1  *Review*

2  # Title: Growth Hormone (GH) and Cardiovascular
3  # system

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13  **Abstract:** This review describes the positive effects of growth hormone on the cardiovascular
14  system. We analyze why the vascular endothelium is a real internal secretion gland, whose
15  inflammation is the first step for developing atherosclerosis, as well as the mechanisms by which
16  GH acts on the vascular endothelium improving its dysfunction. We also report how GH acts on
17  coronary arterial disease and heart failure, and on peripheral arterial disease inducing the
18  generation of new collateral vessels able to bypass a major artery occlusion. We include some
19  preliminary data from a trial in which GH or placebo is given to elder people suffering from critical
20  limb ischemia, showing the effects of the hormone on plasma markers of inflammation, and stating
21  that the administration of GH in short periods of time is safe and effective even in diabetic patients.
22  We also analyze how Klotho may have strong relationships with GH, inducing, after being released
23  from the damaged vascular endothelium, the pituitary secretion of GH to repair the damaged tissue.
24  Lastly, we show how GH induces wound healing by increasing the blood flow to the ischemic tissue.
25  In summary, we postulate that short-time GH administration is useful for treating cardiovascular
26  diseases.

27  **Keywords:** cardiovascular diseases; atherosclerosis; oxidative stress; angiogenesis and
28  arteriogenesis; endothelial dysfunction; growth hormone; IGF-I; wound healing.

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30  **1. Introduction**

31  The hGH gene family is composed by two GH genes (GH-N and GH-V), and three placental
32  genes located in the chromosome 17 [1]. It has been considered that the GH-V gene is expressed only
33  in the placenta, although some studies indicated that this gene, or some other GH gene, still
34  unknown, could also be expressed in the human pituitary gland [2,3]. In the case of GH-N, it is
35  already well known that in addition to its pituitary expression, responsible for the actions of the
36  hormone at the endocrine level, the hormone is also expressed in numerous cells and tissues, where
37  it acts in an auto/paracrine manner [4]. Perhaps the heart is an exception to this peripheral expression
38  of GH, as we will see later.
39  The regulation of GH pituitary expression is very complex, since in the last few years the classical
40  knowledge of a positive regulation by GHRH and negative by somatostatin [5], has been changed
41  after the knowledge of a series of factors that are decisively involved in that regulation [6]. This is the
42  case, for instance, of the orexigenic ghrelin, released by the empty stomach, or the postulated anti-
43  senescence factor Klotho, mainly expressed in the kidney, but also in the brain and in the own
44  somatotroph cells where it would act in an auto/paracrine manner for directly regulating GH
45  secretion [7]; in addition, the growth differentiation factor 15 (GDF15), synthesized and released by
46  cardiomyocytes, inhibits GH-induced hepatic expression of IGF-I, therefore inhibiting the IGF-I effect

47  on hypothalamic somatostatin release and the direct negative effect of IGF-I on pituitary
48  somatotrophs, thus acting as a coordinator between cardiac function and body growth or other IGF-
49  I dependent GH effects on the human body [8].
50      Although the regulation of GH expression is not the aim of this review, perhaps the complexity
51  of its regulation would explain the fact that far beyond of the concept that GH is mainly a metabolic
52  hormone responsible for the longitudinal growth of the organism before puberty ends, the hormone
53  exerts many other actions on practically all the organs and tissues in the human body [4], as
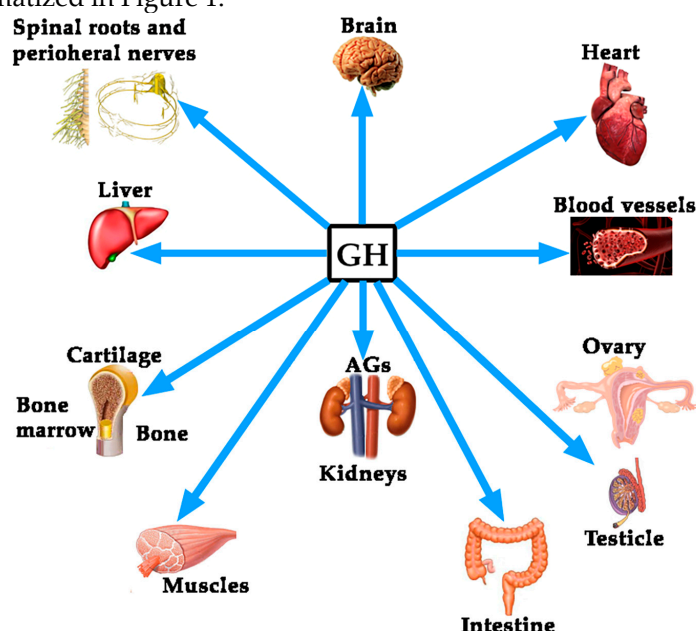54  schematized in Figure 1.



55
56  **Figure 1.** GH is a pleiotropic hormone acting on many tissues and organs in the human organism.
57      Blue arrows show some of the most important territories in which the hormone produces positive
58      effects. For a better understanding of this schema, see reference [4]. AGs: Adrenal glands.

59      In this review, we will focus on the effects of GH on the cardiovascular system; but before it we
60  will analyze the role of the vascular endothelium as an internal secretion gland, as well as the main
61  pathologies that affect the cardiovascular system, to subsequently assess the effect that GH can play
62  in its treatment.

63  *1.1. The role of the vascular endothelium as an internal secretion gland and the effects of GH on it*

64      Histologically, the vascular endothelium is a single unicellular layer that covers the internal
65  surface of blood vessels and forms the wall of capillaries. However, despite its simplicity, this layer
66  is very complex in physiological terms, since its location allows it to be able to detect alterations in
67  the hemodynamic forces acting on the vascular wall (shear stress forces), as well as changes in
68  circulating chemical signals, responding to all this by releasing vasoactive compounds, able to act
69  oppositely depending on the signals received. For instance, at the level of hemostasis, the vascular
70  endothelium can produce both anti-hemostatic factors (protein C, prostacyclin PGl2, tissue
71  plasminogen activator, nitric oxide), or factors that favor hemostasis (von Willebrand factor, tissue
72  Factor III, plasminogen activator inhibitor, thromboxane A2). The same occurs with the vascular tone,
73  since vasodilators such as nitric oxide (NO) or prostacyclin (PGl2), and vasoconstrictors such as
74  angiotensin II, endothelin, thromboxane II and superoxide anion, can be released from the vascular
75  endothelium to contribute to vascular homeostasis. Moreover, the vascular endothelium produces
76  many growth factors, such as vascular endothelial growth factor (VEGF), platelet derived growth
77  factor (PDGF), basic fibroblast growth factor (bFGF), and endothelin; but also inhibitory growth
78  factors, as transforming growth factor-ß (TGF-ß). Even, the vascular endothelium can participate in
79  immunological responses by producing interleukins (Il-1, Il-6 and Il-18), tumor necrosis factor-$\alpha$

80  (TNF-$\alpha$), monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-
81  1), intercellular adhesion molecule-1 (ICAM-1) and selectins E and P.
82        Most of these factors act locally by auto/paracrine mechanisms, so that they allow, as stated
83  above, to regulate the vascular homeostasis.
84        In general, the vascular endothelium decreases the vascular tone, inhibits platelet adhesion and
85  aggregation, decreases the activation of the coagulation system, stimulates fibrinolysis, decreases
86  capillary permeability and inhibits the adhesion and migration of neutrophils and inflammation-
87  generating macrophages. Therefore, endothelial dysfunction, a primary event in the development of
88  atherosclerosis, is associated with increases smooth muscle vascular tone with arterial rigidity and
89  elevated intima-media thickness.
90        Interestingly, while there are clear evidences that the GH-receptor (GHR) is expressed in the
91  vascular endothelium [9,10], the possibility exists that GH itself is expressed in this territory, as in
92  vitro studies reflect [11]. In any case, there are not doubts about the fact that GH plays a very
93  important role on vascular endothelium. This statement is supported by early studies carried out in
94  children with GH-deficiency (GHD), in whom GH replacement therapy recovered existing
95  endothelial dysfunction. This is the case, for instance, of children with renal insufficiency; in them,
96  endothelial dysfunction is quite common finding, but GH therapy reverses it [12]. In addition, an
97  improvement of the arterial response to induced vasodilation were observed in GH-deficiency (GHD)
98  adolescents after GH-replacement therapy [13]; or in obese children, in whom obesity negatively
99  affects the secretion of GH and constitutes a risk of developing atherosclerosis prematurely [14].
100 Similar results have been found in GHD adults (AGHD) after receiving replacement therapy [15],
101 suggesting that GH reduces vascular inflammation, therefore reducing the vascular risk. Another
102 study in AGHD patients showed that GH treatment led to a significant decrease in plasma levels of
103 apolipoprotein B (Apo B) and C-reactive protein (CRP), while no changes were observed in IL-6 or
104 on markers of endothelial function; but in all, GH administration decreased the cardiovascular risk
105 in them [16]. AGHD patients show impaired coronary flow reserve, which is improved after receiving
106 treatment with the hormone, suggesting that GH improves microvascular function and then could
107 reduce cardiovascular morbidity and mortality in these AGHD [17]. A more recent study describes
108 that six months of treatment with GH are enough to decrease cardiovascular risk and improve
109 endothelial dysfunction [18].
110       One of the biomarkers for cardiovascular disease is the loss of circulating CD34+ cells [19]; this
111 has been shown to be corrected after one year of GH treatment in AGHD, since the number of these
112 cells increased and endothelial function improved [20]. This agrees with the fact that GH increases
113 the production and release of endothelial progenitor cells (EPC) in non AGHD subjects, which in the
114 vascular endothelium act as a repair cells [21]. Moreover, GH replacement therapy improves
115 fibrinolysis in AGHD patients, most likely by increasing the release of endothelial tissue plasminogen
116 activator as a response to venous occlusion [22]. On the contrary, other studies found that AGHD
117 patients treated with GH showed increased concentrations of E-selectin, indicative of an uncorrected
118 endothelial dysfunction [23]. These authors conclude that the beneficial effect of GH in these patients
119 may be produced by the effects of the hormone on other mechanisms rather than acting on
120 endothelial dysfunction.
121       The effects of GH administration on E-selectin had not been found in previous studies
122 performed in healthy adults and AGHD patients [24]; instead vascular cell adhesion molecule-1
123 (VCAM-1) significantly increased in AGHD patients during GH treatment. Interestingly, serum from
124 healthy patients treated with GH significantly increased the expression of VCAM-1 in cultured
125 umbilical vein endothelial cells, suggesting that GH might act on VCAM-1 expression by an indirect
126 mechanism, most likely related to the modulation of the expression of other circulating factors [24].
127 This might explain the reported negative effects of the hormone when administered to critically ill
128 patients, since VCAM-1 mediates leukocytes extravasation which can lead to multiple organ failure
129 in sepsis [25], although the increased mortality reported was observed with doses of GH quite higher
130 (10-20 times) than usual treatment doses.

131    To our knowledge only one study reported no positive effects of GH replacement therapy on
132    the endothelial dysfunction in AGHD patients [23], as only one report indicates that GH does not
133    recover the endothelial impairment present in GHD children [26]. Perhaps, the small number of
134    subjects, or the methodology used, or the time during which these studies were carried out justifies
135    the contradictory results.

136    *1.2. GH, IGF-I, Klotho and the vascular endothelium.*

137    Klotho was first described in 1997 as a product of a gene involved in the suppression of several
138    aging phenotypes in mouse. Initially, it was thought that Klotho would be implied in a signaling
139    pathway regulating senescence and the severity of diseases related with the process of aging, such as
140    atherosclerosis [27]. In mice, the gene codifies a membrane protein homologue to β-glucosidases,
141    while in humans the gene has been shown to be composed of five exons and is located on
142    chromosome 13q12. The gene suffers a physiological alternative RNA splicing giving origin to two
143    transcripts, one of them being a membrane protein while the other one is secreted and predominates
144    over the former [28].
145    The possible effects of Klotho on the physiology of the human vascular endothelium were first
146    postulated in 1998, indicating that it protects the cardiovascular system by inducing NO endothelial
147    production [29], although their possible mechanisms of action had not yet been clarified [30]. Further
148    studies in mice of the same group demonstrated that secreted Klotho promoted endothelial increase
149    of NO in aorta and arterioles [31], and that adenovirus-mediated Klotho gene delivery to a typical rat
150    model of multiple atherogenic risk (OLETF rat) improved endothelial dysfunction, increased NO
151    production, reduced increased blood pressure and prevented medial hypertrophy, meaning that
152    Klotho was a clear positive regulator of vascular function [32]. This was confirmed in Klotho mutant
153    mice when observing that in these animals the density of blood capillaries was decreased at the tissue
154    level and angiogenesis was impaired, as it was the release of NO from the vascular endothelium [33].
155    These effects have been related to an action of Klotho on oxidative stress, responsible for inducing
156    apoptosis and senescence in vascular cells [34].
157    While studies in animal models indicate a clear role for Klotho on the vascular endothelium,
158    there are still no clear data on the physiological role that this hormone plays in men on the
159    cardiovascular system [35]. *In vitro* studies demonstrated that Klotho suppress TNF-$\alpha$-induced
160    expression of ICAM-1 and VCAM-1 in human umbilical vein endothelial cells, as well as the
161    inhibition of eNOS phosphorylation induced by the administration of TNF-$\alpha$ [36], effects consistent
162    with its previously postulated role in the modulation of endothelial inflammation.
163    Klotho is mainly produced in kidneys; however, it seems that it could be expressed also in the
164    vascular endothelium, with the only exception of endothelial cells from human brain [37]. In any case,
165    Klotho is a circulating protein that increases NO production and protects the vascular endothelium
166    [38,39].
167    To analyze the role of Klotho on the vascular endothelium nor is the aim of this review, however
168    since it has been shown that this protein plays a role on pituitary GH secretion [7], we think important
169    to try to establish a relationship between Klotho and GH, given both are effective factors to prevent
170    damage to the vascular endothelium and repair it if a damage exists.
171    Mice that do not express Klotho die early than normal mice showing many symptoms of aging,
172    most of them typical of GHD [7]. Plasma levels of Klotho are low in GHD subjects, and the pituitary
173    somatotrophs of Klotho-deficient mice are hypotrophic [7], suggesting that Klotho exerts a trophic
174    effect on them. Besides this, Klotho-deficient mice are smaller than normal mice, and their GH-
175    producing cells in the pituitary show lesser secretory granules [40]. In addition, Klotho strongly
176    inhibits the negative effects of IGF-I on GH secretion, and increases GH secretion in cultured human
177    GH-secreting adenomas [40].
178    All these data indicate that Klotho is a positive active regulator of GH secretion, both in animal
179    models and in humans. However, it is still unknown how GH and Klotho interact to repair a damaged
180    vascular endothelium. For instance, in anorexia nervosa patients, in which the existence of an
181    increased pulsatile secretion of GH is well known, while plasma levels of IGF-I are low or very low,

182    due to malnutrition, plasma levels of Klotho are lower than expected for the age of the patients [41],
183    but they increased significantly after the patients increased their body weight and, concomitantly,
184    IGF-I increased too. This suggests that IGF-I led to the increase of Klotho [41], perhaps for the
185    maintenance of a physiological feedback loop between GH, IGF-I and Klotho. Those supposed
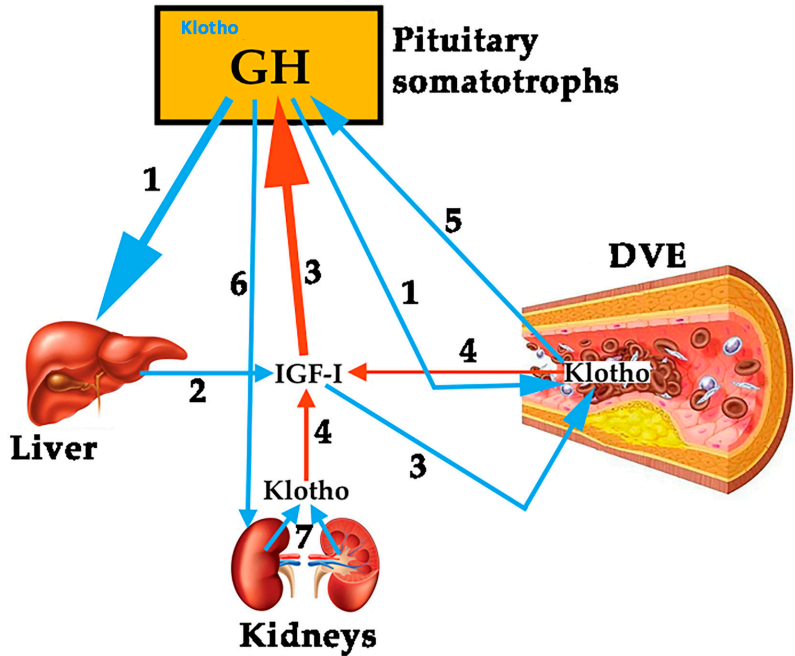186    relationships between the three hormones are schematized in Figure 2.



187
188    **Figure 2.** Schematic representation about the possible relationships between GH, IGF-I and Klotho,
189    and its actions on the vascular endothelium. **1**. Pituitary GH induces the hepatic expression of IGF-I
190    (**2**) and acts on the repair of the damaged vascular endothelium (DVE), although it is also possible
191    that the hormone enhances the production of Klotho by this damaged tissue. **3.** Besides its inhibitory
192    effects on pituitary GH release, IGF-I also contributes to repair DVE, and, as in the case of GH, it could
193    enhance Klotho production in DVE. **4.** DVE secretes Klotho and it inhibits the negative effect of IGF-
194    I on pituitary GH release, but plasma Klotho may also proceed from kidneys (**7**), contributing or being
195    responsible for the inhibition of IGF-I effects on GH secretion. **5.** The possibility exists that Klotho
196    released from DVE stimulates GH secretion for repairing DVE. **6.** GH plays an important role on the
197    physiology of kidneys, being particularly important when there is a chronic kidney disease; since in
198    this pathology there is a state of systemic Klotho deficiency, it is possible that GH tries to correct this
199    problem associated to cardiovascular diseases. Some of these concepts are merely speculative, but
200    existing data lead to think that there is a feedback regulation circuit between GH, IGF-I and Klotho.
201    Blue arrows indicate stimulation and red arrows indicate inhibition.

202    *1.3. Cardiovascular disease as an inflammatory condition.*

203    Several diseases have been related to inflammation since many years, including atherosclerosis
204    [42–46]. It is considered that inflammation plays a key role in atherogenesis, since it is not only
205    involved in the development and progression of this process [46], but also in the associated symptoms
206    [43]. Circulating monocytes and lymphocytes are present in the vascular wall early in atherogenesis,
207    and both are responsible for the formation and complication of the atherosclerotic plaque [46].
208    A current study has demonstrated the high influence of inflammation in cardiovascular disease
209    (CVD) from a clinical point of view. As known, IL-6 has been previously associated with an increased
210    risk of cardiovascular events, with independence of the cholesterol levels in plasma [42]. IL-6
211    amplifies the inflammatory cascade and is the main circulating cytokine linking systemic
212    inflammation with local pathology [47,48]. It stimulates macrophages and promotes proliferation of

213 smooth muscle cells (SMC) in atherosclerotic plaque [47], and stimulates coagulation by increasing
214 messenger ribonucleic acid transcription of tissue factor and factor VIII [49].
215      IL-1β mediates the IL-6 signaling pathway [42], and canakinumab, a fully human monoclonal
216 antibody targeting IL-1β, leads to a marked reduction of both, plasma levels of IL-6 and CRP without
217 lowering the level of low-density lipoprotein (LDL) in patients with diabetes who were at high
218 vascular risk [50]. This drug led to a significant lower rate of recurrent cardiovascular events than
219 placebo [42].
220      The development of the atheromatous plaque is a multi-factorial process. SMC from the middle
221 layer in the elastic arteries show a differentiated phenotype with a low proliferation and migration
222 rate. Unlike the skeletal and cardiac myocyte, mature SMC may suffer a phenotypic modulation,
223 because of an atherogenic stimulus, with a re-entry in the cellular cycle. These activated state makes
224 them proliferate and migrate to the vascular lumen, and synthesize some extracellular matrix (EM)
225 components and proteases that modify the matrix, contributing to the atheromatous plaque [51].
226      The key aspect of the plaque formation is the endothelial dysfunction secondary to some
227 atherogenic stimuli, such as hypercholesterolemia, hypertension, diabetes, tobacco, etc. The
228 consequence of this endothelial dysfunction is the appearance of an inflammatory response.
229      SMC are essential in the stability of these plaques. When there is a scarcity of these cells into the
230 plaque, the atheroma will be highly vulnerable to rupture [52]. Plaque rupture and subsequent
231 thrombus formation can lead to an acute event [53–55], although in the lower extremities this event
232 can be better tolerated because of the numerous and large collateral network.
233      It is well known the role of LDL in this setting. Oxidized LDL (ox-LDL) have been related to the
234 formation and complication of the atherosclerotic plaque [56]. LDL has high susceptibility of being
235 oxidized. But the oxidative environment in the vascular wall may also modified other lipids as HDL.
236 Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leukocyte- and platelet-derived
237 oxidants, and red blood cell-derived iron-rich heme group, are part of the different systems implied
238 in the oxidative modification of lipids, proteins and DNA that in the vascular wall lead to
239 atherosclerosis [56]. All these oxidants maintain the inflammatory response and participate in the
240 arterial wall rupture with platelet aggregation and thrombus formation.
241      Oxidative stress plays a main role in the origin of the pathogenesis of CVD. In a normal vascular
242 wall, oxidative stress activates nuclear defense genes throughout the mediation of the nuclear factor
243 erythroid 2-related factor 2 (Nrf2) 2 [57]. This protects against the formation of foam cells by
244 regulating the expression of antioxidant proteins and scavenger receptors [57]. Nevertheless, its
245 function has not been properly understood, since a pro-atherogenic action has been also associated
246 to Nrf2, because ApoE-null mice, deficient in Nrf2, develop smaller atherosclerotic plaques [58].
247      The recruitment of circulating leukocytes into the blood vessel wall is one of the major
248 etiopathogenic mechanisms of atherosclerosis. This process is predominantly mediated by cellular
249 adhesion molecules (CAM), which are expressed on the vascular endothelium and the leukocytes of
250 the vascular wall, in response to atherogenic stimuli. In patients with peripheral arterial disease
251 (PAD), increased levels of these integrins have been found during exercise, being associated with the
252 severity and the extent of the arterial disease [59]. Antagonists of CAM have shown promise in
253 treating inflammatory disorders in animal models [60,61].
254      Selectins, another group of integrins, are also elevated in PAD population. Studies with the anti-
255 P-selectin antibody inclacumab in coronary arterial disease (CAD) have found a reduction in
256 myocardial damage after percutaneous management [62]. This molecule also reduces elevated
257 circulating platelet-leukocyte aggregates levels in PAD [63].
258      Exercise is associated with an increase in plasma levels of numerous inflammatory mediators in
259 PAD, including thiobarbituric acid–reactive substances (formed as a byproduct of lipid
260 peroxidation), thromboxane, IL-8, TNF-α, ICAM-1, VCAM-1, von Willebrand factor, E-selectin, and
261 thrombomodulin [43].
262      Casual associations between biomarkers and PAD has not been established. However,
263 inflammatory mediators can aggravate endothelial dysfunction, and markers such as IL-6 are
264 inversely correlated with maximum treadmill performance [64]. Although exercise acutely induces

265    oxidative stress in patients with PAD, exercise training has consistently been shown to improve
266    symptoms among patients with PAD. In this sense, GH increases exercise performance improving
267    lean body mass, muscle mass and cardiac output in AGHD patients [65].
268        Interestingly, exercise is a powerful inducer of pituitary GH release [5], most likely by inducing
269    the hypothalamic release of noradrenaline which inhibits somatostatin, the main inhibitor of pituitary
270    GH release [66]; but, as indicated, pituitary secretion of GH decreases while aging [5]. This seems to
271    be contradictory; however, it is important to differentiate the effects of acute and chronic exercise,
272    because both kind of exercises have different effects [43]. Endothelial dysfunction was recently
273    associated with walking impairment independent of the ankle-brachial index (ABI), suggesting that
274    endothelial dysfunction may contribute to the exercise impairment in PAD [67].
275        In addition, inflammatory mediators may also have proangiogenic and antiangiogenic effects,
276    regulating the ischemic response [68]. In fact, patients with PAD have lower circulating VEGF-A and
277    higher circulating inflammatory parameters of TNF-$\alpha$ and IL-8 compared with controls with other
278    comorbid conditions and cardiovascular risk factors [69].
279        On these bases, atherosclerosis is a complex process involving lipid deposition, oxidative stress,
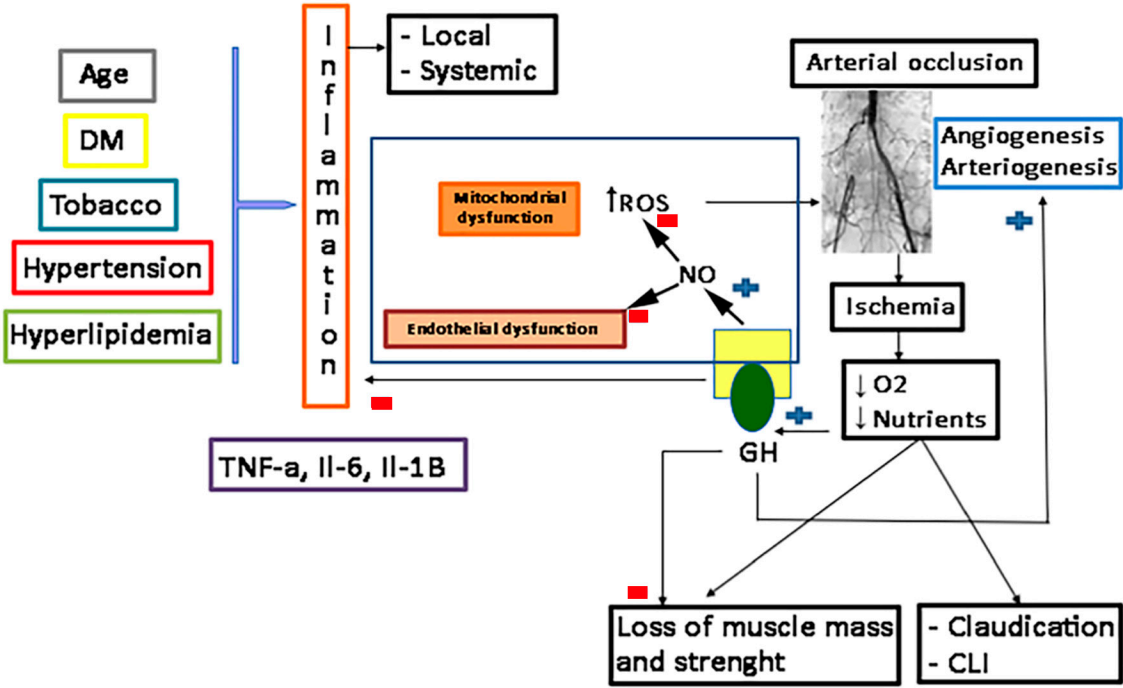280    inflammatory cell recruitment and platelet activation. Figure 3 schematizes these concepts.



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**Figure 3.** Cardiovascular risk factors converge to produce inflammation with increasing of TNF-alpha, IL-6 and IL-1B, which promotes endothelial and mitochondrial dysfunction, with the overload of ROS, all of them being responsible for atheroma plaque formation and arterial occlusion, that leads to hypoxia and decreased nutrition of tissue. Both factors contribute to the loss of muscle mass and strength and symptoms such as intermittent claudication or, critical limb ischemia. GH inhibits all these deleterious effects from cardiovascular risk factors, promoting the NO pathway that compensates redox imbalance, corrects endothelial dysfunction (increasing endothelial-dependent vasodilation), decreases inflammation, and stimulates angiogenesis and arteriogenesis. NO: nitric oxide; ROS: reactive oxygen species; TNF: tumor necrosis factor alpha; IL: Interleukin; GH: growth hormone; O2: oxygen; Blue crosses: stimulation; Red rectangles: inhibition.

292   To find biomarkers that can predict either the risk for suffering CVD or the risk for progression
293   is of high interest, but this is not the aim of this review. Thereby, we will address those biomarkers
294   related to GH that support its action and reduce the risk of CVD.

295   Most knowledge about this issue comes from the studies performed in acromegaly and GHD
296   patients. As mentioned, significant lower levels of VCAM-1 have been found in GHD patients than
297   in healthy subjects; moreover, they increase during GH treatment, as compared with patients treated
298   with placebo [24]. The development of GHD after the treatment of acromegaly affects adversely the
299   body composition and inflammatory biomarkers of cardiovascular risk [70].

300   Visceral adiposity and lipids are one of the best studied markers in CVD. The increase of NO
301   after GH administration lowers lipoxygenase activity and ox-LDL [4]. AGHD patients suffer an
302   elevated risk of CVD because of hyperlipidemia, among other factors. GH therapy in these patients
303   improves the lipid profile and decreases the vascular risk. The visceral fat is elevated in GHD children
304   and adults, perhaps because GH produces lipolysis, and when GH is administered it reverts this
305   increased adiposity [4,71]. Since GH secretion is deficient while aging, the progressive increase in fat
306   stores seen in the elderly population could be due, at least in part, to the insufficient secretion of the
307   hormone.

308   *1.4. Coronary Arterial Disease (CAD) and Heart Failure*

309   CAD is a broad term including several related syndromes caused by myocardial ischemia, an
310   imbalance between cardiac blood supply perfusion and myocardial oxygen and nutritional
311   requirements.

312   Cardiovascular disease (CVD) is the most important cause of death worldwide [72], and a major
313   economic global burden [73]. Despite reductions in CVD mortality in high-income countries, global
314   CVD mortality increased by 41% between 1990 and 2013, largely driven by rises in low-income and
315   lower-middle-income countries [74]. Among CVD, coronary arterial disease (CAD) is the leading
316   cause of death [72–75].

317   The majority of ischemic processes is produced by an alteration in the oxygen supply to the heart
318   due to coronary disease. The obstruction of the coronary arteries is usually of atherosclerotic origin,
319   although there are other infrequent causes such as an anomalous origin of these arteries, its
320   spontaneous dissection or embolisms [76].

321   As indicated before, atherosclerosis implies a degenerative inflammatory process where
322   different risk factors (diabetes, hypertension, dyslipidemia, smoking, obesity, sedentary lifestyle...)
323   damage the endothelium, favoring the entry of LDL particles that oxidize and initiate a complex
324   inflammatory and fibrotic process within the arterial wall that culminates with the development of a
325   plaque that can obstruct the coronary lumen, therefore preventing proper blood flow [76]. Although
326   the atherosclerotic process is usually chronic, abrupt plate instabilities can erode or ulcerate the
327   endothelium giving rise to a thrombotic phenomenon that can obstruct the coronary artery suddenly
328   causing an acute coronary syndrome (unstable angina or acute myocardial infarction). It is estimated
329   that throughout the world these processes are responsible for approximately 7 million deaths per
330   year, being the main cause of mortality in the population of industrialized countries [72–74].

331   The other major disease regarding cardiovascular system is heart failure (HF); it affects about
332   2% of the adult population worldwide. Its prevalence is clearly age-dependent, ranging from less
333   than 2% of people younger than 60 years to more than 10% of those older than 75 years, and it is
334   estimated that it will increase by 25% in the next 20 years [77–79]. The etiology of HF is diverse and
335   most patients have a history of hypertension, coronary artery disease, cardiomyopathies, or valve
336   disease, or a combination of these [77,78]. HF has a poor prognosis, with high rates of hospital
337   admission and mortality; costs related to the treatment of HF encompass 2–3% of the total
338   expenditure of healthcare systems in high-income countries, and it is believed that they will increase
339   by more than 200% in the next 20 years [79].

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*1.5. Peripheral Arterial Disease*

Peripheral arterial disease (PAD) is the term commonly used currently to refer to the atherosclerotic pathology affecting peripheral arteries of the lower extremity and compromising partially or totally the flow in them. Although less frequent that the other two main CVD, cardiac and cerebrovascular, it affects more than 200 million people worldwide [80]. Maybe, the spectrum of symptoms may vary from none (asymptomatic PAD, 3 times more frequent) to critical limb ischemia (CLI), the most severe form that threats the limb. However, the estimated prevalence depends on the tools used for the diagnosis. In people aged 60-70, the prevalence is about 8 % in the Spanish population [81]. For those aged > 70, it is generally accepted that the prevalence rises to 20%. Additionally, PAD is an independent predictor of cardiovascular mortality and morbidity [82].

*1.5.1. Endothelial and mitochondrial dysfunction in PAD: the role of oxidative stress*

As stated above, oxidative stress is the key aspect in producing the endothelial dysfunction that triggers the atherosclerotic process and the aging of the vascular system [56,83]. However, not only vascular risk factors contribute to this phenomenon, but also the own exercise leads to generation of superoxide-anion and other mediators of endothelial dysfunction, that it has been correlated with the clinical severity of PAD [84]. This endothelial dysfunction is not only located in the major arteries, but also in the microcirculation of the skeletal muscle [43]. Patients with PAD suffer a constant ischemia-reperfusion syndrome as they walk and rest, generating reactive oxygen species (ROS) that affect muscle fibers [84], and impairs mitochondrial function, reducing the energy production [85,86]. In fact, higher carbonyl and 4-hydroxy-2-nonenal levels have been found in calf muscle samples indicating the oxidative stress [87].

Skeletal muscle mitochondria release free radicals during the ischemic process, including superoxide-anions and some other ROS derived from the redox cascade [88,89]. Reperfusion also has the same effect, increasing the oxidative stress [89]. These ROS contribute to the endothelial dysfunction and the alteration of proteins in the skeletal muscle, and may lead to mitochondrial DNA injury in the long term [90]. This DNA injury is also seen in less affected limbs of patients with unilateral PAD, suggesting that PAD is not only a local problem, but rather a systemic one [91]

Mitochondrial pathways are vulnerable to free-radical injury [92], and PAD patients show reduced activities of complexes I and III of the mitochondrial respiratory chain [93]. These observations suggest that electron transport chain activity is impaired in PAD, probably because of the ischemia-reperfusion injury and old age, which spreads the oxidative damage and the metabolic dysfunction.

Lactate levels are also significantly elevated in PAD skeletal muscle, because of an incomplete oxidation of glucose, a decreased pyruvate dehydrogenase activity, and exercise performance [94]. At this point it is of interest to remark that GH is a mitochondrial protector [95–97], therefore suggesting that the hormone may play a positive role in this process, since GH restores the redox imbalance, improving mitochondrial respiratory chain and the needed production of energy.

In fact, endothelial dysfunction has been evaluated in Japanese patients with AGHD in the GREAT study. After 24 weeks of GH replacement therapy, the hormone significantly lowered plasma diacron-reactive oxygen metabolites and improved endothelial function measured by reactive hyperemia index [98]. This indicates that GH can exert a protective role in redox balance in AGHD, in which predominates a pro-oxidant environment increasing the atherogenic risk; but this is corrected by short-term GH administration without fully normalizing IGF-I levels [99]. Moreover, GH has a role in stress resistance by altering the functional capacity of the glutathione S-transferase (GST) system through the regulation of specific GST family members in long-living Ames dwarf mice. The hormone also affects the regulation of Thioredoxins (TRX) and glutaredoxins (GRX), factors that regulate post translational modification of proteins and redox balance, thereby further influencing stress resistance [100]. However, the exact role of GH in redox balance has not been completely understood, as in oxidative stress-induced conditions may enhance oxidation [101]. Thereby, both GH overproduction and deficiency are tightly linked with enhanced oxidative stress.

*1.5.2. Endothelin and PAD*

Endothelial dysfunction might be traduced by an imbalance between the endothelium-dependent vasodilation (mediated mainly by NO) and vasoconstriction (mediated by endothelin).

It has been well documented that vascular endothelin (ET) production is elevated in atherosclerosis and influences the development of atherosclerotic lesions through a variety of mechanisms [102]. ET participates in several key steps in the inflammatory component of atherosclerosis, increasing various cytokines from monocytes [103], and enhancing the uptake of LDL by these cells, promoting foam cells [104].

GH has been broadly related to an increase in the production of NO. However, GH is also related to ET, as an increased secretion of GH and ghrelin have been demonstrated in cattle after the injection of ET 1 and 3 [105,106]. Thus, GH increases physiologically in response to the increased level of ET. Despite the relationship between GH and ET has not been well established yet in CVD, it seems that GH may compensate the deleterious effects of ET, as the treatment with the hormone improves ET-induced stroke in adult rats [107]. Perhaps this is due to the actions of GH on NO production.

## 2. Discussion

GH plays a key role for the development of a normal heart during fetal development, and plays a positive role in maintaining the structure and function of the normal adult heart, by stimulating cardiac growth and heart contractility [108–110].

It is known that myocardium and vessels have receptors for GH and IGF-I, and IGF-I can be produced directly in these tissues [111,112]. Therefore, GH may exert endocrine roles on the cardiovascular system, as well as endocrine or auto/paracrine effects of IGF-I can be exerted on it. On the other hand, the GH/IGF-I axis can interact with the vascular system and can regulate the vascular tone and thereby peripheral resistance [113].

The interactions between heart and GH are complex. In fact, it has recently been shown that the heart may influence body growth in pediatric heart disease. In these situations, cardiomyocyte synthesize and release Growth Differentiation Factor 15 (GDF-15), which inhibits liver signaling by GH, therefore impeding the release of IGF-I and affecting body growth [8].

Life expectancy is reduced in patients with hypopituitarism as compared with healthy controls (2-fold higher risk of death for CVD, higher risk in women than in men). The causes of death are probably multifactorial, but GHD has been considered one of the most relevant factors of the increased mortality in these patients [114,115].

On the other hand, patients with acromegaly, despite presenting higher incidence of other cardiovascular risk factors (hypertension, insulin resistance), do not present a clear excess of CAD or stroke in comparison to normal counterparts [4,111,116].

Nowadays, despite multiple studies about the interaction of the GH/IGF-I axis and the cardiovascular system, the clinical importance of effects of GH and local and endocrine IGF-I in adults remains to be clarified.

*2.1. The role of GH in the vascular endothelium*

Two conditions in which the effects of GH on endothelial dysfunction might provide interesting data may be acromegaly and aging.

In the case of acromegaly, plasma levels of two biomarkers of endothelial dysfunction and atherosclerosis, such as endothelin-1 (ET-1) and total homocysteine levels (tHcy), were measured in patients with active acromegaly and cured disease [117]. While tHcy was similar in both groups of patients, ET-1 was significantly higher in active acromegaly, suggesting that it contributes to premature atherosclerosis and cardiovascular affectations observed in this pathology, although the role played by IGF-I on these vascular affectations could not be discarded.

Particularly important, in our opinion, is the case of aging. Important changes in pituitary GH secretion along the life have been widely described (for a more detailed comprehension, see references [4] and [6]. An exponential decline in plasma GH concentrations starts from 18 to 30 years of age, until it is practically imperceptible in elderly subjects, a phenomenon known as somatopause.

443    In this situation, plasma levels of IGF-I are also low, although the liver production of this peptide
444    depends not only on GH but also on the nutritional status of the organism [4].
445        Aging is associated with an increased risk of atherosclerosis, but we know now that this disease
446    can begin earlier, during youthfulness.
447        It has been proposed that the increased risk of atherosclerosis as we age, is due to low production
448    of EPC, which makes unable to repair atherosclerotic vascular walls [118]. Treatment with GH during
449    10 days led, in middle-aged subjects to an increase in plasma levels of EPC which, moreover,
450    improved in its capacity to migrate and incorporate into tube-like structures, and showed increased
451    endothelial NO synthase (eNOS) expression up to levels equivalent to those of healthy young
452    subjects. That is, GH treatment decreased EPC senescence and increased telomerase activity. In the
453    same study, aged mice treated during 7 days with GH or IGF-I increased EPC levels and ameliorated
454    EPC functions. This was not observed when GH treatment was given during only two days. Results
455    from that study attributed to IGF-I, rather than to GH, the reversal of age-dependent EPC dysfunction
456    [118]. We do not know whether these results appear as an IGF-I age-related effect, but other studies,
457    as described before in healthy young people, indicated that GH effects on the vascular system are not
458    dependent on IGF-I, postulating that GH acts directly on GHR and eNOS in the vascular endothelium
459    [9]. These contradictory results led to suggest that GH administration during somatopause does not
460    produce clearly favorable effects on the endothelial dysfunction, while combined treatments with GH
461    plus IGF-I may produce more beneficial effects on the vascular wall in elderly individuals [119];
462    however, we do not think that this combination is advisable.
463        Preclinical studies in hypophysectomized rats also showed that the lack of GH production is
464    associated with the development of atherosclerosis [120], while GH treatment during two weeks
465    reversed several biomarkers indicative of the developing arterial disease. These researchers identified
466    in the aorta of hypophysectomized rats 18 genes regulated by GH, which most likely have a
467    physiological effect on vascular tone and atherogenesis. Among these genes, they found that GH
468    induced an increased expression of the KATP channel, which plays a key role in the regulation of
469    vascular tone, therefore involving GH in this regulation [120]. However, plasma levels of GH must
470    be within normal ranges; since, as it occurs in acromegaly, transgenic mice overexpressing bovine
471    GH develop an endothelial dysfunction, which depends on the age of the animal and the type of
472    blood vessel, indicating that the affectation in endothelial function is most likely produced by
473    increased production of mitochondrial ROS followed by many other affectations in vascular function
474    [121].
475        Curiously, similar results to these shown in transgenic mice overexpressing GH, have been
476    reported in hypopituitary Ames dwarf mice aortas in terms of enhanced production of ROS and
477    lesser expression of antioxidant enzymes (for instance, glutathione peroxidase and eNOS), therefore
478    leading to vascular oxidative stress [122], a first step, as stated above, to develop endothelial
479    dysfunction.
480        Similarly, peripubertal GHD in Lewis dwarf rats leads to a pro-oxidative cellular condition most
481    likely responsible of the development of an altered vascular phenotype (in both structural and
482    functional terms), which leads to vascular affectations, early accelerated, later in the life of these
483    animals [123]. GH treatment reverses these impairments that, interestingly, do not occur equally in
484    the cerebral vessels than in the aorta of these genetically dwarf rats [123].
485        Another model for analyzing the effects of GH on the vascular system comes from studies in
486    which rats are undernourished during pregnancy. Maternal undernutrition produces increased
487    blood pressure and endothelial dysfunction in adult offspring, but if pups receive early pre-weaning
488    GH treatment (from day 3 after birth until weaning in day 21) adult vascular function is normal; this
489    contrasts with what happens in the offspring that received saline during these days before weaning.
490    This indicates that early GH treatment can reverse the vascular alterations resulting from maternal
491    undernutrition during pregnancy, but also that there is a developmental cardiovascular
492    programming, susceptible to be reversed by early treatment with GH after delivery [124].
493        While results from both preclinical and clinical studies clearly indicate that GH plays a key role
494    in the prevention or recovery of endothelial dysfunction, it is not clear at all which of the effects of

GH are due to a direct action of the hormone and which are mediated by IGF-I, because this peptide and its receptors (IGF-IR) are widely expressed in endothelial cells [125]. Moreover, GH induces the expression of IGF-I in many territories, including the fetal brain [126]; however, GH seems to be unable to increase the transcription of IGF-I in endothelial cells, and, in fact, systemic or local infusions of GH lead to a prompt increase in forearm blood flow and NO release in healthy humans without increasing plasma IGF-I concentrations or muscle IGF-I expression [9,127]. The fact that it seems that GH is produced by endothelial cells, and endothelium-derived GH stimulates the proliferation, migration, survival, and capillary formation of endothelial cells in an autocrine manner [11], clearly indicates that the hormone exerts direct effects on the vascular endothelium, although IGF-I is also vasoactive activating eNOS via Pi3K/Akt [127], a signaling pathway also used by GH.

Perhaps some of the apparently contradictory results here reported, in relation to the lack of effects of the administration of GH on the vascular endothelium, in AGHD and GHD [23,26], and the attribution to IGF-I rather than to GH the positive effects on the vascular wall, proceed from the recently described relationships between GH, IGF-I and Klotho [7,40], but also on the effects of Klotho on the vascular endothelium and aging.

The impact of GH on inflammatory processes is not well understood yet. Evidence shows controversial data of both anti- and pro-inflammatory effects of the hormone.

GH therapy reduces the levels of CRP in GHD patients [128], and exerts anti-inflammatory effects in different experimental models of sepsis by lowering TNF-$\alpha$ [129]. Exogenous GH also may improve the effects of sepsis-induced IGF-I resistance [130]. Conversely, a massive increase of GH in GH transgenic mice has a pro-inflammatory effect, rising pro-inflammatory cytokines [130].

As it has been described above, the relationship between GH and CAMs is well known. This action seems to be indirect, mediated by VEGF among others. VEGF has been described as a strong-inducing agent of CAM on endothelial cells during inflammation [131]. Given the fact that GH directly increases VEGF levels after its administration, this peptide could be one of the main mediators of the GH effects. It seems, thereby, that high supraphysiological administration of exogenous GH could increase inflammation, while doses used for treating GHD, or even short-term GH administration to non-GHD patients may represent a protective factor against this issue.

The role of CRP, IL-6 and TNF-$\alpha$ in CVD has also been well established [44,48,132].

In AGHD patients, the administration of GH decreased CRP and IL-6 levels, some that not occurred when treating them with placebo [133]. However, another study in AGHD, showed that GH therapy also reduced CRP, but failed in reducing TNF-$\alpha$ and IL-6 levels [128].

Pregnancy associated plasma protein A (PAPP-A) has been recently included among markers of cardiovascular risk being associated both to the presence of carotid atherosclerosis and acute coronary syndrome [134,135]. PAPP-A is also significantly elevated in AGHD [136], and GH replacement therapy decreases this specific and not generic biomarker of CVD, although not in all GHD patients [137].

At this point, the own GH could be a marker, as the deficit of both GH and IGF-I leads to a more aggressive heart failure, with impaired functional capacity and poor outcomes [138].

All these data support the possible role played by GH in the correction of the state of inflammation in patients suffering from CVD, at least in those with AGHD. The protective effect of GH in inflammation is secondary to its action against oxidative stress, most likely because of its action on NO and extracellular signal-regulated Kinase (ERK) pathway.

Currently, our group is conducting a phase III RCT in patients suffering PAD without options for revascularization: *Growth Hormone Angiogenic Study (GHAS)*, Eudract 2012-002228-34, approved by the Spanish Agency of Drugs and Health Products (AEMPs) and the Autonomic Committee on Research Ethics in Galicia (CAEIG, 2012/378), Spain, in which patients receive GH or placebo.

Although this study has yet finished, all the data have not yet been analyzed and therefore there are no statistical conclusions, early results from the patients enrolled so far show that TNF-$\alpha$ is the marker most frequently elevated in these patients (74%), followed by $\beta$2-microglobulin (B2M) (69%) and C Reactive Protein (CRP) (60%). Figure 4 depicts the graphic tendencies that show these patients depending on the group of treatment.
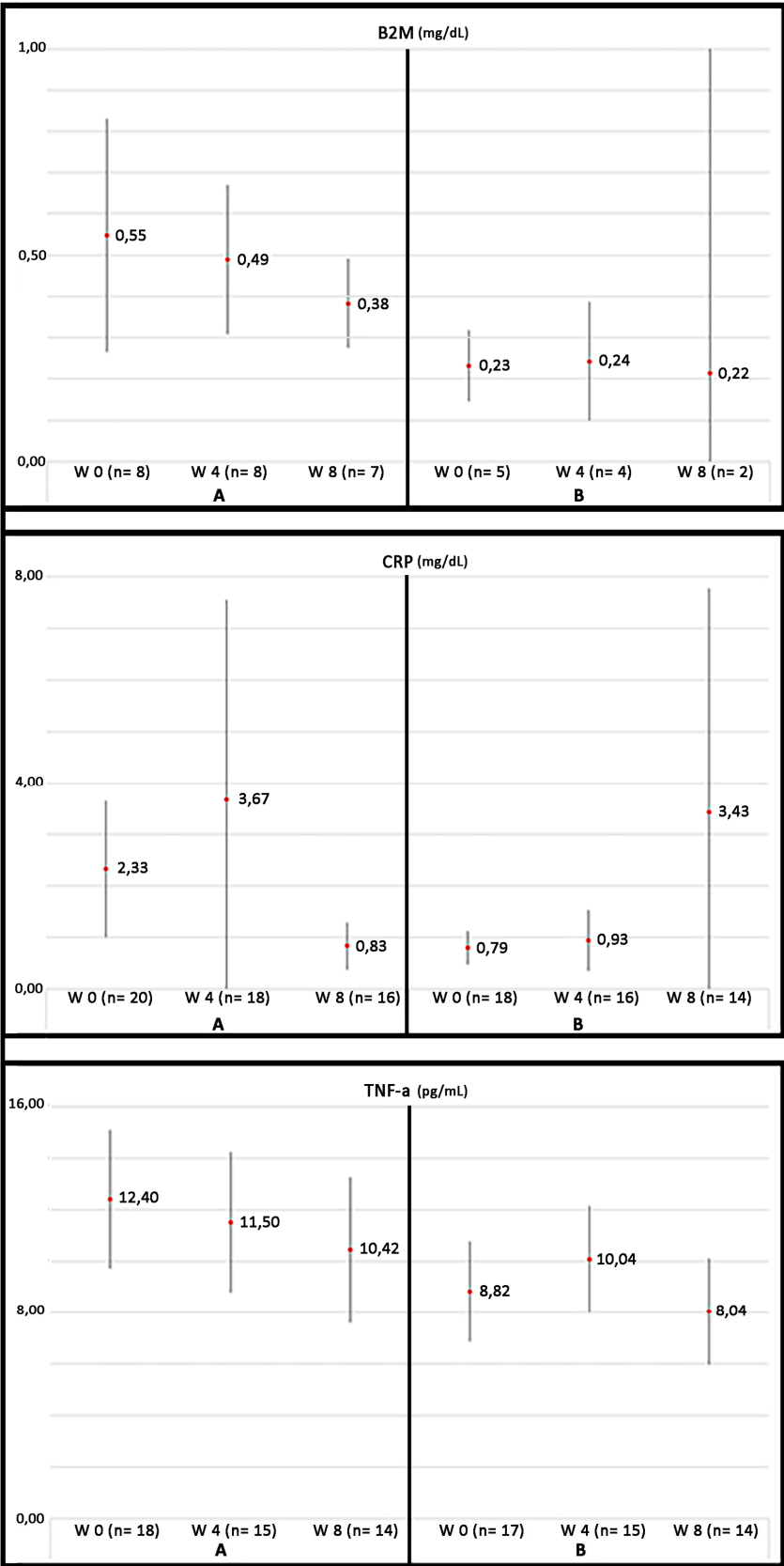
**Figure 4.** Evolution of some biomarkers of inflammation analyzed in the GHAS study. Values are shown as the mean ± SD. B2M (beta-2-microglobulin), CRP (C Reactive Protein) and TNF-$\alpha$. **A** and **B** represent different groups of treatment (GH or placebo, respectively). Note the tendency to decrease in group A as compared with the group B. Patients from group A had significant higher basal levels of markers, indicating that patients in this group suffered from a more severe inflammatory disease

553    as compared with group B. Significant differences in the end of the study have not reached because
554    of the small sample of patients still analyzed (note the differences in n). W = weeks of treatment.

555    *2.2. GH and Coronary Arterial Disease*

556    The effects of GH /IGF-I in the incidence and prognosis of CAD are controversial.
557    As described before, GHD is associated with an increased prevalence of atherosclerosis, CAD
558    and stroke caused by an increased prevalence of atherosclerotic risk factors such as alterations of
559    body composition, lipid profile and coagulation pattern [4,111,116], as shown in Figure 5.

560
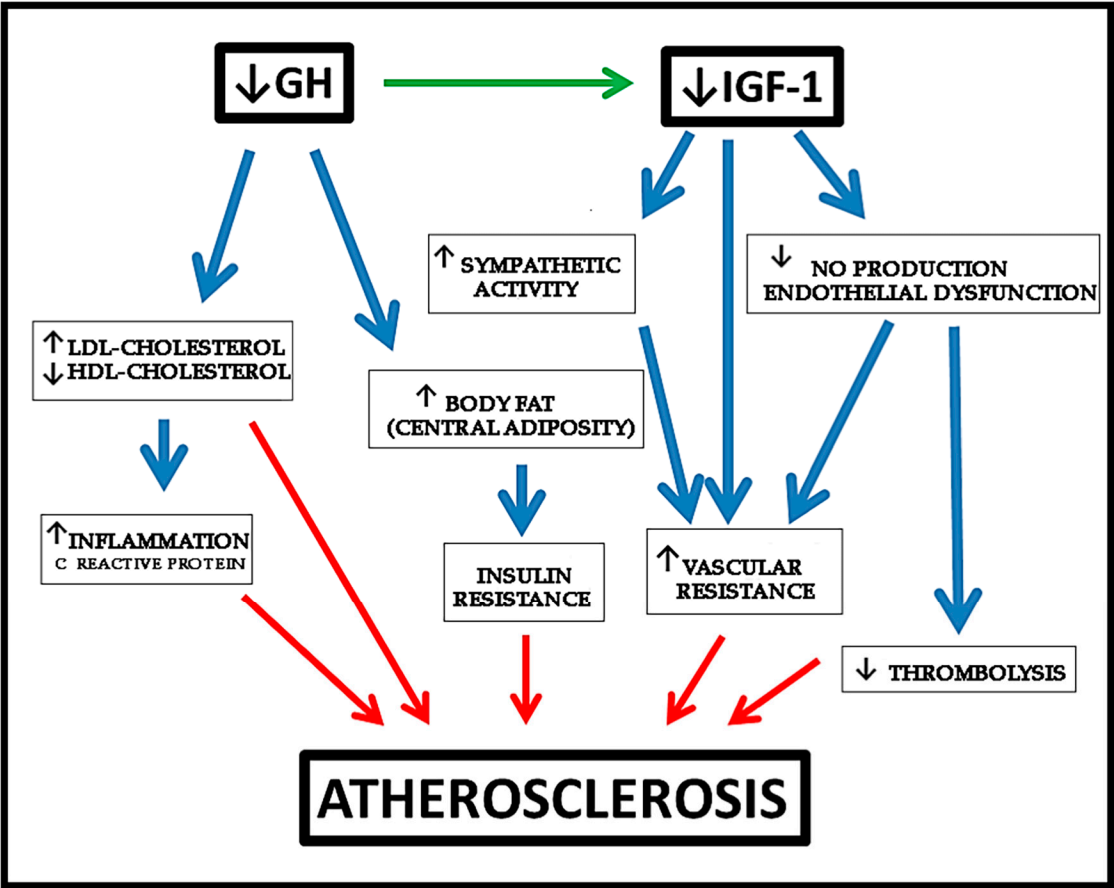


561    **Figure 5.** Effects of GH deficiency on atherosclerosis. GH: Growth Hormone, IGF-1: Insulin growth
562    factor 1. NO: nitric oxide. Blue arrows indicate the effects produced by decreased GH secretion, while
563    red arrows indicate how atherosclerosis is developed.

564    AGHD often have significant changes in their lipid profile with increased LDL, increased
565    triglycerides, decreased high-density lipoprotein (HDL) (the latter observed only in women), with no
566    differences in lipoprotein (a) [139]. GH replacement positively reverses this negative lipid profile in
567    GHD patients, decreasing LDL and total cholesterol, and increasing HDL; in addition, a decrease in
568    CRP has been observed in these GHD after GH replacement therapy, while no clear changes seem to
569    be produced in circulating triglycerides [139–141]. However, no study has determined whether GH
570    has an additive effect that optimizes statin therapy; therefore, this remains an open question.
571    Regarding hypertension and peripheral resistance conflicting results have been reported in the
572    literature [116]. Hypertension is quite frequent in GHD patients, and this condition results in
573    impaired vasodilation responses to stress and/or exercise. As described, the GH–IGF-I axis promotes
574    the synthesis of NO that reduces vascular tone, inhibiting the proliferation and migration of SMC,
575    reduces platelet adhesion, and decreases lipoxygenase activity and ox-LDL [142]. Some vasoactive
576    effects of GH may have central origin. In fact, GHD patients have markedly increased muscle

577  sympathetic nerve activity and GH replacement therapy has been shown to reduce arterial stiffness
578  and to improve vascular endothelial function [143].
579      In some AGHD (patients with high base-line diastolic blood pressure, such as elderly GHD
580  patients or those with previous Cushing´s disease), GH replacement reduces blood pressure, whereas
581  in other patients (specially in young GHD patients) no changes in blood pressure have been shown
582  [116,144].
583      Besides the cardiovascular risk factors mentioned above, GHD patients were shown to have
584  increased blood vessel intima-media thickness (IMT) that is well known to represent one of the
585  earliest morphological changes in the arterial wall in the process of atherogenesis [145].
586      A decrease in IMT has been shown in several studies after the administration of GH to GHD
587  patients [146]. Increases in IMT predict the development of symptomatic coronary disease, thus GH
588  treatment may have a significant improvement in cardiovascular outcome, but this question has not
589  yet been specifically analyzed in patients with GHD.
590      Regarding hard clinical endpoints, we previously commented on the increased risk of
591  cardiovascular mortality in GHD patients. The worse cardiac risk profile (mainly hyperlipidemia) of
592  these patients may explain part of the excess in CAD and mortality, but the studies do not allow to
593  obtain a definitive conclusion. However, as described, GHD patients present an altered body
594  composition with increased fat mass, with a preferential increase in visceral fat that decreases in
595  response to GH therapy. This change occurs within 6 months after the initiation of therapy, and it is
596  maintained if treatment is continued [146].
597      Interventional studies are old, and they did not control cardiac risk factors. There are no
598  prospective, long-term randomized studies in AGHD patients comparing GH treatment to placebo
599  on cardiovascular hard outcomes and mortality, and it is likely that there will never be such a study.
600  A more recent and prospective trial found a lower mortality in GH treated hypopituitary patients
601  compared with a retrospective analysis of patients who had not been treated with GH [147].
602  However, again the different time periods covered also included dramatic changes in the treatment
603  of risk factors such as hypertension, diabetes mellitus and hypercholesterolemia.

604  *2.3. GH and heart failure*

605      As stated, GH plays an important role during myocardial development that can easily be seen
606  in untreated GHD children. They present cardiac atrophy with a reduction in the left ventricle (LV)
607  mass, ejection fraction, and cavity dimensions, as well as reduced cardiac output, high peripheral
608  vascular resistance and reduced functional capacity compared with healthy controls of the same age,
609  sex, and height [148]. When GHD appears in adults, it does not produce a reduction in cardiac mass,
610  but cardiac performance and exercise capacity are impaired [149].
611      On the other hand, GH excess exerts different and opposite effects on the heart. In early-stage it
612  enhances cardiac performance, whereas it causes fibrosis and cardiac dysfunction in the
613  intermediate-late phase. This apparent discrepancy is easily clarified: a physiological GH level, or
614  short-term excess, exert positive inotropic effect; whereas long-term exposure to GH excess induces
615  cardiac dysfunction and progression to heart failure by causing morphological and functional
616  adaptive changes [150]. The most relevant histological abnormalities are interstitial fibrosis, reduced
617  capillary density, increased extracellular collagen deposition, myofibrils derangement, lympho-
618  mononuclear infiltration and myocyte death due to necrosis and apoptosis [150,151].
619      GH acts directly stimulating its own receptors or (mainly) by inducing local synthesis of IGF-I
620  and may regulate cardiac growth and metabolism by increasing protein synthesis (troponin I, myosin
621  light chain-2, and actin), and cardiomyocyte size, increasing collagen synthesis and promoting
622  cardiac hypertrophy [151–153]. There is also evidence about that IGF-I may reduce apoptosis of
623  cardiomyocyte, preventing myocyte loss [152]. The GH/IGF-I axis can also increase cardiac
624  contractility by enhancing calcium sensitivity and reducing vascular resistance [149,151].
625      Chronic heart failure (CHF) patients have a prevalence of 30% in GHD and this fact identifies a
626  subgroup of CHF patients characterized by impaired functional capacity, left ventricle remodeling
627  and elevated natriuretic peptide levels and increased all-cause mortality [154].

628 Several groups have studied the effects of GH and IGF-I in patients with impaired cardiac
629 function. GH replacement trials show an increase in left ventricular mass and improvement in cardiac
630 performance, diastolic filling, and systolic function after GH treatment in children or adults with
631 GHD [65,148,151]. Nevertheless, randomized placebo controlled studies show conflicting results,
632 with an increase in LV mass related to serum IGF-I levels, but no change in LV wall stress, arterial
633 blood pressure, ejection fraction, clinical status or 6-minute walking distance [155].

634 The conflicting results of the clinical trials of GH treatment may be related to the small number
635 of patients enrolled, the different dose and duration of GH treatment, the different cardiac heart
636 failure etiologies, and differences in the patients' clinical characteristics. Besides this, the
637 discrepancies may also reflect the heterogeneity of IGF-I increase in response to GH treatment. In
638 fact, a recent meta-analysis confirms that there is a clear relationship between changes in IGF-I
639 concentrations achieved and the beneficial effects of GH treatment. Only in the trials in which IGF-I
640 increased >89% vs. baseline was there a significant improvement in cardiac output,
641 echocardiographic parameters and exercise capacity, whereas in the trials in which the increase in
642 IGF-I was < 89% beneficial cardiovascular effects were not observed [156].

643 Given its possible positive effects on heart in "responders" patients, it could be speculated that
644 GH treatment might be useful in some patients with heart failure but more investigation is needed in
645 this field.

646 *2.4. GH and molecular aspects of cardiovascular risk factors in PAD*

647 Despite the known negative epidemiological impact of cardiovascular risk factors, its main
648 mechanism of damage is not completely clarified. However, it seems that they may modify redox
649 balance. Since the specific role of each cardiovascular risk factor in redox balance has been widely
650 described, as well as the benefit of their treatment, we will only underline the main aspect related to
651 GH and its possible role and benefit.

652 As described before, GH therapy improves arterial hypertension in GHD patients by acting on
653 the vascular smooth muscle ATP-sensitive potassium (KATP) channel [120], and on the lipid profile
654 with independence of IGF-I [157].

655 Maybe a closer attention would need diabetic patients (DM), in which a dysfunction of eNOS in
656 both endothelial cells and platelets has been found, which attenuates arterial remodeling [158,159].
657 But the latter is also affected because of the lower sensitivity to shear stress that these patients show.
658 This aspect seems to be secondary to the massive calcification and multilevel arterial disease, as well
659 as to the elevated vasomotor tone found that impairs the response to the vasodilator stimuli, and the
660 enlargement of collateral arteries [158–160]. Moreover, a quantitative and qualitative alteration in
661 EPC has been described in DM [158,161]. All these factors explain both the strong atherosclerotic
662 injury and the low capacity of compensating the latter after an arterial occlusion.

663 In addition, a high rate of patients with DM may suffer neuropathy. In these patients, the level
664 of expression of several growth factors, such as neurotrophic factors, insulin-like growth factors,
665 cytokine-like growth factors and VEGF, are altered [161].

666 GH could aid in the recovery of some of these deleterious aspects in diabetic patients, since, once
667 again, the hormone increases eNOS production, decreases vasomotor tone, and may improve the
668 nerve injury, by increasing neurotrophic factors, such as BDNF. Moreover, GH can increase
669 Substance P (SP), one of the main molecules implied in nerve damage and wound healing. SP and
670 GH are strongly related [162,163]. In fact, GH improves wound healing in diabetic rats and mice [164],
671 and SP could be one of the possible mediators. Since a high rate of diabetic patients have small vessels
672 disease, an angiogenic therapy with growth factors might be a good option for them. GH angiogenic
673 stimulation and benefit in patients suffering from PAD will be comprehensively reviewed below.

674 Although GH may cause hyperglycemia or abnormal glucose tolerance, this is not a
675 contraindication for using the hormone in diabetic patients, since clear benefit of GH therapy has
676 been described in these patients.

677

678    *2.5. GH, age, and cardiovascular disease*

679    The relation of GH and age requires a specific section, since age associates with both
680    atherosclerosis and GH deficiency.

681    Aging is a biological process that causes progressive deterioration of structure and function at
682    the cellular level over time. Both cardiovascular structure and function are under a continuous
683    remodeling process as we age.

684    There are two aspects responsible for the pathophysiological changes in the aged vascular bed:
685    the impaired intrinsic cellular mechanisms to resist ischemic injury [165], and the impairment of
686    vascular angiogenic capacity and endothelial function [166]. Evidence from animal model of hind
687    limb ischemia has demonstrated a reduced capability to protect tissues from ischemic insult, and an
688    impaired ability to establish collateral circulation in the aged when a major artery is occluded [167].

689    Aging may attenuate both angiogenesis and arteriogenesis, producing less proangiogenic
690    cytokines or increasing the expression of antiangiogenic factors [167]. For instance, TNF-$\alpha$, that
691    promotes apoptosis in endothelial cells, is upregulated in cultured aged endothelial cells [168], and
692    Thrombospondin (TSP), a substance with antiangiogenic effect, increases its expression in healthy
693    aged tissues [169].

694    As it will be seen latter, EM plays a key role in angiogenesis. Aging alters the expression of
695    Metaloproteinases (MT-MMPs), integrins and structural proteins in EM. Tissue inhibitor of MT-
696    MMPs (TIMP-1 or TIMP-2) selectively inhibits MT-MMPs and has a negative impact on angiogenesis
697    process. Higher levels of TIMP-2 were found in the aged endothelial cell lines than in the young ones
698    [167]. During angiogenesis, EPC are of importance. Both mice and human subjects have an age-
699    dependent impairment of EPC. Middle-aged and elderly subjects had lower circulating
700    CD133+/VEGFR-2+ EPC with impaired function and increased senescence [170].

701    Arteriogenesis is also affected with aging, as collateral blood flow expansion has been seen to be
702    delayed in aged rats with bilateral femoral artery occlusion [171], perhaps because of an attenuated
703    sensitivity of the receptors for shear stress secondary to a less activation of Rho pathway, one of the
704    main arteriogenic signal pathways [167].

705    Vascular aging is also characterized by increased mitochondrial ROS production in endothelial
706    cells, which, in turn, decreases the bioavailability of the vasodilator and anti-apoptotic NO, increases
707    cardiac oxygen demand, and promotes vascular inflammation by inducing nuclear factor kappa- B
708    (NF-kB). eNOS expression or its activity have been shown to be decreased in the cardiovascular
709    system of aged animals [172], and in cultured aged human umbilical vein endothelial cells [168].
710    However, at skin level, aging alters tissue inflammatory response slowing down wound healing
711    [173].

712    Despite all this, elderly people still respond to physical or biochemical stimuli (exercise or
713    exogenous angiogenic growth factors), which improve the angiogenic and arteriogenic responses.
714    NO-donors as nitrates, and angiogenic growth factors, work equally in the ischemic tissues of animals
715    of old age [167].

716    Aging might be a factor that contributes to the unsatisfactory results (not better than the placebo
717    group) of recent clinical trials intended to expand collateral vasculature in the ischemic legs with
718    angiogenic growth factors [174].

719    It has been highlighted before that aging also has a strong impact on GH production, decreasing
720    its pulsatile secretion both in number and amplitude, until it is imperceptible [175]. This fact can
721    contribute to an imbalance between pro- and anti-angiogenic factors favoring the latter. GH
722    administration to old animals and humans raises plasma IGF-I, increases skeletal muscle, improves
723    immune function, and cardiovascular function. Therefore, the relationship between age-related
724    changes in cardiovascular function and the decline in GH levels with age is awakening interest. Either
725    in aged mice and in human, the hormone reverses many of the deficits in cardiovascular function
726    [175].

727    All these are some of the reasons why the administration of GH to elderly people has been
728    proposed [176], though it must be balanced with its possible side effects.

*2.6. GH and neovascularization: experimental and clinical evidences*

That GH has a positive role on the vascular system has been broadly set out here and demonstrated in experimental and clinical studies. One typical example is that cerebral microvasculature decline with age is parallel to that in GH/IGF-I, and the administration of the hormone to aging rats increases the number of cerebral cortical arterioles [177]; or, as mentioned above, the novel role discovered for the hormone in stimulating wound healing, mainly because its angiogenic action and its capacity for promoting myofibroblast differentiation [164,178]. In fact, the skin of GHD patients has reduced capillary density and permeability, which improves after they receive GH treatment [179].

One of the more attractive action of GH related to this issue is the mobilization of EPC into the bloodstream [21,170]. It seems that this action is not a direct stimulation of bone marrow, but an indirect effect via VEGF, SDF-1 or erythropoietin (EPO), among others [21]. It has been demonstrated an impairment of EPC with aging, and that the latter is corrected after GH therapy mediated by IGF-I [170].

GH may have direct, non-IGF-I mediated, actions on endothelial cells, as the promotion of the expression and activity of eNOS [180,181]. Moreover, systemic or local infusions of GH acutely produces vasodilation in the forearm and NO delivery in healthy humans, without producing significant changes in plasma IGF-I levels or in muscle IGF-I mRNA expression [9,182].

In muscle samples of PAD patients from our GHAS study, we found a significant increase of eNOS mRNA expression in patients treated with GH as compared with those treated with placebo [183].

IGF-I may also mediate the proangiogenic actions of GH, as its receptors are expressed in endothelial cells, and it stimulates angiogenesis both in vivo and in vitro [184]. In rats with myocardial infarction, the myocardial overexpression of IGF-I was induced after delivering a human IGF-I gene by means of an adeno-associated viral vector [185]. This study demonstrated that the angiogenic process, measured by micro-SPECT-CT 16 weeks after administering the gene, persisted over time, leading to an improvement of the capillary network in rat hearts, a decreased left ventricle remodeling and an improved cardiac function.

In line with these IGF-I effects, the local infusion of the IGF-I plasmid in skeletal muscle tissue following ligation of the femoral artery in mice leads to angiogenesis and raises the blood flow in the affected muscle [186]. Several studies indicate that IGF-I is a strong inducer of angiogenesis in different tissues, including the brain [187–189], protecting them from ischemia-induced apoptosis and inducing local expression of VEGF, as seen in cultures of ovarian granulosa cells [190].

Nonetheless, the systemic infusion of IGF-I causes negative collateral effects, such as hypoglycemia, hypotension, edema and tachycardia [191], the latter probably occurring because of the onset of hypoglycemia and/or hypotension. Furthermore, IGF-I is a mitogenic hormone with a marked oncogenic potential; hence, its long-term use in myocardial or arterial diseases should be avoided or carefully controlled.

Although the hepatic (and that of many other tissues) expression of IGF-I is mainly dependent on GH, it does not take place if there is not an adequate liver metabolism of glucose [4]. Furthermore, since plasma levels of IGF-binding protein 3 (IGFBP3), a main carrier of IGF-I, are strongly dependent on GH, the bioavailability of IGF-I (free IGF-I), established by the IGF-I/IGFBP3 ratio, would not entail a major problem in the case of GH treatments, whenever it is well controlled.

As mentioned above, the angiogenic effects of GH do not only depend on its direct and IGF-I mediated actions on endothelial cells, or on EPC, but also on its indirect effects mediated by the induction of several growth factors such as VEGF, FGF, EGF, BDNF, EPO, and some cytokines [71]. Besides, GH is capable of interacting with receptors for prolactin (PRL) which can trigger proangiogenic signals [192].

It is of interest, the relationship between GH and C-X-C motif chemokine ligand 12 (CXCL12) or SDF-1, since both molecules orchestrate vasculogenesis, and share CXCR4 receptor present in pituitary somatotrophs [193]. Both also activate the JAK/STAT pathway, responsible for endothelial

migration and differentiation during angiogenesis [193]. GH stimulates the secretion of SDF1 and the latter promotes GH delivery from the anterior pituitary gland [193–195].

GH may also enhance arteriogenesis. Collateral arteries enlargement is mediated by several pathways as Rho and RAS-ERK pathways, both regulating cell proliferation and migration, respectively, and the NO pathway, which partially controls endothelial function and leukocytes adhesion [196]. The GH/IGF-I axis boosts the eNOS enzyme [21,170,179,180,197,198], increasing NO which reduces vascular tone, and in parallel inhibits the proliferation and migration of SMC and reduces platelet adhesion [142,199–202], leading to a control of the angiogenic process. Despite this, some models of PAD have no proven a real depletion of NO, but rather a marked insensitivity to the latter due to redox imbalance [203]. Figure 6 schematizes these events.
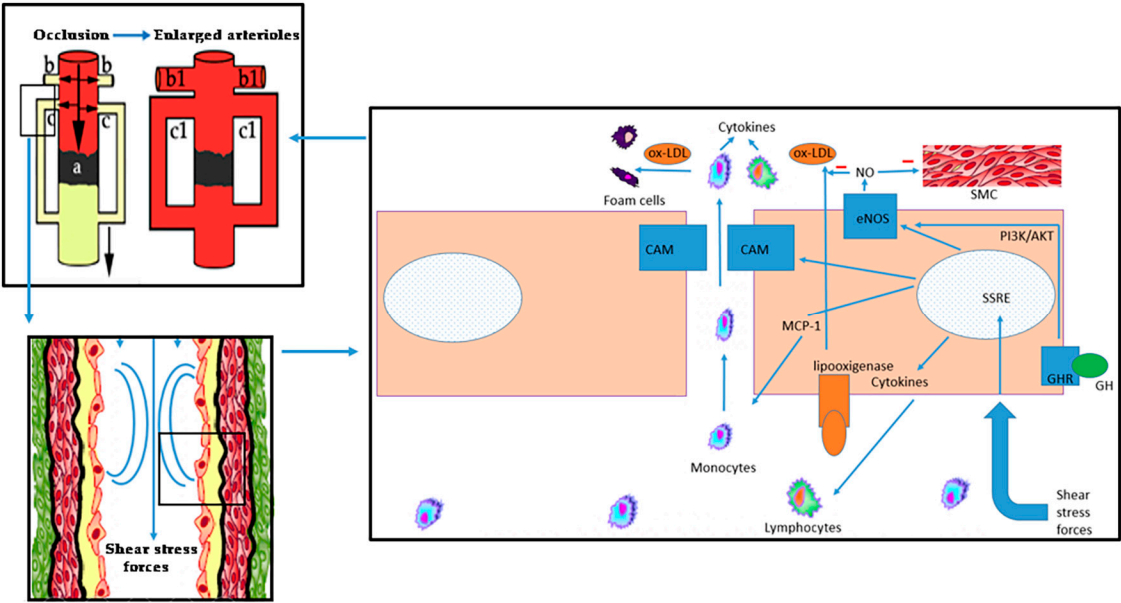


**Figure 6.** Mechanism of arteriogenesis. Oxidative stress at endothelial cell level produces LDL oxidation (ox-LDL) and foam cells formation that compose atheroma plaque, and, in the end, determine the vessels occlusion. After an arterial occlusion (a), the increase in shear stress forces through the collateral vessels (b, c) activates shear stress genes (SSRE) triggering the arteriogenic response. Adhesion molecules (CAM) and some cytokines such as MCP-1 are produced by the endothelial cells, attracting monocytes and lymphocytes from the blood to the vascular wall that start up the vascular remodeling (b1, c1). eNOs is also activated, increasing NO that produces vasodilation, inhibits SMC growing and the oxidation of LDL molecules. These facts lead to the control of the atheroma plaque, lowering oxidative stress. GH contributes to increase NO pathway by activating the Pi3K-AKt pathway. NO: nitric oxide; eNOS: endothelial nitric oxide synthase; ox-LDL: oxidized low density lipoprotein; GH: growth hormone; GHR: GH receptor; SSRE: shear stress response elements (promoter sequences that mediates the responsiveness of endothelial genes to shear stress); SMC: smooth muscle cells. Blue arrows indicate stimulation.

Besides, GH may have vasoactive actions acting on the autonomic nervous system, since GH therapy may reverse sympathetic hyperactivity in patients with GHD [204]. The removal of sympathetic constrictor tone from arterial walls, along with the increased blood flow in the denervated area may stimulate vessel enlargement, consequently leading to arteriogenesis.

The stimulation of VEGF by GH is another fact supporting the possible role of the hormone in arteriogenesis, since VEGF up-regulates CAMs, which are key factors for the development of collateral arteries [199].

812   MCP-1 and T-lymphocytes also mediate vascular remodeling during arteriogenesis [199]. GH
813   strongly induces these cells [4,205,206]; for instance, it has been found that when GH is administered
814   exogenously, there is a rise in MCP-1 mRNA [205]. Moreover, would healing would benefit of the
815   induction of immune system by GH. However, GHD in humans does not frequently associate with a
816   significant affectation in immune system, as GHD in animals does [206], possibly because of the
817   locally produced GH.
818   Both arteriogenesis and angiogenesis, are affected by age, mainly because of the alteration in
819   EPC, and the parallel depletion in GH with aging has been related to the latter impaired processes.
820   Physiological anti-angiogenesis is favored as we age, and a GH therapy for elder people has been
821   proposed to compensate this imbalance [176]. Apart from its metabolic effects, a therapy with this
822   hormone would improve the eNOS system dysfunction and, hence, the arteriogenic and angiogenic
823   mechanisms. If we consider that most patients suffering from PAD are of old age, this therapy could
824   be justified at least as a complementary treatment, provided it has been found that there are no
825   significant contraindications, such as a severe illness, sepsis or a tumor, among others. Maybe it
826   would be necessary to define the most profitable type of therapy with the hormone. In any case, GH
827   treatment does not need to last long time for showing significant benefits. Moreover, they can be
828   interrupted during some months and resumed later.

829   *2.7. Effects of GH on nerve dysfunction and other abnormalities in the ischemic muscle.*

830   Ischemia also damages lower extremity nerves in PAD patients, causing functional impairment.
831   The observation of muscle denervation in these patients supports the idea that arterial flow
832   insufficiency coexists with distal motor neuron neuropathy that worsens muscle function [207].
833   However, cross-sectional studies have shown conflicting results, probably due to small sample sizes.
834   WALCS II study has found an ABI-dependent effect in non-diabetic PAD patients on the nerves.
835   Patients with ABI < 0.5 was associated with poorer peroneal nerve conduction velocity. For those
836   being diabetic, the damage to the nerves was simply related to the fact of suffering or not PAD [84].
837   In this sense, the actions of GH treatment on neurogenesis and peripheral nerve recovery after
838   an injury have been described [208]. Therefore, GH administration might also be useful in this nerve
839   affectations in PAD patients.
840   However, patients with PAD show many other changes in their lower extremities skeletal
841   muscles, among them: muscle apoptosis and atrophy, increased fiber type switching, altered myosin
842   heavy-chain expression, and muscle fiber denervation [209,210]. All these changes impair exercise
843   tolerance and performance, and could be produced by the greater inflammatory response that these
844   patients present [84]. In samples from the gastrocnemius muscle of PAD patients, caspase-3 levels
845   are twice as high as in control patients [210].
846   The role of GH in increasing the skeletal muscle mass has been broadly described. The GH/IGF-
847   I axis constitutes an important physiological regulatory mechanism for coordinating postnatal
848   skeletal muscle expansion and hypertrophy. The administration of GH to both animals and GHD
849   humans improves muscle strength [211,212]. Considering that most of patients with PAD are aged
850   people with a physiologic GH deficiency, and that sarcopenia appears along aging, GH therapy might
851   be useful for recovering muscle mass and performance [4]. GH activates the IGF-I-Akt-mTOR
852   pathway in the skeletal muscle, which mediates both differentiation in myoblasts and hypertrophy
853   in myotubes, and inhibits myostatin-dependent signaling [213,214]. Thereby, in this regard, GH could
854   also help in the recovery of PAD patients.

855   *2.8. Why GH treatment could not work properly in cardiovascular disease?*

856   With independence of all described GH vascular actions, the final vascular effects will depend
857   on the local environment in which the hormone tries to act. In this sense, high levels of GH can be
858   seen without angiogenesis stimulation. In fact, some harvested endothelial cells does not proliferate
859   with GH [192]. The presence of other angiogenic agents, such as IGF-I, NO, VEGF and even the
860   autocrine GH itself may determine the final effect of the exogenous GH. The autocrine hormone could
861   saturate the GHR, thus avoiding the action of endocrine or exogenous GH. Additionally, when GH

862  is internalized, it may suffer a proteolytic cleavage, generating vasoinhibins, being then inactivated.
863  This helps to the balance between growth and regression of blood vessels under physiological
864  conditions, especially in the female reproductive system. The role of vasoinhibins has been reviewed
865  in detail [192].
866       The suppressor of cytokine signaling (SOCS) family is also involved in the regulation of GH
867  signaling. This relationship is not still well understood, since, for example, high levels of SOCS2 up-
868  regulate GH signaling, while less concentrations of the cytokine inhibits GH activity [214]. In PAD
869  patients, pro-inflammatory IL-1$\beta$ or TNF-$\alpha$, and endotoxins, are frequently increased, because of the
870  disease and infection at the level of the foot, and these cytokines may stimulate SOCS proteins,
871  promoting a GH insensibility.

872  *2.9. Adverse effects of GH treatment*

873       Although GH opposes to the effects of insulin and might produce hyperglycemia and diabetes,
874  this is not a clear contraindication for the possible use of the hormone in the case of diabetic patients
875  with PAD. The incidence of diabetes in the GHD population treated with the hormone is very low.
876       Data from more than 23.333 young people aged 10-19 treated with GH for growth disorders for
877  a mean of 2 years showed an incidence of 46.3/100.000/year of GH therapy of type 2 diabetes. In old
878  people and children is even lesser [216]. Although some physicians remain unsure about using GH
879  for this reason and because the incidence of abnormal glucose tolerance is higher, this is not a strong
880  argument to contraindicate the hormone therapy in diabetic patients, as a recent study reported that
881  GH and its receptor regulate the pancreatic $\beta$-cell survival and insulin secretion in rats [217]. GH
882  corrects insulin sensitivity and long-term glycemic control without altering HbA1C levels
883  [216,218,219]. In addition, the plasma half-life of GH is very short (approximately 30 minutes) and
884  the slightly increased glycemia that the hormone produces, may be minimized by exercise, since the
885  working muscle has a great avidity to capture glucose in an insulin-independent manner.
886       In fact, in our GHAS study 65% of the patients suffered diabetes, but we did not find significant
887  differences between patients treated with GH or placebo in the levels of glycemia or HbA1C from the
888  beginning of the study to the end of it. This supports the fact that the treatment with GH during short-
889  time periods does not produce glycemic alteration and may be administrated in diabetic patients with
890  PAD, improving the arterial disease, as Figure 7 shows.

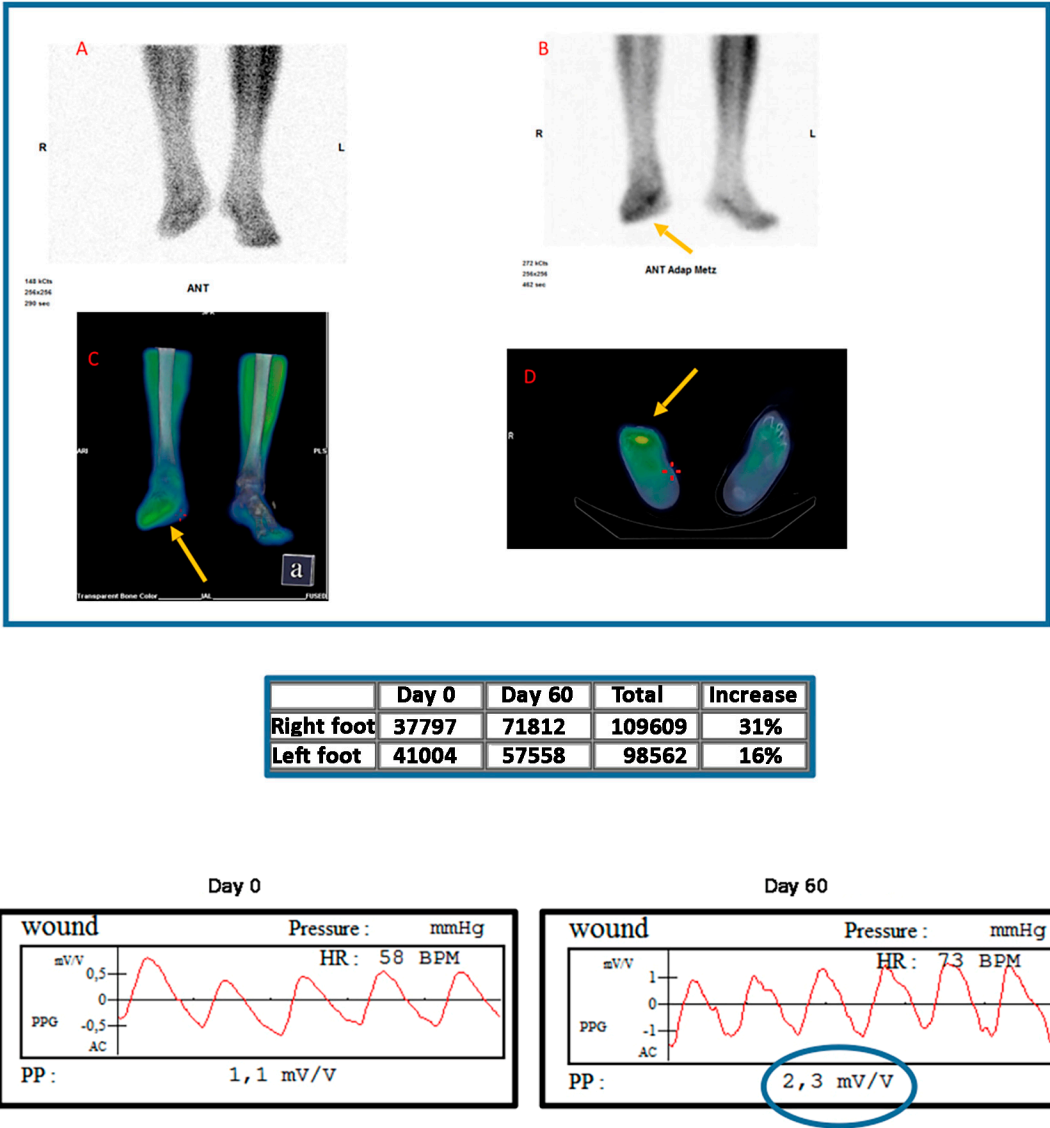| | Day 0 | Day 60 | Total | Increase |
|---|---|---|---|---|
| Right foot | 37797 | 71812 | 109609 | 31% |
| Left foot | 41004 | 57558 | 98562 | 16% |



**Figure 7. (a)** Upper picture: Combination of gammagraphy with leucocytes marked with technetium 99 m (pictures A, B) and single photon emission computed tomography (SPECT)-MIBI (pictures C, D), showing the increasing of flow in the right foot of a diabetic patient suffering from PAD (critical ischemia of the right limb). Before the treatment (picture A; Day 0), and after two months of GH treatment (pictures B, C, D; Day 60). Arrows indicate the blood flow. **(b)** Middle: The table shows the quantification of ROI (region of interest), demonstrating a final increase of flow of 31% of the right foot as compared with the same foot at day 0. **(c)** Lower graph: Photoplethysmography (PPG) from the same patient, showing the improvement in microcirculation at the wound level between the day 0 and the day 60 after GH treatment. Blue circle depicts the increase in pulsatility (PP), measured in mV/V. HR: heart rate.

Concern arises with the oncogenic potential of GH. First, this is something inherent to most of growth factors used for neovascularization. Second, long-term studies in GHD children do not show any increase in the incidence of tumors; moreover, the slight increase in the incidence of a second tumor detected in children with GHD secondary to leukemia treated with associated prophylactic brain radiotherapy, has been shown to be a consequence of the radiotherapy and not of the treatment with GH. Third, although an increased risk of thyroid cancer in acromegaly has been published [220], here we have already described that acromegaly implies a very high and sustained release of GH for many years; therefore, we cannot be sure of the fact that it is GH and no other factors (for example, IGF-I) that are responsible for the slight prevalence of cancer in patients with acromegalia.

911  On the other hand, the association between GH and tumors (breast, colon, prostate, etc), has mainly
912  been established because of the detection of the hormone and its receptor in tumor cells. However, it
913  is necessary to remind that GH is produced in almost any tissue and organ, and therefore, this
914  auto/paracrine GH might be responsible of the development of a tumor in susceptible patients. In our
915  own experience, short-term treatment with a strict control of plasma IGF-I levels is safe and effective.
916  In any case, it is necessary to rule out a neoplasm if GH therapy will be scheduled, especially in the
917  elderly population.
918      Additionally, it has been found that acromegaly also rises the risk of atherosclerosis [221–223] .
919  This argument is contrary to the fact that GH is an atherosclerotic protector, but it must be elucidated
920  whether the latter is a consequence of the increase in cardiovascular risk factors, rather than a pure
921  consequence of the hormone. Another adverse effect of the hormone is the development of carpal
922  tunnel syndrome, but this is unlikely to occur during short-time treatments with GH, as these that
923  we propose for treating CVD and PAD.

924  *2.1.0 Future perspectives*

925      From the concepts described in this review, supported by our GHAS study and previous data
926  from other group [18], it is likely that a short-time GH treatment may be a good alternative for
927  cardiovascular diseases. In fact, eight years ago, one of us (JD) treated with GH (0.4 mg / day) a 72-
928  year-old man who had suffered a stroke 17 years earlier, whose sequelae was a spastic hemiplegia
929  that forced him to remain in a wheelchair. Over the years he developed a critical ischemia of limbs
930  that led him to be programmed for the amputation of his left leg, because gangrene began to develop
931  in his left foot. He was treated for 3-months, followed by 1-month resting and after this another 3-
932  months of GH. Eight years later he still retains his leg, there are no sequels and several vascular
933  studies indicate that the blood flow in his affected leg is conserved at 80 years of age.
934      There are encouraging results obtained in many animal models of lower extremity ischemia, in
935  which an improvement in the perfusion of hypoxic tissues is described using viral-vector delivery
936  growth factors (mainly FGF-2 and VEGF) [224], However, the results in human patients with CLI did
937  not reveal significant benefits when using this type of therapies. Moreover, this type of therapy is not
938  free of adverse effects [225–228].
939      An important reason explaining why VEGF has failed on clinical trials, may be due to that when
940  VEGF is administered, it induces the formation of new vessels in a chaotic, unstable and not
941  functional way. New vessels need of a lumen to be functional, but VEGF alone is insufficient for the
942  lumenogenesis process. In this process, the activation of the protein kinase Akt and R-Ras seems to
943  be the key [229]. That is, Akt1-deficiency compromises endothelial cells sprouting, and impairs the
944  morphogenesis of the new vessels, making these vessels inadequate for re-oxygenating of the
945  ischemic tissues [230]. VEGF activates Akt to induce the sprouting of endothelial cells. Once this
946  phenomenon has been initiated, R-Ras stimulates Akt to reach lumenogenesis. In this second step,
947  the microtubule cytoskeleton is stabilized in the migrated endothelial cells, creating a stable structure
948  and the lumen [231]. The latter is essential for nutrients and oxygen transport and delivery to ischemic
949  tissues. Microtubule network is essential for endothelial cells polarization, lumenization, and
950  stabilization of the endothelial lumen structure [232]. On one side, GTPase protein R-Ras activates
951  Pi3K-Akt signaling [233], facilitating the maturation of vessels in the new vasculature [234], and the
952  association of pericytes with nascent blood vessels. On the other hand, it inhibits an excessive
953  sprouting and branching of angiogenic vessels to control the process.
954      In addition, some studies have described that, rather than a lack of growth factors in patients
955  with PAD, there is an altered response to them. As we stated above, not all studies agree, since in the
956  elderly population it seems to be a real depletion of growth factors, but the differences between VEGF
957  receptor flt-1 could explain the inter-subject differences with respect to the VEGF effect. Apart from
958  those mentioned above, other problem related to human ischemia is the existence of a real endothelial
959  dysfunction. This also impairs the arteriogenic response, which needs to be the ultimate goal in PAD
960  patients.

Vasodilation of collateral vessels and a decreased resistance to blood flow occurs because of arteriogenesis. Paradoxically, in leg ischemia oxygen tension drops in the foot, whilst a collateral network is generated in the thigh, where they are adequately oxygenated tissues.

Therefore, although therapeutic interventions aimed at salvaging a limb affected by critical ischemia may include attempts to stimulate both angiogenesis and arteriogenesis, the latter should be predominant if we seek to find a real clinical benefit in the standard patient with a major artery occlusion.

Taken together, all these facts explain why different growth factors used in PAD have failed in the target: there are not mature angiogenic vessels, there is a lack of stimulation of arteriogenesis, and lack of correction of the main process involved in the pathogenesis of this problem, the endothelial dysfunction. This is corrected by GH administration, independently of the patient is GHD or not.

## 3. Conclusions

From this review and our own data, we can conclude that short-time GH administration may be useful for improving the endothelial dysfunction leading to the development of atherosclerosis, or correcting it when it is established. GH improves the redox imbalance, enhances the appearance of collateral vessels after a major artery occlusion, improves wound healing, and ameliorates cardiac functioning after a myocardial infarction or heart failure. Short-term GH treatment can be safely given to elder patients suffering CVD, regardless they are GHD or not, whenever a severe contraindication does not exist. The relationships between Klotho and GH merit further studies, but the possibility exists that Klotho might be released from the dysfunctional endothelium to induce a secretion of GH able to repair this damaged tissue.

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