Caloric Restriction and Aging

تقييد السعرات الحرارية والشيخوخة

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Abstract

This review focuses on the evidences documented that calorie restriction (CR) without malnutrition slows biological processes of aging and delayed aging signs, improve healthspan, increase average maximal lifespan and reduce the incidence of age-related diseases such as obesity, diabetes mellitus hypertension, cardiovascular disease and cancer in model organisms, rodents and in human and non-human primates. The cell senescence, and age-related diseases involves oxidative stress as an early stage in its development as confirmed by a decrease in antioxidant defenses and an increase in oxidative damage, leading to mitochondrial impairment and mitochondrial DNA damage, these changes have been coupled to increased oxidative damage to DNA, lipids and proteins in tissues with age. Accumulated oxidative stress resulting from a gradual shift in the redox status of cells considered to be a key mechanism underlying the aging process. Calorie restriction an experimental model to extend survival and delay aging and age-related diseases, is recognized to slow the rate of accrual of age-related oxidative stress and preserves

mitochondrial function. The aim of this review research was to discuss recent evidence developments to understand the advances consequences and mechanisms of calorie restriction on mitochondrial functions, reducing of reactive oxygen species and cellular oxidative stress resistance and how the pathways they regulate can delay the aging process, as a potent anti-aging factor in animal cells.

Keywords

Caloric Restriction; aging; cell senescence; delayed aging; oxidative stress; reactive oxygen species; oxidative damage; antioxidant; Sirtuin; anti-aging factor; mitochondrial function.

1.Introduction

Aging is a progressive loss of functions and energy production and accumulation of cellular damages in cells that is accompanied by decreasing fertility and increasing onset age-related diseases and mortality with advancing age (1). Calorie restriction (CR) defined as a 20%-40% reduction in calorie intake below usual ad libitum without malnutrition, slows aging, extend maximal and average life span in animals (2). The anti-ageing effects of Calorie restriction includes delayed onset of the most common chronic diseases, such as type 2 diabetes, cardiovascular diseases, neurogenerative disorder and cancer. In particular, the metabolic and molecular changes induced by CR in humans are similar to those promoting health and prolonging life of CR-treated animals (3).

Abundant experimental evidence indicates that the CR effect on stimulating health impinges several metabolic and stress-resistance pathways. Downstream effects of these pathways include a reduction in cellular damage induced by oxidative stress, enhanced efficiency of mitochondrial functions and maintenance of mitochondrial dynamics and

quality control, thereby attenuating age-related declines in mitochondrial function (4). Caloric restriction, retards the aging process, delays the age-associated decline in physiological fitness, and extends the life span of organisms (5). Calorie restriction trial in healthy humans provide new evidence of persistent metabolic slowing accompanied by reduced oxidative stress, which supports the rate of living and oxidative damage theories of mammalian aging (6). Caloric restriction decreases aging rate and mitochondrial ROS (MitROS) production and oxidative stress in rat post-mitotic tissues. Low levels of these parameters are also typical traits of long-lived mammals and birds (7).

The free radical theory/oxidative stress of aging has provided a theoretical framework for an enormous amount of work leading to significant advances in understanding of aging (8). The oxidative stress theory of aging proposes that free radicals resulting from cellular mitochondrial respiration cause cumulative oxidative damage over time, leading to senescent degeneration and age-related diseases. Functionally, oxidative stress results when the homeostasis between the formation of free radical oxidants and the deactivation of these agents and repair of associated damage through endogenous antioxidant defenses and repair mechanisms tilts in favor of the free radicals (9). The effects of caloric restriction on the mitochondrial free radical theory of aging has been lowering mitROS generation, oxidative stress rate and low sensitivity of membranes to oxidation in calorie restricted animals compared with ad libitum-fed animals (10). Increasing evidence demonstrates that CR can prevent and reverse the physiological and pathophysiological alterations associated with aging (11-14). CR delayed the onset of age-associated pathologies. Specifically, CR reduced the incidence of diabetes, cancer, cardiovascular disease, and brain atrophy. These data demonstrate that CR slows aging in a primate species (15). In this review, we will focus on the current evidence knowledge about the influence of CR regimes on mitochondrial biogenesis and functions, reducing of reactive oxygen species and cellular oxidative stress resistance and how the bio-

mechanisms they regulate can delay the aging process, as a potent anti-aging factor in animal cells.

2. **Caloric restriction attenuates oxidative stress in aging**

A chronic state of oxidative stress exists in cells because of an imbalance between pro-oxidants and antioxidants. The amount of oxidative damage increases in the cells with as an organism ages and is postulated to be a major causal factor of senescence. Support for this hypothesis includes the following observations: (i) Overexpression of antioxidative enzymes retards the age-related accrual of oxidative damage and extends the maximum life-span (ii) Variations in longevity among different species inversely correlate with the rates of mitochondrial generation of the superoxide anion radical (O2) and hydrogen peroxide. (iii) Restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum life-span in mammals (16).

A proposed mechanism of lifespan extension and delayed aging associated with CR is a reduced rate of mitochondrial reactive oxygen species generation (mitROS) and less oxidative damage of mtDNA (17). It has been shown that some long-lived species have markedly lower rates of mitROS production compared to their short-lived counterparts (18, 19). Calorie restriction have a protective effect on oxidative stress and damage are diminished in mice lacking sirtuin-3 **(**SIRT3), a mitochondrial deacetylase. SIRT3 reduces cellular ROS levels dependent on superoxide dismutase 2 (SOD2), a major mitochondrial antioxidant enzyme. SIRT3 deacetylates two critical lysine residues on SOD2 and promotes its antioxidative activity. Importantly, the ability of SOD2 to reduce cellular ROS and promote oxidative stress resistance is greatly enhanced by SIRT3 (Figure:1) (20).

Donato *et al* (21) demonstrated that life-long caloric restriction reduces oxidative stress (Nitrotyrosine and superoxide-dependent suppression) by enhancing NO and increasing in superoxide dismutase (SOD), total SOD,

and catalase activities, CR normalized age-related changes in the critical nutrient-sensing pathways Sirtuin-1(SIRT-1) and mammalian target of rapamycin (mTOR).

Someya et al. (22) describing a new function for the mitochondrial SIRT3 protein in the prevention of age-related hearing loss mediated by caloric restriction during aging. These tantalizing results suggest that SIRT3 might play an important role in providing resistance to oxidative stress, slowing the aging process in mammals and inhibiting the age-related loss of spiral ganglion neurons and hair cells. they discover that SIRT3 regulates the mitochondrial glutathione antioxidant defense system.

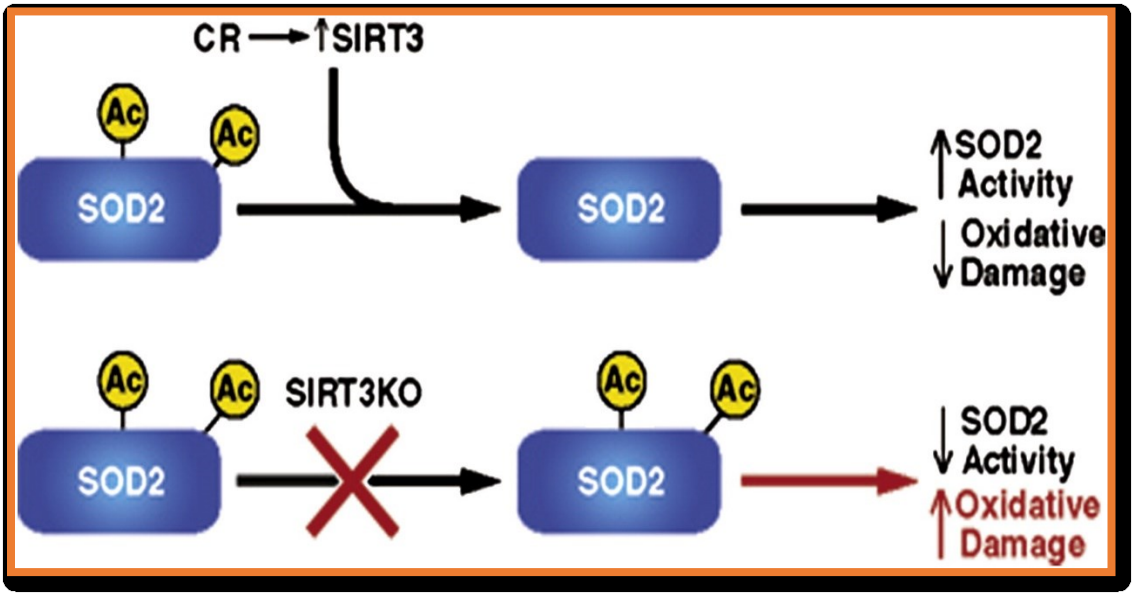

Figure (1): CR reduces oxidative stress by inducing SIRT3

► SIRT3 activates SOD2 via deacetylation ► SIRT3 reduces cellular ROS and promotes stress resistance by deacetylating SOD2 ► SOD2 is activated by SIRT3 via deacetylation during CR (20).

Oxidative stress reduction was proposed to mediate CR effects, decreasing oxidative stress and increasing antioxidant defense has been

hypothesized as one mechanism by which caloric restriction increases longevity in animals (23,24,25). Redman et al (6) found new evidence that prolonged CR enhances resting energy efficiency, resulting in decreased oxidative damage to tissues and organs, which supports the rate of living and oxidative damage theories of mammalian aging (Figure 2) (6). current data support the observation that sustained calorie restriction extends life without chronic disease and promotes a more youthful physical and mental functionality (26). Jang *et al* (27) demonstrate that CR is a powerful mediator of mitochondrial function, mitochondrial ROS production, and oxidative damage, providing a solid protection against oxidative stress–induced neuromuscular defects and muscle atrophy in vivo even under conditions of high oxidative stress.

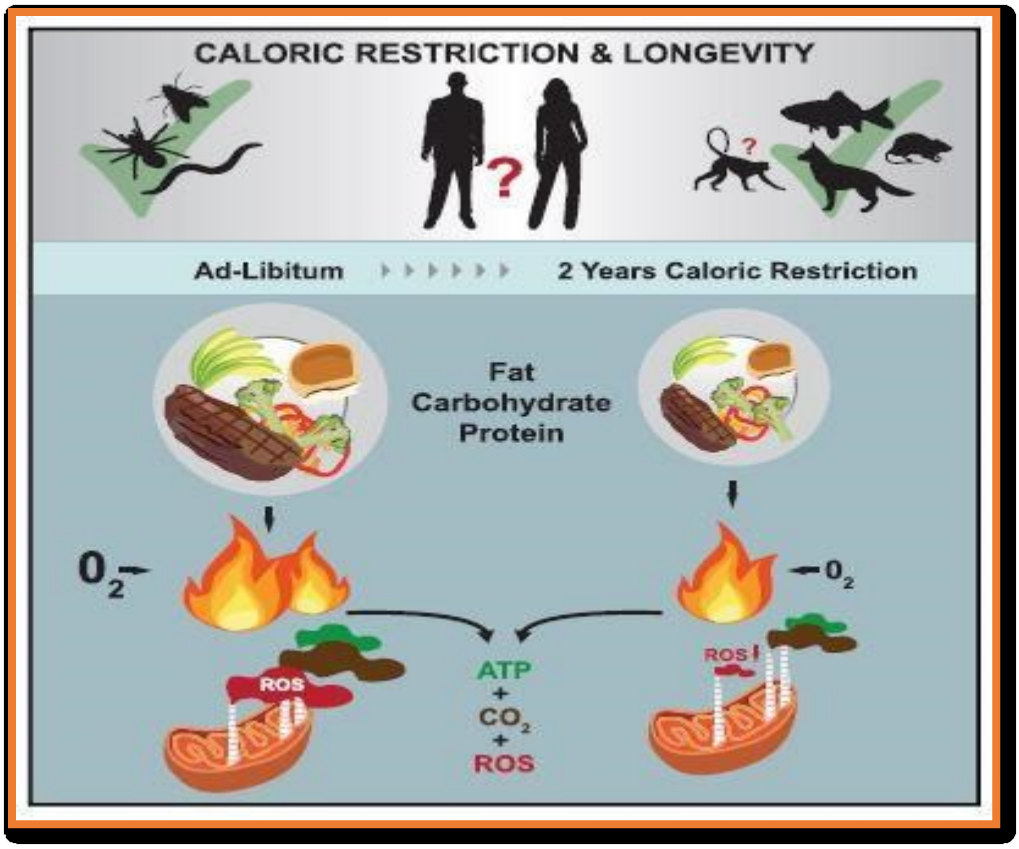

Figure (2): CR enhances resting energy efficiency, resulting in decreased oxidative damage to tissues and organs (6)

3. Caloric Restriction Preserves Mitochondrial Function in aging

Age-related changes in mitochondria are associated with decline in mitochondrial function. With advanced age, mitochondrial DNA volume, integrity and functionality decrease due to accumulation of mutations and oxidative damage induced by reactive oxygen species (ROS). In aged subjects, mitochondria are characterized by impaired function such as lowered oxidative capacity, reduced oxidative phosphorylation, decreased ATP production, significant increase in ROS generation, and diminished antioxidant defense. Mitochondrial biogenesis declines with age due to

alterations in mitochondrial dynamics and inhibition of mitophagy, an autophagy process that removes dysfunctional mitochondria. Age-dependent abnormalities in mitochondrial quality control further weaken and impair mitochondrial function (28,29,30).

Several investigations proved that CR attenuated the levels of ROS production in the mitochondria, restores aged-related mitochondrial dysfunction and attenuates oxidative damage in mitochondria (12,14,31-34). CR increases mitochondrial biogenesis and bioenergetics efficiency (35,36). CR decreases oxidant emission, increases antioxidant scavenging, and minimizes oxidative damage to DNA and protein in the mitochondria, therefore CR preserves mitochondrial function by protecting the integrity and function of existing cellular components (38). Several studies provide the strongest evidence linking the activity of SIRT3 with the beneficial effects of calorie restriction in mammals, particularly effects on mitochondrial ROS production, on mitochondrial function including enzymes of the respiratory chain, tricarboxylic acid cycle, fatty acid β-oxidation and ketogenesis (38,39,40), SIRT3 activity and subsequent control of enzymes involved in energy metabolism is consistent with an overall role of protecting against age-related diseases (41).

Possible mechanisms of attenuate mitochondrial ROS production and preserve Mitochondrial Function by Caloric Restriction summarized in figure 2(4).

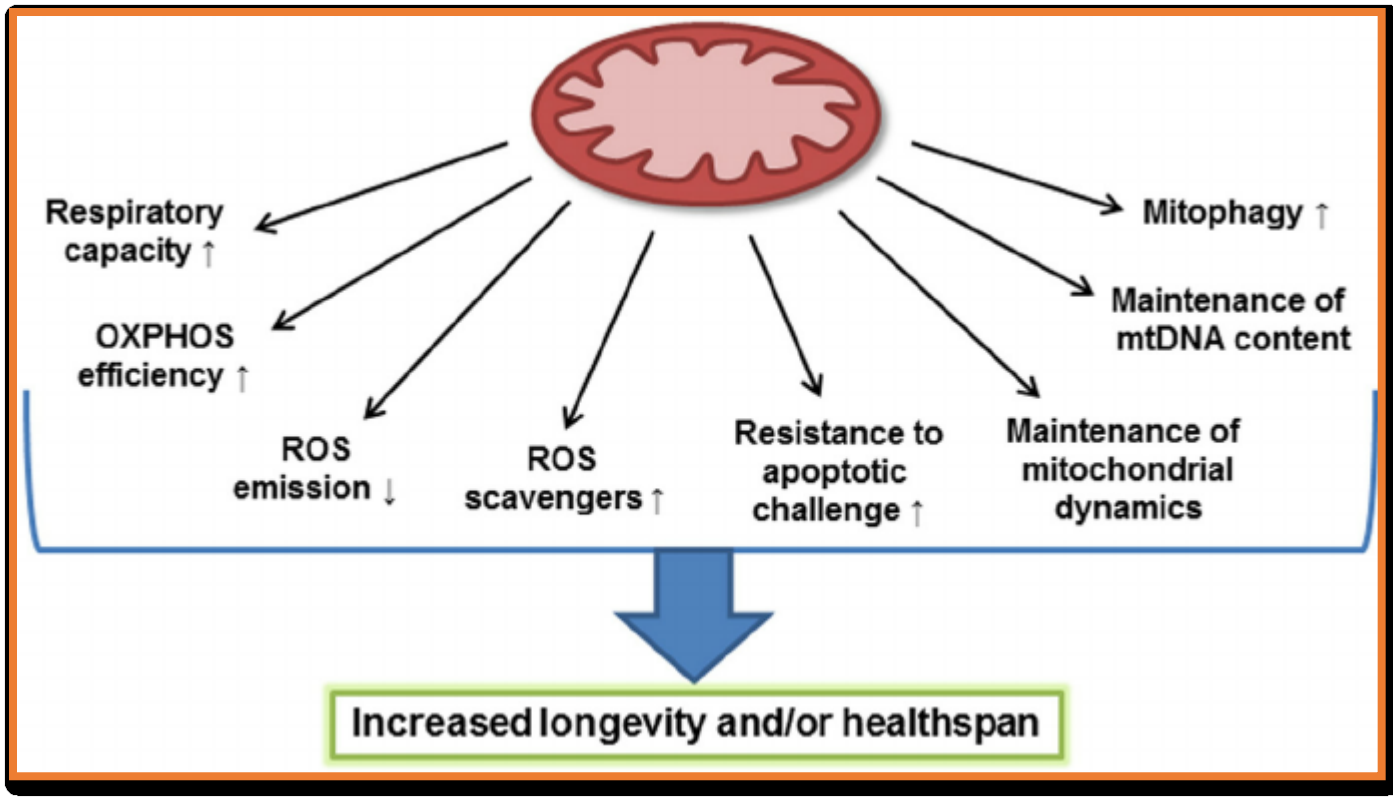

Figure (2) CR-induced alterations in mammalian mitochondrial function implicated in longevity extension (4).

Shinmura *et al* (34) demonstrates that Calorie Restriction primes cardiac mitochondria into a stress-resistant state, through enhances Sirtuin activity which associated with a decrease in the amount of acetylated mitochondrial proteins. they proposed that the beneficial effect of CR on mitochondrial function is mediated by deacetylating specific mitochondrial proteins, deacetylation of NDUFS1 (complex I) and/or Rieske subunit of cytochrome bc1 complex (complex III) plays a key role in the reduced ROS production in the mitochondria (Figure 3). Activation of Sirt1 mitochondrial biogenesis through activation of peroxisome proliferator-activated receptor γ coactivator 1 in the skeletal muscle (38). Nisoli *et al* (39) demonstrated that 3 months of CR enhanced mitochondrial biogenesis in the murine heart and that this phenomenon was dependent on endothelial nitric oxide synthase.

Someya et al. (22) show that caloric restriction leads to an increase in SIRT3 levels in the mitochondria by promoting the deacetylation of isocitrate dehydrogenase 2 (IDH2), SIRT3 promotes the accumulation of NADPH, hence activating glutathione reductase (GSR), which generates reduced glutathione (GSH), a cellular antioxidant (Figure 4).

Qui *et al* (20) demonstrated that CR activated SIRT3 and reduces cellular ROS levels dependent on promotes superoxide dismutase 2, a major mitochondrial antioxidant enzyme. SIRT3 deacetylates two critical lysine residues on SOD2 and promotes its antioxidative activity. Importantly, the ability of SOD2 to reduce cellular ROS and promote oxidative stress resistance is greatly enhanced by SIRT3. Activation of Sirt1 also contributes to decreased oxidative damage in CR-treated hearts by upregulating antioxidant enzymes including MnSOD (40).

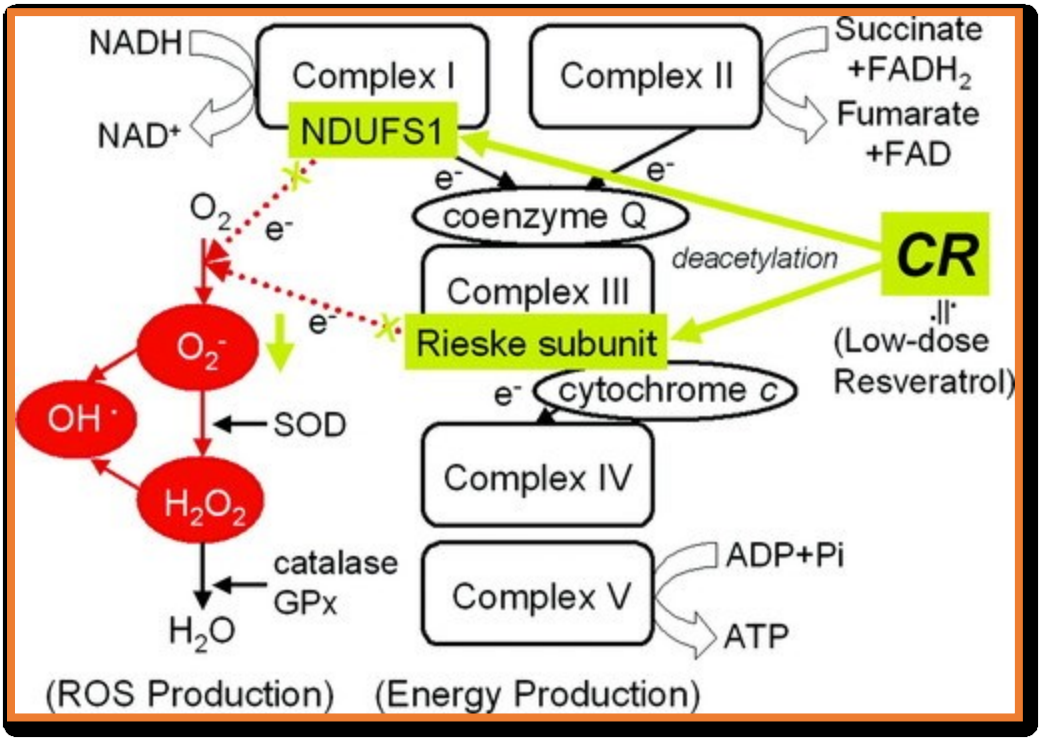

Figure (3): Possible mechanism(s) by which CR attenuates ROS production from mitochondria (34)

Possible mechanism(s) by which CR attenuates ROS production from mitochondria during ischemia/ reperfusion. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; FAD, flavin adenine dinucleotide (oxidized); FADH2, flavin adenine dinucleotide (reduced); GPx, glutathione peroxidase; iP, inorganic phosphate; NAD, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); ROS, reactive oxygen species; and SOD, superoxide dismutase.

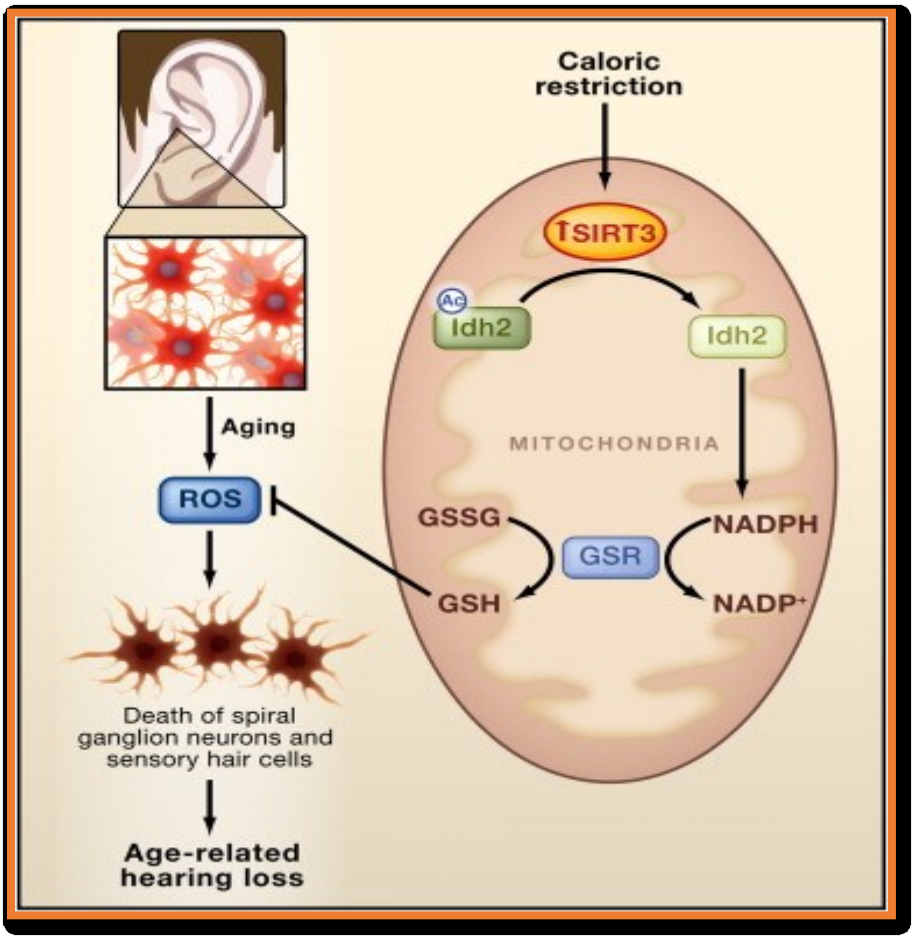

Figure (4): Caloric Restriction, SIRT3, and Age-Related Hearing Loss(22)

(During aging (left), oxidative damage (ROS, reactive oxygen species) leads to the loss of spiral ganglion neurons and sensory hair cells in the ear, leading to age-related hearing

loss. Caloric restriction (right) prevents the age-associated loss of spiral ganglion neurons and sensory hair cells).

4. **Caloric Restriction, Sirtuin (SIRT) and aging**

Sirtuins are a family of of NAD+-dependent enzymes (proteins), mediate a number of metabolic and behavioral responses to CR. Mammals have seven homologs of sir2 enzymes (SIRT1–7). SIRT1, SIR6, and SIR7 are found primarily in the nucleus; SIRT3, SIR4, and SIR5 are mitochondrial; and SIRT2 is cytoplasmic. Sirtuins have NAD+-dependent protein deacetylase activity and ADPribosyltransferase activity. In addition, SIRT5 has desuccinylase and demalonylase activity (41- 45). The physiological effects of deacetylation of sirtuin targets may be classified into two categories; the control of metabolism and the response to oxidative stress. The sum of all of the known activities of sirtuins on transcription factors and metabolic enzymes would program cells for oxidative metabolism in mitochondria. Sirtuins are considered among the most promising targets for modulating aging-associated cellular and molecular processes and disease pathologies (45). Sirtuins are critical mediators of the beneficial effect of CR on signs of ageing and diseases (41). Multiple studies have shown declines in SIRT activity or levels with age in different organisms, tissues, and cells types, SIRT levels and enzymatic activation decline during normal aging, and loss of their protective effects contributes to multiple aging-associated pathologies (50-56).

Calorie restriction increases SIRTs activation which is modulate highly conserved metabolic pathways (insulin-like growth factor (IGF)/insulin, mammalian target of rapamycin/ribosomal protein S6 kinase (mTOR/S6K), AMP-activated protein kinase (AMPK), RAS, and AKT/protein kinase B (AKT/PKB) signaling pathways) and protect cells from multiple stressors; oxidative, genotoxic, proteostatic associated with aging (57,58,59).

Sirtuins (SIRT1-7) have been implicated to mediate the beneficial effects of calorie restriction for healthy aging. At molecular mechanisms (Figure 5) underlying the sirtuin-mediated anti-ageing effects of calorie restriction in cardiac and skeletal muscle. the effects of CR involve nutrient-signaling path ways, such as those of the mammalian target of rapamycin (mTOR), the insulin-like growth factor 1 (IGF-1), and a family of seven proteins named sirtuins (41). Many of the proteins targeted by te acetylation/deacetylation are localized in the mitochondria and are involved in the tricarboxylic acid cycle, β-oxidation, and oxidative phosphorylation (60).

As cellular energy sensors, SIRTs link metabolism and DNA repair, two central inter-dependent aspects of aging (59,61), and SIRT activation by NAD or caloric restriction may promote longevity in part by augmenting DNA repair mechanisms. SIRTs may also link metabolism, DNA repair, and aging by regulating mitochondrial functions, either onsite by protecting cells from oxidative stress in mitochondria (SIRTs 3–5) (62), or remotely by regulating transcription of mitochondrial ribosomal proteins and translation factors (SIRT7) (63). SIRTs also regulate autophagy, another critical aging-associated process that modulates cellular stress responses (64).

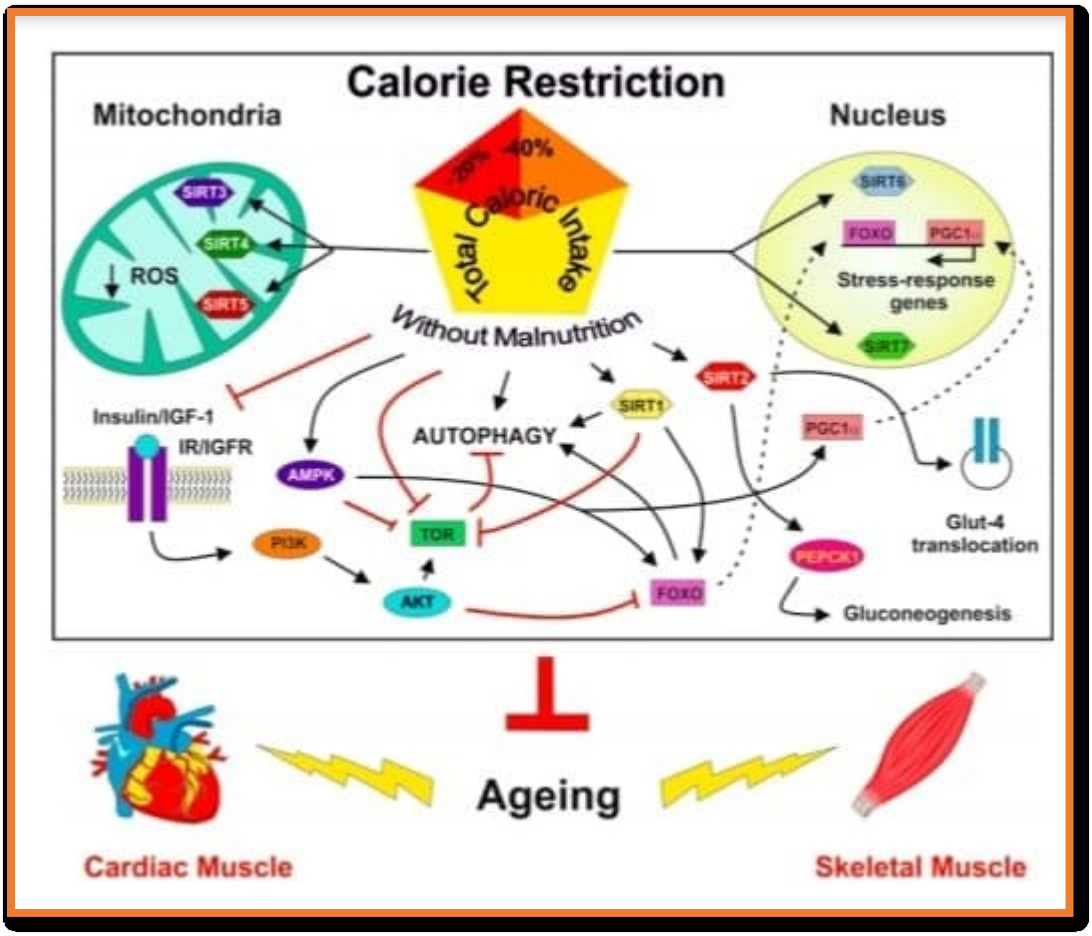

Figure 5. Molecular mechanisms underlying the sirtuin-mediated anti-ageing effects of Calorie Restriction in cardiac and skeletal muscle (41)

Abbreviations: CR, calorie restriction; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; TOR, target of rapamycin; AMPK, AMP-activated protein kinase; FOXO, forkhead box O transcription factor; GLUT-4, glucose transporter type 4; PEPCK1, phosphoenolpyruvate carboxykinase 1; PGC1α, proliferator-activated receptor-gamma coactivator-1α; IR, insulin receptor; IGF1, insulin-like growth factor1;

SIRT1(Silent information regulator-1), also known as sirtuin 1, has been reported to be involved in the regulation of various important biological processes, including inflammation, mitochondrial biogenesis, as well as cell senescence and consequent aging. The level of SIRT1 is decreased in both

transcriptional and postranscriptional conditions during aging, accompanied by attenuated mitochondrial biogenesis, an important component of aging-related diseases. SIRT1 can activate several transcriptional factors, such as peroxisome proliferator activated receptor γ co-activator 1α (PGC-1α) and hypoxia-inducible factor 1α (HIF-1α) resulting in ameliorated mitochondria biogenesis and extended life span (65). Sirt1 plays a crucial role in both cellular senescence and ageing, and it was recognized as modulator of the oxidative stress response by inducing the expression of antioxidant enzymes such as superoxide dismutase and catalase (51,52). SIRT1 declines with aging and its related physiological/pathophysiological processes and it's a key regulator of aging-related metabolic changes, and may be a potent biomarker of aging (53,54).

SIRT1 protein levels are increased in response to CR in many key metabolic tissues, caloric restriction significantly increases SIRT1 activation in mammalians and human (55). SIRT1 also plays a role in the regulation of phenotypes induced by caloric restriction, a diet regimen that delays aging and extends life span in a wide variety of organisms (56,66). extensive studies have clearly revealed that SIRT proteins regulate diverse cell functions and responses to stressors and that SIRT proteins protect against age-related diseases (cardiovascular diseases, diabetes, neurodegenerative diseases, cancer) (67)

CR enhances mitochondrial biogenesis by up-regulating the expression of the endothelial nitric oxide synthase (eNOS), promoting the production of NO, and increasing SIRT1 expression.CR is capable of reducing the levels of NADH or the nicotinamide-degrading enzyme pyrazinamidase/nicotinamidase 1 (PNC1), which consequently activates the Sir2 deacetylase and increases the lifespan (47,68).

SIRT3 regulates mitochondrial biogenesis, and it is correlated with the expression of genes related to mitochondrial function, including PGC1α and UCP1, SIRT3 also appears to be an antioxidant protein, as SIRT3 reduced reactive oxygen species through post-translational regulation of superoxide

dismutase 2 *via* deactylation in response to oxidative stress (69). SIRT3 polymorphisms have been associated with longevity in human populations (70).

Sirtuins (SIRTs), a family of nicotinamide adenine dinucleotide (NAD)-dependent deacetylases, are emerging as key molecules that regulate aging and age-related diseases including cancers, metabolic disorders, and neurodegenerative diseases, SIRTs could be important regulators of beneficial CR-mediated functions in delayed aging and longevity in mammals (Figure 6) (71).

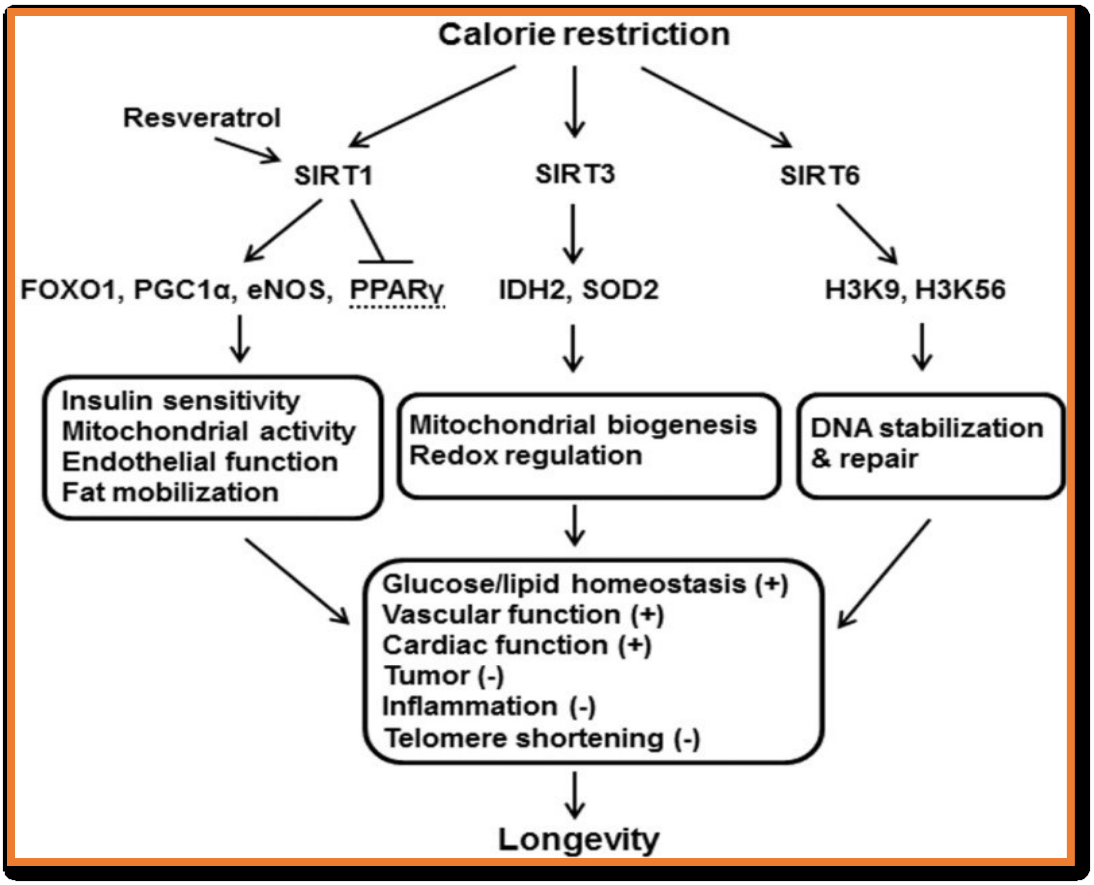

Figure (6): SIRTs-mediated pathways that induce anti-aging effect of CR in mammals (71)

(+), enhancement; (−), inhibition(68):(eNOS, endothelial nitric oxide synthase; FOXO, forkhead box O; H3K9, histone H3 lysine 9; H3K56, histone H3 lysine 56; IDH2, isocitrate dehydrogenase 2; PGC1 α, peroxisome proliferator-activated receptor-γ co-activator 1 α; SOD2, superoxide dismutase 2)

5.Conclutions

The anti-ageing effects of Calorie restriction includes stimulating health impinges several metabolic and stress-resistance pathways, which is leading to delayed onset of the most common age-related diseases. Downstream effects of these pathways include a reduction in cellular damage induced by oxidative stress, enhanced efficiency of mitochondrial functions and

maintenance of mitochondrial dynamics and quality control, thereby attenuating age-related declines in mitochondrial function. Caloric restriction, retards the aging process, delays the age-associated decline in physiological fitness, and extends the life span of organisms. Calorie restriction trial in healthy humans provide new evidence of persistent metabolic slowing accompanied by reduced oxidative stress, which supports the rate of living and oxidative damage theories of mammalian aging. A mechanism of lifespan extension and delayed aging associated with CR is a reduced rate of mitochondrial reactive oxygen species generation (mitROS) and less oxidative damage of mtDNA. CR attenuated the levels of ROS production in the mitochondria, restores aged-related mitochondrial dysfunction, attenuates oxidative damage in mitochondria, increased mitochondrial biogenesis and bioenergetics efficiency. Caloric restriction enhances mitochondrial SIRT3, SIRT4, and SIRT5 activates mitochondria functions and plays an important role in decline ROS production, delay aging and longevity.

Sirtuins are considered among the most promising targets for modulating aging-associated cellular and molecular processes and disease pathologies. Sirtuins are critical mediators of the beneficial effect of CR on signs of ageing and diseases. CR activated mitochondrial SIRTs and reduces cellular ROS levels dependent on promotes mitochondrial antioxidant enzyme. Calorie restriction increases SIRTs activation which is modulate highly conserved metabolic pathways (insulin-like growth factor (IGF)/insulin, mammalian target of rapamycin/ribosomal protein S6 kinase (mTOR/S6K), AMP-activated protein kinase (AMPK), RAS, and AKT/protein kinase B (AKT/PKB) signaling pathways) and protect cells from multiple stressors; oxidative stress, genotoxic, proteostatic associated with aging.

References

1. De A, Ghosh C; Basics of aging theories and disease related aging-an overview; PharmaTutor; 2017; 5(2); 16-23.
2. Bales, C.W.; Kraus, W.E. Caloric Restriction: Implications for Human Cardiometabolic Health. J. Cardiopulm. Rehabil. Prev. 2013, 33, 201–208.
3. (Most, J.; Tosti, V.; Redman, L.M.; Fontana, L. Calorie Restriction in Humans: An Update. Ageing Res. Rev. 2017, 39, 36–45.)
4. Ruetenik A, Barrientos A. Dietary restriction, mitochondrial function and aging: from yeast to humans. Biochim Biophys Acta. 2015 Nov;1847(11):1434-47.
5. Rajindar S.SohalaMichael J.Forsterb. Caloric restriction and the aging process: a critique. Free Radical Biology and Medicine. Volume 73, August 2014, Pages 366-382.
6. Redman ,L M., Steven R. Smith, Jeffrey H. Burton, Corby K. Martin, Dora Il'yasova, Eric Ravussin. Metabolic Slowing and Reduced Oxidative Damage with Sustained Caloric Restriction Support the Rate of Living and Oxidative Damage Theories of Aging. Cell Metabolism, 2018.
7. ReinaldPamplonaaGustavoBarja . Mitochondrial oxidative stress, aging and caloric restriction: The protein and methionine connection. Biochimica et Biophysica Acta (BBA) - Bioenergetics. Volume 1757, Issues 5–6, May–June 2006, Pages 496-508.
8. Jose Vin˜ a,* Consuelo Borras, Kheira M. Abdelaziz, Rebeca Garcia-Valles, and Mari Carmen Gomez-Cabrera .The Free Radical Theory of Aging Revisited: The Cell Signaling Disruption Theory of Aging. ANTIOXIDANTS & REDOX SIGNALING. Volume 19, Number 8, 2013.
9. Shringarpure, R., & Davies, K. J. A. (2009). Free radicals and oxidative stress in aging. In V. L. Bengtson, D. Gans, N. M. Putney, & M. Silverstein (Eds.), *Handbook of theories of aging* (pp. 229–243). New York: Springer Publishing Company.
10. Barja G. The mitochondrial free radical theory of aging .Prog Mol Biol Transl Sci. 2014;127:1-27.
11. Masoro EJ. Overview of caloric restriction and ageing. Mech Ageing Dev. 2005; *126*:913–922.

12.Ungvari Z, Parrado-Fernandez C, Csiszar A, de Cabo R. Mechanisms underlying caloric restriction and lifespan regulation: implications for vascular aging. Circ Res. 2008; *102*:519–528.
13.Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science. 2009; *325*:201–204.
14.Merry BJ. Oxidative stress and mitochondrial function with aging: the effects of calorie restriction. Aging Cell. 2004; *3*:7–12.
15.R.J. Colman, T.M. Beasley, J.W. Kemnitz, S.C. Johnson, R. Weindruch, R.M. AndersonCaloric restriction reduces age-related and all-cause mortality in rhesus monkeys.Nat. Commun., 5 (2014), p. 3557.
16. Sohal RS1, Weindruch R. Oxidative stress, caloric restriction, and aging. Science. 1996 Jul 5;273(5271):59-63.
17. Gredilla R, Barja G. Minireview: the role of oxidative stress in relation to caloric restriction and longevity. *Endocrinology* (2005) 146(9):3713–7. doi:10.1210/en.2005-0378
18. Barja G. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism? *Biol Rev Camb Philos Soc* (2004) 79(2):235–51. doi:10.1017/S1464793103006213
19. Barja G. Free radicals and aging. *Trends Neurosci* (2004) 27(10):595–600. doi:10.1016/j.tins.2004.07.005.
20. Qiu X, Brown K, Hirschey MD, Verdin E, Chen D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metab. 2010 Dec 1;12(6):662-7.
21. Donato AJ1, Walker AE, Magerko KA, Bramwell RC, Black AD, Henson GD, Lawson BR, Lesniewski LA, Seals DR. Life-long caloric restriction reduces oxidative stress and preserves nitric oxide bioavailability and function in arteries of old mice. Aging Cell. 2013 Oct;12(5):772-83.
22.S. Someya, W. Yu, W.C. Hallows, J. Xu, J.M. Vann, C. Leeuwenburg, M . Tanokura, J.M. Denu, T.A. Sirt3 Mediates Reduction of Oxidative Damage

and Prevention of Age-Related Hearing Loss under Caloric RestrictionProlla Cell, 143 (2010), pp. 802-812.
23. Buchowski MS, Hongu N, Acra S, Wang L, Warolin J, Roberts LJ II (2012) Effect of Modest Caloric Restriction on Oxidative Stress in Women, a Randomized Trial. PLoS ONE 7(10): e47079.
24. M. MEYDANI, S. DAS, M. BAND, S. EPSTEIN, and S. ROBERTS. The effect of caloric restriction and glycemic load on measures of oxidative stress and antioxidants in humans: results from the CALERIE Trial of Human Caloric Restriction. . J Nutr Health Aging. 2011; 15(6): 456–460.
25. Il'yasova D, Fontana L, Bhapkar M, Pieper CF, Spasojevic I, Redman LM, Das SK, Huffman KM, Kraus WE . Effects of 2 years of caloric restriction on oxidative status assessed by urinary F2-isoprostanes: The CALERIE 2 randomized clinical trial. Aging Cell. 2018 Apr;17(2).
26. Mattison, J.A., Colman, R.J., Beasley, T.M., Allison, D.B., Kemnitz, J.W., Roth, G.S., Ingram, D.K., Weindruch, R., de Cabo, R., and Anderson, R.M. (2017). Caloric restriction improves health and survival of rhesus monkeys. Nat. Commun. 8, 14063.
27. Jang , Young C. , Yuhong Liu , Christopher R. Hayworth , Arunabh Bhattacharya , Michael S. Lustgarten , Florian L. Muller , Asish Chaudhuri , Wenbo Qi , Yan Li , Jing-Yi Huang , Eric Verdin , Arlan Richardson , Holly Van Remmen. Dietary restriction attenuates age-associated muscle atrophy by lowering oxidative stress in mice even in complete absence of CuZnSOD, Aging cell J. (2012) 11,pp770–782.
28. Dimitry A. Chistiakov, Igor A. Sobenin, Victor V. Revin , Alexander N. Orekhov, and Yuri V. Bobryshev. Mitochondrial Aging and Age-Related Dysfunction of Mitochondria. BioMed Research International.Volume 2014, Article ID 238463, 7 pages.
29. Pierre Theurey and Paola Pizzo.The Aging Mitochondria. Genes 2018, 9, 22; doi:10.3390/ genes9010022 www.mdpi.com/journal/genes.
30. Pénélope A. Andreux, Marcus P. J. van Diemen, Maxime R. Heezen, Johan Auwerx, Chris Rinsch, Geert Jan Groeneveld & Anurag Singh . Mitochondrial function is impaired in the skeletal muscle of pre-frail

elderly. Scientific REPOrTS | (2018) 8:8548 | DOI:10.1038/s41598-018-26944-x 2.

31. Bevilacqua L, Ramsey JJ, Hagopian K, Weindruch R, Harper ME. Long-term caloric restriction increases UCP3 content but decreases proton leak and reactive oxygen species production in rat skeletal muscle mitochondria. Am J Physiol Endocrinol Metab. 2005; *289*:E429–E438.

32.Colom B, Oliver J, Roca P, Garcia-Palmer FJ. Caloric restriction and gender modulate cardiac muscle mitochondrial H2O2 production and oxidative damage. Cardiovasc Res. 2007; 74:456–465.CrossrefMedlineGoogle Scholar

33.Niemann B, Chen Y, Issa H, Silber RE, Rohrbach S. Caloric restriction delays cardiac ageing in rats: role of mitochondria. Cardiovasc Res. 2010; 88:267–276.

34. Shinmura, Ken ., Kayoko Tamaki, Motoaki Sano, Naomi Nakashima-Kamimura, Alexander M. Wolf, Taku Amo, Shigeo Ohta , Yoshinori Katsumata, Keiichi Fukuda , Kyoko Ishiwata, Makoto Suematsu, and Takeshi Adachi. Caloric Restriction Primes Mitochondria for Ischemic Stress by Deacetylating Specific Mitochondrial Proteins of the Electron Transport Chain. Circulation Research. 2011;109:396–406.

35. Civitarese, A. E., Carling, S., Heilbronn, L. K., Hulver, M. H., Ukropcova, B., Deutsch, W. A., et al. (2007). Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med*. 4:e76. doi: 10.1371/journal.pmed.0040076

36. López-Lluch, G., Hunt, N., Jones, B., Zhu, M., Jamieson, H., Hilmer, S., et al. (2006). Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc. Natl. Acad. Sci. U.S.A*. 103, 1768–1773. doi: 10.1073/pnas.0510452103.

37. Ian r. Lanza, Piotrek Zabielski, Katherine a. Klaus, Dawn m. Morse, Carrie j. Heppelmann, H. robert Bergen, Surendra Dasari, Stephane Walrand, Kevin r. Short, Matthew l. Johnson, et al. Chronic Caloric Restriction Preserves Mitochondrial Function in Senescence without

Increasing Mitochondrial Biogenesis. *Cell Metabolism*, Elsevier, 2012, 16 (6), pp.777-788.

38.Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell. 2006;127:1109–1122.

39.Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science. 2005;310:314–317 .

40.Tanno M, Kuno A, Yano T, Miura T, Hisahara S, Ishikawa S, Shimamoto K, Horio Y. Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure. J Biol Chem. 2010;285:8375–8382.

41. Alberto Zullo, Emanuela Simone, Maddalena Grimaldi, Vincenzina Musto and Francesco Paolo Mancini. Sirtuins as Mediator of the Anti-Ageing Effects of Calorie Restriction in Skeletal and Cardiac Muscle. Int. J. Mol. Sci. 2018, 19, 928; doi:10.3390/ijms19040928.

42. A. Giralt and F. Villarroya. a pivotal actor in mitochondrial functions: metabolism, cell death and aging. Biochem. J. (2012) 444, 1–10.

43. van de Ven RAH, Santos D, Haigis MC (2017) Mitochondrial sirtuins and molecular mechanisms of aging. Trends Mol Med 23:320–331.

44. Libert, Sergiy, and Leonard Guarente. “Metabolic and Neuropsychiatric Effects of Calorie Restriction and Sirtuins.” Annual Review of Physiology 75, no. 1 (February 10, 2013): 669- 684.

45. Guarente L. 2011. Sirtuins, aging, and medicine. New Engl J Med 364: 2235–2244.

46. Grabowska W, Sikora E, Bielak-Zmijewska A.Sirtuins, a promising target in slowing down the ageing process. Biogerontology. 2017 Aug; 18(4):447-476.
47. Imai SI, Guarente L. It takes two to tango: NAD(+) and sirtuins in aging/longevity control. NPJ Aging Mech Dis. 2016;2:16017.
48. Kiran S, Chatterjee N, Singh S, Kaul SC, Wadhwa R, Ramakrishna G. Intracellular distribution of human SIRT7 and mapping of the nuclear/nucleolar localization signal. FEBS J. 2013;280(14):3451–66.
49. Mohrin M, Shin J, Liu Y, Brown K, Luo H, Xi Y, et al. Stem cell aging. A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging. Science. 2015;347(6228):1374–7.
50. Mortuza R, Chen S, Feng B, Sen S, Chakrabarti S. High glucose induced alteration of SIRTs in endothelial cells causes rapid aging in a p300 and FOXO regulated pathway. PloS one. 2013;8(1):e54514.
51. Rahman M, Halade GV, Bhattacharya A, Fernandes G. The fat-1 transgene in mice increases antioxidant potential, reduces pro-inflammatory cytokine levels, and enhances PPAR-gamma and SIRT-1 expression on a calorie restricted diet. Oxid Med Cell Longev. 2009;2:307–16.
52.Conti V, Corbi G, Simeon V, Russomanno G, Manzo V, Ferrara N, et al. Aging-related changes in oxidative stress response of human endothelial cells. Aging Clin Exp Res. 2015;27:547–53.
53. Wątroba M, Szukiewicz D.The role of sirtuins in aging and age-related diseases. Adv Med Sci. 2016 Mar; 61(1):52-62.
54. Yuan Y, Cruzat VF, Newsholme P, Cheng J, Chen Y, Lu Y.Regulation of SIRT1 in aging: Roles in mitochondrial function and biogenesis. Mech Ageing Dev. 2016 Apr; 155():10-21.
55. Kitada M, Kume S, Takeda-Watanabe A, Tsuda S, Kanasaki K, Koya D. Calorie restriction in overweight males ameliorates obesity-related metabolic alterations and cellular adaptations through anti-aging effects, possibly including AMPK and SIRT1 activation. Biochim Biophys Acta. 2013 Oct; 1830(10):4820-7.
56. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol. 2010; 5:253-95.
57. Wang Y .Molecular Links between Caloric Restriction and Sir2/SIRT1 Activation. Diabetes Metab J. 2014 Oct; 38(5):321-9.

58. Anne E. Wyman and Sergei P. Atamas. Sirtuins and Accelerated Aging in Scleroderma. Curr Rheumatol Rep. 2018 Mar 17; 20(4): 16.
59. Guarente L. Linking DNA damage, NAD(+)/SIRT1, and aging. Cell Metab. 2014 Nov 4; 20(5):706-707.

60. Yang, L.; Vaitheesvaran, B.; Hartil, K.; Robinson, A.J.; Hoopmann, M.R.; Eng, J.K.; Kurland, I.J.; Bruce, J.E. The Fasted/Fed Mouse Metabolic Acetylome: N6-Acetylation Differences Suggest Acetylation Coordinates Organ-Specific Fuel Switching. J. Proteome Res. 2011, 10, 4134–4149.
61. Choi JE, Mostoslavsky R. Sirtuins, metabolism, and DNA repair. Curr Opin Genet Dev. 2014 Jun; 26():24-32.
62. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol. 2012 Mar 7; 13(4):225-238.
63. Liu JP, Chen R. tressed SIRT7: facing a crossroad of senescence and immortality. Clin Exp Pharmacol Physiol. 2015 Jun; 42(6):567-9.
64. Ng F, Tang BL. Sirtuins' modulation of autophagy.J Cell Physiol. 2013 Dec; 228(12):2262-70.
65. Yuan Y, Cruzat VF, Newsholme P, Cheng J, Chen Y, Lu Y. Regulation of SIRT1 in aging: Roles in mitochondrial function and biogenesis. Mech Ageing Dev. 2016 Apr;155:10-21.
66. Akiko Satoh, Cynthia S. Brace, Nick Rensing, Paul Clifton, David F. Wozniak, ,Kelvin A. Yamada, and Shin-ichiro Imai. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab. 2013 Sep 3; 18(3): 416–430.
67. Franklin H. Epstein Lecture: Sirtuins, aging, and medicine. Guarente L N Engl J Med. 2011 Jun 9; 364(23):2235-44.
68. Chang HC, Guarente L. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell. 2013;153(7):1448–60.

69. Chen Y, Zhang J, Lin Y, Lei Q, Guan KL, Zhao S, Xiong Y. (2011). Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO Rep. 12, 534–41.

70. Albani D, Ateri E, Mazzuco S, Ghilardi A, Rodilossi S, Biella G, Ongaro F, Antuono P, Boldrini P, Di Giorgi E, Frigato A, Durante E, Caberlotto L,

Zanardo A, Siculi M, Gallucci M, Forloni G. Modulation of human longevity by SIRT3 single nucleotide polymorphisms in the prospective study "Treviso Longeva (TRELONG)". Age (Dordr). 2014 Feb; 36(1):469-78.

71. Seongjoon Park, Ryoichi Mori, and Isao Shimokaw. Do Sirtuins Promote Mammalian Longevity?: A Critical Review on Its Relevance to the Longevity Effect Induced by Calorie Restriction. Mol Cells. 2013 Jun 30; 35(6): 474–480.