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REVIEW

At the crossroads of longevity and metabolism: the metabolic syndrome and lifespan determinant pathways

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Summary

The metabolic syndrome is becoming increasingly prevalent in the general population and carries significant incremental morbidity and mortality. It is associated with multi-organ involvement and increased all-cause mortality, resembling a precocious aging process. The mechanisms that account for this phenomenon are incompletely known, but it is becoming clear that longevity genes might be involved. Experiments with overactivation or disruption of key lifespan determinant pathways, such as silent information regulator (SIR)T1, p66Shc, and mammalian target of rapamycin (TOR), lead to development of features of the metabolic syndrome in mice. These genes integrate longevity pathways and metabolic signals in a complex interplay in which lifespan appears to be strictly dependent on substrate and energy bioavailability. Herein, we describe the roles and possible interconnections of selected lifespan determinant molecular networks in the development of the metabolic syndrome and its complications, describing initial available data in humans. Additional pathways are involved in linking nutrient availability and longevity, certainly including insulin and Insulin-like Growth Factor-1 (IGF-1) signaling, as well as FOXO transcription factors. The model described in this viewpoint article is therefore likely to be an oversimplification. Nevertheless, it represents one starting platform for understanding cell biology of lifespan in relation to the metabolic syndrome.

Key words: Integrative biology; metabolic syndrome; morbidity; mortality; oxidative stress.

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Introduction

Metabolic syndrome is a cluster of cardiovascular risk factors that are present together in the same subjects more often than expected by chance combination (Avogaro *et al.*, 1967). According to the most used definition, the revised Adult Treatment Panel-III (ATP-III), the metabolic syndrome is diagnosed when at least three of five of the following alterations are present: visceral obesity (waist circumference ≥ 102 cm in men or ≥ 88 cm in women); raised arterial blood pressure (≥ 130/85 mm Hg); dysglycemia (fasting plasma glucose ≥ 100 mg dL^{-1}); raised triglyceride concentrations (> 150 mg dL^{-1}); low HDL cholesterol (< 40 mg dL^{-1} in men or < 50 mg dL^{-1} in women) (Grundy *et al.*, 2005). Even if it is not clear to what extent a diagnosis of metabolic syndrome helps in clinical practice (Borch-Johnsen & Wareham, 2010), it is recognized that metabolic syndrome represents an important pathophysiological construct to study metabolism in humans and in preclinical models. The presence of metabolic syndrome leads to an increased risk of type 2 diabetes and cardiovascular disease, in the form of coronary or peripheral atherosclerosis and heart failure. In addition, metabolic syndrome is associated with a variety of other systemic complications that affect disparate organs and systems, such as fatty liver disease, respiratory disease, osteoarticular disease, and cancer. As a result, metabolic syndrome patients have an increased all-cause mortality and a shortened lifespan compared with the general population (Guize *et al.*, 2007; Benetos *et al.*, 2008; Zambon *et al.*, 2009). Thus, it is progressively recognized that metabolic syndrome is associated with precocious aging (Nunn *et al.*, 2009), which is of paramount importance in light of the worldwide growing epidemic of metabolic syndrome, because of overnutrition and obesity (Haffner & Taegtmeyer, 2003). With this background, the identification of biochemical mechanisms linking metabolic syndrome alterations to lifespan is of particular interest. In this review, we focus the attention on selected intracellular molecular pathways that integrate nutrient availability, metabolism, and regulation of lifespan, and that may be interconnected with one another. We selected SIRT1, p66Shc, and the mammalian TOR (mTOR)/RSK/AMPK pathways because they integrate nutrient bioavailability, oxidative stress, and metabolism and because biological plausibility supports their reciprocal interconnections. Other relevant molecular pathways, including FOXO transcription factors and the insulin/IGF-1 axis, are important determinants of metabolism and lifespan (Cameron *et al.*, 2008), but are not

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described in detail in the present perspective. Thus, this network likely represents an oversimplification of the biological processes at work, but it represents a starting platform for understanding the cell biology of lifespan in relation to metabolic syndrome.

Sirtuins

Excess caloric intake is one of the most important determinants of metabolic syndrome development. Overnutrition and sedentary lifestyle cause accumulation and inflammation of visceral fat, reduced fatty acid trapping and ectopic fatty acid deposition, hepatic insulin resistance, adrenergic overdrive, and activation of the renin-angiotensin system. These pathophysiological alterations account for the five metabolic syndrome components (as described above) and predispose to metabolic syndrome complications that shorten life expectancy (Neels & Olefsky, 2006). Caloric restriction, however, prevents the development of alterations associated with metabolic syndrome and prolongs lifespan in mammals (Cruzen & Colman, 2009). There are several biochemical pathways activated by caloric restriction in mice, many of which cross-interact with metabolism and insulin signaling (Avogaro *et al.*, 2009). At least in Drosophila, the effects of dietary restriction are modulated by, but not strictly dependent on, the insulin/IGF-1 axis through its most important downstream transcription factor FOXO (Giannakou *et al.*, 2008; Min *et al.*, 2008), which is an important checkpoint of metabolic regulation. The potential regulatory role of insulin/IGF-1 pathway in lifespan determination has support also in humans, because common variants in several genes of this pathway, including FOXO3A, are associated with human longevity (Pawlikowska *et al.*, 2009).

The evolutionary conserved SIR-2 is a NAD^+-dependent histone deacetylase; it regulates lifespan in response to caloric restriction in many organisms. Mammalian homologs of SIR2 comprise a family of seven proteins termed sirtuins (SIRT1–SIRT7). These are implicated in metabolic processes and stress resistance (Imai *et al.*, 2000; Guarente, 2006). Genomic instability and alterations in gene expression are hallmarks of eukaryotic aging. SIRT1 represses repetitive DNA and a functionally diverse set of genes across the mouse genome. In response to DNA damage, SIRT1 dissociates from these loci and re-localizes to DNA breaks to promote repair. Remarkably, increased SIRT1 expression promoted survival in a mouse model of genomic instability and it suppressed age-dependent transcriptional changes (Oberdoerffer *et al.*, 2008). Indeed, chromatin rearrangement is considered one of the most important mechanisms of action of sirtuins (Vaquero, 2009). This occurs through the process of deacetylation, a common reaction that removes an acetyl functional group (CH_3-C = O) from a chemical compound. As part of the gene regulation process, chromatin histones are acetylated and deacetylated on lysine residues in the N-terminal tail. The regulation of transcription factors, effector proteins, molecular chaperones, and cytoskeletal proteins by acetylation/deacetylation is emerging as a significant posttranslational regulatory mechanism analogous to phosphorylation, which might also interact with methylation, ubiquitination, sumoylation, and other biochemical reactions for dynamic control of cellular signaling. It is still unknown whether deacetylation represents a nonspecific evolutionarily conserved mechanism of lifespan regulation or is simply a way to switch on and off relevant molecular targets in a stimulus- and tissue-specific manner. Caloric restriction extends lifespan in a variety of organisms, and there is some evidence that this may be mediated by induction of SIRT1 (Westphal *et al.*, 2007). In yeast, SIR2 is a major determinant of longevity because (i) increased SIR2 gene dosage extends lifespan; (ii) loss-of-function mutations shorten it (Kaeberlein *et al.*, 1999); and iii) caloric restriction did not extend lifespan when SIR2 is absent (Lin *et al.*, 2002). This issue is controversial, however, because SIR2 mutants accumulate extra-chromosomal DNA alterations that might independently shorten yeast lifespan. Moreover, it was subsequently found that caloric restriction can be independent of SIR2 in a different yeast strain (Kaeberlein *et al.*, 2004), suggesting the existence of multiple, albeit similar, pathways that regulate lifespan (Lamming *et al.*, 2005). In Drosophila as well, SIR2 appears to mediate the effects of food restriction on lifespan, because these effects are completely abolished in SIR2 mutant flies and in SIR2 overexpressing flies (Rogina & Helfand, 2004). In mammals, SIRT1 deacetylates many key transcription factors and co-factors, such as the tumor suppressor p53, FOXO proteins, peroxisome proliferation activating receptor (PPAR)-gamma coactivator-1α, and nuclear factor-kB (Motta *et al.*, 2004; Yeung *et al.*, 2004; Rodgers *et al.*, 2005). In *Caenorhabditis elegans*, the effects of caloric restriction are mediated by SIR2 independently of FOXO (Wang & Tissenbaum, 2006), while in mammals deacetylation of FOXO4 by SIRT1 may modulate the effects of caloric restriction (Kobayashi *et al.*, 2005). The effects of SIRT1 appear to be beneficial, as they trigger metabolic changes similar to those observed in caloric restriction. Indeed, caloric restriction increases the levels of SIRT1 in the liver and muscle, which are key insulin-sensitive organs (Cohen *et al.*, 2004). Moreover, $SIRT1^{-/-}$ mice are insensitive to the metabolic effects of caloric restriction (Chen *et al.*, 2005). The mechanisms accounting for this phenomenon range from stress resistance through p53 and FOXO modulation (Luo *et al.*, 2001; Brunet *et al.*, 2004), endocrine regulation by IGF-1, insulin, or yet undefined soluble factors (Cohen *et al.*, 2004). The insulin/IGF-1 axis is crucial to lifespan regulation in a variety of organisms and accumulating evidences demonstrate that SIRT1 modulates the downstream effects of this pathway (Lemieux *et al.*, 2005). In light of these observations, SIRT1 has been proposed as a possible target for the treatment of metabolic syndrome (Jiang, 2008). We have recently shown that SIRT1 expression is reduced in peripheral blood mononuclear cells (PBMCs) of nondiabetic subjects with metabolic syndrome compared with nonmetabolic syndrome subjects. In addition, we found that PBMC SIRT1 expression is directly related to insulin sensitivity and negatively related to carotid intima media thickness, a marker of early atherosclerosis (de Kreutzenberg *et al.*, 2010). Mechanistically,

reduction of SIRT1 expression and activity could be attributed to the negative effects played by excess glucose and saturated fatty acids, through cellular NAD^+ depletion and reduced NAMPT activity, which are essential for SIRT1 functions. This effect, which was in part modulated by the increased oxidative stress induced by exposure to high glucose or fatty acid concentrations, caused downstream c-Jun N-terminal Kinase (JNK) activation and p53 acetylation, events typically linked with cellular activation and inflammation (de Kreutzenberg *et al.*, 2010). Interestingly, many of these negative metabolic effects could be prevented *in vitro* by incubation with resveratrol, a natural plant-derived polyphenolic phytoalexin which is also a constituent of red wine. In THP-1 cells, resveratrol induced SIRT1 expression and prevented SIRT1 downregulation as well as p53 acetylation induced by high glucose and oxidative stress (de Kreutzenberg *et al.*, 2010). Importantly, it was shown that resveratrol has the potential to increase replicative lifespan in the yeast *Saccharomyces cerevisiae* (Howitz *et al.*, 2003). Some evidences indicate that this effect may be mediated by SIRT1: resveratrol lowered the Michaelis constant of SIRT1 for both acetylated substrates and NAD^+ and increased cell survival by stimulating SIRT1-dependent deacetylation of p53. However, there is no definite demonstration that resveratrol is a direct SIRT1 activator: one recent study found that resveratrol, as well as several other putative SIRT1 activators, exhibits multiple off-target activities on receptors, enzymes, transporters, and ion channels that may indirectly determine an effect on SIRT1 and its substrates (Pacholec *et al.*, 2010). In addition, it is possible that the antioxidant resveratrol acts on SIRT1 substrates by preserving SIRT1 from its oxidative stress-induced downregulation, as we have shown (de Kreutzenberg *et al.*, 2010). Despite this inconsistency on the mechanisms that link resveratrol to SIRT1, the longevity-modulating effect of resveratrol was confirmed in other species, including a worm (*C. elegans*) and the fruit fly *Drosophila melanogaster* (Wood *et al.*, 2004) a vertebrate (*Nothobranchius furzeri*), and a short-lived fish (Valenzano *et al.*, 2006). It is still not clear whether the same effects of resveratrol are retained in mammals, including humans, but the well-known *J*-shaped curve describing the relationship between alcohol consumption and all-cause mortality indicates that a moderate alcohol use, especially red wine, might extend human lifespan (Gronbaek, 2002). The concentration of resveratrol found in red wine is many fold lower than pharmacologic levels achieved in animal experimental models (Bertelli, 2007), but long-term exposure in humans might amplify its effect, and our experiments provide indirect support to the hypothesis that resveratrol acts through SIRT1.

So far, SIRT1 alterations in relation to human metabolic syndrome have been demonstrated only in circulating cells. While gene expression in monocytes may represent a surrogate for other relevant metabolically active tissues, it has been recently shown that conditional SIRT1 knockout in the myeloid lineage predisposes mice to inflammation, systemic insulin resistance, and metabolic derangement (Schug *et al.*, 2010). In this light, the finding of a low SIRT1 expression in humans with insulin resistance and metabolic syndrome, which triggers downstream cellular activation and inflammation (de Kreutzenberg *et al.*, 2010), appears of great interest.

Collectively, these data indicate that SIRT1 de-regulation might have a role in both metabolic derangement and cardiovascular complications of human metabolic syndrome. Further studies are needed to elucidate whether these basic notions can be used to devise therapies that counter metabolic syndrome and its morbidity.

p66Shc

Another important lifespan determinant gene that integrates metabolic and longevity pathways is p66Shc. The mammalian Shc locus comprises three isoforms defined by their molecular weight: p52, p46, and p66. While the homologous p52Shc and p46Shc mainly act as adaptor proteins that transduce mitogenic signals from tyrosine kinase receptors to Mitogen Activated protein Kinase (MAP) kinases, p66Shc has a different function, reflected by its different structure. Upon phosphorylation on a specific serine residue (Ser36) in the unique additional N-terminal CH_2 domain by protein kinase C (PKC), p66Shc is imported into mitochondria, oxidizes cytochrome C, and catalyzes the reduction of O_2 to H_2O_2, thus favouring opening of the mitochondrial permeability transition pore, with subsequent release of proapoptotic factors into the cytosol (Giorgio *et al.*, 2005). Given that PKC activated by H_2O_2 is responsible for Ser36 phosphorylation, it appears that p66Shc is both a downstream mediator of oxidative stress and a primary source of oxidative stress. In compliance with the oxidative theory of aging, about a decade ago, it was demonstrated that genetic deletion of p66Shc prolongs lifespan by about 30% in mice, in part through reduction in oxidative damage (Migliaccio *et al.*, 1999; Pinton & Rizzuto, 2008). This prompted researchers to study the effect of p66Shc knockout in diseases which are mediated by oxidative stress, including diabetic complications (Pellegrini & Baldari, 2009). It appeared that $p66Shc^{-/-}$ mice are protected against experimental diabetic glomerulopathy, through reduction in mesangial reactive oxygen species (ROS) levels, extracellular matrix deposition, and glomerular cell apoptosis (Menini *et al.*, 2006). Further, p66Shc deletion prevented development of diabetic cardiomyopathy by reducing cardiomyocyte death and preserving the pool of cardiac stem cells, through inhibition of ROS formation and DNA damage (Rota *et al.*, 2006). $p66Shc^{-/-}$ mice are also protected against hyperglycemia-induced endothelial dysfunction, through reduced peroxynitrite generation and lipid peroxidation and enhanced antioxidant defences (Camici *et al.*, 2008). p66Shc appears to be involved in the mechanisms that impair wound healing in diabetes, as $p66Shc^{-/-}$ diabetic mice have accelerated wound healing and do not develop the typical features of nonhealing diabetic wounds and aged skin characteristics (Fadini *et al.*, 2010). These data highlight p66Shc as part of the signal transduction pathway of hyperglycemic damage, one that is potentially also

related to metabolic syndrome complications. Interestingly, ROS and insulin signaling in the adipose tissue are critical determinants of aging and age-associated diseases. Recent data showed that H_2O_2 is directly implicated in the physiological regulation of different signal transduction pathways, including insulin signaling (Stone & Yang, 2006), based on its ability to induce fully reversible protein modifications. Indirect evidence also suggests that H_2O_2 is involved in the regulation of fat development: for instance, 3T3-L1 preadipocytes treated with H_2O_2 accelerated differentiation, with increased expression of PPAR-γ (Lee *et al.*, 2009), while treatment with antioxidants prevents 3T3-L1 differentiation *in vitro* (Cho *et al.*, 2003). Oxidative stress may be able to activate a similar program in hepatocytes as well (Sekiya *et al.*, 2008), a phenomenon relevant for the development of fatty liver disease, which is very common in subjects with the metabolic syndrome. Unfortunately, there is weak evidence *in vivo* that oxidative stress is related to the development of diet-induced obesity. Indirect support for this hypothesis was provided by Sato *et al.* who showed that absence of metallothionein, which prevents oxidative stress, exacerbates diet-induced obesity in mice (Sato *et al.*, 2010). Relevant to the relations between metabolic syndrome and p66Shc is the observation that insulin activates the redox enzyme activity of p66Shc specifically in adipocytes and that p66Shc-generated H_2O_2 regulates insulin signaling through multiple mechanisms, including AKT phosphorylation, FOXO localization, and regulation of selected insulin target genes. p66Shc$^{-/-}$ mice showed increased mitochondrial uncoupling and reduced triglyceride accumulation in adipocytes and *in vivo* increased metabolic rate and decreased fat mass and resistance to diet-induced obesity (Berniakovich *et al.*, 2008). The relationship between p66Shc signaling and metabolic syndrome has been further explored in leptin/p66Shc double knockout (Ob/Ob-p66Shc$^{-/-}$) mice. Compared with Ob/Ob-p66Shc$^{+/+}$, obese mice lacking p66Shc had reduced fat accumulation starting from 30 weeks of age, paralleled by a shift toward smaller insulin-sensitive adipocytes, and reduced plasma glucose and triglyceride concentrations. The mechanism whereby p66Shc deletion conferred resistance to obesity-induced insulin resistance and development of metabolic syndrome features is probably related to the modulation of the insulin signal via IRS-1 (Ranieri *et al.*, 2010).

As a first-in-human experience, we have reported that p66Shc expression is increased in PBMC of insulin resistance patients with type 2 diabetes when compared to controls. Interestingly, we found a significant linear correlation between p66Shc mRNA levels and plasma total isoprostanes, an index of systemic oxidative stress (Pagnin *et al.*, 2005). These data support the role of p66Shc in regulating oxidative stress in humans and indicate that p66Shc is an attractive target to prevent morbidity associated with type 2 diabetes, obesity, and metabolic syndrome and to counter the resulting lifespan shortening. Unfortunately, there is no definite evidence that p66Shc regulates fat mass development and is involved in obesity in humans. Studies in progress in our laboratories are actively pursuing this hypothesis.

mTOR and AMPK

The target of rapamycin protein signal is another lifespan determinant pathway in mammals. Mammalian TOR is encoded by the human FRAP1 gene. It produces a serine/threonine protein kinase regulating cell growth, proliferation, motility and survival, as well as protein synthesis and transcription. Current research indicates that mTOR integrates the input from multiple upstream pathways, including insulin, growth factors, and mitogens. Mammalian TOR also functions as a sensor of cellular nutrient, energy levels, and redox status (Vander Haar *et al.*, 2007). Rapamycin is a bacterial natural product that can inhibit mTOR through association with its intracellular receptor FKBP12. It has been reported that rapamycin extends median and maximal lifespan of both male and female mice when fed beginning at 600 days of age (Harrison *et al.*, 2009). Although the mechanisms are still unclear, rapamycin may extend lifespan by postponing death from cancer and/or by retarding other mechanisms of aging. Downstream of mTOR, the ribosomal S6 protein kinase (RSK, also known as S6K1) has been identified as a determinant of mammalian aging (Selman *et al.*, 2009). S6K1 is one of two mammalian p70rsk proteins that modulate mRNA translation and protein synthesis in response to mTOR signaling. Phosphorylation of S6K1 decreases in rapamycin fed mice, suggesting that the effect of rapamycin on lifespan likely involves reduced S6K1 activity. Indeed, deletion of S6K1 led to increased lifespan and resistance to age-related pathologies, such as bone, immune, and motor dysfunction and loss of insulin sensitivity. Interestingly, knockout of S6K1 induced gene expression patterns similar to those seen in caloric restriction or with pharmacological activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) and was associated with AMPK activation by phosphorylation (Selman *et al.*, 2009). Collectively, these data appear to suggest that the effects of S6K1 are mediated by AMPK and put AMPK downstream of mTOR. Interestingly, signaling through mTOR and S6K1 phosphorylation is increased in mice lacking FOXO3a (Khatri *et al.*, 2010), although consequences of this network in the setting of metabolic syndrome remain to be explored. Preliminary data support the existence of a link between S6K1 and p66Shc in regulating insulin signaling. The negative effect of p66Shc on insulin-mediated adipogenesis may be modulated by concomitant S6K1 activation by phosphorylation and, indeed, serine-phosphorylated S6K1 is reduced in Ob/Ob-p66Shc$^{-/-}$ compared with Ob/Ob-p66Shc$^{+/+}$ mice (Ranieri *et al.*, 2010).

AMPK acts as a metabolic master switch regulating several intracellular systems, including the cellular uptake of glucose, β-oxidation of fatty acids, and biogenesis of glucose transporter 4 (GLUT4) and mitochondria (Winder & Hardie, 1999). The energy-sensing capability of AMPK can be attributed to its ability to detect and react to fluctuations in the AMP/ATP ratio that

take place during rest and exercise (Winder, 2001). Expressed in key metabolically relevant organs, AMPK is activated in response to a variety of stimuli, including cellular stress, exercise, and a wide range of hormones and agents that impact on cellular metabolism. AMPK indeed integrates energy balance with metabolism and stress resistance and is implicated as a longevity factor in the nematode *C. elegans* (Greer *et al.*, 2009). AMPK activation requires Thr172 phosphorylation of the α-subunit plus a conformational change of the β-subunit, induced by increased concentrations of AMP or of its analog 5-amino-4-imidazolecarboxamide riboside (AICAR). Downstream effects of AMPK activation include glucose uptake through GLUT4 during exercise, stimulation of fatty acid oxidation by modulating malonyl-CoA decarboxylase, inhibition of cholesterol synthesis through inactivation of 3-hydroxy-3-methylglutaryl-CoA reductase, mitochondrial biogenesis, and modulation of adipocytokines production from the adipose tissue. A series of data have also identified inhibition of mTOR as one of the many downstream effects of AMPK, exerted through phosphorylation of two regulatory proteins, namely the TSC2 tumor suppressor and the critical mTOR complex 1 binding subunit Raptor (Shaw, 2009). Interestingly, Raptor acts as a scaffold to recruit downstream substrates of mTOR such as the ribosomal S6 kinase (RSK) (Nojima *et al.*, 2003), which becomes phosphorylated on a Thr residue. These observations appear to be inconsistent with the observation that the lifespan effects of mTOR-target RSK deletion are dependent on AMPK. The observations might be reconciled by the hypothesis that mTOR signal inhibition, by RKS deletion or by AMPK activation itself, differentially affects translation of certain mRNA, potentially increasing AMPK expression (Kaeberlein & Kapahi, 2009). Thus, AMPK is revealed as a core regulator of this pathways and RSK modulation may simply resemble the effects of indirect AMPK activation. In this context, activation of AMPK by pharmacological agents represents a unique challenge, given the complexity of its biology, but holds a considerable potential to reverse the metabolic abnormalities associated with metabolic syndrome (Zhang *et al.*, 2009). Moreover, a novel class of drugs, called exercise mimetics, exploits functions common to the AMPK pathway to switch the metabolic status from a resting-like to an exercised-like state and prevent or treat features of the metabolic syndrome (Richter & Ruderman, 2009). Similarly, the so-called caloric restriction mimetics, drugs targeting metabolic and stress response pathways, including AMPK activation (Ingram *et al.*, 2006), are being tested as hypothetical treatment strategies to tackle metabolic syndrome, and they might translate into improvement of life expectancy in metabolic syndrome patients (Zambon *et al.*, 2009). Clinically, the opportunity to activate AMPK can be met using the antidiabetic drug metformin, which exerts its pharmacodynamic activity mainly by reducing hepatic glucose production and increasing insulin sensitivity, through AMPK stimulation (Zhou *et al.*, 2001). Metformin is the gold standard reference treatment for type 2 diabetes and is well tolerated across several cohort of subjects (Nathan *et al.*, 2009). Given its favorable metabolic effects, metformin has been successfully used for the prevention of type 2 diabetes in subjects at risk, such as those with metabolic syndrome (Petersen & McGuire, 2005; Andreadis *et al.*, 2009). Interestingly, metformin was shown to extend lifespan in the spontaneous hypertensive rat and in other experimental models (Anisimov *et al.*, 2003, 2008).

Pathway integration

We have described three potentially relevant pathways that integrate determinants of longevity with metabolic signals. It is of interest that disruption of these lifespan determinant pathways leads to features of metabolic syndrome in mice. There is also initial indirect evidence that the same molecular mechanisms are active in human diseases as well. We would like to speculatively integrate these signals, hypothesizing that they interplay in a partially redundant system at the crossroads of longevity and metabolism. A hypothetical scheme is described in Fig. 1. It starts from what characterizes the adverse metabolic milieu of metabolic syndrome, high concentrations of glucose and saturated fatty acids. We have shown that these compounds induce SIRT1 downregulation in relation to loss of insulin sensitivity and increased vascular remodeling. The effects of these compounds on SIRT1 are mediated by oxidative stress. Interestingly, most ROS induced after exposure to high glucose come from either mitochondria or NADPH oxidase, both of which are dependent on the presence of and activation of p66Shc (Giorgio *et al.*, 2005; Tomilov *et al.*, 2010). Deletion of p66Shc reduces oxidative stress, dampens detrimental effects of high glucose, impedes development of obesity, and prolongs lifespan. Therefore, it can be argued that the favorable effects of p66Shc knockout are at least in part mediated by preservation of SIRT1 expression and function, even after exposure to an adverse metabolic environment, by sparing SIRT1 from the upstream negative effects of ROS. There is also evidence that SIRT1 and AMPK

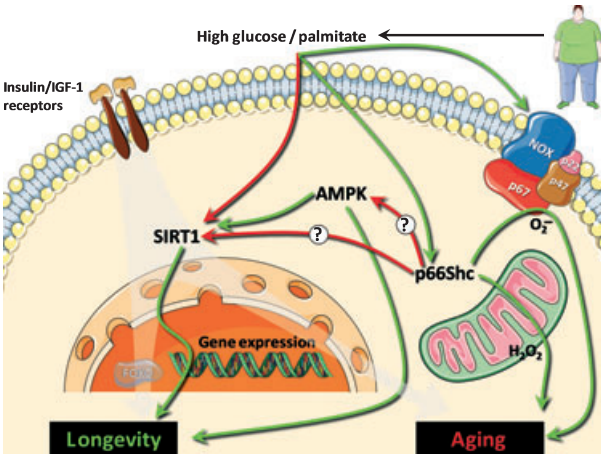

Fig. 1 The hypothetical interplay among selected longevity pathways in the metabolic syndrome. Green arrows indicate stimulation, while red arrows indicate inhibition. The question marks identify hypothetical pathways. A transparent arrow from the insulin/IGF-1 receptor indicates that this pathway may modulate longevity vs. aging signals, mainly through FOXO transcription factors: it is likely that this pathway interacts with the selected longevity networks described in the text.

are co-regulated, interact, and share many common target molecules; as a result, AMPK activation might rescue SIRT1 expression in combination with p66Shc inhibition (Ruderman *et al.*, 2010). We expect that p66Shc influences AMPK phosphorylation as well, in a complex interplay among these three pathways. This model is oversimplified and the interplay between selected pathways needs yet to be substantiated. Moreover, it only describes one of the many possible scenarios resulting from interpretation of the literature. As discussed in the relevant paragraphs, the insulin/IGF-1 axis along with its key downstream transcription factor FOXO represents an important modulator of the effects of SIRT1, p66Shc, and mTOR/AMPK.

Finally, it should be taken into consideration that the net effect of this complex network might turn out to be tissue-specific. Many organs and tissues are likely collectively involved in the final phenotype resulting from modulation of these pathways, such as the liver, adipose tissue, muscle, and central nervous system. To translate the longevity/aging phenotype into relevant cardiovascular readouts, the vasculature itself might be very important. Noteworthy, liver-targeted SIRT1 knockdown was shown to decrease basal hepatic glucose production and increase hepatic insulin sensitivity in type 2 diabetic rats. This unexpected observation is probably related to the tissue-specific vs. systemic effects of SIRT1 modulation, which differ in the regulation of some soluble mediators, such as adiponectin (Erion *et al.*, 2009). In contrast, the favorable effects of p66Shc knockout are likely to be expressed consistently in several tissues, with suppression of adipose tissue growth (Berniakovich *et al.*, 2008), liver inflammation (Koch *et al.*, 2008), muscle damage (Zaccagnini *et al.*, 2004), atherosclerotic plaque growth (Napoli *et al.*, 2003), and cognitive impairment (Berry *et al.*, 2007). However, lineage or tissue-specific gene targeting should be performed to dissect out the local vs. systemic effects of longevity gene modulations on the net organism phenotype.

In conclusion, this new network on which longevity and metabolic pathway converge offers many attractive therapeutic targets with potential applications to counter the spreading epidemics of metabolic syndrome.

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Author contributions

GPF designed and wrote the manuscript; GC and EP reviewed the technical issues in biochemical pathways. SdK and AA contributed to writing of the manuscript and supervised the project.

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