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Mechanistic perspectives of calorie restriction on vascular homeostasis

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Calorie restriction (CR) is a dietary regime based on low calorie intake. CR without malnutrition extends lifespan in a wide range of organisms from yeast to rodents, and CR can prevent and delay the onset of age-related functional decline and diseases in human and non-human primates. CR is a safe and effective intervention to reduce vascular risk factors in humans. In recent years, studies in rodents have provided mechanistic insights into the beneficial effects of CR on vascular homeostasis, including reduced oxidative stress, enhanced nitric oxide (NO) bioactivity, and decreased inflammation. A number of important molecules, including sirtuins, AMP-activated protein kinase, mammalian targets of rapamycin, endothelial nitric oxidase and their regulatory pathways are involved in the maintenance of vascular homeostasis. Evidence has shown that these pathways are responsible for many aspects of CR's effects, and that they may also mediate the effects of CR on vasculature.

calorie restriction (CR), vascular homeostasis, Sirtuin 1 (SIRT1), AMP-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), endothelial nitric oxide synthase (eNOS)

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Calorie restriction (CR), sometimes referred to as dietary restriction (DR), is a dietary regime based on low calorie intake. CR is usually defined as decreasing the calorie intake about 20%–40% of the *ad libitum* (AL) diet without compromising the intake of essential nutrients [1]. Since McCay first reported that CR extended lifespan of rats almost eight decades ago [2], CR is now believed to be the only non-pharmacological intervention to extend lifespan. It has been proven that CR prolongs lifespan in diverse organisms, including yeast, worms, flies and rodents [3]. Long-term CR also significantly improves age-related and all-cause survival in rhesus monkeys implying it also prolongs lifespan in higher mammals [4,5]. Furthermore, studies in humans and monkeys also showed that a reduction in calorie intake without malnutrition prevents and delays the onset of age-related functional decline and also of diseases such as type-2-diabetes, cancer and cardiovascular diseases (CVDs) [3,6].

Vascular homeostasis is a healthy and balanced state, in which endothelial cells (ECs), vascular smooth muscle cells (VSMCs), fibroblasts and other bone marrow-derived cells in the vascular wall coordinate with environmental cues to maintain an appropriate blood pressure and control a proper tissue blood perfusion. Vascular diseases result from a loss of vascular homeostasis. During the past decade, the leading two causes of death worldwide were ischemic heart disease and stroke [7], both of which are directly caused by aberrant vascular homeostasis.

CR exerts protective effects on vascular homeostasis. CR attenuates atherosclerosis and improves age, obesity and diabetes-related vascular dysfunction in rodents [8−11]. In rhesus monkeys, the incidence of CVDs was 50% reduced

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by CR [4]. Studies also suggest beneficial effects of CR on vascular homeostasis in humans. Food restriction during the Second World War in Norway caused a sharp decline in mortality of CVDs, while the mortality began to increase rapidly after the war [12]. In addition, the Japanese Okinawan are naturally calorie restricted due to their traditional low energy-content diet. Their mortality from coronary artery diseases is much lower than that of other Japanese and Americans [13]. These two epidemiological studies of unintentionally induced CR provide evidences that CR may also reduce the mortality of CVDs in humans.

In this review, we introduce the beneficial effects of CR on vascular homeostasis in rodents and primates including humans, and then discuss the possible molecular pathways that mediate its effects on vasculature, which hold a great promise as therapeutic targets in treating vascular diseases.

1 CR preserves vascular homeostasis

1.1 CR reduces risk factors of vascular diseases

The risk factors for CVDs include central obesity, high blood pressure, elevated fasting plasma glucose level, dyslipidemia, and a high level of inflammation. Reducing calorie intake is a very effective and reliable way to reduce the risk for CVDs. CR improves glucose regulation, decreases blood pressure, and reduces circulating inflammatory molecules in rodents [9,14–17]. However, due to the differences in dietary regimes employed by different groups, the effects of CR on blood cholesterol are circumstantial [18–20]. In the absence of a reduction in cholesterol intake, CR does not alter serum cholesterol level in rodents. Nevertheless, an elevated high density lipoprotein-phospholipids/total phospholipids (HDL-PL/T-PL) ratio is still observed, suggesting an enhanced reverse cholesterol transport in calorie-restricted mice [20]. In non-human primates, it was observed that CR could decrease body weight, induces a better serum lipid profile, and improves insulin sensitivity [4,6,21–23]. Consistently, CR also reduces the risk of vascular diseases in humans. Clinical trials of the effects of 6 or 12 months of CR in non-obese humans revealed that short-term CR can effectively induce a better blood lipid profile, improve insulin sensitivity, and reduce C-reactive protein (CRP), a marker of inflammation [24–26]. Data obtained from eight subjects participated in the two-year ecological experiment "Biosphere 2" showed that CR with a low-protein diet can reduce various risk factors for vascular diseases [27]. The effects of long-term CR were examined by studies on 18 members from Calorie Restriction Society who had been on CR diet for an average of six years. Compared with age-matched individuals on western diet, the members of CR Society showed a remarkable reduction in the risk factors for vascular diseases, including lower levels of plasma total cholesterol, low-density lipoprotein-cholesterol (LDL-cholesterol), fasting glucose, and lower blood pressure [28].

Excessive calorie intake results in insulin resistance and an abnormal distribution of lipid, which is characterized by adipocyte expansion, lipid accumulation in liver and skeletal muscle, and hyperlipidemia [29–31]. On the contrary, a restriction of calorie intake makes the body to utilize the energy more efficiently and leads to a reduction of various metabolic factors that are associated with increased CVD risk. In adapting to CR, fatty acid β-oxidation in liver and skeletal muscle is activated, thereby decreasing triglyceride accumulation in these tissues [32,33]. Insulin-stimulated glucose uptake in skeletal muscle is also enhanced by CR, contributing to an improved glucose regulatory function [34,35]. Furthermore, CR reprograms lipid metabolism in adipocyte. Lipogenesis and the sensitivity to lipogenic/lipolytic stimuli of adipocytes are enhanced by CR [36,37]. CR also improves cardiac function in mice by promoting glucose oxidation [38]. These metabolic alterations in the liver, skeletal muscle, adipose tissue, and heart under CR condition coordinately lead to a lower body mass, improved insulin sensitivity, and a better blood lipid profile, which are beneficial for vascular homeostasis.

1.2 CR improves local vascular function

CR retards age-related functional decline of various organs, including the liver, skeletal muscle, and brain [3], and accumulating studies have also demonstrated its beneficial effects on vasculature. Decreased incidence of CVDs in calorie-restricted rhesus monkeys is observed [4]. Results from the study on 11 obese patients showed that a low-calorie diet is an effective treatment for essential hypertension [39]. Because of the difficulties in conducting experiments on humans, most of the studies of CR on vascular function/diseases have been carried out in rodents. These animal studies have revealed that CR could improve local vascular function via reducing oxidative stress, reserving nitric oxide (NO) bioactivity, and inhibiting vascular inflammation [8–11,40–42].

Increased reactive oxygen species (ROS) in vessel wall, which eventually culminates with oxidative stress, is strongly implicated in the development of many vascular diseases such as hypertension, atherosclerosis, and abdominal aortic aneurysm (AAA) [43–45]. Oxidative stress induces lipid peroxidation, protein oxidation, and mitochondrial and nuclear DNA damage, which leads to the activation of redox-sensitive transcription factors and the expression of pro-inflammatory genes [46]. CR attenuates vascular NADPH oxidase (NOX)-dependent ROS production in vasculature by inhibiting both the activity and the expression of NOX [41,42,47]. CR can also reduce mitochondrial ROS generation in the aortas of diabetic rats [9]. Furthermore, CR enhances the anti-oxidative defenses in the vascular system by upregulating the expression and activity

of SOD and catalase [40–42]. Increased glutathione and ascorbate were also observed in the aortas of CR rats [48].

NO is an endogenous vasodilator which can not only stimulate VSMC dilation, but also inhibit platelet adherence and VSMC proliferation, and decrease the expression of pro-inflammatory genes [46]. The reduction of NO bioactivity is closely related to oxidative stress: NO can be inactivated by the ROS superoxide (O_2^-), and the predominant NO-generating enzyme in vessels, endothelial NO synthase (eNOS), will generate O_2^- instead of NO when its function is impaired [49]. In rodents, CR can enhance NO bioactivity and ameliorate endothelial function through upregulating the expression and activity of eNOS, which is revealed as an increase in phospho-eNOS/total-eNOS ratio [8,40–42,47].

Inflammation is a common pathophysiological state observed in vascular diseases [50,51]. CR retards vascular inflammation not only by decreasing systemic inflammation, but also by enhancing the anti-inflammatory function of the local vessel. In rats, CR reduces inflammatory markers in plasma, such as soluble adhesion molecules and CRP [16]. Human coronary arterial endothelial cells (CAECs) treated with serum from CR rats present suppressed nuclear factor κB (NF-κB) activity [48], suggesting the lower inflammatory status of CR serum. ECs obtained from CR mice showed reduced sensitivity to oxidized low-density lipoprotein (ox-LDL), decreased expression of adhesion molecules and less mononuclear cell adhesion [52], implying that local vascular cells have enhanced anti-inflammatory capacity under CR conditions.

In order to maximize the energy obtained from a limited food source, the body reprograms energy metabolism, and the metabolic alterations in the liver, skeletal muscle, adipose tissue and heart lead to an improved function of these organs during CR. But whether CR also reprograms the metabolism of the vascular system remains unclear. Inflammation and oxidative stress are common features of vascular diseases, and they are closely related to the conversion of metabolic state [53]. Aberrant metabolic states of vascular lesions were observed in human and experimental animals [54–57]. Though not thoroughly studied, the present evidences strongly suggest that the metabolic switch is another key trait of aberrant vascular homeostasis. Therefore, we deduce that CR may modulate the metabolic state of vasculature and subsequently reduce inflammation and oxidative stress.

Taken together, CR guards vascular homeostasis by reducing systemic risk factors and improving local vascular functions (Figure 1). CR alters the metabolic state of various organs, including the liver, skeletal muscle, adipose tissue, and heart, thus reducing vascular risk factors. CR also retards the pathological changes of vascular diseases, such as oxidative stress and inflammation, but whether these effects are achieved through reprogramming the metabolism of the vasculature awaits further investigation.

2 CR-responsive pathways in the regulation of vascular homeostasis

Coordinated alterations in gene expression involved in energy metabolism are indicative of metabolic reprogramming and have been shown to be a prominent feature of CR [58,59]. The contributions of CR to vascular homeostasis,

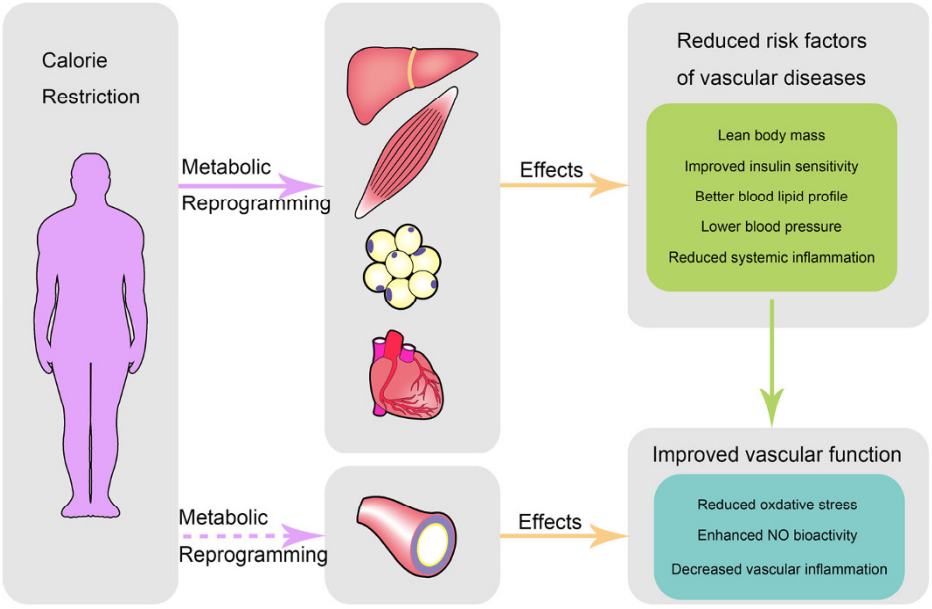

Figure 1 (color online) Calorie restriction preserves vascular homeostasis. CR alters the metabolic state of various organs, including the liver, skeletal muscle, adipose tissue and heart, thereby reducing vascular risk factors. Evidence from various recent studies suggests that CR may also retard the pathological changes of vascular diseases through reprogramming the metabolism of local vessels.

including reduced systemic risk factors and improved local vascular functions, are speculated to be achieved by its function of metabolic reprogramming. Energy-sensing molecules, such as sirtuins, AMP-activated protein kinase (AMPK), and mammalian target of rapamycin (mTOR), can sense the lower energy state caused by reduced calorie intake [60,61]. Therefore, sirtuins, AMPK, mTOR and their regulating pathways play essential roles in mediating the effects of CR. Recent studies also revealed that eNOS plays an important role in modulating whole-body metabolism in response to reduced calorie intake besides its well-known direct role on vascular function.

Here, we summarize the evidence that sirtuins, AMPK, mTOR and eNOS mediate the effects of CR with particular attention paid on their regulatory function on local vascular functions. We then deduce their potential roles in mediating the effects of CR on vascular homeostasis.

2.1 Sirtuins

The mammalian sirtuin family (comprised of seven proteins from SIRT1 to SIRT7) is named after the yeast ortholog of Sir2 (silent information regulator 2), whose enzymatic activity requires NAD^+ as a substrate. Calorie restriction increases the intracellular NAD^+/NADH ratio, and Sir2 is required for CR to extend lifespan in lower organisms [62–66].

2.1.1 SIRT1

SIRT1 participates in regulating a wide variety of biological processes, and many studies have shown that SIRT1 plays a critical protective role in modulating vascular functions via deacetylating different substrates including histones, transcription factors and other proteins (Table 1).

As an interface between the circulating blood and the vascular wall, ECs confront various stimuli, including disturbed shear stress, cytokines and modified lipoproteins. These stimuli can induce oxidative stress, impair NO bioavailability and induce the expression of adhesion molecules which further recruit and activate mononuclear cells transmigrating into the vascular wall. SIRT1 plays an important role in regulating endothelial function, including ROS production, NO production, inflammatory molecules expression, cell senescence and growth. Via suppressing the expression of p66Shc, a protein participates in the generation of mitochondrial ROS, and upregulating the expression of antioxidant enzymes, including MnSOD and catalase, the activation of SIRT1 reduces ROS production and enhances the resistance to oxidative stress in ECs [67,68]. Furthermore, SIRT1 enhances eNOS activity and promotes the production of NO by directly deacetylating eNOS and indirectly activating the liver kinase B1 (LKB1)-AMPK-eNOS signaling pathway [69–71]. Many studies also revealed that SIRT1 has anti-inflammatory effects in ECs by suppressing the transcriptional activity of NF-κB and nuclear factor of activated T cells (NFAT), and subsequently decreasing the expression of inflammatory molecules [72–75]. Moreover, SIRT1 inhibits endothelial cell senescence [76-82], a process involved in endothelial dysfunction and atherogenesis

Table 1 List of described SIRT1 substrates in vascular cells, their downstream molecules, and the result of SIRT1 activation[a)]

Cell type	Substrate		Downstream molecules	Result of SIRT1 activation	References
EC	Histone	H3	p66Shc	Reduces ROS	[67]
		H4	PAI-1	Prevents cell senescence	[82]
	transcription factor	FoxO3a/PGC-1α	MnSOD, Catalase	Reduces ROS	[68]
		NFAT	COX-2	Reduces inflammation	[72]
		p65	ICAM-1, VCAM-1, CD40, E-selectin	Reduces inflammation	[73–75]
		p53	p21	Prevents cell senescence	[76–80]
		FOXO1		Prevents cell senescence	[79]
	others	eNOS		Increases NO production	[70,71]
		LKB1		Enhances eNOS activity, Prevents cell senescence	[69,81]
		NICD		Promotes blood vessel growth	[84]
VSMC	transcription factor	FoxO1	SIRT1	Promotes SIRT1 expression	[85]
		p53	p21	Prevents cell senescence	[87,88]
		p65	TGF-β	Suppresses vascular remodeling	[89]
		AP-1	Cyclin D1, MMP9	Inhibits VSMC migration	[90]
		RFX5	COL1A2	Promotes collagen expression	[91]
	others	NBS-1		Enhances DNA repair	[86]
Macrophage	transcription factor	AP-1	COX-2	Reduces inflammation	[92]
		p65	Lox-1,TNF-α, IL-1β, iNOS	Reduces ox-LDL uptake and inflammation	[93,94]

a) PAI-1, plasminogen-activator inhibitor-1; FoxO3a, forkhead box O3a; PGC-1α, peroxisome proliferator-activated receptor γ-coactivator 1α; NFAT, nuclear factor of activated T cells; COX-2, cyclooxygenase-2; ICAM-1, intracellular adhesion molecule 1; VCAM-1, vascular adhesion molecule 1; FoxO1, forkhead box O1; LKB1, liver kinase B1; NICD, Notch1 intracellular domain; TGF-β, transforming growth factor β; AP-1, activator protein-1; MMP9, matrix metalloproteinase 9; RFX5, regulatory factor for X-box 5; COL1A2, collagen type I; NBS-1, Nijmegen Breakage Syndrom-1; Lox-1, lectin-like ox-LDL receptor 1; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β; iNOS, inducible nitric oxide synthase.

[83], via deacetylating a variety of substrates. It was also found that lacking SIRT1 in ECs causes impaired cell growth and vascular branching as a result of the hyper-acetylated Notch1 intracellular domain (NICD) and enhanced Notch signaling, indicating the importance of SIRT1 in regulating endothelial growth [84].

Pathological changes of VSMCs, including apoptosis, senescence, proliferation and migration, contribute to vascular diseases such as atherosclerosis, post-stent stenosis and hypertension. SIRT1 plays an important role in regulating the function and behavior of VSMCs [85–91]. SIRT1 deacetylates and activates the DNA repair protein Nijmegen Breakage Syndrom-1 (NBS-1), enhancing VSMCs' resistance to apoptosis caused by oxidative stress [86]. Via suppressing p53 and p21, the activation and overexpression of SIRT1 also prevents the senescence of VSMCs [87,88]. In addition, SIRT1 suppresses VSMC migration, proliferation and vascular remodeling by deacetylating a number of transcription factors and modulating the expression of their downstream molecules [89–91].

The infiltration of macrophages into the vascular wall is a key event of vascular inflammation. By modulating macrophage function, SIRT1 suppresses vascular inflammation. Overexpression of SIRT1 in macrophages helps to dampen macrophage function via inhibiting activator protein 1 (AP-1) and NF-κB activity and the expression of inflammatory molecules, such as cyclooxygenase-2 (COX-2) and tumor necrosis factor α (TNF-α) [92,93]. Moreover, by deacetylating RelA/p65, a subunit of NF-κB, SIRT1 diminishes lectin-like ox-LDL receptor 1 (Lox-1) expression in macrophages, thereby reducing ox-LDL uptake and preventing foam cell formation and atherosclerosis in mice [94].

Many scientific evidences demonstrated that SIRT1 mediates the effects of CR in mammals. First, CR increases the expression of SIRT1. In rodents, CR upregulates SIRT1 protein levels in many tissues, including the liver [95], brain [95,96], kidney [95], white and brown adipose tissue [95,97], intestine [98], and the aorta [41,42,48,99]. CR also increases SIRT1 gene expression in skeletal muscles of healthy humans [100]. Reciprocally, a high-fat diet leads to the loss of SIRT1 in adipose tissue of mice [101], and downregulated expression of SIRT1 in adipose tissue was also observed in obese people [102,103]. Second, loss of SIRT1 ablated the outputs of CR. Mice deficient in SIRT1 fail to display some of the responses normally triggered by CR [104]. Moreover, the longevity-promoting effect of CR is blunted in mice lacking SIRT1 [105]. Third, SIRT1 transgenic mice exhibit phenotypes resembling CR: they are leaner and more active than littermate controls, and they have reduced blood cholesterol and improved glucose regulatory function [106]. Furthermore, SIRT1 reprograms the metabolism of the liver, skeletal muscle, and adipose tissue; thereby mitigating diabetes, obesity and hepatic steatosis [107–110]. These effects of SIRT1 resemble closely the effects of CR. Therefore, the activation of SIRT1 by CR triggers a metabolic switch contributing to a lower risk for vascular diseases. Researches also suggest that the upregulation of SIRT1 in vascular cells contributes to the benefits of CR. Treatment of CAECs with serum from CR rats attenuated TNF-α-induced ROS generation and NF-κB activity, while these effects of CR serum were mitigated by knockdown of SIRT1 [48]. CR also leads to deacetylation of eNOS and enhances NO production in mouse aorta, which is at least in part via the activation of SIRT1 [41,47,71]. The effects of SIRT1 in ECs, VSMCs and macrophages may also help explaining the protective role of CR against atherosclerosis and endothelial dysfunction [11,42].

In summary, SIRT1 reprograms whole-body metabolism in response to CR, and also mediates the benefits of CR on vascular function. However, its role in modulating vascular cell metabolism remains unknown and deserves further investigation.

2.1.2 SIRT3

Among the sirtuins, the mitochondrial sirtuin SIRT3 is most similar in sequence to SIRT1 [111]. SIRT3 plays a key role in regulating systemic metabolism by deacetylating a wide range of metabolic enzymes in mitochondria [112,113]. However, studies have showed no obvious vascular dysfunction in *SIRT3*$^{-/-}$ mice. SIRT3 deletion does not augment hypoxia-induced ROS signaling or the development of pulmonary artery hypertension in mice [114]. SIRT3 deficiency in *LDL receptor*$^{-/-}$ (*LDLR*$^{-/-}$) mice results in increases in body weight, plasma glucose level and systemic oxidative stress, but does not accelerate the vascular oxidative stress or the development of atherosclerosis [115]. These findings suggest a potential role of SIRT3 in the development of cardiovascular risk factors and a function of postponing the onset of distinct metabolic risk factors.

SIRT3 also functions as a prominent regulator in CR adaptation. CR upregulates SIRT3 expression in many metabolic active tissues such as the liver, skeletal muscle, and brown and white adipose tissue [116–118]. Moreover, SIRT3 mediates many of the CR's outputs on the regulation of metabolism and oxidative stress, which are supported by evidences derived from *SIRT3*$^{-/-}$ mice. Via deacetylating long-chain acyl-CoA dehydrogenase (LCAD), HMGCS2, and ornithine transcarbamoylase (OTC), SIRT3 increases fatty acid oxidation, amino acid catabolism and ketone body production under CR and fasting conditions [118–120]. Furthermore, in response to CR, SIRT3 deacetylates and activates SOD2 and isocitrate dehydrogenase 2 (IDH2), leading to reduced oxidative stress [121,122]. Acetyl-proteinomic studies further show that CR dramatically alters the mitochondrial protein acetylome, which is partly mediated by activation of SIRT3 [59].

Altogether, SIRT3 is a key molecule that controls the systemic metabolism and oxidative stress, and activation of

SIRT3 under CR leads to metabolic reprogramming. SIRT3 deacetylates and activates SOD in liver and adipose tissue of mice under CR [121]. Enhanced activity of SOD in aorta was also observed in CR mice [40–42]. Therefore, it is possible that the activation of SOD in aorta under CR is also obtained through the activation of SIRT3. Although deletion of SIRT3 in *LDLR*$^{-/-}$ mice does not result in any exacerbation of vascular oxidative stress [115], we should not rule out the possibility that SIRT3 could regulate vascular oxidative stress under other circumstances.

Compared with SIRT1 and SIRT3, other sirtuins, which also sense NAD^+/NADH levels, are involved in the regulation of metabolism as well [123,124]. Whether they are indispensible for the influence of CR is still unclear [125]. Furthermore, little is known about their function in the vascular system. More studies are needed to determine their physiology in vascular homeostasis and CR.

2.2 AMPK

AMP-activated protein kinase (AMPK) is a heterotrimeric kinase composed of a catalytic (α) and two regulatory (β and γ) subunits [126]. The activation of AMPK requires AMP to bind the γ subunit first, and then the phosphorylation of Thr172 on the α subunit to fully activate the enzyme [126]. Once activated, AMPK turns on catabolic pathways to restore ATP levels by promoting glycolysis and fatty acid oxidation and by increasing mitochondrial contents [126]. AMPK is a key sensor and effector of energy status [126], and plays an critical role the regulation of metabolic processes [127]. Furthermore, AMPK is also involved in the regulation vascular function [128].

AMPK plays an essential role in improving endothelial function through regulating eNOS activity, redox status, and lipid metabolism [129]. AMPK activates eNOS and enhances NO bioactivity in ECs by phosphorylating eNOS [130–132]. Loss of AMPKα1 in HUVECs leads to decreased expression of anti-oxidative enzymes, reduced mitochondrial content and increased sensitivity to oxidative stress [133]. On the contrary, activation of AMPK in HUVECs induces mitochondrial biogenesis and enhances the resistance to H_2O_2 [134]. Furthermore, it has been proven that in bovine aortic endothelial cells (BAECs), HAECs and HUVECs, activation of AMPK increases fatty acids oxidation and subsequently reduces hyperglycemia- and linoleic acid-induced cell apoptosis [135–137].

AMPK regulates VSMC function and behavior as well, but different isoforms of AMPKα function differently. Activation of AMPKα1, but not AMPKα2, is able to induce endothelium- and eNOS-independent aortic relaxation in mice [138]. Deletion of AMPKα2—but not AMPKα1—in mice aggravates VSMCs proliferation and neointima formation after mechanical arterial injury [139]. Conversely, in Angiotensin II-induced AAAs, AMPKα2 activation in VSMCs promotes the degradation of tunica media [140].

AMPK also participates in controlling macrophage function. Both genetic and pharmacological activation of AMPK in macrophages results in a decreased inflammatory response, whereas suppressing AMPK increases the secretion of pro-inflammatory factors by macrophages *in vitro* [141–143]. AMPKα1 is crucial for the phenotype transition of macrophages *in vivo* [144]. Moreover, activation of AMPKβ1 reduces macrophage inflammation by enhancing fatty acid oxidation [145].

AMPK is required for many of the adaptations triggered by CR in lower eukaryotes, including lifespan extension [146–148]. In rodents, CR increases the phosphorylation of AMPKα in heart and skeletal muscle [14,149–151]. In addition, many beneficial effects of CR will be abrogated when using AMPK inhibitor to rodents. These effects include protecting hearts from ischemia/reperfusion injury [150,151], preventing hypertension-induced cardiac hypertrophy [14], and promoting revascularization in ischemic muscle [149]. Therefore, AMPK is a sensor and an effector of CR in mammals as well.

AMPK activates and coordinates with SIRT1, the key mediator of CR. First, upon glucose restriction, AMPK activates SIRT1 through the upregulation of nicotinamide phosphoribosyltransferase (Nampt) expression and subsequent increased NAD^+/NADH ratio [152]. SIRT1 also activates AMPK through deacetylating LKB1, the kinase upstream of AMPK [107,153–155]. In addition, AMPK and SIRT1 function synergistically to activate their common substrates. The phosphorylation of peroxisome proliferator-activated receptor γ-coactivator 1α (PGC-1α) and eNOS by AMPK is required for their following deacetylation by SIRT1 [70,156]. It is also noted that SIRT3 and AMPK act coordinately. AMPK can activate SIRT3 by regulating the NAD^+/NADH ratio [157], and conversely, SIRT3 can also activate AMPK by deacetylating LKB1 [116,158].

In summary, the activation of AMPK mediates many effects of CR. The influence of AMPK on the metabolism and energy balance at the whole-body level may provide a lower risk for vascular diseases, and the function of AMPK in ECs and macrophages may help to explain the protective roles of CR in atherosclerosis and in endothelial dysfunction. However, the activation of AMPK in VSMCs can play protective or detrimental roles in different diseases [139,140]. Thus far, no direct evidence has been observed about the expression and activity of AMPK in aortas in response to CR. Increased phosphorylation of eNOS has been observed in the aortas of CR mice [8], which may be due to the activation of AMPK [131,159], while further studies are still required to elucidate whether (and how) CR regulates local vascular AMPK activity.

2.3 mTOR

mTOR is a serine/threonine protein kinase activated by environmental cues, such as growth factors, nutrients, energy

and stress [61,160]. Activation of the mTOR signaling pathway promotes cell growth and division by inducing anabolic metabolism. mTOR functions in two distinct complexes: mTOR complex 1 (mTORC1), which is rapamycin-sensitive, and mTOR complex 2 (mTORC2), which is affected only indirectly by rapamycin [61,160].

The mTOR signaling pathway plays an important role in the regulation of vascular homeostasis. Inhibition of mTOR signaling can attenuate the proliferation and migration of VSMCs [161]. In this manner, everolimus-eluting coronary stent markedly prevents the development of in-stent restenosis in humans [162]. Similarly, 40-O-(2-hydroxyethyl)-rapamycin also attenuates pulmonary artery hypertension in rodents [163]. The mTOR signaling pathway is involved in the regulation of vascular oxidative stress and inflammation as well. Silencing ribosomal S6 protein kinase 1 (S6K1), a component of mTOR signaling pathway, reduces oxidative stress and enhances NO production in senescent HUVECs [164]. In addition, knockdown or pharmacological attenuation of mTOR has been shown to prevent atherogenesis by inhibiting macrophage chemotaxis and the expression of inflammatory molecules in mice [165–169].

Attenuated mTOR signaling is thought to play a part in mediating longevity and health benefits in response to CR [170,171]. CR acts through inhibiting mTOR to regulate lifespan of yeast, worms and flies [172–176]. Pharmacological or genetic disruption of mTORC1 or S6K1 is sufficient to extend lifespan of mice as well [177–180]. Moreover, deletion of S6K1 is able to not only activate AMPK, but also induce a gene expression pattern similar to that seen in CR [180,181]. Inhibition of mTORC1 confers protection against a growing list of age-related pathologies, including obesity, metabolic diseases, neurodegenerative diseases, and cancer [170], and these effects are similar to the effects of CR. Furthermore, mTOR signaling pathway is suppressed by AMPK and SIRT1, which are key molecules in response to CR [182–184].

Increased mTOR signaling has been observed in the aortas of old mice or mice fed with a high-fat diet [41,185]. Conversely, CR inhibits mTOR activity in mouse aortas [41]. However, there is still no evidence proving that CR improves vascular function through inhibiting mTOR activity. More researches shall be made on the role of mTOR in CR and vascular homeostasis.

2.4 eNOS

The molecules discussed above, which sense the energy state, are widely accepted as molecules mediating CR's effects [186]. Although eNOS is less known for sensing the energy state, particular attention is paid to this molecule here because of its central role in modulating vascular function; and also for its emerging importance in the regulation of systemic metabolism under CR.

Endothelial NOS is essential for vascular NO production and ROS clearance, and many reviews have been published concerning the roles of eNOS in vascular homeostasis [46,187,188]. Besides, the importance of eNOS in modulating whole body metabolism and longevity has become more and more prominent. eNOS-knockout mice are hypertensive and insulin-resistant, and they have higher levels of blood cholesterol and triglyceride than wild type mice [189]. On the contrary, eNOS transgenic mice showed lower plasma triglyceride and fatty acids, elevated metabolic activity of adipose tissue and resistance to diet-induced obesity [190]. Furthermore, eNOS also plays an essential role in mediating the effects of CR. First, CR upregulates eNOS expression in the skeletal muscles of healthy humans [100], and also in the brain, liver, heart, and brown adipose tissue of mice [97]. Second, CR promotes mitochondrial biogenesis and SIRT1 expression in male mice, whereas these effects were strongly attenuated in eNOS null-mutant mice [97]. Third, the product of eNOS, NO, has been proved to be an endogenous activator of SIRT1 and AMPK [191–194].

eNOS is critical not only for regulating systemic metabolic state, but also for modulating local vascular function in response to CR. Both life-long and short-term CR enhances arterial eNOS expression and improves endothelium-dependent vascular relaxation in rodents [9,41,42,47]. In addition, enhanced phospho-eNOS (Ser1177) and reduced acetyl-eNOS were also observed in CR mice [8,71], implying the modulation of eNOS by AMPK and SIRT1. Even though, there is still no direct evidence proving the necessity of eNOS in realizing the protective effects of CR on vascular homeostasis.

3 Conclusion and perspectives

In summary, sirtuins, AMPK, mTOR and eNOS and their regulatory pathways are all essential pathways which might mediate CR's effects on vascular homeostasis. CR lowers nutrient supplies and raises NAD^+/NADH and AMP levels, thereby inhibiting mTOR pathway and activating sirtuins and AMPK. CR also upregulates eNOS, despite that the mechanism is still unknown. AMPK activates SIRT1 and SIRT3 through the upregulation of NAD^+-to-NADH ratio, while SIRT1 and SIRT3 also activates AMPK via deacetylating LKB1, the AMPK kinase. SIRT1 and AMPK functions coordinately to activate eNOS, and on the other way around, the eNOS product, NO, could activate SIRT1 and AMPK. Moreover, SIRT1 and AMPK could also inhibit mTOR pathway (Figure 2). These molecules and their regulatory pathways play essential roles not only in regulating the whole-body metabolism, but also in modulating vascular function. Evidences from recent studies suggest that by fine-tuning the activities of sirtuins, AMPK, eNOS, and mTOR, CR contributes to reduced systemic risk factors and improved vascular homeostasis. Drugs targeting these molecules, such as resveratrol, metformin and rapamycin, also

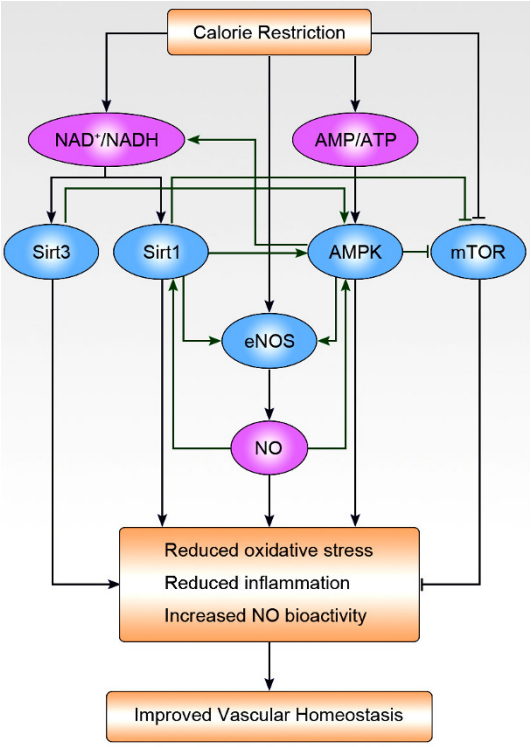

Figure 2 (color online) Molecular pathways which might mediate the effects of CR on vascular homeostasis. CR lowers nutrient supplies and raises NAD^+/NADH and AMP levels, thereby inhibiting the mTOR signaling pathway and activating sirtuins and AMPK. CR also upregulates eNOS, but the mechanism is still unkown. AMPK activates SIRT1 and SIRT3 through the upregulation of NAD^+-to-NADH ratio, while SIRT1 and SIRT3 also activates AMPK via deacetylating LKB1, the AMPK kinase. SIRT1 and AMPK function coordinately to activate eNOS. Conversely, the eNOS product, NO, could activate Sirt1 and AMPK. Moreover, SIRT1 and AMPK could also inhibit the mTOR pathway. By fine-tuning the activities of sirtuins, AMPK, eNOS and mTOR, CR contributes to improved vascular homeostasis.

showed their promising benefits on vascular homeostasis. However, the exact roles of these molecules in local vasculature in response to CR remain unclear and need further investigation. As the employment of CR in human would be hampered by its harsh criteria and long time, targeting these pathways would be more practical for therapeutic uses. Thus, studies on CR not only provide us a life style model good for vascular health, but also open new avenues for searching molecular targets for the treatment of vascular diseases.

CR reprograms metabolism, reduces risk factors for CVDs and maintains vascular homeostasis in experimental animals as well as in humans. Studies in rodents have further yielded with concrete results that CR improves vascular homeostasis via modulating a broad spectrum of pathophysiological processes. However, larger, well-controlled clinical studies are required to determine the efficacy of CR in patients in preventing and treating vascular diseases. Moreover, the effects of CR on vascular metabolism are still an open question.

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