Role of matrix metalloproteinases and their tissue inhibitors as potential biomarkers of left ventricular remodelling in the athlete’s heart

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ABSTRACT

The aim of the present study was to verify whether plasma MMPs (matrix metalloproteinases) and TIMPs (tissue inhibitors of MMPs) could be used as potential markers of paraphysiological remodelling in the athlete’s heart, and to correlate these matrix parameters with echocardiographic signs of LV (left ventricular) remodelling. Plasma MMP-2 and MMP-9 were measured by zymography, and TIMP-1 and TIMP-2 were measured by ELISA in 42 veteran marathoners with AH (athlete’s heart), and in 25 sedentary healthy subjects (CTL). All subjects were submitted to a clinical examination and two-dimensional colour Doppler echocardiography together with the measurement of circulating NT-proBNP (N-terminal pro-B-type natriuretic peptide); GGT (γ-glutamyl transpeptidase) was evaluated as a marker of cardiovascular disease. Veteran athletes had a significant elevation in LV dimensions and calculated LV mass index. Diastolic and systolic functions were normal for both groups. MMP-9 levels were significantly lower in AH than in CTL subjects (56.9 ± 4.3 compared with 119.4 ± 21.5 m-units/l, P < 0.01). There were significant differences in MMP-2 between the two groups, with a down-regulation in the AH subjects (182.5 ± 16.8 units/ml in CTL compared with 117.1 ± 9.1 units/ml in AH, P < 0.01). MMP-2 and MMP-2/TIMP-2 were inversely correlated with myocardial indices of hypertrophy in AH and CTL subjects. AH and CTL subjects showed similar TIMP values. The results of the present study indicate that MMPs and TIMPs could represent potential biomarkers of adaptive heart remodelling in the athletes. In addition, the inverse correlation of the MMP-2/TIMP-2 system with echocardiographic signs of myocardial hypertrophy could represent a new diagnostic and prognostic indicator useful in the evaluation of cardiovascular risk in athletes.

INTRODUCTION

The condition known as AH (athlete’s heart) exhibits paraphysiological remodelling, in particular myocardial parietal hypertrophy and chamber enlargement, due to aerobic endurance sport activity [1]. In this activity, training is accompanied by an elevated inflow of oxygen to the tissues, with a relative shortage of oxygen and an elevated

Key words: athlete’s heart, left ventricular remodelling, matrix metalloproteinase (MMP), sports medicine, tissue inhibitor of MMPs (TIMP).

Abbreviations: A, late diastolic peak velocity; AH, athlete’s heart; CTL, control; E, early diastolic peak velocity; ECM, extracellular matrix; EF, ejection fraction; GGT, γ-glutamyl transpeptidase; HF, heart failure; LV, left ventricular; LVEDD, LV end-diastolic diameter; LVM, LV mass; MMP, matrix metalloproteinase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TIMP, tissue inhibitor of MMPs.

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pre-load under stress, but adequate haemodynamic and tissue compensation under basal conditions [2]. In contrast, patients with pathological remodelling suffer from inadequate oxygen supply to the tissues and elevated filling pressure even at rest. Habitual aerobic activity also reduces coronary heart disease events, although it may increase the risk of sudden cardiac death and acute myocardial infarction in susceptible athletes [3,4]. Paraphysiological and pathological heart remodelling present similar macroscopic, but significantly different microscopic, features; in HF (heart failure) there is marked fibrosis whereas in AH there is none, which makes the remodelling process reversible and preserves diastolic and systolic ventricular function [5].

MMPs (matrix metalloproteinases) and TIMPs (tissue inhibitors of MMPs) are the main determinants of tissue remodelling in both physiological and pathological processes. Evidence suggests that MMP activity can change the structure of the ECM (extracellular matrix), leading to the loss of cardiac contractility through proteolysis [6]. The remodelling of collagen fibres and the loss of myocytes is known to cause ventricular enlargement and progressive contractile dysfunction [7]. LV (left ventricular) remodelling in HF patients is also stimulated by degradation of the ECM by MMPs [8]. Gelatinases A and B (MMP-2 and MMP-9) are particularly active in degrading denatured collagen [9] and have been the focus of pathological heart remodelling studies.

Although previous results suggest that plasma MMP-2 and MMP-9 could provide diagnostic and prognostic information in patients with HF [6], the MMP and TIMP profile in AH has not yet been investigated, even though it presents an excellent model of reversible and adaptive paraphysiological cardiac remodelling [10] that could be useful for comparison studies.

In the present study, we sought to define and compare the plasma profiles of MMP-2 and MMP-9 and their specific inhibitors in veteran marathoners with AH and in a control group of healthy sedentary subjects, in order to determine whether these endopeptidases could serve as potential markers of adaptive remodelling of the heart. The correlations between these parameters of ECM turnover, and laboratory [NT-proBNP (N-terminal pro-B-type natriuretic peptide)] and echocardiographic signs of LV structure and remodelling were analysed. In the present study we also evaluated the values of circulating GGT (γ-glutamyl transpeptidase), emerging as a marker of cardiovascular disease.

### MATERIALS AND METHODS

**Subjects**

Two groups of subjects comparable in age and gender were enrolled in the present study (Table 1): 25 sedentary healthy controls (CTL) and 42 veteran marathoners with AH.

Veteran marathoners with AH were recruited from among athletes who come voluntarily once a year to the AOUP (Azienda Ospedaliera Universitaria Pisana)
for a cardiological examination. All of those enrolled were eligible for competitive sport, with no electrocardiographic or echocardiographic signs of hypertrophic cardiomyopathy, diastolic or systolic dysfunction or arrhythmias. All had been training and competing continuously for at least 5 years and had an LVM (LV mass) > 106 g/m². Plasma samples were obtained 48 h after their most recent training session. None had been under any medical treatment for the last 5 years.

Sedentary controls (CTL) were recruited through primary care physicians. Each subject provided a medical history and underwent a clinical examination, a 12-lead ECG, two-dimensional colour Doppler echocardiography, and an abdominal echography; chest radiography was performed in cases of smoking, professional risk or a family history of lung cancer. Marathoners also underwent cycloergometric and spirometric tests. All of the data were recorded in confidential clinical files.

Fasting blood samples obtained on the same day as the examinations were used for the analyses of gelatinase and TIMP concentrations, NT-proBNP, GGT, inflammation indices, and coagulative, hepatic and renal function. Subjects with evidence of neoplastic disease, acute or chronic inflammatory disease, renal and/or hepatic dysfunction, or haemostatic disease were excluded from the study.

The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The research protocol was reviewed and approved by the AOUP Ethical Committee. All participants gave their written, informed consent on the study procedure and the purpose of the study was explained to them.

Echocardiography
Examinations were carried out by two trained echocardiographers, using a Sonos 5500 system with a 4-MHz transducer. A biplanar evaluation of the EF (ejection fraction) was carried out, and mono-dimensional measurements of the left diastolic and systolic diameters, and the septal and parietal thicknesses were performed at the chordal level just beneath the mitral valve leaflets, following the American Society of Echocardiography criteria. Measurement of the E/A ratio (where A is the late diastolic peak velocity and E is the early diastolic peak velocity) was made in the apical view with a cursor at the mitral valve inflow. The average value based on three consecutive measures (minimum to maximum variation < 5 %) was calculated. The LVM was calculated according to the formula of Devereux et al. [11] and indexed on the basis of individually calculated body surface areas.

Plasma MMP/TIMP measurements
Gelatinase and TIMP concentrations were measured in heparinized plasma. Each sample was immediately separated and stored at −70 °C in multiple aliquots and thawed once, at the time of use.

Gelatinase activity was determined by zymography; proteins at appropriate dilutions were denatured with SDS under non-reducing conditions and then electrophoretically separated in an 8 % polyacrylamide gel containing gelatin at 1 mg/ml. Proteins were renatured by removing SDS, and the gel was incubated for 16–18 h at 37 °C in a buffer that allowed the lytic action of the gelatinases to take place on the gelatin. The gel was then stained with Coomassie Brilliant Blue and gelatinolytic activity appeared as a clear area against the blue background of undegraded gelatin. The images were digitally scanned and the activity was quantified based on the curves of known concentrations of gelatinase standards (MMP-9, Roche Applied Science; MMP-2, Boehringer Mannheim) which were run together with the samples. The lytic areas were measured using QuantityOne software (Bio-Rad Laboratories). Activity was expressed in the same units as the standards. TIMP-1 and TIMP-2 were measured by a commercial ELISA kit (Biotrak; Amersham Bioscience), and the MMP-2/TIMP-2 and MMP-9/TIMP-9 ratios were calculated to obtain a more exact parameter of the net activity of the gelatinases in the plasma.

NT-proBNP and GGT
NT-proBNP was assayed using an Elecsys kit (Roche). GGT was measured spectrophotometrically in a Hitachi 917 automatic analyser (Roche). These were measured as indices of pathological remodelling and cardiovascular risk respectively.

Statistical analysis
Non-parametric tests were applied to analyse the data. Between-group comparisons of gelatinase activity and TIMP levels were carried out using the non-parametric Mann–Whitney test. The significance level was set at $P < 0.05$. Correlations between the parameters under examination and the gelatinases and their inhibitors was determined for the pooled and single group data by the non-parametric Spearman correlation coefficient ($r$). All analyses were performed with Prism 4 (GraphPad Software).

RESULTS
Comparison of echocardiogram and plasma parameters between CTL and AH subjects
Table 1 presents the relevant data for the two groups studied; significant differences emerged on statistical analysis. Both groups showed values of systolic and diastolic function in the normal range. Nonetheless, AH subjects had a significantly lower EF and a higher E/A ratio than CTL subjects. AH subjects had a larger LVM and
significant LV remodelling compared with CTL subjects, with higher values for the LVEDD (LV end-diastolic diameter), septal thickness and posterior parietal thickness. Both MMP-2 and MMP-9 were lower in AH than in CTL subjects. The MMP-2/TIMP-2 ratio was significantly lower in AH subjects. There were no significant differences in TIMP-1, TIMP-2 or the MMP-9/TIMP-1 ratio between the two groups.

Correlations between the plasma MMP/TIMP profiles, and echocardiogram and laboratory parameters
Correlations were tested on the pooled data (Table 2). MMP-2 and the MMP-2/TIMP-2 ratio showed a significant inverse correlation with the septal and posterior parietal thicknesses and (to a lesser extent) with the LVM. A regression curve was designed for each of the mentioned parameters (Figure 1). MMP-9 and the MMP-9/TIMP-1 ratio were inversely correlated with the LVEDD.

No correlation was evident with GGT levels, whereas NT-proBNP positively correlated with the LVM (Spearman’s $r = 0.2832$, $P = 0.0312$).

Analysis of the data by group (Table 3)
Correlation between MMPs, TIMPs and echocardiogram parameters
An inverse correlation between MMP-2 and the septal and posterior parietal thickness (as was seen in the pooled data) was found both in AH and CTL subjects. A similar correlation was found between the MMP-2/TIMP-2 ratio, and septal and posterior parietal thickness in the CTL group, whereas only posterior parietal thickness negatively correlated in the AH subjects.

In AH, but not in CTL, subjects the MMP-9/TIMP-1 ratio was inversely correlated with the LVEDD (as in the pooled data), whereas MMP-9 and the MMP-9/TIMP-1 ratio were positively correlated with posterior parietal thickness.

Correlations between the plasma MMP/TIMP profile and laboratory parameters
In AH subjects, an inverse relationship was found between MMP-2 and MMP-9 levels (Spearman’s $r = -0.4922$, $P = 0.0027$), which was confirmed by an inverse correlation between MMP-2 and the MMP-9/TIMP-1 ratio in AH subjects (Spearman’s $r = -0.4539$, $P = 0.0117$). Moreover, NT-proBNP was positively correlated with MMP-2 (Spearman’s $r = 0.3822$, $P = 0.0235$) and inversely correlated with MMP-9 and MMP-9/TIMP-1 (Spearman’s $r = -0.3583$, $P = 0.0319$ respectively). In CTL subjects, TIMP-1 was inversely correlated with MMP-9 and TIMP-2 (Spearman’s $r = -0.4096$, $P = 0.0469$ and $r = -0.5017$, $P = 0.0125$ respectively).

**DISCUSSION**

The aim of the present study was to investigate whether plasma gelatinases and their tissue inhibitors reflect heart remodelling in athletes compared with controls. Our principle findings were (i) MMP-9 is down-regulated in AH compared with CTL subjects; (ii) MMP-2 and MMP-2/TIMP-2 were lower in AH than in CTL subjects; (iii) both in CTL and AH subjects, MMP-2 and MMP-2/TIMP-2 showed an absolute inverse correlation with echocardiographic indices of myocardial hypertrophy; (iv) AH subjects showed an inverse correlation between MMP-9 and the LV diameter; (v) AH and CTL subjects showed similar TIMP values.

Plasma levels of MMP-2 and MMP-9, as well as the MMP-2/TIMP-2 ratio, were down-regulated in AH compared with sedentary CTL subjects, indicating a reduction in ECM proteolytic activity. Although in AH subjects the levels of both gelatinases were reduced, MMP-2 was inversely correlated with MMP-9 and the MMP-9/TIMP-1 ratio, suggesting differing roles for the two peptidases. Analogous correlations were seen between MMP-2 and MMP-9 and NT-proBNP and the echocardiographic parameters; in athletes NT-proBNP was positively correlated with MMP-2 and inversely correlated with MMP-9/TIMP-1, whereas the opposite held true for parietal thickness.

AH subjects exhibited significantly lower MMP-2 and MMP-2/TIMP-2 values than CTL subjects; moreover, we found MMP-2 levels were correlated with echocardiographic parameters of hypertrophy.
Ahmed et al. [12] showed that the MMP-2 values also are decreased in hypertensive cardiac hypertrophy without HF [12]. AH and hypertensive heart are both characterized by myocardial hypertrophic remodelling. Studies on animal models have shown that MMP-2 up-regulation in the tissues plays a key role in myocardial cardiomyocytic hypertrophy, suggesting that myocardial hypertrophy could be the main determinant of the MMP-2 profile in plasma [13]. Other studies have shown that the MMP-2/TIMP-2 system plays a role in growth and neoangiogenesis processes and that blood flow can modulate MMP-2 expression [14]. At the same time, the high degree of morphological adaptability shown by the capillary network in hypertrophied muscles is well known. Compensatory recapillarization and neoangiogenesis are also seen in atherosclerotic and hypertensive diseases. The presence of MMP-2/TIMP-2 down-regulation in our model of paraphysiological hypertrophic heart remodelling seems to confirm a strong correlation with the processes of cardiomyocytic hypertrophy and cardiovascular neoangiogenesis in the tissues. Further studies to elucidate the apparent discrepancy between the MMP-2 profile in plasma and tissues are needed.

The results of the present study show that aerobic exercise can lead to lower basal MMP-9 values compared with sedentary controls. MMP-9 can be considered an inflammatory marker and we found it positively correlated with other known biomarkers of inflammation such as fibrinogen and IL (interleukin)-6 (results not shown). MMP-9 is now also recognized as a marker of cardiovascular risk [15], and a possible marker of plaque evolution and rupture [16]. Moreover, in vivo studies of the zymographic activity of MMP-9 inside the evolving atherosclerotic plaque confirm its role in vascular remodelling. On the other hand, results have been published on the role of diet and exercise in reducing plasma levels of MMP-9 [17]. Inhibition of MMP-9 activity by statins was demonstrated in biopsy samples of aortic aneurysms and carotid plaques [18,19].
Gullestad et al. [20] confirmed that MMP-9 concentrations are decreased by pharmacological treatment with statins [20]. The lower MMP-9 levels observed in the present study in AH subjects might simply reflect decreased remodelling activity, although it should be noted that under certain conditions MMP-9 in the myocardium has a pro-fibrotic effect since it activates pathways and proteins [such as TGF-β1 (transforming growth factor-β1)] that favour ECM deposition [21]. As a consequence, low levels of MMP-9 may cause a basal down-regulation of pro-fibrotic and pro-inflammatory activity in the matrix system in athletes. Moreover, since strenuous exercise is accompanied by significantly raised MMP-9, troponins and indices of inflammation, the lack of ‘normalization’ of plasmatic MMP-9 and inflammatory markers within 48 h from the end of endurance training could indicate an overtraining cardiovascular syndrome [22].

The present study showed a direct correlation between MMP-9 and MMP-9/TIMP-1 and parietal thickness in AH, and an inverse correlation with the LVDD. In contrast, Yan et al. [23] reported indications of a positive correlation between MMP-9 and LVDD, and a negative correlation between MMP-9 and MMP-9/TIMP-1 and LV function in pathological heart remodelling. Therefore the plasma MMP-9/TIMP-1 ratio could be linked to pathological remodelling and the risk of LV dysfunction in AH as well. To confirm this hypothesis, it would be interesting to compare the MMP-9/TIMP-1 ratio and TIMP-1 values in groups of trained and over-trained athletes.

One finding of the present study was that AH and CTL subjects showed similar TIMP values. On the other hand, there have already been reports of higher TIMP-1 values in patients with hypertension, a condition characterized by interstitial fibrosis and myocardial hypertrophy possibly preceding LV dysfunction [24].

The results of the present study are of particular interest because the two groups studied did not show any difference in NT-proBNP, the most commonly used laboratory index of heart dysfunction [25]. NT-proBNP, a known parameter correlated with HF is similarly distributed in AH or CTL subjects. We also decided to evaluate GGT because it is a laboratory parameter recently proposed to represent a marker of cardiovascular disease [26], therefore it seemed that its evaluation could be of interest, in particular in AH subjects, but we did not observe any significant difference between the two groups.

The results of the present study suggest that AH and CTL subjects have differing plasma gelatinase profiles. The present study does have some limitations, because plasma levels of MMPs and TIMPs may not reflect cardiac tissue remodelling alone; other physiological and pathological conditions are known to affect the concentration and activity of these endopeptidases. Nonetheless, significant differences in these parameters were found between normal subjects and AH subjects. The particularly low basal MMP-9 values detected in AH subjects suggest a mechanism of cardiovascular protection.

Furthermore, this is the first study to show a relationship between plasma matrix biomarkers in AH and echocardiographic parameters of myocardial hypertrophy. It
seems likely that a down-regulation of MMP-2 takes place in conjunction with cardiac parapophysiological hypertrophic remodelling. As specified above, unlike all other laboratory parameters, the plasma profile of gelatinases is quite distinct between AH subjects and reference controls. This could be useful in evaluating the cardiovascular adaptability of athletes before they engage in strenuous exercise, so that high-risk subjects can be screened out. The addition of these remodelling markers to NT-proBNP and electrocardiographic and echocardiographic data could improve protocols for the evaluation of hypertrophic cardiomyopathy in athletes. Monitoring the plasma profile of remodelling markers over time may also be useful in studying the long-term consequences and significance of marked LV remodelling in AH. Finally, integrating echocardiographic imaging parameters with plasma matrix biomarkers could allow clinicians to identify the switching point between parapophysiological and pathological remodelling in athletes, although a larger prospective study will be needed to confirm this.

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REFERENCES


