocytes ». Biosci Rep 12(5):381–386. https://doi.org/10.1007/BF01121501
27. Cairns CB, Walther J, Harken AH, Banerjee A (1998) « Mitochondrial oxidative phosphorylation thermodynamic efficiencies reflect physiological organ roles ». Am J Physiology-Regulatory Integr Comp Physiol 274(5):R1376–R1383. https://doi.org/10.1152/ajpregu.1998.274.5.R1376
28. Chouchani ET, Kazak L, Jedrychowski MP, Gina Z, Lu BK, Erickson J, Szpyt KA, Pierce et al (2016) « Mitochondrial ROS Regulate Thermogenic Energy Expenditure and Sulfenylation of UCP1 ». Nature 532(7597):112–116. https://doi.org/10.1038/nature17399
29. Chouchani ET, Kazak L, Bruce M, Spiegelman (2017) « Mitochondrial Reactive Oxygen Species and Adipose Tissue Thermogenesis: Bridging Physiology and Mechanisms ». J Biol Chem 292(41):16810–16816. https://doi.org/10.1074/jbc.R117.789628
30. ———. « New Advances in Adaptive Thermogenesis: UCP1 and Beyond ». *Cell Metabolism*, novembre. https://doi.org/10.1016/j.cmet.2018.11.002

31. Chrétien D, Benit P, Ha H-H, Keipert S, El-Khoury R, Chang Y-T, Jastroch M, Jacobs HT Pierre Rustin, et Malgorzata Rak. 2018. « Mitochondria are physiologically maintained at close to 50 C ».*PLoS biology*16 (1):e2003992
32. Cimen H, Han M-J, Yang Y, Tong Q, Koc H, Emine C, Koc (2010) « Regulation of Succinate Dehydrogenase Activity by SIRT3 in Mammalian Mitochondria ». Biochemistry 49(2):304–311. https://doi.org/10.1021/bi901627u
33. Clarke SD, Lee K, Andrews ZB, Bischof R, Fahri F, Evans RG, Clarke IJ, Belinda A, Henry (2012) « Postprandial heat production in skeletal muscle is associated with altered mitochondrial function and altered futile calcium cycling ». Am J Physiology-Regulatory Integr Comp Physiol 303(10):R1071–R1079. https://doi.org/10.1152/ajpregu.00036.2012
34. Claypool SM, Carla M, Koehler (2012) « The Complexity of Cardiolipin in Health and Disease ». Trends Biochem Sci 37(1):32–41. https://doi.org/10.1016/j.tibs.2011.09.003
35. Cleri F (2016) The Physics of Living Systems. Springer
36. Cohen P, et Shingo Kajimura (2021). « The Cellular and Functional Complexity of Thermogenic Fat ». *Nature Reviews. Molecular Cell Biology*, mars. https://doi.org/10.1038/s41580-021-00350-0
37. Contreras L, Gomez-Puertas P, Iijima M, Kobayashi K, Saheki T, Jorgina Satrústegui (2007) « Ca2 + Activation Kinetics of the Two Aspartate-Glutamate Mitochondrial Carriers, Aralar and Citrin: Role in the Heart Malate-Aspartate NADH Shuttle ». J Biol Chem 282(10):7098–7106. https://doi.org/10.1074/jbc.M610491200
38. Dawson AG, Gregory J, Cooney (1978) « Reconstruction of the α-Glycerolphosphate Shuttle Using Rat Kidney Mitochondria ». FEBS Lett 91(2):169–172. https://doi.org/10.1016/0014-5793(78)81164-8
39. Denton RM, Randle PJ, Martin BR (1972) « Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. » Biochem J 128(1):161–163
40. Denton RM (2009) « Regulation of Mitochondrial Dehydrogenases by Calcium Ions ». Biochim Biophys Acta 1787(11):1309–1316. https://doi.org/10.1016/j.bbabio.2009.01.005
41. Di X, Wang D, Zhou J, Zhang L, Stenzel MH, Su QP, Dayong Jin (2021) « Quantitatively Monitoring In Situ Mitochondrial Thermal Dynamics by Upconversion Nanoparticles ». Nano Lett 21(4):1651–1658. https://doi.org/10.1021/acs.nanolett.0c04281
42. DosSantos RA, Alfadda A, Eto K, Kadowaki T, Enrique Silva (2003) « Evidence for a Compensated Thermogenic Defect in Transgenic Mice Lacking the Mitochondrial Glycerol-3-Phosphate Dehydrogenase Gene ». Endocrinology 144(12):5469–5479. https://doi.org/10.1210/en.2003-0687
43. Dunn CD (2017) « Some Liked It Hot: A Hypothesis Regarding Establishment of the Proto-Mitochondrial Endosymbiont During Eukaryogenesis ». J Mol Evol 85(3):99–106. https://doi.org/10.1007/s00239-017-9809-5
44. Fahimi P, Chérif F, Matta (2021) « The Hot Mitochondrion Paradox: Reconciling Theory and Experiment », 12

45. Ferguson-Miller S (1996) « Mammalian Cytochrome c Oxidase, a Molecular Monster Subdued ». Sci (New York N Y) 272(5265):1125. https://doi.org/10.1126/science.272.5265.1125
46. Filadi R, Elisa Greotti (2021) « The Yin and Yang of Mitochondrial Ca2 + Signaling in Cell Physiology and Pathology ». Cell Calcium 93:102321. https://doi.org/10.1016/j.ceca.2020.102321
47. Filadi R, Leal NS, Schreiner B, Rossi A, Dentoni G, Pinho CM, Wiehager B et al (2018) « TOM70 Sustains Cell Bioenergetics by Promoting IP3R3-Mediated ER to Mitochondria Ca2 + Transfer ». Curr Biol 28(3):369–382e6. https://doi.org/10.1016/j.cub.2017.12.047
48. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F et al (1997) « Uncoupling Protein-2: A Novel Gene Linked to Obesity and Hyperinsulinemia ». Nat Genet 15(3):269–272. https://doi.org/10.1038/ng0397-269
49. Fontaine EM, Moussa M, Devin A, Garcia J, Ghisolfi J, Rigoulet M, Leverve XM (1996) « Effect of Polyunsaturated Fatty Acids Deficiency on Oxidative Phosphorylation in Rat Liver Mitochondria ». Biochim Biophys Acta 1276(3):181–187. https://doi.org/10.1016/0005-2728(96)00075-8
50. Gamu D, Bombardier E, Smith IC, Fajardo VA et A. Russell Tupling. 2014. « Sarcolipin Provides a Novel Muscle-Based Mechanism for Adaptive Thermogenesis ».*Exercise and Sport Sciences Reviews*42 (3):136–42. https://doi.org/10.1249/JES.0000000000000016
51. Garlid KD, Orosz DE, Modrianský M, Vassanelli S, Jezek etP (1996) « On the Mechanism of Fatty Acid-Induced Proton Transport by Mitochondrial Uncoupling Protein ». J Biol Chem 271(5):2615–2620. https://doi.org/10.1074/jbc.271.5.2615
52. Glancy B, Robert S, Balaban (2012) « Role of Mitochondrial Ca2 + in the Regulation of Cellular Energetics ». Biochemistry 51(14):2959–2973. https://doi.org/10.1021/bi2018909
53. Hansford RG, Zorov etD (1998) « Role of Mitochondrial Calcium Transport in the Control of Substrate Oxidation ». Mol Cell Biochem 184(1–2):359–369
54. Hardie D, Grahame FA, Ross A, Hawley (2012) « AMPK: A Nutrient and Energy Sensor That Maintains Energy Homeostasis ». Nat Rev Mol Cell Biol 13(4):251–262. https://doi.org/10.1038/nrm3311
55. Hatefi Y (1985) « The Mitochondrial Electron Transport and Oxidative Phosphorylation System ». Annu Rev Biochem 54:1015–1069. https://doi.org/10.1146/annurev.bi.54.070185.005055
56. Hinkle PC (2005) « P/O Ratios of Mitochondrial Oxidative Phosphorylation ». Biochim et Biophys Acta (BBA) - Bioenergetics 1706(1):1–11. https://doi.org/10.1016/j.bbabio.2004.09.004
57. Hoch FL (1992) « Cardiolipins and Biomembrane Function ». Biochim Biophys Acta 1113(1):71–133. https://doi.org/10.1016/0304-4157(92)90035-9
58. Hulbert AJ, Else PL (2004) « Basal Metabolic Rate: History, Composition, Regulation, and Usefulness ». Physiological and Biochemical Zoology: PBZ 77(6):869–876. https://doi.org/10.1086/422768
59. Ikeda K, Kang Q, Yoneshiro T, Camporez JP, Maki H, Homma M, Shinoda K et al (2017) « UCP1-independent signaling involving SERCA2b-mediated calcium cycling regulates beige fat thermogenesis and systemic glucose homeostasis ». Nat Med 23(12):1454–1465. https://doi.org/10.1038/nm.4429

60. Jastroch M, Divakaruni AS, Mookerjee S, Treberg JR, Brand MD (2010) « Mitochondrial Proton and Electron Leaks ». Essays Biochem 47:53–67. https://doi.org/10.1042/bse0470053
61. Jekabsons MB, Nicholls DG (2004) « In Situ Respiration and Bioenergetic Status of Mitochondria in Primary Cerebellar Granule Neuronal Cultures Exposed Continuously to Glutamate ». J Biol Chem 279(31):32989–32980. https://doi.org/10.1074/jbc.M401540200
62. Jezek P, Zácková M, Růzicka M, Skobisová E, Jabůrek etM (2004) « Mitochondrial Uncoupling Proteins–Facts and Fantasies ». Physiol Res 53(Suppl 1):S199–211
63. Ježek P, Holendová B, Garlid KD, Jabůrek (2018) « Mitochondrial Uncoupling Proteins: Subtle Regulators of Cellular Redox Signaling ». *Antioxidants* & *Redox Signaling* 29 (7): 667–714. https://doi.org/10.1089/ars.2017.7225
64. Johannsen DL, Jose E, Galgani NM, Johannsen Z, Zhang JD, Covington, Eric Ravussin (2012) « Effect of Short-Term Thyroxine Administration on Energy Metabolism and Mitochondrial Efficiency in Humans ». PLoS ONE 7(7):e40837. https://doi.org/10.1371/journal.pone.0040837
65. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rosario Rizzuto (1999) « Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming ». *Proceedings of the National Academy of Sciences* 96 (24): 13807–13812
66. Joubert F, Nicolas Puff (2021) « Mitochondrial Cristae Architecture and Functions: Lessons from Minimal Model Systems ». Membranes 11(7):465. https://doi.org/10.3390/membranes11070465
67. Kadenbach B (2003) « Intrinsic and extrinsic uncoupling of oxidative phosphorylation ». Biochim et Biophys Acta (BBA)-Bioenergetics 1604(2):77–94
68. Kazak L, Chouchani ET, Gina Z, Lu MP, Jedrychowski, Curtis J, Bare Al, Mina M et al (2017) « Genetic Depletion of Adipocyte Creatine Metabolism Inhibits Diet-Induced Thermogenesis and Drives Obesity ». Cell Metabol 26(4):660–671e3. https://doi.org/10.1016/j.cmet.2017.08.009
69. Kazak L, Chouchani ET, Jedrychowski MP, Brian K, Erickson K, Shinoda P, Cohen R, Vetrivelan et al (2015) « A Creatine-Driven Substrate Cycle Enhances Energy Expenditure and Thermogenesis in Beige Fat ». Cell 163(3):643–655. https://doi.org/10.1016/j.cell.2015.09.035
70. Kudin AP, Bimpong-Buta NYaw-B, Vielhaber S, Elger CE, Wolfram S, Kunz (2004) « Characterization of Superoxide-Producing Sites in Isolated Brain Mitochondria * ». J Biol Chem 279(6):4127–4135. https://doi.org/10.1074/jbc.M310341200
71. Kushnir A, Andrew BW, Marks R (2018) « Ryanodine Receptor Dysfunction in Human Disorders ». Biochim Et Biophys Acta Mol Cell Res 1865:1687–1697 (11 Pt B). https://doi.org/10.1016/j.bbamcr.2018.07.011
72. Laitano O, Murray KO, Lisa R, Leon (2020) « Overlapping Mechanisms of Exertional Heat Stroke and Malignant Hyperthermia: Evidence vs. Conjecture ». Sports Med 50(9):1581–1592. https://doi.org/10.1007/s40279-020-01318-4
73. Lane N (2018) « Hot Mitochondria? ». PLoS Biol 16(1):e2005113. https://doi.org/10.1371/journal.pbio.2005113

74. Legendre LJ, Donald Davesne (2020) « The evolution of mechanisms involved in vertebrate endothermy ». Philosophical Trans Royal Soc B: Biol Sci 375(1793):20190136. https://doi.org/10.1098/rstb.2019.0136
75. Lettieri-Barbato D (2019) « Redox Control of Non-Shivering Thermogenesis ». *Molecular Metabolism* 25 (juillet): 11–19. https://doi.org/10.1016/j.molmet.2019.04.002
76. Lin CS, Klingenberg etM (1980) « Isolation of the Uncoupling Protein from Brown Adipose Tissue Mitochondria ». FEBS Lett 113(2):299–303. https://doi.org/10.1016/0014-5793(80)80613-2
77. Lippe G, Dabbeni Sala F, Sorgato MC (1988) « ATP Synthase Complex from Beef Heart Mitochondria. Role of the Thiol Group of the 25-KDa Subunit of Fo in the Coupling Mechanism between Fo and F1. ». J Biol Chem 263(35):18627–18634. https://doi.org/10.1016/S0021-9258(18)37331-9
78. Lippe G, Coluccino G, Zancani M, Baratta W, Paola Crusiz (2019) « Mitochondrial F-ATP Synthase and Its Transition into an Energy-Dissipating Molecular Machine ». Oxidative Med Cell Longev 2019(avril):e8743257. https://doi.org/10.1155/2019/8743257
79. Lowell BB, Spiegelman BM (2000) « Towards a molecular understanding of adaptive thermogenesis ». Nature 404(6778):652
80. Macherel D, Haraux F, Olivier Bourgeois (2021) « The Conundrum of Hot Mitochondria ». Biochim Et Biophys Acta Bioenergetics 1862(2):148348. https://doi.org/10.1016/j.bbabio.2020.148348
81. Mao W, Yu XX, Zhong A, Li W, Brush J, Sherwood SW, Adams SH, Guohua Pan (1999) « UCP4, a Novel Brain-Specific Mitochondrial Protein That Reduces Membrane Potential in Mammalian Cells ». FEBS Lett 443(3):326–330. https://doi.org/10.1016/S0014-5793(98)01713-X
82. Masson SWC, Christopher P, Hedges, Jules BL, Devaux CS, James JR, Hickey (2017) « Mitochondrial Glycerol 3-Phosphate Facilitates Bumblebee Pre-Flight Thermogenesis ». Sci Rep 7(1):13107. https://doi.org/10.1038/s41598-017-13454-5
83. McCormack JG, Halestrap AP, Denton RM (1990) « Role of calcium ions in regulation of mammalian intramitochondrial metabolism ». Physiol Rev 70(2):391–425. https://doi.org/10.1152/physrev.1990.70.2.391
84. McLaughlin KL, James T, Hagen HS, Coalson, Margaret AM, Nelson KA, Kew AR, Wooten H Fisher-Wellman. 2020. « Novel Approach to Quantify Mitochondrial Content and Intrinsic Bioenergetic Efficiency across Organs ».*Scientific Reports*10 (1):17599. https://doi.org/10.1038/s41598-020-74718-1
85. de Meis L (2002) « Ca2+-ATPases (SERCA): Energy Transduction and Heat Production in Transport ATPases ». J Membr Biol 188(1):1–9. https://doi.org/10.1007/s00232-001-0171-5
86. de Meis L, Ketzer LA, Marlene Benchimol (2010) Rodrigo Madeiro da Costa, Ivone Rosa de Andrade, et. « Fusion of the Endoplasmic Reticulum and Mitochondrial Outer Membrane in Rats Brown Adipose Tissue: Activation of Thermogenesis by Ca2+ ». *PLOS ONE* 5 (3): e9439. https://doi.org/10.1371/journal.pone.0009439
87. Mihara S, Suzuki N, Wakisaka S, Suzuki S, Sekita N, Yamamoto S, Saito N, Hoshino T, Sakane T (1999) « Effects of Thyroid Hormones on Apoptotic Cell Death of Human Lymphocytes ». J Clin

Endocrinol Metab 84(4):1378–1385. https://doi.org/10.1210/jcem.84.4.5598

88. Mills EL, Kerry A, Pierce MP, Jedrychowski R, Garrity S, Winther S, Vidoni T, Yoneshiro et al (2018) « Accumulation of Succinate Controls Activation of Adipose Tissue Thermogenesis ». Nature 560(7716):102–106. https://doi.org/10.1038/s41586-018-0353-2
89. Monternier P-A, Marmillot V, Rouanet J-L, Damien Roussel (2014) « Mitochondrial Phenotypic Flexibility Enhances Energy Savings during Winter Fast in King Penguin Chicks ». J Exp Biol 217(Pt 15):2691–2697. https://doi.org/10.1242/jeb.104505
90. Moreno AJM, Paula I, Moreira JBA, Custódio MS, Santos (2013) « Mechanism of Inhibition of Mitochondrial ATP Synthase by 17β – Estradiol ». J Bioenerg Biomembr 45(3):261–270. https://doi.org/10.1007/s10863-012-9497-1
91. Mráček T, Josef ZD, Houštěk (2013) « The Function and the Role of the Mitochondrial Glycerol-3-Phosphate Dehydrogenase in Mammalian Tissues ». Biochim Biophys Acta 1827(3):401–410. https://doi.org/10.1016/j.bbabio.2012.11.014
92. Murakami A, Nagao K, Sakaguchi R, Kida K, Hara Y, Mori Y, Okabe K, Harada Y, Masato Umeda (2022) « Cell-Autonomous Control of Intracellular Temperature by Unsaturation of Phospholipid Acyl Chains ». Cell Rep 38(11):110487. https://doi.org/10.1016/j.celrep.2022.110487
93. Murphy MP (1989) « Slip and Leak in Mitochondrial Oxidative Phosphorylation ». Biochim Biophys Acta 977(2):123–141. https://doi.org/10.1016/s0005-2728(89)80063-5
94. Murphy MP (2009) « How mitochondria produce reactive oxygen species ». Biochem J 417(1):1–13
95. Nakano M, Arai Y, Kotera I, Okabe K, Kamei Y, Takeharu Nagai (2017) « Genetically Encoded Ratiometric Fluorescent Thermometer with Wide Range and Rapid Response ». Édité par Takuya Matsumoto. PLoS ONE 12(2):e0172344. https://doi.org/10.1371/journal.pone.0172344
96. Nasr MA, Galina I, Dovbeshko SL, Bearne N, El-Badri F, Matta (2019) « Heat Shock Proteins in the “Hot” Mitochondrion: Identity and Putative Roles ». BioEssays: News and Reviews in Molecular Cellular and Developmental Biology 41(9):e1900055. https://doi.org/10.1002/bies.201900055
97. Nath S (2016) « The Thermodynamic Efficiency of ATP Synthesis in Oxidative Phosphorylation ». Biophys Chem 219:69–74. https://doi.org/10.1016/j.bpc.2016.10.002
98. Nedergaard JAN, Cannon B, Olov Lindberg (1977) « Microcalorimetry of isolated mammalian cells ». Nature 267(5611):518
99. Nedergaard J, Lindberg etO (1979) « Norepinephrine-Stimulated Fatty-Acid Release and Oxygen Consumption in Isolated Hamster Brown-Fat Cells. Influence of Buffers, Albumin, Insulin and Mitochondrial Inhibitors ». Eur J Biochem 95(1):139–145. https://doi.org/10.1111/j.1432-1033.1979.tb12948.x
100. Nguyen AD, Nicola J, Lee NKY, Wee L, Zhang RF, Enriquez EC, Khor T et al (2018) « Uncoupling Protein-1 Is Protective of Bone Mass under Mild Cold Stress Conditions ». Bone 106(janvier):167–178. https://doi.org/10.1016/j.bone.2015.05.037
101. Nguyen P, Leray V, Diez M, Serisier S, Bloc’h JL, Siliart B, Dumon etH (2008) « Liver Lipid Metabolism ». J Anim Physiol Anim Nutr 92(3):272–283. https://doi.org/10.1111/j.1439-0396.2007.00752.x

102. Nicholls DG (1974) « Hamster Brown-Adipose-Tissue Mitochondria. The Control of Respiration and the Proton Electrochemical Potential Gradient by Possible Physiological Effectors of the Proton Conductance of the Inner Membrane ». Eur J Biochem 49(3):573–583. https://doi.org/10.1111/j.1432-1033.1974.tb03861.x
103. Nogueiras R, Habegger KM, Chaudhary N, Finan B, Banks AS, Marcelo O, Dietrich TL, Horvath DA, Sinclair PT, Pfluger H Tschöop. 2012. « SIRTUIN 1 AND SIRTUIN 3: PHYSIOLOGICAL MODULATORS OF METABOLISM ».*Physiological reviews*92 (3):1479–1514. https://doi.org/10.1152/physrev.00022.2011
104. O'Rourke B (2007) « Mitochondrial Ion Channels ». Annu Rev Physiol 69:19–49. https://doi.org/10.1146/annurev.physiol.69.031905.163804
105. Pant M, Bal NC, Muthu Periasamy (2016) « Sarcolipin: A Key Thermogenic and Metabolic Regulator in Skeletal Muscle ». Trends Endocrinol Metab 27(12):881–892. https://doi.org/10.1016/j.tem.2016.08.006
106. Papa S, Capitanio N, Capitanio G, De Nitto E, Minuto etM (1991) « The Cytochrome Chain of Mitochondria Exhibits Variable H+/e- Stoichiometry ». FEBS Lett 288(1–2):183–186. https://doi.org/10.1016/0014-5793(91)81030-c
107. Pehowich DJ (1999) « Thyroid Hormone Status and Membrane N-3 Fatty Acid Content Influence Mitochondrial Proton Leak ». Biochim et Biophys Acta (BBA) - Bioenergetics 1411(1):192–200. https://doi.org/10.1016/S0005-2728(99)00041-9
108. Phillips RC, George P, Rutman RJ (1969) « Thermodynamic Data for the Hydrolysis of Adenosine Triphosphate as a Function of PH, Mg2 + Ion Concentration, and Ionic Strength ». J Biol Chem 244(12):3330–3342
109. Porter RK, Brand MD (1995) « Mitochondrial Proton Conductance and H+/O Ratio Are Independent of Electron Transport Rate in Isolated Hepatocytes ». Biochem J 310:379–382. https://doi.org/10.1042/bj3100379
110. Rajagopal MC, Brown JW, Gelda D, Valavala KV, Wang H, Llano DA Rhanor Gillette, et Sanjiv Sinha. 2019. « Transient Heat Release during Induced Mitochondrial Proton Uncoupling ».*Communications Biology*2 (1):1–6. https://doi.org/10.1038/s42003-019-0535-y
111. Ricquier D (2006) « Fundamental mechanisms of thermogenesis ». CR Biol 329(8):578–586
112. ———. « UCP1, the Mitochondrial Uncoupling Protein of Brown Adipocyte: A Personal Contribution and a Historical Perspective ». *Biochimie* 134 (mars): 3–8. https://doi.org/10.1016/j.biochi.2016.10.018
113. Riley CL, Dao C, Alexander Kenaston M, Muto L, Kohno S, Nowinski SM, Solmonson AD et al (2016) « The Complementary and Divergent Roles of Uncoupling Proteins 1 and 3 in Thermoregulation ». J Physiol 594(24):7455–7464. https://doi.org/10.1113/JP272971
114. Rizzuto R, Stefani DD, Raffaello A, Cristina Mammucari (2012) « Mitochondria as Sensors and Regulators of Calcium Signalling ». Nat Rev Mol Cell Biol 13(9):566–578. https://doi.org/10.1038/nrm3412

115. Rolfe DF, Brand MD (1996) « Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate ». Am J Physiology-Cell Physiol 271(4):C1380–C1389. https://doi.org/10.1152/ajpcell.1996.271.4.C1380
116. Rolfe DF, Brown GC (1997) « Cellular Energy Utilization and Molecular Origin of Standard Metabolic Rate in Mammals ». Physiol Rev 77(3):731–758. https://doi.org/10.1152/physrev.1997.77.3.731
117. Rolfe DF, Newman JM, Buckingham JA, Clark MG, Brand MD (1999) « Contribution of Mitochondrial Proton Leak to Respiration Rate in Working Skeletal Muscle and Liver and to SMR ». Am J Physiol 276(3):C692–699. https://doi.org/10.1152/ajpcell.1999.276.3.C692
118. Rolfe DFS, Newman JMB, Buckingham JA, Clark MG, Brand MD (1999) « Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver and to SMR ». Am J Physiology-Cell Physiol 276(3):C692–C699. https://doi.org/10.1152/ajpcell.1999.276.3.C692
119. Rosenberg H, Pollock N, Schiemann A, Bulger T, Kathryn Stowell (2015) « Malignant hyperthermia: a review ». Orphanet J Rare Dis 10(1):93. https://doi.org/10.1186/s13023-015-0310-1
120. Rossi A, Pizzo P, Riccardo Filadi (2019) « Calcium, Mitochondria and Cell Metabolism: A Functional Triangle in Bioenergetics ». Biochim Et Biophys Acta Mol Cell Res 1866(7):1068–1078. https://doi.org/10.1016/j.bbamcr.2018.10.016
121. Roussel D, et Yann Voituron (2020). « Mitochondrial Costs of Being Hot: Effects of Acute Thermal Change on Liver Bioenergetics in Toads (Bufo bufo) ». *Frontiers in Physiology* 11. https://www.frontiersin.org/article/10.3389/fphys.2020.00153
122. Rutter GA, et Denton RM (1988) « Regulation of NAD+-linked isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase by Ca2 + ions within toluene-permeabilized rat heart mitochondria. Interactions with regulation by adenine nucleotides and NADH/NAD + ratios ». Biochem J 252(1):181–189. https://doi.org/10.1042/bj2520181
123. Sahoo SK, Sana A, Shaikh DH, Sopariwala NC, Bal, Muthu Periasamy (2013) « Sarcolipin Protein Interaction with Sarco(Endo)Plasmic Reticulum Ca2 + ATPase (SERCA) Is Distinct from Phospholamban Protein, and Only Sarcolipin Can Promote Uncoupling of the SERCA Pump ». J Biol Chem 288(10):6881–6889. https://doi.org/10.1074/jbc.M112.436915
124. Salway JG (2004) Metabolism at a Glance. Wiley
125. Schlicker C, Gertz M, Papatheodorou P, Kachholz B, Becker CFW, et Clemens Steegborn (2008) « Substrates and Regulation Mechanisms for the Human Mitochondrial Sirtuins Sirt3 and Sirt5 ». J Mol Biol 382(3):790–801. https://doi.org/10.1016/j.jmb.2008.07.048
126. Schneider K, Valdez J, Nguyen J, Vawter M, Galke B, Theodore W, Kurtz Y, Chan (2016) « Increased Energy Expenditure, Ucp1 Expression, and Resistance to Diet-Induced Obesity in Mice Lacking Nuclear Factor-Erythroid-2-Related Transcription Factor-2 (Nrf2) ». J Biol Chem 291(14):7754–7766. https://doi.org/10.1074/jbc.M115.673756
127. Sebaa R, Johnson J, Pileggi C, Norgren M, Xuan J, Sai Y, Tong Q et al (2019) « SIRT3 Controls Brown Fat Thermogenesis by Deacetylation Regulation of Pathways Upstream of UCP1 ». Mol Metabolism 25(juillet):35–49. https://doi.org/10.1016/j.molmet.2019.04.008

128. Shabalina IG, Kramarova TV, Nedergaard J, Cannon B (2006) « Carboxyatractyloside Effects on Brown-Fat Mitochondria Imply That the Adenine Nucleotide Translocator Isoforms ANT1 and ANT2 May Be Responsible for Basal and Fatty-Acid-Induced Uncoupling Respectively ». Biochem J 399(3):405–414. https://doi.org/10.1042/BJ20060706
129. Shi T, Wang F, Stieren E, Qiang Tong (2005) « SIRT3, a Mitochondrial Sirtuin Deacetylase, Regulates Mitochondrial Function and Thermogenesis in Brown Adipocytes ». J Biol Chem 280(14):13560–13567. https://doi.org/10.1074/jbc.M414670200
130. Slimen I, Belhadj T, Najar A, Ghram etM, Abdrrabba (2016) « Heat Stress Effects on Livestock: Molecular, Cellular and Metabolic Aspects, a Review ». J Anim Physiol Anim Nutr 100(3):401–412. https://doi.org/10.1111/jpn.12379
131. Smith WS, Broadbridge R, Malcolm East J, Anthony G, Lee (2002) « Sarcolipin Uncouples Hydrolysis of ATP from Accumulation of Ca2 + by the Ca2+-ATPase of Skeletal-Muscle Sarcoplasmic Reticulum ». Biochem J 361(Pt 2):277–286. https://doi.org/10.1042/0264-6021:3610277
132. Sone N, Nicholls etP (1984) « Effect of heat treatment on oxidase activity and proton-pumping capability of proteoliposome-incorporated beef heart cytochrome aa3 ». Biochemistry 23(26):6550–6554. https://doi.org/10.1021/bi00321a042
133. Sotoma S, Zhong C, Kah JChenY, Yamashita H, Plakhotnik T, Harada Y, Madoka Suzuki (2021) « In situ measurements of intracellular thermal conductivity using heater-thermometer hybrid diamond nanosensors ». Sci Adv 7(3). https://doi.org/10.1126/sciadv.abd7888
134. Steigmiller S, Turina P, et Peter Gräber (2008). « The thermodynamic H+/ATP ratios of the H+-ATPsynthases from chloroplasts and Escherichia coli ». *Proceedings of the National Academy of Sciences* 105 (10): 3745–50. https://doi.org/10.1073/pnas.0708356105
135. Stier A, Bize P, Habold C, Bouillaud F, Massemin S (2014) et François Criscuolo. « Mitochondrial Uncoupling Prevents Cold-Induced Oxidative Stress: A Case Study Using UCP1 Knockout Mice ». *Journal of Experimental Biology* 217 (4): 624–30. https://doi.org/10.1242/jeb.092700
136. St-Pierre J, Buckingham JA, Roebuck SJ, Martin D, Brand (2002) « Topology of Superoxide Production from Different Sites in the Mitochondrial Electron Transport Chain ». J Biol Chem 277(47):44784–44790. https://doi.org/10.1074/jbc.M207217200
137. Sun Y, Rahbani JF, Jedrychowski MP, Riley CL, Vidoni S et al (2021) « Mitochondrial TNAP Controls Thermogenesis by Hydrolysis of Phosphocreatine ». Nature 593(7860):580–585. https://doi.org/10.1038/s41586-021-03533-z
138. Teixeira, Carmen Cabanelas Pazos-Moura (2020) Patrícia de Fátima dos Santos, Patrícia Borges dos Santos, et. « The role of thyroid hormone in metabolism and metabolic syndrome ». *Therapeutic Advances in Endocrinology and Metabolism* 11 (mai): 2042018820917869. https://doi.org/10.1177/2042018820917869
139. Tretter L, et Vera Adam-Vizi (2007) « Uncoupling Is without an Effect on the Production of Reactive Oxygen Species by in Situ Synaptic Mitochondria ». J Neurochem 103(5):1864–1871. https://doi.org/10.1111/j.1471-4159.2007.04891.x

140. Urbani A, Prosdocimi E, Carrer A, Checchetto V, Ildikò S (2021) « Mitochondrial Ion Channels of the Inner Membrane and Their Regulation in Cell Death Signaling ». *Frontiers in Cell and Developmental Biology* 8 (janvier): 620081. https://doi.org/10.3389/fcell.2020.620081
141. Wakatsuki T, Hirata F, Ohno H, Yamamoto M, Sato Y, Ohira Y (1996) « Thermogenic responses to high-energy phosphate contents and/or hindlimb suspension in rats ». Jpn J Physiol 46(2):171–175
142. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM (1992) « Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis ». Biochem J 281(1):21–40. https://doi.org/10.1042/bj2810021
143. Wallimann T, Tokarska-Schlattner M, Neumann D, Epand RM, Raquel F, Epand RH, Andres HR, Widmer et al (2007) « The Phosphocreatine Circuit: Molecular and Cellular Physiology of Creatine Kinases, Sensitivity to Free Radicals, and Enhancement by Creatine Supplementation ». Molecular Systems Bioenergetics. Wiley-VCH Verlag GmbH & Co. KGaA, pp 195–264. https://doi.org/10.1002/9783527621095.ch7.
144. Wallimann T, Tokarska-Schlattner M, Uwe S (2011) « The Creatine Kinase System and Pleiotropic Effects of Creatine ». Amino Acids 40(5):1271–1296. https://doi.org/10.1007/s00726-011-0877-3
145. Wang Q, Sun J, Liu M, Zhou Y, Zhang L, Li Y (2021) « The New Role of AMP-Activated Protein Kinase in Regulating Fat Metabolism and Energy Expenditure in Adipose Tissue ». Biomolecules 11(12):1757. https://doi.org/10.3390/biom11121757
146. Wang, Sheng-Bing DB, Foster J, Rucker, Brian O'Rourke DA, Kass E, Van Eyk (2011) « Redox Regulation of Mitochondrial ATP Synthase ». Circul Res 109(7):750–757. https://doi.org/10.1161/CIRCRESAHA.111.246124
147. Watt IN, Martin G, Montgomery MJ, Runswick AGW, Leslie JE, Walker (2010) « Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria ». *Proceedings of the National Academy of Sciences* 107 (39): 16823–27. https://doi.org/10.1073/pnas.1011099107
148. Wescott AP, Joseph PY, Kao W, Jonathan Lederer, Liron Boyman (2019) « Voltage-Energized Calcium-Sensitive ATP Production by Mitochondria ». Nat Metabolism 1(10):975–984. https://doi.org/10.1038/s42255-019-0126-8
149. Wikström M, et Roger Springett (2020). « Thermodynamic Efficiency, Reversibility, and Degree of Coupling in Energy Conservation by the Mitochondrial Respiratory Chain. » *Communications Biology*. https://doi.org/10.1038/s42003-020-01192-w
150. Yagi T, Youssef Hatefi (1987) « Thiols in Oxidative Phosphorylation: Thiols in the F0 of ATP Synthase Essential for ATPase Activity ». Arch Biochem Biophys 254(1):102–109. https://doi.org/10.1016/0003-9861(87)90085-3
151. Yamada K (2017) « Energetics of Muscle Contraction: Further Trials ». J Physiological Sci 67(1):19–43. https://doi.org/10.1007/s12576-016-0470-3
152. Yamashita H, Ohira Y, Wakatsuki T, Yamamoto M, Kizaki T, Oh-ishi S, Hideki Ohno (1995) « Increased Growth of Brown Adipose Tissue but Its Reduced Thermogenic Activity in Creatine-Depleted Rats Fed

β-Guanidinopropionic Acid ». Biochim et Biophys Acta (BBA) - Bioenergetics 1230(1):69–73. https://doi.org/10.1016/0005-2728(95)00067-S

153. Yehuda-Shnaidman E, Kalderon B, et Jacob Bar-Tana (2014) « Thyroid Hormone, Thyromimetics, and Metabolic Efficiency ». Endocr Rev 35(1):35–58. https://doi.org/10.1210/er.2013-1006
154. Yu X, Xian W, Mao A, Zhong P, Schow J, Brush SW, Sherwood SH, Adams G, Pan (2000) « Characterization of novel UCP5/BMCP1 isoforms and differential regulation of UCP4 and UCP5 expression through dietary or temperature manipulation ». FASEB J 14(11):1611–1618. https://doi.org/10.1096/fj.99-0834com
155. Zanotti F, Guerrieri F, Capozza G, Fiermonte M, Berden J, Sergio Papa (1992) « Role of F0 and F1 Subunits in the Gating and Coupling Function of Mitochondrial H+-ATP Synthase ». Eur J Biochem 208(1):9–16. https://doi.org/10.1111/j.1432-1033.1992.tb17153.x

Box

Box 1: ATP production during OXPHOS

The electron transfer chain (ETC) includes multi-subunits complexes embedded within the inner mitochondrial membrane (IMM), which are functionally and physically linked together: the complex I (CI, NADH Ubiquinone Reductase), complex II (CII, succinate ubiquinone reductase), complex III (CIII, ubiquinol cytochrome c reductase) and complex IV (CIV, cytochrome c reductase). The ETC transfer the electron energetic potential from NADH/NAD+ (CI) and FADH2/FAD+ (CII) to the electrochemical proton gradient known as the proton motive force (Dp). This process involves a series of oxidoreductase reactions in which electron flows sequentially “downhill” along the ETC from a reduced to an oxidized state, ending to molecular oxygen reduction into a water molecule. Free energy release during electron transfer drives the proton pumping across the IMM at complexes CI, CIII, and CIV, resulting in the proton gradient, Dp. The Dp consists in the charge ($\Delta\psi_m$) and chemical (ΔpH) components, and drives the endergonic ATP synthesis when the H^+ flow back to the matrix and many transports through the inner mitochondrial membrane **(Hatefi 1985)**. The ATP synthesis module includes, in addition to the ATP synthase, the adenylate carrier (ANT) to exchange the ADP/ATP and the inorganic phosphate carrier (PiC). When Dp energy potential is used by the ATP synthase, the coupling between substrate oxidation and ATP synthesis is maximized. However, if H^+ leak back across the IMM through alternative H^+ conductance pathways, coupling efficiency is decreased, resulting in heat production at the expense of ATP synthesis.

Box 2: Theoretical estimates of mitochondrial heat production

Theoretically, the oxidation of one mole of glucose, which contains 2871 kJ of energy, produces 38 moles of ATP (2 by glycolysis, 2 by the TCA cycle and 34 by OXPHOS), which represents 1368kJ (≈48%) ($\Delta G°'$ ATP=36kJ/mol at 37°C, pH=7.5 and I 0.2 **(Phillips, George, et Rutman 1969)**, plus ≈1503 kJ (≈52%) dissipated as heat (Figure 1) **(Cleri 2016).** Alternatively, fatty acid breakdown by β-oxidation also provides NADH and $FADH_2$ to the mitochondrial respiratory chain **(P. Nguyen et al. 2008).** On the same principle,

the oxidation of one mole of palmitate, which contains 9800kJ of energy, produces theoretically 129 moles of ATP, which represents 4644kJ (≈47%), plus 5156kJ (≈53%) dissipated as heat. These values are obtained by considering an historical mechanistic P/O ratio, which represent the amount of moles of ADP phosphorylated to ATP per two electrons transferred to oxygen, of 3 per NADH and 2 per $FADH_2$ **(Nath 2016, 20).**

However, it is now widely accepted that the mechanistic efficiencies are lower. Considering the mechanism of F-ATPase and its H^+/ATP ratio of 2.7 in mammals, the calculated P/O ratio is 2.7 for NADH and 1.6 for $FADH_2$ **(Watt et al. 2010)**, which approaches the experimental observed values of 2.5 and 1.5 **(Hinkle 2005).** According to these new P/O values, glucose oxidation result in the generation of only 30 - 32 (≈40%), rather than 38, moles of ATP (Figure 1A) (for a highly readable review, see **(Salway 2004).** The comparable value for palmitate oxidation has been lowered from 129 to 106 ATP.

Moreover, estimates of coupling efficiency in cells and intact tissues indicate an overall effective efficiency in rats of 75%–80% **(M D Brand, Harper, et Taylor 1993; D. F. S. Rolfe et al. 1999)** because of uncoupling mechanisms (detailed in section II.) meaning that effective P/O ratio should be lowered to 1.8 *in vivo*. Thus, ATP/heat production balance is even lower than the predicted 40%/60% in physiological conditions (Figure 1B).

Box 3: Diseases associated to altered control of body temperature involve Ca^{2+} channels.

Malignant hyperthermia (MHT) and exertional heat strokes (EHS) are two human acute diseases related to a hyperpyrexia that are also encountered in other mammalian species, like dog and swine. Both diseases induce body over-heating and severe or even fatal outcomes, almost always associated to rhabdomyolysis and brain damages, and elevated creatine kinase activity in serum. MHT mendelian transmission was evidenced for long, and is today associated to 4 loci (Malignant Hyperthermia Susceptibility; MHS 2, 3, 4 and 6), in addition to variants in 3 genes: *RYR1* (MHS1), *CACNA1S* (MHS5) and *TRPV1***(Rosenberg et al. 2015; Abeele et al. 2018)**. Surprisingly, genetic analysis of EHS predisposition also revealed susceptibility variants in *RYR1* and *TRPV1* genes **(Bosson et al. 2020; Laitano, Murray, et Leon 2020)**, but different from the ones identified in MHT, suggesting an alternative pathological mechanism. Interestingly, the function of RYR1, CACNA1S and TRPV1 proteins are all intimately related to their channel structure involved in calcium trafficking **(Kushnir, Wajsberg, et Marks 2018; Beam, Loudermilk, et Kisor 2017; Abeele et al. 2018)**, suggesting that disruption of Ca^{2+} intracellular fluxes and homeostasis are causative of uncontrolled thermogenesis.

Figures

Figure 1

Theoretical energetic balance of ATP and heat productions in standard biochemical condition.

Under biological operating conditions (pH=7.5, I 0.2, T°=37°C), and considering a P/O ratio of 2.5 per NADH and 1.5 per $FADH_2$, the degradation of one molecule of glucose (2871kJ) successively through the glycolysis in the cytoplasm, the tri-carboxylic acid cycle and the oxidative phosphorylation in mitochondria produces:

(A) 32 ATP (1152kJ ≈40%), including 28 OXPHOS-generated ATP and 1719kJ (≈60%) of heat.

(B) 25 ATP (900 kJ≈30%), including 21 OXPHOS-generated ATP and 1971kJ (≈70%) of heat are produced from glucose oxidation when the mechanistic P/O ratios of NADH and $FADH_2$ are corrected for uncoupling mechanisms, considering a physiological efficiency of 75%–80%.

These values illustrate the major role of mitochondria in heat production linked to the energetic metabolism.

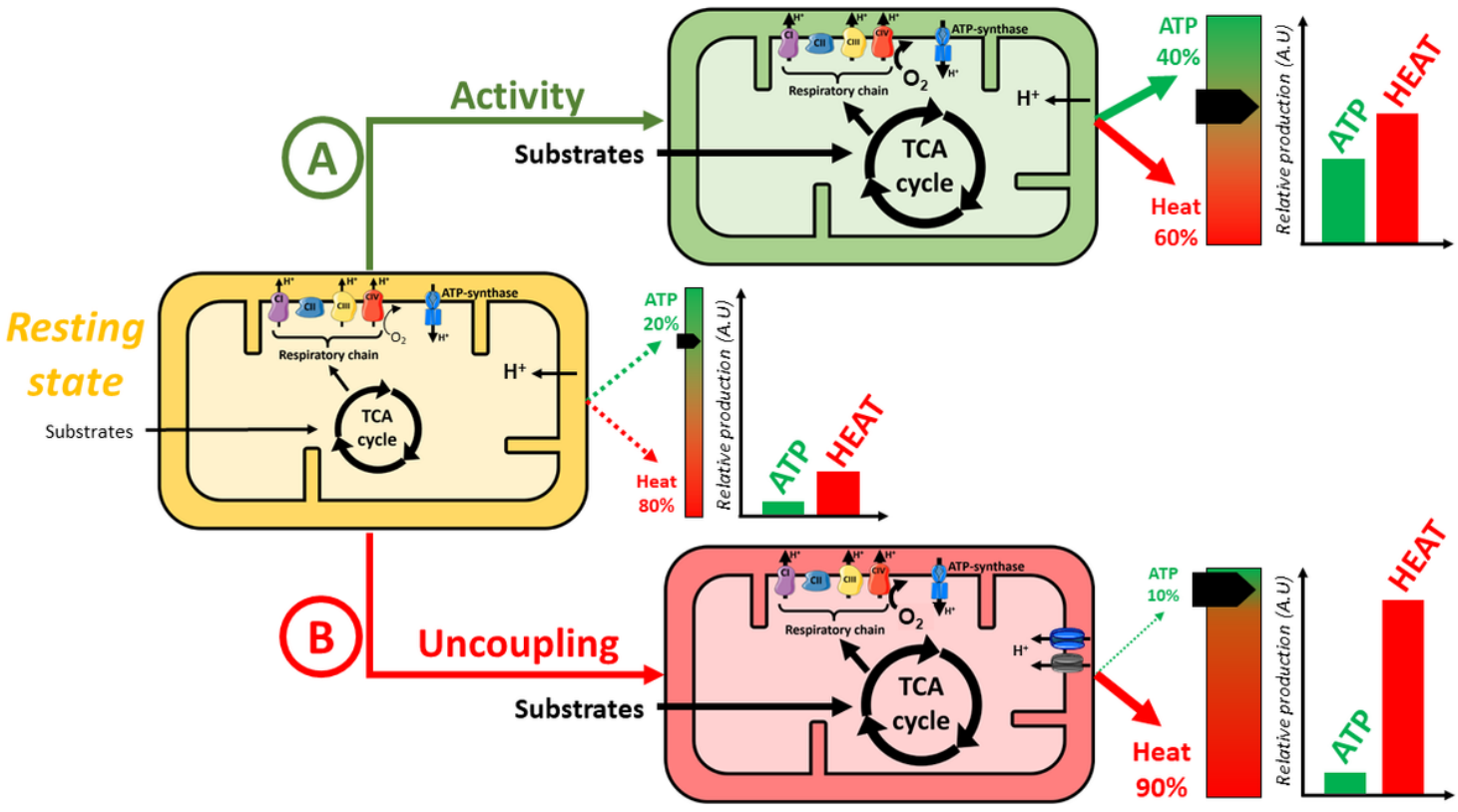

Figure 2

Mitochondrial energetic and thermogenesis balance

In mitochondria, substrate oxidation energy is converted into ATP and heat. The maximal global efficiency of OXPHOS is ~40%/60% for ATP/Heat. However, in resting rat muscle, H^+ leak (detailed in

section II.) could represent ≈50% of O_2 consumption. In this case, the ATP/O is reduced by an half and the ATP/Heat balance increases up to 20%/80%. According to cellular needs, ATP/heat balance in mitochondria can turn to either situations:

(A) a maximum phosphorylating activity, when the H^+ leak represents an insignificant proportion of O_2 consumption. The ATP/Heat balance is close to the theoretical maximal global efficiency of 40%/60%. However, the increased oxidation rate results in an ATP rise and a consecutive heat production.

(B) an uncoupling activity with an increase of the oxidation rate. Thanks to uncoupling mechanisms, the ATP/Heat balance is modulated in favor of heat production.

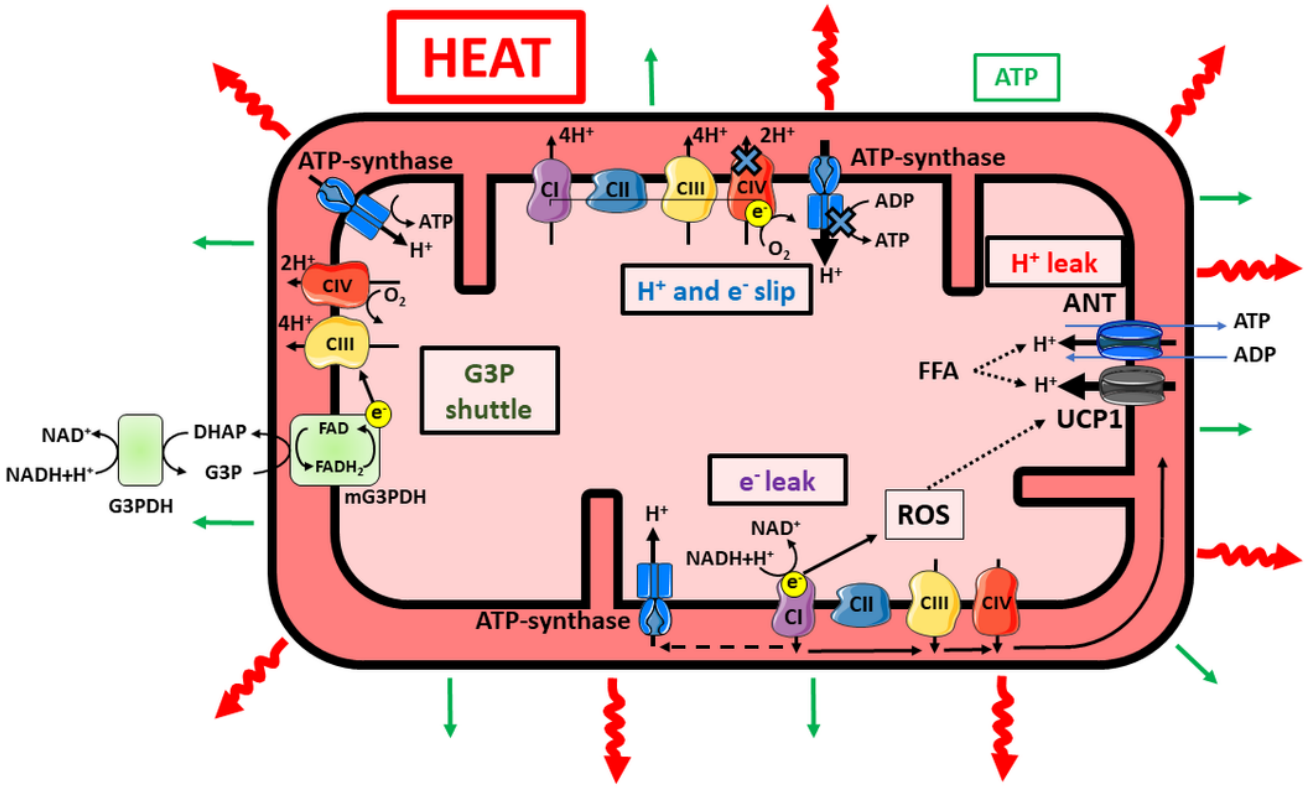

Figure 3

OXPHOS uncoupling mechanisms promote mitochondrial heat production

Mitochondrial uncoupling promotes heat production by reducing the ATP production efficiency. Uncoupling Protein 1 (UCP1) activation induces H^+ leak, which dissipates the Δp thereby uncoupling substrate oxidation from ATP synthesis while increasing thermogenesis. The Adenine nucleotide translocator (ANT), in addition to its main function as ATP/ADP carrier, can also act as H^+ transporter. ANT-mediated H^+ leak requires protonatable Free Fatty Acids (FFA), which would act as cofactors. FFA-dependent H^+ leak competes with nucleotide exchange activity in the ANT translocation pathway,

switching from H^+ leak, promoting heat production, to ATP/ADP translocation, promoting ATP production. e^- slip at CIV and H^+ slip at ATP synthase could lower the P/O ratio. ROS production through e^- leak deflects electron transfer occurring from NADH (CI) or $FADH_2$ (CIII) oxidations. The glycerol-3-phosphate (G3P) shuttle catalyzes an apparent exchange of cytosolic NADH for mitochondrial $FADH_2$, directly transferring electrons to CIII via the ubiquinone. This reduces the H^+/O_2 stoichiometry from $10H^+/2e^-$ for NADH to $6H^+/2e^-$ **(M. D. Brand 2005)**, ultimately reducing the ATP/O stoichiometry and the efficiency of ATP production.

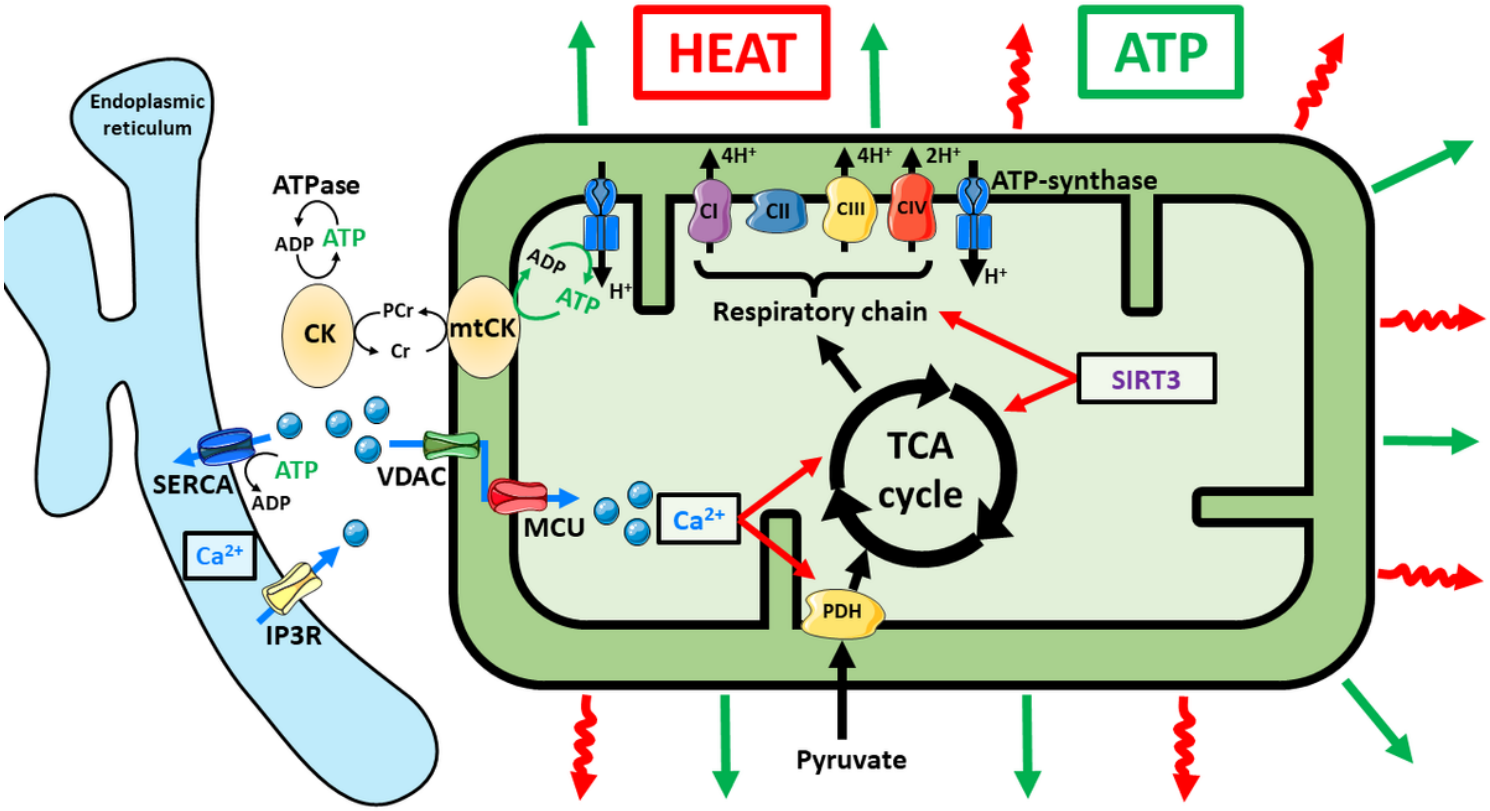

Figure 4

Mitochondrial respiration rate regulators impacting mitochondrial heat production

ATP-dependent futile cycles promote high ATP turnover, thereby increasing OXPHOS rate and inherent energy-loss as heat. Creatine Kinases (CK) connect the ATP-consumption to ATP-production sites through PhosphoCreatine (PCr)/Creatine (Cr) shuttle. Within mitochondria, mtCK in the intermembrane space is coupled to ADP phosphorylation through ANT, maintaining high ADP/ATP ratio within the mitochondrial matrix. Futile cycle of creatine dephosphorylation drives ATP hydrolysis, consequently increasing OXPHOS rate. Ca^{2+} cycling by SERCA in reticulum endoplasmic increases both ATP hydrolysis and Ca^{2+} accumulation in the cytosol. ATP hydrolysis promotes mitochondrial ATP re-synthesis while high substrate oxidation flux is sustained thanks to Ca^{2+} accumulation in mitochondria through mitochondrial

calcium uniporter (MCU). Metabolic energy sensors such as (SIRT3) can also stimulate substrate supply and respiratory chain activity, by modulating the activity of TCA cycle enzymes and respiratory chain complexes.