Original Article

Atheroprotective Properties of Serum IGF-1 in the Carotid and Coronary Territories and Beneficial Role on the Physical Fitness of the Oldest Old

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Abstract

Our aim was to investigate whether physiological levels of soluble insulin-like growth factor-1 (IGF-1) associate with coronary and carotid atherosclerotic burden and physical fitness in the oldest old by means of a cross-sectional study including 100 community-dwelling individuals with no previous cardiovascular events. Linear correlation was found between IGF-1 and intima-media thickness, number of carotid plaques, and walking speed. Individuals in the upper IGF-1 tertile had smaller right and left intima-media thickness compared with the intermediate and lower tertiles, along with reduced atherosclerotic plaques. Also, walking speed was greater in the upper IGF-1 tertile. On the other hand, a nonlinear correlation was observed between IGF-1 and coronary calcification scores, with the intermediate IGF-1 tertile associated to the lowest scores of calcification and participants with lower circulating levels of IGF-1 showing higher frequency of high-risk morphology plaques. All in all, our report supports a territory-dependent, atherorefractory phenotype in the oldest old carrying middle and/or higher serum levels of IGF-1.

Key Words: Carotid atherosclerosis—Coronary calcification—Physical fitness—Risk factor—Insulin-like growth factor-1

People older than 80 years of age—the oldest old—are the fastest growing age group worldwide. By 2020, the number of persons older than 85 years is expected to reach 19 million (1). Epidemiological data suggest that cardiovascular disease will also increase exponentially in this population (2). In developed countries, 50% of deaths are associated with atherosclerosis-related cardiovascular events (3), prompting investigators to look further into this topic.

During aging, the progression of subclinical atherosclerosis is paralleled by a decline in the secretion of growth hormone (GH) and of its primary mediator, insulin-like growth factor-1 (IGF-1). Previous studies have described IGF-1 as either a protective factor (4) or a cardiovascular risk factor (5,6), depending on its levels. A physiological level of IGF-1 in the upper limit has been related to coronary artery disease (7), carotid intima-media thickness (IMT) (8), and cardiovascular mortality (9). Conversely, other studies suggest that lower serum levels of IGF-1 may be related to increased incidence of coronary calcification (10), carotid atherosclerotic plaques (11), and ischemic stroke (12). Thus, the exact atherogenic role of IGF-1 has not been elucidated (13–16).

Inconclusive results have also been obtained in the older adults for the association between physical fitness, which is per se an independent predictor of mortality (17), and IGF-1 serum levels; both positive and negative associations have been reported, for instance, between scores of handgrip strength and walking speed scores with circulating IGF-1 (18–20). A better understanding of these phenomena would improve cardiovascular risk assessments and help design primary interventions for the oldest old based on nonpharmacological, life-style measures to promote cardiovascular health.
Therefore, the aim of the present study was to investigate the relationship between serum levels of IGF-1 and markers of carotid and coronary artery atherosclerosis in asymptomatic community-dwelling individuals older than 80 years of age. A second aim was to investigate the role of physical fitness in these relationships.

**Methods**

**Patients**

Participants were recruited from the Brazilian Study on Healthy Aging cohort, established in 2008 with the aim of identifying cardiovascular risk factors in community-dwelling volunteers aged 80 years or older, with no clinical evidence of acute myocardial infarction, stroke, or peripheral vascular disease and receiving primary preventive care. Additional selection criteria were absence of the following: autoimmune disease, current or previous neoplastic disease, chronic obstructive pulmonary disorder, creatinine clearance ≤ 25 mL/min/1.73 m², chronic or recurrent infection, liver disease, heart failure (left ventricular ejection fraction < 50%), or use of steroidal and non-steroidal anti-inflammatory drugs in the past 30 days. For the present cross-sectional study, we took into account baseline clinical and biochemical records (medical history, blood biochemistry, current use of therapeutic drugs, and carotid coronary imaging), as well as physical/psychological and laboratory data (anthropometry, physical fitness, quality of life, IGF-1, and immune mediators) obtained from a subgroup of the cohort who volunteered for the latter assessments.

Blood samples were collected after 12-hour fasting for routine biochemical tests. Serum was stored at ~8°C for later analysis. Cardiovascular ultrasound examination (echocardiography) was performed immediately after collection of blood samples, and computed tomography (CT) was performed in the following week. Assessment of quality of life based on the WHOQOL-BREF instrument and on the Mini-Mental State Examination (MMSE) is described elsewhere (21). Approval by Research Ethics Committee for the analyses presented herein was obtained on 2011 at the institutional Medicine Faculty, and all participants signed an informed consent form prior to the evaluations.

**Carotid Artery Imaging**

IMT and presence of carotid plaques were determined using high-resolution B-mode ultrasound (Philips, model IE 33, 3–9 MHz linear transducer, Philips Medical Systems, Andover, MA) following American Association of Echocardiography recommendations (22). Bilateral measurements were made at the far wall of the carotid bulb and at the internal carotid artery using a computerized edge detection system (QLAB version 6.0). Carotid plaque was defined as focal thickening of the wall of at least 50% as compared with the surrounding vessel walls or as focal region with IMT > 1.5 mm and distinct adjacent edges.

**Cardiac CT**

Cardiac CT scans were performed using a 64-slice scanner (Aquilllion 64, Toshiba, Ottawa, Japan). Axial slices of 3-mm thickness with 3-mm table-feed were acquired at 70% of the R–R interval with prospective electrocardiographic triggering. Coronary artery calcification (CAC) was defined as a minimum of three contiguous pixels with a peak Hounsfield unit density > 130. CAC was evaluated by a certified radiologist using the Agatston score (AS).

**Cardiac CT**

Cardiac CT was performed for analysis of coronary calcium score on a 64-detector CT scanner (Aquilllion 64). Axial slices of 3-mm thickness were acquired in synchrony with prospective electrocardiographic tracing in 70% of the R–R interval. Coronary calcifications were measured and analyzed using the Agatston method. Contrast-enhanced CT angiograms were reconstructed from data acquisition windows with temporal resolution of 250 ms. In line, presence and characteristics of coronary atherosclerotic plaques were analyzed with the aid of a workstation (Vitrea, Vital Image) based on previously defined criteria (23). In these analyses, an spotty calcification was defined as a finding less than 3 mm in size that occupied only one side on successive cross-sectional images. Low-attenuation plaque (LAP) was defined as noncalcified plaque with less than 30 HU. Also, occurrence of coronary arterial remodeling was assessed and defined when the diameter at the plaque site was at least 10% larger than the reference segment.

**Ten-year Cardiovascular Risk**

A multivariate model of the Framingham risk score (FS) was used to estimate the 10-year probability of a coronary event based on age and other risk factors according to the American College Foundation Clinical Expert Consensus Task Force (24). Participants were later grouped into the following cardiovascular risk categories: low (FS < 10%), intermediate (10% ≤ FS < 20%), or high (FS ≥ 20%).

**Biochemical Analyses**

After collection, blood was mixed with EDTA and centrifuged at 4,500 rpm for 15 minutes at 5°C for plasma separation and measurement of glucose (GOD-PAP, Roche Diagnostics, Mannheim, Germany), total cholesterol (CHOD-PAP, Roche Diagnostics), triglycerides (GPO-PAP, Roche Diagnostics), HDL-C (Roche Diagnostics), C-reactive protein (high-sensitivity CRP, CardioPhase, DadeBehring, Marburg, Germany), urea and creatinine (GLDH, Hitachi, Tokyo, Japan), fibrinogen (Sysmex CA 1500, Siemens, Munich, Germany), interleukin (IL)-10, and tumor necrosis factor-alpha (eBioscience, San Diego, CA). Parathyroid hormone and calcitonin (Immulate 2000, Siemens, Los Angeles, CA), alkaline phosphatase (bone fraction; Hitachi), calcium (Hitachi), and apolipoprotein (apo) A and apo B (Behring Nephelometer BNII, Dade Behring, Marburg, Germany) were also measured. Creatinine clearance was estimated using serum creatinine and the equation from Cockcroft and Gault. Serum levels of GH and IGF-1 were determined using the automated Immulite 2000-Siemens system (Los Angeles, CA) with analytic sensitivity of 0.01 ng/mL and 20 ng/mL, respectively. All samples were analyzed in duplicate.

**Walking Speed**

The walking speed test was performed by trained physical educators. A 2.44-m-long track was marked on the floor with tape. Participants were instructed to walk at their usual pace, from a standing start with feet together on the ground. A stopwatch was activated by a physical educator when the first foot touched the ground immediately after the start line and paused as a soon as the participant’s foot touched the ground beyond the finish line. The test was performed three times, and the mean duration was used to estimate walking speed (m/s). None of the participants used walking aids for the test (walkers, crutches, etc.). We also calculated the coefficient of variation (% CV) for the three measurements.

**Muscle Strength**

Participants were instructed to remain seated and to keep the dominant arm close to the trunk, with the elbow flexed to a right angle (90°). A mechanical hand dynamometer (Crown, São Paulo, Brazil)
was used, with grip strength (kgf) expressed as the mean of three measurements with 2-minute rest periods intervening. Grip size was individually adjusted for comfort.

Statistical Analyses

Serum IGF-1 levels were stratified into tertiles (1st, <79.1 ng/mL; 2nd, 79.1–107.7 ng/mL; and 3rd, >107.7 ng/mL). The number of atherosclerotic plaques was categorized (0; 1; > 1) for analysis. Normality was tested using the Kolmogorov–Smirnov test. Continuous variables with a distribution close to normal were expressed as means ± SD, whereas variables with asymmetric distribution were expressed as medians (interquartile range). To reduce skewness, AS were log-transformed after adding 1 [log10(AS + 1)]. Analysis of covariance (ANCOVA) was used to evaluate differences between variables with normal or close to normal distribution, with standard adjustment to age and sex for all analyses and further adjustment to body mass index, LDL-cholesterol (LDL-C), and diabetes when inferential examination tested main study variables (vascular phenotypes, muscle strength, walking speed, and Framingham scores) across IGF-1 levels. ANCOVA assumptions of linearity, normality, and homogeneity of variance were verified using normal probability plots and residual dispersion. For multiple comparisons, significant values were adjusted using the Bonferroni method. Mathematical-statistical modeling was used to build a predictive model for the relationship between CAC scores and IGF-1 levels. Categorical variables were expressed as percentage and compared using the $\chi^2$ test. Cohen’s effect size ($'d'$) was calculated whenever appropriate. A $p$ value less than .05 in a bilateral hypothesis test was defined as statistically significant. Data analysis was carried out using the Statistical Package for the Social Sciences 8.0 (SPSS Inc, Chicago, IL).

Results

Laboratory Profile and Clinical Characteristics of Participants

Of the entire cohort, 107 participants were eligible and agreed to participate in this cross-sectional study. Seven were excluded from the analyses because of missing biochemical from the baseline databank ($n = 3$) or functional data from our recent assessments ($n = 4$). Therefore, the present results refer to a sample of 100 asymptomatic older adults (mean age = 86.5 ± 4.0 years), of which 74% were women, 2% were smokers, 74% were physically unfit, 14% had diabetes, and 77% had arterial hypertension. Quality of life was good to very good in 87% of the sample. MMSE scores (median = 26; range: 22–28) were negatively associated with age ($r = −0.38$, $p < .001$) and not associated with any of the other variables investigated.

Table 1 describes the main characteristics of individuals according to IGF-1 tertile. Only IGF-1 ($p < .01$) and LDL cholesterol ($p = .03$) were different between tertiles.

As shown in Figure 1, our findings suggest greater structural integrity of the carotid vascular bed in the upper IGF-1 tertile as compared with the first tertile, as expressed by lower mean values for maximum right (1.4 ± 0.5 mm vs 2.1 ± 0.7 mm, $p = .03$) and left IMT (1.5 ± 0.6 mm vs 2.3 ± 0.9 mm, $p = .02$). There were no significant differences between the first and second tertiles. In addition, participants with 1 and >1 atherosclerotic plaques had lower levels of IGF-1 (99 ± 36 ng/mL).

Table 1. Clinical and Laboratory Characteristics According to IGF-1 Tertiles

<table>
<thead>
<tr>
<th></th>
<th>First tertile</th>
<th>Second tertile</th>
<th>Third tertile</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>33</td>
<td>33</td>
<td>34</td>
<td>—</td>
</tr>
<tr>
<td><strong>Female, %</strong></td>
<td>85</td>
<td>72</td>
<td>65</td>
<td>.7</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>87 ± 4</td>
<td>86 ± 4</td>
<td>86 ± 4</td>
<td>.9</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>26 ± 4</td>
<td>27 ± 4</td>
<td>27 ± 4</td>
<td>.6</td>
</tr>
<tr>
<td><strong>Body fat, %</strong></td>
<td>16 ± 4</td>
<td>17 ± 4</td>
<td>17 ± 4</td>
<td>.3</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, %</strong></td>
<td>15</td>
<td>9</td>
<td>17</td>
<td>.7</td>
</tr>
<tr>
<td><strong>GH, ng/mL</strong></td>
<td>0.8 (0.8)</td>
<td>1.5 (1.6)</td>
<td>0.9 (0.8)</td>
<td>.2</td>
</tr>
<tr>
<td><strong>IGF-1, ng/mL</strong></td>
<td>59 ± 15</td>
<td>93 ± 8</td>
<td>137 ± 29</td>
<td>&lt;.01</td>
</tr>
<tr>
<td><strong>TSH, mU/L</strong></td>
<td>2.2 (2.7)</td>
<td>2.5 (1.5)</td>
<td>1.9 (2.6)</td>
<td>.4</td>
</tr>
<tr>
<td><strong>T4, ng/L</strong></td>
<td>1.4 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>.6</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>89 ± 10</td>
<td>92 ± 10</td>
<td>93 ± 11</td>
<td>.3</td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td>6.0 (0.7)</td>
<td>5.9 (0.5)</td>
<td>6.0 (0.6)</td>
<td>.9</td>
</tr>
<tr>
<td><strong>Hypertension, %</strong></td>
<td>32</td>
<td>34</td>
<td>34</td>
<td>.9</td>
</tr>
<tr>
<td><strong>Hypertension duration, years</strong></td>
<td>23 (21)</td>
<td>10 (17)</td>
<td>23 (19)</td>
<td>.1</td>
</tr>
<tr>
<td><strong>Menopause duration, years</strong></td>
<td>34 ± 4</td>
<td>38 ± 5</td>
<td>36 ± 6</td>
<td>.1</td>
</tr>
<tr>
<td><strong>Creatinine clearance, mL/min</strong></td>
<td>44 ± 15</td>
<td>49 ± 15</td>
<td>51 ± 15</td>
<td>.2</td>
</tr>
<tr>
<td><strong>Fibrinogen, mg/dL</strong></td>
<td>327 (71)</td>
<td>325 (118)</td>
<td>338 (60)</td>
<td>.9</td>
</tr>
<tr>
<td><strong>ApoA, mg/dL</strong></td>
<td>148 ± 25</td>
<td>156 ± 44</td>
<td>152 ± 33</td>
<td>.4</td>
</tr>
<tr>
<td><strong>ApoB, mg/dL</strong></td>
<td>85 ± 21</td>
<td>88 ± 22</td>
<td>75 ± 21</td>
<td>.1</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>194 ± 30</td>
<td>203 ± 43</td>
<td>192 ± 38</td>
<td>.1</td>
</tr>
<tr>
<td><strong>Triglyceride, mg/dL</strong></td>
<td>121 ± 42</td>
<td>131 ± 67</td>
<td>124 ± 54</td>
<td>.6</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mg/dL</strong></td>
<td>53 ± 13</td>
<td>54 ± 17</td>
<td>54 ± 15</td>
<td>.6</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mg/dL</strong></td>
<td>116 ± 31</td>
<td>111 ± 35</td>
<td>107 ± 33</td>
<td>.03</td>
</tr>
<tr>
<td><strong>Statin use, %</strong></td>
<td>32</td>
<td>28</td>
<td>40</td>
<td>.5</td>
</tr>
<tr>
<td><strong>Statin use duration, months</strong></td>
<td>72 (78)</td>
<td>42 (36)</td>
<td>60 (84)</td>
<td>.2</td>
</tr>
<tr>
<td><strong>IL-10, pg/mL</strong></td>
<td>2.2 (4.3)</td>
<td>1.9 (2.6)</td>
<td>1.0 (3.0)</td>
<td>.5</td>
</tr>
<tr>
<td><strong>CRP, mg/L</strong></td>
<td>2.2 (1.7)</td>
<td>1.1 (1.7)</td>
<td>1.7 (1.6)</td>
<td>.4</td>
</tr>
</tbody>
</table>

Notes: CRP = C-reactive protein; GH = growth hormone; IGF-1 = insulin-like growth factor-1; IL = interleukin; TSH = thyroid-stimulating hormone. Continuous variables having normal or close to normal distribution were expressed as means ± SD, whereas variables with asymmetric distribution were expressed as medians (interquartile range). Analysis of covariance adjusted for age and sex.
and 88±14 ng/mL, respectively) as compared with the group without plaques (140±38 ng/mL, p = .003; Figure 1C). The only significant association revealed by [one-tailed] partial correlation tests was between IGF-1 and maximum left carotid IMT (r = −0.31, p = .04).

Association Between IGF-1 and Measures of Physical Function

ANOVA revealed a relationship between higher circulating IGF-1 levels and physical fitness expressed as walking speed (Figure 2A), with individuals in the third IGF-1 tertile showing the best performance (>1.0 m/s) when compared with those in the second (p = .001) and first tertiles (p = .02). Despite the significant association (p < .03) between walking speed and hand grip strength (r = .36, r = .72, r = .55, for first, second, and third tertiles, respectively), a direct association was not observed between hand grip strength and IGF-1 levels. Partial correlation tests also revealed positive associations between IGF-1 levels and walking speed measures (r = .30, p = .003).

Association Between IGF-1, CAC, and High-risk Morphology Plaques

Agatston scores ≥ 100 were recorded in 52% of the sample. There was significant variation in CAC means [log_{10}(AS+1)] among IGF-1 tertiles (p < .001, Figure 4). Individuals in the first (lower) and third (upper) IGF-1 tertiles had higher CAC scores as compared with individuals in the second tertile (2.03±.7 vs 1.43±.9, p = .004 and 2.22±.5 vs 1.43±.9, p = .001, respectively), with no difference between the first and third IGF-1 tertiles (p = .90). When a simple nonlinear model was used to describe the relationship between CAC and IGF-1 (y = 0.0001x^2 − 0.02x + 2.5, r = .30, p = .02), the very old individuals in the upper and lower IGF-1 tertiles (1st+3rd) had higher CAC scores as compared with those in the intermediate level (2nd, y > 0, x = 100 ng/mL). ANOVA did not reveal significant variation in log-transformed AS according to FS category (p = .63).

No significant correlations were observed between CAC scores and right (1st, r = .25; 2nd, r = .04; 3rd, r = .31) or left maximum IMT (1st, r = .14; 2nd, r = .32; 3rd, r = .08) in carotid arteries after a partial correlation test with adjustment for sex and age (Figure 4A). After characterization of all plaques according to positive remodeling (PR), low-attenuation (LAP), and spotty calcification (Ca), it was observed that 32 individuals exhibited plaques in statuses compatible with an elevated degree of vulnerability (PR + LAP + Ca). The intermediate IGF-1 tertile displayed a prevalence of vulnerable plaque twice as low (18.2%) as that observed in the higher (35.5%) and lower tertiles (42.4%) (Figure 4B). And comparison between the mean numbers of coronary branches with high-risk morphology across IGF-1 tertiles revealed a significantly greater plaque instability in the 1st compared with the intermediate (p = .01) tertile (Figure 4 C).

Discussion

Association of IGF-1 With IMT and Carotid Plaques

Very old individuals without atherosclerotic plaques had higher circulating levels of IGF-1 as compared with those with one or more plaques. In addition, the upper IGF-1 tertile was associated with the lowest mean IMT. These relationships remained significant even after adjustment for clinical and sociodemographic variables. Considering that a 0.10-mm increase in carotid IMT has been shown to provide a relevant contribution to the risk of myocardial infarction (hazards ratio = 1.14) and stroke (hazards ratio = 1.17) (25), the difference of 0.70 mm (right) and 0.80 mm (left) in maximum IMT between the 1st and 3rd IGF-1 tertiles observed in the present study indicates a substantial increase in cardiovascular risk among individuals in the lower tertile.

Our findings are consistent with those of studies with asymptomatic older adults in which negative associations were observed between circulating IGF-1 levels and the number of atherosclerotic plaques (11,26) or carotid IMT (27). A randomized study in which traditional risk factors were controlled demonstrated potent inhibitory effects of the GH/IGF-1 axis on the progression of carotid IMT in hypopituitary adults (28). Data in humans support the concept that physiological levels of IGF-1 are important for the maintenance of a healthy endothelium, contributing to maintenance of functional and structural integrity of the microcirculation, to increased NO bioavailability and to decreased reactive oxygen species production, also exerting anti-inflammatory, antiapoptotic, and proangiogenic effects (13,29,30).

Despite a large body of evidence that indicates the importance of adequate levels of circulating GH/IGF-1 for healthy aging, others support the view that normal levels of IGF-1 accelerate the aging process whereas low levels exert antiaging effects (31). However, this belief is mostly based on studies on invertebrate models that do not bear a cardiovascular system (eg, Caenorhabditis elegans). In addition, those studies conducted in animal models that draw conclusions about IGF-1 effects on aging and life span are usually based on experimental participants devoid of underlying clinical disorders so to hinder assuming their conclusions as valid in pathological scenarios (30,31) Therefore, the data available on humans do not reflect the increased life span found in animal model, suggesting that IGF-1 signaling in later life cannot be considered part of an evolutionary conserved mechanism for life-span constraint.

Association of IGF-1 with Physical Fitness

Taken together, our results suggest that very old individuals with the highest circulating levels of IGF-1 had the highest level of physical fitness. In particular, the very old in the third IGF-1 tertile were significantly faster (individual scores > 1.0 m/s) than those in other tertiles. The comparison of muscle strength among tertiles based on the handgrip test did not reveal significant differences, despite a trend to higher strength in the upper IGF-1 tertile. Elsewhere, slower pace walking speed (<1.0 m/s) has been associated with subclinical atherosclerosis without overt cardiovascular events (32), and also with increased risk of cardiovascular-related mortality (20,22,29,33–36).

Worthy of note is the considerable difference in physical capacity markers between the different groups of very old individuals. The absolute difference in walking speed between the upper IGF-1 tertile and the two lower tertiles was of 0.20 m/s and 0.24 m/s, respectively.

As shown in a recent meta-analysis, for each 0.10 m/s increment in walking speed, there is a decrease in mortality risk estimated at 12% (36). In this scenario, a delta of 0.20 m/s indicates a substantial difference in risk, adding clinical relevance to our observation. In addition, about 30% of the total levels of IGF-1 are determined by exogenous factors, such as life-style factors and increased physical activity (34,37). To the best of our knowledge, there have been no prospective studies
investigating the relationship between the IGF-1 system and physical performance in geriatric populations, with further studies needed. The correlations between handgrip strength and walking speed suggest moderate to large size effects (0.36 < \(d\) < 0.72). The fact that greater handgrip strength was not observed in the group with the highest levels of IGF-1 may be explained by the fact that handgrip represents global muscle strength (38), whereas the walking speed test is more sensitive to detect the effects of lower limb sarcopenia. Studies with centenarians have shown marked changes in the ratio of upper to lower limb maximum strength (39,40). Thus, our results suggest that the maintenance of physiological levels of IGF-1 may benefit specific muscle sectors, especially in the lower body. We do not rule out the hypothesis that, given the small to moderate effect size observed (partial eta squared = 0.03), a larger number of participants would be required to provide more power to the test.

**Figure 1.** Maximum right (A) and left (B) scores of intima-media thickness across insulin-like growth factor-1 (IGF-1) tertiles. The third tertile showed scores significantly lower compared with the first tertile (*\(p\) < .05) in both branches. (C) Individuals with no atherosclerotic plaques had significantly higher circulating levels of IGF-1 (*\(p\) < .01) compared with individuals with ≥1 plaque. Analysis of covariance adjusted for age, sex, body mass index, LDL-cholesterol, and diabetes.

**Figure 2.** (A) Walking speed according to insulin-like growth factor-1 (IGF-1) tertiles. Participants in the upper tertile were significantly faster than those in the two lower tertiles (*\(p\) < .01). Dashed line represents the threshold of 1 m/s. (B) Handgrip strength among IGF-1 tertiles, with no difference detected. Analysis of covariance adjusted for age, sex, body mass index, LDL-cholesterol, and diabetes.

**Association of IGF-1 With CAC and High-risk Morphology Plaques**

The present results indicate an association between circulating levels of IGF-1 and CAC scores in a sample of very old individuals with no previous cardiovascular events. CAC is an indicator of overall coronary atherosclerotic burden (41) and also a robust predictor of coronary events in elderly patients (42). Interestingly, the lowest CAC scores were associated with intermediate physiological levels of IGF-1, indicating a nonlinear relationship between IGF-1 and CAC. The higher CAC scores observed in both lower and upper IGF-1 tertiles suggest a J- or U-shaped relationship. In this context, it is plausible to hypothesize that individuals grouped in extreme IGF-1 tertiles have a higher likelihood of future coronary events, because the mean CAC scores were 30% to 36% higher than the scores recorded for the intermediate tertile. Despite CAC comprises a useful tool to yield atherosclerotic burden and prognostics in clinical care of asymptomatic participants, this score bears limited utility on what concerns assessing presence, width and severity of noncalcified plaques, and characterization of contributors to the onset of plaques with greater instability might evolve into an important aspect of risk stratification (23). Having that in mind, our study also enclosed an analysis on the association of IGF-1 levels with high-risk plaque morphology, having found that only the very old participants with the lowest levels of this soluble mediator showed a greater number of unstable plaques (RP + LAP + Ca) compared with counterparts in the intermediary tertile.

The possibility of a J-shaped relationship is supported by evidence from clinical and epidemiologic studies associating both high (7,43)
and reduced (10) levels of IGF-1 with calcification and coronary heart disease. Further support for the biological plausibility of our finding is provided by a cross-sectional study with 3,980 community-dwelling older adults, which showed a U-shaped association of the middle-range of the distribution of IGF-1 with the lowest risk of metabolic syndrome in men aged (≥70 years) (44). van Bunderen and colleagues (45) observed a U-shaped relationship between IGF-1 levels and mortality, including fatal cardiovascular disease in a population-based study with 1,273 older persons. Curve-shaped relationships have also been observed in works investigating the association between levels of insulin (a peptide that is structurally similar to IGF-1), coronary heart disease (46), and CAC (47). Tanaka and colleagues (47) propose that the process of coronary calcification occurs in a milieu of cell insulinization resulting from an effect of either insulin resistance or insulin sensitivity on the phenotype of vascular smooth muscle cells (VSMCs).

This scenario raises the question of which mechanisms underlie the observed association between IGF-1 levels and CAC. Experimental studies suggest that IGF-1 has a dual effect on the vascular wall, that is, both unfavorable and protective effects, depending on the availability of the IGF-1 mediator (8, 29, 48, 49). In the transition from initial to more advanced atherosclerotic stages in the vascular milieu, higher physiological levels of IGF-1 may favor the proliferation/migration of VSMCs (13) and consequently accelerate the process of coronary calcification (50, 51). In turn, reduced circulating IGF-1 levels may promote coronary mineralization (10) by inhibiting osteoblastic conversion of calcifying vascular cells and VSMC apoptosis (52, 53). Based on such evidence, it is plausible to infer that within a narrow physiological window IGF-1 may contribute to both endothelial turnover and phenotypic changes in VSMCs (54).

Another clinically interesting result was the observed association between FS coronary risk and circulating levels of IGF-1. Many older adults at intermediate (10% ≤ FS < 20%) and high (FS ≥ 20%) coronary risk had IGF-1 levels in the upper or lower tertiles, again suggesting a nonlinear relationship. However, these results must be interpreted with care, because the predictive power of this algorithm declines with aging as a result of selective survival bias and of the influence of comorbidities that usually affect the very old (55).

Other Aspects and Limitations of the Study

The notion that IGF-1 levels in the upper physiological range provide cardiovascular protection should be viewed with caution (29). Because of the nature of our study, we cannot rule out the possibility of a chance finding, and therefore prospective studies should be carried out to investigate the clinical value of serum concentrations of IGF-1 as a potential biomarker to stratify vascular risk in populations of asymptomatic, very old individuals.

No differences were observed in metabolic data or inflammatory markers according to IGF-1 tertiles, exception to LDL-C, for which reduced serum titers associated with decreased levels of the mediator, suggesting a more favorable lipid profile for carriers of low IGF-1 levels. However, inclusion of LDL-C as a covariate did not produce significant adjustments to the model of association found between IGF-1 and the vascular phenotypes, nor did the inclusion of the diabetic condition. Moreover, we believe that our
findings do not reflect pharmaceutically influenced results because the chi-square test failed to reveal quantitative variances in the distribution of users and nonusers of the most frequent pharmaceutical products (lipid-lowering, glucose-lowering, or antihypertensive agents) across tertiles \( (p > .05) \). CRP determined with high sensitivity was also unable to reveal significant differences in CAC scores between IGF-1 tertiles, suggesting that the systemic proinflammatory environment did not determine variance of the investigated parameters, with other pathophysiological pathways leading to atherosclerosis and subsequent calcification \( (56) \). These results support the hypothesis that risk evaluation of cardiovascular events based on traditional factors loses strength in the oldest old \( (21) \) and corroborate the findings of studies \( (7,10,57,58) \) that show IGF-1 as an independent cardiovascular risk factor.

We did not observe significant associations between markers of coronary and carotid atherosclerotic burden. Despite the generally accepted views of atherosclerosis as a systemic condition and of a relationship between coronary and carotid disease, the different susceptibility of these two arteries to proatherogenic stimuli may reflect different disease triggers.

**Conclusions**

In the present study including oldest old individuals, linear models were able to explain the relationship between circulating levels of IGF-1 and changes that are representative of the atherosclerotic process in the carotid vascular bed, showing a negative association between atherogenic factors and median physiological levels of IGF-1. Along this line, very old individuals with the highest circulating IGF-1 levels were significantly faster in the walking speed test in relation to the lower IGF-1 tertiles.

Conversely, a nonlinear relationship was observed between systemic levels of IGF-1 and CAC scores in very old persons independently of other cardiovascular risk factors, where participants with lower circulating levels of IGF-1 showing higher frequency of high-risk morphology plaques were compared with participants from the intermediate tertile. All in all, our report suggests atheroprotective properties of middle and/or higher serum levels of IGF-1 in the oldest old, in a territory-sensitive manner.

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**Conflict of Interest**

The authors report no relationships that could be construed as conflict of interest.

**References**

23. Motoyama S, Sarai M, Harigaya H, et al. Computed tomographic angiography characteristics of atherosclerotic plaques subsequently result-


