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Serum fetuin-A levels and abdominal aortic calcification in healthy men — The STRAMBO study

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ABSTRACT

Vascular calcification results from an imbalance between increased extracellular levels of calcium and phosphate, reduced solubility, and low levels of calcification inhibitors in blood or the vascular wall. Fetuin-A is a major circulating calcification inhibitor. Rodent models of fetuin-A deficit indicate its calcification inhibiting potential. Clinical studies suggest its role as a biomarker in vascular disease. This cross-sectional study was performed in a cohort of 974 men aged ≥40 years (average 68 years) consisting of men holding health insurance cover with Mutuelle des Travailleurs de la Région Lyonnaise. Abdominal aortic calcification (AAC) was assessed semi-quantitatively on lateral dual energy X-ray absorptiometry (DXA) spine scans. Serum fetuin-A was measured by an immunoassay. After adjustment for confounders (age, lifestyle, body composition, health status, treatment, glomerular filtration rate [GFR], hormones, and cytokines), prevalence of severe AAC (AAC score > 4) decreased with increasing fetuin-A levels (OR = 0.68 per SD increase, 95% CI: 0.54–0.84, $p < 0.001$). After adjustment for confounders, low fetuin-A and hypertension were each associated with higher odds of AAC > 4. Coexistence of low serum fetuin-A levels and heavy smoking, elevated fibroblast growth factor 23 levels or low serum dickkopf-1 levels were associated with higher odds of AAC > 4. Similar results were obtained for 789 men with GFR > 60 mL/min/1.73 m^2. Similar results were obtained when severe AAC was defined as AAC score >3 or AAC > 5.

Thus, lower serum fetuin-A levels are associated with severe AAC, suggesting that poor calcification inhibitory potential contributes to vascular calcification, independently of renal impairment.

Introduction

Vascular calcification results from abnormal mineral homeostasis and a deficit in calcification inhibitors [1]. Severe abdominal aortic calcification (AAC) is associated with cardiovascular morbidity and mortality independent of other cardiovascular risk factors [2,3]. Some patient groups have a high risk of AAC, cardiovascular morbidity and mortality, e.g. patients with chronic kidney disease or diabetes mellitus [4–6]. Thus, characterizing determinants of severe AAC may improve the early identification of subjects at high cardiovascular risk, who may benefit from lifestyle modification, or treatment of cardiovascular risk factors.

Rodent models have revealed a number of calcification inhibitors, acting in the circulation or in the vascular wall. Fetuin-A, synthesized mainly in the liver as a negative acute phase reactant, is a potent calcification inhibitor which acts by binding small clusters of calcium and phosphate [7]. In mice, targeted ablation of fetuin-A gene (on a genetic background prone to calcification or on a mineral- and vitamin D-rich diet) leads to a phenotype of heterotopic calcification in most organs (heart, kidney, lung) and renal arterioles, but not in large arteries [8]. In another animal model, crossing fetuin-A −/− mice with ApoE −/− mice in the context of hyperphosphatemia and chronic kidney disease increased intimal calcification (typical for atherosclerosis), rather than medial calcification (typical for large arteries) [9].

Clinical studies on the association of fetuin-A and atherosclerosis have yielded ambiguous results. Lower fetuin-A levels were associated with higher cardiovascular mortality, but not with future cardiovascular events in patients with prevalent coronary artery disease [10–12]. One-year cardiovascular mortality was highest in patients with low fetuin-A serum levels associated with high C-reactive protein (CRP) [13]. In

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contrast, high circulating fetuin-A levels were associated with the risk of myocardial infarction, or metabolic syndrome and an atherogenic lipid profile in patients with coronary artery disease [10,14,15]. Finally, findings on the association between fetuin-A levels and the risk of stroke were discordant [13,16].

Previous studies have mainly focused on the association of fetuin-A and vascular calcification in patients with chronic kidney disease (CKD) [17,18]. By contrast, data in the general population without CKD are limited. In a small prospective study lower fetuin-A levels were associated with faster progression of calcification of aortic and mitral valves [19]. In a group of diabetic patients, calcified plaques in carotid or femoral arteries were associated with lower fetuin-A levels [20]. The aim of our study was to assess the association between circulating fetuin-A and AAC in a cohort of home-dwelling men who did not self-report CKD.

Subjects and methods

Participants

The STRAMBO study is a single center cohort study of skeletal fragility and its determinants in men performed as a collaboration between Institut National de la Santé et de la Recherche Médicale and Mutuelle des Travailleurs de la Région Lyonnaise (MTRL) [21]. The study was approved by the local ethics committee and is conducted in agreement with the Helsinki Declarations of 1975 and 1983. Letters inviting participation in the study were sent to a randomly selected sample of men aged 20–87 years from the MTRL lists living in greater Lyon. During the recruitment in 2006–2008, 1169 men provided informed consent to participate in the study. All men able to give informed consent, to answer the questionnaire, and to participate in the diagnostic exams were included. No specific exclusion criteria were used. As very few men aged <40 years had AAC, this analysis was performed in 974 men aged ≥40 years who had AAC assessment and fetuin-A measurements.

Serum measurements

Non-fasting serum was collected at 1:00 p.m. and stored at −80 °C. Fetuin-A was measured in duplicate by a human fetuin-A ELISA kit (Epitope Diagnostics Inc., San Diego, CA) after appropriate dilution. The recovery of a known concentration of recombinant fetuin-A in a sample buffer (spike recovery) is 97–107%. The assay utilizes the two-site sandwich technique with two selected goat anti-human fetuin-A polyclonal antibodies that bind to different epitopes of human fetuin-A. Detection limit is 5 ng/mL (concentration directly measurable). The intra- and inter-assay coefficients of variations are <10%.

Serum total osteocalcin (tOC) was measured by a human-specific immunochemiluminescence assay (ELECSYS, Roche, Indianapolis, IN) [21]. Serum calcium and phosphate were measured by standard laboratory methods [21]. Serum 25-hydroxycholecalciferol (25OHD) was measured by radioimmunoassay (RIA) after acetonitrile extraction (DiaSorin, Stillwater, MN) [22]. Serum parathyroid hormone (PTH) was measured by a human-specific immunochemiluminescence assay (ELECSYS; Roche Diagnostics, Mannheim, Germany) [23]. Serum testosterone was measured by a tritiated RIA after diethylether extraction [24]. Serum 17β-estradiol was measured using an ultrasensitive RIA (CISBio-International, Gif sur Yvette, France) [24]. Fibroblast growth factor 23 (FGF23) levels were measured using an ELISA which recognizes the intact molecule and carboxy-terminal fragments of FGF23 (Immutopics, San Clemente, CA) [25]. Myostatin was measured by a competitive ELISA (Immundiagnostik AG, Bensheim, Germany) based on polyclonal antibodies raised in a rabbit against recombinant human myostatin [26]. Osteoprotegerin (OPG) was measured by ELISA (Biomedica, Vienna, Austria) [27]. High-sensitivity CRP was measured by an immunoturbidimetric assay (Cobas; Roche Diagnostics) [28]. Human DKK-1 was measured with a commercial assay from Biomedica (Vienna, Austria) [29]. The compensated Jaffé method was used for detection of creatinine. Glomerular filtration rate (GFR) was estimated by the CKD-EPI equation [30].

Dual energy X-ray absorptiometry (DXA)

Body composition was assessed by whole body DXA performed using a Hologic Discovery A device equipped with a rotatory C-arm (HOLOGIC Inc., Waltham, MA) [31]. Lateral single-energy scans of the spine were obtained in the dorsal decubitus position.

Assessment of the abdominal aortic calcification (AAC) score

The AAC score was assessed on the lateral DXA spine scans using a semiquantitative method [32]. Calcific deposits in the abdominal aorta adjacent to the first four lumbar vertebrae were assessed for the posterior and anterior aortic walls using the midpoint of the intervertebral space above and below the vertebra as boundaries (8 segments). Individual severity scores for each segment (0 to 3) were added to yield an AAC score ranging from 0 to 24. Intra- and interrater reproducibility was assessed by two readers on 76 scans. Intra- and interrater agreement for continuous AAC score assessed by intraclass correlation coefficient was 0.95 and 0.90. The agreement for AAC dichotomized as AAC > 4 vs. AAC ≤ 4 (the cut-point used in this study) was calculated using the κ score. The intra- and interrater agreement score was excellent: $\kappa = 0.92$ (95% CI: 0.86; 0.99) and $\kappa = 0.84$ (95% CI: 0.70; 0.95), respectively.

Covariates

Participants completed interviewer-administered questionnaire. Lifestyle and medical history were self-reported without formal ascertainment. Smoking was categorized as current, former smoker, and never smoker, and lifelong smoking was calculated. Alcohol intake was evaluated by adding up the current average weekly intakes of wine, beer, and spirits, and the sum of the collected values was divided into quartiles. Caffeine intake was defined as the total amount of cups of coffee, cups of tea, and glasses of cola drunk per week; the data for the total values were divided into quartiles. The time spent outdoors was assessed as the total time spent per week walking, gardening, and practicing outdoor leisure physical activity (including seasonal activities). Participants self-reported chronic diseases (ischemic heart disease, hypertension, diabetes mellitus) and their pharmacological treatment. No participant self-reported CKD. Weight and height were measured in light clothes without shoes using standard clinical equipment.

Statistical analysis

Statistical analyses were performed using the SAS 9.1 software (SAS, Cary, NC). Data are presented as the mean ± SD or median (interquartile [IQ] range). Variables with non-Gaussian distribution were log-transformed. Associations between linear variables were assessed by simple and partial Pearson's correlation coefficient. Comparisons between groups were performed using analysis of covariance adjusted for confounders. Prevalence of severe AAC across quartiles of serum fetuin-A was assessed using the chi^2 test with the evaluation of trend. Multivariable adjusted association between fetuin A levels and dichotomized AAC score was assessed using logistic regression adjusted progressively for age, lifestyle factors (smoking categorized as never smokers and quartiles of lifelong smoking; alcohol intake, caffeine intake and leisure physical activity divided into quartiles), height, body composition (lean mass, fat mass), health status (ischemic heart disease, hypertension, diabetes mellitus, history of stroke, Parkinson's disease — dichotomized as yes vs. no), treatments (vitamin D supplementation, vitamin K antagonists — dichotomized as yes vs. no), hormones, cytokines (25OHD, OPG, FGF23, tOC, testosterone,