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Mitochondrial redox state, bioenergetics, and calcium transport in caloric restriction: A metabolic nexus

Eloisa A. Vilas-Boas [a,**], Alicia J. Kowaltowski [b,*]

[a] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Brazil
[b] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil

A B S T R A C T

Mitochondria congregate central reactions in energy metabolism, many of which involve electron transfer. As such, they are expected to both respond to changes in nutrient supply and demand and also provide signals that integrate energy metabolism intracellularly. In this review, we discuss how mitochondrial bioenergetics and reactive oxygen species production is impacted by dietary interventions that change nutrient availability and impact on aging, such as calorie restriction. We also discuss how dietary interventions alter mitochondrial Ca^{2+} transport, regulating both mitochondrial and cytosolic processes modulated by this ion. Overall, a plethora of literature data support the idea that mitochondrial oxidants and calcium transport act as integrating signals coordinating the response to changes in nutritional supply and demand in cells, tissues, and animals.

1. Mitochondria, calcium, and caloric intake: the nexus of metabolic regulation

Mitochondria stand out amid cellular organelles as the central coordinators of energy metabolism, orchestrating intricate biochemical reactions and producing most of the ATP essential to sustain life in complex organisms. These organelles have the canonic function of generating ATP with high capacity and efficiency through oxidative phosphorylation [1,2]. Additionally, their metabolic contributions are much more widespread, as the location of the tricarboxylic acid cycle [3], as well as fatty acid metabolism [4], and harboring important steps in amino acid oxidation [5].

Mitochondria are not restricted to metabolic activity, and also serve as signaling hubs, responding to cellular cues and influencing the activation of diverse processes, including the regulation of metabolism and other cell functions. As an example, mitochondria can determine cell fate, both modulating differentiation and participating in the events leading to apoptotic and necrotic cell death. Plastic changes in mitochondrial metabolism govern cancer cells, modifying anabolic abilities, as well as the capacity to grow in tumor environments and metastasize [6,7]. Changes in mitochondrial function and mitochondrially-produced metabolites also regulate immune responses and the activation of immune cells such as macrophages and T lymphocytes [8,9]. This dual role of mitochondria as energetic sources and arbiters of cell fate underscores their significance in cellular physiology and multiple pathologies.

In addition to mitochondrial mechanisms, localized dynamic changes in intracellular calcium concentrations are also important regulatory mechanisms in metabolism [10,11]. Calcium ions act as intracellular and intramitochondrial mediators of hormonal effects in different cell types, directly modulating key metabolic enzymes through allosteric interactions or promoting enzymatic post-translational modifications such as changes in protein phosphorylation, by modulating key kinases and phosphatases [12–14].

A final and central regulatory aspect in metabolism is caloric availability and intake. The quantity, periodicity, and types of nutrients ingested and available in different tissues will promote plastic changes in energy metabolism, and specifically in mitochondrial activities, morphology, dynamics, and function [15–18]. This modulation can occur directly or indirectly, by changing hormonal signaling. Interestingly, our finding that preventing obesity in rodents through caloric restriction promotes changes in mitochondrial calcium uptake [19] brings together the three central regulatory hubs in metabolism: mitochondria, calcium, and nutrient availability. This review will discuss the complex relationships between different mitochondrial properties (bioenergetics, redox state, and ion transport) and changes in intracellular calcium signaling in caloric restriction and other dietary interventions, as well as how these relationships impact on health and disease.

* Corresponding author.
** Corresponding author.
E-mail addresses: elovilasboas@usp.br (E.A. Vilas-Boas), alicia@iq.usp.br (A.J. Kowaltowski).

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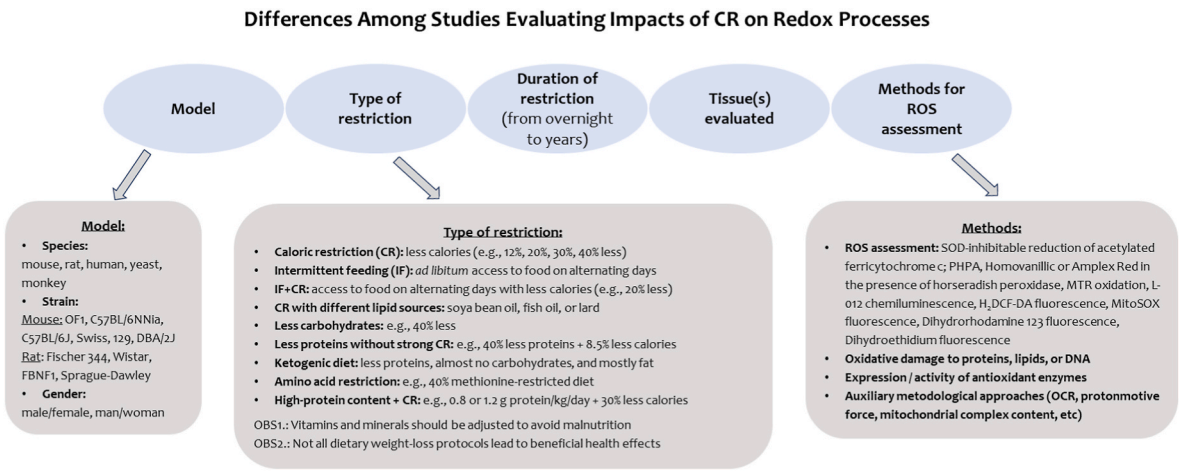

Fig. 1. Differences among studies evaluating impacts of CR on redox processes. Dietary restriction protocols vary largely in the literature. Most differences are due to the model used, type of restriction adopted, duration of restriction, tissue(s) evaluated, and methods for ROS assessment.

2. Caloric restriction

In 1935, McCay and co-authors [20] demonstrated that the mean lifespans of laboratory rats could be extended by up to 50 % through a dietary intervention they described as "caloric restriction" (CR), reducing daily calorie food offerings to limit spontaneous ingestion by approximately 40 %. The results were later reproduced in many different animal models. Indeed, CR is currently the most well-studied non-genetic intervention that robustly extends life expectancy and health spans in a large variety of species [21–24]. While the original group suggested the effects of CR on lifespan were related to growth restriction, it was later shown to be effective in animal models without continued growth as adults, and when applied past their growth stage [21]. Thus, the intervention is more widely believed today to prevent overfeeding and the complex ensuing changes in metabolism and disease onset promoted by *ad libitum* (AL) diets in control laboratory animals. Indeed, we and others consider control AL-fed rodents, typical of research facilities, as a model of diet-induced obesity [25], while CR animals maintain healthy body weight and adiposity, modeling the health and lifespan aspects of a lean human. This is compatible with the finding that CR is effective only in rodent models that develop obesity on AL diets [26].

It should be noted that the "caloric restriction" nomenclature should, but does not always, allude to what the name specifically states: a restriction of calories alone, or undernutrition relative to AL diets, without malnutrition [27,28]. We find, however, that diets labelled as "CR" often describe different interventions, and may involve other restrictions such as time-limited feeding (e.g., intermittent fasting) or total food

Table 1
Effects of CR on mitochondrial bioenergetics.

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on mitochondrial biogenesis
Nisoli et al., 2005 [33]	8 week male C57BLK/6 mice	Alternate day feeding/fasting with 30–40 % restriction	3 or 12 months	WAT, brain, heart, liver, BAT	eNOS-dependent increase in PGC1a, COX-IV, cytochrome *c* and OCRs
Amigo et al., 2017 [19]	2 month male Sprague Dawley rats	CR: 40 % less calories than AL	14 weeks	Brain	• No change in intact mitochondrial OCR • Higher activities of complexes I + III and complex IV • Increase in UQCRC2, Drp-1, and Mfn-2 proteins • Increased cardiolipin
Cerqueira et al., 2012 [34]	4 week female Swiss mice	CR: 40 % less calories than AL	6 months	Brain	• Increased cytochrome *c* oxidase and mitofusin 1 protein • Increased citrate synthase activity
Chausse et al., 2015 [35]	8 week male Sprague Dawley rats	Alternate day feeding/fasting (IF)	1 month	Liver, brain, skeletal muscle, heart	• Increased OCR in liver; no changes in brain, muscle, or heart • Increased COX IV in liver
Lambert et al., 2005 [36]	60 day male Brown-Norway rats	body weights were maintained at 55 % of the fully fed rats	4 months	Liver	No changes in OCRs or membrane potentials
Price et al., 2012 [37]	male C57/BL6 mice	CR: 40 % less calories than AL	16 months	Liver	CR reduced synthesis and breakdown rates of almost all measured proteins and prolonged the half-lives of most, particularly mitochondrial proteins
Picca et al., 2013 [38]	3.5 month male Fischer 344 x Brown Norway rats	CR: 40 % less calories than AL	18 or 28 months	Liver	CR prevents age-related decrease in TFAM and mtDNA
Sohal et al. [40],	4 month male C57BL/6NNia mice	FR: 40 % less food	5, 11, or 18 months	Brain, heart, kidney	Decreased state 4 respiration in brain, heart, and kidney with FR
Lal et al., 2001 [41]	10 month male Wistar rats	CR: 40 % less calories than AL	23 months	Skeletal muscle	CR decreased State 4 respiration
López-Lluch et al., 2006 [42]	Weaned Fischer 344 male rats	CR: 40 % less calories than AL	12 months	Liver	• Decrease in membrane potentials indicated by JC1 fluorescence • Increased mitochondrial mass/number of mitochondrial per cell
Cui et al., 2013 [46]	3 month male Fischer 344 rats	CR: 30 % less calories than AL	21 months	Kidney	CR decreased Pink1 in kidney mitochondria

restriction, in which vitamins and minerals are not supplemented (see Fig. 1). This is of importance, since the impact of these alternative diets on life and health spans is not as well established. Indeed, insulin signaling and redox results of every other day feeding or total food restriction in rodents are quite opposite: while CR leads to prevention of insulin receptor nitration in the adipose tissue and muscle, total food restriction (without vitamin and mineral supplementation) and every other day time-restricted feeding significantly increase this oxidative modification relative to AL animals [29].

While the more recent literature has avoided labeling time-restricted dietary interventions as CR, we find many studies still use total food restriction, without micronutrient supplementation, in lieu of specific caloric restriction. Notably, a 40 % restriction of the standard AIN-93 rodent diet without vitamin or mineral supplementation leads to malnutrition (as set by standards of the National Research Council for laboratory rats and mice), with suboptimal amounts of redox- and bioenergetically-important components such as copper, iron, calcium, menadione, riboflavin, and thiamin [27]. Thus, checking the exact dietary intervention adopted is essential when considering the literature, and may explain significant differences in findings between different laboratories.

3. Caloric restriction and mitochondrial bioenergetics

While mitochondrial oxidative phosphorylation is an essential part of aerobic metabolism, and pivotal for the maintenance of ATP flux, the amount of mitochondrial components in cells must be carefully controlled through the regulation of biogenesis and removal/turnover of these organelles [30,31]. Synthesizing excessive mitochondrial mass is metabolically costly for a cell, and has the added danger that it may lead to excessive production of damaging mitochondrial oxidants. On the flip side, lack of sufficient mitochondrial components can limit important metabolic steps, in particular ATP generation, either under basal conditions or when a rapid increase in ATP turnover occurs due to stress or perturbance. For these reasons, the synthesis and turnover of mitochondrial components is tightly regulated. It stands to reason that this process is also affected by aging and nutritional interventions, including CR.

Interestingly, biogenesis of mitochondrial components is redox-regulated through the activity of endothelial nitric oxide synthase (eNOS) [32]. An alternate-day fasting protocol in mice, that results in caloric restriction, activates eNOS and increases the expression of mitochondrial proteins and oxygen consumption in brain, liver, heart, and brown adipose tissue [33], indicating that obesity prevention can be accompanied by increased mitochondrial mass. Consistently, CR mice and rats present increased cerebral eNOS, nNOS, cytochrome *c* oxidase, and citrate synthase levels, as well as Complex I + III and IV activities, although, surprisingly, oxygen consumption rates were not significantly affected [19,34]. Intermittent fasting in rats did not change mitochondrial oxygen consumption rates in the brain significantly [35], and oxygen consumption rates in livers of CR rats were also unchanged [36]. Indeed, a dynamic proteomics study in CR liver samples indicates that the diet is associated to lower mitochondrial biogenesis [37], although a separate study found that CR increased mtDNA content [38]. Overall, these results (summarized in Table 1, in the order presented here) indicate that specific components of mitochondrial oxidative phosphorylation machinery are differentially affected by distinct dietary interventions, and that the changes that happen may not be those that are rate limiting for oxidative phosphorylation. Other associated functional changes, such in reactive oxygen species (ROS) release may occur, as will be discussed below. These findings also indicate that changes in the synthesis of mitochondrial components are not equally regulated in a concerted fashion; indeed, in an organelle that contains ~1500 proteins related to a large variety of metabolic functions, it would be surprising if differences in control of distinct pathways did not occur.

Mitochondria also have a significant amount of membrane lipid components, the regulation and synthesis of which is even less understood. There is evidence that CR affects mitochondrial membrane composition, with a lower unsaturation/saturation index and impact on the proton leak and inner membrane potentials [39]. Indeed, CR preserves lower leak-dependent O_2 consumption in skeletal muscle, brain, heart, and kidney mitochondria from aged animals, maintaining it similar to young animals [40,41]. Contrastingly, hepatocytes cultured in the presence of sera from CR rats seemingly exhibited lower inner mitochondrial inner membrane potentials, associated with lower oxygen consumption rates and higher number of mitochondria per cell [42]. A cautionary note here is that inner membrane potentials in this study were not calibrated, and fluorescent measurements such as those conducted have notorious artifacts, particularly under conditions known to change mitochondrial mass and morphology [43,44]. Overall, changes in leak-dependent O_2 consumption measurements suggests that CR often prevents age-associated loss of inner mitochondrial membrane integrity and the increase in proton leak. This may be related to the lipid saturation index alterations and modulation of mitochondrial ROS production and removal.

Another aspect that importantly affects mitochondrial mass and bioenergetic functional integrity is turnover of its components. Typically, CR and other obesity-preventing dietary interventions are believed to be associated with enhanced mitophagy, although direct evidence is often lacking [45]. At least one quantitative study shows lower mitochondrial protein turnover in livers from CR animals [37], demonstrating this belief should be more carefully assessed. The result may be tissue-specific and indicative that the liver responds differently in terms of mitochondrial bioenergetics, mass and turnover in CR. Indeed, renal CR mitochondria display enhanced autophagy and mitophagy markers [46]; this may be a result of enhanced basal mitochondrial H_2O_2 production [47].

Overall, while tissue-specific results may vary, most studies indicate that CR either increases components of mitochondrial oxidative phosphorylation machinery or preserves their quantity and function during aging. This may be associated with changes in mitochondrial membrane lipid composition and mitochondrial turnover, that still remain to be better studied.

4. Mitochondrial reactive oxygen species and aging

Biological activities require an energy conversion source, typically in the form of ATP, which is mostly produced in mitochondria through oxidative phosphorylation, a process that involves a series of redox reactions culminating in the reduction of oxygen to water. Given the ubiquity and magnitude of these redox reactions in mitochondria, it is to be expected that a small amount of oxygen is not fully reduced to water and can generate intermediately reduced products such as the superoxide radical ($O_2^{\cdot-}$), a product of one-electron reduction of O_2. This process is known as the electron leak within the electron transport chain (ETC), and, while quite small, consists of a constant and unavoidable source of oxidants [48] that makes it the main source of reactive oxygen species (ROS) in most tissues.

Indeed, $O_2^{\cdot-}$ and derived oxidants such as H_2O_2 are formed in a tissue-specific manner within different ETC components, especially within complexes I and III, as well as reverse electron transfer from complex II to complex I [49]. Mitochondria can also generate significant amounts of $O_2^{\cdot-}$ And hydrogen peroxide (H_2O_2) from flavoenzymes that are not part of the ETC, including pyruvate and ketoglutarate dehydrogenases [50] and acyl-CoA dehydrogenases [51]. Given the constant flux that is in the nature of energy metabolism, these oxidant sources are the most important oxidant origins in most cells, although there are several others, including peroxisomes, the endoplasmic reticulum (ER), cytochrome P450, xanthine oxidase, and NAD(P)H oxidases [52].

While superoxide radicals are often the primary oxidant formed in mitochondria, and H_2O_2 is often the most detected oxidant in mitochondria due to its diffusibility, it is important to emphasize the vast

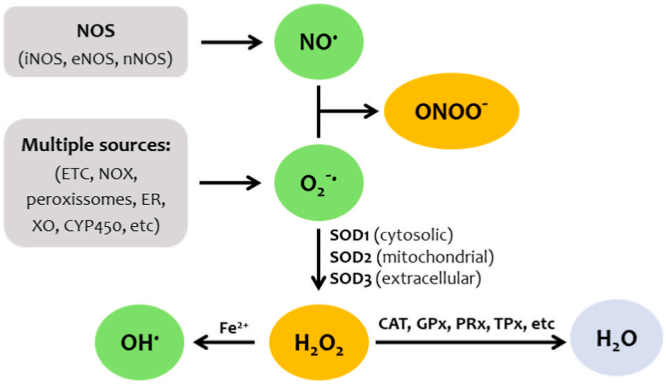

Fig. 2. Different reactive oxygen species (ROS) and how they are formed. Nitric oxide ($NO^{\cdot}$) is formed by nitric oxide synthases (NOS) isoforms. Superoxide ($O_2^{\cdot-}$) can be formed by multiple sources, including the mitochondrial electron transport chain (ETC), NADPH oxidases (NOX), peroxissomes, endoplasmic reticulum (ER), xanthine oxidase (XO), cytochrome P450 (CYP450), among others. $O_2^{\cdot-}$ can react with $NO^{\cdot}$ to form peroxynitrite ($ONOO^-$). $O_2^{\cdot-}$ can also be converted into hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD), found in different mitochondrial and cellular compartments. H_2O_2 can form the hydroxyl radical ($OH^{\cdot}$) by the Fenton reaction in the presence of Fe^{2+}, or can be removed by antioxidant systems, such as catalase (CAT), glutathione peroxidases (GPx), peroxiredoxins (PRx), thioredoxin peroxidases (TPx), among others. ROS in green are radicals; ROS in yellow are non-radicals.

differences in reactivity of these oxidants [53]. Indeed, the blanket-term "ROS" includes oxygen radicals and non-radicals that are chemically reactive, such as $O_2^{\cdot-}$, H_2O_2, and the hydroxyl radical (see Fig. 2). These molecules display a vast difference in reactivity rates, affinities, and stability [53,54].

Irreversible and cumulative modifications to proteins and nucleic acids promoted by the more reactive species of ROS throughout life can lead to oxidative damage, age-related tissue deterioration, and the development of pathological conditions [55,56]. Indeed, cells possess many specialized intracellular antioxidant systems to protect against ROS overproduction and remove these undesirable oxidants. In mitochondria, antioxidant systems involve superoxide dismutases (SODs) in the matrix and intermembrane space, removing $O_2^{\cdot-}$; as well as catalases (CAT) and thiol peroxidase systems (glutathione and thioredoxin) to eliminate H_2O_2 [57]. Importantly, these mitochondrial thiol peroxidase/reductase systems use NADPH in the mitochondrial matrix, a separate pool of the electron donor found in the cytosol. This is relevant in rodent studies, as much of the NADPH in mitochondria is reduced by the mitochondrial NAD(P) transhydrogenase (NNT), which was discovered to be absent in C57BL/6J mice due to a spontaneous mutation [58] associated with redox imbalance [59]. The lack of physiological mitochondrial H_2O_2 removal in these animals should be considered in studies that use this highly common animal background [60], although it is often difficult to determine if this strain was used, as in many studies this is unspecified.

Oxidative stress and redox imbalance are a result of ROS overproduction and/or reduced removal of oxidants by antioxidant systems, with a disruption of redox signaling and molecular damage [61,62]. However, while often associated with damaging effects, the reactivity of ROS can be useful, such as for the elimination of pathogens by immune cells [63,64]. Additionally, as we evolved with the production of these species, many ROS (particularly those with higher stability and diffusion) are also important signals in non-destructive intracellular pathways [65,66]. Perhaps the most known example of free radical-mediated signaling is nitric oxide ($NO^{\cdot}$), produced by different nitric oxide synthases (NOS) and involved in a myriad of cellular and physiological responses, including mitochondrial biogenesis [32]. Metabolically, $NO^{\cdot}$ signaling results in regulation of mitochondrial mass in response to alternate day feeding [33].

While these effects of less reactive ROS may promote signaling, more reactive and accumulation of ROS is frequently associated with tissue damage and dysfunction. The free radical theory of aging, which was first suggested by Harman in the 1950s [67] and later perfected [68,69], states that cumulative oxidative damage to cells as a result of aerobic metabolism leads to aging. Indeed, many studies, including those with model organisms such as *Drosophila*, show increased ROS production during aging, correlating with mitochondrial dysfunction [70]. However, in an indication that ROS effects are complex and depend on the species, location, quantities, and targets, the authors of this work also show that mitochondrial ROS produced by reverse electron transfer in Complex I generate signaling effects that actually enhance lifespans. This is compatible with the finding that reverse electron transfer through complex I in Drosophila is an important beneficial stress response pathway [71]. These studies thus highlight the complex roles of ROS in aging, in which there is also ample evidence of many different oxidative modifications and redox signaling involved in pathological and degenerative changes associated with more advanced ages [66].

5. Caloric restriction, oxidants, and antioxidants

Weight loss, such as that promoted by CR, has proven to be beneficial for animals and humans in many aspects, including life span extension. Numerous studies in animal models and preliminary human studies have explored the impacts of CR during aging and in preventing age-related diseases, as well as the intracellular mechanisms involved, which are undoubtedly related in a large part to changes in metabolic processes. We will discuss this literature in terms of the various animal models, different CR protocols, and the different tissues evaluated, in order to unravel the impacts of CR on redox processes. Of note, for all literature mentioned here, we have included, in order of appearance in the text, details of the specific dietary intervention used, animal model, and redox methodologies adopted in Table 2, given that methodological specificities are of utmost importance in these studies [72].

Several lines of evidence indicate that CR leads to changes in mitochondrial metabolism and the generation of oxidants, decreasing oxidative stress markers. Early studies showed that a CR protocol led to decreased mitochondrial superoxide and H_2O_2 production and lower carbonyl content relative to AL in homogenates of the brain, heart, and kidney throughout the life span of mice [40]. There was no clear pattern of age-related or dietary-related changes in activities of the antioxidant enzymes SOD, catalase, and glutathione peroxidase (GPx) in this study [40].

In addition to preventing the increase in lipid peroxidation markers, a CR diet also prevented age-induced increases in apoptosis markers in rat kidneys [73]. A similar CR protocol in mice reversed the age-associated increases in carbonyl and sulfhydryl protein oxidation markers for different brain regions, while retarding age-associated decline in sensorimotor coordination [74]. A decrease of carbonylated protein markers in heart mitochondria was seen with CR, although no differences in H_2O_2 production relative to AL diets were observed [75]. In this sense, a CR diet was also shown to protect from cardiac hypertrophy in mice exposed to isoproterenol [76], isoproterenol-induced H_2O_2 release, and isoproterenol-induced decrease in antioxidant enzyme activities [76].

The aging process leads to an oxidizing shift in the glutathione redox state and an overall more pro-oxidizing environment, with attenuation by a CR diet [77]. These authors saw large variations in the concentrations of reduced glutathione (GSH), oxidized glutathione (GSSG), and protein-glutathione mixed disulfides (protein-SSG) from different tissues analyzed (liver, kidney, heart, brain, eye, and testis). During aging, the GSH/GSSG ratio in mitochondria and tissue homogenates decreased due to increased GSSG content, while the protein-SSG content increased significantly. CR induced a decrease in GSSG and protein-SSG contents. In this sense, microarray analysis results indicate that weight loss in obese postmenopausal women decreased the expression of GSTA4, a

Table 2
Effects of CR on redox state.

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on ROS	Methods for ROS measurement	Effects of CR on other relevant parameters
Rojas et al., 1993 [100]	13 week male OF1 mice	• CR: 40 % less calories than AL OR • 40 % less carbohydrates than AL	8 weeks	Liver	N/A	N/A	• No changes in activities of antioxidant enzymes (SOD, catalase, GPx) • CR: increased GSSG and decreased GSH/GSSG ratio • Carb restriction, but not CR, increased sensitivity to peroxidation
Sohal et al., 1994 [40]	4 month male C57BL/6NNia mice	40 % less calories than AL	Up to 9, 16 and 23 months	Brain, heart, and kidney	Decreased mitochondrial superoxide and H_2O_2 production	• Superoxide: SOD-inhibitable reduction of acetylated ferricyto-chrome c • H_2O_2: PHPA in the presence of horseradish peroxidase	• Lower carbonyl content • No clear pattern of age-related or dietary-related changes in activities in antioxidant en-zymes (SOD, cata-lase, and GPx)
Cadenas et al., 1994 [101]	13 week male OF1 mice	• CR: 40 % less calories than AL OR • 40 % less carbohydrates than AL	8 weeks	Kidney	N/A	N/A	• Carb restriction: increased GPx activity, no changes in SOD and catalase • CR and carb restriction: decreased GSSG and increased GSH/GSSG ratio • Carb restriction: decreased lipid peroxidation
Dubey et al., 1996 [74]	4 month male C57BL/6Nnia mice	40 % less calories than AL	Up to 8, 21 and 27 months	Different brain regions	N/A	N/A	• Prevention of increased carbonyl and sulfhydryl levels
Lass et al., 1998 [89]	4 month male C57BL/6Nnia mice	40 % less calories than AL	Up to 20 months, then reversed to AL for 6 weeks	Skeletal muscle	Protection from age-induced increase in mitochondrial superoxide	SOD-inhibitable reduction of acetylated ferricytochrome c	• Protection from age-induced oxidative damage to mito-chondrial proteins and lipids • Increased catalase activity • Mitochondrial oxidative damage could not be reversed by CR or induced by AL diet
Lee et al., 1999 [85]	1.5 month male Fischer 344 rats	40 % less calories than AL	Up to 6 and 24 months	Heart	Decreased ROS induced by *tert*-butyl hydroperoxide	H_2DCF-DA fluorescence	Prevented age-induced decrease in mitochondrial membrane fluidity and alteration of fatty acid composition
Lass et al., 1999 [80]	4 month male C57BL/6Nnia mice	40 % less calories than AL	Up to 6, 12 and 24 months	Skeletal muscle	N/A	N/A	Prevention against age-induced decrease of the antioxidant CoQ9 in mitochondria
Pamplona et al., 2002 [75]	2 month male Wistar rats	40 % less calories than AL	4 months	Heart	No differences in H_2O_2 production	Homovanillic acid in the presence of horseradish peroxidase	• Decreased carbonylated protein levels
Lin et al., 2002 [108]	*S. cerevisiae*	Reduced glucose content in the media (from 2 % to 0.5 %)		N/A	N/A	N/A	• Increased oxygen consumption rates • Metabolic shift toward respiration is necessary for CR-induced life span extension
Rebrin et al., 2003 [77]	4 month male C57BL/6Nnia mice	40 % less calories than AL	Up to 26 months	Liver, kidney, heart, brain, eye, and testis	N/A	N/A	Decreased GSSG and protein-SSG contents (protection from age-induced pro-oxidizing environment)

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Table 2 (*continued*)

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on ROS	Methods for ROS measurement	Effects of CR on other relevant parameters
Bevilacqua et al., 2004 [86]	Male $FBNF_1$ rats	40 % less calories than AL	Up to 2 weeks, 2 and 6 months	Skeletal muscle	Decreased mitochondrial H_2O_2 release	PHPA in the presence of horseradish peroxidase	• 2 week CR: decreased protonmotive force • 2 month CR: increased protonmotive force • 6 month CR: decreased whole body oxygen consumption (open-circuit indirect calorimeter) and increased UCP3 protein expression
Sanz et al., 2004 [97]	Male Wistar rats	40 % less proteins than AL without strong CR (8.5 % less calories than AL)	7 weeks	Liver	Decreased mitochondrial H_2O_2 release	Homovanillic acid in the presence of horseradish peroxidase	• No changes in mitochondrial oxygen consumption rates • Decreased oxidative damage to nuclear and mitochondrial DNA
Bua et al., 2004 [106]	14 week Fischer Brown Norway hybrid ($FBNF_1$) rats	40 % less calories than AL	Up to 36 months	Skeletal muscle	N/A	N/A	• CR limited the onset of electron transport system abnormalities during aging (associated with fiber loss) • CR was not able to impact on the progression once the abnormality was already established
De Cabo et al., 2004 [82]	1 month male Fischer 344 rats	40 % less calories than AL	Up to 8 and 25 months	Liver plasma membrane	N/A	N/A	Attenuated age-related decrease in CoQ10/CoQ9 ratio and α-tocopherol
Kamzalov & Sohal, 2004 [81]	4 month Fischer 344 rats	40 % less calories than AL	Up to 19 months	Liver, heart, and kidney	N/A	N/A	• Prevention from age-induced decrease of mitochondrial CoQ • Decreased α-tocopherol content
Lee et al., 2004 [73]	6 week male Fischer 344 rats	40 % less calories than AL	Up to 12 and 24 months	Kidney	N/A	N/A	• Prevention of increased lipid peroxidation (MDA + HNE) in cytosolic and mitochondrial fractions • Prevention of age-induced apoptotis (Bax protein and cytochrome *c* release)
Bevilacqua et al., 2005 [87]	6 month male $FBNF_1$ rats	40 % less calories than AL	Up to 12 and 18 months	Skeletal muscle	Decreased mitochondrial H_2O_2 release	PHPA in the presence of horseradish peroxidase	• Decreased whole body oxygen consumption (open-circuit indirect calorimeter) • Decreased mitochondrial lipid peroxidation (TBARS) • Increased UCP3 protein expression
Heilbronn et al., 2005 [92]	20–55 year men and women	Alternate day fasting: *ad libitum* access to food on alternating days. Feasting days: double the usual intake to avoid losing body weight	3 weeks	Skeletal muscle biopsies	N/A	N/A	• Glucose response to a meal: slightly impaired in women, but no changes in men • Insulin response to a meal: unchanged in women and reduced in men • Increased SIRT1 mRNA expression in muscle biopsies

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Table 2 (*continued*)

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on ROS	Methods for ROS measurement	Effects of CR on other relevant parameters
López-Lluch et al., 2005 [83]	Male C57BL/6J mice (just after weaning)	40 % less calories than AL	Up to 3 and 23 months	Liver plasma membrane	N/A	N/A	Attenuated age-related decrease in CoQ10/CoQ9 ratio and α-tocopherol
Tirosh et al., 2005 [112]	6–7 month female αMUPA mice	Spontaneously eat less and live longer	N/A	Liver	N/A	N/A	• Decreased SOD2 (expression and activity) and GPx activity • Increased mitochondrial aconitase activity and reduced lipid peroxidation
Hyun et al., 2006 [84]	Male Fischer 344 rats	40 % less calories than AL	Up to 6, 12, 18 and 24 months	Brain plasma membrane	N/A	N/A	• Enhanced CoQ10 levels and α-tocopherol • Decreased age-induced oxidative damage to proteins and lipids
Rohrbach et al., 2006 [79]	4 and 23 month male Sprague-Dawley rats	~12 % less calories than AL	2 months	Heart and skeletal muscle	Increased mitochondrial H_2O_2 release after TrxR2 silencing (siRNA) in C2C12 myoblasts exposed to ceramide or TNF	Amplex Red in the presence of horseradish peroxidase	• Prevented age-induced decrease of TrxR2 levels • Thioredoxins from both mitochondria (Trx2) and the cytosol (Trx1) were not affected by CR
Minamiyama et al., 2007 [121]	4 week male OLEFT rats (T2D model)	30 % less calories than AL	Up to 24 and 42 weeks	Heart and aorta	Prevention of diabetes-induced ROS increase	• Heart sections: MTR oxidation by ROS (oxidized product binds to thiol groups and proteins within mitochondria) • Aorta sections: L-012 chemiluminescence (luminol derivate with a high sensitivity for ROS)	Prevention of diabetes-induced increase in UCP2 expression, increase in lipid peroxidation markers, and increase in eNOS
Ayala et al., 2007 [98]	Male Wistar rats	40 % less proteins than AL without strong CR (8.5 % less calories than AL)	7 weeks	Liver	N/A	N/A	• Decreased protein oxidative damage • Altered fatty acid composition (total liver and liver mitochondria)
Tahara et al., 2007 [50]	*S. cerevisiae*	Reduced glucose content of the media (from 2 % to 0.5 %)	16 h or 64 h	N/A	Decreased mitochondrial H_2O_2 release	Amplex Red in the presence of horseradish peroxidase	Increased oxygen consumption rates
Caldeira da Silva et al., 2008 [113]	18 week female Swiss mice	CR-like phenotype: low doses of DNP in the drinking water (1 mg/L)	1 or 5 months	Brain, liver, and heart	Reduced mitochondrial H_2O_2 release	Amplex Red in the presence of horseradish peroxidase	• Increased oxygen consumption rates • Reduced oxidative stress markers (protein carbonyls and 8-OHdG)
Asami et al., 2008 [88]	2 month female C57BL/6J and UCP3 knockout mice	30 % less calories than AL	Up to 8, 19, and 26 months	Skeletal muscle	N/A	N/A	• CR: decreased lipid peroxidation • UCP3 KO: Increased lipid peroxidation (TBARS)
Caro et al., 2009 [99]	7 week male Wistar rats	40 % methionine-restricted diet	7 weeks	Brain and kidney	Decreased mitochondrial H_2O_2 release	Homovanillic acid in the presence of horseradish peroxidase	• Decreased oxidative damage to kidney mitochondrial DNA • Decreased markers of mitochondrial protein oxidation, lipoxidation, and glycoxidation in both tissues
Hirschey et al., 2010 [120]	12 week SIRT3−/− 129Sv male mice	Fasting	24 h	Liver and brown adipose tissue	N/A	N/A	WT: increased SIRT3 expression SIRT3−/−: reduced ATP levels and intolerance to cold exposure

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Table 2 (*continued*)

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on ROS	Methods for ROS measurement	Effects of CR on other relevant parameters
Hayashida et al., 2011 [94]	6 week male Wistar rats	30 % less calories than AL	Up to 6 and 24 months	Liver	Long-term IF: decreased mitochondrial ROS release with samples collected on a feasting day, but no changes with samples collected on fasted days	H_2DCF-DA fluorescence	Differences between IF-fed and IF-fasted were observed in liver mitochondrial GSH and GSSG levels, GSH/GSSG ratio and SOD2 activity
Cerqueira et al., 2011 a [29]	4 week male Sprague-Dawley rats	• CR: 40 % less calories than AL • Intermittent feeding (IF): *ad libitum* access to food on alternating days • Food restriction (FR): CR without micronutrient supplementation	4 weeks or 8 months	Adipose tissue and skeletal muscle	• CR and AL: similar H_2O_2 release in both tissues • long-term IF: increased H_2O_2 in abdominal adipose tissue and skeletal muscle • long-term FR: increased H_2O_2 release in skeletal muscle	Amplex Red in the presence of horseradish peroxidase	• All restricted diets in short-term: decreased body weight, but no differences in glucose tolerance • Long-term IF: glucose intolerance and increased insulin receptor inactivation (insulin receptor nitration) • All restricted diets: enhanced iNOS levels
Cerqueira et al., 2011 b [114]	4 week female Swiss mice	• CR-like phenotype: low doses of DNP in the drinking water (1 mg/L) • CR: 30 % less calories than AL	6 months	Skeletal muscle and visceral adipose tissue	N/A	N/A	DNP and CR: increased citrate synthase activity, cytochrome *c* oxidase, mitofusin-2, PGC-1α expression, and eNOS phosphorylation
McKiernan et al., 2011 [107]	8 and 14 year male rhesus monkeys	Food allotments were reduced 10 % per month for 3 months to reach a 30 % less calories than AL	6, 9, and 12 years	Skeletal muscle	N/A	N/A	Preservation of skeletal muscle fibers during aging, with protection against age-related increases in electron transport system abnormalities
Da Cunha et al., 2011 [111]	*S. cerevisiae*	Reduced glucose content of the media (from 2 % to 0.5 %)	16 h or 28 days	N/A	N/A	N/A	• Increased oxygen consumption rates • Improved GSSG/GSH ratio at 16 h (not maintained after 28 days) • Increased H_2O_2 removal (more efficient than control at 16h; without differences after 28 days) • Decreased protein oxidation markers (protein carbonyls) • Preserved ubiquitin-proteasome system activity
Chen et al., 2012 [95]	2 month male C57BL/6J mice	3 CR groups with 40 % less calories than AL, but with three different lipid sources: soya bean oil, fish oil, or lard	1 month	Liver	CR-fish oil: decreased mitochondrial ROS production	PHPA in the presence of horseradish peroxidase	Different profiles of liver mitochondrial phospholipid fatty acid composition according to the dietary lipid source
Finley et al., 2012 [117]	Mixed C57/BL6 and 129 background mice with a skeletal muscle-specific PGC-1α deletion	20 % less calories than AL for the first week and 40 % less calories than AL thereafter	11 weeks	Skeletal muscle	N/A	N/A	• PGC-1α: required for full CR-induced increases in mitochondrial gene expression and mitochondrial density • Muscle PGC-1α: not necessary for whole-body benefits of CR
Li et al., 2012 [102]	14 week male C57BL/6J and DBA/2J mice	10 % less calories than AL during the first week, 20 % in the	Up to 6 and 23 months	Skeletal muscle and liver	N/A	N/A	C57BL/6: CR attenuated age-induced protein

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Table 2 (*continued*)

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on ROS	Methods for ROS measurement	Effects of CR on other relevant parameters
		second week and 40 % in the third week and thereafter					damage in kidney and liver mitochondria DBA/2J: CR attenuated age-induced protein damage only in liver mitochondria
Cui et al., 2013 [46]	3 month male Fischer 344 rats	30 % less calories than AL	20 months	Kidney	N/A	N/A	• Decreased oxidative DNA damage (8-OHdG levels) • Protected against abnormal mitochondrial morphology and fewer autolysosomes induced by high caloric intake • Increased expression of autophagy and mitophagy markers
Hagopian et al., 2013 [104]	4 month male C57BL/6J mice	40 % less calories than AL	2 months	Different liver mitochondrial subpopulation	N/A	N/A	Different changes in enzyme activities and in oxidative damage markers in different mitochondrial subpopulations
Choi & Lee, 2013 [110]	*S. cerevisiae*	Reduced glucose content of the media (from 2 % to 0.5 %)	Up to 30 days	N/A	Reduced total ROS and mitochondrial superoxide	• Total ROS: H_2DCF-DA fluorescence • Mitochondrial ROS: MitoSOX fluorescence	• Sustained increase in ATP levels • No changes in oxidative damage in proteins and DNA
Cerqueira et al., 2016 [122]	Serum from male Sprague-Dawley rats	40 % less calories than AL	26 weeks	Pancreatic beta cell lines and primary islets exposed to the serum of the CR animals	N/A	N/A	• Increased glucose-stimulated oxygen consumption rates, ATP-linked respiration, and maximal respiration • Increased eNOS expression • Increased mitochondrial fusion rates, length, and connectivity • Protection from glucolipotoxicity
Smith et al., 2016 [78]	50–65 year obese postmenopausal women	Hypocaloric diet (30 % less calories) with high-protein content (0.8 or 1.2 g protein/kg/day)	Until 10 % weight loss, then energy intake modified to maintain stable body weight for 3–4 weeks	Skeletal muscle	N/A	N/A	Prevented beneficial adaptations in redox state: decreased GSTA4 expression (oxidative stress marker, member of the glutathione S-transferase family)
Menezes-Filho et al., 2017 [140]	8 week male Swiss mice	40 % less calories than AL	4 months	Liver	Decreased mitochondrial H_2O_2 release	Amplex Red in the presence of horseradish peroxidase	• No changes in OCR • Decreased NAD(P)H content • Protection against ischemia/reperfusion injury
Wang et al., 2017 [105]	14 week male $FBNF_1$ rats	10 % restriction at 14 weeks, 25 % restriction at 15 weeks, 40 % restriction at 16 weeks and thereafter	4–7 months	Different skeletal muscle fibers	N/A	N/A	Results dependent on the specific skeletal muscle fiber analyzed: • Increased insulin-stimulated glucose uptake • Reduced abundance of several mitochondrial electron transport and oxidative phosphorylation proteins
David et al., 2018 [76]	2 month male Swiss mice	40 % less calories than AL	3 weeks	Heart	Protection from isoproterenol-	Amplex Red in the presence of horseradish peroxidase	• Protection from isoproterenol-induced decrease in

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Table 2 (*continued*)

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on ROS	Methods for ROS measurement	Effects of CR on other relevant parameters
					induced H_2O_2 release		antioxidant enzymes (catalase, SOD and GPx) activities
Gültekin et al., 2018 [91]	3 month male Wistar rats	• IF: food available for 2 short periods of the day • IF + CR: IF with 20 % less calories	20 weeks	Hippocampal (HIPPON) and dorsal root ganglion (DRGN) neurons	• IF: increased ROS production • IF + CR: protection from IF-induced increase in ROS in HIPPON, but not in DRGN neurons	Dihydrorhodamine 123 fluorescence	IF: increased lipid peroxidation in HIPPON; protection by IF + CR • IF: decreased GPx activity; attenuation by IF + CR
Munhoz et al., 2020 [90]	4 week female Wistar rats	IF: *ad libitum* access to food on alternating days	3 months	Pancreatic islets	Increased superoxide release	Dihydroethidium fluorescence	Increased intra-abdominal adiposity, increased insulinemia, increased insulin resistance indexes
Serna et al., 2020 [103]	2 month male Sprague-Dawley rats	40 % less calories than AL	6 months	Skeletal muscle and heart	Decreased mitochondrial H_2O_2 release in the heart, with no changes in the skeletal muscle	Amplex Red in the presence of horseradish peroxidase	Decreased mitochondrial oxygen consumption rates in the heart (states 3, 4, and 3u) and skeletal muscle (only in state 3)
Liu et al., 2021 [93]	35–70 year overweight or obese women	• CR: 30 % less calories than AL • IF: 32 % of calories at breakfast on fasting days and 100 % on fed days	8 weeks	Skeletal muscle biopsies	N/A	N/A	• IF led to greater weight and fat loss than CR, while also reducing antioxidant enzymes (GPX1, SOD1 and SOD2) mRNA levels • Decreased insulin sensitivity when samples were collected after 24 h fasting, compared to an overnight fasting
Wallace et al., 2021 [96]	12 month male C57BL/6J mice	Ketogenic diet (KD): less proteins, almost no carbohydrates, and mostly fat	Up to 16 and 26 months	Skeletal muscle	N/A	N/A	• Fiber type shift and neuromuscular junction remodeling • Increased mitochondrial complexes levels • Increased citrate synthase activity, PGC-1α, SIRT1, and SIRT3 levels • Increased antioxidant enzyme (SOD2 and catalase) expression
Serna et al., 2022 [47]	2 month male Sprague-Dawley rats	40 % less calories than AL	6 months	Kidney	Increased H_2O_2 release	Amplex Red in the presence of horseradish peroxidase	Increased mitochondrial oxygen consumption rates (states 4 and 3u)
Munhoz et al., 2023 [123]	• Serum from male Sprague-Dawley rats • Plasma from male and female obese patients • Serum from C57BL/6J Adiponectin KO mice	• 40 % less calories than AL • N/A • N/A	26 weeks	Pancreatic beta cell lines and primary islets exposed to the serum or plasma	N/A	N/A	• CR serum/lean plasma: increased OCR • Adiponectin KO serum: decreased OCR • Adiponectin protected from the OCR decrease and insulin secretion failure induced by obese patients' plasma

member of the glutathione S-transferase family and an oxidative stress marker in skeletal muscle [78].

Skeletal and cardiac muscle mitochondria have lower thioredoxin reductase (TrxR2) levels during aging [79]. A CR diet was able to normalize TrxR2 levels to those similar to young individuals. Interestingly, thioredoxins from both mitochondria (Trx2) and the cytosol (Trx1) were not affected by CR. The use of TrxR2 siRNA in myoblasts followed by exposure to ceramide or TNF leads to an increase in nucleosomal DNA cleavage, caspase 9 activation, and mitochondrial H_2O_2 release.

Interestingly, while AL feeding in mice leads to a decrease in total coenzyme Q (CoQ) content in skeletal muscle mitochondria during

aging, CR increases CoQ9 [80], an important antioxidant, known to prevent oxidative damage to membranes. Indeed, CoQ content loss in skeletal muscle is a suggested aspect of aging [80]. Similarly, decrease of CoQ9 amounts in mitochondria from liver, heart, and kidneys of rats during aging has been detected, in a manner prevented by CR [81]. CR was also able to attenuate age-related declines in the CoQ10/CoQ9 ratio in the liver plasma membrane of rats [82] and mice [83], promoting increased membrane resistance to oxidative stress-induced lipid peroxidation. A similar CR protocol also protective of the deleterious effects of aging in rat neurons, by enhancing CoQ10 levels and decreasing age-induced oxidative damage to proteins and lipids in brain plasma membranes [84].

Besides altering oxidant production, antioxidant activity, and expression of oxidative stress markers, CR also seems to impact on mitochondrial membrane composition. AL diets lead to a decrease in membrane fluidity of heart mitochondria, with significant differences in fatty acid composition of the mitochondrial membranes during aging, while no differences in membrane fluidity nor in the composition of the mitochondrial membranes over time were observed in CR animals [85]. This may alter membrane responses to oxidative damage, as discussed above. Indeed, this group saw decreased ROS detection induced by *tert*-butyl hydroperoxide in the CR group.

6. Different dietary protocols and redox state

The length of time the dietary intervention is maintained can influence the outcomes. A medium-term CR diet decreased whole body cellular oxygen consumption [86]. Additionally, CR decreased H_2O_2 release of skeletal muscle mitochondria after 2 weeks, 2 months, and 6 months of the diet [86]. Interestingly, while short-term CR decreased protonmotive force, medium-term CR led to increases [86]. Consistently with a protection against ROS-induced damage, they also found increased uncoupling protein (UCP3) expression in skeletal muscle mitochondria after a long-term (18 months of diet) CR diet [87]. In this sense, UCP3 knockout mice have been shown to have greater concentrations of lipid peroxidation markers in skeletal muscle homogenates [88].

An interesting approach established a reversible protocol, where mice that received an AL or CR diet for 20 weeks were later fed the opposite diet for six weeks. The CR protocol protected from AL-induced increase in superoxide formation and from oxidative damage to proteins and lipids in the skeletal muscle [89]. Interestingly, the only effects that could be reversed were body weights; mitochondrial oxidative damage could not be reversed by CR or induced by the AL diet at the later time point evaluated.

As already mentioned, metabolic effects of several restriction protocols different from CR have been described. It is important to note that depending on the protocol, different redox results may be achieved, and these results may not always be beneficial. For instance, we have compared the short- and long-term effects (one and eight months of diet, respectively) of three different restriction protocols: a CR diet, intermittent feeding (IF, with AL access to food on alternating days), and food restriction (FR) without micronutrient supplementation, leading to malnutrition [29]. All restricted diets promoted a decrease in body weight and no differences were found in glucose tolerance following short-term protocols. However, long-term IF led to glucose intolerance, with an increase in insulin receptor inactivation (insulin receptor nitration) in both intra-abdominal adipose tissue and muscle. We also found that all three restriction protocols promoted enhanced nitric oxide synthase (iNOS) levels in the adipose tissue and skeletal muscle. However, while CR and AL had similar results regarding H_2O_2 release in both tissues, long-term IF significantly increased H_2O_2 production in abdominal adipose tissue and skeletal muscle, and long-term FR led to increased H_2O_2 release in skeletal muscle, possibly indicating the mechanisms involved in enhanced insulin receptor nitration, through formation of peroxynitrite. Those results point to potential long-term detrimental effects on glucose tolerance and redox status, depending on the restriction protocol implemented.

Similar detrimental effects have been found with a 3-month IF diet, with increases in intra-abdominal adiposity, increases in insulinemia, insulin resistance indexes (HOMA-IR and HOMA-β), and increased superoxide radical production in pancreatic islets [90]. Using a type of IF protocol where food was available for two short periods of the day for five months, ROS production was increased in primary hippocampal and dorsal root ganglion neurons [91]. Interestingly, the increase in hippocampal, but not dorsal root neurons, was prevented when animals were subjected to the same IF protocol, but with 20 % less calories [91].

In humans, different results were found in men and women following three weeks of an alternate day fasting protocol [92]. Patients were between 20 and 55 years old and were told to eat whatever they wanted on feasting days, but to double their usual intake to avoid losing body weight. Glucose response to a meal was slightly impaired in women after the protocol, but no changes were observed in men. Insulin responses to a meal were unchanged in women and reduced in men. Comparing a CR and an IF diet (30 % less calories than AL for 8 weeks) in overweight or obese women between 35 and 70 years old, IF led to greater weight and fat loss than CR, while also reducing antioxidant enzyme mRNA levels (GPX1, SOD1, and SOD2) [93].

Results obtained with IF protocols can also vary greatly depending on when the samples are processed. In rats, liver mitochondrial ROS release is decreased by long-term IF (6 and 24 months) when samples were collected on a feasting day (IF-fed), but no changes were observed between IF and AL when samples were collected on fasted days (IF-fasted) [94]. Differences between IF-fed and IF-fasted were also observed for mitochondrial GSH and GSSG levels, GSH/GSSG ratios, and SOD2 activity. Similarly, women exposed to an IF diet had decreased insulin sensitivity in skeletal muscle when samples were collected after 24 h fasting, compared to overnight fasting [93].

Several different restriction protocols have been tested, with some restricting the total amount of calories, while others restrict only one macronutrient (carbohydrate, protein, or lipid). Mice have been shown to present different liver mitochondrial phospholipid fatty acid profiles depending on the dietary lipid source [95]. Moreover, different lipid sources also influenced ROS production; fish oil, for example, decreased ROS production by complexes I and III.

A ketogenic diet with less proteins, almost no carbohydrates, and mostly fat has also been suggested to have beneficial effects on age-induced skeletal muscle deterioration [96]. The diet was linked to a fiber type shift and neuromuscular junction remodeling, in addition to increased mitochondrial complex levels and antioxidant enzyme expression (SOD2 and catalase) [96]. It is important to note that enzyme expression cannot be directly translated into activity.

While the lipid source seems to greatly impact on the effects of CR, protein intake may also be crucial. A protein-restriction diet (40 % less proteins than AL) without strong caloric restriction (8.5 % less calories than AL) leads to decreased mitochondrial H_2O_2 release, specifically at complex I, with no changes in mitochondrial oxygen consumption rates and a decreased proton leak, in addition to decreasing oxidative damage to nuclear and mitochondrial DNA [97] and decreasing protein oxidative damage [98]. A 40 % methionine-restricted diet promotes similar weight gain as control animals, but decreased mitochondrial H_2O_2 release in both brain and kidney mitochondria, together with lower oxidative damage to kidney mitochondrial DNA [99]. These results point to methionine as a central regulator of longevity extension by CR.

On the other hand, high protein intake in obese postmenopausal women avoided some of the weight-loss benefits in muscle insulin signaling. A microarray analysis indicated that a high protein intake prevents beneficial adaptations in redox state in muscle, such as increased skeletal muscle gene expression of PRDX3, a mitochondrial antioxidant member of the peroxiredoxin family which is increased during oxidative stress induction [78].

The group of Prof. Barja compared the effects of eight weeks of a CR

diet and pure carbohydrate restriction on the livers and kidneys from mice. No changes were observed in the activities of antioxidant enzymes (SOD and CAT) between both restrictions in the liver [100] and kidney [101]. However, CR and carbohydrate restriction increased kidney GSH/GSSG ratios, and carbohydrate restriction decreased *in vivo* lipid oxidation [101]. On the other hand, carbohydrate restriction, but not CR, increased sensitivity to lipid oxidation in the liver [100], showing that the protection observed in the CR diet may be due to a reduction in calories, and not to a reduction in carbohydrates. Moreover, their results point to the existence of important tissue-specific differences.

7. Organism- and tissue-specific effects of dietary interventions

Tissue specificity of CR effects was observed in DBA/2 mice, an animal model in which CR has relatively minor or no impact on life spans. Markers for oxidative damage to proteins were barely altered in skeletal muscle mitochondria from DBA/2 mice during aging or CR [102]. On the other hand, protein-HNE levels in liver mitochondria showed a significant increase with aging, with a protection by CR [102]. In this regard, we have also shown important tissue-specific effects of a CR diet in rat skeletal muscle, heart, and kidney [47,103]. CR led to decreased oxygen consumption rates in skeletal muscle and heart mitochondria [103], while increases were observed in kidney mitochondria [47]. Moreover, CR led to decreased mitochondrial H_2O_2 release in the heart, with no changes observed in the skeletal muscle [103], and increased H_2O_2 release in kidney mitochondria [47]. Those results point to unexpected deleterious redox effects in the kidney, and thus further indicate that beneficial effects of CR are highly tissue-dependent. Additionally, CR leads to different changes in enzyme activities and in oxidative damage markers in different mitochondrial subpopulations, and thus more subtleties can be found according to the mitochondrial subpopulation used in the assays [104], adding another layer to the complexity of understanding effects of CR on mitochondrial redox state.

Depending on the specific skeletal muscle fiber analyzed, CR seems to have multiple effects including increases in glucose uptake, reduction in the abundance of several mitochondrial electron transport components, as well as oxidative phosphorylation proteins [105]. In rats, CR limits the onset of electron transport system abnormalities during aging, which are associated with fiber loss [106]. However, CR was not able to impact on the progression once the abnormality was already established [106]. In male rhesus monkeys, years of a CR diet preserved skeletal muscle fibers during aging, with protection against age-related increases in electron transport system abnormalities [107].

CR protocols have also been explored in less complex model organisms. In *Saccharomyces cerevisiae* (*S. cerevisiae*), a CR protocol (reducing the glucose content of the media from 2 % to 0.5 %) originally questioned the idea that the extension of lifespan by CR is due to decreased oxidative metabolism and decreased ROS. The authors suggest that CR increased mitochondrial respiration (through a shift in metabolism that favors the tricarboxylic acid cycle), and therefore should increase ROS formation [108]. In this sense, we have shown that a CR protocol increases respiration in *S. cerevisiae*, but decreases mitochondrial H_2O_2 release, which in this model is mainly formed by matrix dihydrolipoyl dehydrogenases in an NADH-sensitive manner [50]. Interestingly, CR seems to induce *S. cerevisiae* life span extension through a hysteretic mechanism, or the tendency of a system to conserve properties in the absence of the time-limited stimulus that generated them [109]. Many hours after the complete exhaustion of glucose from the media, increased oxygen consumption rates were still present in CR yeast [109], as CR prepared the cells for earlier, faster, and more efficient metabolism of respiratory substrates. CR also leads to a sustained increase in ATP levels in *S. cerevisiae* during aging [110], and reduces the generation of total and mitochondrial oxidants, but without changes in oxidative damage to proteins and DNA [110]. Moreover, CR was also able to decrease protein oxidation markers in *S. cerevisiae*, together with a preservation of the ubiquitin-proteasome system activity, with great impact on protein modification and turnover [111], similarly to the effects of CR on animal autophagy and mitophagy discussed above [46].

A CR-like phenotype occurs in αMUPA mice, a transgenic model that spontaneously eats less and lives longer. Liver homogenates from female αMUPA mice have reduced SOD2 mRNA, protein, and activity under basal conditions, while maintaining the capacity to produce high levels of SOD2 when exposed to a proinflammatory stimulus [112]. Additionally, liver mitochondria have increased aconitase activity and reduced lipid oxidation, indicating protection against oxidative damage [112]. Other strategies, such as a mild mitochondrial uncoupling with low doses of 2,4-dinitrophenol (DNP) for 1- to 2-months, have also been found to promote a CR-like phenotype, with increased life span and reduced body weight, serum glucose, and triglyceride levels [113]. DNP also increased brain and liver mitochondrial oxygen consumption rates, while markedly reducing mitochondrial H_2O_2 release and oxidative stress markers [113]. Moreover, this protocol was found to impact on mitochondrial biogenesis similarly to CR [114]. Both CR and DNP increased citrate synthase activity, cytochrome *c* oxidase, mitofusin-2, and PGC-1α expression, as well as skeletal muscle and visceral adipose tissue eNOS phosphorylation and fasting plasma levels of $NO^{\cdot}$ products [114]. Of note, $NO^{\cdot}$ has been shown to increase mitochondrial biogenesis in various cell types [115], as discussed above.

Similarly, a ketogenic diet was linked to increased mitochondrial biogenesis, with augmented mitochondrial complexes levels, citrate synthase activity, PGC-1α, SIRT1, and SIRT3 levels [96]. PGC-1α, a transcriptional coactivator that regulates mitochondrial function [42,116], is known to be induced by CR [42]. To check if mitochondrial and metabolic benefits of CR were due to increased PGC-1α activity, Finley et al. [117] treated PGC-1α knockout mice with a CR diet and saw that although PGC-1α is essential for the CR-induced increases in mitochondrial gene expression and density in skeletal muscle, it is not necessary for the whole-body benefits of CR, because animals presented normal glucose homeostasis in response to CR in its absence. Importantly, a role for SIRT in the regulation of PGC-1α during CR and fasting has been shown [118,119]. In humans exposed to an IF protocol, there was an increase of SIRT1 mRNA expression in muscle biopsies [92]. As sirtuins (NAD^+-dependent protein deacetylases) normally mediate adaptive responses to stress, these results indicate prevention of apoptosis initiation, and thus increased stress resistance. In line with those results, SIRT3 expressed in the mitochondrial matrix is upregulated in the liver and brown adipose tissues of mice after a 24 h fasting protocol [120]. Furthermore, livers from SIRT3 knockout mice exhibit signs of defective fatty acid oxidation during fasting, such as reduced ATP levels as well as intolerance to cold exposure. A role for SIRT2 in the CR-induced increase in life span has also been suggested in *S. cerevisae* [108].

Using a type 2 diabetes animal model, CR was shown to prevent diabetes-induced oxidative stress in cardiovascular tissues [121]. Rat hearts and aortas from type 2 diabetic animals display increases in mitochondrial ROS production, increased UCP2 expression, increased lipid peroxidation markers, and increased endothelial nitric-oxide synthase (eNOS); CR was able to protect from all these oxidative stress markers.

We have also shown that pancreatic β cells exposed to serum from rats on a CR diet were protected from mitochondrial fusion arrest and reduced mitochondrial respiration promoted by glucolipotoxicity conditions, a metabolic syndrome model [122]. Pancreatic β cells exposed to serum from rats that received a CR diet under basal conditions also displayed increased glucose-stimulated oxygen consumption rates, ATP-linked respiration, and maximal respiration [122]. We recently demonstrated the central role of the adipose-tissue derived hormone adiponectin in CR-induced β cell protection [123]. While serum from adiponectin knockout mice decreased mitochondrial oxygen consumption, hormone supplementation protected from the decrease in cells exposed to plasma from obese human patients. Surprisingly, adiponectin alone improved glucose-stimulated insulin secretion and viability, in the

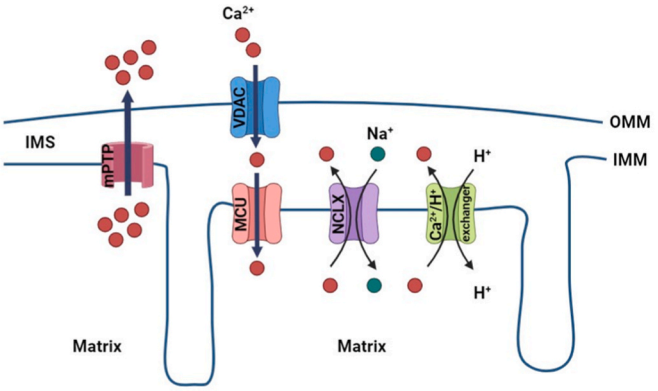

Fig. 3. Mitochondrial Ca^{2+} transport and dynamics. In mammalian cells, Ca^{2+} can transverse the outer mitochondrial membrane (OMM) through the voltage-dependent anion channel (VDAC). Ca^{2+} transport across the inner mitochondrial membrane (IMM) into the mitochondrial matrix is mediated by specific ion transporters. Ca^{2+} uptake is driven by the mitochondrial calcium uniporter (MCU), part of the mitochondrial Ca^{2+} uniporter complex MCUc. Removal of Ca^{2+} ions from the matrix occurs in exchange for Na^{+} ions, mediated by NCLX, or in exchange for protons (H^{+}). Mitochondria may also release large amounts of Ca^{2+} through a non-selective pathway, the mitochondrial permeability transition pore (mPTP). IMS: intermembrane space.

absence of any other serological factor [123]. The intracellular mechanisms are still undetermined, but may involve mitochondrial biogenesis and dynamics, since β cells exposed to CR serum had increased eNOS expression, mitochondrial fusion rates, length, and connectivity [122].

8. Mitochondrial calcium transport

Calcium ions are not only important intracellular signaling molecules due to changes in ion transport in the ER and plasma membrane, the canonic Ca^{2+}-modulatory transporters in the cell. These ions are also transported across the outer and inner mitochondrial membrane in mammals, and can be actively accumulated in the mitochondrial matrix [13,124–126] (see Fig. 3). Although mitochondrial Ca^{2+} uptake pathways have low affinity for the ion if compared to average intracellular levels, the uptake is facilitated by close association with ER membranes, creating a Ca^{2+}-enriched microenvironment. Once sufficient concentrations are present to allow for mitochondrial Ca^{2+} uptake in the cell, the capacity to accumulate these ions in mitochondria is very large when compared to the ER, due to matrix volume and buffering by phosphate. Due to the inner membrane potential and distinct transporters, regulatory properties governing uptake and release of Ca^{2+} from mitochondria are distinct from other cellular Ca^{2+} control sites. Ca^{2+} uptake and controlled release from mitochondria thus impacts both on mitochondrial function, as well as on cellular and subcellular Ca^{2+} dynamics.

Like many other small molecules and ions, Ca^{2+} can transverse the outer mitochondrial membrane through the voltage-dependent anion channel (VDAC). However, the inner mitochondrial membrane is inherently impermeable, a characteristic necessary to maintain membrane potentials and, hence, oxidative phosphorylation. Therefore, Ca^{2+} transport through the inner membrane into the mitochondrial matrix is mediated by specific ion transporters. Uptake of the ion, driven by the negatively-charged matrix, occurs primarily through the mitochondrial calcium uniporter (MCU) [127,128], part of the mitochondrial calcium uniporter complex (MCUc), which includes other modulatory components such as EMRE and MICU proteins [129–131]. Removal of Ca^{2+} ions from the matrix occurs in exchange for Na^{+} ions, mediated by NCLX, or in exchange for protons, mediated either directly by LETM1 [131,132] or by TMBIM5, an interactor of LETM1 recently shown to display Ca^{2+}/H^{+} exchange activity in mitochondria [133].

Mitochondria may also release Ca^{2+} through a non-selective pathway, the mitochondrial permeability transition pore (mPTP). This pore releases not only ions but any small molecule, and therefore is incompatible with the maintenance of functional oxidative phosphorylation. It is inhibited by cyclosporin A, which acts by binding and inhibiting mitochondrial cyclophilin D, a peptidyl-prolyl *cis*-trans isomerase involved in mPTP formation. Although temporarily reversible under specific conditions, mPTP is most often associated with a loss of function of individual mitochondria or more widespread cellular loss of bioenergetic function under pathological conditions. Indeed, it is opened by conditions of mitochondrial Ca^{2+} overload associated with oxidative imbalance, oxidation and cross-linkage of inner mitochondrial membrane protein thiols [57,134–138].

Table 3
Effects of CR on mitochondrial Ca^{2+} transport.

Reference	Model	Type of restriction	Duration of restriction	Tissue(s) evaluated	Effects of CR on Ca^{2+}	Effects of CR on other relevant parameters
Amigo et al., 2017 [19]	• 2 month male Swiss mice • 2 month male Sprague-Dawley rats	40 % less calories than AL	14 weeks	Brain mitochondria	Increased CRC	• Increased antioxidant enzyme activities (GPx-1, GR and SOD2) • Increased complex I + III and IV activities • Increased protein expression of Drp-1, Mfn-2, Sirt3
Menezes-Filho et al., 2017 [140]	8 week male Swiss mice	40 % less calories than AL	4 months	Liver mitochondria	Increased CRC	• No changes in OCR • Decreased mitochondrial H_2O_2 release
Menezes-Filho et al., 2019 [141]	8 week male C57BL/6 N mice	Fasting	Overnight (16 h) or 4 h	Liver mitochondria	Decreased CRC	• Decreased OCR (states 3 and 3u) • Decreased mitochondrial membrane potentials
Serna et al., 2020 [103]	2 month male Sprague-Dawley rats	40 % less calories than AL	6 months	Skeletal muscle and heart mitochondria	No changes in CRC	• Decreased mitochondrial H_2O_2 release in the heart, with no changes in the skeletal muscle • Decreased mitochondrial OCR in the heart (states 3, 4, and 3u) and skeletal muscle (only in state 3)
Serna et al., 2022 [192]	2 month male Sprague-Dawley rats	40 % less calories than AL	6 months	Kidney mitochondria	Decreased CRC and MICU2 protein expression	• Increased H_2O_2 release • Increased mitochondrial OCR (states 4 and 3u)
Ramos et al., 2024 [168]	8 week male Swiss mice	40 % less calories than AL	4 weeks	Liver mitocondria	Increased NCLX expression	• Hepatocyte cell line (AML12) exposed to a condition of serum and amino acid starvation had decreased NCLX expression • siNCLX or CGP (NCLX inhibitor) led to decreased autophagy activity in AML12 cells

9. Caloric restriction changes mitochondrial Ca^{2+} transport

In 2017, while investigating mechanisms through which CR prevents cerebral pathological conditions, we found that the dietary intervention strongly protected neurons against excitotoxic insults [19] (see a summary for this section in Table 3). As excitotoxicity involves intracellular Ca^{2+} overload [139], which can be prevented by the significant Ca^{2+} uptake capacity of mitochondria, we investigated if rodents maintained on a CR diet had changes in mitochondrial Ca^{2+} transport, and found that the same amount of mitochondria were capable of taking up more Ca^{2+} at a faster rate when isolated from CR forebrains. This increase in Ca^{2+} uptake speed and capacity was related to changes in Sirt3 expression, altering cyclophilin D acetylation and activity, as well as enhanced activity of mitochondrial antioxidant enzymes, overall resulting in mPTP prevention [19].

In addition to explaining mechanisms in which CR prevents excitotoxicity, this finding was, to our knowledge, the first indication in the literature that a physiological nutritional change modulated mitochondrial Ca^{2+} transport properties. It thus provided a new link between three important metabolic hubs: caloric supply, mitochondria, and calcium. Interestingly, changes in mitochondrial calcium uptake were not limited to the brain, but were also found in liver and kidney mitochondria [47,140], but not heart and skeletal muscle [103]. Not only was maximal Ca^{2+} uptake capacity modulated by CR, but also uptake rates, in a cyclosporin A-insensitive manner, indicating that not only the mPTP, but also MCUc activity was modulated by diet. While liver mitochondria from CR animals displayed both increases in Ca^{2+} uptake rates and capacity [140], CR kidney mitochondria displayed higher Ca^{2+} uptake rates related to changes in MICU2 expression, but lower Ca^{2+} retention capacity due to premature mPTP opening, associated with enhanced H_2O_2 production [47]. Not only were results tissue-specific, but also different with modified dietary interventions. While chronic CR increased Ca^{2+} uptake in liver mitochondria, acute overnight fasting decreased uptake capacity and increased mPTP opening probability [141].

Changes in supply and demand of substrates are also well known to impact on mitochondrial plasticity, altering organellar dynamics, trafficking within the cell, and morphology [17,142]. Indeed, rather than isolated individual organelles, mitochondria are shape-changing entities which are constantly undergoing fission and fusion as well as changing locations and exchanging components over time, forming a dynamic network. A well-studied *in vitro* condition that promotes changes in mitochondrial dynamics is nutrient overload, such as incubation of cells with excess fatty acids (lipotoxicity) and/or glucose (glucotoxicity), often promoting extensive fragmentation of the mitochondrial network, hampering the dynamic exchange of mitochondrial components over time, and resulting in functional impairment [143–149]. While fatty acids can impact on mitochondrial morphology, intriguingly the opposite is also true: multiple interventions that promote changes in mitochondrial shape impact on fatty acid oxidation by modulating carnitine palmitoyltransferase 1 (CPT1) by malonyl-CoA. Fragmented mitochondria present lower CPT inhibition, and therefore higher mitochondrial fatty acid oxidation [150]. Interestingly, lipotoxicity can also modulate glycolytic rates, in a manner dependent on mitochondrial redox signaling, but not changes in morphology [148].

In β cells, mitochondrial fragmentation is a mechanism in which glucolipotoxicity promotes cell death, as preventing fragmentation rescues cell survival [143]. Interestingly, serum from CR rats used in substitution for typical cell culture sera was able to preserve β cell mitochondrial morphology with fatty acid and glucose overload [122]. As discussed above, incubation of primary or β cell lines with sera from obese humans or AL-fed obese rats leads to inadequate mitochondrial oxidative phosphorylation and insulin secretion [123], which could be fully restored by replenishing adiponectin, a hormone which is decreased in obesity. Surprisingly, adiponectin alone, in the absence of other serological components, was capable of maintaining β cell integrity, oxidative phosphorylation, and glucose-stimulated insulin secretion [123], demonstrating the importance of this hormone in mitochondrial morphology, oxidative phosphorylation, and β cell viability in response to changes in food intake and obesity.

Interestingly, mitochondrial morphology is strongly related to Ca^{2+} signaling. Changes in intracellular calcium can arrest mitochondrial motility and change morphology by modulating human dynamin-related protein Drp1, required for fission [151]. Indeed, DRP1-mediated mitochondrial fission occurs at positions in which the ER contacts mitochondria [152,153], sites in which Ca^{2+} released from the ER can be taken up by mitochondria [154–157]. Conversely, changes in mitochondrial morphology also affect both mitochondrial Ca^{2+} uptake and cellular Ca^{2+} homeostasis, modulating store-operated calcium entry and ER Ca^{2+} levels [43,158]. Overall, these studies show a net of interactions connecting substrate availability, Ca^{2+} signaling, mitochondrial structure and function.

10. Consequences of changes in mitochondrial Ca^{2+} transport

The ability of mitochondria to take up Ca^{2+} was recognized early on as a means to activate matrix enzymes such as pyruvate, isocitrate, and α-ketoglutarate dehydrogenases, relaying to mitochondrial metabolic pathways responses to hormones and other extracellular messengers that modulate intracellular Ca^{2+} levels [159,160]. Activation of pyruvate dehydrogenase occurs through interaction of Ca^{2+} with its phosphatase, removing the inhibitory phosphorylation sites [161], while isocitrate and α-ketoglutarate dehydrogenases present higher affinity for their substrates in the presence of Ca^{2+} [162,163]. Ca^{2+} ions can also increase the activity of respiratory chain complexes and substrate transporters, as determined by flux analysis in skeletal muscle mitochondria [164].

Using modern flux analysis techniques in isolated liver mitochondria and intact hepatic cells, we find that uptake of Ca^{2+} in mitochondria increases electron transfer capacity in a substrate and quantity-dependent manner [165]. However, the positive effect occurs within a limited window of Ca^{2+} quantities, since higher mitochondrial Ca^{2+} loads hamper oxidative phosphorylation due to mPTP opening, in a Goldilocks-type effect. Interestingly, in intact cells, we find that baseline mitochondrial Ca^{2+} concentrations coincide with the Goldilocks setpoint to maintain maximum electron transport capacity. While cells are expected to keep mitochondrial Ca^{2+} below the threshold that induces mPTP, it is interesting to see they maintain maximized electron transport capacity, with no reserve for induced enhanced Ca^{2+} signaling. Compatibly, liver MCU deletion reduces oxidative phosphorylation, while also disrupting cytosolic Ca^{2+} homeostasis, resulting in enhanced hepatic lipid accumulation [166]. Also in liver, CR can modulate Ca^{2+} uptake activity and increase the threshold of mitochondrial Ca^{2+} necessary to induce mPTP. As a result, the dietary intervention strongly prevents damage due to mPTP-mediated ischemia-reperfusion in this organ [140].

An important aspect of CR is that it upregulates autophagy, while obesity attenuates this important mechanism for the removal of damaged cell components and nutrient availability [167]. We find that CR and autophagy-inducing conditions such as nutrient deprivation strongly induce the expression of mitochondrial Ca^{2+} removal pathway NCLX in liver and hepatic cells [168]. Conversely, knockdown or inhibition of NCLX strongly impairs autophagy, by changing intracellular Ca^{2+} levels, while oxidative phosphorylation was unchanged, maintained within the "Goldilocks threshold". On the other hand, decreases in mitochondrial Ca^{2+} uptake limiting oxidative phosphorylation and ATP production activate macroautophagy through the AMPK pathway [169].

In brain, enhanced mitochondrial Ca^{2+} uptake promoted by CR diets is associated with prevention of excitotoxic neuronal damage [19], given its property to prevent cytosolic Ca^{2+} overload. Ca^{2+} uptake in mitochondria is important in synaptic modulation, switching from

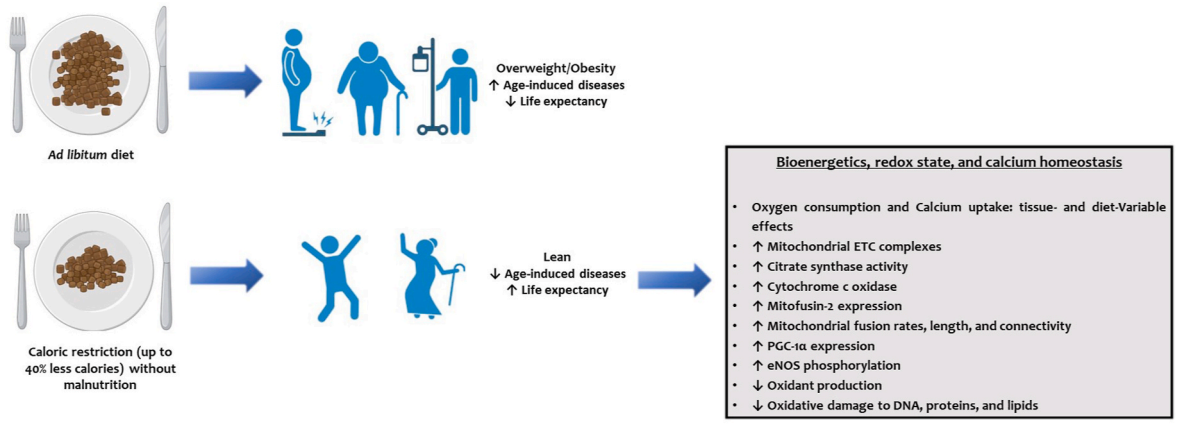

Fig. 4. Maintenance of healthy body weights is associated with multiple bioenergetic and redox state changes, increased life- and health-spans.

glycolytic to oxidative metabolism [170]. Interestingly, cognitive ability can also be modulated by changes in astrocyte mitochondrial Ca^{2+} transport: inhibiting NCLX in these cells can increase lactate secretion, and improve novel object recognition [171]. The effect is compatible with the important effects of astrocyte Ca^{2+} homeostasis uncovered in the recent literature [172], and occurs without significant changes in electron transport in the astrocytes, which therefore maintain mitochondrial Ca^{2+} within the "Goldilocks range". Indeed, the most important effect of NCLX inhibition in this model seems to be its impact on cytosolic Ca^{2+} regulation, as well as activation of the glycolytic pathway [171], underlying the importance of mitochondrial Ca^{2+} transport and more specifically NCLX in the regulation of cytosolic Ca^{2+} homeostasis [173]. NCLX is also critically important for neuronal function, as demonstrated by the fact that a human recessive variant with impaired activity is associated with severe cognitive impairment, and knockout mice display disrupted synaptic activity [174]. In neurons from these animals, the Goldilocks mitochondrial Ca^{2+} threshold was surpassed, since membrane potentials and mitochondrial Ca^{2+} influx were disrupted. Indeed, neuronal protection against excitotoxic insults depends on NCLX, which also mediates the enhanced novel object recognition effects of phosphodiesterase 2 blocker Bay 60–7550 [175].

NCLX loss of activity can also lead to mitochondrial Ca^{2+} overload and mPTP opening in heart, while cardiomyocyte-specific NCLX overexpression is protective against heart failure in some models, although the animals displayed other deleterious characteristics [176,177]. Others have found NCLX inhibition to be protective in heart failure [178,179], indicating the effects are model- and context-specific. Although contrasting, these results reinforce an important role for mitochondrial Ca^{2+} homeostasis in the heart. Indeed, mitochondrial Ca^{2+} uptake through the MCU supports stimulated increased heart contractility and regulates metabolic substrate selection [180,181]. But MCU activity is not always desirable in the heart: because excess Ca^{2+} uptake promotes mPTP, in ischemia reperfusion, loss of MCU activity can be protective [182,183].

Finally, and coming full circle, changes in mitochondrial Ca^{2+} transport, as well as changes in mitochondrial redox state discussed previously, regulate pancreatic β cell function, which is by nature related to responses to nutrient availability and CR. Beta cells respond to increases in circulating glucose concentrations with enhanced mitochondrial ATP production, which promotes ATP-sensitive plasma membrane K^+ channel closure and activates Ca^{2+} influx through plasma membrane voltage-gated channels, resulting in insulin release. Silencing MCU inhibits sustained cytosolic increases in cellular ATP and attenuates glucose-stimulated insulin secretion, demonstrating that mitochondrial Ca^{2+} uptake participates in β cell nutrient responses [184–187]. Indeed, β cell-specific MCU-deleted mice display abnormal glucose-stimulated insulin secretion [188]. NCLX silencing or pharmacological inhibition also modulates cytosolic ATP and insulin secretion in these cells [186,189]. Indeed, intracellular metabolic responses and Ca^{2+} signaling in β cells are modulated by Na^+ and NCLX [190]. Finally, altered expression of the NCLX and regulatory elements of the MCU have been documented in β cells exposed to glucotoxicity [191]. Overall, these intricate relationships between mitochondrial metabolism, Ca^{2+} signals and whole body nutrient responses seen in β cells underly and emphasize the importance of the interplay between these central metabolic regulatory systems in all tissues discussed here.

11. Conclusions

Overall, although some specific findings differ, a large body of data indicate that prevention of obesity through caloric restriction avoids the loss of mitochondrial bioenergetic functions over time in aging protocols. This prevention is related to changes in mitochondrial phospholipid content, ROS production, and antioxidant systems (see Fig. 4). Indeed, most studies find a significant decrease promoted by caloric restriction in markers of oxidation in mitochondria in aged animals, indicating that the prevention of obesity significantly improves mitochondrial redox state over time. Some studies also show an increase in oxidative phosphorylation capacity in young animals induced by caloric restriction diets, although this effect seems to be diet and animal model-dependent.

The more recent finding that prevention of obesity also changes mitochondrial calcium uptake rates is related to many of the bioenergetic and redox effects seen previously, as mitochondrial calcium regulates oxidative phosphorylation, ROS production, and consequences of oxidative imbalance in mitochondria such as the mPTP. Furthermore, as mitochondrial calcium uptake significantly impacts on cellular calcium homeostasis and metabolism, we believe many new connections still remain to be uncovered in the complex mechanisms interrelating calorie restriction, mitochondrial bioenergetics, redox state, and calcium signaling.

CRediT authorship contribution statement

Eloisa A. Vilas-Boas: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Alicia J. Kowaltowski:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of competing interest

Authors declare no conflict of interests.

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Abbreviations

DNP)	2,4-dinitrophenol
HNE	4-hydroxynonenal
8-OHdG	8-hydroxydeoxyguanosine
AL	*ad libitum*
CRC	calcium retention capacity
CR	caloric restriction
CPT1	carnitine palmitoyltransferase 1
CoQ	coenzyme Q
DRGN	dorsal root ganglion neuron
ETC	electron transport chain
ER	endoplasmic reticulum
eNOS	endothelial nitric oxide synthase
FR	food restriction
$FBNF_1$	Fischer Brown Norway hybrid rat
GPx	glutathione peroxidase
GSTA4	GSTA4 glutathione S-transferase alpha 4
HIPPON	hippocampal neuron
H_2O_2	hydrogen peroxide
IF	intermittent feeding
KD	ketogenic diet
MDA	malondialdehyde
MCU	mitochondrial calcium uniporter
MCUc	mitochondrial calcium uniporter complex
NCLX	mitochondrial calcium/sodium (lithium) exchanger
mtDNA	mitochondrial DNA
mPTP	mitochondrial permeability transition pore
nitric oxide	NO
NOS	nitric oxide synthases
GSSG	oxidized glutathione
oxygen consumption rate	OCR
OLEFT	Otsuka Long Evans Tokushima Fatty Rat
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PHPA	p-hydroxyphenylacetate
protein-SSG	protein-glutathione mixed disulfides
ROS	reactive oxygen species
GSH	reduced glutathione
SIRT	sirtuin
SOD	superoxide dismutase
$O_2^{\cdot-}$	superoxide radical
TBARS	thiobarbituric acid reactive substances
Trx	thioredoxin
TrxR	thioredoxin reductase
UCP	uncoupling protein
VDAC)	voltage-dependent anion channel

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