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IGF-1, oxidative stress, and atheroprotection

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Abstract

Atherosclerosis is a chronic inflammatory disease in which early endothelial dysfunction and subintimal modified lipoprotein deposition progress to complex, advanced lesions that are predisposed to erosion, rupture and thrombosis. Oxidative stress plays a critical role not only in initial lesion formation but also in lesion progression and destabilization. While growth factors are thought to promote vascular smooth muscle cell proliferation and migration, thereby increasing neointima, recent animal studies indicate that IGF-1 exerts pleiotropic anti-oxidant effects along with anti-inflammatory effects that together reduce atherosclerotic burden. This review discusses the effects of IGF-1 in vascular injury and atherosclerosis models, emphasizing the relationship between oxidative stress and potential atheroprotective actions of IGF-1.

Oxidative Modification of Lipoproteins and Atherosclerosis

A complete understanding of the pathogenesis of atherosclerosis remains elusive. However, the oxidation hypothesis of atherosclerosis, which has been updated and thus evolved from its original premise, is currently the most widely accepted mechanism for the development and progression of this disease [1]. The original hypothesis proposed that oxidatively modified lipoproteins cause lipid-laden macrophage, or foam cell, accumulation in the fatty streak, leading to initiation of atherosclerotic lesion formation (Figure 1). Subsequent recruitment of immune cells establishes a pro-inflammatory state, further increasing oxidative stress, which in turn triggers a series of pro-atherogenic events such as apoptotic or necrotic cell death of vascular cells. Traditionally, the role of growth factors in atherosclerosis has been thought to be permissive, for instance, in stimulating vascular smooth muscle cell (VSMC) migration and proliferation, thereby promoting neointima formation [2–4]. However, recent findings from our group [5] and others [6,7] suggest that some growth factors may have unexpected anti-atherogenic effects. Tang *et al* showed that the absence of platelet derived growth factor-B (PDGF-B) in circulating cells, which are a major source of PDGF in atherosclerotic lesions, led to a phenotypic change in lesion composition, namely, enhanced inflammatory cell infiltration [6]; intriguingly, data from the same group indicates that elimination of PDGF-B in circulating cells or blockade of PDGF receptors delayed but did not inhibit smooth muscle accumulation in lesions [8]. Their data suggests a modest contribution of PDGF to atheroprotection, but a major inhibitory effect of PDGF on inflammatory responses and on monocyte accumulation, potentially limiting lesion expansion. Recently, cardiac specific overexpression of transforming growth factor – β (TGF- β), a growth factor that promotes VSMC proliferation and matrix protein production (reviewed in [9]), was reported to limit atherosclerotic plaque burden [7]. These plaques were characterized by fewer T-lymphocytes,

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more collagen, less lipid, and lower expression of inflammatory cytokines [7]. In our recent study, insulin-like growth factor-1 (IGF-1) demonstrated anti-atherogenic effects via anti-inflammatory and pro-repair mechanisms, both of which are mechanisms coupled to changes in oxidative stress in the vasculature [5]. Undoubtedly, the effects of growth factors are pleiotropic, particularly in the setting of a complex pathology such as atherosclerosis, where many different types of cells are involved. In this review, we provide a novel paradigm for the relation between IGF-1, oxidative stress and atherosclerosis.

The IGF-1 system and the vasculature

IGF-1 is synthesized by almost all tissues and is an important mediator of cell growth, differentiation, and transformation [10–12]. In view of its pleiotropic effects and involvement in multiple disease processes, IGF-1 has become the focus of research by an increasing number of investigators.

IGF-1 exerts its physiologic effects by binding to the IGF-1 receptor (IGF-1R) and the insulin/IGF-1 hybrid receptor consisting of a half insulin receptor (α - and β -subunits) and a half IGF-1 receptor (α - and β -subunits). In VSMCs, both IGF-1 and insulin receptor subunits are expressed, but expression of the former is higher than the latter, resulting in dominant expression of IGF-1R and insulin/IGF-1 hybrid receptors [13,14]. Recently, Engberding *et al* described that VSMC are insensitive to insulin because the hybrid receptor predominantly mediates IGF-1 signaling [15]; however, these cells can be sensitized to insulin by downregulating IGF-1R using silencing RNA, thereby unmasking anti-inflammatory and glucose uptake effects of insulin. This group also suggested that circulating IGF-1 (via negative regulation of IGF-1R expression) alters VSMC insulin sensitivity [15].

In vascular endothelial cells, both IGF-1R and insulin receptor subunits are expressed, and IGF-1R is more abundant than the insulin receptor [16]. Thus, endothelial cells express IGF-1R, insulin/IGF-1 hybrid receptor as well as insulin receptor. However, endothelial cells appear more sensitive to insulin than VSMCs; in fact, insulin at physiological concentrations activates insulin but not IGF-1 or hybrid receptors [17]. IGF-1 binding to these receptors is modulated by six different IGF binding proteins (IGFBPs). In addition to this already complicated system, a variety of hormones and growth factors regulate IGF-1, IGF-1R and IGFBP expression in most tissues. Moreover, there is cross-talk between IGF-1 signaling pathways and those of other growth factors and hormones. Thus, physiological consequences of IGF-1 action are potentially altered by multiple mechanisms. It is outside the scope of this review to discuss all physiological functions of IGF-1 and its related peptides in the vasculature and readers are referred to recent reviews [12,18,19].

IGF-1 is a potent mitogen and antiapoptotic factor in vascular cells, including VSMC and endothelial cells (EC), and is also pro-migratory [20]. Thus, there is reason to speculate that IGF-1 is pro-atherogenic by stimulating VSMC migration and proliferation [2–4], enhancing chemotactic macrophage migration [21], and promoting cell adherent molecule expression [22,23]. Potential reductions in IGF-1 effects could be beneficial in certain pathologic conditions in the vasculature, such as hypertension and early stages of atherosclerotic plaque formation characterized by hypertrophy/hyperplasia of VSMC. However, it has also been postulated that reduced IGF-1 pro-survival effects could be detrimental in other conditions in which loss of VSMC contributes to the disease process, such as destabilization of atherosclerotic plaques [24].

IGF-1 and atherosclerosis animal models

Multiple animal models have been exploited to determine IGF-1 effects on vascular lesion formation (Table 1). Localized arterial injury models have been primarily used to study the

restenotic process. These models attempt to mimic the vascular repair responses to angioplasty, but several important differences must be recognized; for instance, the injury is in general to non-diseased vessels with no pre-existing neointimal cell populations and thus responses come predominantly from proliferating VSMC. Since IGF-1 is a potent VSMC mitogen, the majority of studies exploring mechanical injury models and direct [25,26] or indirect [27–30] mechanisms to alter vascular IGF-1 signaling reported that increased IGF-1 or IGF-1 signaling correlates with increased neointimal burden, suggesting that IGF-1 promotes vascular hyperplasia. In particular, targeted overexpression of IGF-1 in VSMC increases neointimal area size [25]. Leukocyte antigen-related protein-tyrosine phosphatase (LAR) physically associates with IGF-1R and diminishes its signaling activity by dephosphorylating IGF-1R. Deletion of the LAR gene increased neointima size in a mechanical injury model, consistent with a positive effect of IGF-1 on VSMC migration and proliferation [31].

Other animal models used to investigate atherosclerosis include genetic hyperlipidemia models, namely, apolipoprotein E (*Apoe*) deficient and LDL receptor (*Ldlr*) deficient mice. A high fat diet, commonly called a Western diet, is often supplied to these animals to produce even more pronounced hyperlipidemia, accelerating atherogenesis. These hyperlipidemic animals develop atherosclerotic lesions that parallel those of human subjects, characterized by lesion development at vascular branch points and progression from a foam cell stage to a fibroproliferative stage with well-defined fibrous caps and necrotic lipid cores [32]. However, there are significant differences between human and mouse lesions at advanced stages, and caution should be taken when extrapolating observations in these animal models to humans with respect to acute events associated with plaque vulnerability (interested readers are referred to recent reviews discussing the usefulness and limitations of various mouse atherosclerosis models [33,34]). Regardless, their reproducibility and extensively characterized phenotype make these genetically modified animal models an important tool to study mechanisms of atherosclerosis.

To date, few studies have evaluated IGF-1 effects on atherosclerosis using *Apoe*-deficient mice. Harrington et al. created double-knockout mice deficient in *Apoe* and pregnancy-associated plasma protein-A (*Pappa*), a metalloproteinase which degrades IGFBP-4 [29]. *Pappa*^{-/-}/*Apoe*^{-/-} mice fed a Western diet had decreased lesion size, and this correlated with reduced expression of IGFBP-5, which is positively regulated by IGF-1 [35–37]. Since *Pappa* deficiency increases IGFBP-4 levels, thereby reducing IGF-1 bioavailability, these data suggest potential pro-atherogenic effects of PAPPA mediated by IGF-1. However, direct effects of PAPPA on atherosclerosis cannot be excluded.

We investigated IGF-1's effect on atherosclerosis by infusing human recombinant IGF-1 into *Apoe* deficient mice fed a Western diet. Our study protocol provided an approximate 2-fold increase in total serum IGF-1 levels associated with enhanced IGF-1 signaling activity, confirmed by elevation in tissue Akt phosphorylation [5] (Figure 2). We did not measure free IGF-1, thought to be the fraction of serum IGF-1 immediately active for binding to its receptor (reviewed in [38]). Therefore, the increase of free IGF-1 in this model may be higher than the 2-fold increase in total IGF-1 measured. IGF-1 infusion was associated with reduced atherosclerotic lesion size. This effect was accompanied by a significant reduction in urinary excretion of 8-isoprostane, a systemic index of oxidative stress. Other potential beneficial effects included a decrease in lesion macrophage infiltration, reduced aortic expression of pro-inflammatory cytokines, and increased levels of circulating endothelial progenitor cells. IGF-1-infused animals had decreased vascular superoxide levels, upregulated expression of vascular endothelial nitric oxide synthase, and increased nitric oxide bioavailability (Figure 2). However, infusion of *Apoe* deficient mice with growth hormone releasing peptide-2 (GHRP-2), a synthetic peptide that increases circulating GH and IGF-I levels, did not reduce atherosclerotic burden despite a significant increase in serum IGF-1 levels associated with

decreased reactive oxygen species (ROS) in the vasculature [39]. Increased growth hormone levels may explain the discrepancy between the apparent beneficial effect observed in the IGF-1 infusion model and lack of effectiveness in the GHRP-2 infusion model [40]. Our findings suggest that the potential anti-atherogenic effect of IGF-1 is closely associated with reduced oxidative stress in the vasculature. Further investigation is required to characterize underlying mechanisms.

Atheroprotective effects of IGF-1

IGF-1 and inflammation

Inflammatory cells have a predominant role in the pathogenesis of atherosclerosis [41]. At an early stage of atherogenesis, phagocytic cells such as monocytes/macrophages are recruited and infiltrate into a fatty streak, scavenging accumulated low-density lipoproteins (LDL) which have been denatured or modified (e.g. aggregated, or oxidized) locally in the tissue (Figure 1). Modified-LDL binding to cell surface receptors and subsequent internalization induces a variety of pro-inflammatory responses in these cells, triggering feed-forward reactions to promote atherosclerosis [41]. Inflammatory responses and oxidative stress are closely related; in activated inflammatory cells, oxidant-generating enzymes are upregulated, such as myeloperoxidase, NADPH-oxidase and 12/15-lipoxygenase (LOX), causing oxidative damage to surrounding tissue, further inducing inflammatory responses. Several reports suggest that IGF-1 is anti-inflammatory. For instance, there is an inverse relation between serum IL-6 and IGF-1 levels [42], and IGF-1/BP-3 administration to patients suffering from severe burn injury induced an anti-inflammatory effect and reduced IL-6 and TNF- α [43,44]. Furthermore, low IGF-1 and high IL-6 and TNF- α levels are associated with higher mortality in elderly subjects [45,46]. Consistent with these clinical observations, data from our group shows that IGF-1 modulates macrophage function. This represents a key mechanism mediating IGF-1's anti-inflammatory and anti-atherogenic effects in the vasculature. Indeed, human recombinant IGF-1 infusion into *Apoe*-deficient mice markedly suppressed macrophage infiltration into atherosclerotic lesions by a mechanism that is not yet elucidated but is associated with downregulation of TNF- α expression [5]. In human subjects with growth hormone (GH) deficiency, low serum IGF-1 levels are associated with increased lipoprotein lipase (LPL) and TNF- α expression in macrophages [47]. Intriguingly, macrophages from these subjects are more susceptible to foam cell formation in vitro; in fact, uptake of oxidized LDL was enhanced in these cells [47] consistent with the known ability of LPL to facilitate binding and uptake of modified LDL. IGF-1 reduces macrophage LPL expression. These observations in animal models and human subjects support an anti-inflammatory, thus potentially anti-oxidant effect of IGF-1, which contributes to atheroprotection. However, other reports suggest that IGF-1 has pro-inflammatory effects. For instance, in monocytes/macrophages, IGF-1 enhanced chemotactic macrophage migration [21], stimulated TNF- α expression [21], and enhanced LDL uptake and cholesterol esterification [48]. Moreover, proatherogenic factors such as advanced glycosylation end products [49] and TNF- α [50] increase IGF-1 synthesis. Some of these effects of IGF-1 on macrophages may not necessarily be proatherogenic; for instance, IGF-1-mediated stimulation of cellular uptake of LDL could contribute to reducing plasma LDL-cholesterol levels [48]. Additional studies are required to clarify IGF-1's action on inflammatory processes in atherosclerosis.

IGF-1 and vascular smooth muscle cell apoptosis

Moderate oxidative stress stimulates IGF-1 [51] and IGF-1R [52] expression in VSMC. In fact, IGF-1R tyrosine kinase activity mediates hydrogen peroxide activation of mitogen-activated protein kinase [53] and Akt [54]. In addition, IGF-1 itself moderately elevates ROS generation in VSMC [55], suggesting that IGF-1 amplifies its activity through ROS generation. ROS can enhance insulin signaling by inhibiting protein tyrosine phosphatase activity [56]. Considering

the substantial similarity between IGF-1 and insulin signaling pathways, it is possible that ROS similarly enhance IGF-1 signaling. These reports support the hypothesis that moderate oxidative stress and IGF-1 coordinately promote VSMC growth and hence atherosclerosis, especially at the initial stage of lesion formation where VSMC migration and proliferation predominate. On the other hand, it is also known that elevated ROS inhibits insulin/IGF-1 signaling [57–59] and induces apoptotic cell death [60]. It is likely that the balance between ROS elevation and activity of anti-oxidant systems (e.g. enzymes such as glutathione peroxidase), and presumably a specific location of ROS generation (e.g., plasma membrane vs. mitochondrion), will determine the dominant downstream vascular effects of oxidant stress.

In advanced atherosclerotic plaques, a balance between cell death and survival of cells within the fibrous cap, primarily composed of VSMC and extracellular matrix, appears to correlate with plaque instability or stability [61]. Moreover, VSMC apoptosis itself can accelerate atherosclerosis [62], consistent with its significant role in the transition from early to advanced plaques. VSMC apoptosis is controlled by growth factors and cytokines, including IGF-1. A key pro-atherogenic molecule, oxidized low density lipoprotein (OxLDL), co-localizes with apoptotic VSMC in human atherosclerotic plaques, and these cells have reduced IGF-1 and IGF1R levels [63–65]. OxLDL (Box 1) induces apoptosis of cultured VSMC through a redox-sensitive mechanism which includes activation of two major oxidases, LOX and NADPH oxidase. The enhanced oxidative stress correlates with reduced IGF1R levels [66,67].

Box 1

Oxidized LDL (OxLDL)

It is important to note that there is significant debate about the usage of OxLDL for *in vitro* study of oxidative stress. Specifically, the optimal oxidation method and dose of OxLDL that would best mimic intrinsic oxidized LDL action in the vasculature is not known. Physiologically oxidized LDL in tissue is likely highly heterogeneous in terms of its oxidation status, in part because of its location (e.g. in a fatty streak or in an advanced plaque [141]). One can speculate that OxLDL prepared under moderate conditions (so called minimally modified LDL), such as by ferrous ion-catalyzed oxidation at 4°C for 96 hours [142], can best mimic the modified LDL found in early fatty streaks. Alternately, OxLDL prepared with relatively abrasive conditions (e.g. 5 μ M CuSO₄ at 37°C for 3 hours [143] or 50 μ M CuSO₄ at 37°C for 24 hours [143]) likely better mimics OxLDL found in advanced plaque. Because resultant oxidation adducts differ depending on oxidation status [144], differently prepared OxLDL can exert different biological responses. Studies from our group used OxLDL prepared with 5 μ M CuSO₄ at 37°C for 3 hours and are intended to examine responses of VSMC exposed to highly oxidized LDL. In addition to levels of oxidation, tissue concentration of oxidatively modified LDL can vary widely, by ~10-fold [145,146]. Our studies [66,67,70] used higher doses of OxLDL to mimic the situation presumably present in advanced plaques.

Pharmacological suppression of 5-lipoxygenase and 12/15-LOX activities abolishes OxLDL-induced VSMC apoptosis [66]. Interestingly, 12-hydroxyeicosatetraenoic acid, a 12/15-LOX product, upregulates TNF- α expression and inhibits Akt phosphorylation in cultured adipocytes [68], and LOX inhibitors prevent apoptosis-inducing factor-mediated cell death [69]. IGF-1 is a potent survival factor for VSMC, and increased IGF-1 signaling prevents OxLDL-induced VSMC apoptosis via a PI3K/Akt dependent pathway [70]. In addition to its anti-apoptotic effects, IGF-1 also suppresses autophagic cell death of plaque-derived VSMC via Akt-dependent inhibition of MAPLC-3 expression [71]. In summary, our findings indicate that OxLDL induced VSMC death involves LOX and NADPH oxidase and is blocked by IGF-1.

IGF-1 and endothelial dysfunction

Nitric oxide availability, oxidative stress and IGF-1

Endothelial dysfunction is implicated in the pathogenesis of cardiovascular diseases including atherosclerosis [72] and is closely associated with oxidative stress. Nitric oxide (NO) plays a critical role because of its potent vasodilator effect and sensitivity to the redox status of the endothelium. NO reacts with ROS, particularly superoxides, forming peroxynitrite, which is highly reactive and thus potentially highly deleterious. The ability of peroxynitrite to nitrate protein tyrosine residues can alter multiple cellular pathways that are dependent on tyrosine phosphorylation, such as protein kinase C, Akt, the MAP kinases, Nuclear Factor Kappa B, as well as signaling in response to IGF-1/insulin and the sympatho-adrenergic system. Thus, increasing ROS production causes not only perturbations in vasodilation by decomposing NO, but also potentially inhibits multiple signaling pathways that are critical for maintaining normal endothelial function. Oxidative stress also lowers NO bioavailability by blocking its production. Endothelial NO synthase (eNOS) is the main enzymatic source that produces NO constitutively as well as in response to vasodilators such as acetylcholine. eNOS catalyzes L-Arginine oxidation to produce L-Citrulline and NO by transferring electrons provided from NADPH and tetrahydrobiopterin (BH4). BH4, an essential cofactor of eNOS activity, is susceptible to oxidative modification by ROS such as peroxynitrite, which results in conversion of BH4 to biologically inactive BH2. An additional mechanism for BH4 depletion caused by oxidative stress was described by Zhen et al. who found that an increase in ROS leads to downregulation of guanosine 5'-triphosphate cyclohydrolase I, which is a rate limiting enzyme catalysing the initial step of BH4 biosynthesis [73] (Figure 2). Insufficient availability of BH4 causes "uncoupling" of eNOS activity, resulting in superoxide and hydrogen peroxide formation instead of NO. Thus there is likely a feed-forward system for lowering NO bioavailability, once enhanced oxidative stress occurs.

Limited information is available on IGF-1 regulation of vascular tone. Increased cardiovascular morbidity and mortality has been reported with GH deficiency [74]; in fact, it has been shown that flow-mediated arterial dilation, thus endothelial NO-dependent vasodilation, is impaired in GH deficiency [75]. Because GH is a primary regulator of circulating IGF-1, and GH deficiency leads to low IGF-1, it is reasonable to speculate that IGF-1 plays a major role in vasodilatory responses by regulating NO production in the endothelium. This hypothesis is supported by a recent publication showing that low plasma IGF-1 levels are associated with impaired endothelium-dependent vasodilation [76]. In diabetic patients, vascular tone regulation is impaired and IGF-1 supplementation improves vascular responses to vasodilators, potentially by enhancing eNOS activity [77,78]. These observations in humans are supported by animal and cell culture studies. In mice fed a high-fat diet (an animal model of type II diabetes), for example, IGF-1 resistance exists at the level of endothelium, which in turn blunts eNOS dependent vasorelaxation [79]. This finding is consistent with IGF-1's role in endothelium function. However, this model was characterized by increased serum IGF-1 levels [79], whereas patients with poorly controlled diabetes generally have low serum IGF-1 levels [80]. In endothelial cell culture systems, IGF-1 acutely enhances eNOS dependent NO production by increasing phosphorylation at Ser1177 via a PI3K and Akt pathway [81]. In addition to acute effects of IGF-1 on eNOS activity, a link has been suggested between BH4 biosynthesis and IGF-1. One of the key enzymes involved in BH4 biosynthesis is 6-pyruvoyltetrahydropterin synthase; its deficiency in mice causes dwarfism with markedly reduced serum IGF-1 levels [82], suggesting that a functional BH4 biosynthetic pathway is essential for maintenance of IGF-1 levels and normal growth. Vice versa, in pheochromocytoma-12 (PC12) cells, IGF-1 elevates BH4 levels potentially by enhancing its biosynthesis through a PI3K dependent mechanism [83]. It would be interesting to determine

if the potential IGF-1 effect on BH4 biosynthesis can be generalized to other tissues with NO synthase activity.

In *Apoe*-deficient mice fed a high-fat diet, we observed enhanced eNOS gene expression in aorta [5]. Csizsar *et al* reported that in Ames dwarf mice, low GH and IGF-1 serum concentrations were associated with high oxidative stress in the vasculature, potentially leading to endothelial dysfunction [84]. They observed reduced eNOS gene and protein expression in aortae of Ames dwarf mice, in which acetylcholine-induced vasorelaxation was impaired. Their observation is consistent with a stimulatory role of GH/IGF-1 on eNOS expression and function. It is noteworthy that both GH and IGF-1 enhanced expression of antioxidant enzymes such as Mn-SOD, Cu/Zn-SOD, glutathione peroxidase-1 in explants of mouse aorta and human coronary arterial endothelial cells [84]. Although it is unclear if these are direct effects of GH and IGF-1, these data suggest that GH and/or IGF-1 may have antioxidant effects that are eNOS independent. However, definitive conclusions on mechanisms whereby IGF-1 regulates endothelial function will require further studies.

Redox-mediated endothelin-1 effect and IGF-1

Endothelin-1 is a powerful vasoconstrictor, and a link has been suggested between IGF-1 and endothelin-1 effects. For instance, IGF-1 attenuates endothelin-1-induced contractile responses in porcine aorta, potentially by altering endothelin-1/endothelin receptor type A (ETA) signaling activity in smooth muscle cells [85]. IGF-1 may directly or indirectly regulate endothelin-1 gene expression; for example, in liver specific IGF-1 deficient mice, endothelin-1 gene expression was upregulated in the aorta, and this was associated with elevated systolic blood pressure and impaired vasorelaxation [86]. IGF-1 enhancement of NO bioavailability could explain the ability of IGF-1 to antagonize endothelin-1 contractile responses; in fact, endothelin-1 increases vascular superoxide by enhancing NADPH-oxidase activity and thus lowers NO bioavailability [87–89], suggesting that endothelin-1-induced endothelial dysfunction is at least in part mediated by increased oxidative stress. Interestingly, insulin and IGF-1 upregulate ETA levels in VSMC [90,91], indicating a complex interaction between insulin/IGF-1 and endothelin effects. Further studies are required to understand the interplay between IGF-1 and endothelin-1 with respect to vascular tone regulation.

IGF-1 and endothelial repair

Endothelial progenitor cells (EPCs) derived from bone marrow and/or the vascular wall contribute to neovascularization in response to ischemia [92], and their levels are related to cardiovascular disease outcome [93]. EPCs promote endothelial repair, thereby preventing endothelial dysfunction [94,95]. Interestingly, factors which positively or negatively alter endothelial function affect EPC function. eNOS, which is critical for maintenance of endothelial function, is also involved in mobilization of EPCs from bone marrow [96]; vice versa, eNOS uncoupling impairs EPC mobilization and function [97]. Intrinsic high expression of anti-oxidant enzymes has been shown in EPC [98,99], suggesting that they are resistant to oxidative stress. However, there are conflicting reports on the effects of ROS on EPC function in the setting of neovascularization induced by hind limb ischemia [100–102]. Perhaps appropriate levels of ROS, i.e. “fine tuning” of the level of oxidative stress, is essential for EPC integrity. On the other hand, it has been shown in human subjects that an increase in circulating IGF-1 in response to GH or IGF-1 administration raised circulating EPC numbers, and this was associated with increased NO bioavailability [103,104]. Similar findings in an animal model of atherosclerosis [5,105–107] imply that IGF-1 influences EPC mobilization and function by altering NO bioavailability as it does in mature endothelial cells. However, there are many remaining questions about potential IGF-1 effects on EPC, and these are complicated by the continuing debate about the definition of EPC [108]. For instance, potential

IGF-1 regulation of specific EPC niches in bone marrow and vascular wall [109] and of EPC mobilization and homing remains to be determined.

Clinical Studies

Available literature on the association of IGF-1 with cardiovascular disease as an independent cardiovascular risk factor remains inconclusive. Some cross-sectional and prospective studies [105–107,110–113] suggest a positive association between IGF-1 (and in some cases IGFBP-3) and atherosclerosis. Others [45,114–122] found that low IGF-1 is a predictor of ischemic heart disease and ischemic heart disease mortality consistent with the potential anti-apoptotic, anti-oxidant and plaque stabilization actions of IGF-1. Several large prospective cohort studies failed to systematically confirm these findings [123–128]. Multiple methodological constraints can explain this variance. So far, most *in vivo* studies have used total extractable IGF-1 as an estimate for IGF-1 activity *in vivo*. However, this provides only a crude estimate of the active hormone, as less than 1% is in its free form. Indeed, only free IGF-1 is believed to be active as it can readily cross the endothelium and interact with its own receptor. Of note, IGFBPs, in addition to their potential IGF-1 independent actions, may modulate IGF-1 bioactivity without any changes in the extractable concentrations of total IGF-1. Furthermore the activity of IGFBPs is modulated by several IGFBP-proteases, complicating the analysis of IGF-1 bioactivity.

Brugts *et al* recently introduced a new method to assess IGF-1 bioactivity [129]. Instead of measuring immunoreactive IGF-1, this kinase-receptor assay measures IGF-1 bioactivity of serum by its ability to activate IGF-1 receptor auto-phosphorylation. Thus this assay accounts for IGFBPs and IGFBP-protease modulation of ligand and receptor interactions. Using this technique, IGF-1 bioactivity was evaluated in relation to survival in an elderly male population in the Netherlands. Individuals in the highest IGF-bioactivity quartile survived significantly longer than those in the lowest quartile, both in the total population and in subgroups with a high inflammatory risk profile or a medical history of cardiovascular disease. Taken together with results from two other studies [106,130] that demonstrated an association between low free IGF-1 and risk of carotid and coronary artery disease, these findings suggest that high bioactive IGF-1 is associated with lower atherosclerosis risk and cardiovascular mortality. Further support for this concept comes from studies investigating a polymorphism of the IGF-1 gene promoter, namely a variable length of a cytosine-adenosine repeat sequence, shown to influence circulating IGF-1 levels. In the population-based Rotterdam study [131], individuals without the wild-type 192bp allele had 18% significantly lower circulating IGF-1 and were at increased risk for type II diabetes, myocardial infarction and presence of left ventricular hypertrophy [132]. In the presence of hypertension, these individuals also had higher carotid intima-media thickness and higher aortic pulse wave velocity [133]. Additional analyses from this study demonstrated that subjects heterozygous for the 192bp or 194bp alleles or non-carriers of these 2 alleles had lower IGF-1 and higher myocardial infarction-related mortality, particularly in the case of co-existing diabetes [134,135]. However, others have not replicated these studies [136] and in a United Kingdom study, the opposite association was found between the 192bp allele and IGF-1 levels [137]. It is also relevant to point out that loss-of-function mutations in genes encoding components of the insulin/IGF-1 pathway are associated with extension of life in many species. Recently, mutations in the IGF-1R gene that result in reduced IGF-1 signaling have been identified in centenarians [138]. Additional studies are required to determine whether genetically determined low IGF-1 levels or low bioactive IGF-1 is an important risk factor for atherosclerotic burden and a negative determinant of survival.

Oxidative stress plays a critical role in atherosclerosis initiation and progression. While the role of growth factors in lesion development has traditionally been thought to be permissive, recent findings indicate that circulating IGF-1 has pleiotropic anti-inflammatory, anti-apoptotic

and anti-oxidant effects that reduce atherosclerotic burden in an animal model. These effects appear distinct from the ability of locally synthesized IGF-1 to increase neointimal responses following mechanical arterial injury. Further studies will be required to determine specific molecular pathways mediating the anti-atherosclerotic effects of IGF-1.

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Figure 1. Anti-atherosclerotic effects of IGF-1

(a) Mechanisms of atherogenesis. As a result of endothelial dysfunction and/or damage, circulating monocytes and T cells are recruited to the subintimal space where LDL is oxidized (OxLDL). OxLDL induces pro-oxidant and pro-inflammatory effects, including activation of monocytes, secretion of multiple chemokines and cytokines such as IL-6 and TNF- α , from various cells including macrophages and induction of smooth muscle migration and proliferation. Both macrophages and smooth muscle cells become foam cells after internalizing and accumulating modified LDLs. (b) IGF-1 decreases atherosclerotic lesion size in aortae of *Apoe*-deficient mice. An increase in circulating IGF-1 reduces Oil Red-positive foam cells, decreases plaque macrophage infiltration and downregulates TNF- α and IL-6 levels. IGF-1 also decreases expression of lipoprotein lipase (LPL) in macrophages (LPL facilitates uptake of modified LDL) and increases expression of endothelial nitric oxide synthase (eNOS) and phosphorylated Akt. IGF-1 upregulates the number of endothelial progenitor cells (EPCs) in the circulation, potentially facilitating endothelial repair.

Figure 2. Potential mechanisms whereby IGF-1 reduces oxidative stress and atherosclerosis
Oxidative stress causes enhanced inflammatory responses, foam cell formation, smooth muscle cell apoptosis, and endothelial dysfunction, which trigger and/or promote atherosclerosis in the vasculature. IGF-1 potentially counteracts these detrimental effects, serving as an anti-atherogenic factor. (a) In endothelial cells, IGF-1 enhances nitric oxide (NO) production by endothelial NO synthase (eNOS) via two potential mechanisms; phosphorylation and activation of eNOS and upregulation of tetrahydrobiopterin (BH4) production. IGF-1 also upregulates anti-oxidant enzymes, such as glutathione peroxidase (GPX) and superoxide dismutase (SOD). (b) In smooth muscle cells, IGF-1 prevents oxidative stress-induced apoptotic cell death by activating a cell-survival pathway involving BAD phosphorylation via a phosphoinositide 3-kinase (PI3-K)/Akt dependent pathway. (c) In atherosclerotic plaques, IGF-1 reduces pro-inflammatory cytokine production (TNF- α and IL-6) presumably from infiltrated macrophages. IGF-1 also reduces expression of lipoprotein lipase (LPL) in macrophages. LPL facilitates binding and uptake of modified LDL in macrophages.

Table 1

Animal studies of IGF-1 effect on mechanical injury- or proatherogenic diet-induced lesion formation

Mechanism to regulate IGF-1 levels	Effect on IGF-1 or on marker protein levels	Animal model	Effect on lesion size	Reference
Infusion with protease-resistant IGFBP4	Decrease in IGFBP5 levels, IGF-1 marker protein	Hypercholesterolemic pigs with balloon injured carotid and femoral arteries	Reduction in neointimal area	[30]
Injections with cyclolignan, IGF1R inhibitor	Decrease in IGF1R phosphorylation	Rats with balloon injured carotid arteries	Reduction in neointimal area	[27]
Genetically modified mice with severe systemic IGF-1 deficiency	Decrease in tissue and serum IGF-1 levels	Mice with cuff-induced carotid artery injury	Reduction in neointimal area	[26]
Perivascular microsphere implantation to release IGF-1	Increase in PCNA levels, cell proliferation marker	Rabbits with balloon injured common femoral arteries	Reduction in neointimal area	[139]
Injections with glargine, long acting insulin analog	Increase in serum IGF-1 levels	Zukker fatty rats with balloon injured carotid arteries	Reduction in neointimal area	[140]
Targeted overexpression of IGF-1 in VSMC	Increase in VSMC-specific IGF-1 levels	Mice with resin probe- injured carotid arteries	Increase in neointimal area	[25]
Targeted disruption of Pappa, IGFBP4 protease	Decrease in IGFBP5 mRNA levels, IGF-1 marker protein	Mice with ligation- injured carotid arteries	Reduction in neointimal area	[28]
Infusion with human recombinant IGF-1	Increase in circulating IGF-1 and tissue pAkt levels	Hyperlipidemic <i>Apoe</i> -deficient mice	Reduction in aortic lesion size	[5]
Deficiency in LAR protein, modulator of IGF-1-induced signaling	Decrease in serum IGF-1 levels and increase in tissue IGF1R phosphorylation	Mice with injury- induced neointimal hyperplasia in femoral arteries	Increase in neointimal area	[31]
Targeted disruption of Pappa, IGFBP4 protease	Decrease in aortic IGFBP5 mRNA levels, IGF-1 marker protein	Hyperlipidemic <i>Apoe</i> -deficient mice	Decrease in aortic lesion size	[29]