Positive Association of High Leptin Level and Abdominal Aortic Calcification in Men
— The Prospective MINOS Study —

Pawel Szulc, MD, PhD; Ez Zoubir Amri, PhD; Annie Varennes, PhD; Patricia Panaia-Ferrari, MD, PhD; Eric Fontas, MD PhD; Joëlle Goudable, MD, PhD; Roland Chapurlat, MD, PhD; Véronique Breuil, MD, PhD

Background: Severe abdominal aortic calcification (AAC) points to high cardiovascular risk and leptin stimulates arterial calcification; however, clinical data on their association are scarce. We studied the link between serum leptin and AAC severity and progression, and the effect of smoking and lipid levels, on this association in men.

Methods and Results: At baseline, 548 community-dwelling men aged 50–85 years underwent blood collection and lateral lumbar spine radiography. In 448 men, X-ray was repeated after 3 and 7.5 years. AAC was assessed using Kauppila’s semiquantitative score. In multivariable models, high leptin was associated with higher odds of severe AAC (odds ratio [OR]=1.71 per SD, 95% confidence interval [CI]: 1.22–2.40). The odds of severe AAC were the highest in men who had elevated leptin levels and either were ever-smokers (OR=9.22, 95% CI: 3.43–24.78) or had hypertriglyceridemia (vs. men without these characteristics). Higher leptin was associated with greater AAC progression (OR=1.34 per SD, 95% CI: 1.04–1.74). The risk of AAC progression was the highest in men who had elevated leptin levels and either were current smokers or had high low-density lipoprotein-cholesterol levels (OR=5.91, 95% CI: 2.46–14.16 vs. men without these characteristics). These links remained significant after adjustment for baseline AAC and in subgroups defined according to smoking and low-density lipoprotein-cholesterol levels.

Conclusions: In older men, high leptin levels are associated with greater severity and rapid progression of AAC independent of smoking, low-density lipoprotein-cholesterol or triglycerides.

Key Words: Abdominal aortic calcification; Leptin; Low-density lipoprotein-cholesterol; Smoking; Triglycerides
Serum Leptin and Aortic Calcification

Regarding blood levels of leptin and lipids, positive correlations have been observed. Lipid accumulation in the aortic wall increases osteogenic differentiation of VSMC and calcification. However, the link between serum lipids and AAC has been poorly studied. Obesity is associated with AAC and high levels of leptin and lipids. As leptin may influence AAC or be a bystander in hyperlipidemia, study of the link between leptin and AAC should account for lipids.

Thus, the linkage of serum leptin with AAC severity and progression and the potential effect of smoking and lipids on this relation are poorly studied. Therefore, our primary aim was to study the association of serum leptin with AAC severity and progression in older men. The secondary aim was to analyze the additive effects of leptin and other factors (smoking, lipids) in their associations with AAC severity and progression.

## Methods

### The MINOS Cohort

The MINOS study is a prospective single-center cohort study of osteoporosis in men aged 50–85 years. This collaborative project involves the Institut National de la Santé et de la Recherche Médicale and Société de Secours Minière de Bourgogne, a health insurance service for families of mineworkers living in Montceau-les-Mines. The study was approved by the Ethical Board and performed according to the Declaration of Helsinki (1983). The men (n=783) provided informed consent and blood was collected in 1995–1996. The cross-sectional study was performed in 548 men who had enough serum for leptin assays. They did not differ from the other men (Table S1) and were followed for 7.5 years. The prospective study was performed in 448 men who had follow-up spine X-rays (100 men dropped out because of death or health status).

### Leptin Measurements

Fasting blood samples were collected at 08.00 hours. Blood was centrifuged and separated. Serum aliquots were stored at −80°C without thawing until the assay in 2011. In order to limit the interassay variability, measurements were performed using kits from a single series (Table 1). AAC progression was calculated as the difference between the score on the last X-ray (3 or 7.5 years) and the baseline score divided by the follow-up duration. AAC was assessed in 2005–2006 (thus, without knowing leptin levels) by P.S. who evaluated the baseline and the follow-up X-rays side.

### Table 1. Biological Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Method</th>
<th>Provider</th>
<th>Detection limit</th>
<th>Interassay CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>ELISA</td>
<td>TECO-Medical, Switzerland</td>
<td>0.2 ng/mL</td>
<td>7.6%</td>
</tr>
<tr>
<td>Glucose</td>
<td>Hexokinase assay</td>
<td>Modular Analyzer, Roche, Meylan, France</td>
<td>2 mg/dL</td>
<td>1.0%</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Enzymatic colorimetry</td>
<td></td>
<td>4 mg/dL</td>
<td>1.5%</td>
</tr>
<tr>
<td>Cholesterol, HDL-C</td>
<td>Enzymatic colorimetry</td>
<td></td>
<td>3 mg/dL</td>
<td>0.6–0.9%</td>
</tr>
<tr>
<td>Testosterone</td>
<td>RIA</td>
<td></td>
<td>0.06 nmol/L</td>
<td>7.8%</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>RIA</td>
<td></td>
<td>11 pmol/L</td>
<td>6–8%</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>Immunochemoluminometry</td>
<td>Magic Lite; Ciba Corning Diagnostic, Medfield, MA, USA</td>
<td>0.2 pmol/L</td>
<td>7%</td>
</tr>
<tr>
<td>25-hydroxycholecalciferol</td>
<td>Acetonitrile extraction, RIA</td>
<td>Incstar Corp., Stillwater, MN, USA</td>
<td>3 ng/mL</td>
<td>10%</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>IRMA assay</td>
<td>CISBioInternational, Bagnols, France</td>
<td>0.4 ng/mL</td>
<td>6%</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; HDL-C, high-density lipoprotein-cholesterol; IRMA, immunoradiometric assay; RIA, radioimmunoassay

---

Blood levels of leptin and lipids correlate positively. Lipid accumulation in the aortic wall increases osteogenic differentiation of VSMC and calcification. However, the link between serum lipids and AAC has been poorly studied. Obesity is associated with AAC and high levels of leptin and lipids. As leptin may influence AAC or be a bystander in hyperlipidemia, study of the link between leptin and AAC should account for lipids.

Thus, the linkage of serum leptin with AAC severity and progression and the potential effect of smoking and lipids on this relation are poorly studied. Therefore, our primary aim was to study the association of serum leptin with AAC severity and progression in older men. The secondary aim was to analyze the additive effects of leptin and other factors (smoking, lipids) in their associations with AAC severity and progression.

### Methods

#### The MINOS Cohort

The MINOS study is a prospective single-center cohort study of osteoporosis in men aged 50–85 years. This collaborative project involves the Institut National de la Santé et de la Recherche Médicale and Société de Secours Minière de Bourgogne, a health insurance service for families of mineworkers living in Montceau-les-Mines. The study was approved by the Ethical Board and performed according to the Declaration of Helsinki (1983). The men (n=783) provided informed consent and blood was collected in 1995–1996. The cross-sectional study was performed in 548 men who had enough serum for leptin assays. They did not differ from the other men (Table S1) and were followed for 7.5 years. The prospective study was performed in 448 men who had follow-up spine X-rays (100 men dropped out because of death or health status).

### Leptin Measurements

Fasting blood samples were collected at 08.00 hours. Blood was centrifuged and separated. Serum aliquots were stored at −80°C without thawing until the assay in 2011. In order to limit the interassay variability, measurements were performed using kits from a single series (Table 1). AAC progression was calculated as the difference between the score on the last X-ray (3 or 7.5 years) and the baseline score divided by the follow-up duration. AAC was assessed in 2005–2006 (thus, without knowing leptin levels) by P.S. who evaluated the baseline and the follow-up X-rays side.

---

**Figure.** Abdominal aortic calcification (AAC) assessed using Kauppila’s score: (Left) no AAC (score=0); (Middle) mild AAC (score=4); (Right) severe AAC (score=21).
as total weekly intake of wine, beer, and spirits, expressed in g/week. Daily calcium intake was assessed by food questionnaire that was adapted for French dietary habits. Education level was categorized as >8 vs. ≤ 8 years of school. Occupational physical activity was assessed by a self-reported scale (low, medium, hard, very hard). Leisure current physical activity was calculated as the overall amount of time (hours/week) spent walking, gardening, and participating in sports activities. Diagnoses were self-reported as present vs. absent. They included ischemic heart disease, hypertension, diabetes mellitus. Participants self-reported current medications (vitamin K antagonists, fibrates and statins). Weight, height, and waist and hip circumference were measured by P.S.

### Biological Assays

Glucose, triglycerides, cholesterol and high-density lipoprotein-cholesterol were measured by side, knowing their order. Reproducibility was assessed on 30 X-rays. The short- and long-term intra-observer reproducibility was excellent: intraclass correlation coefficient (ICC) of 0.95 and 0.91, respectively. The interobserver reproducibility was also excellent: ICC=0.92. As the difference between 2 readings was ≤1 point, a difference ≥2 points between the last and the first X-ray (>0.25 point/year for 7.5 years) was defined as meaningful AAC progression. This cutoff was selected arbitrarily because it was considered to exclude false positives and did not exclude moderate progression.

### Questionnaire

Lifestyle and health status were assessed using interviewer-administered questionnaires at baseline. Smoking was categorized as current, former, and never. Alcohol intake was assessed as total weekly intake of wine, beer, and spirits, expressed in g/week. Daily calcium intake was assessed by food questionnaire that was adapted for French dietary habits. Education level was categorized as >8 vs. ≤ 8 years of school. Occupational physical activity was assessed by a self-reported scale (low, medium, hard, very hard). Leisure current physical activity was calculated as the overall amount of time (hours/week) spent walking, gardening, and participating in sports activities. Diagnoses were self-reported as present vs. absent. They included ischemic heart disease, hypertension, diabetes mellitus. Participants self-reported current medications (vitamin K antagonists, fibrates and statins). Weight, height, and waist and hip circumference were measured by P.S.

### Table 2. Description of the Participants According to the Leptin Quartiles

<table>
<thead>
<tr>
<th>Leptin Quartile</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>Waist (cm)</th>
<th>Hip (cm)</th>
<th>Waist-hip ratio</th>
<th>Smokers (n, %)</th>
<th>Alcohol intake (g/week)</th>
<th>Calcium intake (mg/day)</th>
<th>Education level (&gt;8 years, n, %)</th>
<th>Occupational activity (weak, n, %)</th>
<th>Leisure physical activity (g/week)</th>
<th>IHD (n, %)</th>
<th>Hypertension (n, %)</th>
<th>Diabetes mellitus (n, %)</th>
<th>AAC score</th>
<th>VKA (n, %)</th>
<th>Statins (n, %)</th>
<th>Fibrates (n, %)</th>
<th>Leptin (ng/mL)</th>
<th>Glycemia (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>Testosterone (nmol/L)</th>
<th>17β-estradiol (pmol/L)</th>
<th>25-hydroxycholecalciferol (ng/mL)</th>
<th>PTH (pg/mL)</th>
<th>Osteocalcin (ng/mL)</th>
<th>GFR (mL/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (&lt;3.86ng/mL)</td>
<td>65±8</td>
<td>64±7</td>
<td>168.9±5.6</td>
<td>24.86±2.59</td>
<td>91±8</td>
<td>97±6</td>
<td>0.95±0.07</td>
<td>Ccurrent 26 (19)</td>
<td>219 [31; 359]</td>
<td>741±254</td>
<td>34 (25)</td>
<td>10 (7)</td>
<td>21 [12; 30]</td>
<td>17 (12)</td>
<td>25 (18)</td>
<td>5 (4)</td>
<td>2 (0; 5)</td>
<td>5 (4)</td>
<td>2 (1)</td>
<td>13 (9)</td>
<td>2.08±1.15</td>
<td>104.1±27.2</td>
<td>227±37</td>
<td>55.0±16.4</td>
<td>137±60</td>
<td>144±36</td>
<td>116±30</td>
<td>28±12</td>
<td>20.4±6.7</td>
<td>14.8±6.9</td>
<td></td>
</tr>
<tr>
<td>Q2 (3.86–6.43ng/mL)</td>
<td>64±7</td>
<td>80.9±10.2</td>
<td>169.3±6.5</td>
<td>27.26±2.46</td>
<td>97±6</td>
<td>99±6</td>
<td>0.99±0.07</td>
<td>Former 64 (47)</td>
<td>234 [78; 437]</td>
<td>716±210</td>
<td>29 (21)</td>
<td>5 (4)</td>
<td>22 [13; 28]</td>
<td>14 (10)</td>
<td>28 (21)</td>
<td>5 (4)</td>
<td>1 [0; 4]</td>
<td>5 (4)</td>
<td>7 (5)</td>
<td>24 (18)</td>
<td>5.22±0.88</td>
<td>107.0±21.5</td>
<td>231±40</td>
<td>50.4±13.8</td>
<td>175±107</td>
<td>146±39</td>
<td>116±27</td>
<td>29±11</td>
<td>19.1±7.0</td>
<td>16.4±6.4</td>
<td></td>
</tr>
<tr>
<td>Q3 (6.43–10.15ng/mL)</td>
<td>65±7</td>
<td>89.9±14.9</td>
<td>168.7±6.1</td>
<td>28.40±3.07</td>
<td>100±8</td>
<td>99±6</td>
<td>1.01±0.08</td>
<td>Never 47 (34)</td>
<td>219 [47; 344]</td>
<td>732±229</td>
<td>36 (26)</td>
<td>3 (2)</td>
<td>21 [15; 30]</td>
<td>18 (10)</td>
<td>34 (25)</td>
<td>13 (9)</td>
<td>2 [0; 7]</td>
<td>2 (1)</td>
<td>7 (5)</td>
<td>16 (12)</td>
<td>8.12±1.09</td>
<td>111.3±27.0</td>
<td>230±36</td>
<td>48.4±13.8</td>
<td>77±97</td>
<td>146±35</td>
<td>111±27</td>
<td>26±10</td>
<td>19.7±13.7</td>
<td>14.8±6.4</td>
<td></td>
</tr>
<tr>
<td>Q4 (&gt;10.15ng/mL)</td>
<td>65±7</td>
<td>88.9±14.9</td>
<td>169.1±7.0</td>
<td>30.94±3.90</td>
<td>107±9</td>
<td>103±7</td>
<td>1.04±0.08</td>
<td></td>
<td>234 [109; 437]</td>
<td>736±235</td>
<td>40 (29)</td>
<td>9 (6)</td>
<td>18 [12; 27]</td>
<td>24 (17)</td>
<td>47 (34)</td>
<td>18 (13)</td>
<td>4 [1; 8]</td>
<td>2 (1)</td>
<td>7 (5)</td>
<td>23 (17)</td>
<td>15.44±5.49</td>
<td>133.2±24.0</td>
<td>226±41</td>
<td>47±15.2</td>
<td>92±110</td>
<td>141±36</td>
<td>110±29</td>
<td>26±10</td>
<td>18.4±6.5</td>
<td>14.8±6.9</td>
<td></td>
</tr>
</tbody>
</table>

aAnalysis of variance. Age-adjusted. AAC, abdominal aortic calcification; BMI, body mass index; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein-cholesterol; IHD, ischemic heart disease; LDL-C, low-density lipoprotein-cholesterol; PTH, parathyroid hormone; VKA, vitamin K antagonist.
protein-cholesterol (HDL-C) were measured as previously described. Low-density lipoprotein-cholesterol (LDL-C) was calculated by Friedewald’s equation. Testosterone, 17β-estradiol, 25-hydroxycholecalciferol (25OHD) parathyroid hormone (PTH) and osteocalcin were measured as previously described. Thyroid hormone (PTH) and osteocalcin were measured as previously described. β Estradiol, 25-hydroxycholecalciferol (25OHD) were measured as previously described.

LDL-cholesterol, and osteocalcin, and GFR. Variables associated with AAC at P<0.15 or changing the odds ratio (OR) by 5% were retained (see Results). The most relevant index of obesity was selected based on the AIC criterion and on the Hesmer-Lemeshow goodness-of-fit test. The most discriminating thresholds were established using Youden’s index. The same approach was used for the development of the model for the meaningful AAC progression (>0.25 vs. ≤0.25 point/year). The final model was further adjusted for baseline AAC severity.

### Results

#### Descriptive Analysis

Weight, BMI, waist, hip circumference, waist/hip ratio, prevalence of hypertension and diabetes, AAC score and serum triglyceride and glucose levels increased across the leptin quartiles (Table 2). Serum HDL-C, osteocalcin, and testosterone levels, and GFR decreased across the leptin quartiles. The trends remained significant after adjustment for age.

#### Cross-Sectional Study

The percentage of men with severe AAC (>6) increased...
levels vs. the reference group (normal triglycerides, lower in the men who had hypertriglyceridemia and higher leptin). In the multivariable models, hypertriglyceridemia (OR=2.45, 95% CI: 1.43–4.18, P<0.001). High triglyceride (>164 mg/dL obtained by Youden index, or therapy with fibrates) was associated with higher odds of severe AAC (Table 4). In a multivariable model high leptin levels). Higher leptin tended to be associated with severe AAC in the subgroups analyzed separately, but the link was not always significant because of poor statistical power. The interactions of leptin with smoking and hypertriglyceridemia were not significant (P=0.33 and P=0.47, respectively).

In all the models, abdominal circumference and other alternative indices of obesity were not significant (P>0.30). Adjustment for other measures of obesity did not change the link of leptin with severe AAC (e.g., with BMI: OR=1.66 per SD, 95% CI: 1.16–2.30, P=0.0017; highest vs. lowest quartile: OR=3.25, 95% CI: 1.40–7.48, P=0.0042).

Prospective Study
Men lost to follow-up were older and more often reported hypertension and ischemic heart disease (Table S2). They had higher waist-hip ratio, AAC and PTH, but less physical activity and 25OHD. Most differences lost significance after adjustment for age.

The median increase in AAC was 0.27 points/year (interquartile range: 0.13; 0.67 points/year). The percentage of men who had meaningful progression in AAC severity (>0.25 points/year) increased across the leptin quartiles (trend P<0.001) (Table 3). In the multivariable models higher leptin level was associated with higher odds of severe AAC. The most discriminating threshold of leptin was 8.93 ng/mL.

Leptin levels did not differ among the current, former and never smokers (P=0.15). As predicted probabilities of severe AAC were similar for current and former smokers, men were divided into ever-smokers (former/current) and never-smokers. Ever-smokers had higher odds of severe AAC vs. never-smokers in the multivariable models (OR=2.67, 95% CI: 1.51–4.74, P<0.001). In the multivariable models, ever-smoking and high leptin contributed jointly to severe AAC (e.g., with BMI: OR=1.66 per SD, 95% CI: 1.16–2.30, P=0.0017; highest vs. lowest quartile: OR=3.25, 95% CI: 1.40–7.48, P=0.0042).

Prospective Study
Men lost to follow-up were older and more often reported hypertension and ischemic heart disease (Table S2). They had higher waist-hip ratio, AAC and PTH, but less physical activity and 25OHD. Most differences lost significance after adjustment for age.

The median increase in AAC was 0.27 points/year (interquartile range: 0.13; 0.67 points/year). The percentage of men who had meaningful progression in AAC severity (>0.25 points/year) increased across the leptin quartiles (trend P<0.001) (Table 5). In a multivariable model high

### Table 5. Association Between Serum Leptin Concentration and Risk of Meaningful AAC Progression

<table>
<thead>
<tr>
<th>Serum leptin</th>
<th>Prevalence</th>
<th>Analysis in the entire cohort</th>
<th>Analysis in the subgroups of the covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>≤9.75 ng/mL</td>
<td>No</td>
<td>165/293 (56%)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>No</td>
<td>77/103 (75%)</td>
<td>2.09 (1.14–3.64)</td>
</tr>
<tr>
<td>≤9.75 ng/mL</td>
<td>Yes</td>
<td>25/ 39 (64%)</td>
<td>1.91 (0.90–4.06)</td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>Yes</td>
<td>12/ 13 (92%)</td>
<td>14.95 (1.83–122.2)</td>
</tr>
<tr>
<td>High LDL</td>
<td>No</td>
<td>83/165 (50%)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>No</td>
<td>47/ 66 (72%)</td>
<td>2.08 (1.04–4.19)</td>
</tr>
<tr>
<td>≤9.75 ng/mL</td>
<td>Yes</td>
<td>107/167 (64%)</td>
<td>1.98 (1.24–3.15)</td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>Yes</td>
<td>42/ 50 (84%)</td>
<td>5.91 (2.46–14.16)</td>
</tr>
</tbody>
</table>

### Table 6. Odds of Meaningful AAC Progression Associated With Serum Leptin Concentrations and Other Risk Factors

<table>
<thead>
<tr>
<th>Leptin*</th>
<th>Covariate</th>
<th>Prevalence</th>
<th>Analysis in the entire cohort</th>
<th>Analysis in the subgroups of the covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>Current smoking</td>
</tr>
<tr>
<td>≤9.75 ng/mL</td>
<td>No</td>
<td>165/293 (56%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>No</td>
<td>77/103 (75%)</td>
<td>2.09 (1.14–3.64)</td>
<td>0.0327</td>
</tr>
<tr>
<td>≤9.75 ng/mL</td>
<td>Yes</td>
<td>25/ 39 (64%)</td>
<td>1.91 (0.90–4.06)</td>
<td>0.07</td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>Yes</td>
<td>12/ 13 (92%)</td>
<td>14.95 (1.83–122.2)</td>
<td>0.0154</td>
</tr>
<tr>
<td>High LDL</td>
<td>No</td>
<td>83/165 (50%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>No</td>
<td>47/ 66 (72%)</td>
<td>2.08 (1.04–4.19)</td>
<td>0.0414</td>
</tr>
<tr>
<td>≤9.75 ng/mL</td>
<td>Yes</td>
<td>107/167 (64%)</td>
<td>1.98 (1.24–3.15)</td>
<td>0.0156</td>
</tr>
<tr>
<td>&gt;9.75 ng/mL</td>
<td>Yes</td>
<td>42/ 50 (84%)</td>
<td>5.91 (2.46–14.16)</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

Note: Adjusted for age, weight, alcohol intake, education level, ischemic heart disease, hypertension, diabetes mellitus, fibrates, parathyroid hormone, triglycerides, glomerular filtration rate, and mutually exclusively for high LDL-C (>146 mg/dL or treatment with statins) or current smoking. *Established using Youden’s index. Index of obesity selected using the AIC criterion and Hosmer-Lemeshow’s goodness-of-fit test. AAC, abdominal aortic calcification; CI, confidence interval; LDL-C, low-density lipoprotein-cholesterol; OR, odds ratio.
leptin was associated with higher risk of AAC progression for all the approaches (continuous, quartiles, threshold). The relation persisted after adjustment for baseline AAC.

Current smokers had a higher risk of AAC progression vs. never-smokers (OR=3.00, 95% CI: 1.43–6.30, P<0.01), whereas former smokers did not (OR=1.36, 95% CI: 0.87–2.12, P=0.40). Current smokers had a higher risk of AAC progression vs. non-smokers (former and never): OR=2.53, 95% CI: 1.27–5.03, P<0.01. The interaction between smoking and leptin was not significant (P=0.31), but the risk of AAC progression was higher in current smokers with high leptin vs. the reference group (non-smokers, low leptin) (Table 6). This link persisted after adjustment for baseline AAC (OR=15.58, 95% CI: 1.83–132.30, P=0.0305). Moreover, higher leptin was associated with AAC progression in the subgroups analyzed separately.

Men with high LDL-C (>146 mg/dl by Youden’s index, or with statins) had a higher risk of AAC progression vs. men with normal LDL (OR=1.84, 95% CI: 1.22–2.79, P=0.0083). Higher leptin and LDL-C contributed jointly to the higher risk of AAC progression. The interaction between leptin and LDL-C was not significant (P=0.29), but the risk of AAC progression was higher in men with high LDL-C and leptin levels vs. the reference group (normal LDL-C, low leptin). This pattern remained significant after further adjustment for baseline AAC (OR=5.83, 95% CI: 2.41–14.13, P=0.0013). Moreover, higher leptin was associated with AAC progression in the subgroups analyzed separately.

In all the models, none of the alternative indices of obesity were significant (P>0.40). After adjustment for other measures of obesity (e.g., BMI), leptin remained predictive of rapid AAC progression (OR=1.28 per SD, 95% CI: 1.00–1.59, P=0.0500; highest vs. lowest quartile: OR=1.98, 95% CI: 1.01–4.02, P=0.0480).

Discussion

In a cohort of older men, high serum leptin levels were associated with higher odds of severe AAC and of meaningful AAC progression after adjustment for confounders. The associations of leptin with AAC were additive with those of smoking habit and serum levels of triglycerides and LDL-C.

Data on the association between serum leptin and AAC are scarce: a weak negative relation in 1 study, no link in others. Those studies did not provide the prevalence of severe AAC. They analyzed AAC as present/absent, which is not specific enough to study its associations with other factors. The link between leptin and AAC severity/progression has been poorly studied; it may be bystander of the link between obesity and AAC, but in the present study it was significant after adjustment for all indices of obesity, which themselves were non-significant in the models, including leptin.

The arterial wall may be a target for leptin. Leptin acts via its receptor on calcifying vascular cells in the calcifying aortic media. Leptin may stimulate osteoblastic differentiation. It activated transcription factor, Runx2, induces phosphorylation of the kinases Erk-1 and Erk-2, stimulates expression of osteoblast-specific proteins and calcification. It exerts these effects by enhancing the expression of RANKL and bone morphogenetic protein 4 (BMP4). A high fat diet increases leptin expression and the development of vascular lesions in the carotid arteries. Thus, our results are consistent with experimental data showing that leptin contributes to the development of calcification in the aortic media.

High leptin levels and smoking are associated with greater AAC severity and progression independently of each other. As smoking cessation cannot reverse calcific deposits, severe AAC is found in ever-smokers. Only current smoking is associated with AAC progression. Severe AAC has been found mainly in heavy smokers. Data on the effect of smoking and its cessation on leptin levels are discordant, even after adjustment for weight. Several mechanisms of the effect of tobacco on AAC are raised: insulin resistance, chronic inflammation, oxidative stress. The link between leptin, smoking and AAC is unclear; however, our data suggested that tobacco and leptin contribute additively to AAC development and independently of each other, being involved in different mechanisms.

Experimental studies suggest distinct pathways for the effect of leptin and lipids on AAC. Leptin-deficient mice fed a high fat diet had smaller atherosclerotic lesions (despite high triglyceride levels) vs. wild-type mice. In these mice, exogenous leptin decreased fat mass and the levels of serum cholesterol and triglycerides, but accelerated the progression of vascular lesions. In LDL receptor-deficient mice, a high fat diet induced vascular calcification by mechanisms involving BMP2 and reactive oxygen species. Thus, leptin, triglycerides, and LDL may stimulate AAC by distinct pathways. LDL can enter dysfunctional arterial endothelium and induce the formation of calcifying atherosclerotic plaques. This process, found mainly in the coronary arteries, may contribute to AAC progression, which is in line with our data that high LDL-C was associated with a greater increase in the AAC score. Kauppila’s score cannot measure the increase in calcium content in existing deposits. The increase in the AAC score reflects formation of new calcific foci, in line with the possible role of LDL.

Leptin-deficient mice had few atherosclerotic lesions and a high HDL-C level. In such mice, leptin injection lowered the HDL-C level. Exogenous HDL inhibited osteoblastic differentiation and calcification of calcifying vascular cells. However, in our study HDL-C was not associated with AAC severity/progression (data not shown). Because the data on the link between HDL-C and vascular calcification are discordant, the relationships between leptin, HDL and AAC need further study.

Our data have clinical importance. High leptin levels are associated with AAC severity and progression, after adjustment for measures of obesity. The link is significant regardless of the model, which suggests that leptin is an independent predictor of AAC progression. Regardless of its scientific novelty, our finding may stimulate further studies; for example, the possible use of leptin measurement to improve identification of men at high risk of rapid AAC progression.

High levels of serum leptin have been associated with measures of cardiovascular risk (calcification of aortic valve, carotid artery or coronary arteries, number of stenotic coronary vessels). but the data are limited, inconsistent and mainly from cross-sectional studies. AAC is interesting because severe AAC and rapid AAC progression strongly predict cardiovascular morbidity and mortality. Identification of men at high risk of rapid AAC progression enables earlier treatment and thus more efficacious reduction of their cardiovascular risk. AAC may be detected on
an X-ray ordered for another reason, so its assessment is easily available and inexpensive.

The potential effects of interventional modification of leptin levels (weight loss), of LDL levels (statins) or smoking cessation on AAC progression remain to be studied. Leptin and its analogs have been used in the treatment of obesity, but their side effects on AAC need studies; AAC may be increased by leptin directly or inhibited because of the improved metabolic status.

Adiponectin is an anti-inflammatory adipokin secreted by adipocytes and involved in the regulation of vascular metabolism. It inhibits VSMC proliferation and platelet aggregation. Low serum adiponectin levels are associated with higher vascular wall thickness in some, but not all studies. Thus, unlike leptin, serum adiponectin does not seem to be clinically useful for the identification of individuals at high risk of AAC or its progression.

Study Limitations
The study cohort including mainly low–middle-class community-dwelling men is not representative of the French population. We cannot extrapolate our results to women. Leptin was assayed in sera stored for 16 years, but unavoidable partial loss of leptin during the storage was probably similar in all samples (systematic error). A single assay may not reflect metabolic status. Information on lifestyle and diseases was self-reported at baseline. Duration and severity of diseases may produce residual confounding. Kaupilla’s score provides less accurate assessment of AAC than computed tomography (CT). It depends mainly on the length of deposits in the anterior and posterior aortic walls. CT results also reflect thickness, calcium density and calcified deposits in lateral areas. However, AAC assessed using this score predicts cardiovascular risk in the general population and in patients with chronic kidney disease.

We assessed AAC progression in men who returned for follow-up visits. The sickest men who did not return may have had higher AAC progression. In prospective studies, an increase in Kaupilla’s score reflects new calcific deposits, rather than thickening or mineralization of the existing ones. Thus, for the assessment of aggravation of AAC in longitudinal studies, the semiquantitative score is more specific but less sensitive than CT. The advantages of this score vs. CT include availability, affordability and low radiation dose, which is important, especially for the repeated measures in longitudinal studies. The choice of the AAC threshold (4th quartile, ≥2 points increase) was arbitrary; however, no guidelines define the threshold of clinically relevant AAC severity or progression. As the normal AAC score is zero and higher cardiovascular risk is found in patients with severe AAC, the highest quartile is a tradeoff between sensitivity and specificity. The 2-point increase in AAC score exceeds a change in AAC score related to a random error of the rater. We did not assess other metabolic measures (e.g., insulin, adipokines) and it is no longer possible to do so.

In conclusion, we showed that high leptin levels were associated with greater severity and rapid progression of AAC in older men. The clinical data confirmed experimental studies showing an effect of leptin on arterial calcification. Leptin may be involved in pathways that are distinct from those associated with smoking or lipids. The robust association between leptin levels and AAC progression showed that leptin was a strong predictor of rapid AAC progression and invites further studies on this topic. Our findings suggested that studies of the therapeutic use of leptin or its analogs should include an assessment of arterial calcification.

Conflict of Interest
The authors report no conflicts of interest.

Author Contributions
P.S. was responsible for the study, assessed aortic calcification, made the statistical analyses and wrote the manuscript. E.Z.A. performed measurements of leptin and contributed to data interpretation and the discussion. A.V. was responsible for the measurement of glycemia and lipids. P.P.-F. performed the assays of leptin and contributed to data interpretation and the discussion. E.F. contributed to data interpretation and the discussion. J.G. supervised the measurement of glycemia and lipids, reviewed the manuscript and contributed to the discussion. R.C. reviewed the manuscript and contributed to the discussion. V.B. was responsible for the measurement of leptin (including funding), contributed to data interpretation and the discussion, and revised the manuscript. P.S. is the guarantor of this work, had full access to all the data in the study and takes responsibility for the integrity and accuracy of the data analysis.

Funding

References
13. Zachariah JP, Hwang S, Hamburg NM, Benjamin EJ, Larson...
Serum Leptin and Aortic Calcification


Supplementary Files

Supplementary File 1

Table S1. Comparison of baseline characteristics in men who did or did not have leptin measurements

Table S2. Comparison of baseline characteristics in men who were or were not followed up

Please find supplementary file(s) at http://dx.doi.org/10.1253/circj.CJ-18-0517