Received: 8 June 2023 | Accepted: 3 August 2023

DOI: 10.1111/pin.13371

REVIEW ARTICLE

Pathology International WILEY

Effect of mitochondrial quantity and quality controls in white adipose tissue on healthy lifespan: Essential roles of GH/IGF-1-independent pathways in caloric restriction-mediated metabolic remodeling

Yuina Otani[1] | Yuka Nozaki[1] | Yuhei Mizunoe[1] | Masaki Kobayashi[2,3] | Yoshikazu Higami[1,4]

[1]Laboratory of Molecular Pathology and Metabolic Disease, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan

[2]Department of Nutrition and Food Science, Graduate School of Humanities and Sciences, Ochanomizu University, Tokyo, Japan

[3]Institute for Human Life Innovation, Ochanomizu University, Tokyo, Japan

[4]Research Institute for Biomedical Sciences (RIBS), Tokyo University of Science, Chiba, Japan

Correspondence

Yoshikazu Higami, MD, PhD, Laboratory of Molecular Pathology and Metabolic Disease, Faculty of Pharmaceutical Sciences, Research Institute for Biomedical Sciences (RIBS), Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan.
Email: higami@rs.tus.ac.jp

Funding information

Ministry of Education, Culture, Sports, Science and Technology,
Grant/Award Numbers: Fostering Joint International Research (B) / 20KK0, Grant-in-Aid for Scientific Research (B) / 17H0217, Grant-in-Aid for Scientific Research

Abstract

Long-term caloric restriction is a conventional and reproducible dietary intervention to improve whole body metabolism, suppress age-related pathophysiology, and extend lifespan. The beneficial actions of caloric restriction are widely accepted to be regulated in both growth hormone/insulin-like growth factor 1-dependent and -independent manners. Although growth hormone/insulin-like growth factor 1-dependent regulatory mechanisms are well described, those occurring independent of growth hormone/insulin-like growth factor 1 are poorly understood. In this review, we focus on molecular mechanisms of caloric restriction regulated in a growth hormone/insulin-like growth factor 1-independent manner. Caloric restriction increases mitochondrial quantity and improves mitochondrial quality by activating an axis involving sterol regulatory element binding protein-c/peroxisome proliferator-activated receptor γ coactivator-1α/mitochondrial intermediate peptidase in a growth hormone/insulin-like growth factor 1-independent manner, particularly in white adipose tissue. Fibroblast growth factor 21 is also involved in this axis. Moreover, the axis may be regulated by lower leptin signaling. Thus, caloric restriction appears to induce beneficial actions partially by regulating mitochondrial quantity and quality in white adipose tissue in a growth hormone/insulin-like growth factor 1-independent manner.

Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; AL, *ad libitum*; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; COX4, cytochrome c oxidase subunit 4; CR, caloric restriction; df/df, Ames dwarf mice; eNOS, endothelial nitric oxide synthase; eWAT, epididymal white adipose tissue; FA, fatty acid; FASN, FA synthase; FGF21, Fibroblast growth factor 21; FGFR1, FGF receptor 2; FOXO, forkhead box O; GH, growth hormone; GHR, growth hormone receptor/binding protein; GHRH, growth hormone-releasing hormone; GSSG/GSH, oxidized glutathione to reduced glutathione; HSL, hormone sensitive lipase; IGF-1, insulin-like growth factor 1; KLB, beta-klotho; LEPR, leptin receptor; MDH2, malate dehydrogenase 2; ME-1, malic enzyme 1; MEFs, mouse embryonic fibroblasts; MIPEP, mitochondrial intermediate peptidase; MnSOD, Manganese superoxide dismutase; MPP, mitochondrial processing peptidase; mTORC1, mechanistic target of rapamycin complex 1; MtSPases, mitochondrial signal peptidases; NRF, nuclear respiratory factor; *ob*, obese gene; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; pHSL, phosphorylated hormone sensitive lipase; QFM, quadriceps femoris muscle; rWAT, retroperitoneal white adipose tissue; Sir2, silent information regulator 2; SREBPs, sterol regulatory element binding proteins; sWAT, subcutaneous white adipose tissue; Tg, heterozygous transgenic dwarf rats bearing an anti-sense GH transgene; TOM20, translocase of outer mitochondrial membranes 20 kDa; vWAT, visceral white adipose tissue; WAT, white adipose tissue; Wd, wild-type; γ-GCS, γ-glutamylcysteine synthetase.

(B) / 20H0413, Japan Society for the Promotion of Science

KEYWORDS
aging, caloric restriction, fibroblast growth factor 21, growth hormone/insulin-like growth factor 1, mitochondria, mitochondrial intermediate peptidase, peroxisome proliferator-activated receptor γ coactivator-1α, sterol regulatory element binding protein-c, white adipose tissue

INTRODUCTION

Obesity is a serious problem for human health in the modern world. In 2016, the World Health Organization reported that more than 1.9 billion adults over 18 years of age are overweight, of whom 650 million are obese. The number of obese people in 2016 is nearly three times the number in 1975. Overweight and obesity cause and promote age-related diseases including type 2 diabetes, atherosclerosis, cardiovascular diseases, and certain cancers.[1]

Aging, also called senescence, involves irreversible and progressive morphological and functional deteriorative processes that occur with time. Age-related diseases are widely accepted to occur based on the aging process. Therefore, to prevent age-related diseases, we must understand the mechanisms of aging. Recent advances in molecular, genetic, and reproductive technologies provide several clues to understand aging. Recently, 12 aging hallmarks (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis) were proposed;[2] however, the mechanism of aging has yet to be fully clarified.

In 1935, McCay and colleagues reported that long-term caloric restriction (CR) after weaning extends the lifespan of rats.[3] Thereafter, a number of studies using rodents suggested that CR improves whole body metabolism, suppresses age-related pathophysiological changes, and extends lifespan. Indeed, the beneficial actions of CR are observed in various species from yeast and *Caenorhabditis elegans* to mammals.[4,5] In the late 1980s, two parallel CR studies were initiated using rhesus monkeys. The University of Wisconsin study reported a significant positive impact of CR on survival, but the National Institute on Aging study did not detect it. Thereafter, two studies were comprehensively analyzed and concluded that CR is effective in nonhuman primates as well.[6] Therefore, the beneficial action of CR may also be effective for humans.

In 1996, Ames dwarf mice (df/df) were reported to live longer than wild-type (Wd) mice.[7] Ames dwarf mice, which bear a *Prop1* mutation, lack growth hormone (GH), prolactin, and thyroid-stimulating hormone. This was the first report of a prolongevity effect induced by a single gene mutation in mammals. Thereafter, more than 40 rodents bearing a single gene mutation or genetic modification have been reported to live longer than Wd rodents. Approximately one-third of reported rodents showing a prolongevity phenotype are involved in GH/insulin-like growth factor 1 (IGF-1) signaling. Because CR also suppresses GH/IGF-1 signaling, it has been suggested that the beneficial action of CR might be largely regulated in a GH/IGF-1-dependent manner. However, the longevity of various long-lived rodents exhibiting suppressed GH/IGF-1-signaling is further extended by CR. The findings suggest that the beneficial action of CR may be regulated by both GH/IGF-1-dependent and -independent mechanisms.[8] GH/IGF-1 signaling and sirtuin are well researched; in contrast, the beneficial actions of CR regulated in a GH/IGF-1-independent manner are not well understood.

In this review, we first discuss the impacts of GH/IGF-1 signaling and sirtuin on the beneficial actions of CR, then focus on novel GH/IGF-1-independent signaling mechanisms.

GH/IGF-1 signaling in the beneficial actions of CR

GH/IGF-1 signaling, which is involved in anabolic processes, is important for insulin sensitivity and lifespan regulation.[9,10] Ames dwarf mice, mini rats bearing an anti-sense GH transgene, *growth hormone-releasing hormone* (*GHRH*)-knockout mice, and *GH receptor/binding protein* (*GHR*)-knockout mice live longer than Wd rodents.[11–14] CR further extends the longevity of Ames dwarf mice, mini rats and *GHRH*-knockout mice.[11–13] On the other hand, the significant positive impact of CR on lifespan disappears in *GHR*-knockout mice.[14] Thus, GH/IGF-1 signaling is well researched, but the conflicting results on lifespan regulation by CR are not well understood.

Forkhead box O (FOXO) signaling and mechanistic target of rapamycin complex 1 (mTORC1) signaling are well-known factors involved in aging processes in a GH/IGF-1-dependent manner. FOXO family members FOXO1, FOXO3, FOXO4, and FOXO6 are negatively regulated by the GH/IGF-1/phosphoinsitide-3 kinase/Akt signaling pathway.[15] Under CR conditions, FOXO1 and FOXO3 contribute to inhibition of tumorigenesis and lifespan extension, respectively.[16,17] mTOR is a serine/threonine kinase formed by two complexes,

mTORC1 and mTORC2, which are composed of special binding partners. mTORC1 is sensitive to rapamycin and consists of RAPTOR, PRAS40, DEPTOR and mLST8 proteins. mTORC2 is relatively insensitive to rapamycin and consists of RICTOR, PROTOR, DEPTOR, mLST8, and mSIN1. mTORC1 regulates protein synthesis and cell growth through downstream molecules, 4E-BP1 and S6K. mTORC2 plays a crucial role in maintenance of normal and cancer cells through downstream molecules, AKT, SGK-1 and PKCα.[18] Interestingly, it has been reported that rapamycin-mediated inhibition of mTORC1 extends the median and maximal lifespan of mice.[19]

Sirtuin in the beneficial actions of CR

Sir2 (silent information regulator 2) was first discovered as a gene involved in transcriptional silencing of budding yeast mating type loci.[20] Sir2 is required for CR-associated prolongevity in yeast.[21] In mammals, seven Sir2 orthologs (the Sirtuin family) have been identified; of these, SIRT1, SIRT3, and SIRT6 have important roles in age-related phenotypes.[22] In addition, cell survival under high oxidative stress requires FOXO1, FOXO3a, and FOXO4; SIRT1 regulates the transcriptional activity of these FOXO family members through its deacetylase activity.[23,24] Increases of SIRT3, a major deacetylase in the mitochondrial matrix, reportedly enhance mitochondrial glutathione antioxidants to extend lifespan in CR conditions.[25] Moreover, *Sirt6* transgenic mice exhibit alterations of key pathways in lifespan regulation, including lower serum levels of IGF-1, and live longer than Wd mice.[26]

A novel molecular target of the GH/IGF-1-independent beneficial actions of CR

To identify the molecular targets regulated in a GH/IGF-1-independent manner that underlie the beneficial actions of CR, we first analyzed the white adipose tissue (WAT) morphology of Wd rats fed *ad libitum* (WdAL) or CR (WdCR), as well as those of heterozygous transgenic mini rats bearing an anti-sense GH transgene fed AL (TgAL) or CR (TgCR). The findings show that the size of adipocytes is markedly reduced in WdCR and TgAL compared with WdAL, although the effect is less significant in TgAL. In addition, we evaluated gene expression profiles using high-density oligonucleotide microarrays, which indicates marked changes in WdCR compared with WdAL but not TgAL. Genes involved in fatty acid (FA) biosynthesis, particularly those regulated by sterol regulatory element binding proteins (SREBPs), display the largest changes by CR but not Tg.[27] SREBPs are transcription factors belonging to the basic helix-loop-helix/leucine zipper family that are known to be master regulators of FA and cholesterol metabolism. The SREBP family has three isoforms: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a and SREBP-1c are master regulators of FA biosynthesis, while SREBP-2 regulates cholesterol synthesis. SREBP-1c localizes in insulin-sensitive tissues such as liver, WAT, and muscle; in contrast, SREBP-1a is more widely distributed than SREBP-1c in the jejunum, ileum, thymus, and spleen as well.[28–30] The SREBPs-SREBP cleavage-activating protein (SCAP) complex is retained in the endoplasmic reticulum together with insulin-induced gene proteins (INSIGs). INSIGs are ubiquitylated and rapidly degraded when sterols are absent. In this condition, the SREBPs-SCAP complex is transported to the Golgi. Site-1 and site-2 proteases, two proteolytic enzymes that cleave and release the N-terminal domain of SREBPs, are located at the Golgi membrane. Thereafter, the N-terminal domain of SREBPs is transported to the nucleus and binds to SRE. Subsequently, SREBPs promote transcription of their target genes.[31] Among SREBPs, CR increases *Srebp-1c* expression the most, followed by *Srebp-1a* and *Srebp-2*. CR markedly upregulates expression of *Srebp-1c* and its downstream targets including FA synthase (*Fasn*) and acetyl-CoA carboxylase (*Acc*), whereas Tg does not.[27] These findings suggest that CR can promote FA biosynthesis by increasing SREBP-1c in a GH/IGF-1-independent manner.

SREBP-1c is a key regulator of the GH/IGF-1-independent beneficial actions of CR

As mentioned above, SREBP-1c acts as a key regulator of FA biosynthesis in a GH/IGF-1-independent manner. Therefore, to analyze mechanisms of CR, we used *Srebp-1c*-knockout mice. SREBP-1c binds with sterol regulatory element to activate transcription of its downstream genes, some of which are involved in FA biosynthesis. As shown in Figure 1, CR upregulates expression of genes and/or proteins involved in FA biosynthesis including FASN, ACC, ATP citrate lyase (ACLY), and malic enzyme 1 (ME-1) in the WAT of Wd mice but not *Srebp-1c*-knockout mice.[32] SREBP-1c and its downstream factors increase with adipocyte differentiation. Thus, we consider that CR-associated upregulation of genes involved in FA biosynthesis occurs in mature adipocytes in WAT.[33] This CR-associated phenotypic alteration, which might be regulated by Srebp-1c, is observed in WAT but not liver, heart, quadriceps femoris muscle (QFM), or kidney.[32] Our findings support a previous report that CR promotes *de novo* FA biosynthesis in WAT rather than liver.[34]

In general, CR suppresses oxidative stress.[35] Both aconitase activity and the ratio of oxidized glutathione

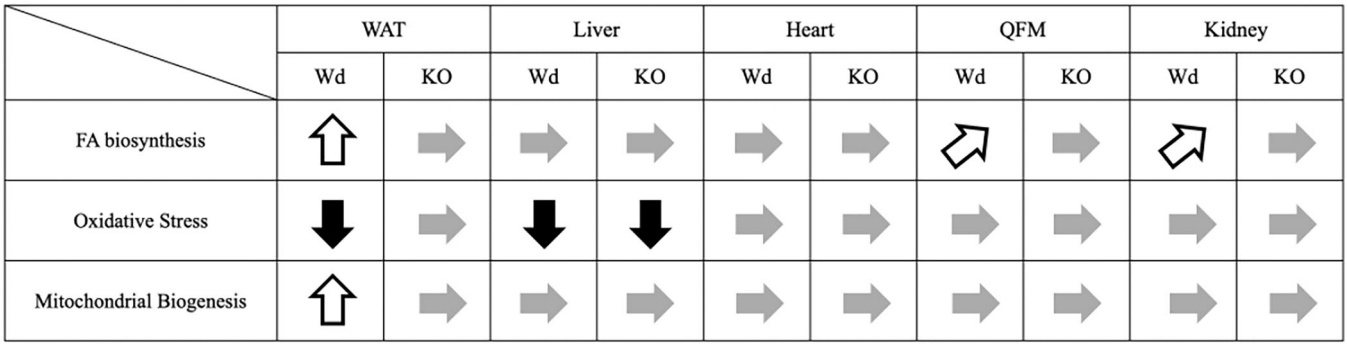

FIGURE 1 Differential responses of caloric restriction (CR) among various tissues. Based on our previous published report, the CR-associated changes involved in fatty acid (FA) biosynthesis, oxidative stress, and mitochondrial biogenesis are summarized.[32] All results were compared among five tissues, white adipose tissue (WAT), liver, heart, quadriceps femoris muscle (QFM) and kidney between wild-type mice (Wd) and *Srebp-1c*-knockout mice (KO). Up arrows show "increase," diagonally up arrows show "increased tendency," and right arrows show "no change" in the levels of FA biosynthesis, oxidative stress, and mitochondrial biogenesis.

to reduced glutathione (GSSG/GSH) are useful biomarkers for oxidative stress.[36,37] CR increases both aconitase activity and GSSG/GSH ratios in the WAT of Wd mice but not *Srebp-1c*-knockout mice. γ-glutamylcysteine synthetase (γ-GCS), a rate-limiting enzyme for GSH biosynthesis,[38] is slightly upregulated by CR in Wd, but not in *Srebp-1c*-knockout mice. CR-associated phenotypic alterations, which may be regulated by Srebp-1c, are observed in WAT but no other tissues (Figure 1).[32] These results indicate that CR suppresses oxidative stress through the regulation of GSH biosynthesis, which is mediated by γ-GCS expression in a *Srebp-1c*-dependent manner.

It is reported that CR enhances mitochondrial biogenesis by upregulating peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α).[39–41] PGC-1α was originally identified as a PPARγ-interacting protein that is expressed preferentially in brown adipose tissue, a key thermogenic tissue.[42] Currently, it is recognized as a master transcriptional regulator of mitochondrial biogenesis. PGC-1α stimulates mitochondrial biogenesis and respiration by binding to and activating nuclear respiratory factor (NRF)-1 and NRF-2, which enhance the transcription of various mitochondria-related genes.[43] In addition, PGC-1α induces the expression of transcription factor A mitochondria (TFAM), which is a mitochondrial transcriptional factor required for the replication and transcription of mitochondrial DNA.[43] SIRT3, a mitochondria deacetylase, activates various mitochondrial matrix enzymes *via* its deacetylase activity.[22] Cytochrome c oxidase subunit 4 (COX4) is a component of the mitochondrial respiratory chain (electron transport chain),[44] while translocase of outer mitochondrial membranes 20 kDa (Tom20) imports mitochondrial proteins into mitochondria from the cytosol.[45] Thus, SIRT3, COX4, and Tom20 are important proteins in mitochondria and its functions. CR increases expression levels of SIRT3, COX4, and Tom20 in the WAT of Wd mice but not *Srebp-1c*-knockout mice. In addition, no changes in protein expression levels of these factors occur in other organs of *Srebp-1c*-knockout mice.[32] We found that CR upregulates *Pgc-1α* expression in WAT of Wd mice but not *Srebp-1c*-knockout mice. In addition, a chromatin immunoprecipitation assay using adipocytes derived from mouse embryonic fibroblasts (MEFs) demonstrates that SREBP-1c directly binds the promoter region of *Pgc-1α*.[32] The findings indicate CR activates mitochondrial biogenesis via *Pgc-1α* regulated by SREBP-1c in adipocytes but not in other tissues and cells (Figure 1).

As expected, CR extends the lifespan of Wd mice but not *Srebp-1c*-knockout mice. Elovl6, which is highly expressed in liver and adipose tissue, is involved in elongation of C16 saturated and monounsaturated FAs to form C18 FAs.[46,47] *Elovl6*-knockout mice display obesity and low fatty acid oxidation in the liver.[46] These findings implicate the altered quality of FAs due to Elovl6 deficiency in age-related pathologies. Thus, CR likely increases FA biosynthesis and improves FA quality via SREBP-1c/ELOVL6. According to our findings, the CR-associated prolongevity effect and metabolic remodeling observed in WAT may be regulated by SREBP-1c in a GH/IGF-1-independent manner. In addition, metabolic remodeling in WAT, which is regulated by SREBP-1c, likely includes enhanced FA biosynthesis via FASN and other markers, conversion of FA quality via ELOVL6, reduced oxidative stress via γ-GCS, and activated mitochondrial biogenesis via PGC-1α. Furthermore, Nisoli et al. (2005) reported that CR induces expression of endothelial nitric oxide synthase (eNOS) and enhances mitochondrial biogenesis in various tissues including WAT of mice. These effects are strongly attenuated in *eNos*-knockout mice.[48] Thus, mitochondrial biogenesis, which plays a fundamental role in the

processes regulated by CR, is activated by SREBP-1c as well as eNOS.

Srebp-1c upregulates expression of *Pgc-1α* and *Fgf21*

Fibroblast growth factor 21 (FGF21) is a member of the endocrine FGF superfamily. FGF21 binds to the FGF receptor 2 (FGFR1)/beta-klotho (KLB) complex to activate local downstream signaling in WAT and muscle. FGF21 was originally identified as a hepatokine; since then, FGF21-FGFR1/KLB signaling has been shown to upregulate expression levels of both glucose uptake- and lipogenesis-related gene.[49–52] Because few studies have investigated the impact of FGF21 on the effect of CR, we focused on FGF21 in WAT under CR conditions. At adult ages, CR upregulates messenger RNA (mRNA) and protein expression levels of FGF21 in WAT but not liver. To reveal the effects of CR-induced upregulation of *Fgf21* expression in WAT, we generated and analyzed 3T3-L1 adipocytes overexpressing FGF21. Our findings suggest that FGF21 induces Pgc-1α mRNA expression in adipocytes. Thus, FGF21 positively regulates Pgc-1α expression, leading to CR activating the FGF21-FGFR1/KLB-GLUT1/PGC-1α pathway in WAT.[53]

Fgf21 expression is negatively regulated by SREBP-1c in the liver.[54] CR upregulates Fgf21 mRNA expression in the WAT of Wd mice but not *Srebp-1c*-knockout mice. In 3T3-L1 adipocytes overexpressing SREBP-1c, *Fgf21* mRNA expression is upregulated. Unexpectedly, unlike regulation in the liver, evidence suggests that SREBP-1c positively regulates Fgf21 expression in WAT and adipocytes.[55] Because *Srebp-1c* and *Pgc-1α* appear to participate in mitochondrial biosynthesis, we evaluated the involvement of FGF21 in mitochondrial biosynthesis. In 3T3-L1 adipocytes overexpressing FGF21, expression of both Pgc-1α mRNA and protein is increased. In contrast, expression of *Pgc-1α* mRNA is decreased in *Fgf21*-knockout adipocytes differentiated from MEFs. However, in 3T3-L1 adipocytes overexpressing Fgf21, FGFR inhibitor does not change expression levels of Pgc-1α mRNA or protein. Therefore, it is likely that FGF21 and SREBP-1c upregulate PGC-1α expression, and SREBP-1c-associated upregulation of PGC-1α expression may be predominant under CR conditions.[53,55]

CR upregulates Srebp-1c expression by suppressing leptin signaling

Leptin, an adipokine, regulates appetite and energy expenditure *via* the central nervous system. Leptin is a product of the obese (*ob*) gene. In *ob*/*ob* mice bearing a mutation in *ob* gene, hyperphagia occurs due to defective leptin, leading to obesity.[56,57] Zucker fatty rats, which carry a mutation of the leptin receptor (*Lepr*) gene, also display obesity due to hyperphagia.[58]

The disappearance of CR-induced upregulation of proteins involved in FA biosynthesis is not observed in WAT of obese Zucker fatty rats. In *Lepr*-knockdown cells, mRNA levels of *Srebp-1c* are upregulated, suggesting that *Srebp-1c* expression levels are negatively regulated by leptin signaling.[59] Therefore, we consider that the CR-associated upregulation of *Srebp-1c* is regulated by lower leptin signaling.

A novel mechanism of SIRT3 activation by CR

As mentioned above, SIRT3, a representative mitochondrial sirtuin, activates various enzymes in the mitochondrial matrix *via* its deacetylase activity.[22,25] Therefore, SIRT3 is a key regulator of mitochondrial quality. We found that immunoblotting with an anti-SIRT3 antibody detects two bands of different molecular weights in WAT, and CR increases the low-molecular-weight band and decreases the high-molecular-weight band in Wd mice but not *Srebp-1c*-knockout mice (Figure 2). Perico and colleagues described a short isoform (28 kDa) and long isoform (44 kDa) of SIRT3.[61] However, our data show an upper band under 37 kDa and lower band of approximately 28 kDa (Figure 2a). Moreover, three bands of varying molecular weights are distinguished by immunoblotting of 3T3-L1 adipocytes overexpressing SIRT3 after treatment with a proteasome inhibitor.[60] These results suggest that the upper band under 37 kDa in Figure 2a is intermediate form of SIRT3 and the intermediate form is cleaved by some enzymes into a 28 kDa mature form in the mitochondrial matrix. CR increases proportions of the lower band to upper band in WAT of Wd mice but not *Srebp-1c*-knockout mice (Figure 2b). Thus, SIRT3 cleavage and maturation are regulated by SREBP-1c.

Most mitochondrial matrix proteins are encoded in the nuclear genome and translated in the cytosol. To transfer mitochondria matrix proteins from the cytosol to mitochondria, these proteins contain an N-terminal mitochondrial targeting signal sequence, most of which are cleaved by one or two mitochondrial signal peptidases (MtSPases) to produce functional mature forms.[62] Mitochondrial processing peptidase (MPP) is a representative MtSPase for which SIRT3 is a substrate.[63] Mitochondrial intermediate peptidase (MIPEP), another MtSPase, sequentially cleaves eight amino acids from the N-terminus after cleavage by MPP.[64] Both COX4 and malate dehydrogenase 2 (MDH2) are reported substrates of MIPEP.[65] Knockdown of Mipep reduces protein expression levels of

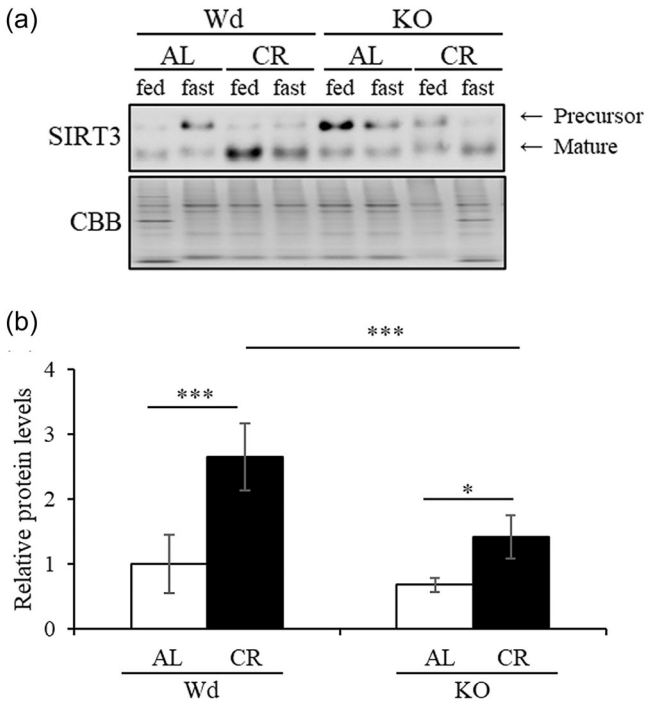

FIGURE 2 Maturation of SIRTUIN 3 (SIRT3) protein by caloric restriction (CR). Analysis of SIRT3 in wild-type mice (Wd) and *Srebp-1c*-knockout mice (KO) by western blotting. (a) Coomassie Brilliant Blue (CBB) was used as a loading control. CR increased the mature form of SIRT3 in Wd mice but not KO mice. (b) Relative protein levels of lower/upper bands are shown. Data derived from Figure 1a in Kobayashi et al. (2017)[60] were recalculated. Values represent mean ± SEM. $*p < 0.05$, $***p < 0.001$ versus AL or KO, analyzed using Tukey's test. AL: fed *ad libitum*, CR: caloric restriction.

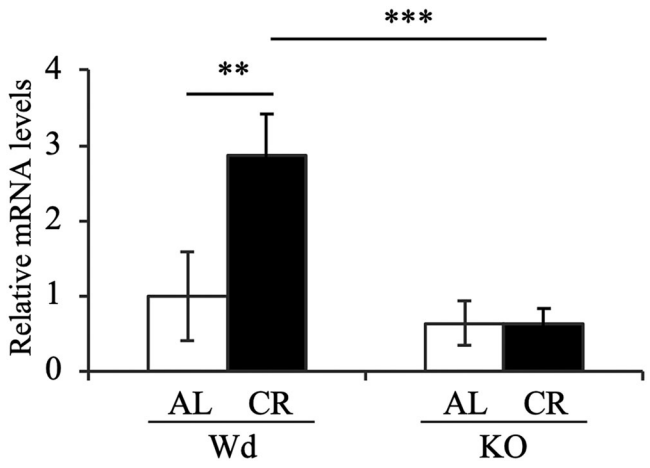

FIGURE 3 Effect of Srebp-1c on changes in mitochondrial intermediate peptidase (Mipep) messenger RNA (mRNA) expression induced by CR. Analysis of *Mipep* mRNA level in wild-type mice (Wd) and *Srebp-1c*-knockout mice (KO) by real-time reverse transcription – polymerase chain reaction. Values represent mean ± SEM. $**p < 0.01$, $***p < 0.001$ versus WdAL or WdCR, analyzed using Tukey's test. AL: fed *ad libitum*, CR: caloric restriction. Part of the Figure was reported in our previously published report.[55]

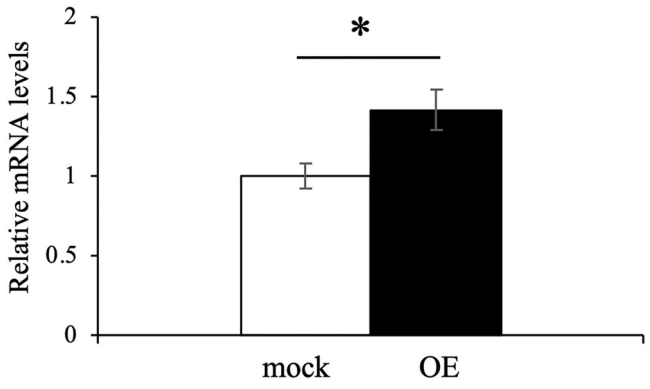

FIGURE 4 Expression levels of *mitochondrial intermediate peptidase* (*Mipep*) messenger RNA (mRNA) in 3T3-L1 adipocytes overexpressing sterol regulatory element binding protein 1c (SREBP-1c). Mock and 3T3-L1 adipocytes overexpressing SREBP-1c were differentiated into adipocytes and evaluated at day 8. Total RNA was extracted from adipocytes and *Mipep* mRNA expression was quantified using real-time reverse transcription – polymerase chain reaction (RT-PCR) ($n = 3$). *Rps18* was used as a reference gene to normalize target gene expression. 3T3-L1 adipocytes overexpressing SREBP-1c and methods of RT-PCR with primer information have been described in our previous reports.[55,60] Values represent mean ± SEM. $*p < 0.05$, analyzed using Student's *t*-test.

SIRT3, COX4, and MDH2. Therefore, SIRT3 may be cleaved by both MPP and MIPEP, as well as COX4 and MDH2.[64] In fact, CR increases expression of *Mipep* mRNA in Wd mice but not *Srebp-1c*-knockout mice (Figure 3). *Mipep* mRNA expression levels are upregulated in 3T3-L1 adipocytes overexpressing SREBP-1c (Figure 4). Moreover, CR upregulates expression of SIRT3 protein, as well as known MIPEP substrates COX4 and MDH2.[60]

Manganese superoxide dismutase (MnSOD), which is deacetylated by SIRT3, detoxicates superoxide radicals in mitochondria.[66] The ratio of acetylated MnSOD compared with total MnSOD is increased in *Mipep*-knockdown cells, indicating defective SIRT3 function.[60] To further confirm whether SIRT3 is a substrate of MIPEP, we generated adipose-specific *Mipep*-knockout mice. As expected, SIRT3 is down-regulated and the ratio of acetylated MnSOD to total MnSOD is upregulated (unpublished data). This result reinforces the earlier idea that, after cleavage by MPP, SIRT3 is cleaved by MIPEP into its mature form.[60] Thus, MIPEP upregulation is a novel SIRT3 activation mechanism and CR upregulates Mipep expression in an *Srebp-1c*-dependent manner.

CR-associated alteration of metabolic remodeling is location-dependent in WAT

The characteristics of WAT depend on the location of each depot. For example, lipolytic activity and FA turnover is more readily activated in visceral WAT (vWAT) than subcutaneous WAT (sWAT). vWAT includes retro-peritoneal WAT (rWAT) and epididymal WAT (eWAT). In

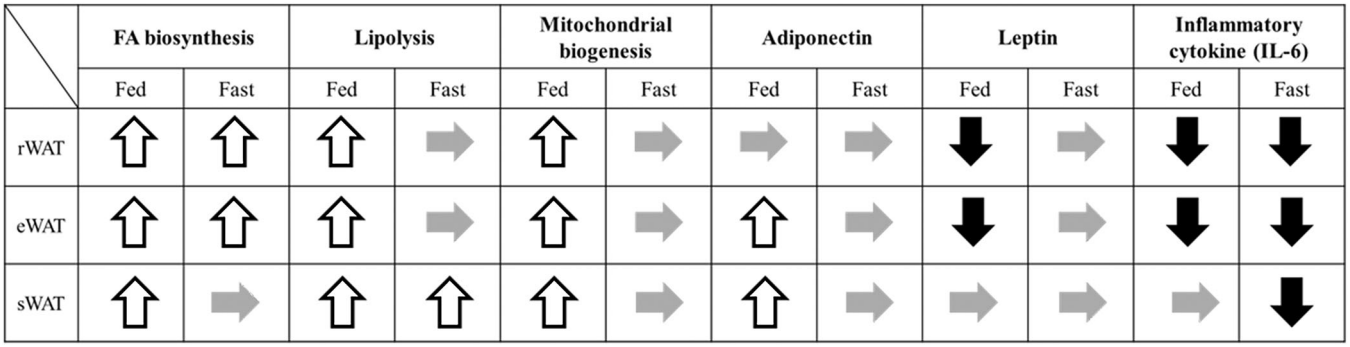

FIGURE 5 Differential responses of caloric restriction (CR) among white adipose tissue (WAT) with different locations. Based on our previous published report, the representative metabolic changes of CR in retroperitoneal WAT (rWAT), epididymal WAT (eWAT) and subcutaneous WAT (sWAT) are summarized.[69] Upwards arrow shows "upregulating," rightwards arrow shows "no change," downwards arrow shows "downregulating." Fed mice sacrificed without removing food, fast mice sacrificed 20 h after removal of food from their cage.

addition, expression levels of adiponectin and leptin are lower in vWAT compared with sWAT.[67,68] Therefore, CR effects may vary across different fat depots. As shown in Figure 5,[69] expression of proteins involved in *de novo* FA biosynthesis, ACLY and ME-1, is upregulated in CR-fed rats compared with AL-fed and AL-fasted rats, but decreased in CR-fasted rats compared with CR-fed rats. These phenotypes of ACLY and ME-1 are identical in rWAT, eWAT, and sWAT.

Regarding lipolytic proteins, phosphorylated HSL (Ser563) (pHSL) is an activated form of hormone sensitive lipase (HSL), and the ratio of pHSL to total HSL protein serves as an indicator of activated HSL levels. pHSL/total HSL protein levels are increased in rWAT and eWAT of CR-fed rats compared with AL-fed and AL-fasted rats, whereas there is no change in CR-fasted rats. In contrast, no notable change of pHSL/total HSL protein levels occurs in sWAT. In addition, the lipolytic protein adipose triglyceride lipase (ATGL) is upregulated in all three WAT depots of CR-fasted rats compared with AL-fed and AL-fasted rats, whereas CR-fed rats display no change (Figure 5).[69] Because *Atgl* mRNA is negatively regulated by insulin signaling, ATGL might regulate CR-enhanced lipolysis in place of insulin signaling during fasting.[69]

As mentioned above, CR increases *Pgc-1α* mRNA expression levels to induce mitochondrial biogenesis under CR conditions. In addition, mitochondrial DNA content and citrate synthase activity are increased by CR.[31] CR increases adiponectin mRNA expression levels in sWAT and tends to increase levels in eWAT, but no change occurs in rWAT. mRNA levels of leptin, another adipocytokine, are increased in rWAT and eWAT, but unchanged in sWAT. Thus, adipocytokine expression levels in CR conditions may differ between vWAT (rWAT and eWAT) and sWAT. Finally, regarding inflammatory cytokines, CR reduces mRNA expression of *Il-6* in rWAT and eWAT, but not sWAT (Figure 5).[69]

CONCLUSIONS AND PROSPECTS

Various compounds, molecules, and signaling pathways including CR mimetics have been proposed to contribute to healthy life expectancy over the past decade.[70] Recently, a senescence-associated secretory phenotype, so-called SASP, was identified as a specific condition by which senescent cells induce paracrine senescence by secreting inflammatory cytokines, chemokines, matrix-remodeling proteases, and growth factors to inflict damage on surrounding cells. Acosta and colleagues reported that understanding the importance and regulation of paracrine senescence will provide a new avenue for therapeutic benefit.[71,72] Recently, senescent cells are shown to accumulate in aged individuals, while their removal improves age-related pathologies and extends lifespan.[73] Accordingly, compounds to remove senescent cells through induction of apoptosis (senolytics) have been developed. For example, the Src/tyrosine kinase inhibitor dasatinib and natural flavonoids quercetin and fisetin (as well as their combination), which can activate senescent cell antiapoptotic pathways, are under current or planned clinical trials for various age-related diseases including idiopathic pulmonary fibrosis and bone loss.[74,75]

CR mimetics might also contribute to healthy life expectancy. mTORC inhibitors, AMP-activated protein kinase (AMPK) activators, and sirtuin activators are potentially effective candidates. For example, rapamycin inhibits mTORC signaling to extend lifespan.[19,76] AMPK regulates metabolic homeostasis as a metabolic checkpoint.[77] Metformin activates AMPK and extends healthy lifespan.[78] Moreover, sodium/glucose cotransporter 2 inhibitors, another CR mimetic, can promote glycose excretion in proximal tubules, are used as anti-diabetic medicines, and can extend lifespan.[79,80] Accordingly, we believe that development of CR mimetics and senolytics will lead

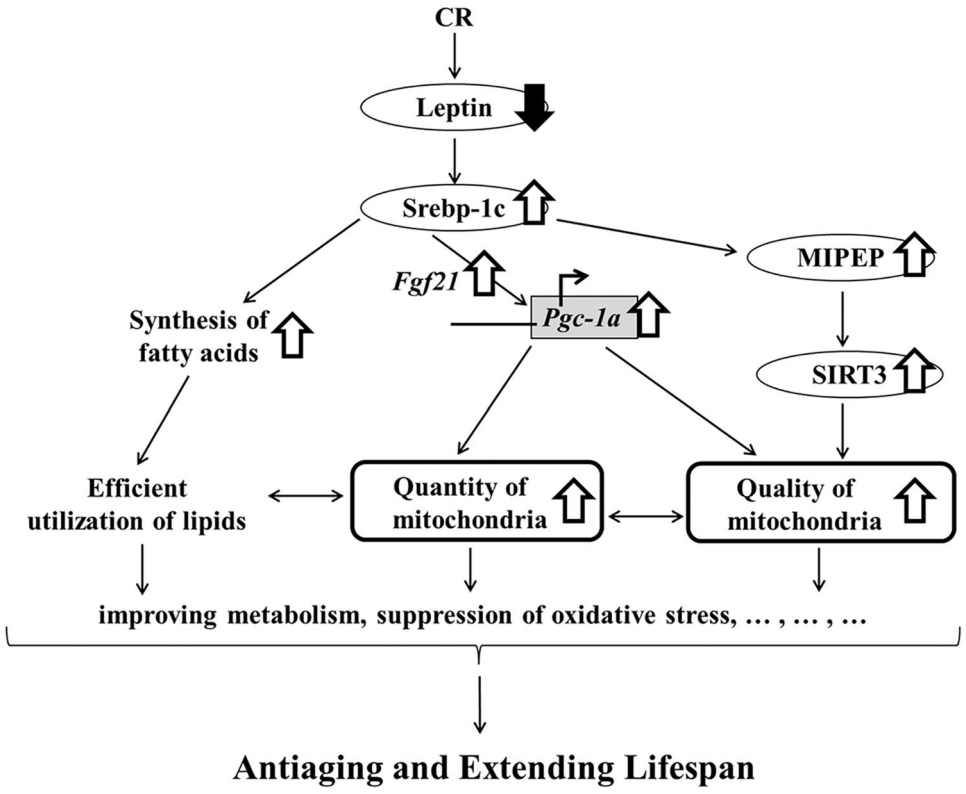

FIGURE 6 Diagram demonstrating the proposed molecular mechanism of caloric restriction (CR)-associated metabolic remodeling in white adipose tissue (WAT). CR downregulates leptin signaling, subsequently inducing upregulation of *sterol regulatory element binding protein 1c* (*Srebp-1c*). SREBP-1c activates synthesis of fatty acids and increases messenger RNA (mRNA) expression of both *peroxisome proliferator-activated receptor γ coactivator-1α* (*Pgc-1α*) and *mitochondrial intermediate peptidase* (*Mipep*). SREBP-1c also upregulates *Pgc-1α* expression by upregulating *fibroblast growth factor 21* (*Fgf21*). MIPEP increases SIRTUIN 3 (SIRT3) mature form by processing. PGC-1α and MIPEP/SIRT3 improve mitochondrial functions by increasing quantity and quality, respectively. These effects of CR improve metabolism and suppress oxidative stress, probably leading to antiaging and prolongevity effects.

to successful scientifically based antiaging medicine in the near future.

In this review, we demonstrate the molecular mechanism of CR-associated metabolic remodeling in WAT (Figure 6). (1) CR increases mitochondrial quantity *via* PGC-1α and improves its quality *via* MIPEP, particularly in WAT. (2) Expression of PGC-1α and FGF21 increase in a mutually complementary manner, and both are regulated by SREBP-1c. (3) Elevated expression of MIPEP activates SIRT3. (4) Similar CR-associated mitochondrial alterations occur in different parts of WAT. (5) CR-associated mitochondrial alterations are regulated by SREBP-1c under conditions with downregulated leptin signaling.

Finally, because better characteristics of WAT are associated with longer healthy life expectancy, we propose that mitochondria in WAT are a better target to use in the development of CR-mimetic medicines.

AUTHOR CONTRIBUTIONS

Y.O. wrote the manuscript. Y.N., Y.M. and M.K. helped in writing the manuscript. Y.H. conceived and wrote the manuscript.

ACKNOWLEDGMENTS

Y.H. has been announced as the winner of The Japanese Society of Pathology; Japan Pathology Research Award in 2022. We thank Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript. We also would like to thank all faculty members and students who have been enrolled in the laboratory and engaged in the research.

CONFLICT OF INTEREST STATEMENT

None declared.

ORCID

Yoshikazu Higami http://orcid.org/0000-0003-1381-0777

REFERENCES

1. World Health Organization. Obesity and overweight [Internet]. Geneva Switzerland: World Health Organization; 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
2. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. Cell. 2023;186(2):243–78.

3. McCay CM, Crowell MF, Maynard LA. The effect of retarded growth upon the length of life span and upon the ultimate body size. J Nutr. 1935;10(1):63–79.
4. Masoro EJ. Overview of caloric restriction and ageing. Mech Ageing Dev. 2005;126:913–22.
5. Sinclair DA. Toward a unified theory of caloric restriction and longevity regulation. Mech Ageing Dev. 2005;126(9):987–1002.
6. Mattison JA, Colman RJ, Beasley TM, Allison DB, Kemnitz JW, Roth GS, et al. Caloric restriction improves health and survival of rhesus monkeys. Nat Commun. 2017;8:14063.
7. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. Nature. 1996;384:33.
8. Hoshino S, Kobayashi M, Higami Y. Mechanisms of the anti-aging and prolongevity effects of caloric restriction: evidence from studies of genetically modified animals. Aging. 2018;10(9): 2243–51.
9. Brown-Borg HM. Hormonal control of aging in rodents: the somatotropic axis. Mol Cell Endocrinol. 2009;299(1):64–71.
10. Coschigano KT, Holland AN, Riders ME, List EO, Flyvbjerg A, Kopchick JJ. Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. Endocrinology. 2003;144(9):3799–810.
11. Mattison JA, Wright C, Bronson RT, Roth GS, Ingram DK, Bartke A. Studies of aging in Ames dwarf mice: effects of caloric restriction. Age. 2000;23:9–16.
12. Shimokawa I, Higami Y, Tsuchiya T, Otani H, Komatsu T, Chiba T, et al. Lifespan extension by reduction of the growth hormone-insulin-like growth factor-1 axis: relation to caloric restriction. FASEB J. 2003;17(9):1108–9.
13. Sun LY, Spong A, Swindell WR, Fang Y, Hill C, Huber JA, et al. Growth hormone-releasing hormone disruption extends lifespan and regulates response to caloric restriction in mice. eLife. 2013;2:e01098.
14. Bonkowski MS, Rocha JS, Masternak MM, Al Regaiey KA, Bartke A. Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. Proc Nat Acad Sci. 2006;103(20):7901–5.
15. Martins R, Lithgow GJ, Link W. Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. Aging cell. 2016;15(2):196–207.
16. Yamaza H, Komatsu T, Wakita S, Kijogi C, Park S, Hayashi H, et al. FoxO1 is involved in the antineoplastic effect of calorie restriction. Aging cell. 2010;9(3):372–82.
17. Shimokawa I, Komatsu T, Hayashi N, Kim SE, Kawata T, Park S, et al. The life-extending effect of dietary restriction requires Foxo3 in mice. Aging cell. 2015;14(4):707–9.
18. Szwed A, Kim E, Jacinto E. Regulation and metabolic functions of mTORC1 and mTORC2. Physiol Rev. 2021;101(3): 1371–426.
19. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature. 2009;460:392–5.
20. Gottlieb S, Esposito RE. A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. Cell. 1989;56(5):771–6.
21. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science. 2000;289(5487):2126–8.
22. Giblin W, Skinner ME, Lombard DB. Sirtuins: guardians of mammalian healthspan. TIG. 2014;30(7):271–86.
23. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science. 2004;303(5666): 2011–5.
24. Kobayashi Y, Furukawa-Hibi Y, Chen C, Horio Y, Isobe K, Ikeda K, et al. SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. Int J Mol Med. 2005;16(2):237–43.
25. Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell. 2010;143(5):802–12.
26. Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, et al. The sirtuin SIRT6 regulates lifespan in male mice. Nature. 2012;483:218–21.
27. Chujo Y, Fujii N, Okita N, Konishi T, Narita T, Yamada A, et al. Caloric restriction-associated remodeling of rat white adipose tissue: effects on the growth hormone/insulin-like growth factor-1 axis, sterol regulatory element binding protein-1, and macrophage infiltration. Age. 2013;35:1143–56.
28. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002;109(9):1125–31.
29. Shimano H. SREBPs: physiology and pathophysiology of the SREBP family. FEBS J. 2009;276(3):616–21.
30. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest. 1997;99(5):838–45.
31. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology—divergent pathophysiology. Nat Rev Endocrinol. 2017;13:710–30.
32. Fujii N, Narita T, Okita N, Kobayashi M, Furuta Y, Chujo Y, et al. Sterol regulatory element-binding protein-1c orchestrates metabolic remodeling of white adipose tissue by caloric restriction. Aging cell. 2017;16(3):508–17.
33. Price NL, Holtrup B, Kwei SL, Wabitsch M, Rodeheffer M, Bianchini L, et al. SREBP-1c/MicroRNA 33b genomic loci control adipocyte differentiation. Mol Cell Biol. 2016;36(7): 1180–93.
34. Bruss MD, Khambatta CF, Ruby MA, Aggarwal I, Hellerstein MK. Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. Am J Physiol Endocrinol Metabol. 2010;298(1):E108–16.
35. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science. 1996;273(5271):59–63.
36. Murakami K, Yoshino M. Inactivation of aconitase in yeast exposed to oxidative stress. IUBMB Life. 1997;41(3):481–6.
37. Dudley RWR, Khairallah M, Mohammed S, Lands L, Des Rosiers C, Petrof BJ. Dynamic responses of the glutathione system to acute oxidative stress indystrophic mouse (mdx) muscles. Am J Physiol Regul Integr Compar Physiol. 2006;291(3):R704–10.
38. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; Applications in research and therapy. Pharmacol Ther. 1991;51(2):155–94.
39. Martin-Montalvo A, de Cabo R. Mitochondrial metabolic reprogramming induced by calorie restriction. Antioxid Redox Signaling. 2013;19(3):310–20.
40. López-Lluch G, Hunt N, Jones B, Zhu M, Jamieson H, Hilmer S, et al. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. Proc Nat Acad Sci. 2006;103(6): 1768–73.
41. Anderson RM, Barger JL, Edwards MG, Braun KH, O'Connor CE, Prolla TA, et al. Dynamic regulation of PGC-1α localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell. 2008;7(1):101–11.
42. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 1998;92(6):829–39.
43. Scarpulla RC. Nuclear control of respiratory gene expression in mammalian cells. J Cell Biochem. 2006;97(4):673–83.

44. Oliva CR, Zhang W, Langford C, Suto MJ, Griguer CE. Repositioning chlorpromazine for treating chemoresistant glioma through the inhibition of cytochrome c oxidase bearing the COX4-1 regulatory subunit. Oncotarget. 2017;8(23):37568–83.
45. Eliyahu E, Pnueli L, Melamed D, Scherrer T, Gerber AP, Pines O, et al. Tom20 mediates localization of mrnas to mitochondria in a translation-dependent manner. Mol Cell Biol. 2010;30(1):284–94.
46. Matsuzaka T, Shimano H, Yahagi N, Kato T, Atsumi A, Yamamoto T, et al. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. Nature Med. 2007;13(10):1193–202.
47. Moon YA, Shah NA, Mohapatra S, Warrington JA, Horton JD. Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. J Biol Chem. 2001;276(48):45358–66.
48. Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science. 2005;310(5746): 314–7.
49. Ding X, Boney-Montoya J, Owen BM, Bookout AL, Coate KC, Mangelsdorf DJ, et al. βKlotho is required for fibroblast growth factor 21 effects on growth and metabolism. Cell Metab. 2012;16(3):387–93.
50. Kurosu H, Choi M, Ogawa Y, Dickson AS, Goetz R, Eliseenkova AV, et al. Tissue-specific expression of βKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem. 2007;282(37):26687–95.
51. Ogawa Y, Kurosu H, Yamamoto M, Nandi A, Rosenblatt KP, Goetz R, et al. βKlotho is required for metabolic activityof fibroblast growth factor 21. Proc Nat Acad Sci. 2007;104(18): 7432–7.
52. Véniant MM, Sivits G, Helmering J, Komorowski R, Lee J, Fan W, et al. Pharmacologic effects of FGF21 are independent of the "Browning" of white adipose tissue. Cell Metab. 2015;21(5):731–8.
53. Fujii N, Uta S, Kobayashi M, Sato T, Okita N, Higami Y. Impact of aging and caloric restriction on fibroblast growth factor 21 signaling in rat white adipose tissue. Exp Geront. 2019;118: 55–64.
54. Zhang Y, Lei T, Huang JF, Wang SB, Zhou LL, Yang ZQ, et al. The link between fibroblast growth factor 21 and sterol regulatory element binding protein 1c during lipogenesis in hepatocytes. Mol Cell Endocrinol. 2011;342(1–2):41–7.
55. Kobayashi M, Uta S, Otsubo M, Deguchi Y, Tagawa R, Mizunoe Y, et al. Srebp-1c/Fgf21/Pgc-1α axis regulated by leptin signaling in adipocytes -possible mechanism of caloric restriction-associated metabolic remodeling of white adipose tissue. Nutrients. 2020;12(7):2054.
56. Ozata M, Ozdemir IC, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab. 1999;84(10):3686–95.
57. Klein S, Coppack SW, Mohamed-Ali V, Landt M. Adipose tissue leptin production and plasma leptin kinetics in humans. Diabetes. 1996;45(7):984–7.
58. Iida M, Murakami T, Ishida K, Mizuno A, Kuwajima M, Shima K. Substitution at codon 269 (glutamine --> proline) of the leptin receptor (OB-R) cDNA is the only mutation found in the Zucker fatty (fa/fa) rat. Biochem Biophys Res Commun. 1996;224(2): 597–604.
59. Okita N, Tsuchiya T, Fukushima M, Itakura K, Yuguchi K, Narita T, et al. Chronological analysis of caloric restriction-induced alteration of fatty acid biosynthesis in white adipose tissue of rats. Exp Geront. 2015;63:59–66.
60. Kobayashi M, Takeda K, Narita T, Nagai K, Okita N, Sudo Y, et al. Mitochondrial intermediate peptidase is a novel regulator of sirtuin-3 activation by caloric restriction. FEBS Lett. 2017;591(24):4067–73.
61. Perico L, Morigi M, Pezzotta A, Corna D, Brizi V, Conti S, et al. Post-translational modifications by SIRT3 de-2-hydroxyisobutyrylase activity regulate glycolysis and enable nephrogenesis. Sci Rep. 2021;11:23580.
62. Mossmann D, Meisinger C, Vögtle FN. Processing of mitochondrial presequences. Biochimica et Biophysica Acta (BBA). 2012;1819(9–10):1098–106.
63. Schwer B, North BJ, Frye RA, Ott M, Verdin E. The human silent information regulator (Sir) 2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. J Cell Biol. 2002;158(4):647–57.
64. Isaya G, Kalousek F, Fenton WA, Rosenberg LE. Cleavage of precursors by the mitochondrial processing peptidase requires a compatible mature protein or an intermediate octapeptide. J Cell Biol. 1991;113(1):65–76.
65. Vögtle FN, Prinz C, Kellermann J, Lottspeich F, Pfanner N, Meisinger C. Mitochondrial protein turnover: role of the precursor intermediate peptidase Oct1 in protein stabilization. Mol Biol Cell. 2011;22(13):2135–43.
66. Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H, et al. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. Mol Cell. 2010;40(6):893–904.
67. Fisher FM, McTernan PG, Valsamakis G, Chetty R, Harte AL, Anwar AJ, et al. Differences in adiponectin protein expression: effect of fat depots and type 2 diabetic status. Horm Metab Res. 2002;34(11/12):650–4.
68. Guglielmi V, Sbraccia P. Obesity phenotypes: depot-differences in adipose tissue and their clinical implications. Eat Weight Disord. 2018;23(1):3–14.
69. Narita T, Kobayashi M, Itakura K, Itagawa R, Kabaya R, Sudo Y, et al. Differential response to caloric restriction of retroperitoneal, epididymal, and subcutaneous adipose tissue depots in rats. Exp Geront. 2018;104:127–37.
70. Hofer SJ, Davinelli S, Bergmann M, Scapagnini G, Madeo F. Caloric restriction mimetics in nutrition and clinical trials. Front Nutr. 2021;8:717343.
71. Ovadya Y, Krizhanovsky V. Strategies targeting cellular senescence. J Clin Invest. 2018;128(4):1247–54.
72. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nature Cell Biol. 2013;15(8):978–90.
73. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature. 2016;530(7589):184–9.
74. Kirkland JL, Tchkonia T, Zhu Y, Niedernhofer LJ, Robbins PD. The clinical potential of senolytic drugs. J Am Geriatr Soc. 2017;65(10):2297–301.
75. Chaib S, Tchkonia T, Kirkland JL. Cellular senescence and senolytics: the path to the clinic. Nature Med. 2022;28: 1556–68.
76. Dai DF, Karunadharma PP, Chiao YA, Basisty N, Crispin D, Hsieh EJ, et al. Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. Aging cell. 2014;13(3):529–39.
77. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell. 2008;30(2):214–26.
78. Onken B, Sedore CA, Coleman-Hulbert AL, Hall D, Johnson E, Jones EG, et al. Metformin treatment of diverse Caenorhabditis

species reveals the importance of genetic background in longevity and healthspan extension outcomes. Aging cell. 2022;21(1):e13488.
79. Miller RA, Harrison DE, Allison DB, Bogue M, Debarba L, Diaz V, et al. Canagliflozin extends life span in genetically heterogeneous male but not female mice. JCI Insight. 2020;5(21):e140019.
80. Hoong CWS, Chua MWJ. SGLT2 inhibitors as calorie restriction mimetics: insights on longevity pathways and age-related diseases. Endocrinology. 2021;162(8):bqab079.

How to cite this article: Otani Y, Nozaki Y, Mizunoe Y, Kobayashi M, Higami Y. Effect of mitochondrial quantity and quality controls in white adipose tissue on healthy lifespan: essential roles of GH/IGF-1-independent pathways in caloric restriction-mediated metabolic remodeling. Pathol Int. 2023;73:479–489.
https://doi.org/10.1111/pin.13371