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Mitochondria and Cardiovascular Aging [*Circ Res.* 2012;110:1109–1124]

The Contribution of Impaired Mitochondrial Autophagy to Cardiac Aging: Mechanisms and Therapeutic Opportunities [*Circ Res.* 2012;110:1125–1138]

Growth Factors, Nutrient Signaling, and Cardiovascular Aging

Telomeres and Mitochondria in the Aging Heart

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Nonmammalian Models of Cardiovascular Aging

David Sinclair & Brian North, Guest Editors

## Growth Factors, Nutrient Signaling, and Cardiovascular Aging

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**Abstract:** Growth factors regulated by specific macronutrients have been shown to promote aging and accelerate mortality in the majority of the organisms studied. In particular, the enzymes activated by growth hormone, insulin, and insulin-like growth factor-1 in mammals and their orthologs in simple model organisms represent perhaps the best-understood proteins involved in the aging process. Dietary restriction, which reduces the level of insulin-like growth factor-1 and of other growth factors, has been associated with protection from diabetes, cancer, and cardiovascular diseases, and deficiencies in growth hormone signaling and insulin-like growth factor-1 are strongly associated with protection from cancer and diabetes in both mice and humans; however, their role in cardiac function and cardiovascular diseases is controversial. Here, we review the link between growth factors, cardiac function, and heart disease with focus on the cardioprotective and sensitizing effect of growth factors in both model organisms and humans. (*Circ Res.* 2012;110:1139-1150.)

**Key Words:** insulin-like growth factor-I ■ nutrition ■ signaling ■ stress resistance

Aging results in a progressive functional and structural decline in multiple organs and, in particular, has profound effects on the heart and arterial system. Age-related cardiac and vascular changes include impaired endothelial function and intimal proliferation,<sup>1</sup> increased arterial stiffness,<sup>2–7</sup> left ventricular (LV) diastolic dysfunction,<sup>8–10</sup> LV pathological hypertrophy,<sup>11</sup> diminished LV systolic reverse capacity,<sup>9,10</sup> decreased heart rate variabil-

ity,<sup>12–14</sup> and a reduction in maximal heart rate.<sup>15</sup> Furthermore, as a consequence of aging, the interaction between the heart and arterial system is altered to preserve ventricle–arterial homeostasis. Therefore, the age-associated LV structural and functional deterioration attributable to the intrinsic effects of aging on the myocardium in conjunction with the compensatory reactive cardiac modifications in response to the progressive increase of systolic load

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**Non-standard Abbreviations and Acronyms**

<b>Ang II</b>	angiotensin II
<b>CSC</b>	cardiac stem cells
<b>DR</b>	dietary restriction
<b>GH</b>	growth hormone
<b>GHR</b>	growth hormone receptor
<b>GHRD</b>	growth hormone receptor deficient
<b>HOMA-IR</b>	homeostatic model assessment–insulin resistance
<b>IGF-I</b>	insulin growth factor 1
<b>InR</b>	insulin receptor
<b>mTOR</b>	mammalian target of rapamycin
<b>PI3K</b>	phosphatidylinositol 3-kinase
<b>PKA</b>	protein kinase A
<b>SIRT1</b>	sirtuin 1
<b>S6K</b>	p70 S6 ribosomal kinase

imposed by increased arterial stiffness<sup>4,9</sup> can have a significant detrimental effect on the senescent heart.

### Dietary Restriction and Cardiovascular Aging

Dietary restriction (DR), a 20% to 40% reduction in calorie intake, which reduces the levels of insulin-like growth factor-1 (IGF-I) and other growth factors, has been consistently shown to increase life span and to prevent the development of age-associated cardiovascular functional and structural changes in several model organisms.<sup>16–23</sup> In particular, DR has been shown to improve arterial flow-mediated vasodilation<sup>24,25</sup> and to delay the development of atherosclerotic lesions in rodents.<sup>18</sup> DR significantly ameliorates LV diastolic function of the aging heart and reduces arterial stiffness.<sup>17,20,21,24</sup> Moreover, long-term DR has been shown to improve autonomic function and, in particular, to increase the high-frequency component of the heart rate variability spectra, a marker for parasympathetic activity in rats.<sup>22</sup> Finally, long-term DR has a powerful effect in preventing/delaying the age-related increase in the severity of cardiomyopathy in rodents as well as in monkeys.<sup>23,26</sup>

There are a number of hypotheses regarding the mechanisms by which DR mediates its beneficial effects on aging in lower organisms that could have relevance to slowing cardiovascular aging in humans. These include a decrease in chronic inflammation, a reduction in the levels of various hormones and growth factors, an increased resistance to oxidative stress, as well as the potentiation of antioxidant defense mechanisms (Figure 2).<sup>27</sup> The protection from free radical-induced tissue damage is presumably conferred, at least in part, by the DR-mediated reduction in growth factors signaling. In the long-lived dwarf, GHR-knockout, *klotho* transgenic, and *p66shc*<sup>-/-</sup> mice, the suppression of intracellular mitogenic signaling pathways increases the expression of reactive oxygen species scavenging enzymes, such as catalase and superoxide dismutase, thereby facilitating removal of these toxic oxygen species.<sup>28</sup> Also, human cells exposed to IGF-I–deficient human serum are more protected against oxidative DNA damage.<sup>29</sup> In the heart of rats, these

DR-dependent effects lead to a decrease in oxidative stress and an improved functional recovery after ischemia.<sup>30–32</sup> These effects and the reduced heart hypertrophy in aging rats may be attributable to improvements in mitochondrial function.<sup>33</sup> In fact, DR is well-known to delay the deterioration of mitochondrial respiratory function, a main source of reactive oxygen species, by preserving enzymatic activities of the electron transport system and controlling proton leak.<sup>34–36</sup>

The protection against inflammation-mediated tissue damage and aging-associated deterioration in immune function also may play an important role in preventing/delaying cardiovascular aging, because it could lower the levels of inflammatory cytokines and oxidative stress involved in cardiovascular disease progression.<sup>37</sup> In fact, DR has been shown to lower the circulating levels of inflammatory cytokines (eg, IL-6 and tumor necrosis factor- $\alpha$ ) and to increase plasma adiponectin and cortisol concentration.<sup>38–43</sup> In addition, DR simultaneously affects multiple processes that are involved in cardiovascular aging, including more rapid removal of damaged proteins and oxidized lipids and lipoproteins, decreased protein glycation and collagen cross-linking,<sup>44–46</sup> effects that suggest the involvement of autophagy.

Long-term DR in human volunteers causes profound reductions in several cardiometabolic risk factors for coronary heart disease, including lowering of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, and a large increase in high-density lipoprotein cholesterol concentrations, lower fasting glycemia, and homeostatic model assessment for insulin resistance index, and a remarkable lowering effect on systolic and diastolic blood pressure.<sup>27,47,48</sup> Long-term DR in humans also has a powerful antiinflammatory effect reflected by very low circulating levels of C-reactive protein and tumor necrosis factor. This decrease in systemic inflammation and other cardiometabolic markers was accompanied by a significantly lower thickness of the carotid artery intima-media thickness and by an improved LV diastolic function in the DR individuals compared to the age- and sex-matched control group.<sup>47,49</sup>

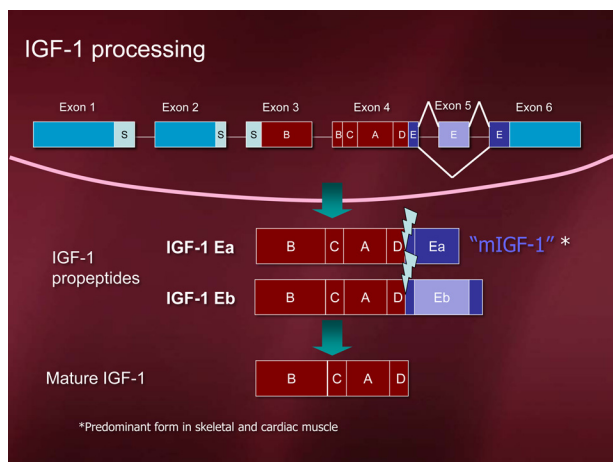
Recent studies have provided evidence for the role of inactivation of GH and IGF-I signaling pathways in the protective effects of DR on cellular resistance and aging. In agreement with this link, downregulation of the insulin/IGF-I pathway slows aging and protects against several metabolic alterations that promote cardiovascular disease. However, the role of growth factors in modulating age-associated cardiovascular dysfunction has not been clearly defined.

Among the pathways whose inactivation is believed to mediate part of the protective effects of DR are the PI3K-AKT, Ras, and TOR-S6K pathways, all regulated by insulin and IGF-I.<sup>16</sup> The adenylate cyclase/PKA pathway is also emerging as a sensitizing and aging-promoting pathway in yeast and mice, but its connection with growth factor signaling is still poorly understood.<sup>16,50</sup>

## Growth Signaling, Stress Resistance, and Heart Function in Model Organisms

### IGF-I Signaling and Function

IGF-I acts as an intermediate of several growth hormone (GH) responses and affects multiple signaling cascades,



**Figure 1. Mammalian IGF-I transcript processing.** The insulin-like growth factor-1 (IGF-I) gene contains six exons that encode multiple isoforms, all of which include the core IGF-I protein body (dark red boxes). Exons 1 and 2 contain multiple transcription start sites and code for the N-terminal signal peptide of precursor IGF-I (class 1 and class 2). Exons 5 and 6 each encode distinct portions of the E-peptides. Class 1 IGF-I Ea is also termed mIGF-I.

resulting in a potent proliferative signal that blocks apoptosis and stimulates growth in many different cells and organs.<sup>51</sup> IGF-I actions are mediated through its receptor, a heterotetrameric transmembrane glycoprotein complex belonging to the receptor tyrosine kinase family (Figure 2). The IGF-I receptor is closely related to the insulin receptor, although each has significantly different affinities for its cognate ligand.<sup>52</sup> One branch of the mitogenic signaling of IGF-I involves the association of the receptor tyrosine kinase with Shc, Grb2, and Sos-1 to activate Ras and the MAP kinase cascades (Raf, Mek, Erk).<sup>53</sup> One of the endpoints of the MAP kinase pathways is the modification of ELK transcription factors, serum response factor, and AP-1. Another important branch of IGF-I signaling involves the phosphorylation of IRS-1 and the activation of the PI3K/Akt signaling cascade, which is similarly activated by insulin, and which is known to sensitize cells in part by the phosphorylation and inactivation of FOXO transcription factors (Figure 1).<sup>53,54</sup> These kinases and transcription factors have profound effects on cell survival, cell cycle, and lipid and glucose metabolism by regulating downstream effectors such as NF- $\kappa$ B, BAD, and many others, and the expression of protective genes including superoxide dismutases and catalase.<sup>16,55</sup>

The IGF-I core peptide is released by the liver into the bloodstream and it has a half-life of <10 minutes. It is usually stabilized by forming complexes with carrier proteins, the IGF-BPs, which serve not only to transport IGF-I in circulation but also to prolong its half-life, modulate its tissue specificity, and strengthen or neutralize its biological actions.<sup>56</sup> Studies on mice lacking the IGF-I gene have shown that normal IGF-I expression is critical for normal growth and tissue development; both prenatally and postnatally, IGF-I contributes more to the total body weight than GH alone (35% versus 14%). Mice deficient for IGF-I have a birth

weight of only 60% of that of their wild-type littermates and most of them die near the time of birth because of respiratory failure. Depending on the genetic background, only a small percentage survives until adulthood, and adult IGF-I knockout mice are infertile and severely growth-restricted, reaching only 30% of the normal adult body weight.<sup>57,58</sup> When the IGF-IR is genetically removed, mice are even more profoundly affected; the birth weight of IGF-IR knockout animals is only 45% of that of their wild-type littermates, and they all die immediately after birth because of respiratory failure. The essential role IGF-I plays in somatic growth is also illustrated by the finding that one single-nucleotide polymorphism in the IGF-I gene can be responsible for the size difference between strains of small and large dogs.<sup>59</sup> Although these results firmly establish the importance of IGF-I in somatic growth and its interplay with GH in this process, it remains unclear how the participation of endocrine liver-derived IGF-I in the promotion of growth differs from the effects of locally produced IGF-I, which acts in an autocrine and paracrine fashion in skeletal and heart muscle. Because of these central effects on growth, survival, and metabolism, IGF-I and insulin signaling pathways are connected to the aging process in many organisms.<sup>16,60–62</sup>

### IGF-II Signaling and Function

Whereas IGF-I is perhaps the most important GH-induced growth factor produced postnatally, IGF-II is considered the main IGF for the regulation of fetal and placental growth.<sup>63</sup> IGF-II signals through the IGF-I receptor, triggering similar signaling cascades of IGF-I,<sup>64</sup> and by binding to the IGF-II receptor, which is believed to signal through G-protein-related mechanisms.<sup>65</sup> Mice with disrupted IGF-II are growth-deficient but they do not have alterations in life span.<sup>66</sup> Elevated levels of IGF-II in the postnatal period do not rescue the body and skeletal growth defects in the absence of IGF-I.<sup>67</sup> There is evidence that IGF-II is induced by hypertrophic and apoptotic stressors in the heart<sup>68,69</sup> and that it triggers cardiac hypertrophy *in vitro*.<sup>70</sup> Given its role during development, it has been suggested that a finely tuned IGF-II signaling is crucial for a balanced heart growth.<sup>71</sup> Polymorphisms in the human IGF-II gene have been positively associated with body mass index in adult males.<sup>72</sup> Despite these observations, to date, a role for IGF-II role in cardiovascular aging and development remains unproven.

### Growth Signaling and Stress Resistance in Simple Organisms

The understanding of the basic mechanisms of cellular protection and the identification of the factors that regulate this protection are important to counteract cardiovascular aging and diseases. Yeast do not express insulin/IGF-I-like growth factors but respond to the presence of glucose, amino acids, and other macromolecules by directly activating orthologs of genes that function in the mammalian IGF-I pathway including Tor/S6K and Ras. Inactivation of the yeast Tor/Sch9 amino acid-response pathway or of the Ras/adenylate cyclase/PKA glucose-response pathway extends life span and also causes a major increase in the resistance to a variety of stresses, including oxidative and heat stress.<sup>50,73</sup>

These effects are mediated in large part by stress resistance transcription factors *Gis1* and *Msn2/Msn4*, which promote extensive gene expression changes, including increased expression of the mitochondrial superoxide scavenger *SOD2*.<sup>16,50,74,75</sup> Similarly, the downregulation of the PI3K *age-1* gene or of the upstream *daf-2* insulin/IGF-I-like receptor is associated with resistance to a variety of stresses in worms,<sup>76</sup> which, analogously to what is observed in yeast, is often mediated by the activation of forkhead transcription factor *DAF-16*, which regulates the expression of a number of protective genes, including heat shock proteins and antioxidant enzymes.<sup>61</sup>

Fruit flies (*Drosophila melanogaster*) have been proposed as a valuable and simple model organism to study cardiac aging.<sup>77</sup> In *Drosophila*, the insulin-like receptor (*InR*) and its downstream substrate *Chico* regulate longevity and stress resistance. Loss of the activity of *Chico*, the insulin receptor substrate that regulates cell size and metabolism,<sup>78</sup> increases life span and provides some resistance to paraquat.<sup>79,80</sup> Interfering with *InR* signaling by overexpressing the phosphatase *dPTEN* or the forkhead transcription factor *dFOXO*, analogous to the worm *DAF-16* transcription factor, prevents the age-dependent decline in cardiac performance.<sup>81</sup> *Drosophila* has a simple “heart”/cardiovascular system composed of a cardiac tube or dorsal vessel, made of single layer of cardiomyocytes directly in contact with the internal and external environments.<sup>99</sup> This constitutes the entire cardiovascular system of the organism that operates in an open circulation and that has been often regarded as a useful model of cardiac organogenesis environments.<sup>99</sup> The fruit fly has also a simple and unique insulin/IGF-I signaling pathway without variant isoforms or complex gene regulation by alternative splicing. In this organism, a mutation in the *InR*, the only receptor homologous to mammalian insulin/IGF-I receptors or *Chico* (the only homologous to the 3 insulin/IGF-I substrate *IRS1–4*) or other experimental interventions that dampen this pathway reduces the progressive and detrimental changes in heart function observed when the fly ages (decrease in heart rate and heart failure).<sup>81</sup> As introduced previously, this evidence confirms that reduced IGF-I signaling can have both a positive and a negative impact on cardiovascular function during stress and aging, a dichotomy that may be explained by the well-established role of IGF-I and of IGF-I signaling proteins in acting acutely to block apoptosis but in promoting cellular sensitivity to stress in response to chronic exposure (see Growth Signaling and Stress Resistance in Mammals).

### Growth Signaling and Stress Resistance in Mammals

As anticipated by the conserved effect of progrowth signaling on increasing sensitivity to stress in simple organisms, the downregulation of nutrient signaling and cellular protection are also linked in mammals. Fibroblasts removed from different IGF-I-deficient mouse models are resistant to a variety of toxins, including  $H_2O_2$ , paraquat, UV, methylmethanesulfonate, heat, and cadmium.<sup>28,82</sup> Conversely, the levels of superoxide dismutases and catalase activity are reduced after exposure of hepatocytes to GH or IGF-I or overexpres-

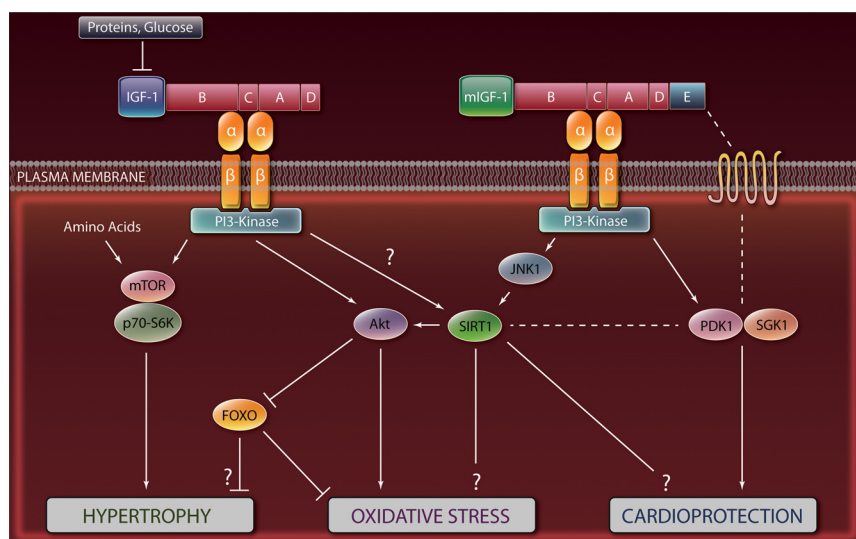
sion of GH in transgenic mice.<sup>83,84</sup> Also, IGF-I signaling sensitizes rat primary neurons to oxidative stress by a SIRT1 and Ras/Erk-dependent mechanism<sup>85</sup> and sensitizes glial cells against chemotherapy drugs.<sup>86</sup> Furthermore, mice with a 70% to 80% deficiency in IGF-I (*LID*) mice have been shown to be resistant to multiple toxins, including doxorubicin, which is well-known to promote cardiac damage.<sup>86</sup> Thus, IGF-I signaling is likely to promote a chronic aging-promoting effect on cardiomyocytes. These sensitizing effects of growth factor signaling genes in various mammalian cell types may be mediated in part by the inactivation of FOXO forkhead stress resistance transcription factors, which, analogously to *Msn2/4* in yeast and *DAF-16* in worms, regulate cellular protection in part by modulating the expression of antioxidant enzymes such as *SOD2*.<sup>54</sup>

Mitochondria play an important role during cardiac stress resistance and aging.<sup>11</sup> Short-term (24 hours) IGF-I subcutaneous administration in rats confers cardioprotection from ischemic stress by enhancing mitochondrial function, boosting anti-apoptotic mechanisms, inhibiting  $Ca^{2+}$ -induced mitochondrial swelling and cytochrome c release, and increasing ATP synthesis.<sup>87</sup> In mice with hypopituitary dwarfism (Ames dwarf), low plasma GH/IGF-I levels are associated with increased cardiac mitochondrial oxidative stress.<sup>88</sup> Nonetheless, Ames dwarf mice have a significantly increased life span and are less sensitive to dobutamine-induced heart stress.<sup>88,89</sup>

Although IGF-I and adenylate cyclase/PKA are not closely associated in mammals, mice lacking adenylate cyclase 5, an ortholog of the proaging and stress-sensitizing yeast adenylate cyclase, are resistant to oxidative stress and protected against cardiac damage, including ventricular hypertrophy, apoptosis, and fibrosis.<sup>90</sup> Similar results were obtained for mice with deficiencies in PKA, downstream of adenylate cyclase 5, which are resistant to age-related changes in diastolic dysfunction, and myocardial performance.<sup>91</sup> These results raise the possibility that some of the fundamental mechanisms of cellular sensitization by specific IGF-I signaling-like and adenylate cyclase 5/PKA signaling pathways may be conserved from nondividing yeast to heart cells. However, the relationship between IGF-I/progrowth signaling and stress resistance is not straightforward, particularly in nondividing cells. In fact, *GHRD* mice are more susceptible to the pro-oxidant paraquat, possibly because IGF-I can be a strong temporary protector of cardiomyocytes against acute damage such as that caused by oxidative and other forms of damage.<sup>92</sup> This dual role of IGF-I is not surprising considering the variety of enzymes that it activates and considering that some of them, such as AKT, are well-known to activate blockers of apoptosis (*Bcl-2* and others).

### IGF-I and Stress Resistance in Cardiomyocytes

Decades of studies pointed to IGF-I as a potential therapeutic agent. Paradoxically, as discussed previously, decades of studies also pointed to reduced insulin and IGF-I signaling as a strategy to protect cells and organs against aging and diseases. The picture is complicated by recent evidence about the cardioprotective, cardiosensitizing, and regenerative effects exerted by different IGF-I isoforms, and by the newly



**Figure 2.** A simplified model representing the effect of nutrients and insulin-like growth factor-1 (IGF-I) on cellular protection and the differences between the intracellular signaling cascades triggered by the circulating IGF-I and the cardiomyocyte-produced mIGF-I isoform. Because studies on the effect and role of the mIGF-I isoform are relatively new, the **right panel** represents only a potential model for mIGF-I effects on cardioprotection. Additional studies are necessary to confirm this role. Question marks indicate mechanisms not fully clarified. Dashed lines indicate hypothetical interactions. See text for details and references. Illustration credit: Cosmocycle/Ben Smith.

characterized molecular cross-talk between IGF-I and other signaling pathways involved in aging-associated damage and diseases in animal models.

In some reports, overexpression of IGF-I in the heart protected and prevented myocardial cell death after infarction, limiting ventricular dilation, hypertrophy, and diabetic cardiomyopathy.<sup>93–96</sup> In another study, overexpression of a different IGF-I transgene in the heart produced hypertrophy and failure, despite an initial physiological hypertrophy.<sup>97</sup> As for oxidative stress, the role of circulating IGF-I is also debated; cardiomyocyte-specific overexpression of IGF-I has been shown to protect from Ang II-mediated oxidative stress,<sup>94</sup> but severe lack of IGF-I in hepatocyte-specific IGF-I knockout mice antagonizes oxidative stress and cell death in cardiomyocytes induced by the potent oxidant agent paraquat,<sup>98</sup> in agreement with a number of studies showing a pro-oxidation effect of IGF-I in a number of cells and tissue (see Growth Signaling and Stress Resistance in Mammals).

Unlike *Drosophila*, mammals display a complex IGF-I signaling system with different isoforms that have distinct effects on cardiovascular function (Figures 1, 2). The IGF-I gene spans >70 kb and has six exons, giving rise to multiple splice variants. These variants share a common core peptide, flanked by varying termini (class 1 and 2 N-terminal peptides, and E peptides; Figure 1). IGF-I is a systemic growth factor produced mainly by the liver in response to GH and a local growth factor acting in an autocrine/paracrine manner in organs such as the heart.<sup>51</sup> Posttranscriptionally, IGF-I isoforms are cleaved to give a mature 70-amino-acid core hormone (identical for all isoforms) devoid of both the signal peptide and the extension peptide (Figure 1). Although there are little data available on the tissue-specific expression of the IGF-I splicing isoforms in mammals, several IGF-I variants have been discovered, resulting in complex speculative models to describe IGF-I function and regulation. The locally acting mIGF-I isoform comprises a class 1 signal peptide and a C-terminal Ea extension peptide (Figure 1).<sup>51</sup> The mIGF-I is highly expressed in neonatal tissues and in the adult liver, but decreases during aging in the heart and skeletal muscle,

where it is expressed only transiently in response to local damage.<sup>51,100,101</sup> Over the past decade, mouse genetics have been used to show that enhancement of the mIGF-I signaling pathway is highly effective in countering tissue decline, possibly by its regenerative properties and its promotion of cell survival and renewal demonstrated in senescent skeletal muscle.<sup>102–104</sup> Continuous cardiac expression of this isoform throughout postnatal life does not perturb cardiac physiology and does not evolve into a pathological phenotype.<sup>105</sup> The mIGF-I overexpression is able to recover heart functionality after heart failure-inducing injuries (infarct induced by ligation of the left coronary artery or after cardiotoxin-induced injury),<sup>105</sup> demonstrating a restoration of cardiac function in postinfarct and injury-challenged mIGF-I transgenic mice that is facilitated by modulation of the inflammatory response. Molecular analysis further revealed that mIGF-I enhances antioxidative cell defenses by upregulating a subset of genes that display antioxidant antiapoptotic properties and are inversely correlated with cardiac adiposity (adiponectin, uncoupling protein-1 and methallothionein 2).<sup>105–108</sup> Moreover, mIGF-I ventricular tissue exhibits increased proliferative activity in the affected areas several weeks after injury. The canonical signaling pathway involving Akt, mTOR, and p70S6 kinase is not induced in mIGF-I hearts, which instead displays activated PDK1 and SGK1 signaling intermediates (Figure 2).<sup>105</sup> This is in agreement with the conserved role of Akt, mTOR, and p70S6 kinase in sensitizing different model organisms against stress and aging,<sup>50,109–110</sup> and may explain part of the dichotomy underscored previously. The robust response achieved with the mIGF-I isoform suggests a potential mechanistic basis for therapeutic strategies to improve the outcome of heart disease.

Against a cardioprotective role for mIGF-I, a recent *ex vivo* study showed that mIGF-I overexpression in cardiomyocytes diminishes heart functional recovery after acute ischemic challenge was applied.<sup>111</sup> It is possible that the cardioprotective effects of mIGF-I overexpression occur only on prolonged stress, triggering an *in vivo* response of the immune system. In fact, mIGF-I may repair the heart from

injury through production of specific cytokines, cross-talk with the bone marrow, and recruitment of endothelial-primed cells for de novo vascularization of the myocardium.<sup>112</sup> These finely tuned immune responses are not taken into account during ex vivo analyses, possibly leading to confounding results. A main limitation of the in vivo data obtained with mIGF-I cardiac-restricted transgenic mice is the lack of comparison with control core peptide IGF-I cardiac-restricted transgenics to study if the observed cardioprotection is attributable to the peculiar properties of mIGF-I isoform (class I signal peptide and a C-terminal Ea extension peptide; Figure 1) in the same experimental settings. In the skeletal muscle tissue it is well-known that the mIGF-I isoform promotes cell survival and renewal by paracrine mechanisms; its local supplementation orchestrates efficient repair of injured skeletal muscle tissues without scar formation, prevents age-related muscle atrophy, and enhances bone marrow stem cell recruitment to the damaged tissue. Importantly, neither fully processed IGF-I nor systemically administered IGF-I counteracts muscle loss.<sup>102–104</sup>

It also would be of great interest to explore the protective properties of cardiac-restricted mIGF-I versus IGF-I signaling against fat-induced cardiac adiposity and lipotoxicity, which have not been addressed to date. This is relevant to cardiovascular disease because when the storing capacity of cardiomyocytes is exceeded, lipotoxicity, which includes cellular dysfunction or cell death, gradually leads to heart failure.<sup>113</sup>

### IGF-I, Stem Cells, and Myocardial Regeneration

Great effort has been devoted to exploring the potential of adult stem cells using different approaches (cellular reprogramming, tissue engineering) to provide new therapeutic options for myocardial regeneration, although many challenges remain to be solved.<sup>114</sup> Early work from Anversa, Nadal-Ginard, and others provided evidence for the possibility to isolate and utilize a pool of resident cardiac stem cells (CSC) both in humans and in rodent models for research and therapeutic purposes.<sup>115,116</sup> In humans, these cells can amplify and likely commit to the myocyte lineage in response to increased workload, thereby contributing to the generation of new fibers during stress-induced hypertrophy.<sup>116</sup> Further, the existence of CSC was confirmed in the adult rat myocardium, and it was demonstrated that they are self-renewing, clonogenic, multipotent, and can give rise to several different cardiogenic cell lines.<sup>115</sup> CSC could regenerate myocardium on infarct by functionally differentiating into adult cardiomyocytes.<sup>115</sup> More recently, a study from Anversa et al on human hearts collected from patients 19 to 104 years of age who died from causes other than cardiovascular diseases showed that from 20 to 100 years of age, the cardiomyocyte compartment is replaced 15 times in women and 11 times in men, and this is regulated by a pool of resident CSC modulating cardiac homeostasis and aging.<sup>117</sup>

A mechanistic link between the IGF-I signaling system and CSC function in the heart was discovered; IGF-I signaling induces division of CSC by upregulating telomerase activity and hindering replicative senescence, thereby preserving the

pool of functionally competent CSC.<sup>118–121</sup> In dogs, circulating IGF-I was injected after infarction to stimulate resident CPC; this intervention led to the formation of cardiomyocytes and coronary vessels within the infarct and resulted in a marked recovery of contractility of the infarcted heart.<sup>120</sup> Similar results were obtained in mice.<sup>119</sup> With chronological aging, CPC undergo telomere shortening, which generates a differentiated progeny acquiring a senescent phenotype. CPC aging was mediated by attenuation of the IGF-I system in rats.<sup>118</sup> Activation by IGF-I injection led CPC to migrate to the regions of damage, partly reversing the aging cardiomyopathy. Consequently, heart aging and failure were partially corrected, leading to an extension of the maximum life span.<sup>118</sup> CPC can be activated locally and induced to proliferate by administration of IGF-I, but they could also be isolated from myocardial biopsies and, after their expansion in vitro, administered back to the myocardium to improve the response to the challenge.<sup>122</sup> In this respect, the most impressive protective effects and reduction of tissue damage from myocardial infarct induced in rats were observed with a combination therapy comprising simultaneous injection of CPC and circulating IGF-I tethered to peptide nanofibers to prolong its release.<sup>123</sup> Although this wealth of data needs to be confirmed independently, the prominent emerging notion is that stem cell–based cell reprogramming and organismal life span share some common regulatory pathways.<sup>124,125</sup> Because in some cases, such as for IGF-I, stem cell regeneration and aging may have opposite responses (IGF-I promotes regeneration but also aging), it will be essential to understand how the positive effects of regeneration can be combined with the antiaging effects of reduced growth factor signaling. It also will be important to understand how IGF-I and most likely the locally generated mIGF-I can have a protective and not sensitizing effect on the myocardium, particularly in the periods after ischemic injury.

### IGF-I and SIRT1

At least three reports indicate that IGF-I and SIRT1 can influence the same signaling pathways: (1) SIRT1 modulates the IGF-I signaling critical for both growth regulation and mammary gland development in mice<sup>126</sup>; (2) in neurons, the inhibition of SIRT1 reduces IGF-I signaling through deacetylation of IRS-2 and thereby protects them<sup>85</sup>; and (3) SIRT1 is needed for IGF-I–mediated hypertrophy of cardiomyocytes.<sup>127</sup>

The Sirtuins family of enzymes plays a role in the regulation of organismal life span.<sup>128</sup> In mammals, Sirtuins are composed of seven members of class III nicotinamide adenine dinucleotide–dependent protein deacetylases (SIRT1 to 7). SIRT1, the largest and best-characterized among them, is the mammalian orthologue of yeast “longevity” gene *Sir2*.<sup>129</sup> SIRT1 activation displays pleiotropic effects<sup>128</sup> and, in fact, both protective and sensitizing effects for *Sir2* and *SirT1* have been described.<sup>130,131</sup> Interventions capable of activating SIRT1 enzymatic activity have been shown to increase life span in some model organisms. These include chronic treatment with the polyphenol resveratrol<sup>132,133</sup> and a DR regimen.<sup>134</sup> In contrast, SIRT1 knockout mice die prenatally or perinatally.<sup>135,136</sup> In one report, moderate

(approximately 3- to 8-fold) SIRT1 overexpression protected mice from paraquat-induced cardiac stress and delayed the onset of age-dependent heart fibrosis and apoptosis.<sup>137</sup> In parallel, *in vitro* findings on cultured or primary cardiomyocytes models expanded our understanding of the cardioprotective effects of SIRT1, suggesting that pharmacological SIRT1 activation might be beneficial for the treatment of some cardiac diseases.<sup>128</sup> Two other sirtuins, SIRT3 and SIRT7, may also play an important protective role against age-related, genotoxic stress-induced, hypertrophic stress-induced, and oxidative stress-induced cardiac pathology,<sup>138</sup> indicating a diversified and nonredundant conserved role of the Sirtuin family members. Interestingly, recent evidence shows that mice lacking SIRT3 have development of severe age-related pathologies in the heart, such as hypertrophy, attributable to mitochondrial swelling and dysfunction, possibly because SIRT3 can modulate directly the mitochondrial permeability transition pore, a multiprotein complex involved in age-related mitochondrial diseases.<sup>139</sup>

SIRT1 also has been shown to have the opposite effect on the heart; it sensitizes cardiomyocytes to stress. For example, higher increases in SIRT1 levels (approximately 13-fold) induced oxidative stress and apoptosis, ultimately leading to cardiomyopathy.<sup>137</sup> In agreement with these results, the lack of SIRT1 has been shown to promote the acetylation and reduced activity of Akt, leading to protection against cardiac hypertrophy.<sup>127</sup> These studies indicate that SIRT1 activation can be either beneficial or deleterious in the heart in agreement with the role of its yeast ortholog Sir2 in promoting either proaging or antiaging effects,<sup>128,131</sup> and also in agreement with the dual role of IGF-I described previously. The disparate effects of different doses of cardiac SIRT1 could represent an example of the concept of “hormesis,” according to which an exogenous perturbation or stress can be protective or induce a damage according to the dose and the length of administration.<sup>140</sup>

Other than for a few studies listed, there is scarce information about the molecular mechanisms of cross-talk between IGF-I and SIRT1 in the heart and, in particular, about the impact of separate IGF-I isoforms, acting locally or systemically, on cardiac SIRT1, although it is evident that IGF-I and SIRT1 share molecular downstream targets in cardiomyocytes such as AKT and FOXOs, and this in turn may affect cardiovascular function.<sup>127,141</sup> In a recent study, the role of the IGF-I core protein isoform and the locally acting mIGF-I isoform were tested to determine if they could display distinct effects in the protection from cardiac stress.<sup>142</sup> Using Ang II and paraquat as hypertrophic/oxidative stressors, an important signaling pathway that protects cardiomyocytes and that relies on the activation of SIRT1 by the locally acting mIGF-I isoform was identified.<sup>142</sup> Heart-specific transgenic mIGF-I mice displayed a two-fold increase in SIRT1 expression compared with wild-type mice, and this correlated with a downregulation in the acetylation of SIRT1 targets (H1, p53) in mouse hearts.<sup>142</sup> *In vitro*, mIGF-I overexpression protected cardiomyocytes from cell damage induced by hypertrophic and oxidative stressors in a specific

SIRT1-dependent fashion, again in agreement with the function of SIRT1 in IGF-I signaling described previously.<sup>142</sup> The beneficial activity of mIGF-I was mediated by SIRT1-dependent activation of the protective molecules uncoupling protein-1, adiponectin, and MT2, through their respective gene promoters. Interestingly, the circulating IGF-I isoform, either added exogenously or produced by plasmid overexpression in cardiomyocytes, did not regulate SIRT1 expression and activity, and it was not beneficial during hypertrophy and oxidative stress conditions.<sup>142,143</sup>

Cardiac-specific mIGF-I transgenic mice in which SIRT1 was depleted from adult cardiomyocytes in a tamoxifen-inducible and conditional fashion were recently generated to evaluate the role of mIGF-I/SIRT1 signaling *in vivo*. Analysis of these mice confirmed that mIGF-I-induced SIRT1 activity is necessary to protect the heart from paraquat-induced oxidative stress and lethality.<sup>143</sup> In cultured cardiomyocytes, mIGF-I increases SIRT1 expression through a JNK1-dependent signaling mechanism, whereas circulating core peptide IGF-I activated preferentially JNK2.<sup>143</sup> Thus, mIGF-I can protect the heart from oxidative stress via SIRT1/JNK1 enzymatic activities (Figure 2). Considering the studies listed that indicate that SIRT1 facilitates IGF-I-dependent cardiomyocyte hypertrophy, it is possible that the mIGF-I isoform causes a set of signaling changes that turns SIRT1 from a promoter of cardiotoxicity to a protective deacetylase (Figure 2).

The field of study of the different signaling and cross-talk between the distinct IGF-I isoforms in the heart is in its infancy. Intriguingly, exogenous recombinant IGF-I administration could be beneficial for heart diseases in patients, but only during precise temporal windows and duration of IGF-I administration.<sup>144,145</sup> Understanding the cellular signaling networks, the epigenetic mechanisms (such as SIRT1 activation and chromatin remodeling processes) and the temporal windows through which the distinct IGF-I isoforms signal into the cardiomyocytes using animal models during growth and aging could lead to the identification of therapeutic entry points to fight oxidative stress and cardiac hypertrophy. In addition, this will provide us with a framework to understand the mechanistic basis of the modulation of the immune system and of the proposed recruitment of cardiac stem cells by IGF-I signaling.

## Growth Factors and Cardiovascular Aging in Humans

### IGF-I and Heart Disease: Good or Bad?

The relationship between IGF-I and cardiovascular diseases in humans is complex. In mammals, aging is negatively associated with serum concentrations of several growth factors and anabolic hormones. For example, the circulating levels of GH, IGF-I, sex hormones, and DHEAs progressively decline between 20 and 80 years of age in humans, whereas serum concentrations of proinflammatory cytokines increase with age.<sup>146</sup> Data from epidemiological data show an association between low serum IGF-I concentrations and increased cardiovascular mortality.<sup>147,148</sup> In particular, a low serum IGF-I concentration is associated with coronary artery disease and diabetic vascular lesions.<sup>149–151</sup> However, asso-

ciation is not equal to causation, as indicated by recent data showing that several metabolic and hormonal factors play a crucial role in modulating the risk of development of chronic diseases and survival in mammals.<sup>16,29</sup>

As discussed previously, the downregulation of IGF-I or similar signaling pathways by several dietary or genetic interventions has been shown to improve health and prolong life span in model organisms including mice.<sup>16</sup> In agreement with these findings and in contrast to the epidemiological data described, humans with mutations that cause constitutively very low levels of IGF-I do not display increased atherosclerosis but appear to be protected against diabetes and cancer.<sup>29</sup> Conversely, subjects with elevated GH/IGF-I attributable to acromegaly have a two- to three-fold increase in mortality attributable mostly to vascular disease but also to cancer and a variety of other diseases.<sup>152</sup> The discrepancy between the aforementioned studies on the effects of IGF-I on cardiovascular morbidity and mortality in humans may be attributable to confounding factors. Patients with coronary artery disease are exposed for many years to harmful cardiometabolic risk factors (ie, type 2 diabetes, high blood pressure, dyslipidemia, and, most importantly, chronic systemic inflammation).<sup>153</sup> It has been shown that an antagonistic relationship exists between the elevated proinflammatory cytokines and serum IGF-I concentration during degenerative conditions.<sup>154–157</sup> Therefore, it is likely that inflammation (or other metabolic alterations), rather than low IGF-I, is responsible for the increased cardiovascular mortality in patients affected by coronary heart disease and that low IGF-I is another consequence of inflammation. In fact, the dramatic decline in serum IGF-I concentration induced by infections, trauma, and critical illness is not reversed by treatment with GH.<sup>158–161</sup> Further, growth hormone infusion increases serum IGF-I concentration and nitrogen balance in healthy control subjects, but septic patients treated with GH maintain low IGF-I levels and a negative nitrogen balance.<sup>160–162</sup> A proof of our scarce and possibly misguided understanding of the implications of upregulating the GH/IGF-I signaling pathway on health came from two prospective, multicenter, double-blind, randomized, placebo-controlled trials that were ended prematurely because of increased morbidity and mortality in critically ill patients treated with GH.<sup>163</sup>

Taken together, these data strongly suggest that the association of low circulating IGF-I levels with diseases is likely to represent a protective response to conditions that can promote heart disease and not a risk factor for the disease. Because of the increasing trend of GH supplementation in older adults to increase muscle and bone mass, a stress-sensitizing effect of circulating GH/IGF-I in cardiomyocytes could increase the risk for cardiac diseases, in addition to diabetes and cancer.

### **GH/IGF-I Deficiencies and Cardiovascular Diseases**

Obesity is a major risk factor for the metabolic syndrome, which in addition to playing a key role in diabetes is associated with other chronic diseases, including coronary artery disease and stroke. Thus, GH and IGF-I deficiencies, which promote fat deposition in mice and humans, would be

expected to also increase the incidence of cardiovascular diseases. However, the studies performed failed to provide evidence for either premature atherosclerosis or cardiovascular disease in subjects with deficiencies in GHs/growth factors. Salvatori and colleagues<sup>162</sup> reported that whereas subjects from Brazil with homozygous mutations in the growth hormone-releasing hormone receptor gene were obese and had higher low-density lipoprotein and C-reactive protein levels, they did not have development of premature atherosclerosis as evaluated by exercise echocardiography. Furthermore, a 6-month treatment with GH in these GH-deficient subjects caused weight loss, but also a progressive increase in the number of atherosclerotic plaques and in the carotid intima-media thickness.<sup>163</sup> GH replacement therapy instead resulted in an increase in diastolic and systolic blood pressure.<sup>163</sup> Results consistent with those from the GH-releasing hormone receptor-deficient subjects in Brazil were obtained for the GH receptor-deficient (GHRD) subjects living in Ecuador. Whereas 27% of the deaths of GHRD subjects were reported to be caused by cardiac disease and 3% were caused by stroke, in the control population comprising first- to fourth-degree relatives, in the GHRD subjects 21% of the deaths were reported to be caused by cardiac disease and 12% were caused by stroke, providing evidence that cardiovascular disease deaths are approximately the same for the GHRD subjects and normal relatives.<sup>29</sup>

Although the protection against both diabetes and cancer in GHRD subjects<sup>29</sup> is in agreement with studies in mice, the normal longevity of GH receptor-deficient and IGF-I-deficient humans does not reflect the record mammalian life span extension observed in GH receptor-deficient and IGF-I-deficient rodents.<sup>164,165</sup> One possible explanation for this disparity is the finding that many GHRD subjects die young from a variety of unusual causes.<sup>29,166</sup> For example, 17% of the deaths in GHRD subjects were reported to be caused by convulsive disorders, whereas 13% were alcohol-related, 20% were caused by accidents, and 17% were caused by unknown causes, representing a combined total of 67% of the deaths caused by these non age-related causes versus 34% unknown causes of death and 2% accidental deaths in the relatives.<sup>29</sup> Whereas neither GHR nor GH-releasing hormone receptor-deficient subjects appear to be long-lived, mutations that reduce the activity of the IGF-IR protein were overrepresented among centenarians, suggesting that lower activity but not severe deficiency in GH/IGF-I signaling may be more beneficial for longevity extension.<sup>167</sup>

### **Conclusions**

In conclusion, the studies in simple model organisms, mice, and humans reviewed here point to a number of conclusions. First, DR causes a decrease in growth factors and anabolic hormones, including IGF-I, which may mediate many of its protective effects, including those on heart disease. Second, low IGF-I is associated with aging and cardiovascular disease in humans, but the studies discussed here indicate that this may be a consequence and not a cause of aging and heart disease. Third, different IGF-I isoforms can have protective and sensitizing effects on cardiomyocytes. Also, IGF-I may have a temporary protective effect based on inhibition of



apoptosis, but a chronic cellular sensitizing and aging promoting effect. The IGF-I expressed by heart cells and not the circulating IGF-I appears to be the protective factor.

Additional studies are needed in both rodents and humans to test the hypothesis that the severe IGF-I deficiency associated with reduced cancer and diabetes incidence rates does not contribute to cardiovascular disease, and to determine whether certain isoforms of IGF-I can, in fact, promote cardioprotection without activating the proaging signaling pathways. Because several drugs targeting the GHRH, GH, and GHR axes are Food and Drug Administration–approved and widely used to treat acromegaly and other diseases, it will be important to monitor their effects on cardiovascular diseases in patients already being treated. More studies are also urgently needed to clarify the relationship between IGF-I signaling and cardiovascular health, particularly in older individuals eating high-calorie Western diets that promote chronic inflammation, insulin resistance, and dyslipidemia.

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### Disclosures

None.

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