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Correlation of Endogenous Mineralization-Inhibitory Activity of Arterial Conduits With Gensini Score in Patients Undergoing Coronary Artery Bypass Grafting: A Cross-Sectional Study

Monica Kakkar [1], Rajiv Kumar [2], Dilrag Dhindsa [2], Rakesh Kakkar [3], Disha Sharma [1], Davinder Singh [1]

1. Biochemistry, All India Institute of Medical Sciences, Bathinda, Bathinda, IND 2. Cardiothoracic and Vascular Surgery, All India Institute of Medical Sciences, Bathinda, Bathinda, IND 3. Community and Family Medicine, All India Institute of Medical Sciences, Bathinda, Bathinda, IND

Corresponding author: Monica Kakkar, drmonicakakkar@gmail.com

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Abstract

Introduction

Arterial conduits used in coronary artery bypass grafting (CABG) harbour loosely associated, matrix-bound biomolecules that inhibit calcium-phosphate mineralization in vitro. Whether the potency of this endogenous inhibitory activity varies with the angiographic severity of the underlying coronary artery disease (CAD) has not been examined. We investigated the relationship between the mineralization-inhibitory activity of arterial-conduit extraction fluid and the Gensini score in patients undergoing CABG.

Methods

In this cross-sectional study, arterial conduit segments surplus to CABG requirement were prospectively collected from 60 male patients at All India Institute of Medical Sciences (AIIMS) Bathinda, India, and stratified into three groups of 20 each by Gensini score tertile (Group I - severe, 140-258; Group II - moderate, 90-136; Group III - mild, 51-85). To prepare the organic matrices from arterial conduits for in vitro mineralization studies, conduits were incubated in extraction fluids for the release of loosely bound biomolecules. Extraction fluids were tested in homogeneous (matrix-free) and heterogeneous (matrix-dependent) in vitro mineralization assay systems; percentage inhibition of Ca^{2+} and HPO_4^{2-} precipitation was calculated relative to controls without extraction fluid. Patient-level one-way ANOVA (with Welch's correction where variances were unequal), Tukey post-hoc comparisons, and Pearson correlation against each patient's exact Gensini score were applied; $p < 0.05$ was considered significant.

Results

Extraction-fluid inhibitory potency showed a significant, monotonic inverse relationship with Gensini score in both assay systems (all $p < 0.001$). Heterogeneous-system Ca^{2+} inhibition rose from 3.88 ± 0.70% in Group I to 12.86 ± 1.97% in Group III; homogeneous-system Ca^{2+} inhibition rose correspondingly from 8.92 ± 1.00% to 18.07 ± 1.83%. In both systems, inhibition of Ca^{2+} precipitation exceeded that of HPO_4^{2-} precipitation, and inhibitory potency was consistently greater in the homogeneous than the heterogeneous system. Pearson correlation coefficients against each patient's exact Gensini score ranged from −0.84 to −0.89 across the four inhibition parameters (all $p < 0.001$).

Conclusions

The inverse gradient between arterial-conduit mineralization-inhibitory activity and angiographic disease severity supports the hypothesis that depletion of loosely associated endogenous inhibitors accompanies, and may contribute mechanistically to, the atherosclerotic calcification process reflected by the Gensini score. These findings are hypothesis-generating and require confirmation with molecular characterisation of the responsible inhibitors and multivariable analysis.

Categories: Cardiology, Cardiac/Thoracic/Vascular Surgery
Keywords: atherosclerosis, calcification inhibitors, calcinosis, coronary artery bypass grafting, gensini score, in vitro mineralization assay, vascular calcification

Introduction

Coronary artery bypass grafting (CABG) remains a cornerstone of revascularisation for complex, multi-vessel coronary artery disease (CAD) [1,2]. The severity of the underlying atherosclerotic burden, most commonly quantified angiographically by the Gensini score, which weights the degree of luminal narrowing at each coronary segment by a factor reflecting its functional importance, remains the principal determinant of the indication for, and anticipated benefit from, surgical revascularisation [3].

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Atherosclerosis is a chronic inflammatory disease of the arterial wall that begins with endothelial dysfunction and subendothelial retention of apolipoprotein-B-containing lipoproteins, principally low-density lipoprotein (LDL) [4], within the arterial intima, initiating the inflammatory cascade of foam-cell formation, plaque growth, vascular calcification, and downstream cardiovascular risk [5-7]. For research and clinical decision-making, several angiographic and tomographic scoring systems have been developed to quantify CAD severity. The Synergy Between Percutaneous Coronary Intervention With Taxus and Cardiac Surgery (SYNTAX) score, developed for the SYNTAX trial, is a lesion-based system that grades the complexity of coronary disease by combining the number and location of significant (≥50%) stenoses with anatomical features including calcification [8]. The Gensini score, in contrast, is a purely stenosis- and location-weighted system. In the Gensini score, the percentage luminal narrowing at each coronary segment is converted to a geometric severity coefficient (1-32), multiplied by a factor reflecting the functional importance of the myocardial territory supplied by that segment, and the products are summed across all diseased segments to yield a single continuous measure of cumulative atherosclerotic burden [3]. A third widely used modality, the coronary artery calcium (CAC) score, is derived by the Agatston method from non-contrast computed tomography and is used extensively for cardiovascular risk stratification in asymptomatic populations [9]. Unlike Gensini and SYNTAX, which are obtained from angiography and reflect luminal stenosis, CAC scoring measures the mineral phase itself. CAC correlates significantly, though only moderately, with both Gensini and SYNTAX scores in direct comparison (r≈0.40-0.46) [10]. Friesinger and the Duke Jeopardy score are other systems rarely used, as their resolution is coarser than that of Gensini, SYNTAX, or CAC.

We selected the Gensini score for this study for four reasons. First, it is a more direct surrogate of cumulative atherosclerotic plaque burden - the process our extraction-fluid mineralization assay is designed to probe - whereas the SYNTAX score, based on anatomical complexity and revascularisation difficulty, is comparatively less efficient for tracking plaque burden [11]. Second, the Gensini score requires only the standard diagnostic coronary angiogram and a validated segment-weighting table, without a dedicated online calculator or the additional anatomical variables required for the SYNTAX score. Third, although CAC scoring measures calcified mineral directly and might appear the more intuitive comparator for a study of mineralization-inhibitor biology, it requires a dedicated non-contrast CT acquisition and radiation exposure that falls outside the standard pre-operative work-up for CABG; the Gensini score, by contrast, is derived from the diagnostic angiogram that every patient in this cohort undergoes as a mandatory pre-operative investigation [10]. Fourth, the Gensini score is well validated as a research tool that enables standardised comparison across studies and populations [12] and has been used extensively to correlate angiographic severity with circulating calcification-inhibitor levels [13-16].

Vascular calcification is physiologically restrained by endogenous calcification inhibitors, including matrix Gla protein (MGP), fetuin-A, osteopontin, osteoprotegerin, inorganic pyrophosphate (PPi) [7,13,14] and uncarboxylated MGP fractions [15,16]. Circulating levels of several of these inhibitors track with coronary disease severity. In parallel, a distinct pool of inhibitors has been shown to exist in a loosely, non-covalently associated state within connective-tissue matrices, originally characterised in rabbit tendon and rabbit aorta, potently suppressing hydroxyapatite formation in standardised in vitro mineralization assays [17,18]. The inhibitory activity can be assessed either indirectly by radiography, which measures plaque burden, or by determining concentration in serum/plasma, as reported for fetuin-A, MGP fractions, and Gla-rich protein [14-16], or directly by assessing the inhibitory potency of the extraction fluids obtained from the processing of the tendons/arteries/veins by using the standardized in vitro assay system of mineralization. Because the tissue-extraction approach measures inhibitory activity where atherosclerotic calcification actually occurs, it offers a mechanistically distinct advantage over imaging modalities.

Whether the abundance of these loosely associated inhibitors in human arterial conduits varies with the angiographic severity of the coronary atherosclerosis for which the conduit is being harvested has not previously been examined.

We hypothesised that the release or progressive depletion of such inhibitors from the vessel wall is mechanistically linked to the calcification that accompanies atherosclerotic plaque progression, and hence that extraction-fluid inhibitory potency would fall as angiographic disease severity, and by extension Gensini score, rises. To test this, we prospectively compared the in vitro mineralization-inhibitory activity of arterial conduit extraction fluid across three Gensini-score-defined tertiles of patients undergoing elective CABG. The primary objective of this study was therefore to determine whether the mineralization-inhibitory potency of arterial-conduit extraction fluid correlates inversely with angiographic CAD severity, as quantified by the Gensini score, in patients undergoing CABG.

Materials And Methods

Study design and ethics

This was a prospective, cross-sectional, comparative study conducted at a tertiary-care academic centre in India (All India Institute of Medical Sciences (AIIMS) Bathinda), reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for cross-sectional studies. Extraction fluid inhibitory-capacity testing and Gensini scoring were performed

independently and in parallel by two separate teams, each blinded to the other's results, as detailed below.

Study population

Over a 17-month period (April 2025 to August 2026), 60 male patients undergoing elective CABG at AIIMS Bathinda were enrolled. Patients with a prior history of myocardial infarction, stroke, chronic kidney disease, diabetes mellitus, tobacco smoking, or a mineral-metabolism disorder were excluded to limit confounding of calcium-phosphate handling by these conditions. Only male patients were enrolled, as it has not been established whether sex modifies either extraction-fluid inhibitory capacity or the Gensini score; restricting the cohort to one sex removed this as a potential confounder, at the cost of generalisability to women, which is addressed as a limitation below. Sample size was determined pragmatically by the number of consecutive eligible patients meeting these criteria over the enrolment period, without a pre-specified sample-size calculation. For transparency, a post-hoc power calculation based on the effect sizes actually observed indicated that this sample (n = 20/group) was well powered (>99.9%) to detect the reported between-group differences at $\alpha = 0.05$. As this calculation uses the study's own observed effect sizes rather than an independently anticipated effect, it does not retrospectively justify the sample size and is reported for transparency only, not as a substitute for a priori power estimation, which should be incorporated in future prospective studies.

Tissue collection

Arterial conduits (left internal mammary artery (LIMA) in 60 patients) surplus to CABG requirement were harvested using a standard no-touch technique to minimise pre-analytical mechanical injury, and anastomosed to the left anterior descending artery in the conventional manner. A segment of each harvested arterial conduit was excised, collected into a sterile container at 4°C, and processed for extraction-fluid preparation within approximately 20 minutes, before construction of the distal anastomosis. This approach yielded a single, consistently timed arterial specimen per patient across all 60 patients, minimising confounding from variable handling or ischaemic time. Laboratory personnel performing tissue processing and the mineralization assays described below had no access to any patient's Gensini score or angiographic findings at any stage of sample handling or analysis.

Extraction fluids and assay systems

To release loosely bound biomolecules, tissue samples were incubated (incubator shaker: S.M. Scientific Instruments Pvt. Ltd., Delhi/Ghaziabad, India) in an extraction medium containing 3% Na_2HPO_4 and 0.1 mM $CaCl_2$ at pH 7.4, using 1 mL of extraction fluid per 10 mg of tissue. To induce spontaneous mineralization, the homogeneous (matrix-free) system consisted of 5.0 mM $CaCl_2$ and 5.0 mM KH_2PO_4 in Tris-HCl buffer with 105 mM NaCl, pH 7.4; the heterogeneous (matrix-dependent) system had an identical composition to the homogeneous system, except for the addition of 1 mg of lyophilised conduit tissue (lyophiliser: iGene Labserve Private Limited, New Delhi, India). All reagents (Na_2HPO_4, $CaCl_2$, KH_2PO_4, Tris-HCl, NaCl) were of the American Chemical Society (ACS) grade, obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India. A 200 µL aliquot of extraction fluid was added to each assay system to test its effect on mineralization. Before addition of extraction fluid, the calcium and phosphate content of each fluid was determined and compensated for, so that control and test systems contained identical starting concentrations of these ions. Test tubes were incubated at 37°C for 15 minutes (incubator shaker: Neuation iShak, Gandhinagar, Gujarat, India) and then centrifuged at 5,000 rpm for 10 minutes (Thermo Fisher Scientific, Waltham, MA) to obtain the mineral-phase precipitates. Precipitates were dissolved in 5 mL of 0.1 N HCl for calcium and phosphate determination. Recovery of more than 98% of the added calcium and phosphate from the combined precipitate and supernatant confirmed the mass balance, integrity, and validity of the assay system used in this study. In each assay batch, calcium and phosphate determinations were referenced against standard curves prepared from serial dilutions of calcium chloride and potassium phosphate solutions of known concentration, run concurrently with the test and control systems, to ensure quantification accuracy across batches.

Biochemical measurements and outcomes

Calcium and phosphate concentrations were determined by ion-selective electrode (Orion Star, Thermo Fisher Scientific, Waltham, MA) [19] and the Malachite Green assay (Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India) [20], respectively. Percentage inhibition caused by each extraction fluid was calculated relative to the ion concentration in the corresponding control system (without extraction fluid). The Ca^{2+}/HPO_4^{2-} ratio in the mineral phase formed was consistent with the 1.67:1 ratio characteristic of hydroxyapatite.

Gensini scoring

Independently of, and in parallel with, the extraction-fluid inhibitory-capacity testing described above, the Gensini score of each patient was calculated from the invasive coronary angiogram [3] obtained as part of routine pre-operative work-up. Angiograms were scored independently by two surgeons, each blinded to every patient's inhibitory-capacity results. A third surgeon was designated to adjudicate any discordant scores, although no such discordance occurred in this cohort. Raw inter-observer agreement between the

two independent, blinded evaluators was therefore complete (100%, 60/60 patients); because no discordant pairs occurred, Cohen's kappa is mathematically undefined (it is computed from the pattern of disagreement) and is not reported, and the absence of any discordant case is presented instead as the direct measure of concordance achieved in this cohort.

Group stratification

Once Gensini scores were available for all 60 patients, scores were ranked in descending order, and the cohort was divided into three equal groups of 20: the 20 highest scores (Group I), the middle 20 (Group II), and the 20 lowest scores (Group III). Because groups were formed by rank rather than by pre-specified score thresholds, the resulting Gensini-score ranges are of unequal width and reflect this cohort's actual score distribution rather than fixed clinical cut-offs: Group I, 140-258 (severe disease); Group II, 90-136 (moderate disease); Group III, 51-85 (mild disease). Only after group membership was fixed on this basis were the separately measured, blinded inhibitory-capacity results merged in for between-group comparison.

Statistical analysis

Data were analysed in Statistical Product and Service Solutions (SPSS, version 29.0; IBM SPSS Statistics for Windows, Armonk, NY). Each patient contributed a single reading per parameter (four extraction-fluid inhibition parameters), giving a total analytic sample of 60 patients (n = 20/Gensini-score tertile). Continuous variables are expressed as mean ± standard deviation (SD) for each tertile. Normality of the distribution of each parameter within each group was assessed using the Shapiro-Wilk test, and homogeneity of variance across the three groups was assessed using Levene's test. Group differences across the three Gensini-score tertiles were tested by one-way ANOVA (F test, two and 57 degrees of freedom); where Levene's test indicated significant heterogeneity of variance, the Welch-corrected ANOVA was used in place of, and reported alongside, the standard F test, as this correction does not assume equal group variances. Where the overall ANOVA was significant, pairwise between-group comparisons (Group I vs II, I vs III, and II vs III) were performed using Tukey's honestly significant difference (HSD) post-hoc test. Patient-level Gensini scores were retained for this analysis, so the association between each parameter and angiographic severity was quantified using Pearson correlation coefficients computed against each patient's exact Gensini score, rather than a tertile-level approximation. A two-tailed $p < 0.05$ was considered statistically significant for all tests.

Results

The study cohort comprised 60 male patients undergoing elective CABG at AIIMS Bathinda, stratified into three Gensini score tertiles of 20 patients each (Table *1*).

Characteristic	Group I (severe, n=20)	Group II (moderate, n=20)	Group III (mild, n=20)	Total (n=60)
Age, years (mean ± SD)	58.40 ± 3.62	57.50 ± 4.30	55.55 ± 3.43	57.15 ± 3.92
Sex, male, n (%)	20 (100)	20 (100)	20 (100)	60 (100)
Cardiovascular disease status, n (%)	20 (100)	20 (100)	20 (100)	60 (100)
Hypertension (medication-controlled), n (%)	17 (85.0)	16 (80.0)	16 (80.0)	49 (81.7)
Dyslipidaemia, n (%)	19 (95.0)	18 (90.0)	19 (95.0)	56 (93.3)
Antiplatelet therapy, n (%)	20 (100)	20 (100)	20 (100)	60 (100)
Statin therapy, n (%)	20 (100)	20 (100)	20 (100)	60 (100)
Antihypertensive medication, n (%)	17 (85.0)	16 (80.0)	16 (80.0)	49 (81.7)

TABLE 1: Baseline demographic and clinical characteristics of the study cohort, by Gensini score tertile

Values are mean ± SD or n (%) as indicated; n = 20 patients per group. Sex is 100% male by design (see Methods, Study population). Note: Patients with a history of myocardial infarction (MI), ischaemic stroke, diabetes mellitus, tobacco smoking, chronic kidney disease (CKD), or mineral metabolism disorders referable to calcium-phosphate pathways were strictly excluded by protocol. All hypertensive patients were on guideline-directed antihypertensive therapy at enrolment.

Mineralization-inhibitory activity of arterial conduit exraction fluid (Table *2*): Extraction-fluid inhibitory potency showed a graded, statistically significant inverse relationship with Gensini score in both assay systems (Table *3*; all ANOVA $p < 0.001$; Pearson r ranged from −0.84 to −0.89 versus each patient's exact

Gensini score across the four inhibition parameters, all $p < 0.001$). In the heterogeneous system, Ca^{2+} precipitation was inhibited by 3.88 ± 0.70% in Group I (severe), 6.62 ± 1.28% in Group II (moderate), and 12.86 ± 1.97% in Group III (mild); HPO_4^{2-} inhibition followed the same pattern (2.90 ± 0.79%, 5.05 ± 1.65%, and 9.95 ± 1.80%, respectively). Levene's test indicated significant heterogeneity of variance for all four inhibition parameters (Table 3); Welch's ANOVA, robust to this violation, confirmed the same conclusion in every case (all $p < 0.001$). Shapiro-Wilk testing showed an approximately normal distribution within each group for all parameters (all $p > 0.05$) except homogeneous HPO_4^{2-} inhibition in Group I ($p = 0.042$), a minor deviation that does not affect the ANOVA/Welch conclusions given the concordant, robust Welch-corrected result. Tukey post-hoc comparisons were significant for every pairwise contrast (Group I vs II, I vs III, and II vs III) for all four inhibition parameters (Table 3). Inhibitory potency was consistently greater in the homogeneous (matrix-free) system than in the heterogeneous (matrix-dependent) system across all three groups. Ca^{2+} inhibition in Group III rose from 12.86 ± 1.97% (heterogeneous) to 18.07 ± 1.83% (homogeneous), suggesting that some inhibitory biomolecules become inactive or lose their inhibitory potency in the presence of the organic matrix, perhaps by binding to the non- specific sites of the matrix. In both assay systems and across all three groups, inhibition of Ca^{2+} precipitation consistently exceeded inhibition of HPO_4^{2-} precipitation, suggesting that the responsible biomolecules are predominantly acidic (net negatively charged) species with preferential affinity for the cationic calcium ion.

Gensini Score Group	Heterogeneous Assay System (% inhibition)		Homogeneous Assay System (% inhibition)	
	Ca^{2+}	HPO_4^{2-}	Ca^{2+}	HPO_4^{2-}
Group I (severe) Gensini, 140-258	3.88 ± 0.70	2.90 ± 0.79	8.92 ± 1.00	5.54 ± 0.54
Group II (moderate) Gensini, 90-136	6.62 ± 1.28	5.05 ± 1.65	11.83 ± 1.50	7.47 ± 1.15
Group III (mild) Gensini, 51-85	12.86 ± 1.97	9.95 ± 1.80	18.07 ± 1.83	12.04 ± 1.48

TABLE 2: Relationship between the Gensini score and the mineralization-inhibitory activity of arterial conduit extraction fluid

Values are mean ± SD, n = 20 patients per group. Groups were defined by the Gensini score tertile (Group I, severe, 140-258; Group II, moderate, 90-136; Group III, mild, 51-85). See Table 3 for inferential statistics.

Parameter	One-way ANOVA F (df 2,57), P	Welch's ANOVA F, P	Levene's test P (variance)	Pearson r vs Gensini score (95% CI)	Tukey post-hoc P (I-II/I-III/II-III)
Heterogeneous Ca^{2+} inhibition (%)	F=210.71, P<0.001	F=194.48, P<0.001	P<0.001	−0.882 (−0.928, −0.809)	<0.001/<0.001/<0.001
Heterogeneous HPO_4^{2-} inhibition (%)	F=118.49, P<0.001	F=128.55, P<0.001	P=0.0035	−0.840 (−0.902, −0.745)	<0.001/<0.001/<0.001
Homogeneous Ca^{2+} inhibition (%)	F=199.28, P<0.001	F=191.52, P<0.001	P=0.0385	−0.886 (−0.931, −0.816)	<0.001/<0.001/<0.001
Homogeneous HPO_4^{2-} inhibition (%)	F=175.82, P<0.001	F=175.19, P<0.001	P=0.0057	−0.859 (−0.914, −0.774)	<0.001/<0.001/<0.001

TABLE 3: Statistical comparison of extraction-fluid inhibitory activity across Gensini score tertiles

Values were derived from patient-level raw data (n = 60; 20/group). One-way ANOVA and Welch's ANOVA F statistics (df = 2, 57 for ANOVA; df = 2, variable for Welch's, computed per parameter) were calculated from the individual patient values. Levene's test P values reflect the corresponding test for homogeneity of group variances. Pearson r denotes the correlation of each parameter with each patient's exact Gensini score (n = 60); the accompanying 95% CI was computed by Fisher z-transformation. Tukey post-hoc P values are given in the order Group I vs II/Group I vs III/Group II vs III. P<0.05 was considered significant for all tests.

Discussion

Principal findings

This study shows that the mineralization-inhibitory activity of loosely associated biomolecules extracted from arterial CABG conduits falls in a graded, statistically robust manner as angiographic disease severity, quantified by the Gensini score, rises, in both a matrix-dependent (heterogeneous) and a matrix-free

(homogeneous) in vitro assay system, with patient-level Pearson correlations against each patient's exact Gensini score as strong as r=−0.89 (homogeneous Ca^{2+} inhibition) and confirmed by Welch's ANOVA, where group variances were unequal.

Comparison with previous literature and biological plausibility

Loosely associated, matrix-bound mineralization inhibitors were first characterised in animal connective tissue [17,18]. The present findings demonstrate that the inhibitory potency of the loosely bound inhibitor associated with arterial conduits varies inversely with the angiographic severity of the atherosclerotic process for which the conduit is being harvested. This is consistent with the broader concept in which circulating, well-characterised calcification-regulatory proteins like fetuin-A, MGP fractions, and Gla-rich protein, among others, correlate with coronary calcification burden and disease severity [14-16]. This theory can further be extended to a distinct, loosely matrix-bound pool of inhibitors that has not previously been benchmarked against angiographic severity. A plausible mechanistic sequence, consistent with our working hypothesis, is that progressive loss or consumption of these loosely bound inhibitors from the arterial wall permits calcium-phosphate deposition to proceed unopposed, contributing to plaque calcification, luminal narrowing, and the resulting rise in the Gensini score. The cross-sectional design of this study, however, cannot establish this temporal sequence, and the observed association is equally compatible with depletion of inhibitors being a consequence, rather than a cause, of advancing atherosclerotic calcification.

Strengths

This study benefits from the use of two complementary, previously validated in vitro assay systems, a well-defined tertile stratification by a widely used and reproducible angiographic severity score [3], and correlation analysis performed against each patient's exact Gensini score rather than a group-level approximation. A further methodological strength is that extraction-fluid inhibitory-capacity testing and Gensini scoring were conducted independently and in parallel by two separate teams, each blinded to the other's results, with group allocation fixed by Gensini-score rank before the two datasets were merged. This design minimises the possibility that either assessment was influenced, consciously or otherwise, by knowledge of the other.

Limitations

Several limitations warrant emphasis. First, the cross-sectional, single-centre design with a modest sample size (n = 60) precludes causal inference and limits generalisability. Second, the identity of the loosely associated inhibitory biomolecule(s) remains uncharacterised; proteomic profiling (e.g., liquid chromatography-tandem mass spectrometry (LC-MS/MS)) of the extraction fluid, as recommended for the companion arterial-versus-venous conduit study, would be required to determine whether the gradient observed here reflects established inhibitors (fetuin-A, MGP fragments, osteopontin, PPi) or a novel species. Third, only male patients were enrolled during the study period, so these findings cannot be generalised to women; sex-related differences in vascular calcification biology are recognised in the literature and should be examined in future work. Fourth, the exclusion criteria applied here (prior myocardial infarction, stroke, chronic kidney disease, diabetes mellitus, tobacco smoking, or a mineral-metabolism disorder) were deliberately strict, in order to isolate the relationship of interest from these well-recognised confounders of calcium-phosphate handling; this strengthens the internal validity of the primary comparison but correspondingly narrows the pool of eligible patients, which may limit both the generalisability of these findings to a less highly selected, real-world CABG population and the ease of replicating this specific cohort composition in future single-centre studies. Finally, because chronic kidney disease, diabetes mellitus and mineral-metabolism disorders were excluded by design rather than statistically adjusted for, and because statin therapy and dyslipidaemia were present in 100% and 93.3% of patients respectively across all three tertiles (Table *1*), leaving minimal residual between-group variability in these exposures, a multivariable adjusted analysis was not performed in this cohort; with only 60 patients, such a model would in any case have been substantially underpowered and at risk of overfitting. Larger, multicentric cohorts with more heterogeneous comorbidity and drug-exposure profiles will be required to formally adjust for these and other systemic confounders.

Future directions

Future work should prioritise: (i) proteomic/metabolomic characterisation of the inhibitory biomolecule(s); (ii) prospective, multicentric validation, including female patients, incorporating an a priori sample-size/power calculation and multivariable adjustment for systemic confounders (including chronic kidney disease, diabetes mellitus, and statin exposure) in a cohort with sufficient heterogeneity in these exposures; and (iii) mechanistic studies testing whether restoring inhibitory capacity (e.g., via pyrophosphate analogues or fetuin-A supplementation) decreases calcification in ex vivo arterial tissue with high baseline Gensini scores.

Conclusions

Arterial conduits from patients with more severe angiographic CAD harbour significantly less potent loosely associated mineralization-inhibitory activity than conduits from patients with milder disease, providing

preliminary support for the hypothesis that depletion of these inhibitors accompanies, and may mechanistically contribute to, the atherosclerotic calcification reflected in the Gensini score. This finding is hypothesis-generating rather than confirmatory and warrants molecular characterisation and further patient-level validation before its clinical implications for conduit selection or as a marker of calcification risk can be assessed.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Monica Kakkar, Rajiv Kumar, Rakesh Kakkar

Acquisition, analysis, or interpretation of data: Monica Kakkar, Dilrag Dhindsa, Rakesh Kakkar, Disha Sharma, Davinder Singh

Drafting of the manuscript: Monica Kakkar, Rakesh Kakkar, Disha Sharma, Davinder Singh

Critical review of the manuscript for important intellectual content: Monica Kakkar, Rajiv Kumar, Dilrag Dhindsa

Supervision: Monica Kakkar, Rajiv Kumar

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. AIIMS Bathinda Institutional Ethics Committee issued approval IEC/AIIMS/BTI/454, dated 27.01.2024. The project had the approval of the Indian Council of Medical Research (ICMR project ID: IPR-2023-6157) and the AIIMS Bathinda Institutional Ethics Committee (approval number IEC/AIIMS/BTI/454, dated 27.01.2024). Written informed consent was obtained from all patients. All procedures were in conformity with the Declaration of Helsinki. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** The authors express their sincere gratitude to the Indian Council of Medical Research (ICMR), New Delhi, India, for providing the financial support to conduct this study (Project ID: IRP-2023-6157; GAP no. 0041). **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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