Clinical research

Relations of plasma total TIMP-1 levels to cardiovascular risk factors and echocardiographic measures: the Framingham heart study


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Aims Tissue inhibitor of metalloproteinases-1 (TIMP-1) is a key regulator of extracellular matrix degradation. We examined relations of plasma total TIMP-1 to cardiovascular risk factors and echocardiographic left ventricular (LV) structure and function in a community-based sample.

Methods and results We studied 1069 Framingham Heart Study participants (mean age 56 years, 58% women) free of heart failure and previous myocardial infarction. Plasma TIMP-1 was higher in men compared with women, and increased with age, body mass index and total/HDL–cholesterol ratio, but decreased with alcohol intake. Plasma TIMP-1 was also directly related to smoking, diabetes and use of anti-hypertensive treatment. Adjusting for age, sex and height, plasma TIMP-1 was positively associated with LV mass, wall thickness, relative wall thickness, end-systolic diameter, and left atrial diameter and the risk of having increased LV end-diastolic diameter or increased wall thickness, and negatively correlated with fractional shortening. Additional adjustment for clinical covariates attenuated the relations of plasma TIMP-1 to most echocardiographic measures.

Conclusions In our cross-sectional investigation, plasma total TIMP-1 was related to major cardiovascular risk factors and to indices of LV hypertrophy and systolic dysfunction. This raises the possibility that cardiovascular risk factors may influence cardiovascular remodelling via extracellular matrix degradation, which may be reflected in plasma TIMP-1 levels.

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KEYWORDS
Heart failure;
Left ventricular hypertrophy;
Metalloproteinases;
Remodelling;
Echocardiography

Introduction

Left ventricular (LV) dilation and LV hypertrophy (LHV) are markers of LV remodelling, a key precursor of heart failure. LV remodelling is associated with
The design and selection criteria of the Framingham Offspring participants. 

We hypothesised that cardiovascular disease (CVD) risk factors may influence plasma TIMP-1 levels. Additionally, we theorised that remodelling of the cardiac extracellular matrix may be associated with LV dilation and dysfunction, and that this process may be reflected in plasma levels of TIMP-1. Accordingly, we examined the clinical correlates of plasma TIMP-1 and investigated the cross-sectional relations of plasma TIMP-1 to cardiac structure and function in Framingham Heart Study participants.

Methods

Study sample

The design and selection criteria of the Framingham Offspring Study have been described previously. Participants in this cohort are examined every four years and are mostly Caucasian. The 3512 participants at the sixth examination cycle (1995–1998) were considered eligible for the present study. Also eligible were the 506 participants attending the first examination of the minority Omni cohort (58% women; 36% African American, 40% Hispanic). The Omni cohort is a part of the multi-centre Sleep Health Heart Study and was recruited between 1994 and 1996 from among Framingham residents 40–75 years old who identified themselves as being members of a minority group. A multi-modality recruitment approach was used to invite all age-eligible minority residents via mailings and telephone calls. Priority was given to recruitment of both men and women.

We examined the sex-specific distributions of echocardiographic end-diastolic LV internal diameter (LVEDD) and wall thickness (LVWT) among eligible participants (Fig. 1). We sampled all subjects with measurements of LVEDD and LVWT below median (“referent group”, n = 724) and all those with values equal to or exceeding the 90th percentile of LVEDD (“increased LVEDD group”, n = 276) or LVWT (“increased LVWT group”, n = 276). Thus, we investigated 1044 Offspring cohort participants and 200 Omni cohort participants according to the criteria noted above, in order to maximise statistical and blood sample use efficiency.

Participants were excluded if they had congestive heart failure or a history of myocardial infarction (n = 52), serum creatinine >2 mg/dl or missing (n = 37), or missing covariates (n = 74). Twelve subjects with TIMP-1 levels >3 standard deviations (SD) above sex-specific means were considered “outliers” and were excluded. After these exclusions, 1069 participants remained eligible (Offspring cohort n = 930, 58% women; Omni n = 139, 59% women; referent group n = 656, increased LVEDD group, n = 221 and increased LVWT group n = 217; 26 subjects had both increased LVEDD and increased LVWT, Fig. 1). Clinical characteristics were similar for sampled and non-sampled participants (Appendix A). Subjects with overt CVD (n = 65) were excluded from analyses of clinical correlates of TIMP-1. For analyses examining the relations of plasma TIMP-1 to a composite coronary disease risk score (the Framingham Risk Score), 969 participants free of CVD and with complete information on risk score variables were eligible. The study was approved by the Institutional Review Board at Boston Medical Center and all subjects gave written informed consent.

Clinical examination

Participants underwent a standardised medical history and physical examination including measurements of blood pressure;
Blood samples were drawn from fasting participants in a supine position, centrifuged, and the plasma frozen at -70 °C until assay. Plasma TIMP-1 was measured in duplicate using a two-site sandwich ELISA (Amersham Pharmacia Biotech), which measures free TIMP-1, and TIMP-1 complexed with various MMPs (MMP-1/ TIMP-1 complexes, etc.). The intra-assay coefficient of variation for TIMP-1 was <5%.

**Plasma total TIMP-1 measurements**

Blood samples were drawn from fasting participants in a supine position, and the plasma frozen at -70 °C until assay. Plasma TIMP-1 was measured in duplicate using a two-site sandwich ELISA (Amersham Pharmacia Biotech), which measures free TIMP-1, and TIMP-1 complexed with various MMPs (MMP-1/ TIMP-1 complexes, etc.). The intra-assay coefficient of variation for TIMP-1 was <5%.

**Echocardiographic methods**

All participants underwent routine transthoracic echocardiography with Doppler colour flow imaging. M-mode measurements of LV dimensions were obtained using the leading edge-to-leading edge technique. The interventricular septum thickness (IVS), posterior LV wall thickness (PW) and LVEDD were measured at end-diasstole. Left atrial diameter was measured at end-systole, as was the LV internal diameter (LVESD). LVWT was calculated as IVS + PW, and LV relative wall thickness was calculated as (IVS + PW)/LVEDD. LVM was calculated as 0.8[1.04 (IVS + LVEDD + PW)3 – (LVEDD)3] g.23 Endocardial LV fractional shortening (LVFS) was calculated as (LVEDD-LVESD)/LVEDD. Mid-wall LVFS, calculated as previously described, was used in additional analyses. Valve disease was defined as ≥mild degree of stenosis or regurgitation of the aortic or mitral valves on Doppler echocardiography. Reproducibility of echocardiographic measurements was excellent.25

**Statistical analyses**

We used graphical analysis and descriptive statistics to assess the distributional properties of each of our analytical variables (including means, standard deviations, medians, quartiles and ranges). We used natural logarithmic transformation for variables with skewed distributions (TIMP-1, LVM, LVWT, relative wall thickness, LVEDD, LVEDD, left atrial diameter and alcoholic drinks/week).

Step-wise multiple linear regression models were used to investigate the relation of select clinical variables (age, sex, ethnicity, body mass index, smoking, alcohol intake, diabetes, total/HDL–cholesterol ratio, systolic blood pressure, anti-hypertensive treatment, and heart rate) to plasma TIMP-1 levels [log-transformed, ln(TIMP-1)] in the total sample, and in the referent group. We evaluated clinical correlates in both groups to understand whether determinants of TIMP-1 in a “healthier” subset of individuals with normal LV dimensions differed from that observed in the larger sample. A p value of <0.05 was used for retaining variables in the stepwise models. Self-reported ethnicity was used as a dichotomous variable (Caucasian vs. non-Caucasian). In secondary analyses, we forced LVWT and LVEDD into the models evaluating clinical correlates of TIMP-1.

We evaluated the relations of ln(TIMP-1) to the Framingham Risk Score (modelled as a continuous variable) in multivariable models adjusting for age, sex, body mass index, and other correlates identified above. We also used analysis of variance to examine trends in ln(TIMP-1) across sex-specific quartiles of the Framingham Risk Score.

We used multiple logistic regression to examine the associations of ln(TIMP-1) with increased LVEDD or increased LVWT (separate models for each). Subjects with both increased LVEDD and increased LVWT were included in the increased LVEDD and increased LVWT groups in the separate models. Two models were examined in a hierarchical fashion: (A) Adjusted for age, sex and height. (B) Adjusted for age, sex, height, weight, ethnicity, smoking, alcohol intake, diabetes, total/HDL–cholesterol ratio, systolic blood pressure, anti-hypertensive treatment, valve disease and heart rate.

We used analysis of covariance to examine trends in covariate-adjusted means of echocardiographic variables across sex-specific quartiles of plasma TIMP-1 levels. In the multivariable-adjusted models, relations between plasma TIMP-1 and left atrial diameter were also adjusted for the presence of atrial fibrillation and valve disease. We initially tested for statistical interactions between plasma TIMP-1 levels and sex, but because no interaction with sex was noted, sex-pooled analyses are presented.

Regression models using log-transformed echocardiographic (dependent) variables better met the assumptions of homoscedasticity, independence, and normality of residuals with the exception of fractional shortening, for which the untransformed residuals were preferable. TIMP-1 and continuous covariates were log transformed to reduce skewness and kurtosis that would otherwise influence the slope of the relationship with the dependent variables. Although log-transformed echocardiographic variables (with the exception of fractional shortening) were used as dependent variables in regression models, least squares means were computed in original units by back-transforming log-adjusted means into the original units.

Because the analyses of echocardiographic measurements as continuous variables pooled data for individuals in the three LV groups, we performed several additional analyses to: (a) test the hypotheses that the slope of the ln(TIMP-1) and LV relations were similar in the referent group versus the group with increased LV measurements; and (b) examine effect modification by group status by incorporating interaction terms [LV group × ln(TIMP-1)] in the multivariable models. A two-sided p value <0.05 was considered statistically significant for all analyses.

**Results**

Means ± SD plasma TIMP-1 was 781 ± 133 ng/ml (range 447–1410) in the total sample. Clinical characteristics of the study subjects are shown in Table 1. None of the participants were known to have cancer within the 6 months preceding the examination.

**Clinical correlates of plasma total TIMP-1 in participants without clinical evidence of cardiovascular disease**

In a multivariable linear regression analysis (Table 2), plasma TIMP-1 was higher in men compared with women (p = 0.0001), and increased with age (p = 0.0001), body mass index and total/HDL–cholesterol ratio (both p = 0.001). Plasma TIMP-1 was also positively related to smoking, anti-hypertensive treatment (both p < 0.01) and diabetes (p = 0.04). Plasma TIMP-1 was inversely
related to alcohol consumption \( (p = 0.008) \). Systolic blood pressure, heart rate and ethnicity were not significantly related to plasma TIMP-1. Clinical covariates explained 22% of the inter-individual variation in plasma TIMP-1 levels. The \( \beta \)-coefficients did not change when LVWT and LVEDD were forced into the model (data not shown). In multivariable models adjusting for age, sex, body mass index, and other correlates, the Framingham Risk Score was significantly related to \( \ln \) (TIMP-1) \( (p < 0.0001) \). Plasma TIMP-1 rose with increasing number
TIMP-1 levels were significantly higher in the increased individuals receiving change when analyses were repeated for subgroups of (Table 3). The relations of TIMP-1 to LV measures did not justed models, but not in multivariable-adjusted ones quartile increase in TIMP-1 in age-, sex- and height-adjusted ones (β = −0.02 per 1 SD, p = 0.008) and ethnicity (β = 0.04 Caucasian vs. non-Caucasian, p = 0.02).

Echocardiographic correlates of plasma total TIMP-1 in entire sample

TIMP-1 levels were significantly higher in the increased LVEDD and increased LVWT groups compared to the referent group adjusting for age, sex and height, but not in multivariable-adjusted models. The odds of having increased LVEDD or increased LVWT increased significantly by 20–28% per SD increase in ln(TIMP-1) or a quartile increase in TIMP-1 in age-, sex- and height-adjusted models, but not in multivariable-adjusted ones (Table 3). The relations of TIMP-1 to LV measures did not change when analyses were repeated for subgroups of individuals receiving (n = 141) and not using (n = 748) anti-hypertensive treatment.

Table 3 Plasma total TIMP-1 levels and increased left ventricular diastolic dimensions and increased wall thickness: multiple logistic regression models

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds ratio for increased LVEDD (95% CI)</th>
<th>p Value</th>
<th>Odds ratio for increased LVWT (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Age, sex and height adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 SD increase in ln(TIMP-1)</td>
<td>1.24 (1.03–1.48)</td>
<td>0.02</td>
<td>1.28 (1.07–1.54)</td>
<td>0.007</td>
</tr>
<tr>
<td>Trend across TIMP-1 quartiles</td>
<td>1.20 (1.02–1.40)</td>
<td>0.03</td>
<td>1.23 (1.04–1.44)</td>
<td>0.013</td>
</tr>
<tr>
<td>(B) Multivariable-adjusted</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 SD increase in ln(TIMP-1)</td>
<td>1.09 (0.88–1.36)</td>
<td>0.43</td>
<td>0.92 (0.72–1.16)</td>
<td>0.46</td>
</tr>
<tr>
<td>Trend across TIMP-1 quartiles</td>
<td>1.07 (0.89–1.29)</td>
<td>0.49</td>
<td>0.95 (0.78–1.16)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Quartiles of TIMP-1 are sex-specific. 1 SD in ln(TIMP-1) = 0.165. Model B adjusted for age, sex, ethnicity, height, weight, smoking, alcohol intake, diabetes, total/HDL-cholesterol ratio, systolic blood pressure, antihypertensive treatment, valve disease and heart rate. Ranges of TIMP-1 (ng/ml) in the quartiles were 476–710, 714–788, 790–871 and 872–1410 for men, and 447–670, 671–743, 744–838 and 839–1289 for women. LV, left ventricular; EDD, end-diastolic diameter; WT, wall thickness.

In models examining linear trends of echocardiographic variables across TIMP-1 quartiles (Table 4), plasma TIMP-1 was related positively to LVM, LVWT, relative wall thickness, LVESD and left atrial diameter but inversely to LVFS, adjusting for age, sex and height. Additional adjustment for clinical covariates attenuated the relations of plasma TIMP-1 to LVM, LVWT, relative wall thickness and left atrial diameter. In the latter models, inverse relations of plasma TIMP-1 to LVFS were maintained, and direct relations to LVESD were of borderline statistical significance.

Additional analyses evaluated whether the slope of the relations of TIMP-1 to LV measurements differed across the LV groups. For fractional shortening, the only echocardiographic measure that was significantly related to TIMP-1 in multivariable models, the parameter estimates were −0.036 for the referent group (p = 0.01), and −0.038 (p = 0.03) in the increased LVWT/LVDD group, and the null hypothesis of no difference in slopes between the groups was accepted. Also none of the interaction terms (TIMP-1 × echo group) was statistically significant (all p values exceeded 0.50). In secondary analyses, mid-wall LVFS was inversely related to TIMP-1 quartiles (p < 0.01 in both models).

Discussion

The present investigation reports on the cross-sectional association of plasma total TIMP-1 levels with established CVD risk factors and echocardiographic measurements in a large community-based sample. Our principal findings are that plasma total TIMP-1 was directly related to major CVD risk factors and to echocardiographic indices of LVH, and inversely to systolic function.

Plasma total TIMP-1 and risk factors for atherosclerotic disease

The direct relations of plasma TIMP-1 levels to all major CVD risk factors is a striking finding of the present study. The observation that plasma TIMP-1 was increased in diabetics and participants using anti-hypertensive treatment is in agreement with previous studies using smaller
We observed a direct relationship between plasma TIMP-1 levels and LVM, LVWT, relative wall thickness and odds for increased LVWT, consistent with most previous experimental\(^9\) and small human\(^{15}\) studies. Additionally, plasma TIMP-1 levels were related to LVESD and risk of increased LVEDD. These associations were attenuated in multivariate models. The relations between TIMP-1 and left atrial diameter may in part be explained by the close relation between left atrial size and LVM.\(^{27}\)

Several traditional CVD risk factors are known to predict LVH,\(^{28,29}\) and we noted their associations with plasma TIMP-1 levels. Therefore, we performed additional analyses to examine whether plasma TIMP-1 was related to LV measures after adjusting for these known risk factors as potential confounding factors. The finding that relations between TIMP-1 and LVH indices were attenuated by adjustment for traditional CVD risk factors is consistent with the hypothesis that the risk factors may influence LV structure through effects on TIMP-1 levels. Alternatively, it is possible that the CVD risk factors influencing LV remodelling directly, and TIMP-1 levels are a marker of the remodelling process. A third possibility is that TIMP-1 is a marker of vascular remodelling, which may result in increased LV afterload and consequent LVH.

The association of plasma TIMP-1 with increased LVWT merits comment. The major biological role of TIMP-1 appears to be to balance the activity of MMPs. In the first half of the study, TIMP-1 levels would inhibit myocardial MMP activity, leading to increased myocardial collagen deposition and increased LVWT, as we have seen here. TIMP-1 also has growth-promoting effects independent of its MMP inhibitory effects,\(^{30}\) and this may also play a role in our observations.

### Plasma total TIMP-1 and cardiac structure and function

We observed a direct relationship between plasma TIMP-1 levels and LVM, LVWT, relative wall thickness and odds for increased LVWT, consistent with most previous experimental\(^9\) and small human\(^{15}\) studies. Additionally, plasma TIMP-1 levels were related to LVESD and risk of increased LVEDD. These associations were attenuated in multivariate models. The relations between TIMP-1 and left atrial diameter may in part be explained by the close relation between left atrial size and LVM.\(^{27}\)

#### Table 4 Relations of plasma total TIMP-1 to echocardiographic measures

<table>
<thead>
<tr>
<th>Continuous ln(TIMP-1)</th>
<th>TIMP-1 quartile</th>
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<tbody>
<tr>
<td></td>
<td>Q1</td>
</tr>
<tr>
<td>(A) Age-, sex- and height-adjusted</td>
<td></td>
</tr>
<tr>
<td>LVM (g)</td>
<td>0.03</td>
</tr>
<tr>
<td>LVWT (cm)</td>
<td>0.02</td>
</tr>
<tr>
<td>LV RWT (%)</td>
<td>0.01</td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>0.006</td>
</tr>
<tr>
<td>LVESD (cm)</td>
<td>0.016</td>
</tr>
<tr>
<td>LVFS (%)</td>
<td>-0.007</td>
</tr>
<tr>
<td>LA (cm)</td>
<td>0.014</td>
</tr>
<tr>
<td>(B) Multivariable-adjusted</td>
<td></td>
</tr>
<tr>
<td>LVM (g)</td>
<td>0.005</td>
</tr>
<tr>
<td>LVWT (cm)</td>
<td>0.002</td>
</tr>
<tr>
<td>LV RWT (%)</td>
<td>0.001</td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>0.001</td>
</tr>
<tr>
<td>LVESD (cm)</td>
<td>0.011</td>
</tr>
<tr>
<td>LVFS (%)</td>
<td>-0.006</td>
</tr>
<tr>
<td>LA (cm)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

\(^{a}\) is the regression coefficient that indicates change in log-transformed LV measurements per 1 SD increment in ln(TIMP-1). 1 SD ln(TIMP-1) = 0.165. For the TIMP-1 quartiles columns, least squares means are presented (with 95% confidence intervals in parentheses), adjusted for age, sex and height in the upper panel (Model A) and adjusted for age, sex, ethnicity, height, weight, smoking, alcohol intake, diabetes, total/HDL–cholesterol ratio, systolic blood pressure, anti-hypertensive treatment, valve disease and heart rate in the lower panel (Model B). Left atrial diameter values are also adjusted for atrial fibrillation. Sex-specific TIMP-1 quartiles are as in Table 3. LV, left ventricular; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening.
their close correlate LVESD were independent of traditional risk factors, which could support an effect of TIMP-1 above and beyond the effects of these risk factors on LV systolic function. Demonstration of an association between TIMP-1 and LVFS within the normal range is an intriguing finding from a mechanistic point of view. One possible explanation for this observation is that the increased total TIMP-1 measured is indicative of increased MMP activity and increased matrix degradation, which may result in a disrupted extracellular matrix architecture, and associated LV dysfunction.\(^2\) Another is that higher TIMP-1 levels may indicate an increased collagen content of the cardiac extracellular matrix, and such an increase may adversely impact LV systolic function.

**Strengths and limitations**

Some differences in findings between the present and previous studies may be attributed to differences in study subjects (our sample was relatively healthy) and to the sample sizes studied (we used a large community-based sample with greater statistical power to detect modest associations with risk factors). We also assessed the associations of TIMP-1 and LV measurements in relation to known CVD risk factors in hierarchical statistical models, which has not been done previously.\(^4\)–\(^6\) Several limitations of our study merit description. We did not assess LV diastolic function, arterial compliance or arrhythmias. A comprehensive assessment of these characteristics would have provided valuable additional insights. We also had few non-Caucasian participants in this study and hence limited power to examine the impact of ethnicity on TIMP-1 levels or LV effects. We examined plasma total TIMP-1, which gives limited information on myocardial TIMP-1 levels and also combines information on both free TIMP-1, and TIMP-1 complexed with various MMPs. The interpretation might be aided by measures of MMP activity, MMP/TIMP-1 complexes and pro-collagen peptides. Plasma TIMP-1 arises from several organ systems, including but not limited to cardiovascular tissues. Some caution in the interpretation of clinical correlates of TIMP-1 may be warranted due to the sampling strategy based on the distribution of LV measurements. Lastly, it is important to note that we examined the relations of several echocardiographic measurements with plasma TIMP-1. While we did not adjust for multiple comparisons, it is important to note that all analyses were specified *a priori*. We believe it is unlikely that the association of plasma TIMP-1 with fractional shortening is solely due to chance as a result of multiple testing given the degree of statistical significance observed in our study \((p = 0.003, \text{Table 4})\), the consistency of results in age- and sex-adjusted and multivariable models, and biological plausibility.

**Conclusions**

In our community-based sample, plasma total TIMP-1 was directly related to major CVD risk factors and to indices of LVH, and inversely to systolic function. These observations raise the possibility that CVD risk factors may influence vascular and cardiac remodelling via extracellular matrix degradation, which may be reflected in plasma TIMP-1 levels.

**Acknowledgements**

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**Appendix A**

See Table 5.

**References**


