Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Serum Calcification Propensity Is Increased in Myocardial Infarction and Hints at a Pathophysiological Role Independent of Classical Cardiovascular Risk Factors

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BACKGROUND: Vascular calcification is associated with increased mortality in patients with cardiovascular disease. Secondary calciprotein particles are believed to play a causal role in the pathophysiology of vascular calcification. The maturation time (T_{50}) of calciprotein particles provides a measure of serum calcification propensity. We compared T_{50} between patients with ST-segment–elevated myocardial infarction and control subjects and studied the association of T_{50} with cardiovascular risk factors and outcome.

METHODS: T_{50} was measured by nephelometry in 347 patients from the GIPS-III trial (Metabolic Modulation With Metformin to Reduce Heart Failure After Acute Myocardial Infarction: Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction: a Randomized Controlled Trial) and in 254 matched general population controls from PREVEND (Prevention of Renal and Vascular End-Stage Disease). We also assessed the association between T_{50} and left ventricular ejection fraction, as well as infarct size, the incidence of ischemia-driven reintervention during 5 years of follow-up, and serum nitrite as a marker of endothelial dysfunction.

RESULTS: Patients with ST-segment–elevated myocardial infarction had a significantly lower T_{50} (ie, higher serum calcification propensity) compared with controls (T_{50}: 289±63 versus 338±56 minutes; $P<0.001$). In patients with ST-segment–elevated myocardial infarction, lower T_{50} was associated with female sex, lower systolic blood pressure, lower total cholesterol, lower LDL (low-density lipoprotein) cholesterol, lower triglycerides, and higher HDL (high-density lipoprotein) cholesterol but not with circulating nitrite or nitrate. Ischemia-driven reintervention was associated with higher LDL ($P=0.03$) and had a significant interaction term for T_{50} and sex ($P=0.005$), indicating a correlation between ischemia-driven reintervention and T_{50} above the median in men and below the median in women, between 150 days and 5 years of follow-up.

CONCLUSIONS: Serum calcification propensity is increased in patients with ST-segment–elevated myocardial infarction compared with the general population, and its contribution is more pronounced in women than in men. Its lack of/inverse association with nitrite and blood pressure confirms T_{50} to be orthogonal to traditional cardiovascular disease risk factors. Lower T_{50} was associated with a more favorable serum lipid profile, suggesting the involvement of divergent pathways of calcification stress and lipid stress in the pathophysiology of myocardial infarction.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: atherosclerosis ■ heart disease risk factors ■ myocardial infarction ■ nephelometry and turbidimetry ■ ST elevation myocardial infarction ■ triglycerides ■ vascular calcification

Nonstandard Abbreviations and Acronyms

CPP	calciprotein particle
HDL	high-density lipoprotein
IDR	ischemia-driven reintervention
LDL	low-density lipoprotein
MI	myocardial infarction
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PREVEND	Prevention of Renal and Vascular End-Stage Disease
STEMI	ST-segment–elevation myocardial infarction

Highlights

- Serum calcification propensity is increased in patients with ST-segment–elevated myocardial infarction as compared with the general population.
- A higher serum calcification propensity is associated with a more favorable serum lipid profile in patients presenting with an ST-segment–elevated myocardial infarction, suggesting the involvement of divergent pathways of calcification stress and lipid stress in the pathophysiology of myocardial infarction.
- The association of calcification propensity with coronary artery disease is more pronounced in women than in men, and calcification propensity predicts the need for reintervention.
- Previous studies have shown that calcification propensity can be modified using low-cost and widely available treatments; therefore, our findings carry implications for future clinical studies on the prevention of coronary artery disease, especially in women.

Vascular calcification is a common pathological process that can manifest as 2 morphological entities: (1) medial calcification (Mönckeberg sclerosis), often encountered in aging and in chronic kidney disease, and (2) intimal calcification, often found in atherosclerotic cardiovascular disease. Vascular calcification is a strong predictor of cardiovascular mortality, independent of traditional cardiovascular risk factors.[1–6] Our understanding of the pathophysiology of vascular calcification continues to evolve, and recent data suggest that calcification not only involves passive deposition of calcium phosphate crystals but also an active, cell-mediated component.[7,8] Experimental data concerning the active cell-mediated component indicate that vascular smooth muscle cells can dedifferentiate from their normal, contractile phenotype and start to express osteoblast-related genes (eg, *Runx2*, *alkaline phosphatase*, and *osteopontin*) leading to active mineralization.[8–14] Calciprotein particles (CPPs) are highly abundant in animal models and patients affected by exaggerated vascular calcification[15,16] and are thought to be causally involved in the pathogenetic process of vascular calcification. CPPs are generated by aggregation of the soluble anticalcification protein fetuin A with calcium and phosphate, among other factors.[17,18] These aggregates spontaneously mature over time from primary CPPs (containing amorphous calcium phosphate) to secondary CPPs (containing crystalline calcium phosphate).[19–22] While timely clearance of primary CPPs constitutes an effective anticalcification mechanism, secondary CPPs have been found to induce vascular smooth muscle cell calcification,[7] and the rate at which primary CPPs mature into secondary CPPs is considered to be a reflection of the anticalcification buffering capacity of serum. Recently, a functional serum calcification propensity test has been developed to measure the half-time (T_{50}) speed of maturation of primary to secondary CPPs.[18,23–26] T_{50} values in patients with chronic kidney disease[27] or after renal transplantation[25,26] were inversely associated with mortality. We hypothesize that CPPs are involved in the etiology of vascular calcification in coronary artery disease in the absence of renal function impairment. Therefore, we studied whether T_{50} values differ between a cohort with advanced coronary artery disease and the general population and whether T_{50} values are associated with established cardiovascular risk factors, disease severity, and outcomes in patients with ST-segment–elevation myocardial infarction (STEMI).

MATERIALS AND METHODS

Availability of Data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Cohorts

Included patients with myocardial infarction (MI) were taken from the GIPS-III trial (Metabolic Modulation With Metformin to Reduce Heart Failure After Acute Myocardial Infarction: Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction: a Randomized Controlled Trial). In brief, the GIPS-III trial was a single-center, randomized, double-blind, placebo-controlled trial (URL: https://www.clinicaltrials.gov; unique identifier: NCT01217307) that evaluated the effect of metformin on left ventricular ejection fraction. Design, baseline characteristics, and primary outcomes of the trial have been reported previously. Metformin did not have an effect on left ventricular ejection fraction, diastolic function, or MI size.[28–30] Therefore, both treatment groups (placebo and metformin) were included in this study. Patients presenting with STEMI who underwent successful percutaneous coronary intervention were included. Important exclusion criteria were previous MI, known history of diabetes, inability to undergo magnetic resonance imaging, and severe renal dysfunction. On hospital admission, (baseline) blood samples were obtained, either anticoagulated with EDTA

or not. These samples were spun down, and collected plasma and serum samples were kept frozen at −80 °C until further analysis. After inclusion, patients were randomized to receive either 4 months of metformin 500 mg BID or visually matching placebo. Treatment was started within 3 hours after percutaneous coronary intervention. All patients provided informed consent before any study procedure.

Outcomes

Left ventricular ejection fraction and infarct size were determined 4 months after randomization by magnetic resonance imaging using a 3.0T scanner (Achieva; Philips) using a phased array cardiac receiver coil. ECG-gated cine steady-state free precession magnetic resonance images were acquired in contiguous short-axis slices of 1 cm covering the entire left ventricle, during repeated breath holds. Using identical slice locations, late contrast-enhanced images were acquired 10 minutes after intravenous administration of a gadolinium-based contrast agent. The magnetic resonance imaging scans were evaluated by an independent core laboratory (Image Analysis Center, VU University Medical Center, Amsterdam, the Netherlands), blinded for treatment allocation and clinical patient data. The borders of the endocardium were outlined in end-diastolic and end-systolic images. Left ventricular end-diastolic volumes and left ventricular end-systolic volumes were calculated using the summation of slice method multiplied by slice thickness. Infarct size was calculated by summation of the volumes per slice of areas of hyperenhancement.

In the GIPS-III trial, follow-up data were gathered during 5 years after randomization on a set of predefined clinical end points including death, reinfarction, recurrent coronary intervention, stroke, hospitalization for heart failure or chest pain, implantable cardioverter defibrillator implantation, and new-onset diabetes (defined as either receiving antidiabetic medication or a glycated hemoglobin level of ≥48 mmol/mol [≥6.5%] or a fasting glucose level of ≥11.1 mmol/L compatible with this diagnosis). Each outcome was assessed by an independent adjudication committee. For the present study, the incidence of ischemia-driven reintervention (IDR), defined as the occurrence of either percutaneous coronary intervention or coronary artery bypass grafting during 5 years of follow-up, was used as a secondary outcome.

To compare T_{50} in post-MI patients with T_{50} in the general population, a control group was matched on age, sex, history of hypertension, history of dyslipidemia, and history of diabetes from individuals participating in the PREVEND (Prevention of Renal and Vascular End-Stage Disease) cohort, which had recruited individuals in the same geographic area. Design, baseline characteristics, and primary outcomes of the trial have been reported previously.[31,32] For the measurement of serum T_{50} in PREVEND, serum samples were available from the second examination round, which took place between April 2001 and November 2003. Fasting blood samples were taken and kept frozen at −80 °C until further processing. Both study protocols were approved by the local ethics committee (University Medical Center Groningen, Groningen, the Netherlands) and were in accordance with the Declaration of Helsinki and Dutch laws.

T_{50} and Nitrite and Nitrate Measurements

T_{50} was measured as described previously.[18,26] Briefly, baseline sera stored at −80 °C were thawed at 4 °C overnight,

before vortexing and centrifugation. Supersaturated calcium (10 mmol/L, 35 µL) and phosphate (6 mmol/L, 25 µL) stock solutions, both pH 7.40 at 37 °C, were mixed with 40 µL of serum, and nephelometry was performed for 600 minutes in a Nephelostar nephelometer (BMG Labtech, Germany). Nonlinear regression analysis was performed to determine the half-maximal precipitation time. Samples from the GIPS-III cohort were from the baseline collection time point, that is, before coronary intervention and administration of either metformin or placebo.

Circulating concentrations of nitrite and nitrate were determined in serum aliquots of the GIPS-III and PREVEND cohorts using a dedicated high-performance liquid chromatography system for simultaneous nitrite/nitrate quantification (ENO-30 analyzer with AS-700 INSIGHT autosampler and Clarity Envision software; Amuza, Inc, USA/Eicom, Japan). Frozen samples were processed in batches of 10, thawed on ice, deproteinized with methanol (1:1 v/v) at room temperature, cleared by centrifugation, and added to a 96-well plate kept at 4 °C, followed by automated analysis injecting 20 µL of deproteinized sample onto the column (EICOM NO-PAK separation column followed by EICOM NO-RED reduction column). The system was calibrated daily using combined nitrite/nitrate standards (0.1−50 µmol/L), with additional validation of nitrite levels using spiked quality controls.

All measurements were performed in a blinded manner, using serum aliquots that had been kept frozen at −80 °C.

Statistical Analysis

Continuous variables are presented as mean±SD or median (interquartile range) for normally and non-normally distributed data, respectively. Differences between groups were tested using 2-tailed Student t test for normally distributed data and Wilcoxon rank-sum for non-normally distributed data. To identify possible associations of clinical and angiographic variables, as well as biomarkers with T_{50}, multivariable linear regression models correcting for age and sex were constructed. The candidate sets of variables measured at baseline ($P<0.05$) were checked for multicollinearity using variance inflation factor with a cutoff of 10, and when appropriate, the variable with the weakest relation to the outcome was excluded from further analysis. Bootstrapping stepwise regression was used to narrow the remaining candidate set of variables that were associated with T_{50}. The bootstrap sample size was 344 (99.1% of the entire data set). Variables selected >600× were assumed to be accurate and included in the final multivariable model. Each variable in the final model was tested for interaction with every other variable in the model.

Kaplan-Meier graphs and Cox proportional hazards models were used to determine whether T_{50} influenced IDR. Each individual variable of the final multivariable regression model for T_{50} was analyzed to determine whether it should be added to a multivariable Cox proportional hazards model. A multivariable Cox proportional hazards model for IDR was constructed, and each variable in the model was tested for interaction with every other variable in the model. The assumption of proportionality was tested in the final model by including time-dependent covariates and using the (scaled) Schoenfeld residuals. A time-dependent analysis was performed if the model violated the assumption of proportionality. Survival analyses were repeated with T_{50} as

a continuous and as a logarithmically transformed variable. All reported P values are 2 sided, and a P value of <0.05 was considered to indicate statistical significance. All analyses were performed using Stata (StataCorp. 2015. Stata Statistical Software: Release 14; StataCorp LP, College Station, TX).

RESULTS

Of the 379 participants with STEMI of the GIPS-III trial (the STEMI cohort), T_{50} was measured in 347 participants (92%; Figure S1) because of insufficient serum of 32 participants. Mean age was 58.7 (±11.7) years, 26% were women, and the mean T_{50} was 291 (±63) minutes in the STEMI cohort (Table 1). Baseline characteristics of the STEMI cohort patients were compared with individuals with T_{50} below (thus higher serum calcification propensity) versus above the median (Table 1). Patients with T_{50} below the median were more likely to be women, had lower systolic and diastolic blood pressures, had lower hemoglobin levels, had lower LDL (low-density lipoprotein) cholesterol levels, and had higher HDL (high-density lipoprotein) cholesterol levels.

From the STEMI cohort, 254 patients with measured T_{50} were matched 1:1 with 254 patients from the PRE-VEND cohort (the general population), based on age, sex, history of hypertension, history of dyslipidemia, and history of diabetes (Table 2). An analysis of these 254 patients from the STEMI cohort revealed a mean T_{50} of 289 (±63) minutes compared with 338 (±56) minutes in the 254 matched participants of the general population (P<0.001; Figure 1; Table 2). We then compared serum nitrite and nitrate levels, as a reflection of endothelial NO formation and availability. Serum nitrite levels were found to be lower in STEMI patients compared with the matched PRE-VEND cohort (1.1±0.8 versus 1.5±1.0 µmol/L; P<0.001; Figure 2A; Table S1). Serum nitrate levels, however, were not significantly different (33.5±17.4 [GIPS-III] versus 36.4±17.3 [PREVEND] µmol/L; P=0.08; Figure 2B). Linear regression analysis did not find any association between T_{50} and either nitrite or nitrate levels or between nitrite or nitrate levels and patient-related factors, such as smoking status, history of hypertension, history of dyslipidemia, or infarct-related parameters (data not shown).

Multivariable linear regression analyses of the baseline variables of the full STEMI cohort (n=347) showed an age- and sex-independent positive association of T_{50} with systolic blood pressure, total cholesterol, triglycerides, and LDL and a negative association with plasma urea, HDL, aspartate transaminase, NT-proBNP (N-terminal pro-B-type natriuretic peptide), and peak NT-proBNP (Table 3). Calculation of the variance inflation factor revealed collinearity between LDL and total cholesterol, resulting in the exclusion of total cholesterol from further regression analyses. Sex, systolic blood pressure, triglycerides, LDL, and HDL were highly selected in a bootstrap of the model for T_{50}. The multivariable adjusted

association of these variables is presented in Table 4. Notably, we did not find any association between randomization to metformin and T_{50} (Tables 1 and 3).

At 4 months of follow-up, magnetic resonance imaging scans were performed in 259 (75%) patients (Figure S1). Regression analyses suggested no association between T_{50} on admission with STEMI and left ventricular ejection fraction or T_{50} and infarct size.

After 5 years of follow-up, 42 (12%) patients had experienced an IDR event. Kaplan-Meier graphs and Cox proportional hazard models for IDR and the individual variables that were independently associated with T_{50} did not show a significant association with IDR events. After testing the proportionality assumption, we constructed a multivariable and time-dependent Cox proportional hazards model for IDR events, including age, sex, LDL, and T_{50} (above versus below median). Additional testing for interactions showed a significant interaction between sex and T_{50} for the association with IDR events. IDR events that occurred during the first 150 days after STEMI had no significant association with T_{50} (Table S2). However, IDR events occurring after 150 days were associated with higher LDL (P=0.03) and had a significant interaction term for T_{50} and sex (P=0.005), indicating an association between IDR and T_{50} above median in men and below median in women. (Table S3). Repeat analyses with T_{50} as a continuous and a logarithmically transformed variable produced similar results (Tables S4 through S7). Competing risk analyses for all-cause mortality did not change these associations.

DISCUSSION

This study revealed that serum calcification propensity was significantly increased in patients with STEMI compared with matched general population controls. This suggests that a reduction in T_{50} might indeed be linked to a preponderance toward calcification in coronary atherosclerosis. Previous work on T_{50} was mainly performed on vascular calcification in patients with renal function decline, including predialysis,[27,33,34] hemodialysis,[24,35] and renal transplantation cohorts,[25,26] as well as in the general population.[36] Likewise, increased serum calcification propensity has also been reported in patients with diabetes and ischemic heart failure.[37,38] We here show that the serum calcification propensity is increased in a population with acute MI independent of renal function decline. The STEMI cohort T_{50} values in our study are generally in line with values reported in other populations afflicted with vascular conditions, but we are cautious with the interpretation of absolute values, considering the overlap in T_{50} distribution.

Similar to the chronic kidney disease cohort described by Smith et al[27] and Mencke et al,[37] women in our cohort had a significantly higher serum calcification propensity (lower T_{50}) when compared with men. This suggests that the role of anticalcification buffering capacity of serum in the pathophysiology of vascular calcification and the

Table 1.   Baseline Characteristics for Patients With Myocardial Infarction and Stratified by T_{50} Above or Below Median

Characteristics	Total (n=347)	T_{50}≤median (n=177)	T_{50}>median (n=170)	P value
T_{50}, min	291±63	247±37	337±49	<0.001*
Age, y	58.7±11.7	59.3±11.9	58.1±11.5	0.34
Women	89 (25.6%)	55 (31.1%)	34 (20.0%)	0.02*
Metformin treatment	173 (49.9%)	86 (48.6%)	87 (51.2%)	0.63
Body weight, kg	84.4±14.4	83.2±14.5	85.6±14.2	0.12
Body mass index, kg/m²	27.0±3.8	27.0±4.2	27.1±3.3	0.86
Race/ethnicity				
White	333 (96.0%)	169 (95.5%)	164 (96.5%)	0.85
Asian	10 (2.9%)	6 (3.4%)	4 (2.4%)	
Black	4 (1.2%)	2 (1.1%)	2 (1.2%)	
Cardiovascular-related history				
Hypertension	105 (30.3%)	53 (29.9%)	52 (30.6%)	0.90
Dyslipidemia	216 (62.2%)	106 (59.9%)	110 (64.7%)	0.35
Current smoking	193 (55.6%)	99 (55.9%)	94 (55.3%)	0.90
Stroke	2 (0.6%)	1 (0.6%)	1 (0.6%)	1.00
Previous PCI	4 (1.2%)	2 (1.1%)	2 (1.2%)	0.97
Vital signs				
Systolic BP, mm Hg	133.7±23.3	130.1±23.2	137.5±22.8	0.003*
Diastolic BP, mm Hg	84.0±14.8	82.0±14.7	86.1±14.6	0.01*
Heart rate, bpm	75.6±16.4	74.6±17.4	76.7±15.4	0.23
Infarct-related factors				
Ischemic time, min	161 (109, 245)	167 (114, 266)	158 (109, 216)	0.07
Multivessel disease	237 (68.3%)	123 (69.5%)	114 (67.1%)	0.63
Anterior infarction*	137 (39.5%)	75 (42.4%)	62 (36.5%)	0.26
Intervention-related assessments				
TIMI flow grade pre-PCI ≤1	213 (61.4%)	109 (61.6%)	104 (61.2%)	0.94
TIMI flow grade post-PCI <3	32 (9.2%)	16 (9.0%)	16 (9.4%)	0.90
Myocardial blush grade ≤1	38 (11.0%)	22 (12.6%)	16 (9.5%)	0.36
Laboratory values at admission				
Glucose, mmol/L	8.2 (7, 9.5)	8.2 (6.9, 9.5)	8.3 (7.2, 9.4)	0.68
HbA1c, %	5.8 (5.6, 6)	5.8 (5.6, 6)	5.8 (5.6, 6.1)	0.17
Hemoglobin, mmol/L	8.9 (8.4, 9.4)	8.9 (8.3, 9.3)	9 (8.5, 9.5)	0.02*
Magnesium, mmol/L	0.8 (0.7, 0.8)	0.7 (0.7, 0.8)	0.8 (0.7, 0.8)	0.18
Creatinine, µmol/L	72 (62, 82)	71 (62, 81)	73 (62, 82)	0.42
eGFR, mL/min per 1.73 m²	95 (85, 103)	94 (84, 103)	96 (86, 103)	0.43
NT-proBNP, ng/L	80 (40, 188)	94 (42, 220)	66 (37, 178)	0.09
CK, U/L	131 (85, 213)	131 (83, 213)	132 (91, 209)	0.89
Myocardial band of CK, U/L	16 (13, 24)	16 (12, 24)	16 (13, 23)	0.75
Troponin T, ng/L	49 (23, 136)	52 (22, 160)	45 (26, 131)	0.81
Total cholesterol, mmol/L	5.3 (4.7, 6.1)	5.3 (4.6, 6)	5.4 (4.8, 6.1)	0.07
LDL cholesterol, mmol/L	3.8 (3.2, 4.4)	3.7 (3.1, 4.3)	3.9 (3.3, 4.5)	0.01*
HDL cholesterol, mmol/L	1.1 (0.9, 1.3)	1.1 (1.0, 1.4)	1.1 (0.9, 1.3)	0.02*

Values are displayed as mean±SD, n (%), or median (interquartile range). BP indicates blood pressure; CK, creatine kinase; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PCI, percutaneous coronary intervention; and TIMI, thrombolysis in myocardial infarction.

*$P<0.05$.

Table 2. Comparison of T_{50} in Patients With Myocardial Infarction With T_{50} in the General Population

| Characteristics	GIPS-III (n=254)	PREVEND (n=254)	P value
Age, y	58.6±10.2	58.6±10.2	1.00
Women	75 (30%)	75 (30%)	1.00
Hypertension	80 (32%)	80 (32%)	1.00
Hypercholesterolemia	132 (50%)	132 (50%)	1.00
Diabetes	0 (0%)	0 (0%)	1.00
T_{50}, min	289±63	338±56	<0.001*

Values are displayed as mean±SD or n (%). GIPS-III indicates Metabolic Modulation With Metformin to Reduce Heart Failure After Acute Myocardial Infarction: Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction: a Randomized Controlled Trial; and PREVEND, Prevention of Renal and Vascular End-Stage Disease.

*$P<0.05$.

development of acute coronary syndromes shows sexual dimorphism. Calcification might prognostically be more important in women, compared with men. This theory is supported further by our findings regarding the occurrence of reinfarction within 5 years after STEMI. Our data show that higher serum calcification propensity is associated with higher occurrence of IDR in women. However, in men, higher serum calcification propensity was associated with lower occurrence of IDR. We believe that the difference in findings before and after 150 days after intervention may also reflect a pathophysiological discrepancy between early or more technical complications (like in-stent thrombosis), compared with the later development of new occlusive lesions. Given the relatively small size of the group that underwent IDR and the short follow-up of 5 years, a larger study may provide more definitive answers to this question.
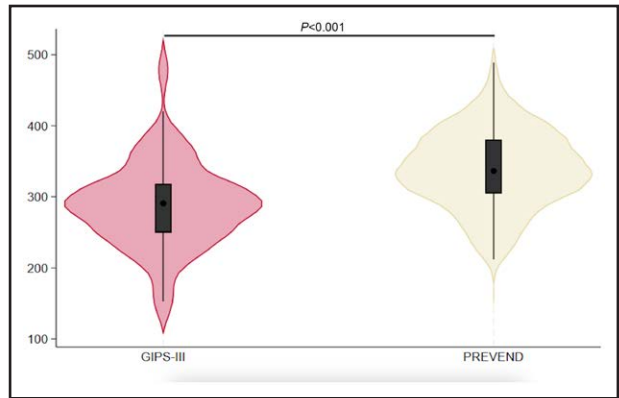
Figure 1. Distribution of T_{50} compared between patients in GIPS-III (Metabolic Modulation With Metformin to Reduce Heart Failure After Acute Myocardial Infarction: Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction: a Randomized Controlled Trial) and PREVEND (Prevention of Renal and Vascular End-Stage Disease).
T_{50} was significantly lower in patients with myocardial infarction (n=254) compared with the general population (n=254; $P<0.001$).
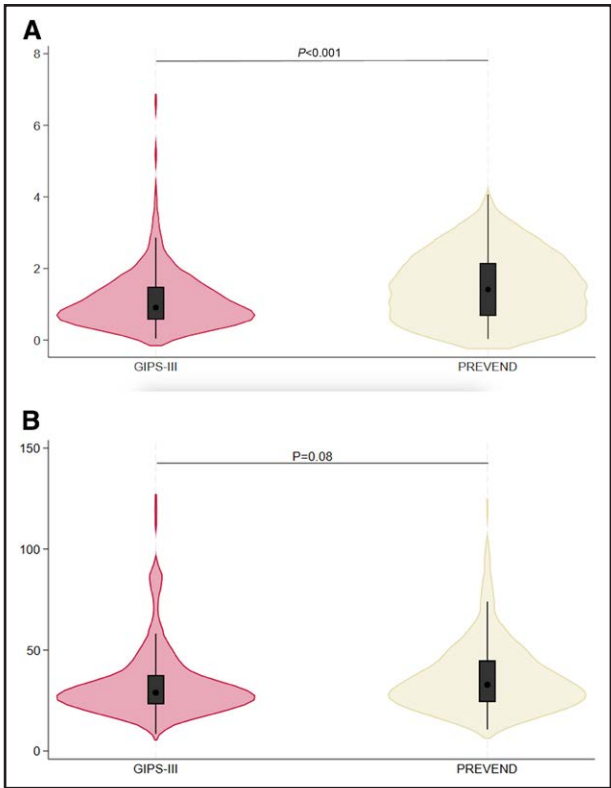
Figure 2. Serum nitrite and nitrate levels (in µmol/L) compared between patients in GIPS-III (Metabolic Modulation With Metformin to Reduce Heart Failure After Acute Myocardial Infarction: Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction: a Randomized Controlled Trial; n=215) and PREVEND (Prevention of Renal and Vascular End-Stage Disease; n=215).
A, Nitrite ($P<0.001$). **B**, Nitrate ($P=0.08$).

Interestingly, a higher systolic blood pressure, which is a traditional cardiovascular risk factor, was associated with a lower serum calcification propensity. Similarly, the finding that patients with a lower T_{50} have a significantly higher HDL cholesterol and lower LDL and total cholesterol levels, as well as lower serum triglycerides, may indicate that patients with a lower T_{50} have a more favorable traditional cardiovascular risk profile. Together, the observations of a lower T_{50} in STEMI patients and a lower T_{50} in patients with a favorable lipid and risk profile may indicate that calcification stress and lipid stress may act as complementary pathways in the pathophysiology of MI. CPPs are internalized in lipid plaques by endothelial cells and macrophages and there they contribute to the inflammatory and oxidative phenotype of endothelial cells.[39,40] Therefore, both calcification stress and lipid stress may be driving factors in the progression toward cardiac events but possibly inversely proportionate to each other (schematically depicted in Figure 3). What etiological factors determine these discrete subpopulations is currently unknown. To this end, we measured serum nitrite and nitrate levels to gain insight into the

Table 3. Association of T_{50} With Baseline Characteristics in the Myocardial Infarction Group (n=347)

Characteristics	Unadjusted			Adjusted for age and sex		
	β	SE	*P* value	β	SE	*P* value
Age, y	−0.32	0.29	0.26	−0.28	0.28	0.33
Female sex	−25.65	7.57	0.001*	−25.30	7.58	0.001*
Metformin treatment	2.80	6.72	0.68	2.27	6.63	0.73
Body weight, kg	0.60	0.23	0.01*	0.35	0.25	0.16
Body mass index, kg/m²	0.29	0.89	0.75	0.33	0.89	0.71
Hypertension	−2.49	7.32	0.73	3.41	7.54	0.65
Dyslipidemia	14.45	6.89	0.04*	13.65	6.86	0.05
Smoking	−1.52	6.77	0.82	−3.67	7.40	0.62
Systolic blood pressure, per 10 mm Hg	3.19	1.44	0.03*	3.42	1.41	0.02*
Diastolic blood pressure, per 10 mm Hg	2.93	2.28	0.20	2.09	2.27	0.36
Heart rate, bpm	0.02	0.20	0.90	−0.02	0.20	0.91
Ischemic time, min	−0.04	0.02	0.08	−0.03	0.02	0.15
Multivessel disease	0.44	7.22	0.95	0.80	7.16	0.91
TIMI flow grade pre-PCI ≤1	−1.06	6.90	0.88	−0.95	6.84	0.89
TIMI flow grade post-PCI <3	6.58	11.61	0.57	9.01	11.55	0.44
Myocardial blush grade ≤1	−13.38	10.70	0.21	−10.41	10.64	0.33
Hemoglobin, mmol/L	11.69	4.25	0.01*	6.73	4.62	0.15
Thrombocytes, per doubling	9.64	10.06	0.34	17.41	10.41	0.10
Leucocytes, 10⁹/L	−0.66	0.89	0.46	−0.78	0.91	0.39
Glucose, mmol/L	−2.34	1.36	0.09	−1.91	1.36	0.16
HbA1c, %	−4.04	4.15	0.33	−2.28	4.13	0.58
Creatinine, µmol/L	0.19	0.22	0.37	−0.10	0.24	0.67
eGFR, mL/min per 1.73 m²	0.26	0.22	0.22	0.15	0.27	0.59
Urea, mmol/L	−5.07	2.16	0.02*	−5.06	2.22	0.02*
LDH, per 100 U/L	−7.35	3.60	0.04*	−6.50	3.56	0.07
Sodium, mmol/L	1.65	1.38	0.23	1.62	1.36	0.24
Potassium, mmol/L	−1.22	8.02	0.88	−4.50	7.95	0.57
Magnesium, mmol/L	78.30	54.42	0.15	65.31	54.06	0.23
Total cholesterol, mmol/L	7.58	3.08	0.01*	7.09	3.14	0.02*
Triglyceride, mmol/L	8.18	2.35	0.001*	7.12	2.39	0.003*
LDL, mmol/L	9.02	3.26	0.01*	8.09	3.31	0.02*
HDL, mmol/L	−40.25	10.32	<0.001*	−33.50	10.78	0.002*
ASAT, per doubling	−7.54	3.66	0.04*	−8.37	3.62	0.02*
ALAT, per doubling	−4.22	3.87	0.28	−6.97	3.88	0.07
CK, per doubling	1.86	2.80	0.51	0.83	2.78	0.77
CK-MB, per doubling	−1.17	3.13	0.71	−1.09	3.10	0.72
NT-proBNP, per doubling	−5.21	1.78	<0.001*	−3.99	1.89	0.04*
Troponin T, per doubling	−2.23	1.82	0.22	−1.84	1.83	0.32
Peak CK-total, per doubling	0.66	2.03	0.75	0.18	2.01	0.93
Peak CK-MB, per doubling	0.02	2.27	0.99	−0.25	2.24	0.91
Peak NT-proBNP, per doubling	−7.25	2.15	0.001*	−5.72	2.26	0.01*
Peak troponin T, per doubling	0.52	1.73	0.76	0.46	1.71	0.79

These analyses were performed in the entire STEMI cohort with an available measurement of T_{50} (n=347). ALAT indicates alanine transaminase; ASAT, aspartate transaminase; CK, creatine kinase; CK-MB, creatine kinase myocardial band; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PCI, percutaneous coronary intervention; STEMI, ST-segment–elevation myocardial infarction; and TIMI, thrombolysis in myocardial infarction.

*$P < 0.05$.

**Table 4. Multivariable Association of Baseline Markers With T_{50} (n=347)**

Predictor	Multivariate model for T_{50} *		
	Unstandardized β	SE	P value†
Female sex	−17.51	7.61	0.02
Systolic blood pressure, mm Hg	0.35	0.14	0.01
Triglycerides, mmol/L	5.20	2.44	0.03
LDL, mmol/L	7.96	3.14	0.01
HDL, mmol/L	−26.17	11.01	0.02

HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.
*Explained variance of the model: 0.09.
†P<0.05.

degree of endothelial dysfunction and whole-body formation of NO in our cohorts. NO is generated from the amino acid L-arginine by the activity of NO synthase, and in humans, nitrate is the end product of oxidative NO metabolism.[41] We found no indication that total NO production, as assessed by circulating nitrate levels, was different in our STEMI patients as compared with the general population (Figure 2; Table S1). Oxidative stress accompanied by an enhanced production of superoxide (O_2^-) is known to impair endothelial NO formation while also lowering its bioavailability. Plasma/serum nitrite concentrations reflect constitutive NO synthase activity in the vasculature.[42] We found circulating nitrite levels to be lower in the MI cohort when compared with the general population (Figure 2; Table S1), reflecting a higher degree of endothelial dysfunction.[43] While CPPs were reported to induce endothelial dysfunction,[44] we did not find an association between serum nitrite or nitrate levels and T_{50}. We see this as an additional indication for the notion that serum calcification propensity represents
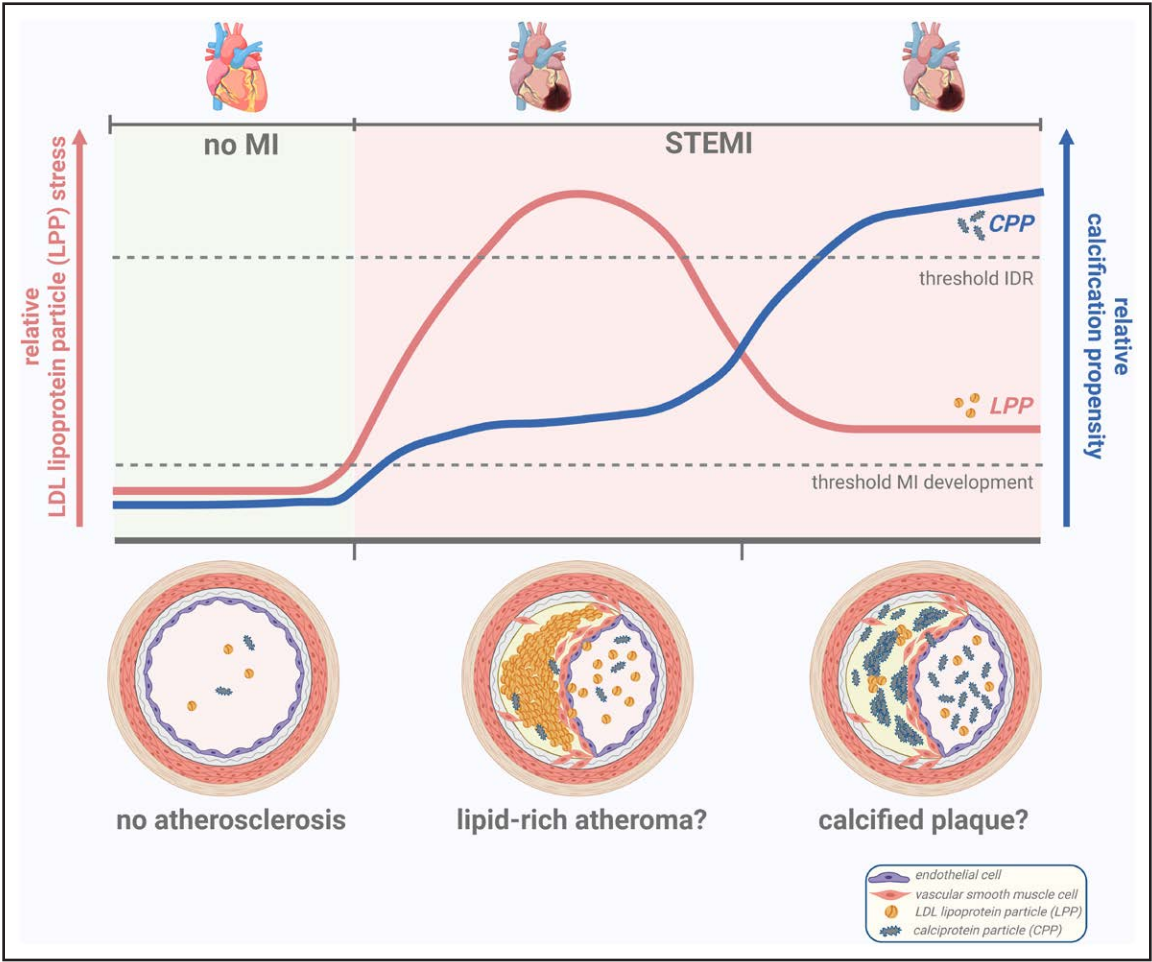
Figure 3. Schematic paradigm of calciprotein particle (CPP)–induced and lipoprotein particle (LPP)–induced vascular abnormalities underlying the pathophysiology of myocardial infarction (MI).
In our cohort of patients with MI, patients with a low serum calcification propensity (ie, high T_{50}) presented with traditional cardiovascular risk factors (ie, male sex, high systolic blood pressure, high total cholesterol level, high LDL [low-density lipoprotein] level, and high triglycerides level, combined with a low HDL [high-density lipoprotein] level). Conversely, patients with a high serum calcification propensity (ie, low T_{50}) were women, had a lower systolic blood pressure, had low total cholesterol, LDL, and triglyceride levels, and had a high HDL level, suggesting that calcification stress serves as an independent pathway to an MI. Serum calcification propensity in the MI cohort as a whole was higher than in the general population, which may suggest that patients protected by a favorable traditional cardiovascular risk profile may precipitate a MI in the presence of a higher calcification burden. STEMI indicates ST-segment–elevated myocardial infarction. Created with BioRender.com.

a pathophysiological mechanism that precipitates major cardiac events, especially in women, independently of traditional cardiovascular risk factors.

Future studies should be directed toward determining whether treatment for MI increases the T_{50} values during follow-up. As other studies have indicated, the T_{50} is a variable that can be modified by treatment, such as calcium carbonate,[45] magnesium,[23,46,47] or hemodialysis,[35] while it has not been found to change after kidney donation.[48] These studies suggest that the T_{50} may be a promising test for follow-up over the course of treatment for patients at risk for or presenting with a disease related to vascular calcification, like coronary artery disease or chronic kidney disease but also hypertension[33] and especially in those presenting with less pronounced serum lipid abnormalities.

Study Strengths and Limitations

Strengths of our study included the relatively high availability of serum samples and completeness of follow-up data in the STEMI cohort. Another strength is the comparison of T_{50} measurements with general population-based control samples matched for age, sex, and history of hypertension and dyslipidemia. A limitation of our study is that the GIPS-III cohort did not include patients with diabetes, while diabetes has been shown to modify associations between T_{50} and clinical outcomes.[36] Therefore, the generalizability of our results to the STEMI population at large should be interpreted with caution. Furthermore, the event rate for IDR during the 5-year follow-up was relatively low. Definitive answers regarding the associations with clinical outcomes remain to be determined.

Conclusions

Serum calcification propensity is increased in patients with STEMI compared with the general population. A higher serum calcification propensity was associated with a more favorable serum lipid profile, suggesting the involvement of divergent pathways of calcification stress and lipid stress in the pathophysiology of MI. Moreover, the contribution of calcification propensity in the pathogenesis of coronary artery disease was more pronounced in women than in men, and calcification propensity predicts the need for reintervention.

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