IGF-1 and atherothrombosis: relevance to pathophysiology and therapy

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ABSTRACT

IGF-1 (insulin-like growth factor-1) plays a unique role in the cell protection of multiple systems, where its fine-tuned signal transduction helps to preserve tissues from hypoxia, ischaemia and oxidative stress, thus mediating functional homeostatic adjustments. In contrast, its deprivation results in apoptosis and dysfunction. Many prospective epidemiological surveys have associated low IGF-1 levels with late mortality, MI (myocardial infarction), HF (heart failure) and diabetes. Interventional studies suggest that IGF-1 has anti-atherogenic actions, owing to its multifaceted impact on cardiovascular risk factors and diseases. The metabolic ability of IGF-1 in coupling vasodilation with improved function plays a key role in these actions. The endothelial-protective, anti-platelet and anti-thrombotic activities of IGF-1 exert critical effects in preventing both vascular damage and mechanisms that lead to unstable coronary plaques and syndromes. The pro-survival and anti-inflammatory short-term properties of IGF-1 appear to reduce infarct size and improve LV (left ventricular) remodelling after MI. An immune-modulatory ability, which is able to suppress ‘friendly fire’ and autoreactivity, is a proposed important additional mechanism explaining the anti-thrombotic and anti-remodelling activities of IGF-1. The concern of cancer risk raised by long-term therapy with IGF-1, however, deserves further study. In the present review, we discuss the large body of published evidence and review data on rhIGF-1 (recombinant human IGF-1) administration in cardiovascular disease and diabetes, with a focus on dosage and safety issues. Perhaps the time has come for the regenerative properties of IGF-1 to be assessed as a new pharmacological tool in cardiovascular medicine.

INTRODUCTION

Two peptide hormones, namely IGF (insulin-like growth factor)-1 and IGF-2, two types of cell-surface receptors and six IGFBPs (IGF-binding proteins) form the IGF-1 axis, which helps to keep a highly steady and conserved individual-specific ‘reservoir’ of serum IGF-1 concentrations, from which IGF-1 tissue delivery is...
fine-tuned. From serum to interstitial fluid, IGF-1 contributes to regulate cellular growth, proliferation, differentiation and survival against apoptosis, and hence tissue remodelling and homeostasis, endocrine function, energy metabolism, brain activity and brain—environment networking, which ultimately influence an organism’s health.

Among these effects, the metabolic function of IGF-1 appears more central in cardiovascular health and disease and related illnesses. This is achieved by enabling glycolipid homeostasis, vascular health, rescue from ischaemia/reperfusion damage and by fuelling organ function with a favourable pro-inflammatory/anti-inflammatory balance. The peptide thus performs a role as an antidote to multiple organ failure [1,2], and regulates the synthesis of many growth factors [3–5], as in a hub model, through a central growth factor stimulus, represented by HIF (hypoxia-inducible factor) synthesis [6]. Conversely, evidence for the stimulation of IGF-1 by other growth factors is lacking.

Owing to its crucial activities, evidence has accrued showing a possible causative role of decreased IGF-1 levels in several cardiovascular pathologies, including atherothrombosis, MI (myocardial infarction), HF (heart failure) and diabetes, as well as hypertension and renal dysfunction. These findings are described in the present review according to areas of interest.

THE IGF-1 NETWORK, BIOACTIVITY AND DETERMINANTS

IGF-1 and IGF-2 are 70- and 67-amino-acid single-chain polypeptides, so named as their A and B domains share 50% homology with the B and A chains of insulin. Despite their structural similarity, each ligand results in unique signalling outcomes. In particular, IGF-1 has been shown to share functional homology with insulin, participating in glucose homeostasis, but also to reduce serum insulin concentrations with the same levels of glucose metabolism efficiency [7].

IGF-1 and IGF-2 are encoded on the long arm of chromosome 12 (12q22-q24.1) and the short arm of chromosome 11 (11p15.5) respectively [8]. IGF-1 is expressed, under GH (growth hormone) control, by the liver in an ‘endocrine form’ or ubiquitously in a relevant ‘paracrine/autocrine’ fashion [9]. Under resting conditions, the two forms of IGF-1 appear to be balanced, with no hepatosplanchnic difference in basal secretion, and similar IGF-1 concentrations found in the femoral and hepatic vein [10]. Besides the liver, important contributions to serum IGF-1 levels come from bone, vascular endothelium and exercising skeletal muscle (60 μg/min after 30 min of exercise) [11] to give a total daily secretion of approx. 3–10 mg/day [12]. The highly preserved serum IGF-1 buffer is accounted for by minimal renal daily clearance (10 000-fold lower than the estimated daily production rate of 0.063 μg·m⁻²·day⁻¹), demonstrating free IGF-1 tissue delivery as the main IGF-1 clearance process. Although largely modifiable by the environment, IGF-1 concentrations are also affected by genetic factors (approx. 38–63%), as described by adult twin studies [13].

Daily IGF-1 serum preservation/tissue delivery is supervised by six different specific high-affinity binding proteins (IGFBP-1—IGFBP-6), synthesized mainly by the liver, which can bind IGF-1 in biological fluids, regulating its movement between intravascular and extravascular compartments, increasing its half-life, and managing IGF delivery to tissues by fine-tuning its concentrations in the interstitial fluid and its affinity for receptors.

All IGFBPs share a common domain organization and a high degree of similarity. The highest conservation is in the N- and C-terminal IGF-binding regions. The variable L-domain contains cleavage sites for proteases, acting as a hinge, and promoting tertiary structure and high-affinity binding to IGFs [14].

The C-terminal region in IGFBP-3, IGFBP-5 and IGFBP-6 contains a heparin-binding sequence. This region reduces the affinity of IGFBPs for IGF-1, increases free IGF-1 [15] and associates with proteoglycans, enhancing localization to the cell surface and ECM (extracellular matrix). This region is also able to reduce IGF-1 bioavailability by inhibiting protease-degrading IGFBP-4 [16] and by binding ALS (acid-labile subunit) and endothelial cells. In IGFBP-1 and IGFBP-2, the central domain features an RGD sequence, which interacts with integrins, regulating cell—cell and cell—ECM interactions, and providing a reservoir of IGFs [14].

The IGF-1-dependent and -independent actions of IGFBPs have also been studied in transgenic mouse models [17]. IGFBP-1 overexpression causes brain and growth retardation, hyperglycaemia, impaired fertility, proteinuria and glomerular lesions. IGFBP-2 transgenic mice had reduced growth and body weight. IGFBP-3 overexpression was associated with organomegaly, suggesting an IGF-independent role. Tissue-specific overexpression of IGFBP-4 resulted in hypoplasia and reduced weight of smooth-muscle-rich tissues, such as the bladder aorta, and stomach [17].

By binding IGF-1 with greater affinities than type 1 IGF-1Rs (IGF-1 receptors), IGFBPs may sequester IGF-1 away from the IGF-1R and inhibit its actions. On the other hand, modification by IGFBPs or interaction with the cell or matrix components may concentrate IGF-1 near the IGF-1R, thus enhancing IGF-1 bioactivity [18]. Approx. 80% of total IGF-1 is bound in serum by IGFBP-3, complexed with ALS into a ternary complex, enhancing localization to the cell surface and ECM interactions, and providing a reservoir of IGFs. Although largely modifiable by the environment, IGF-1 concentrations are also affected by genetic factors (approx. 38–63%), as described by adult twin studies [13].

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with IGF-1. IGFBP-1 has confirmed inhibitory actions, despite acting as a partial agonist, thus potentiating IGF-1 actions, when dephosphorylation or binding with proteoglycans occurs, which decrease its affinity for IGF-1 and thus enhance IGF-1 delivery to target tissues [13]. IGFBP-1 and IGFBP-2, whose hepatic synthesis is reduced by insulin, bind IGF-1 and IGF-2 respectively with high affinity. IGFBP-4, controlled by parathyroid hormone, binds both IGF-1 and IGF-2 [19] and regulates bone turnover. It is the most abundant IGFBP expressed in arterial walls and the dominant IGFBP secreted by rat VSMCs (vascular smooth muscle cells) in vitro [15]. IGFBP-5 and IGFBP-6 bind mainly IGF-2. IGFBP-5, regulated by glucocorticoids, is also involved in bone physiology [17].

Post-translational modification of IGFBPs, such as proteolytic cleavage, phosphorylation or binding to the cell surface or ECM, are involved in the regulation of IGF release: by reducing their affinity for IGFs, they make IGFs more (via cleavage or binding to matrix) or less (via phosphorylation) available to cell receptors [20]. One of the well-known factors involved in IGFBP proteolysis is PAPP-A (pregnancy-associated plasma protein-A), which is specific for IGFBP-4 cleavage [13].

Bound IGF-1 circulates in peripheral blood in nanomolar concentrations, with median values in a middle-aged man of 150–200 ng/ml, approx. 1000 times higher than those of insulin, which is at picomolar levels. IGF-1 bioactivity is related to its free fraction, which represents less than 1% of the circulating total amount (0.2–0.6 ng/ml), but is 20-fold higher in the interstitial fluid (3.6–10 ng/ml) in close proximity to target tissues.

IGF-1 has a half-life of 15 min in the free form, accounting for its immediate availability [21]. IGFBP binding prevents IGF-1 proteolysis, thus extending its half-life up to several hours. Conversely, IGFBP cleavage by proteases enables IGF-1 to cross the vessel barrier and access the extravascular space and tissues. IGFBP disposal to specific sites, after tissue secretion, in contrast functions as a local reservoir for tissue requirements. The ternary complex of IGFBP-3, ALs and IGF-1 has a half-life of 16 h and mediates the long-term availability, stabilizing and buffering of IGF-1 in the intravascular space [13]. The binary complex formed by IGF-1 with IGFBP-2, IGFBP-4, IGFBP-5 or IGFBP-6 has a half-life of 90 min, thus regulating medium-term availability [13]. IGFBP-1 is responsible for the short-term availability of IGF-1 [22], as it is rapidly produced by the liver and it is equally as quickly reduced, under the control of insulin, thereby resulting in a half-life of free IGF-1 approaching approx. 30 min.

For clinical purposes, the most used and reproducible measure is that of total IGF-1, for which different assays have been tested and compared [23]. Although presenting technical problems [24,25], assays of free or easily dissociable IGF-1 are also useful, as these reflect IGF-1 bioactivity better than circulating total IGF-1 levels in secondary GH disorders. A surrogate measure of free IGF-1 may be derived from the molar ratio of total IGF-1 and IGFBP-3 [24]. Acute stress conditions, ILs (interleukins), acidosis [26,27], HD (haemodialysis) and also the Kd of the erythrocyte IGF-1R are known to profoundly affect [28,29] free IGF-1 levels. These are also linked to the clearance rate of IGF-1, mainly explained by free IGF-1 tissue uptake [13], which varies with different pathophysiological conditions [27]. Therefore combined measurement of total and free IGF-1 should be chosen to reflect the different pieces of information.

IGF-2 promotes cell growth, survival, migration and differentiation via the IGF-1R. Compared with IGF-1, it plays a key role in fetal and placental growth [30], resulting in the majority of IGFBP-4 proteolysis in this setting [31]. It is also involved in adult differentiation processes in muscle, brain and brown adipose tissue [32], and in angiogenesis [33]. Its median serum levels in humans, which are normally distributed, are approx. 500 ng/ml, and are similar by gender, increasing from birth to puberty and then remaining unchanged through the rest of life [34,35].

Monoallelic expression regulates tissue IGF-2 precisely and its overexpression is associated with tumour development. Mature IGF-2 and two ‘big’ variants (10–20% of total IGF-2 in healthy humans, and up to 60% in tumour-induced hypoglycaemia) are synthesized by a pro-peptide. Big IGF-2 potentiates its action via a ‘vicious’ circle by inhibiting insulin and GH, which in turn decreases IGF-1, IGFBP-3 and ALS, enhancing the effects of circulating IGF-2 [36].

IGFs can bind two different cell-surface receptors: IGF-1Rs and IGF-2Rs (IGF-2 receptors). IGF-1R shares 40–84% sequence homology with the IR (insulin receptor), is a hetero-tetramer consisting of two extracellular α-subunits, containing the binding pocket for IGFs, and two transmembrane β-subunits that exert tyrosine kinase activity. IGF-1R is ubiquitously expressed, has a high affinity both for IGF-1 and IGF-2, and a lower affinity for insulin [9].

IGF-2R is a monomeric protein, identical with the mannose 6-phosphate receptor, that binds IGF-2 with a higher affinity than IGF-1, but not insulin. It is considered a ‘scavenger’ receptor as it has no role in IGF signal transduction and primarily acts to sequester IGF-2 from potential receptor-activating interactions, internalizing and degrading it [37].

Approx. 90–95% of the receptor is located intracellularly, with 5–10% on the cell surface. Intracellular IGF-2R facilitates the trafficking of lysosomal enzymes between the trans-Golgi network, endosomes and lysosomes. By operating as an IGF-2 antagonist, the IGF-2R has tumour-suppressor-like properties, a suggestion consistent with reports of loss of heterozygosity at the IGF-2R locus in a variety of human malignancies [37].
After cleavage from the cell surface, a soluble form of IGF-2R arises, acting as a carrier for IGF-2 in serum, and regulating its bioavailability and activity [32].

Hybrid IGF-1Rs/IRs made of one IGF-1R heterodimer and one IR heterodimer have also been found. They are believed to provide additional binding sites for IGF-1, increasing cell sensitivity to the polypeptide [38]. Indeed, hybrid receptors are reported to behave as IGF-1Rs, rather than IRs, with respect to ligand-binding affinity, receptor autophosphorylation, hormone internalization and biological action [38,39].

The signal transduction pathways operating when IGFs bind to the extracellular α-subunit of its receptors lead to the activation of the tyrosine kinases of the β-subunit (Figure 1). Trans-phosphorylation involving tyrosine residues leads to the full activation of kinase activity. This serves to recruit multiple substrates, including IRS (IR substrate) family proteins (IRS-1–IRS-4), which activate the PI3K (phosphoinositide 3-kinase) signalling pathway, whose main actions are to synthesize NO, to counteract pro-apoptotic molecules such as Bad or caspase 3, and to prevent mitochondrial cytochrome c release, thereby inhibiting apoptosis [40]. By activating IRS-2 and Akt2, IGF-1 promotes GLUT4 (glucose transporter 4) translocation to the plasma membrane and activates glycogen synthase.

After receptor interaction, activated Shc (Src homology and collagen homology) leads to the sequential activation of Ras, Raf and MAPK (mitogen-activated protein kinase), which increases cellular proliferation, differentiation and motility through cell-cycle progression, and modulation of cyclins and related proteins, thus inhibiting apoptosis [41]. All of these actions are related to the suggested carcinogenic activity of IGF-1. However, via Raf phosphorylation [42], which abrogates Raf activity on downstream substrates [43], such as MAPK, a dissociation between PI3K-like and MAPK-like activities of IGF-1 is possible, which can be enhanced by metformin [44] or adenosine [42]. When considering the potential therapeutic impact of IGF-1, such cross-talk between signalling pathways needs to be investigated more thoroughly, as many of the positive actions of IGF-1 are mediated by PI3K, in contrast with many of the unfavourable effects being mediated by MAPK (Figure 1).

Additional useful actions of IGF-1 include cell differentiation with phenotype induction, such as myelin production by neuronal cells [45], or hormone synthesis by endocrine tissues [46], inhibition of protein synthesis and proteolysis, calcium accretion, and fatty acid and glucose transport [13]. In relation to the abundance of metabolic, anti-apoptotic [47], NO-mediated [48], vasodilator and anti-inflammatory [49] properties of IGF-1, it appears to be able to counteract endothelial dysfunction, atherosclerosis plaque development and ischaemic myocardial damage [50,51].

**IGF-1 FROM BENCH TO BEDSIDE AND BEYOND**

The development of an IGF-1 transgenic model has been hindered by the fact that total IGF-1-knockout mice are not viable and die perinatally due to respiratory
failure [52]. Some lessons, however, have arisen from animals with selective liver-inactivated IGF-1 (LID mice) or muscle-inactivated IGF-1, which exhibit different grades of insulin resistance. Conversely, overexpression of liver IGF-1 in IGF-1-null mice, having a feature of abolished autocrine/paracrine IGF-1, restored glucose metabolism and body growth, but not reproductive function, by increasing serum IGF-1 levels [53]. General problems in the translation of knockout animals models to human physiology also apply to this area, due to the compensatory mechanisms established, such as increased paracrine and autocrine IGF-1 expression, higher IGF-1R phosphorylation and increased GH levels [52]. Conversely, the initial use of IGF-1R inhibitors in human cancer trials resulted in the observation of hyperglycaemia as the most common and dose-limiting side effect, which required anti-diabetic therapy; in addition, proteinuria and venous thrombosis (10%) were also reported [54, 55]. Continuing follow-up will provide information as to whether the association with hypertension, arterial or venous thrombotic events, and atherothrombosis in the long term will become a serious drawback for this drug class, as has been reported for bevacizumab (see below).

Different disease states are associated with changes in IGF-1 levels. In acute intensive care illnesses, such as sepsis or multiple injury, IGF-1 is generally low and it is lower in non-survivors [56]. Rheumatic disease and osteoporosis [13], as well as obstructive sleep apnoea syndrome, are also characterized by reduced IGF-1 levels [13] due to impaired GH sensitivity [57].

Thyroid hormone and insulin increase IGF-1 levels [58], whereas glucocorticoids induce IGF-1 resistance through a TNF-α (tumour necrosis factor-α)-modulated mechanism [59]. Oral, but not transdermal, oestrogen therapy suppresses serum IGF-1 levels [60]. In addition, acidosis has been shown to reduce IGF-1 levels [27]. On the other side, low IGF-1 levels predicted low haemoglobin levels [61], a high total cholesterol/HDL (high-density lipoprotein)-cholesterol ratio as well as the number of carotid plaques [62].

Furthermore, many studies have investigated IGF-1 levels and determinants in adult physiology. IGF-1 has been shown to be inversely related to age, and independent of gender and GH levels, if within normal ranges [13].

Different environmental determinants of IGF-1 concentrations have been identified. A positive correlation between serum IGF-1, physical fitness and mean intake of several nutrients, such as protein, zinc, red meat, fish and seafood [63], or dairy protein and calcium [64], has been shown. Dietary manipulation with fat deprivation was shown to increase IGF-1 levels [65], and IGF-1 has been shown to be inversely associated with meal glycaemic index and BMI (body mass index) [66]. Large surveys have found that gender, age, BMI, waist circumference, increasing numbers of metabolic syndrome components, smoking in white men and alcohol intake in black men [67] were inversely related to serum IGF-1 levels [68], although no association with diet and physical exercise was proven. Many cardiovascular risk factors, such as oxLDL [oxidized LDL (low-density lipoprotein)], obesity, waist/hip ratio, diabetes, smoking, sedentary lifestyle, psychological distress and reduced coronary flow reserve, have been associated with reduced IGF-1 levels [50, 69], and similar findings have also been reported in a recent re-analysis of the large Framingham database [70].

Therefore recent attention has focused on the relationship between the IGF-1 system and cardiovascular disease, in particular on the IGF-1-operated link between atherogenic dyslipidaemia and diabetes, which are markers of insulin resistance and form the basis of the so-called metabolic syndrome, and of atherothrombosis.

**FLOW—METABOLISM COUPLING AS A ROLE OF IGF-1: FROM THE METABOLIC SYNDROME TO DIABETES**

The recognition of a close relationship between insulin sensitivity and basal NO production in healthy individuals [71] has led to multiple experimental and human studies showing a tight interdependence between blood flow, vascular surface area and glucose delivery in skeletal muscle. A mixed meal or oral glucose load are able to expand capillary surface area and to increase muscle blood flow, thus substantially improving glucose and insulin delivery [72]. Variations in insulin-mediated capillary recruitment and glucose disposal appear to be strictly related [72], whereas NOS (NO synthase) inhibitors, by suppressing insulin-mediated capillary recruitment, cause a simultaneous 40% reduction in glucose disposal [73]. This suggests that NO is the mediator of the two concurrent effects. PI3K-dependent vascular actions of insulin appear to be crucial in substantially increasing blood flow and capillary recruitment, therefore coupling metabolic and haemodynamic homeostasis and promoting healthy glucose disposal.

Like insulin, IGF-1 co-operates in this metabolic and vascular role, with an established ‘rescue’ effect in insulin-resistance conditions [48] (Figure 2). Indeed serum IGF-1 concentrations and its vascular receptors are several-fold more abundant than insulin [74]. Moreover hybrid IGF-1Rs/IRs appear to be activated by physiological concentrations of IGF-1, but not those of insulin [38]. The hybrid receptor produces a greater PI3K activation upon IGF-1 stimulation compared with insulin at equivalent doses [75]. Therefore IGF-1 deals with this vascular/metabolic role in a much more significant way than insulin. Indeed, mouse models overexpressing a dominant-negative form of muscle IGF-1R, having
Figure 2  Proposed mechanisms of IGF-1 metabolic cardiovascular protective actions, and windows of opportunity for rhIGF-1, described through intermediates and final effects of IGF-1 action on cardiovascular risk factors/diseases and on renal function
CV, cardiovascular; ACS, acute coronary syndromes.

blunted IGF-1R and hybrid IGF-1R/IR activity, developed early severe insulin resistance/diabetes similar to that in muscle-specific GLUT4-deleted models [76], whereas models with deleted [77] or dominant-negative [78] muscle IR resulted in only a relatively mild phenotype with no major metabolic consequences.

In muscle interstitial fluid, the free IGF-1 concentration relates closely and directly to lactate concentration, being 20-fold higher than that in plasma. This large gradient from plasma to the interstitium clearly explains the relevant IGF-1 local actions [79], which are proportional to the metabolic requirements. An experimental model of acute systemic acidosis produced a gradual and significant reduction in plasma IGF-1 (by 30%), paralleled by a significant increase in tissue IGF-1 concentration in kidneys (63%) and liver (35%) [27]. Similarly, a human study of venous-arterial extraction of IGF-1 in ischaemic limbs of patients with peripheral artery disease shows a high ischaemic tissue extraction of IGF-1, which is inversely related to CRP (C-reactive protein) transfemoral production [80]. Therefore tissue IGF-1 extraction appears to be enhanced by ischaemia/acute acidosis: in this way, exercise recruits tissue IGF-1 by muscle extraction as the prototypical mechanism [81]. On the other hand, exercising human muscle has been shown to induce a 2-fold increase in IGF-1 [82], which also increases serum IGF-1 levels [13] and networks muscle activity with brain functional efficiency and vascularization [5]. Therefore improved peripheral (muscle) efficiency together with a well-matched energy supply (vasodilation) and expenditure (metabolism) results in insulin-sensitivity, with reduced post-challenge dyslipidaemia and inflammation, and the ultimate vascular correlate of preserved endothelial function. This IGF-1-connected network is also centrally organized by brain-focused insulin sensitivity promotion, which is achieved by the release of IGF-1 by exercising muscle taken up from blood into the hypothalamus [11].

On the other hand the metabolic syndrome is a cluster of risk factors related to cardiovascular risk, of which the common factor is insulin resistance [83]. These factors include hypertension, hyperglycaemia, hypertriglyceridaemia, low HDL-cholesterol and abdominal obesity, which are catalysed by overfeeding and inactivity. All of these are associated with reduced IGF-1 levels, suggesting that IGF-1 appears to be the ideal putative causal agent of this syndrome as it co-ordinately enhances insulin sensitivity, suppresses plasma NEFAs (non-esterified ‘free’ fatty acids), reduces fasting plasma triacylglycerols (triglycerides) and the total cholesterol/HDL-cholesterol ratio [62], increases glucose uptake [84] and metabolism [85], and exerts anti-platelet effects partly as a direct effect of NOS activation [86]. After a first seminal report on serum IGF-1 levels suggesting that it is an important human determinant of glucose homeostasis, with reduced levels in healthy subjects being predictive of later development of glucose intolerance and diabetes [22], a more recent...
IGF-1 and cardiovascular health

Figure 3  Known favourable cardiovascular effects of IGF-1, according to the target site and biological mechanisms involved

study [87] in a cohort of approx. 400 non-diabetic subjects with different degrees of glucose tolerance showed that IGF-1 levels are inversely and independently associated with insulin sensitivity and are significantly lower in subjects with WHO (World Health Organization)-defined metabolic syndrome compared with those subjects without the metabolic syndrome. Each unit increase in log-transformed IGF-1 concentration was associated with a 90.5 % reduction in the risk of WHO-defined metabolic syndrome [87]. In another cohort, low IGF-1 and testosterone, as well as high CRP, conferred a 13-fold increased risk of the metabolic syndrome [88]. Moreover, in non-diabetic subjects, low IGF-1 levels were inversely related to IL-6 [89] and CRP [90] levels. Reduced IGF-1 was also shown to be predictive of insulin resistance, arterial hypertension and increased waist circumference, as opposed to acute-phase proteins. Genetic polymorphisms of IGF-1R have also been associated with insulin resistance, as well as with arterial hypertension, independent of known acquired modifiers [91].

In addition, in overt human diabetes, IGF-1 was found to be a much more potent enhancer of glucose transport in skeletal muscle cells than insulin itself [92]. rhIGF-1 (recombinant human IGF-1) administration could indeed reduce the dose of insulin required by as much as 50 % and blood glucose levels by 23 %, while improving hyperinsulinaemia and hypertriglyceridaemia [92]. IGF-1 is also able to reduce serum lipids [93] and glucose and lipid-related oxidative stress, by normalizing ROS (reactive oxygen species) and glutathione cell concentrations [94]. Likewise, a recent study [95] has shown that the islet β-cell-sparing properties of GLP-1 (glucagon-like peptide-1), a precursor of incretins, which are a family of new promising anti-diabetic drugs, is mainly mediated by IGF-1 and reversed by IGF-1 antagonism. The triple action of IGF-1 on vascular recruitment, lipid metabolism and glucose disposal appears crucial in linking metabolism to vascular protection from clinical ischaemic events.

FROM RISK FACTORS TO ATHEROTHROMBOSIS THROUGH LOW IGF-1-MEDIATED ENDOTHELIAL BREACH

Since the 2000s, the relationship between the IGF-1 system and cardiovascular disease has been a topic of interest. Indeed, multiple endothelial-protective activities of IGF-1 are pivotal in linking reduced IGF-1 levels to glucose derangements and, subsequently, in associating these with thrombosis, dyslipidaemia, vascular damage and, ultimately, atherothrombotic events. Furthermore, plaque destabilization, due to VSMC apoptosis, and an immediate herald to clinical events appears to be mediated by low plaque tissue IGF-1 and IGF-1R expression [96] (Figure 3) (see below).

Despite its association with many other cardiovascular risk factors [50], low IGF-1 levels have been shown to be an important predictor of cardiovascular disease, even
after correcting for BMI, smoking, blood cholesterol, alcohol intake, physical activity, gender, age, past history of diabetes and a family history of IHD (ischaemic heart disease) [97]. Both traditional cardiovascular risk factors and low IGF-1 levels have been shown to reduce endothelial function, as well as the progenitor cell reservoir, the latter remaining as the main determinant of endothelial function [98] has also recently been shown to be directly associated with IGF-1 levels [99].

EPCs (endothelial progenitor cells) are known to play a role as suitable predictors of event-free survival [100] and cardiovascular outcomes [101], and as a correlate for cardiovascular disease activity (unstable compared with stable coronary syndromes) [98].

Two relevant risk factors for cardiovascular disease, namely age [13] and diabetes [102], are known to be associated with reduced IGF-1 levels [13,103] and are strictly associated with an impaired proliferative potential of endogenous CPCs (cardiac progenitor cells) and EPCs [104,105].

Conversely, an increase in IGF-1 levels has been shown in vivo, ex vivo and in vitro to reverse age-related EPC dysfunction and senescence [106]. It has also been shown to induce EPC differentiation, colony forming and migratory capacity, as well as telomerase activity, whereas this has not been shown with GH [106]. The most striking evidence in experimental models of atherosclerosis [107] shows that rhIGF-1 infusion causes plaque regression and has anti-inflammatory actions. Direct rhIGF-1 infusion in apoE (apolipoprotein E)-deficient mice, resulting in a doubling of serum IGF-1 concentrations, was shown in vivo to directly reduce atherosclerosis plaque progression, as well as lesion macrophage infiltration. At the same time, rhIGF-1 infusion reconstituted EPC numbers, resulting in a constellation of beneficial effects, including decreased vascular inflammation and macrophage accumulation within the atherosclerotic lesions, as well as suppression of oxidative stress. This shows, in a single model of atherosclerosis, that rhIGF-1 infusion exerts in vivo vascular anti-inflammatory, antioxidant and pro-repair abilities of IGF-1. Again, GH administration, at doses able to double serum IGF-1 levels, was shown to increase circulating EPC number and improve their colony forming and migratory capacity in an IGF-1-dependent manner, which was completely abrogated by IGF-1R blockade [106]. In addition, EPCs have been suggested to exert their endothelial-protective effects through direct IGF-1 and other growth factor release, acting on local sites as IGF-1 factories or ‘mini-pumps’, rather than being used for direct cell engraftment. Therefore the mainstay of ‘regenerative therapy’ is not the cells, but the growth factors released by them, which suggests the use of the term ‘regenerative pharmacology’ [108]. With age and an increase in risk factors, the ability of bone-marrow-derived EPCs to repair endothelial and vascular damage could be exhausted [109], leading to a disequilibrium between vascular injury and repair, thus ultimately contributing to the onset of atherothrombosis in animals [107] and to clinically unstable coronary syndromes in humans [98].

Direct EC (endothelial cell)-protective properties of IGF-1 have been described including: (i) anti-apoptosis [110] and reduced EC detachment from the endothelial monolayer and related anoikis [111], (ii) expression of eNOS (endothelial NOS) and NO production with endothelium-dependent vasodilatation [112], (iii) re-endothelialization through activated EPCs [113], (iv) oxygen free radical scavenging to stabilize atherosclerotic plaques [114–117], and (v) anti-platelet activity [112]. IGF-1 is the constitutive endothelial response to damage and repair mechanisms, increasing 2-fold after endothelial damage [118], thus preventing angiotensin-mediated damage and tissue factor-mediated thrombosis [119]. Another valuable IGF-1-protective activity is the induction of prostacyclin (PGI2) synthesis in endothelial cells through the activation of PLA2 (phospholipase A2). PGI2 has important anti-platelet effects and increases cAMP in VSMCs, thereby inducing growth and vasodilatation [120]. Moreover, IGF-1 has been shown to increase PT (prothrombin time) and PTT (partial thromboplastin time) [121], without influencing circulating mediators of fibrinolysis [122]. Indeed, recent evidence has highlighted the arterial and venous prothrombotic side effects of bevacizumab, an anti-cancer monoclonal antibody inhibitor of VEGF (vascular endothelial growth factor), which is a known downstream effector of IGF-1 [123,124]. All of these endothelial-protective actions are mediated by a well-represented abundance of specific endothelial IGF-1Rs, which are much more numerous than those of insulin [74] in micro- and macro-vascular ECs. They mediate the action of both serum IGF-1 coming from blood and of autocrine IGF-1 secreted by various cell types, including MSCs (mesenchymal stem cells). In this setting, IGF-1 confers the most relevant anti-apoptotic effect of MSCs, accounting for more than half of the anti-apoptotic and angiogenetic effect, without MSC transdifferentiation [125].

The vasodilator activity of IGF-1 is another significant vessel-targeted IGF-1 action, which is mediated by NO synthesis. It exerts its effect on VSMCs of epicardial coronary arteries and microvessels through different pathways: NO in the former and K+-channel agonism [126] in the latter. By comparing insulin-resistant experimental [127] and human [128] settings with normal ones, it has been observed that a significantly blunted insulin-induced aorta vasorelaxation appears to be replaced by a compensatory increase in serum IGF-1 and IGF-1-induced relaxation, independent of treatment for classical cardiovascular risk factors.

Moreover, IGF-1 levels have been described to be doubled by compensatory mechanisms in order to
normalize the reduction in myocardial capillary density described in insulin resistance, whereas eclampsia, a human disease whose prominent pathophysiology is as a local and systemic vascular disorder, is characterized by markedly reduced serum and placental IGF-1 levels [129].

VSMCs are able to produce IGF-1 and IGFBPs. The most frequent co-secretion is that of IGF-1 and IGFBP-5, the latter increasing the biological activity of IGF-1. Co-secretion with IGFBP-1, IGFBP-2 [130] and IGFBP-4 [131] conversely reduces IGF-1 levels and activity. The putative role of IGF-1 in VSMCs is to arrange vascular reparative responses to damage [132] or mechanical stretch [133] by promoting vascular recruitment in normal tissue growth and regeneration, as well as by down-regulating pro-inflammatory cytokines and fibrosis [134]. This process is preserved even in states of severe IGF-1 deficiency [135], and is active in normal and atherosclerotic vessels. In the latter, it is mediated by up-regulated IGF-1 activity [136] or IGF-1R expression [137] as compensatory responses to acute rather than chronic ischaemia, de novo more than restenotic lesions, and MI more than stable angina [138], independently of the type of mechanical or pharmacological reperfusion [139]. The degree of IGF-1-induced mitogenic action appears to be mainly regulated by the level of MAPK activation [140], which is weaker and transient compared with that achieved by other mitogenic factors [141]. Its clinical relevance to human processes of neointimal hyperplasia and coronary restenosis is equivocal [142] or unknown for definite due to methodological drawbacks (i.e. inaccurate correction for confounders such as glycaemia or insulin therapy doses [143]). Moreover, clinical studies using GH/IGF-1 inhibitors have been shown to be ineffective in preventing plaque progression [50].

Conversely, direct systemic IGF-1 administration reduces atherosclerosis progression [107] by activating positive PI3K, rather than negative MAPK, actions. In addition, adenosine produced during cardiac ischaemia [144] and drugs interacting with the adenosine receptor, such as metformin [145], are able to block mitogenic effects and MAPK activity downstream of IGF-1, rather promoting the PI3K pathway and its favourable anti-ischaemic and anti-atherogenic effects.

PAPP-A, as a modulator of IGFBP4 action [146] and enhancer of IGF-1 bioavailability, has been claimed to be pro-atherogenic, as it associated with larger aortic lesion areas in high-fat-fed transgenic mice [147]. The relevance of this finding was hampered by a higher body weight, or body weight gain, in the transgenic animals (up to 2-fold) [147,148] for which aortic lesion areas were not corrected. Moreover, aortic VSMC-restricted IGFBP-4 overexpression models, even without changes in total body weight, have selectively increased the weight of the aorta, to which atherosclerotic lesions have to be related [149]. Compensatory increased substitute pathways of IGF-1 activation are also turned on in mice lacking the PAPP-A gene, thus confounding the comparison of the transgenic and wild-type animals [150]. Finally, as stated by the authors [148], control apoE-knockout mice had less lesion development than observed in previous studies, due to a genetic background less prone to atherosclerosis. Therefore the hypothesis that IGF-1 plays a noxious or pro-atherogenic role does not appear to be categorically shown by the results of these studies.

Conversely, experimental studies are available on the non-harmful and even atheroprotective effect of IGF-1. A recent study with apoE-knockout mice overexpressing IGF-1 in smooth muscle cells [151] has shown that, in the transgenic animals compared with wild-type, a comparable plaque burden on a Western diet was observed, but with more features of plaque stability in the presence of similar body weight, blood pressure, cholesterol and IGF-1 levels, vascular oxidative stress, and aortic apoptotic/mitogenic indexes. Likewise, and more markedly, when the significantly lower weight of the transgenic animals was taken into account, a study in which Akt, the downstream signalling kinase of PI3K and IGF-1, was knocked out showed the development of severe atherosclerosis (up to 30% of thoracic aortic surface) and occlusive coronary artery disease, due to increased vascular endothelial and macrophage apoptosis [152]. Moreover, in this model, features of plaque vulnerability, MI and cardiac dysfunction developed spontaneously [153]. These findings, according to the authors, suggest that VSMC accumulation can be considered a repair mechanism, and that inhibiting it is not an optimal atheroprotective strategy [152,153].

Moreover, IGF-1 synthesis emerges as a relevant step in the processes that promote reverse cholesterol transport, so crucial in counteracting atherogenesis [154], such as preventing the pro-atheroprotic effects of oxLDL mediated by LOX-1 (lipoxygenase-1) [154], and reducing oxidative stress [51,94,107,115].

The same atheroprotective mechanisms appear to be enforced in the carotid atherosclerotic process, where, with the development of plaques, increased compensatory IGF-1R expression has been detected, which was significantly correlated with the percentage of VSMC apoptosis recorded. This expression favoured an increase in radiolabelled IGF-1 binding, whose function was well described by the fact that it was 4-fold higher in asymptomatic than in symptomatic patients [155]. Similarly, higher serum IGFBP-3, an inhibitor of IGF-1 action, was independently associated with a 9-fold higher risk of carotid plaque formation in a small cohort of hypertensive patients [156].

Interestingly, an IGF-1 gene promoter polymorphism is also associated with both low circulating IGF-1 levels and increased carotid IMT (intima-media thickness) [157], confirming the crucial role of low levels of this hormone in the pathogenesis of vascular damage.
In conclusion, these findings suggest that, by promoting vasodilation and inhibiting platelet aggregation, and by protecting ECs and VSMCs from several mechanisms of death, IGF-1 does not promote atherogenesis, but prevents plaque formation and counteracts plaque destabilization and thrombosis [158] and its clinical correlates [153]. Indeed, it has been shown that oxLDL decreases VSMC IGF-1R expression and IGF-1 levels, thus inducing VSCM apoptosis, which is a hallmark of advanced unstable atherosclerotic plaques present in 60% of autopsied MI subjects [159]. Similarly, reduced IGF-1 expression and binding exposes ECs to death, enabling endothelial plaque erosion [113,118] to be present in the remaining 40% of subjects [159].

**ISCHAEMIA AND CARDIOVASCULAR DISEASE: ROLE OF IGF-1 BETWEEN RAS (RENIN—ANGIOTENSIN SYSTEM) AND IMMUNE MECHANISMS**

Owing to the effects on cardiomyocyte viability and contractility, IGF-1 has also been shown to play a critical role in the reduction of ischaemia/reperfusion damage [160], LV (left ventricular) dysfunction remodelling and in the recovery of ischaemic cardiomyopathy through a reduction in apoptosis and apoptosis-induced inflammation [161] (Figure 2). Indeed, ischaemia has been shown to teleologically increase IGF-1 levels through the activation of the HIF and RISK [162] pathways, thereby achieving ischaemic pre- [162] and post- [163] conditioning. By optimizing the heart workload, and improving glycolipid metabolism, traditional cardiovascular risk factors and platelet aggregation/thrombosis, IGF-1 can prevent or limit ischaemia/reperfusion damage and ventricular dilation. An interesting interlinking of the opposing activities of the IGF-1 and RAS axes contributes to achieving these actions. IGF-1 and AngII (angiotensin II) counterbalance each other, as their anti-apoptotic and vasodilatative properties and their signal transduction pathways cross at different levels [164]. Low IGF-1 levels have been shown to predict the onset of high blood pressure. Indeed, early liver-derived IGF-1-inactivated mice have increased systolic blood pressure at 4 months, secondary to increased peripheral resistance due to impaired vasorelaxation of resistance vessels [165]. Similarly, a large cross-sectional population study has described a 50% reduction in the probability of prevalent hypertension in patients in the fourth compared with the first quartile of serum IGF-1 levels [166]. A later increase in IGF-1 levels has been inconsistently described with the progression of hypertension, accounting for the compensatory aim of hypertensive/hypertrophic responses. Several findings indeed associate IGF-1 and reactive and physiological LV hypertrophy [167], although conflicting evidence is available. Recently, one large cross-sectional study and two small case-control studies have shown opposing results, indicating inverse [168] or no association between LVM (LV mass) and IGF-1 levels in hypertension [169] or diabetes [170]. Non-functionally active IGF-1R polymorphisms have been reported to be associated with LVM [171]. In addition, a decline in IGF-1 sensitivity and effects have been described in spontaneously hypertensive rats [172], with age [173] and dysmetabolic conditions [174] due to reduced NO generation, which was overcome by aerobic exercise in an endothelium-dependent manner [175]. This defect appears similar to the IGF-1-resistant state that develops late in CRF (chronic renal failure) (see below).

Within this view, a complex cross-talk between IGF-1 and the RAS cardiovascular axes includes both positive- and negative-feedback regulation. In the whole-animal setting, IGF-1 overexpression exhibits RAS-inhibiting properties [176] and, conversely, AngII stimulates cardiac IGF-1 and IGF-1R expression in a compensatory and total pressor-dependent manner [177]. Again, AT1R (AngII type 1 receptor) antagonism increases cardiac, renal and plasma IGF-1 production by stimulating sensory neurons in spontaneously hypertensive rats [178], but did not affect it in another study [179]. A few reports describe some biological effects shared by AngII and IGF-1, for example both receptors activate MAPK in the heart, producing cardiomyocyte hypertrophy and myocardial interstitial fibrosis instead of cell proliferation [180]. However, owing to the inconsistent and not obligatory co-activation of PI3K and MAPK pathways downstream of IGF-1, the RAS and IGF-1 axes have been described to split elsewhere in the activation of ‘physiological’ compensatory hypertrophy. A study has shown indeed that ACEIs (angiotensin-converting enzyme inhibitors) are able to reverse experimental hypertension and hypertrophy, while enhancing ventricular tissue IGF-1 expression, and that increasing workloads up-regulated IGF-1 in a linear manner to counteract acute ventricular dysfunction [181]. In addition, myocardial IGF-1R expression was up-regulated by ACEIs, thus improving post-MI LV remodelling [176,182,183]. Moreover, the reduction in IGF-1 levels measured in HF patients was normalized by chronic enalapril therapy, with a parallel significant increase in ejection fraction and peak maximal oxygen consumption [184].

Other evidence in isolated VSMCs suggests a deleterious AngII-mediated IGF-1 effect with up-regulated [185] and transactivated IGF-1Rs, ROS generation and activation of both PI3K and MAPK [186]. Whether these latter two processes are really relevant to plaque growth (by mitogenesis activation, rather the role of MAPK) more than to plaque stabilization (by reduced VSMCs apoptosis, rather the role of PI3K) [187] is uncertain. More importantly, IGF-1-mediated ROS generation at high AngII levels is not necessarily
mediated AT1R activation interferes with IRSs and the and IGF-1 appear to be in opposition. Indeed, AngII- [198].

by IGF-1 administration in a progeroid animal model by the anti-aging and life span-extending effects exerted final biological effects of IGF-1 are again demonstrated compensatory hypertrophy upon challenge [181]. The net reactive hypertrophy [197], and to mediate the same IGF-1 on LV hypertrophy. Indeed, IGF-1 is able to both effect. A similar duality occurs concerning the effect of oxidant/antioxidant activities, with a net latter beneficial

patients [196].

Thus a dual IGF-1 action emerges with respect to its oxidant/antioxidant activities, with a net latter beneficial effect. A similar duality occurs concerning the effect of IGF-1 on LV hypertrophy. Indeed, IGF-1 is able to both inhibit myocyte apoptosis/necrosis and the consequent reactive hypertrophy [197], and to mediate the same compensatory hypertrophy upon challenge [181]. The net final biological effects of IGF-1 are again demonstrated by the anti-aging and life span-extending effects exerted by IGF-1 administration in a progeroid animal model [198].

In the area of glucose and energy metabolism, AngII and IGF-1 appear to be in opposition. Indeed, AngII-mediated AT1R activation interferes with IRSs and the PI3K/Akt pathway by producing ROS via endothelial NADPH oxidase. Secondly, AngII-activated Rho-kinase inhibits eNOS as well, thus reducing NO synthesis and Na+/K+-ATPase activity, inducing cytoplasmic calcium overload and vasoconstriction. Thirdly, skeletal muscle AT1Rs inhibit GLUT4 expression through Rho-kinase, reducing IGF-1-promoted blood glucose uptake and insulin sensitivity, whereas adipocytes and hepatocytes, in the presence of low IGF-1 levels, produce excessive angiotensinogen and AngII levels, which in turn lead to blood pressure elevation, sodium retention and impaired vasodilation [199].

Thus most of the anti-IGF-1 effects of AT1Rs are caused by ROS and Rho-kinase. Conversely, increased IGF-1 levels have been shown to be able to reduce Rho-kinase activation, as well as AngII synthesis, p53 and its related genes [176], angiotensinogen and renin levels, and AT1R and pro-apoptotic Bax protein expression, with a final effective anti-apoptotic action [182]; this is also exerted through a powerful negative influence on myocyte RAS, mediated by p53 modulation. AngII infusion in pre-clinical studies produces a marked reduction in body weight, which is matched to decreased serum and muscle IGF-1 levels, whereas overexpressed muscle-specific IGF-1 diminishes AngII-induced muscle loss [183]. All of the above can partly explain the improved insulin sensitivity and vasodilation observed during ACEI therapy. Indeed, the interference with AT1R activation allows the IGF-1-activated PI3K/Akt pathway to work in its usual manner, increasing vasodilation and reducing oxidative stress.

Another example of the interlinking activities of the IGF-1 and RAS axes is that of immune modulation during both atherothrombosis and ischaemia/reperfusion damage. RAS is closely related to the innate immune system, and its components are expressed on inflammatory cells, such as AT1Rs on T-cells and macrophages [200]. Moreover, AngII modulates the inflammatory response by increasing T-cell activation, homing marker expression and cytokine production [201]. Conversely, ACEI therapy, which has recently been suggested in the treatment of sepsis [202], is able to dampen the immune response, decreasing the synthesis of inflammatory cytokines [203] and the expression of the MHC molecule [204]. Finally, experimental and clinical use of ACEI has been shown to increase both IGF-1 tissue bioactivity [205] and serum [206,207] levels.

By itself, IGF-1, which is known to be expressed on lymphocytes and APCs (antigen-presenting cells), is able to improve the immune response on one hand by increasing the T-cell count and function against non-self antigens, and on the other hand by preserving self-tolerance and immune privilege by down-regulating the MHC class I antigen expression [208], as well as MHC system-linked antigen handling [209]. IGF-1 reduces pancreatic β-cell autoimmune damage in early Type 1 diabetes by maintaining peripheral tolerance through regulatory T-cells, by reducing β-cell antigen (β2-microglobulin) expression, by lowering CD4-positive T-cells [210] and by increasing thymic T-cell manipulation, which specializes in additional negative selection of autoreactive T-cells from the mature repertoire and tolerance development [211]. Moreover, low IGF-1 is also known to contribute to bacterial translocation in sepsis [212], while rhIGF-1 administration has been shown to increase the percentage of peripheral B-cells and CD8 cells, with a fall in the CD4/CD8 ratio [213]. It also exerts anti-inflammatory actions and suppresses Th1-dependent immune responses, increasing anti-inflammatory IL-10 production by T-cells [214].

These properties reduce ischaemia-linked inflammation (‘friendly fire’) [215] and autoimmune activation [160–162], limit plaque instability and ischaemia/reperfusion damage [216] and hasten tissue repair [217].
IGF-1-mediated immune modulation is therefore likely to act in concert with the activation of ischaemia/reperfusion salvage pathways [162] and to save cardiomyocytes.

Moreover, IGF-1 enhances cardiomyocyte contractility and relaxation [218]. A deficient GH/IGF-1 axis, conversely, worsens cardiac contractility and the exercise-induced increase in cardiac output [219], whereas the IGF-1/PI3K/Akt axis appears to be able to increase Ca$^{2+}$ channel activity, with an overall increase in the influx and re-uptake of Ca$^{2+}$ ions, which has a consequence of improving inotropism and lusitropism [220]. In a mouse model of diabetic cardiomyopathy, the induced expression of the IGF-1R gene prevented the onset of diastolic dysfunction, fibrosis and preserved cardiac function [221].

Acute IGF-1 administration in patients with CHF (chronic HF) increased the cardiac index and stroke volume [222], and, through eNOS stimulation, reduced the afterload, suggesting a possible therapeutic role in these patients. These positive IGF-1 properties prompted GH administration trials in HF; however, these have turned out to be disappointing. These trials showed inconsistencies due to different degrees of disease severity [223], different individual efficacy of GH in the achievement of increased IGF-1 levels [224], and different study design and treatment duration. The main reason for failure appears, however, to be the diverging effects of GH and IGF-1 on glycolipid metabolism, due to the known pro-arrhythmic [225], diabetogenic and pro-oxidant [226] effects of GH. A recent study has described low IGF-1 levels as a significant predictor of clinical outcomes in HF at 6 year of follow-up, which was inversely associated with serum adiponectin levels [227]. A current trial of short-term GH administration in patients with HF showed that as many as 40% were GH deficient, as determined using a GH stimulation test, and that GH replacement therapy in this proportion of patients improved exercise capacity, vascular reactivity, LV function and quality of life [228], although information on outcomes was not provided.

Taken together, all of these properties of the interconnected IGF-1 and ACEI-mediated activities emphasize the vascular- and myocardial-protective actions of IGF-1 and support the outcome results of ACEI administration trials and the epidemiological studies on the association between serum IGF-1 levels and cardiovascular events.

Indeed, many genetic and long-term prospective observational studies conducted on approx. 15000 human subjects have consistently found that low serum IGF-1 levels are able to predict the future onset of IHD, HF, and cardiovascular and all-cause mortality [69,70,97,229–237]. Two isolated exceptions have described no ($n = 1122$) [238] or inverse ($n = 642$) [239] prospective associations between IGF-1 levels and all-cause and cardiovascular mortality. A third case is that of a very recent prospective study ($n = 1273$) [230] describing a more complex relation, in which low IGF-1 levels predicted all-cause mortality, and both low-normal and high-normal IGF-1 levels predicted IHD mortality [240].

Another cross-sectional study ($n = 6773$) has previously shown fairly similar results: a significant positive association between both low and high IGF-1 levels and IHD in women was demonstrated, which also resulted in a positive association between high IGF-1 levels and IHD in the whole population (odd ratio, 1.5) [241]. However, this gender-specific association has been contradicted elsewhere [242].

Two possible reasons can be suggested to explain this infrequent ‘J-shaped’ relationship reported. One is the phenomenon of vascular IGF-1 resistance in dysmetabolic conditions [172–174], which is better known in CRF states and is associated with increased compensatory serum IGF-1 levels aiming to overcome IGF-1 signalling defects [243]. Another reason is related to the compensatory increase in IGF-1 after ischaemia/necrosis [162,163], merely statistically observed by cross-sectional designs examining an association with outcome, and therefore possibly indicative of reverse, and not direct, causality with events.

However, both prospective and cross-sectional studies have consistently shown associations between reduced IGF-1 levels and the metabolic syndrome [87,244], diabetes mellitus [22,87,245], LV dysfunction [184], and the presence and number of silent and symptomatic unstable carotid atherosclerotic plaques [62,146,156,246,247]. Moreover, cross-sectional studies have shown that reduced IGF-1 levels are present in patients with documented reduced coronary flow reserve [248], in patients with angina and normal coronary arteries [249] and IHD [250], as well as in patients in the acute phase of MI [251], or with worse post-acute MI outcome [50,252]. To complete this IGF-1-protective view, both prospective and cross-sectional studies have described a direct relationship between high IGF-1 and cognitive function or intellectual quotient at all age ranges [253,254], as well as prospective associations with reduced mortality and longevity [234,237,245,255], and also in the very elderly [255].

**IGF-1, HEART AND KIDNEY**

Large studies on patients with acute coronary syndromes have consistently shown a reduction in creatinine clearance as an independent predictor of 1-year cardiovascular mortality. Evidence suggests that renal dysfunction may increase the risk of death and MI in these patients, at least in part through an associated reduction in NO and IGF-1 levels [256]. Indeed, although endothelial dysfunction has been shown as an early
manifestation in CRF [257], it is strongly predictive of an increased overall and cardiovascular mortality. Therefore IGF-1 could represent the major intermediate between the two diseases, as it is able, both in patients with moderate renal dysfunction [258] and with end-stage CRF [259], to increase capillary and total renal plasma flow and GFR (glomerular filtration rate), and to lower renal vascular resistances. These effects are achieved via NO; indeed, NOS inhibitors prevented IGF-1-induced renal vasodilation [260]. Moreover, IGF-1 appears to play a central role in the MSC-operated limitation of ischaemia/reperfusion damage and repair by counteracting inflammation [1] in experimental models of ARF (acute renal failure) [217].

A large prospective study in Type 1 diabetic patients with a median follow-up time of 10 years has described a reduction in basal IGF-1 levels to be predictive of later microalbuminuria development [261]; moreover, a case-control study in non-diabetic non-dialysed patients has described significantly reduced, by almost 50 %, IGF-1 levels in patients with CRF, compared with controls, with ‘free’ IGF-1 being directly related to creatinine clearance measurements [262]. Strikingly, in a consecutive cohort of ICU (intensive care unit) patients admitted for ARF, low IGF-1 levels were shown to be significantly lower in non-surviving compared with surviving patients, and it was strongly predictive of a 7-fold higher ARF mortality, independently of other known confounders, thus making it a suitable candidate as an early and sensitive predictor of ARF mortality in the ICU [263].

An intravenous IGF-1 infusion for 3 h in healthy subjects produced a potent renal vasodilation and antinatriuretic effect [264]. Experimental studies indicate that, in ischaemic ARF, IGF-1 administration enhances the recovery of renal function and suppresses protein catabolism, normalizing post-acute renal injury histology and reducing mortality through stimulated anabolism, thus helping to maintain GFR and enhance tubular regeneration. Human data are available for IGF-1 treatment in advanced CRF patients, showing safety of administration, and enhancement of GFR and renal blood flow by 14–45 % [265]. One short-term high-dose IGF-1 administration trial showed an enhanced GFR in end-stage CRF for the time of drug treatment [266]. Different regimens with a lower (50 μg·kg⁻¹·body weight·day⁻¹) intermittent dose of IGF-1 in a randomized placebo-controlled administration in patients with end-stage CRF scheduled to initiate HD resulted in a significant and sustained increase in GFR in the absence of side effects, effectively delaying the beginning of HD [267]. Despite these findings, other IGF-1 administration trials in human contrast-induced nephropathy [268], in some CRF patient series [269] and in ARF with substantial co-morbidities [270] yielded negative results, even when the drug was given early in the course of the disease. The reason for the loss of responsiveness to IGF-1 administration appears to be the state of IGF-1 resistance known to be present in advanced renal failure [271]. Although not irreversible, since resistance training has been shown to simultaneously improve IGF-1 sensitivity [175] and to reduce IGF-1 levels in CRF patients [272], the state of IGF-1 resistance shown may account for the recently described cross-sectional association between higher IGF-1 levels and CRF [273].

In HD patients, inconsistent results have shown that reduced IGF-1 levels are often linked to HD-induced catabolism. Careful studies have described a marginal decrease in total serum IGF-1 (<12 %) at the very end of HD. In contrast, a large (35 %) and persistent reduction in ‘free’ and bioactive IGF-1 [274], as well as a strong and inverse correlation between low IGF-1 and systolic or diastolic blood pressure, was observed, which was independent of age. Although not clearly causal, this interesting relationship among CRF, coronary risk factors and IGF-1 in HD patients confirms the link between IGF-1 and chronic renal disease, together with mechanisms of its increased cardiovascular mortality [275].

**rhIGF-1 Administration**

rhIGF-1 was cloned in 1983 and subsequently its injectable form (mecasermin) was made available. Since then, more than 1000 published papers have appeared in PubMed on rhIGF-1 in the topical form, the biomaterial-releasing form, the engineered tissue-secreting form or the intravenous form in both children and adults. After studies in healthy volunteers, a large variety of diseases have been targeted in the long- and short-term, including GH-insensitivity syndrome in children and insulin-resistance states for which rhIGF-1 is an orphan drug, diabetes, AIDS and burn-associated wasting and malabsorption associated with short bowel syndrome or Crohn’s disease, chronic liver disease, rheumatological or orthopaedic conditions, X-linked severe combined immunodeficiency, Alzheimer’s disease, muscular dystrophy, amyotrophic lateral sclerosis and spinal cord injury [276].

On the basis of the evidence reported, the therapeutic use of IGF-1 in cardiovascular disease would be intended to address endothelial function through direct and EPC-mediated survival, reducing cardiovascular risk factors and ischaemia/reperfusion damage, and increasing cardiomyocyte function and survival.

Despite the availability of a large body of positive experimental results, rhIGF-1 administration has not been reported in human studies of ischaemia/reperfusion injury in MI or IHD. Conversely, positive initial data on VEGF [277] and erythropoietin [278] administration in human MI and coronary artery disease do exist, and
Administration in healthy subjects

Glucose metabolism
Upon its intravenous infusion, rhIGF-1 causes acute hypoglycaemia by stimulating peripheral glucose uptake, glycosylation and glycogen synthesis. A single intravenous dose of 100 μg/kg of body weight, which is equipotent to an insulin dose of 0.15 unit/kg of body weight, results in the rapid onset of symptomatic hypoglycaemia. When administered by subcutaneous injection to healthy volunteers, the peak IGF-1 concentration is reached after approx. 7 h, and IGF-1 is cleared with a half-life of approx. 20 h [280]. Intravenous rhIGF-1 infusions (7 and 14 μg·kg⁻¹·h⁻¹ of body weight) have been shown to decrease serum levels of insulin and C-peptide without affecting plasma glucose levels: the decrease in the insulin/glucose ratio was related to tissue insulin sensitivity enhanced by IGF-1 [281,282]. Metabolic and cardiovascular effects of rhIGF-1 (20 μg·kg⁻¹·h⁻¹ of body weight) and insulin (0.5 m-unit·kg⁻¹·min⁻¹ of body weight) were compared: IGF-1 infusion decreased Ra (glucose appearance rate) and increased Rd (glucose utilization rate), without changes in NEFA levels. It also caused increases in cardiac output, heart rate and stroke volume. Insulin infusion caused similar results, except for decreased NEFAs and no significant changes in cardiovascular variables [283].

Lipid metabolism
rhIGF-1 administration profoundly affects lipoprotein kinetics, reducing triacylglycerol and VLDL (very-low-density lipoprotein) levels by almost 50%, as well as decreasing total cholesterol, LDL-cholesterol and lipoprotein(a) [282–286], by increasing HDL-cholesterol and insulin sensitivity, inhibiting insulin and GH secretion [287], and reducing postprandial serum triacylglycerol-rich particles levels, which have been shown to be able to prevent premature atherosclerosis and insulin resistance [288].

Energy metabolism and cardiovascular function
Subcutaneous administration of IGF-1 (at 60 μg/kg of body weight) in healthy volunteers, when compared with saline administration, resulted in positive inotropism with a significant increase in stroke volume, cardiac output and ejection fraction, but unchanged maximal exercise duration and peak oxygen consumption [289]. Low doses of rhIGF-1 (20 μg/kg of body weight, subcutaneously) in healthy adults increased cardiac inotropism, as shown by radionuclide angiocardiography, demonstrating that even this low subcutaneous dose was able to maintain circulating IGF-1 levels within the normal range of basal IGF-1 in adulthood [290].

Therapy in cardiovascular and metabolic diseases

Atherosclerosis and MI
Direct rhIGF-1 infusion in apoE-deficient mice decreased the progression rate of the atherosclerotic process [107]. This effect appeared to be related to a decrease in IL-6 and TNF-α levels, and in macrophage accumulation within atherosclerotic lesions, as well as to suppressed oxidative stress, up-regulated vascular eNOS expression and increased circulating EPCs, which play an important role in vascular repair.

Evidence from animal models of MI have demonstrated that rhIGF-1 administration reduces infarct size, ischaemia/reperfusion injury, apoptosis, polymorphonuclear cell accumulation and leucocyte-induced amplification of cardiac necrosis [107,108,291]. Experimental MI models overexpressing IGF-1 or being subjected to IGF-1 administration have decreased levels of ischaemia/reperfusion injury and cardiomyocyte apoptosis. These effects were achieved by activation of the PI3K/Akt pathway [292], improved post-ischaemic recovery and stroke volume [293], which contributed to short- and long-term ventricular remodelling [294], blunted ventricular dilation, wall stress and cardiac hypertrophy [295], obtained by reducing peripheral resistance and arterial pressure, and by preserving myocardial structure [296]. Injection of clonogenic CPCs and NF-IGF-1 (nanofibre-assembling-IGF-1) in infarcted animals [297] enhanced the activation and differentiation of resident and delivered CPCs, improving cardiac repair, ejection fraction and diastolic wall stress. Owing to the reported potent vascular IGF-1 effect [125], cardiomyocyte protection has been related to increased IGF-1-mediated angiogenesis, as assessed by microSPECT-CT (single-photon emission computed tomography-computed tomography) in adenovirus-delivered human IGF-1 gene models. In these models, a sustained IGF-1 expression in the peri-infarct area, reduced LV remodelling and improved cardiac function were also described [298]. Moreover, in injured muscle, IGF-1 has been shown to promote progenitor cell recruitment and to subsequently resolve the inflammatory response, thus enhancing local repair mechanisms [134].

Congestive HF
Different favourable IGF-1 effects are described in animal models of HF in which it enhances cardiac performance both in vivo and in vitro [299], with increased Ca²⁺ responsiveness, contractility and cardiac output [300]. IGF-1 injection in a canine model of dilated cardiomyopathy, obtained by pacing at a rate of 220 beats/min, showed an improvement in cardiac
output, stroke volume, LV end-diastolic volume, LV end-systolic volume, pulmonary wedge pressure, systemic vascular resistance and LV wall stress [301]. The cardioprotective effects of IGF-1 were also shown in doxorubicin-treated rats, which had higher cardiac output and stroke volume than controls [302], and in diabetic cardiomyopathy, where it reduced cardiac fibrosis and diastolic dysfunction. Therefore targeting the IGF-1R/Akt signalling pathway may also represent a therapeutic tool for the treatment of diabetic myocardial disease [303]. The acute haemodynamic effects of an intravenous rhIGF-1 dose of 60 μg/kg of body weight in patients with CHF were also investigated. As in the healthy human volunteers, IGF-1 increased cardiac output and stroke volume, and reduced left and right heart filling pressures and peripheral vascular resistance, thus matching the increased cardiac output to the reduction in cardiac afterload. During IGF-1 treatment, no side effects were apparent [222].

**Insulin resistance and diabetes**

rhIGF-1 has also been studied as a potential therapy for diabetes, as it can influence the glucose, lipid and metabolic profile, due to its ability to achieve hypoglycaemia, stimulate glucose uptake, glycolysis and glycogen synthesis, and suppress gluconeogenesis, while at the same time inhibiting insulin secretion [304].

Genetic syndromes of severe insulin resistance have been deemed an orphan indication for rhIGF-1, such as GH insensitivity. Indeed, because being unresponsive to insulin treatment, these patients may benefit from rhIGF-1 treatment, which has been shown to ‘shortcut’ the insulin signalling pathway blockade. Subcutaneous rhIGF-1 administration in selected patients with a type A phenotype of severe insulin resistance and diabetes mellitus enhances insulin sensitivity, as shown by the decrease in endogenous insulin levels, normalizing the response to intravenous insulin and reducing plasma glucose, as well as HbA1c (glycated haemoglobin) [305,306]. A comparison of two rhIGF-1 dose regimens in severely insulin-resistant insulin-treated patients has shown that a short protocol with a high dose of rhIGF-1 (80 μg/kg of body weight, twice a day) was more effective in lowering fasting plasma glucose and serum insulin levels [307].

Thus, in Type 2 diabetes, where insulin resistance is the main pathophysiological mechanism and the greatest hindrance to successful therapy, rhIGF-1 therapy has appeared as an attractive possibility. Subcutaneous rhIGF-1 administration (100 μg/kg of body weight, twice a day) in insulin-resistant Type 2 diabetic patients improved short- and long-term indexes of glycaemic control and insulin sensitivity, therefore decreasing insulin levels. Co-administration of IGFBP-3 and IGF-1 in Type 2 diabetic patients has been shown to produce a low number of side effects [308], with the same degree of efficacy also being shown in a more recent study [309].

A similar rationale for rhIGF-1-replacement therapy exists in Type 1 diabetes, as this disease is associated with insulin resistance, reduced IGF-1 concentrations and hepatic GH resistance, and with higher GH levels. Indeed, in these patients, rhIGF-1 administration reduced GH levels, restoring the negative feedback on the pituitary, but also exerted an independent effect on glucose metabolism, thereby reducing hepatic gluconeogenesis and increasing peripheral glucose utilization and metabolic rate [310]. Subcutaneous low-dose rhIGF-1 (20 and 40 μg/kg of body weight) in young insulin-dependent diabetic patients reduced insulin levels and overnight hyperglycaemia, and also decreased overnight GH [311] and glucagon [312] levels. rhIGF-1 was then formally investigated [313,314], at a dose of 40 μg/kg of body weight (twice a day), as an adjunct to standard insulin therapy and resulted in a significant decrease in HbA1c and a reduction in the insulin doses required. This dose of rhIGF-1 was well tolerated, but higher doses were hindered by an increased incidence of adverse events, including oedema, jaw pain and worsening of retinopathy. The incidence of side effects in Type 1 diabetic patients was reduced in trials with co-administration of IGFBP-3 and IGF-1 [315].

Severe insulin-resistance syndromes are a confirmed indication for systemic rhIGF-1 administration. Although the insulin-sensitizing IGF-1 effect may benefit both Type 1 and Type 2 diabetes, there are no ongoing clinical trials because of the concern of a risk of retinopathy in long-term systemic administration [316]. Owing to the favourable pancreatic and metabolic effects, IGF-1 has instead been considered as a candidate gene in gene therapy and ‘prevention’ of Type 1 diabetes [317]. However, the potential benefits of IGF-1 therapy in diabetes mellitus have yet to be realized.

**Side effects**

**Short-term side effects**

The immediate adverse effects of the pure rhIGF-1 preparation reported include hypoglycaemia, jaw pain, headache, myalgias and fluid retention. The equimolar complexed form of rhIGF-1 and rhIGFBP-3 (recombinant human IGFBP-3), which is more readily tolerated, but higher doses were hindered by an increased incidence of adverse events, including oedema, jaw pain and worsening of retinopathy. The incidence of side effects in Type 1 diabetic patients was reduced in trials with co-administration of IGFBP-3 and IGF-1 [315].

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**Long-term side effects**

The reported long-term adverse effects in children (up to 12 years of administration) include increased tonsillar and adenoidal tissue growth during treatment, which rapidly recovers after discontinuation of treatment. Unproven
concerns remain with regard to the potential development of acromegaly-like syndrome and cancer.

Post-marketing pharmacosurveillance is still too short to draw any definitive conclusions regarding the long-term side effects of IGF-1. Animal toxicology studies with maximally tolerated lifetime drug doses, corresponding to many times the human dose, have been designed to assess the possibility of rare carcinogenic events in humans. Very high doses of rhIGF-1 (61 mg·kg^{-1}·day^{-1}) administered to rodents over their lifetime [318] have resulted in an increase in benign adrenal and epithelial skin neoplasms, as well as benign and malignant breast neoplasms in females. Therefore a direct mitogenic effect of rhIGF-1 upon these sorts of tumours cannot be overlooked, despite the fact that the carcinogenic effect in these animals could be due more to chronic hypoglycaemia and large increases in body weight and food consumption as a result of the very high drug doses, rather than on an effect of the drug itself. Indeed, interesting comparisons have been made between insulin and rhIGF-1 toxicologies. Long-term toxicology and lifetime carcinogenicity studies on insulin and insulin analogues have reported an increase in the incidence of breast tumours similar to rhIGF-1 models [319].

However, human data particularly reassuring on the safety of short-term administration courses are available. After 12 weeks of rhIGF-1 administration as an immuno-enhancer in HIV infection [320] or in HIV-related wasting [321], no cancer developed in these immunocompromised patients who are prone to carcinogenesis. Likewise, in a pilot study in liver cirrhosis patients [322], who are known to be prone to cancer development, and even in patients with hepatocellular carcinoma [323], rhIGF-1 has been shown to be safe, without an increase in the incidence of cancer cases or progression.

Recent observational evidence [324] has shown a small association between human endogenous serum IGF-1 levels and tumour proliferation/survival (breast, prostate, lung and colorectal cancer). Given that IGF-1-deficient patients have subnormal IGF-1 serum levels, which is only normalized by rhIGF-1 therapy [325], it could be anticipated that their cancer risk during rhIGF-1 therapy should not exceed that of the normal population. Thus rhIGF-1 treatment could be accepted for short-term courses in specific diseases, for definite indications, with the assistance of frequent serum monitoring to ascertain whether serum IGF-1 levels remain within the normal range.

**CONCLUSIONS**

The evidence outlined in the present review suggests that the IGF-1 axis is a very important endogenous tool that is daily recruited for cardiovascular and metabolic health, owing to its complete range of anti-atherogenetic abilities, and the versatile actions on many cardiovascular risk factors and diseases. Human intervention studies are needed to better clarify the possible specific indications for its exogenous administration, as well as time windows of opportunity and safety.

**REFERENCES**


