ORIGINAL RESEARCH

Circulating Biomarkers Predicting Longitudinal Changes in Left Ventricular Structure and Function in a General Population

Nicholas Cauwenberghs, MSc; Susana Ravassa, PhD; Lutgarde Thijs, MSc; Francois Haddad, MD; Wen-Yi Yang, MD, PhD; Fang-Fei Wei, MD, PhD; Begoña López, PhD; Arantxa González, PhD; Javier Díez, MD, PhD; Jan A. Staessen, MD, PhD; Tatiana Kuznetsova, MD, PhD

***Background*—**Serial imaging studies in the general population remain important to evaluate the usefulness of pathophysiologically relevant biomarkers in predicting progression of left ventricular (LV) remodeling and dysfunction. Here, we assessed in a general population whether these circulating biomarkers at baseline predict longitudinal changes in LV structure and function.

***Methods and Results*—**In 592 participants (mean age, 50.8 years; 51.4% women; 40.5% hypertensive), we derived echocardiographic indexes reflecting LV structure and function at baseline and after 4.7 years. At baseline, we measured alkaline phosphatase, markers of collagen turnover (procollagen type I, C-terminal telopeptide, matrix metalloproteinase-1) and high-sensitivity cardiac troponin T. We regressed longitudinal changes in LV indexes on baseline biomarker levels and reported standardized effect sizes as a fraction of the standard deviation of LV change. After full adjustment, a decline in LV longitudinal strain (−14.2%) and increase in E/e′ ratio over time (+18.9%; $P \leq 0.019$) was associated with higher alkaline phosphatase activity at baseline. Furthermore, longitudinal strain decreased with higher levels of collagen I production and degradation at baseline (procollagen type I, −14.2%; C-terminal telopeptide, −16.4%; $P \leq 0.029$). An increase in E/e′ ratio over time was borderline associated with lower matrix metalloproteinase-1 (+9.8%) and lower matrix metalloproteinase-1/tissue inhibitor of metalloproteinase-1 ratio (+11.9%; $P \leq 0.041$). Higher high-sensitivity cardiac troponin T levels at baseline correlated significantly with an increase in relative wall thickness (+23.1%) and LV mass index (+18.3%) during follow-up ($P \leq 0.035$).

***Conclusions*—**We identified a set of biomarkers predicting adverse changes in LV structure and function over time. Circulating biomarkers reflecting LV stiffness, injury, and collagen composition might improve the identification of subjects at risk for subclinical cardiac maladaptation. **(*J Am Heart Assoc*. 2019;8:e010430. DOI: 10.1161/JAHA.118.010430)**

Key Words: cardiac biomarkers • cardiac dysfunction • phosphatase • population studies • remodeling

Increasing life expectancy and prevalence of cardiovascular risk factors contribute to the surging epidemic of symptomatic heart failure (HF).[1,2] However, adverse maladaptation of the heart starts years to decades before HF symptoms occur.[3] Subclinical or asymptomatic HF (ie, stage B) refers to the state in which the heart remodeling (left ventricular [LV] hypertrophy/remodeling) and/or cardiac systolic and/or diastolic dysfunction have been detected but without exerting the typical HF symptoms used for clinical diagnosis. Recent guidelines emphasized the need for timely identification and management of subjects at risk for early LV maladaptive processes that precede HF symptoms.[3]

Within this context, the identification of prognostic and pathophysiologically informative biomarkers will advance our understanding of mechanisms of early LV remodeling and dysfunction that might lead to better risk stratification and to novel therapeutics. Up to now, several biomarkers reflecting vascular calcification, cardiomyocyte stiffness, extracellular

From the Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium (N.C., L.T., W.-Y.Y., F.-F.W., J.A.S., T.K.); Program of Cardiovascular Diseases, Centre for Applied Medical Research (S.R., B.L., A.G., J.D.), Departments of Cardiology and Cardiac Surgery (J.D.), and Nephrology (J.D.), University of Navarra, Pamplona, Spain; IdiSNA, Navarra Institute for Health Research, Pamplona, Spain (S.R., B.L., A.G., J.D.); CIBERCV, Carlos III Institute of Health, Madrid, Spain (S.R., B.L., A.G., J.D.); Division of Cardiovascular Medicine, Stanford University School of Medicine and Stanford Cardiovascular Institute, Stanford, CA (F.H.).

Accompanying Data S1, Tables S1, S2, and Figure S1 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.010430

Correspondence to: Tatiana Kuznetsova, MD, PhD, Research Unit of Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Campus Sint Rafaël, Kapucijnenvoer 35, block D, box 7001, B-3000 Leuven, Belgium. E-mail: tatiana.kouznetsova@med.kuleuven.be

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Clinical Perspective

What Is New?

- In this longitudinal population study, we identified a set of circulating biomarkers predicting adverse changes in left ventricular (LV) structure and function.
- Higher alkaline phosphatase activity predicted worsening in LV systolic performance (assessed by ejection fraction and longitudinal strain) and in diastolic function (by E/e′) during follow-up.
- Higher collagen I production (procollagen type I) and degradation (C-terminal telopeptide) at baseline associated with future decline in LV systolic function.
- Signs of myocardial injury (high-sensitivity cardiac troponin T) at baseline predicted LV concentric remodeling and hypertrophy over follow-up.

What Are the Clinical Implications?

- Pathophysiologically relevant biomarkers reflecting vascular calcification and LV stiffness, injury, and collagen composition might improve the early identification of subjects at risk for early cardiac maladaptation processes leading to symptomatic heart failure.

matrix collagen turnover and LV injury have been proposed for diagnosis and prognosis of both subclinical and symptomatic HF.[4–6]

For instance, recent experimental studies demonstrated that alkaline phosphatase (ALP) can consecutively induce vascular calcification, blood pressure elevations, and adverse LV remodeling.[7,8] Moreover, ALP might directly affect cardiomyocyte stiffness and contractility via dephosphorylation of sarcomeric proteins such as titin.[9,10] In the extracellular matrix, excessive deposition and cross linking of collagen fibers adversely affect the LV mechanics, electrical activity, and coronary microcirculation.[11] Higher serum levels of C-terminal propeptide of procollagen type 1 (PICP) and C-terminal telopeptide (CITP) reflecting collagen I synthesis and degradation, respectively, were associated with greater myocardial collagen content and higher risk for clinically overt HF.[12] Of note, in a cross-sectional population study, CITP was already increased in participants with subclinical LV dysfunction.[13] Moreover, previous community-based studies identified biomarkers of cardiomyocyte injury such as high-sensitivity cardiac troponin T (hs-cTnT) as independent predictors of incident HF.[14] Along with other researchers, we previously demonstrated in a cross-sectional study that hs-cTNT is associated with LV hypertrophy in the general population.[5]

On the other hand, serial imaging studies in the general population remain crucial to evaluate the usefulness of these circulating biomarkers in predicting early LV maladaptation. To the best of our knowledge, population data on the longitudinal changes of LV structure and function in relation to these biomarkers are sparse. Therefore, in this follow-up study, we investigated whether and to what extent pathophysiologically informative biomarkers can predict subclinical yet adverse changes in echocardiographic indexes of LV structure and function in the general population.

Methods

The Ethics Committee of the University of Leuven approved FLEMENGHO (the Flemish Study on Environment, Genes and Health Outcomes), and the subjects gave informed consent. Consent given by study participants did not include data sharing with third parties. Anonymized data, analytic methods, and study materials can be made available to investigators for purposes of reproducing the results or replicating the procedure based on a motivated request to be addressed to the corresponding author.

Study Population

From August 1985 until December 2005, we randomly recruited a family-based population sample stratified by sex and age from a geographically defined area in northern Belgium as described elsewhere.[15] Seven Belgian municipalities provided listings of all inhabitants sorted by address. Households, defined as those who lived at the same address, were the sampling unit. We numbered households consecutively, and generated a random-number list by use of SAS software's random function (SAS Institute, Cary, NC). Households with a number matching the list were invited; household members older than 18 years were eligible. From 2005 to 2009, we invited 1031 former participants for an

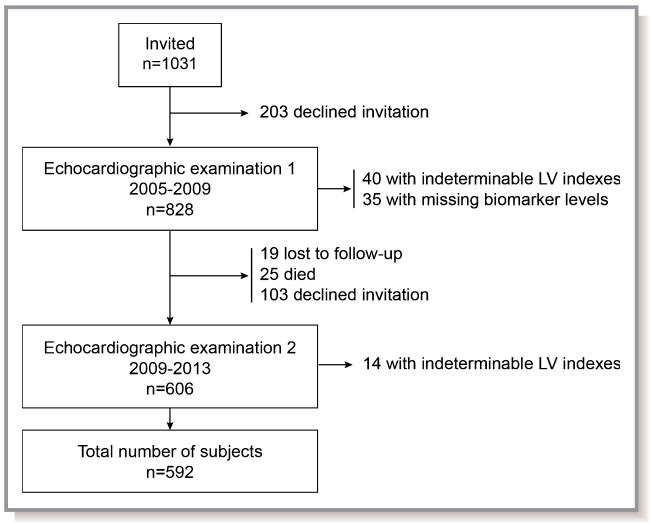

Figure 1. Flow chart for participants in the FLEMENGHO study. FLEMENGHO indicates the Flemish Study on Environment, Genes and Health Outcomes.

echocardiographic examination at our field center (Figure 1). We obtained written informed consent from 828 subjects (participation rate, 80.3%). To study changes in LV structure and function, we invited these people for a follow-up examination, on average 5 years after their first cardiovascular examination. We excluded 147 participants because they died (n=25), were lost to follow-up (n=19), or declined the follow-up invitation (n=103). We additionally excluded 54 subjects because of atrial fibrillation at baseline (n=8) or at follow-up (n=4), an artificial pacemaker (n=4), or insufficient quality of the echocardiographic recordings (n=38). We also discarded 35 participants from analysis because of missing biomarker levels at baseline. Overall, the current analysis thus included 592 participants (Figure 1).

Echocardiography

All participants refrained from smoking, heavy exercise, and drinking alcoholic or caffeinated beverages at least 3 hours before echocardiography. Echocardiography was performed after the subjects had rested for at least 15 minutes in the supine position.

Data acquisition

A detailed echocardiographic protocol is provided in Data S1. Briefly, 1 experienced physician (T.K.) performed both ultrasound examinations in accordance with clinical recommendations[16] and as described previously[17] using a Vivid7 Pro and Vivid E9 (GE Vingmed, Horten, Norway) interfaced with a 2.5- to 3.5-MHz phased-array probe. With the subjects in partial left decubitus, the observer obtained conventional images along the parasternal long and short axes and from the apical 4- and 2-chamber long-axis views, simultaneous with an electrocardiographic signal. All digital recordings included at least 5 cardiac cycles.

Offline analysis

One observer (T.K.) analyzed the echocardiograms blinded to the participants' characteristics. Digitally stored images were postprocessed using EchoPac software (GE Vingmed, Horten, Norway). Measurements were averaged over at least 3 heart cycles for statistical analysis. From the long-axis parasternal view, LV internal diameter and interventricular septal and posterior wall thickness were measured at end-diastole from 2-dimensionally guided M-mode tracings. Relative wall thickness (RWT) was calculated as 0.5 × (interventricular septal+posterior wall thickness) / LV internal diameter at end-diastole. End-diastolic LV dimensions were also used to derive LV mass using an anatomically validated formula. LV concentric remodeling was defined as RWT >0.42. We defined LV hypertrophy as LV mass index >115 g/m² in men and 95 g/m² in women.

Two experienced observers (N.C., T.K.) measured LV global longitudinal strain (LS), reflecting LV systolic function, using myocardial speckle-tracking software (Q-analysis, GE Vingmed) at default settings. The LV endocardial border was manually traced at the end-systolic frame of the 2-dimensional 4-chamber view. The software automatically tracked myocardial speckle motion while dividing the region of interest in basal, mid, and apical levels. We adjusted the region of interest after visual evaluation of the tracking. Images were rejected if tracking was inadequate in ≥2 segments. We used absolute values of peak systolic, midwall global LS for statistical analysis. Relative intra- and interobserver reproducibility of global LS were 6.1% and 7.3%, respectively.[18]

Transmitral Doppler flow signals were used to measure peak early (E) and late (A) diastolic velocities and E/A ratio. From pulsed-wave tissue Doppler imaging recordings, we measured the early (e′) and late (a′) diastolic peak velocities of the mitral annulus displacement at 4 acquisition sites. We calculated the E/e′ ratio, a noninvasive surrogate for LV filling pressure, by dividing transmitral E peak by e′ averaged from the 4 acquisition sites. We combined the mitral inflow and tissue Doppler imaging velocities to classify the grade of LV diastolic dysfunction at baseline and follow-up as described previously.[19]

Biomarker Measurements

A detailed description of the biochemical measurements, including information on inter- and intra-assay variations, is provided in Data S1. On the day of the baseline echocardiographic examination, fasting venous blood samples were collected into serum and ethylenediaminetetraacetic acid tubes. After centrifuging, plasma and serum were separated, aliquoted, and stored at −80°C.

Using commercially available enzymatic assays, enzyme immunoassays, and sandwich ELISA, we determined plasma activity of ALP (BioVision, Milpitas, CA) and serum levels of: PICP, a marker of collagen I synthesis (METRA EIA kit, Quidel Corporation, San Diego, CA); CITP, a marker of collagen I degradation (Orion Diagnostica, Espoo, Finland); matrix metalloproteinase-1 (MMP-1), a collagenase (MMP-1 Biotrak ELISA System; GE Healthcare, Little Chalfont, UK); free tissue inhibitor of metalloproteinase-1 (TIMP-1), a marker of inhibition of collagen degradation (TIMP-1 Human Biotrak ELISA System, GE Healthcare); and amino-terminal peptide of procollagen type III, a marker of collagen III synthesis (PIIINP ELISA kit; MyBioSource, San Diego, CA). Serum hs-cTnT levels were measured using a highly sensitive assay (Troponin T hs STAT; Roche Diagnostics, Rotkreuz, Switzerland) optimized on the Cobas 8000 modular analyzer series (Roche Diagnostics). The presence of hs-cTnT was considered in all participants with values >3 ng/L.

Other Measurements

At both visits, we administered a standardized questionnaire to collect detailed information on each subject's medical history,

smoking and drinking habits, and medication intake. Participants also completed the standardized London School of Hygiene Cardiovascular Questionnaire for detection of HF symptoms such as chest pain and breathlessness on the day of the examination. In addition, during the technical examination and before echocardiography, an experienced physician assessed signs and symptoms of HF. We collected medical information by medical records provided by the participant's general practitioner and regional hospitals. Self-reported diseases were ascertained against the medical records of general practitioners and regional hospitals. From the type and number of alcoholic beverages consumed each day, we calculated the alcohol consumption in grams per day. To exclude occasional drinkers, we defined current alcohol drinking as a consumption of ≥5 g of ethanol per day. Conventional blood pressure was the average of 5 auscultatory readings obtained with the patient in a seated position. Hypertension was defined as a BP of at least 140 mmHg systolic or 90 mmHg diastolic or by the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the height in square meters. Serum creatinine, serum insulin, and total cholesterol were measured in venous blood samples.

Statistical Analysis

For database management and statistical analysis, we used SAS software version 9.4 (SAS Institute, Cary, NC). We compared changes in means and proportions by means of a paired t test and McNemar test, respectively. Statistical significance was a 2-sided significance level <0.05. We calculated longitudinal changes in echocardiographic indexes by subtracting the baseline from the follow-up measurement. All variables were normally distributed or log-transformed to achieve normality before statistical analysis.

By use of a mixed model, we assessed multivariable-adjusted associations between longitudinal changes in LV structural and functional indexes and the biomarker levels at baseline. All statistical models were adjusted for follow-up duration, baseline LV index, age, sex, body height and weight, heart rate, and mean arterial pressure (MAP). We additionally adjusted for changes in these covariables. All covariables were identified based on stepwise regression models reported previously.[17,18] We reported effect sizes on a relative scale as a percentage of the standardized effect size (ie, the absolute effect size per doubling in biomarker level divided by the SD of the echocardiographic change and multiplied by 100). Using multiple logistic regression models including the covariables mentioned above, we explored whether baseline ALP activity could predict development or worsening in LV diastolic dysfunction and whether baseline hs-cTnT levels were associated with the development of LV concentric remodeling and/or hypertrophy.

Results

Characteristics of Participants

At baseline, the 592 participants (51.4% women) included 240 hypertensives (40.5%), of whom 145 (60.4% of the hypertensives) were on antihypertensive drug treatment. The mean age at baseline was 50.8±14.5 years. The median follow-up was 4.7 years (5th to 95th percentile, 3.8–5.4 years). Tables 1 and 2 list the clinical and echocardiographic characteristics of the study participants by examination phase. RWT and LV mass index increased significantly during follow-up (P<0.0001; Table 2). Transmitral and tissue Doppler imaging peak velocities decreased and E/e′ ratio increased over time (P<0.0001 for all; Table 2).

Associations Between Change in LV Structure and Function Indexes and Baseline Serum ALP

Table 3 presents the multivariable-adjusted estimates (95% CI) for temporal changes in LV structure and function indexes associated with a doubling in baseline ALP activity. After full adjustment, longitudinal decreases in LV ejection fraction (−14.5%; P=0.0074) and global LS (−14.2%; P=0.019; Table 3) were significantly associated with higher ALP activity at baseline. We also observed a greater increase in E/e′ ratio during follow-up with elevated ALP activity at baseline (+18.9%; P=0.0078; Table 3). Figure 2 and Figure S1 illustrate the multivariable-adjusted changes in LV ejection fraction, global LS, and E/e′ by quartiles of baseline ALP. Participants belonging to the fourth quartile of ALP (>2.7 U/mL) experienced worsening of EF, global LS, and E/e′ ratio over time (P≤0.047; Figure 2) as compared with the average changes in these LV indexes in the whole cohort. Furthermore, in multivariable-adjusted logistic regression, baseline ALP tended to predict worsening of LV diastolic function (odds ratio, 2.01; P=0.071; Table S1).

In contrast, ALP activity at baseline did not significantly predict the longitudinal changes in structural LV indexes such as RWT and LV mass index after full adjustment (P≥0.15; Table 3). Additional adjustment for use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers at baseline and during follow-up did not alter our findings (data not shown).

Associations Between Change in LV Structure and Function Indexes and Baseline Collagen Markers

We present the multivariable-adjusted estimates (95% CI) for temporal changes in echocardiographic indexes of LV function (Table 4; Table S2) and structure (Table 5) associated with a doubling in baseline biomarkers levels reflecting collagen metabolism and myocardial injury.

Table 1. Clinical Characteristics of 592 Participants at Baseline and Follow-Up Examination

Characteristic	Examination 1 (2005–2009)	Examination 2 (2009–2013)	Δ	*P* Value
Anthropometrics				
Age, y	50.8±14.5	55.5±14.4	+4.78±0.50	<0.001
Body mass index, kg/m²	26.4±4.15	27.1±4.17	+0.72±1.83	<0.001
Systolic BP, mm Hg	128.6±16.7	132.0±17.0	+3.40±13.3	<0.001
Diastolic BP, mm Hg	79.7±9.3	82.2±9.6	+2.48±8.66	<0.001
Pulse pressure, mm Hg	48.9±14.1	49.8±15.7	+0.92±11.2	0.048
MAP, mm Hg	96.0±10.3	98.8±10.1	+2.79±9.02	<0.001
Heart rate, bpm	62.6±9.2	62.3±9.7	−0.28±9.44	0.46
Questionnaire and medical data				
Current smoking, n (%)	119 (20.1)	97 (16.4)	−3.7%	<0.001
Drinking alcohol, n (%)	250 (42.2)	231 (39.0)	−3.2%	<0.001
Hypertensive, n (%)	240 (40.5)	297 (50.2)	+9.7%	<0.001
Treated for HT, n (%)	145 (24.5)	189 (31.9)	+7.4%	<0.001
β-blockers, n (%)	88 (14.9)	100 (16.9)	+2.0%	<0.001
ACE-I or ARB, n (%)	47 (7.9)	78 (13.2)	+5.3%	<0.001
CCB or α-blockers, n (%)	25 (4.2)	50 (8.5)	+4.3%	<0.001
Diuretics, n (%)	51 (8.6)	66 (11.2)	+2.6%	<0.001
History of CHD, n (%)	16 (2.7)	31 (5.2)	+2.5%	0.0009
History of diabetes mellitus, n (%)	20 (3.4)	47 (7.9)	+4.5%	<0.001
Biochemical data				
Serum creatinine, μmol/L	85.9±15.7	89.6±23.3	+3.66±14.3	<0.001
Total cholesterol, mmol/L	5.28±0.96	5.01±0.96	−0.26±0.91	<0.001
Serum insulin, μmol/L	4.66 (2.00 to 10.0)	5.04 (2.00 to 12.0)	+1.08 (−1.45 to 2.00)	0.004
Alkaline phosphatase, U/mL	2.03 (1.17 to 3.65)	…	…	…
PICP, μg/L	94.5 (61.0 to 157.7)	…	…	…
CITP, μg/L	5.22 (3.29 to 7.91)	…	…	…
MMP-1, μg/L	8.18 (3.89 to 16.9)	…	…	…
PIIINP, ng/L	524.6 (242.8 to 1182.1)	…	…	…
TIMP-1, μg/L	667.7±181.6	…	…	…
Hs-cTnT, ng/L	6.18 (4.00 to 10.0)	…	…	…

Values are mean (±SD), number of subjects (%) or geometric mean (10%–90% percentile interval). For longitudinal changes (Δ), values are mean (±SD), or percentage change. None of the participants was using antimineralocorticoids such as spironolactone at baseline or during follow-up. ACE-I indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BP, blood pressure; CCB, calcium channel blockers; CHD, coronary heart disease; CITP, carboxyl-terminal telopeptide of collagen type I; hs-cTnT, high-sensitivity cardiac troponin T; HT, hypertension; MAP, mean arterial pressure; MMP-1, matrix metalloproteinase-1; PICP, carboxyl-terminal propeptide of type I collagen; PIIINP, amino-terminal peptide of procollagen type III; TIMP-1, tissue inhibitor of metalloproteinases-1.

After full adjustment, a stronger decrease in global LS during follow-up was significantly related to higher PICP (−14.2%) and CITP (−16.4%) ($P \leq 0.029$ for all; Table 4). Furthermore, a stronger increase in E/e′ ratio over time was borderline associated with lower MMP-1 (+9.8%) and lower MMP-1/TIMP-1 ratio (+11.9%) ($P \leq 0.041$; Table 4). For the LV structure indexes, only an increase in RWT over time correlated significantly with a higher CITP level at baseline ($P=0.015$; Table 5). Additional adjustment for use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers did not alter these findings (data not shown).

Associations Between Change in LV Structure and Function Indexes and Cardiac Troponin T at Baseline

In multivariable-adjusted models, higher hs-cTnT levels at baseline predicted stronger increase in RWT (+23.1%) and LV

Table 2. Echocardiographic Characteristics of 592 Participants at Baseline and Follow-Up Examination

Characteristic	Examination 1 (2005–2009)	Examination 2 (2009–2013)	Δ±SD	*P* Value
LV structure				
Relative wall thickness	0.37±0.06	0.39±0.05	+0.018±0.053	<0.001
Mass index, g/m²	92.0±20.8	95.6±21.1	+3.67±13.1	<0.001
LV systolic function				
Ejection fraction, %	63.4±6.4	61.2±6.5	−2.26±8.26	<0.001
Global LS, %	19.7±2.4	19.5±2.4	−0.17±2.32	0.067
LV diastolic function				
E peak, cm/s	75.9±16.0	66.9±15.7	−8.99±11.7	<0.001
A peak, cm/s	64.7±17.0	61.0±15.2	−3.71±9.26	<0.001
E/A ratio	1.27±0.47	1.18±0.45	−0.086±0.27	<0.001
TDI e′ peak, cm/s*	11.5±3.54	9.77±3.34	−1.72±1.56	<0.001
TDI a′ peak, cm/s*	10.2±2.03	9.56±2.11	−0.63±1.53	<0.001
E/e′ ratio*	7.04±2.12	7.41±2.48	+0.37±1.45	<0.001

Values are mean (±SD). LS indicates longitudinal strain; LV, left ventricular; TDI, tissue Doppler imaging.
*Average of septal, lateral, inferior, and posterior mitral annulus sites.

mass index (+18.3%; $P \leq 0.035$; Table 5). Figure 3 illustrates the multivariable-adjusted changes in RWT and LV mass index by tertiles of baseline hs-cTnT. Participants belonging to the third tertile of baseline hs-cTnT (>7.0 ng/L) experienced a stronger increase in RWT and LV mass index during follow-up as compared with the average changes in these LV indexes in the whole cohort ($P \leq 0.041$; Figure 3).

Table 3. Multivariable-Adjusted Associations Between 4.7 Years Change in LV Structure and Function Indexes and Baseline Serum Alkaline Phosphatase

	Alkaline Phosphatase, per Doubling	
	Parameter Estimate (95% CI)	*P* Value
LV structure		
Δ Relative wall thickness	8.46% (−3.08, 19.2)	0.15
Δ Mass index	2.71% (−10.9, 16.4)	0.69
LV systolic function		
Δ Ejection fraction	−14.5% (−25.2, −3.91)	0.007
Δ Global LS	−14.2% (−25.9, −2.41)	0.019
LV diastolic function		
Δ E peak	7.01% (−4.88, 18.9)	0.25
Δ TDI e′ peak	−0.67% (−13.0, 11.7)	0.92
Δ E/A	7.78% (−3.71, 18.9)	0.19
Δ E/e′	18.9% (4.97, 32.2)	0.008

Parameter estimates (95% CI) are for a doubling in baseline level of alkaline phosphatase and are expressed as percentage of SD of longitudinal change (Δ) in LV index. Analyses were adjusted for follow-up duration, sex, baseline LV index, age, heart rate, body height and weight, and mean arterial pressure as well as changes in these variables. All covariables were identified based on stepwise regression analyses. For LV mass index, models did not include body mass index. LS indicates longitudinal strain; LV, left ventricular; TDI, tissue Doppler imaging.

At the follow-up examination, the prevalence of LV concentric remodeling increased significantly from 18.4% (n=109) to 24.2% (n=143; $P<0.0001$). Similarly, the prevalence of LV hypertrophy raised significantly from 20.8% (n=123) to 26.9% (n=159) during the follow-up period ($P<0.0001$). Of note, participants with higher baseline hs-cTnT had a greater risk to develop or retain LV concentric remodeling during the follow-up period (odds ratio, 1.84 [1.18–2.86]; $P=0.0071$). Similarly, higher hs-cTnT at baseline implied a greater risk to develop or retain LV hypertrophy over time (odds ratio, 1.61 [1.02–2.52]; $P=0.040$).

In contrast, the level of hs-cTnT at baseline did not correlate with any of the longitudinal changes in the LV systolic and diastolic function indexes ($P \geq 0.15$; Table 4). Additional adjustment for use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers did not alter these findings (data not shown).

Discussion

In this longitudinal population study, we investigated to what extent circulating biomarkers reflecting LV stiffness and injury predict changes in echocardiographic indexes of LV structure and function. In summary, we observed that (1) the plasma activity of ALP at baseline was associated with a stronger decline in LV systolic and diastolic function over time, (2) markers of collagen I synthesis and degradation predicted

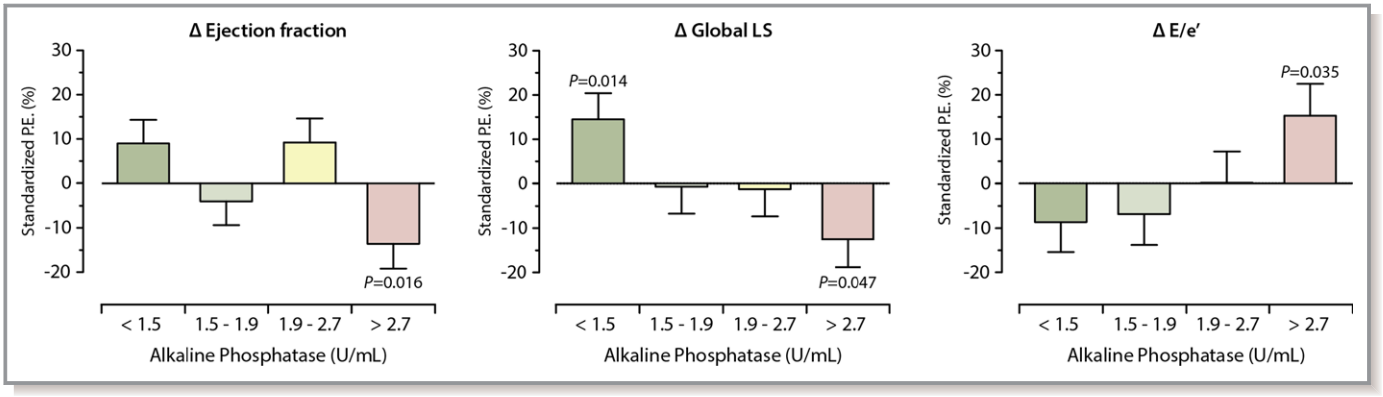

Figure 2. Multivariable-adjusted parameter estimates (PEs; ±SE) for 4.7 years of change (Δ) in left ventricular ejection fraction, longitudinal strain (LS), and E/e′ ratio per alkaline phosphatase quartile. Number of participants per quartile: Q1, n=168; Q2, n=142; Q3, n=140; Q4, n=140. Adjusted PEs are expressed as percentage of SD of the longitudinal change in the whole cohort. *P* values are for comparisons with the average LV changes within the whole cohort. Analyses were adjusted for follow-up duration, sex, baseline LV index, age, heart rate, body height and weight, and mean arterial pressure. We additionally adjusted for longitudinal changes in these risk factors.

adverse changes in LV structure and function during follow-up, and (3) higher hs-cTnT at baseline predicted the development of LV concentric remodeling and hypertrophy during follow-up.

ALP is a plasma enzyme responsible for the release of phosphate groups (dephosphorylation) from different types of molecules. Elevated ALP levels are mainly associated with high-turnover bone disease and liver disorders.[20] Recently, population-based studies reported an independent association between elevated ALP and an increased risk of cardiovascular mortality and morbidity.[20] Along this line, in our study we observed for the first time that higher serum ALP at baseline predicted worsening of both LV systolic (ie, decrease in global LS) and diastolic (ie, increase in E/e′ ratio) function. The possible mechanism by which an elevated ALP impacts on cardiac function might be related to an involvement of ALP in vascular calcification processes by hydrolyzing pyrophosphate in the vascular wall. Indeed, an experimental study demonstrated that transgenic mice overexpressing the *ALPL* gene (encoding human tissue–nonspecific ALP) developed severe arterial calcification and cardiac remodeling and had a reduced life span.[8]

Another possible mechanism of ALP affecting cardiomyocyte function might be related to its ability to dephosphorylate sarcomeric proteins such as titin[10,21,22] and myosin light chains,[23] which adversely affects cardiomyocyte stiffness and contractility. In support, total titin phosphorylation

Table 4. Multivariable-Adjusted Associations between 4.7 Years Change in LV Function Indexes and Baseline Markers of Collagen and Myocardial Injury and Stress

	Δ Ejection Fraction		Δ Global LS		Δ E/e′	
Explanatory Variable	Parameter Estimate (95% CI)	*P* Value	Parameter Estimate (95% CI)	*P* Value	Parameter Estimate (95% CI)	*P* Value
Collagen turnover						
PICP, doubling	−9.87% (−21.2, 1.43)	0.087	−14.2% (−26.7, −1.47)	0.029	−2.10% (−16.8, 12.6)	0.78
CITP, doubling	−7.43% (−19.9, 5.07)	0.24	−16.4% (−30.2, −2.80)	0.018	5.31 (−11.2, 21.7)	0.52
MMP-1, doubling	−0.33% (−7.81, 7.15)	0.93	7.33% (−0.86, 15.5)	0.079	−9.79% (−19.6, −0.42)	0.041
PIIINP, doubling	−2.97% (−8.70, 2.75)	0.31	−1.94% (−8.19, 4.31)	0.54	−2.94% (−10.5, 4.76)	0.46
TIMP-1, +180 μg/L	0.69% (−5.88, 7.26)	0.84	−0.043% (−7.33, 7.33)	0.99	6.01% (−2.38, 14.7)	0.16
MMP-1/TIMP-1, doubling	0.48% (−6.86, 7.83)	0.90	7.62% (−0.48, 15.7)	0.065	−11.9% (−21.6, −2.17)	0.017
Cardiomyocyte injury						
hs-cTnT, doubling	−0.70% (−14.2, 12.8)	0.92	−3.23% (−18.1, 11.7)	0.67	12.1% (−5.77, 30.0)	0.18

Parameter estimates (95% CI) are for a 1-SD increase (TIMP-1) or doubling in baseline biomarker level and are expressed as percent of SD of the longitudinal change in LV index. Analyses were adjusted for follow-up duration, age, sex, baseline LV index, heart rate, body height and weight, and mean arterial pressure as well as changes in these variables. All covariables were identified based on stepwise regression analyses. CITP indicates carboxyl-terminal telopeptide of collagen type I; hs-cTnT, high-sensitivity cardiac troponin T; LV, left ventricular; MMP-1, matrix metalloproteinase-1; PICP, carboxyl-terminal propeptide of type I collagen; PIIINP, amino-terminal peptide of procollagen type III; TIMP-1, tissue inhibitor of metalloproteinases-1.

Table 5. Multivariable-Adjusted Associations Between 4.7 Years Change in LV Structure Indexes and Baseline Markers of Collagen and Myocardial Injury and Stress

	Δ Relative Wall Thickness		Δ LV Mass Index	
Explanatory Variable	Parameter Estimate (95% CI)	*P* Value	Parameter Estimate (95% CI)	*P* Value
Collagen turnover				
PICP, doubling	4.62% (−7.47, 16.9)	0.45	2.64% (−11.9, 17.1)	0.73
CITP, doubling	16.6% (3.24, 29.9)	0.015	13.1% (−2.87, 29.1)	0.11
MMP-1, doubling	−4.23% (−11.5, 3.08)	0.26	−1.32% (−10.6, 8.06)	0.79
PIIINP, doubling	−3.27% (−9.23, 2.88)	0.30	−1.47% (−8.68, 5.74)	0.69
TIMP-1, +180 μg/L	−2.88% (−10.4, 4.81)	0.55	−2.64% (−11.0, 5.66)	0.53
MMP-1/TIMP-1, doubling	−4.73% (−12.1, 2.63)	0.21	−0.32% (−9.36, 8.73)	0.95
Myocardial injury and stress				
Hs-cTnT, doubling	23.1% (8.85, 36.5)	0.001	18.3% (1.32, 35.3)	0.035

Parameter estimates (95% CI) are for a 1-SD increase (TIMP-1) or doubling in baseline biomarker level and are expressed as percent of SD of the longitudinal change in LV index. Analyses were adjusted for follow-up duration, sex, baseline LV index, age, heart rate, body height and weight, and mean arterial pressure as well as changes in these variables. All covariables were identified based on stepwise regression analyses. CITP indicates carboxyl-terminal telopeptide of collagen type I; hs-cTnT, high-sensitivity cardiac troponin T; MMP1, matrix metalloproteinase-1; PICP, carboxyl-terminal propeptide of type I collagen; PIIINP, amino-terminal peptide of procollagen type III; TIMP-1, tissue inhibitor of metalloproteinases-1.

was reduced in animal models of HF with preserved ejection fraction,[24,25] in mice exposed to volume overload,[26] and in cardiac biopsies of HF patients.[27,28] Although ALP has been used ex vivo to dephosphorylate titin, thereby increasing the overall cardiomyocyte stiffness,[10] its effects on cardiomyocyte properties in vivo remain unknown.

LV properties are also determined by the composition of the extracellular matrix surrounding the cardiomyocytes.[4,11] A diffuse deposition of collagen fibers (mainly type 1) in the interstitial and perivascular space characterizes the myocardial fibrosis seen in HF. Indeed, the severity of myocardial collagen deposition is associated with serum levels of PICP (collagen I synthesis) and CITP (collagen I degradation) in symptomatic HF patients.[29,30] In previous cross-sectional analysis including 782 FLEMENGHO participants, CITP levels were already increased in participants with subclinical LV diastolic dysfunction.[13] In the present longitudinal analysis, both biomarkers of higher collagen I synthesis (PICP) and degradation (CITP) at baseline significantly predicted a stronger decline in LV systolic function as assessed by global LS during follow-up. These direct associations of PICP and CITP with worsening in LV function over time might indicate

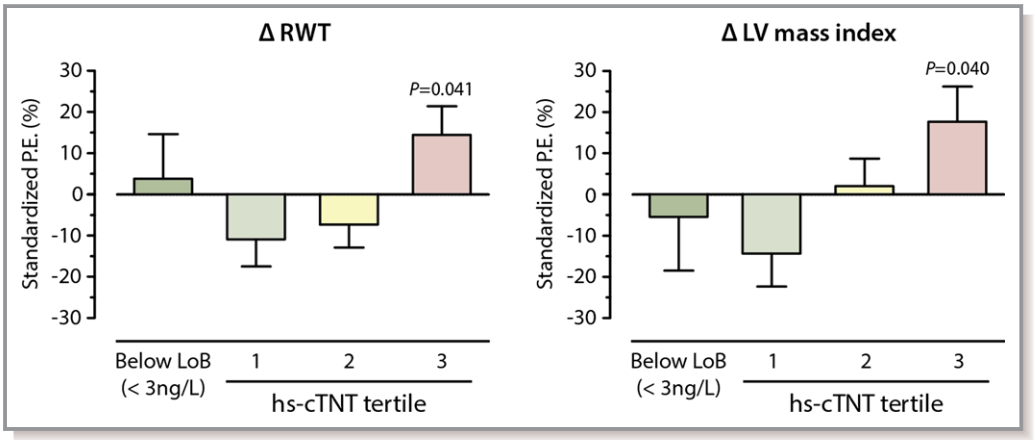

Figure 3. Multivariable-adjusted parameter estimates (PEs; ±SE) for 4.7 years of change (Δ) in left ventricular relative wall thickness (RWT) and mass index for participants with a hs-cTnT level below the level of blank (LoB, <3 ng/L) and per high-sensitivity cardiac troponin T (hs-cTnT) tertile. Number of participants below LoB: n=32. Number of participants per tertile: T1, n=124; T2, n=242; T3, n=160. Adjusted PEs are expressed as percentage of SD of the longitudinal change in the whole cohort. *P* values are for comparisons with the average LV changes within the whole cohort. Analyses were adjusted for follow-up duration, sex, baseline LV index, age, heart rate, body height and weight, and mean arterial pressure. We additionally adjusted for longitudinal changes in these risk factors. Adjustments for Δ LV mass index did not include body height and weight.

that collagen metabolism is overactivated in response to mechanical and neurohumoral stimuli that lead to LV dysfunction. On the other hand, a lower level of MMP1, a main enzyme for collagen degradation, and MMP-1/TIMP-1 ratio at baseline significantly predicted stronger worsening in LV diastolic function (ie, stronger increase in E/e′ ratio) over time. In support, as reflected by decreased levels of MMP-1 and increased levels of its inhibitor (TIMP-1), collagen I degradation was depressed in patients with hypertension[31] and moderate chronic HF.[32] As such, the mechanisms of collagen degradation might already be inhibited in subjects developing early LV dysfunction.

Cardiac-specific troponin T is released from cardiomyocytes in the blood following myocardial stress, ischemia, and injury.[33] Previous community-based studies already identified hs-cTnT as an independent predictor of incident HF.[14] In line with cross-sectional[5,34] and longitudinal population data,[6] our study demonstrated that elevated hs-cTnT levels at baseline were associated with a greater risk to develop or maintain LV hypertrophy over time. Moreover, subjects with higher hs-cTnT levels at baseline had an increased risk to present LV concentric remodeling at follow-up. In addition, cardiomyocyte injury and following release of hs-cTnT can induce alterations in myocardial collagen metabolism and vice versa. Indeed, tissue-repairing processes activated by LV wall injury and stress might alter the myocardial collagen network and contribute to early LV remodeling.[5]

The set of biomarkers identified in our study might be useful for diagnosis and prognosis of subclinical LV maladaptation in subjects at risk. Moreover, the phosphorylation status of myocardial proteins as well as cross linked collagen networks are promising targets to improve myocardial stiffness and contractility and, as such, prevent or delay LV remodeling and dysfunction.[4,35] Future studies in large multiethnic cohorts should further elucidate to what extent a combination of circulating LV biomarkers is useful for risk stratification and therapy guidance in populations at risk for HF.

Study Limitations

Our study must be interpreted within the context of its potential limitations and strengths. First, echocardiographic measurements are prone to measurement errors due to signal noise, acoustic artifacts, and angle dependency. However, one experienced observer recorded all echocardiographic images using a standardized imaging protocol. Moreover, 2 experienced observers post-processed all the echocardiographic images with good intra- and interobserver reproducibility. Second, due to scarce longitudinal population data, most of our observations could only be interpreted in the light of previous cross-sectional findings. Third, we did not investigate the association between the studied biomarkers and concomitant diseases that might be linked to progression of LV remodeling and dysfunction. Finally, our study population included only white Europeans, limiting the generalizability of our findings to other ethnicities.

Conclusions

In a general population sample, we identified a set of circulating biomarkers predicting adverse changes in LV structure and function over time. Reflecting LV stiffness, injury, and collagen composition, these biomarkers might improve the identification of subjects at risk for subclinical cardiac remodeling and dysfunction and, in the long run, symptomatic HF.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

ECHOCARDIOGRAPHY. All participants refrained from smoking, heavy exercise and drinking alcoholic or caffeinated beverages at least 3 hours before echocardiography. Echocardiography was performed after the subjects had rested for at least 15 min in supine position.

Data acquisition. One experienced physician (T.K.) performed both ultrasound examinations in accordance to clinical recommendations[1] and as described previously[2] using a Vivid7 Pro and Vivid E9 (GE Vingmed, Horten, Norway) interfaced with a 2.5- to 3.5-MHz phased-array probe. With the subjects in partial left decubitus and breathing normally, the observer obtained conventional images along the parasternal long and short axes and from the apical 4- and 2-chamber long-axis views, simultaneous with an electrocardiographic signal. All digital recordings included at least 5 cardiac cycles. M-mode tracings were recorded from the LV parasternal long-axis view under guidance of the 2D image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae. To record pulsed wave transmitral Doppler waveforms from the apical window, the observer positioned a 1- to 3-mm Doppler sample volume at the mitral valve tips. Using Tissue Doppler Imaging (TDI), the observer recorded low-velocity, high-intensity myocardial signals at a high frame rate (>190 FPS), while ensuring a parallel alignment of the ultrasound beam with the myocardial segment of interest. The sonographer placed a 5-mm pulsed Doppler sample at the septal, lateral, inferior and posterior sites of the mitral annulus to record mitral annulus velocities from the apical window.

Off-line analysis. In a few weeks after the initial and follow-up examinations, one observer (T.K.) analyzed the echocardiograms blinded to the participants' characteristics. Digitally stored images were post-processed using EchoPac software (GE Vingmed, Horten, Norway). Measurements were averaged over at least three heart cycles for statistical analysis. From the long-axis parasternal view, LV internal diameter and interventricular septal and posterior wall thickness were measured at end-diastole from two-dimensionally guided M-mode tracings. When optimal orientation of M-mode ultrasound beam could not be obtained, the reader performed linear measurements on correctly oriented two-dimensional images. End-diastolic LV dimensions were also used to derive LV mass using an anatomically validated formula as recommended by the American Society of Echocardiography.[3] Relative wall thickness (RWT) was calculated as 0.5 x (interventricular septal + posterior wall thickness)/LV internal diameter at end-diastole. End-diastolic LV dimensions were also used to derive LV mass using an anatomically validated formula. LV concentric remodeling was defined as RWT>0.42. We defined LV hypertrophy as LV mass index exceeding 125 g/m² in men and 110 g/m² in women. We calculated LV ejection fraction (EF) from LV end-systolic and end-diastolic volumes measured from the apical 4- and 2-chambers views, using the standard Simpson's method.

Two experienced observers (N.C and T.K.) measured LV global longitudinal strain (LS) using myocardial speckle-tracking software (Q-analysis, GE Vingmed) at default settings. The LV endocardial border was manually traced at the end-systolic frame of the two-dimensional 4 chamber view. The software automatically tracked myocardial speckle motion while dividing the region of interest in basal, mid and apical levels. We adjusted the region of interest after visual evaluation of the tracking. Images were rejected if tracking was inadequate in ≥2 segments. We used absolute values of peak systolic, mid-wall global LS for statistical analysis.

Transmitral Doppler flow signals were used to measure peak early (E) and late (A) diastolic velocities and E/A ratio. From pulsed-wave Tissue Doppler Imaging (TDI) recordings, we measured the early (e') and late (a') diastolic peak velocities of the mitral annulus displacement

at 4 acquisition sites. We calculated the E/e' ratio by dividing transmitral E peak by e' averaged from the 4 acquisition sites.

We combined the mitral inflow and TDI velocities to classify the grade of LV diastolic dysfunction at baseline and follow-up as previously described.[4] The first group included study participants with an abnormally low age-specific transmitral E/A ratio indicative of impaired relaxation, but without evidence of increased LV filling pressures (E/e'≤8.5). The second group had mild-to-moderate elevated LV filling pressure (E/e'>8.5) and E/A ratio within the normal age-specific range. We also used the differences in durations between the mitral A flow and the reverse pulmonary veins flow during atrial systole (Ad less than ARd+10) or left atrial volume index (≥29 ml/m²) to confirm possible elevation of the LV filling pressures in group 2. Group three had an elevated E/e' ratio and an abnormally low age-specific E/A ratio (combined dysfunction). We previously demonstrated that these thresholds were consistent and reproducible across population cohorts[5] and were validated in prospective studies as predictor of cardiovascular complications.[6]

Reproducibility - As reported before,[7] the physician (T.K.) analyzed the conventional echocardiograms of 17 subjects twice to determine intra-observer reproducibility. Intra-observer reproducibility coefficient of a measurement was the 2SD interval about the mean of the relative differences across pairwise readings. The intra-observer reproducibility was 2.2% for LV internal end-diastolic diameter, 4.6% for LV wall thickness and 4.3% for LV mass, whereas the intra-observer reproducibility for the tissue Doppler velocities ranged from 4.5% to 5.3% for e' velocities and from 4.0% to 4.5% for a' velocities across the four sampling sites. Relative intra- and interobserver reproducibility of global LS was 6.1% and 7.3%, respectively.[8]

BIOMARKER MEASUREMENTS. On the day of the baseline echocardiographic examination, fasting venous blood samples were collected into serum and EDTA tubes, after the subjects had

rested in the supine position for at least 45 min. After centrifuging, plasma and serum were separated, aliquoted and stored at -80°C.

Using commercially available enzymatic essays, enzyme immunoassays (EIA) and sandwich enzyme linked immunosorbent assays (ELISA), we determined plasma activity of ALP (BioVision, USA) and serum levels of: PICP, a marker of collagen I synthesis (METRA EIA kit, Quidel Corporation, USA); CITP, a marker of collagen I degradation (Orion Diagnostica, Finland); MMP-1, a collagenase (MMP-1 Biotrak ELISA System, GE Healthcare, UK); free TIMP-1, a marker of inhibition of collagen degradation (TIMP-1 Human Biotrak ELISA System, GE Healthcare); and PIIINP, a marker of collagen III synthesis (PIIINP ELISA kit, MyBioSource, USA). The inter-assay and intra-assay variations for determining ALP were 8.6% and 13.4%, respectively. the lower detection limit was 0.25 U/mL. The inter-assay and intra-assay variations for determining PICP were 6.4% and 4.5 %, respectively. The lower limit of detection was 0.2 µg of PICP/L. The inter- and intra-assay variations for determining CITP were 13.1% and 10%, respectively. The lower detection limit was 0.30 µg of CITP/L. The inter-assay and intra-assay variations for determining TIMP-1 were 12.8% and 2.6%, respectively. The lower detection limit was 1.25 ng of TIMP-1/mL. Inter- and intra-assay coefficients for PIIINP were both < 15%. The limit of detection was 31.3 pg of PIIINP/mL.

Serum hs-cTnT levels were measured using a highly sensitive assay (Troponin T hs STAT, Roche Diagnostics, Switzerland) optimized on the Cobas 8000 modular analyzer series (Roche Diagnostics, Switzerland). The limit of blank of the assay has been established in 3 ng of cTnT/L and the inter-assay coefficient was 4.6%. The presence of hs-cTnT was considered in all participants with values above 3 ng/L. The upper reference limit (99th percentile) provided by the manufacturer for hs-cTnT was 14 ng/L, 95% confidence interval (12.7 to 24.9 ng/L).

Table S1. Baseline Alkaline Phosphatase Levels and Development or Worsening in LV Diastolic Dysfunction from Examination 1 to 2.

	Alkaline Phosphatase, doubling	
	Adjusted OR (95% CI)	*P* value
Development of LVDDF		
Normal ➔ Any LVDDF (n=38)	1.37 (0.73 to 2.55)	0.33
Normal ➔ Impaired LV relaxation (n=31)	1.26 (0.63 to 2.53)	0.51
Worsening of LVDDF		
Normal/impaired LV relaxation ➔ Increased LV filling pressure* (n=23)	2.01 (0.94 to 4.28)	0.071

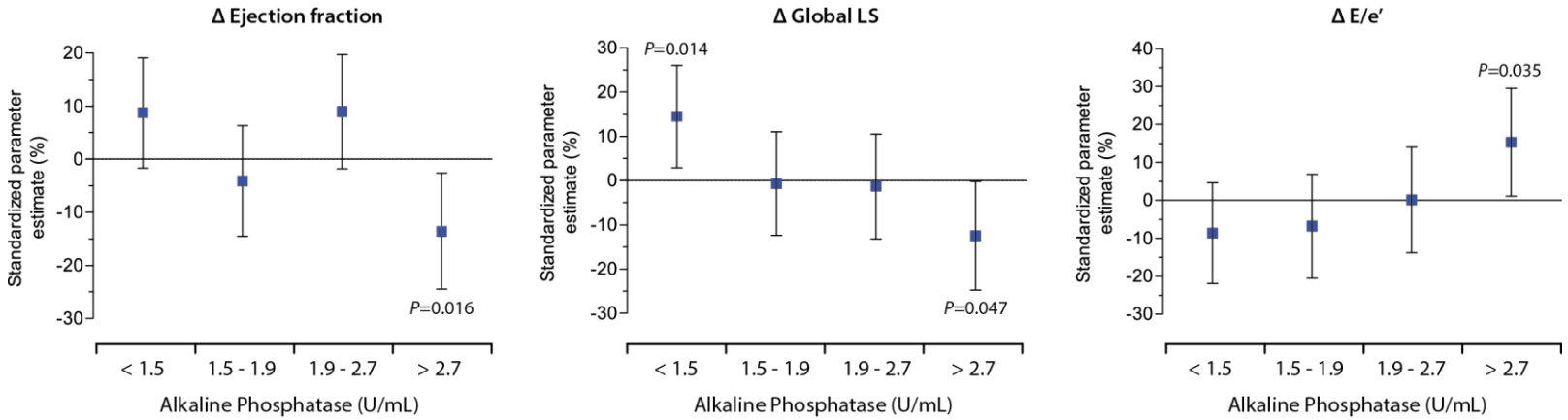
We performed logistic regression to assess the independent correlation of change in LV diastolic dysfunction (LVDDF) state with baseline alkaline phosphatase levels. Odds ratios (OR) express the risk for worsening or development of LVDDF with higher baseline alkaline phosphatase as compared to normal LV diastolic function at both examinations. OR are expressed per doubling in alkaline phosphatase, while adjusting for years of follow-up, age, sex, body mass index, mean arterial pressure. *Increased LV filling pressure group included subjects with combined LVDDF.

Table S2. Multivariable-Adjusted Associations between 4.7 Years Changes in LV Diastolic Function Indexes and Baseline Biomarkers of Collagen Turnover and Myocardial Injury and Stress.

	Δ E/A		Δ E peak		Δ TDI e' peak	
Explanatory variable	Parameter estimate (95% CI)	*P* value	Parameter estimate (95% CI)	*P* value	Parameter estimate (95% CI)	*P* value
Collagen turnover						
PICP, doubling	1.81% (-10.4, 14.1)	0.77	0.42% (-12.3, 13.1)	0.95	5.30% (-8.00, 18.6)	0.43
CITP, doubling	7.41% (-5.93, 20.7)	0.27	2.50% (-11.4, 16.4)	0.72	3.67% (-10.9, 18.2)	0.62
MMP-1, doubling	3.70% (-3.70, 11.5)	0.33	-4.51% (-12.6, 3.57)	0.27	6.40% (-1.71, 14.5)	0.12
PIIINP, doubling	3.15% (-3.04, 9.26)	0.32	0.42% (-6.01, 6.86)	0.90	4.81% (-1.99, 11.6)	0.17
TIMP-1, +180 µg/L	-2.67% (-9.26, 4.44)	0.50	0.17% (-7.14, 7.48)	0.96	-2.77% (-10.3, 4.87)	0.48
MMP-1/TIMP-1, doubling	5.26 (-2.30, 12.9)	0.17	-3.16 (-11.1, 4.75)	0.43	8.73 (0.48, 16.0)	0.038
Cardiomyocyte injury and stress						
Hs-cTnT, doubling	1.00% (-13.3, 15.2)	0.89	-3.05% (-17.9, 11.8)	0.69	-10.1% (-25.7, 5.48)	0.20

Parameter estimates (95% confidence interval) are for a 1-SD increase (TIMP-1) or doubling in baseline biomarker level and are expressed as percent of SD of the longitudinal change in LV index. Analyses were adjusted for follow-up duration, age, sex, baseline LV index, heart rate, body height and weight, and MAP as well as changes in these variables. All covariables were identified based on stepwise regression analyses.

Figure S1. Multivariable-adjusted parameter estimates (P.E. and 95% confidence intervals) for 4.7 years of change (Δ) in left ventricular ejection fraction, longitudinal strain (LS) and E/e' ratio per alkaline phosphatase quartile.



Number of participants per quartile: Q1, n=168; Q2, n=142; Q3, n=140; Q4, n=140. Adjusted PEs are expressed as percentage of SD of the longitudinal change in the whole cohort. *P* values are for comparisons with the average LV changes within the whole cohort. Analyses were adjusted for follow-up duration, sex, baseline LV index, age, heart rate, body height and weight, and MAP. We additionally adjusted for longitudinal changes in these risk factors.

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