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Gelatinases and physical exercise

A systematic review of evidence from human studies

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Abstract

Background: Matrix metalloproteinases (MMPs), particularly gelatinase A (MMP-2) and gelatinase B (MMP-9), as well as their tissue inhibitors (TIMP-1 and TIMP-2), are involved in the development of skeletal muscle tissue, in the repair process after muscle injury and in the adaptive modifications induced by physical exercise in skeletal muscle. This paper aims at reviewing results from human studies that investigated the role of gelatinases and their inhibitors in skeletal muscle response to acute physical exercise or training.

Methods: Electronic databases PubMed/MEDLINE, Scopus and Web of Science were searched for papers published between January 2000 and February 2017. The papers were eligible when reporting human studies in which MMP-2 and/or MMP-9 and/or the inhibitors TIMP-1/TIMP-2 were evaluated, in blood or muscular tissue, before and after acute physical exercise or before and after a period of structured physical training. We included studies on healthy subjects and patients with chronic metabolic diseases (obesity, diabetes mellitus, metabolic syndrome—MS) or asymptomatic coronary artery disease. We excluded studies on patients with neurological, rheumatologic or neoplastic diseases.

Results: Studies conducted on muscle biopsies showed an early stimulation of MMP-9 gene transcription as a result of acute exercise, whereas MMP-2 and TIMP transcription resulted from regular repetition of exercise over time. Studies on serum or plasma level of gelatinases and their inhibitors showed an early release of MMP-9 after acute exercise of sufficient intensity, while data on MMP-2 and TIMP were more contrasting. Most of the studies dealing with the effect of training indicated a trend toward reduction in blood gelatinase levels, once again more clear for MMP-9. This result was related to an anti-inflammatory effect of regular exercise and was more evident when training consisted of aerobic activities. This study has limitations: as the initial selection was done through titles and abstracts, incomplete retrieval cannot be excluded, as well as we cannot exclude bias due to selective reporting within studies.

Conclusion: A better knowledge of the molecular events activated by different types of acute exercise and regular training could be of great relevance in order to maximize the benefits of physical activity in healthy subjects and patients.

Abbreviations: CK = creatine kinase, ECM = extracellular matrix, ELISA = enzyme-linked immunosorbent assay, MMP = matrix metalloproteinase, MS = metabolic syndrome, MT-MMP = membrane-type matrix metalloproteinase, TIMP = tissue inhibitor of metalloproteinase, VO_2 = oxygen uptake, VO_2max = maximal oxygen consumption.

Keywords: aerobic fitness, matrix metalloproteinase, physical exercise, physical training, skeletal muscle tissue

1. Introduction

The effects of exercise on skeletal muscle have been extensively studied, but many biochemical aspects are not yet fully elucidated. A better knowledge of the mechanisms through which exercise modifies the structure and function of muscular tissue is of crucial importance not only to sports medicine but also as a guidance for exercise prescription in human diseases.

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Matrix metalloproteinases (MMPs) are a large and heterogeneous family of zinc-dependent endopeptidases whose activity basically consists in degrading extracellular matrix (ECM) proteins by cleavage of internal peptide bonds, although evidence is growing that also many intracellular molecules are potential substrates for MMPs.

MMPs are categorized by their structure or substrate, but MMPs belonging to different classes often share the same substrate specificities. The main MMP categories are collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs.[1,2] Collagenases, including MMP-1, -8, -13, and -18, cleave interstitial collagen I, II, and III at specific sites and other ECM and non-ECM molecules. Gelatinases A (MMP-2) and B (MMP-9), defined according to their affinity for denatured collagen (gelatin), are responsible for degradation of several types of collagen, in particular type IV collagen, a major component of basal lamina. Stromelysins include stromelysin 1 (MMP-3), stromelysin 2 (MMP-10), and stromelysin 3 (MMP-11). MMP-3 and -10 degrade collagen of several types, fibronectin, laminin, gelatins-I, whereas MMP-11, involved in cancer cell survival and spreading, has a less known substrate specificity.[2,3] Matrilysins, including matrilysin-1 (MMP-7) and matrilysin-2 (MMP-26), hydrolyze fibronectin, gelatins, and plasminogen. Membrane-type MMPs (MT-MMPs) include 4 transmembrane proteins (MMP-14, -15, -16, and -24) and 2 glycosylphosphatidylinositol

(GPI)-anchored proteins (MMP-17 and -25). They degrade type I, II, and III collagen and other components of ECM, and can activate pro-MMPs to MMPs. In fact, most MMPs are secreted as precursors (zymogens) and then activated in the extracellular space by several proteases, including plasmin and other MMPs.[2]

MMPs are produced by inflammatory cells and many other cell types, and their production is regulated by cytokines.[1,2] MMPs participate in several biological processes, such as cell migration, growth and differentiation, tissue remodeling, and angiogenesis. They can be considered mediators of inflammation and play a role in the pathophysiology of atherosclerosis, cancer, and several other human diseases.[1,2]

MMP activity is regulated by 4 tissue inhibitors of MMPs (TIMPs).[4] All the TIMPs are able to inhibit MMPs, but with different specificities. TIMP-1 affinity range is more restricted in comparison with the other TIMPs, but MMP-1, -3, -7, and -9 are inhibited effectively. Also the plasma protein α_2-macroglobulin is an inhibitor of MMPs.[2]

As regulators of ECM and cell function, MMPs participate in the adaptive modifications induced by physical exercise in skeletal muscle. Studies on myoblast migration and differentiation in vitro and on models of muscle injury and repair have demonstrated the involvement of MMPs and TIMPs, and especially the key role played by gelatinases, able to hydrolyze the components of the basal lamina surrounding the myofiber sarcolemma: type IV collagen, laminin, elastin, fibronectin. This activity contributes to maintenance of muscular fiber integrity, to muscle development and repair after injury.[5,6] Gelatinase A (MMP-2) is constitutively expressed on the surface of myofibers and fibroblasts in normal skeletal muscle, and can also be demonstrated within skeletal muscle fibers by immunochemistry.[7] It is inducible by stimuli to a lesser extent than other MMPs, even though its activity is enhanced in inflammatory conditions.[8] Gelatinase B (MMP-9) is secreted by endothelial cells, satellite cells, neutrophils, and monocyte macrophages. It is stored in intracellular secretory granules and is inducible by exogenous stimuli such as cytokines, growth factors, and altered cell–matrix contacts.[9] As regards gelatinase inhibition by TIMPs, the mechanisms are relatively specific: TIMP-1 forms a complex with the active form of MMP-9 while TIMP-2 interacts with pro-MMP-2. The latter interaction is also part of the activation process of pro-MMP, because it allows the binding of the complex MMP-2/TIMP-2 to MT-1-MMP (MMP-14), a key step in the generation of the active form of MMP-2. At higher levels of TIMP-2, pro-MMP-2 activation is prevented.[6]

Exercise is essential to maintain skeletal muscle homeostasis and is known to have beneficial effects on cardiovascular health. But in certain conditions, when the exercise intensity is very high and especially if repeated eccentric contractions are performed, muscle damage ensues. An acute exhausting exercise can induce the breaking of sarcomeres, cell death, infiltration of inflammatory cells, and enrolling of satellite cells to replace lost fibers.

Besides the type and intensity of exercise, other factors come into play to determine its effects on muscular tissue. The external conditions under which the exercise is performed, including room temperature and oxygen supply, can influence the degree of muscle damage. Another discriminating element is the training state of the subject who performs the exercise. Some evidence exists that not only sedentary subjects but also those who regularly practice concentric exercise are more susceptible to damage from an eccentric exercise than subjects who regularly perform eccentric exercise.[10] For the latter, the so-called "repeated bout effect" is observed.[11]

The aim of this study was a comprehensive review of data obtained in humans about the behavior of gelatinases and their inhibitors as an effect of acute exercise or physical training.

2. Methods

We searched the electronic databases PubMed/MEDLINE, Scopus and Web of Science by using the terms "matrix metalloproteinase" and "gelatinase" associated with "exercise," "resistance training," and "strength training." The period was 2000 to present and the last search was conducted in February 2017. After eliminating duplicates, a total of 327 papers were retrieved. Considering titles and abstracts, we selected those reporting human studies in which MMP-2 and/or MMP-9 and/or the inhibitors TIMP-1/TIMP-2 were evaluated, in blood or muscular tissue, before and after acute physical exercise or before and after a period of structured physical training. We included studies on healthy subjects or patients with chronic metabolic diseases (obesity, diabetes mellitus, metabolic syndrome—MS) or asymptomatic coronary artery disease. We excluded studies on patients with neurological, rheumatologic, or neoplastic diseases.

Some papers investigated the effect of antioxidants or other substances on the gelatinase response to physical exercise. Lack of significant effects in such studies might be cause of publication bias. For this reason and in order to stay focused on the main topic, we did not consider this type of studies.

The search and selection were performed separately by 2 of the authors, to keep inaccuracies to a minimum. At the end of the process, the papers eligible for qualitative review were 28.

As the initial selection was done through titles and abstracts, incomplete retrieval cannot be excluded, as well as we cannot exclude bias due to selective reporting within studies.

We were not able to perform any statistical analysis of the data reviewed, because of the extreme variability of the parameters investigated, techniques employed, and experimental conditions. Our review was only descriptive and qualitative.

Ethical approval was not necessary because the study did not involve patients.

We separately describe studies on muscular tissue, measurements of blood gelatinases before and after acute exercise and before and after physical training, respectively. Summary tables for each section are provided.

3. Results

3.1. Gelatinases and exercise: studies on muscular tissue

A few studies, summarized in Table 1, have explored the effect of exercise on MMP synthesis and activity in human muscle biopsies. All the studies included small numbers of subjects, which is a significant limitation to the relevance of results.

Gelatinase activity was evaluated by zymography on biopsies taken from the vastus lateralis of 9 healthy subjects, 3 days before and on day 4 and 22 after an exercise consisting in a single bout of 100 maximum voluntary eccentric contractions of the knee extensors.[12] This kind of exercise is known to cause muscle damage, which was confirmed in the study by several indices (muscle soreness, force decline and increase in serum creatine kinase—CK). Gelatinase activity did not change significantly postexercise, with a large variability in values.

In another study 10 healthy subjects performed a cycle incremental exercise until the highest sustainable intensity, and biopsies of the vastus lateralis muscle were taken at rest,

Table 1

Summary of the results from studies on the behavior of gelatinases and their inhibitors in muscle tissue after acute exercise or training.

	Subjects	Type of exercise	MMP-9	MMP-2	TIMP
Mackey et al[12]	9 healthy subjects (5 men and 4 women)	Eccentric exercise of the leg (100 repetitions)	↔ at 4 d and 22 d (activity)	↔ at 4 d and 22 d (activity)	
Rullman et al[13]	10 healthy men	Incremental cycling until the highest sustainable intensity	↑ at T0 and 2 h (mRNA and activity)	↔ at T0 and 2 h (mRNA and activity)	↔ TIMP-1 (mRNA)
Rullman et al[7]	10 healthy men	45 min 1-legged exercise at the highest tolerable workload, repeated 4 times a week for 5 wk	↑ at T0 after the first bout of exercise (mRNA and activity), ↑ at 10 d and 5 wk (mRNA only)	↔ at T0 after the first bout of exercise (mRNA and activity), ↑ at 10 d (mRNA and activity), ↔ at 5 wk (mRNA and activity)	↔ TIMP-1 at T0 after the first bout of exercise (mRNA), ↑ at 10 d TIMP-1 (mRNA)
Hoier et al[14]	14 healthy men	60 min cycling at 60–68% of VO_2max, repeated 3 times per week for 4 wk	↑ (mRNA) at 1 h and 3 h after both the first and the last exercise session	↔ (mRNA)	↑ TIMP-1 (mRNA) at 1 h and 3 h only after the last exercise session
Scheede-Bergdahl et al[15]	9 healthy men	8 wk training on a row ergometer (30 min every other day at 65–70% of VO_2 peak)		↑ at 2 wk and 8 wk (mRNA), ↔ at 8 wk (protein)	↑ TIMP-2 (mRNA) at 2 wk and 8 wk, ↔ at 8 wk (protein)
	12 male subjects with type 2 diabetes			↑ at 2 wk and 8 wk (mRNA), ↑ at 8 wk (protein)	↑ TIMP-2 (mRNA) at 2 wk and 8 wk, ↔ at 8 wk (protein)

↑ = increase, ↔ = no significant variation, T0 = immediately postexercise, 1 h–2 h–3 h = 1–2–3 h postexercise, 4 d–10 d–22 d = respectively 4–10–22 days postexercise, 2 wk–5 wk–8 wk = respectively 2–5–8 weeks postexercise, MMP = matrix metalloproteinase, TIMP = tissue inhibitor of metalloproteinase.

immediately and 120 minutes after the exercise.[13] MMP-9 mRNA and MMP-9 activity increased postexercise, while MMP-2 mRNA and MMP-2 activity did not change, neither did TIMP-1 mRNA. In a following study by the same authors,[7] gelatinase mRNAs and activities were studied on muscular biopsies before and after an acute exercise (45 minutes 1-legged exercise at the highest tolerable workload) and during a 5-week period in which the same exercise was regularly repeated 4 times per week. Whereas MMP-9 mRNA and activity increased significantly after the first bout of exercise and MMP-9 mRNA was still elevated after 10 days and 5 weeks, MMP-2 mRNA and activity did not change after the acute exercise and were elevated only after 10 days. The paper also reported a significant increase in TIMP-1 mRNA after 10 days.

Hoier et al[14] studied 14 young male subjects who were physically active but did not undergo any type of training; they exercised on a cycle ergometer for 60 minutes at 60% of the maximal oxygen consumption (VO_2max), 3 times per week for 4 weeks. Before and after the first pretraining and the last posttraining session MMP-2, MMP-9, and TIMP-1 mRNA were determined on biopsies from vastus lateralis muscle. No difference was observed in mRNA basal levels for any of the molecules between pre- and posttraining evaluation. MMP-9 mRNA increased significantly after acute exercise on both occasions, whereas TIMP-1 mRNA rose only after acute exercise performed posttraining and MMP-2 mRNA did not change on any occasion.

Scheede-Bergdahl et al[15] investigated the effect of training on gelatinase transcription and protein concentration in muscle biopsies from 12 type 2 diabetic patients and 9 healthy control subjects, who exercised on a rowing ergometer, for 30 minutes every other day at 65% to 70% of VO_2 peak, for 8 weeks. Before training there was no difference between diabetic subjects and controls in active MMP-2 and TIMP-2 concentrations or mRNA levels. MMP-2 and TIMP-2 mRNA increased as an effect of training, without differences between diabetic patients and controls. After training active MMP-2 was higher than at baseline only in diabetic patients, but no change in TIMP-2 concentrations was observed in any group. MMP-9 was not investigated.

Taken together, these results pose a challenge to interpretation, mainly because 3 different aspects are investigated in different studies: gene transcription, protein concentration, and enzyme activity. Focusing on mRNA evaluation, MMP-9 gene transcription appears as an early event after acute exercise,[7,13,14] whereas MMP-2 transcription seems to be a delayed effect of exercise repetition.[7,15] Similarly, TIMP gene transcription seems to result from training rather than from acute exercise.[7,14,15] As regards the site of MMP-9 gene transcription within the muscular tissue, the cells likely to be involved are endothelial and satellite cells, while MMP-2 is synthesized by muscular fibers.[7]

The only study in which type 2 diabetics, at an early stage of the disease, were studied and compared to healthy controls showed that the adaptive response of skeletal muscle to exercise was preserved.[15]

3.2. Circulating gelatinases and acute exercise

The results described in this section are summarized in Table 2. Circulating gelatinase concentrations were measured after acute exercise mostly in healthy volunteers, with males greatly outnumbering females. The studies considerably differed from each other in the type and intensity of exercise, time of sampling and features of the enrolled subjects, which can partly explain the variability of results. The measurement of gelatinase concentration was performed on serum in the majority of the studies. There is evidence that determination on serum, compared to plasma, tends to overestimate gelatinase concentrations, because of the release from leukocytes and platelets during clot formation.[16] Although a significant correlation was observed between MMP levels in plasma and serum,[17] the most recent studies recommend the use of plasma samples.[18,19] The techniques employed by different researchers included enzyme-linked immunosorbent assay (ELISA) or others immunological methods. In a few studies gelatinase activity was evaluated by zymography.

The most commonly observed effect of a single bout of exercise was an increase in MMP-9 level. When eccentric exercise of the lower limbs (downhill running) was performed by both sedentary and active subjects, MMP-9 increased early[18,20] and the elevation persisted after 4 days.[18] In other studies eccentric exercise of the leg was followed by a MMP-9 increase only after 8 days[12] or by an early rise only when the exercise was performed at low temperature (5°C), a condition imposing further stress on

Table 2

Summary of the results from studies on the behavior of blood gelatinases and their inhibitors after acute exercise.

	Subjects	Type of exercise	MMP-9	MMP-2	TIMP
Koskinen et al[21]	14 healthy men	45 min downhill running at 5 and 22°C	↑ at T0 (5°C), ↔ (22°C)	↔	TIMP-1 ↑ at T0 (22°C), TIMP-2 ↑ at 7 d (5°C)
Mackey et al[12]	9 healthy subjects (5 men and 4 women)	Eccentric exercise of the leg (100 repetitions)	↑ at 8 d		TIMP-1 ↑ at 1 d, 2 d, 3 d, 4 d, 14 d TIMP-2 ↔
Tayebjee et al[32]	20 healthy subjects (gender not specified)	Maximal incremental test on treadmill	↔ at T0	↔ at T0	TIMP-1 ↑ at T0, TIMP-2 ↑ at T0
Saenz et al[33]	30 male and female nonelite runners	Marathon	↑ at T0		
Suhr et al[25]	12 male cyclists with different performance backgrounds	90 min cycling at different intensities	↑ at 1 h and 4 h	↑ at T0	
Urso et al[23]	16 recreationally active healthy men	Resistance exercise (60 repetitions)	↑ at 30 min	↔	
Suhr et al[30]	13 male highly trained runners	Maximal incremental test on treadmill	↑ at T0 in short-track runners, ↔ in long-track runners	↑ at 1 h in long-track runners, ↔ in short-track runners	
Madden et al[22]	14 sedentary men	Eccentric exercise of the arm (60 repetitions)	↔ at T0, 1 d, 2 d, 4 d, 7 d (concentration and activity)		TIMP-1 ↔ at T0, 1 d, 2 d, 4 d, 7 d
Nourshahi et al[28]	15 men and 15 women	1-h cycling at submaximal intensity	↔ at T0 and 2 h	↓ at T0, ↔ at 2 h	
Reihmane et al[31]	26 professional male ice hockey players	Maximal exercise on a cycloergometer	↑ at T0		
Reihmane et al[34]	22 half-marathon and 18 marathon male amateur runners	Half-marathon marathon	↑ at T0		
Rullman et al[27]	10 healthy men	60 min cycling	↑ at 27 min, 57 min, and 2 h (concentration) ↑ at 27 min (activity)		
Ross et al[24]	13 trained men	Endurance-resistance exercise	↑ at T0 and 2 h	↑ at T0 and 2 h	
Welsh et al[18]	12 sedentary subjects (4 males, 8 females); 9 "concentrically active" subjects (4 males, 5 females)	Downhill running (30 min)	↑ at T0 and 4 d		TIMP-1 ↔
Rocha et al[29]	9 healthy subjects (6 men, 3 women)	40 min cycling at submaximal intensity	↔ at T0 (concentration and activity)	↑ at T0 (activity)	
	15 subjects with early metabolic syndrome (12 men, 3 women)		↔ at T0 (concentration), ↑ at T0 (activity)	↔ at T0 (activity)	
van de Vyver et al[20]	12 recreationally active healthy men	Downhill running (60 min)	↑ at T0 and 4 h		
Nascimento Dda et al[36]	10 elderly obese women	Eccentric exercise	↓ at T0, 3 h, 1 d, 2 d (activity)	↓ at T0 and 1 d (activity)	
Schild et al[26]	19 endurance exercise trained men	50 min cycling at 80% of VO_2max	↑ at T0 and 3 h	↑ at T0 and 3 h	TIMP-1 ↑ at T0, ↔ at 3 h
	17 sedentary men		↑ at T0 and 3 h	↔ at T0 and 3 h	TIMP-1 ↑ at T0, ↔ at 3 h

↑ = increase, ↓ = decrease, ↔ = no significant variation, T0 = immediately or within 15 min postexercise, 27 min–30 min–57 min = respectively 27–30–57 min postexercise, 1 h–2 h–4 h = respectively 1–2–4 h postexercise, 1 d–2 d–3 d–4 d–7 d–14 d = respectively 1–2–3–4–7–14 days postexercise, MMP = matrix metalloproteinase, TIMP = tissue inhibitor of metalloproteinase.
Data on gelatinase and inhibitors refer to protein concentration in plasma or serum unless otherwise specified.

muscles.[21] Eccentric exercise of the arms did not elicit any significant variation in MMP serum concentration or activity during a 7 days' follow-up in sedentary men.[22]

A significantly higher serum concentration of MMP-9 was detected after acute resistance exercise in groups of trained men, after 30 minutes[23] and after 10 minutes and 2 hours.[24] In the latter study, the increase was no longer detectable after 24 hours.

In trained men a 90 minutes cycling session, consisting of alternate high-load bouts (at 80–85% of VO_2max) and brief recovery intervals (at 55–60% of VO_2max), caused a rise in MMP-9 level, already significant after 1 hour and peaking at 4 hours, when follow-up was discontinued.[25] Both in endurance exercise trained and in sedentary men 50 minutes cycling at 80% of VO_2max caused a rise of MMP-9 immediately and 3 hours after the exercise was completed.[26] A 1-hour cycling, consisting of 20 minutes at 50% and 40 minutes at 65% of VO_2max, induced an increase in MMP-9 concentration already detectable halfway during exercise and persisting 2 hours postexercise.[27] A 1-hour cycling session at submaximal intensity (70% of VO_2max), performed by 50 healthy subjects, did not cause any variation in MMP-9 level[28]; 40 minutes cycling at submaximal intensity had no effect on MMP-9 concentration either in healthy subjects or in subjects with early MS.[29] In the same study MMP-9 activity rose postexercise only in MS subjects.

A maximal incremental test on treadmill or cycloergometer induced an immediate MMP-9 rise in short-track elite runners[30] and in professional ice hockey players[31]; it was unable to induce any change in long-track elite runners[30] and in a group of healthy volunteers not specified as active or sedentary.[32] No study explored the effect of a maximal incremental test later than 1 hour after the exercise was completed.

After a marathon or half-marathon race, a huge increase in MMP-9 blood level was demonstrated.[33,34] When half-marathon was compared to marathon, the rise in MMP-9 was significantly higher after marathon; the concentrations were back to the prerace levels 28 hours later.[34]

The rise in MMP-9 following an acute exercise has been related to muscle damage, and thus a possible relation between MMP-9 response and markers of muscle damage has been investigated.

Muscle damage causes soreness, whose quantitative assessment, even if made with the best accuracy, is too prone to

subjectivity; loss of muscle strength can be more reliable, but blood biochemical markers should be the most useful objective indices. Among the blood markers, CK is the most commonly used, but its levels show a great interindividual variation and the interpretation of exercise-related changes is still under discussion.[35]

Serum CK levels were assessed alongside gelatinases in healthy subjects performing eccentric exercise[12,18,21,22] or strenuous endurance exercise.[34] In these studies, with only 1 exception,[22] MMP-9 rose after exercise, but in no case with a pattern parallel to the CK increase. When the correlation between MMP-9 and CK was evaluated, no statistical significance emerged.[21] Overall, these data suggest that MMP-9 is not a good quantitative indicator of muscle damage.

An early stimulation of MMP-9 gene transcription was demonstrated by studies on muscle biopsies,[7,13,14] but the rise in MMP-9 blood level detected immediately after exercise is attributable to the release of presynthesized molecules. Leukocytes are known to accumulate MMP-9 in their secretory granules, so they are the most likely source of the MMP-9 rise. Admitting this, it is not clear where the release takes place and which leukocyte subtype is responsible for it. In the study by Rullman et al[27] blood was drawn from femoral artery and vein simultaneously in subjects performing a 60 minutes cycle exercise. MMP-9 concentration increased significantly during and after exercise but with no difference between artery and vein, which seemed to rule out a release from exercising muscle. At 27 minutes into the exercise bout there was an increase also in MMP-9 activity. In the same study, elastase and neutrophil-associated lipocalin were measured as markers of neutrophil degranulation, but no significant change was demonstrated in their levels, leaving mononuclear cells as the most plausible candidate for MMP-9 release induced by exercise.

In some of the studies previously described, MMP-2 concentration in blood was measured along with that of MMP-9. It was significantly increased after 90 minutes cycling,[25] after a maximal incremental test on treadmill in long-track elite runners[30] and after a strenuous resistance exercise.[24] When both endurance exercise trained men and sedentary men performed 50 minutes cycling at 80% of VO_2max, plasma MMP-2 level increased only in trained subjects.[26] In all these studies the MMP-2 rise was detected soon after the exercise or within 3 hours. When the follow-up was prolonged until 24 hours, the increase was not detectable any more.[24] MMP-2 did not change after various types of exercise in other studies,[21,23,32] the longest follow-up being of 7 days after downhill running.[21] After 1-hour cycling at submaximal intensity (70% of VO_2max), MMP-2 transiently decreased but was back to baseline levels at 2 hours.[28] In the study by Rocha et al[29] serum MMP-2 activity was measured in 9 healthy subjects and 12 subjects with early MS before and after 40 minutes cycling at submaximal intensity; it was increased after exercise only in healthy subjects.

The few studies in which blood gelatinase activity was evaluated along with gelatinase concentration[22,27,29] have not always provided evidence of a parallel trend. A recent study conducted in obese elderly women addressed the effect of an acute eccentric exercise only on plasma gelatinase activity.[36] Rather surprisingly, there was a reduction in MMP-2 and -9 activity immediately after the exercise, which persisted up to 48 hours. Discussing this finding, the authors mention the state of low-grade inflammation associated with obesity and old age that may be responsible for chronically enhanced gelatinase activity in comparison with healthy young subjects. However, the mechanism by which a single bout of eccentric exercise can promptly blunt the inflammatory state detectable in blood remains obscure. As regards studies in healthy individuals, only the paper by Nourshahi et al[28] reported a transient decrease in MMP-2 serum concentration (without changes in MMP-9) after acute exercise, but the experimental conditions were too different from the study by Nascimento Dda et al[36] to suggest a unifying hypothesis.

Even fewer and less enlightening are the data on TIMP concentration in circulating blood after exercise. TIMP-1 transiently rose in serum soon after downhill running at room temperature, in experimental conditions that did not induce any change in gelatinase levels.[21] TIMP-1 exhibited an early and prolonged increase after eccentric exercise of the leg, a test followed by a MMP-9 rise only after 8 days[12]; TIMP-1 rose soon after a maximal incremental test on treadmill that did not cause changes in MMP-9 or MMP-2 level.[32] In the latter study an early increase of TIMP-2 was observed as well, while downhill running at low temperature induced, besides an early increase in MMP-9, a rise in TIMP-2 after 7 days.[21] More recent studies failed to detect changes in TIMP-1 concentrations after eccentric exercise of the arm[22] or downhill running,[18] but 50 minutes cycling at 80% of VO_2max induced an early increase of TIMP-1, not detectable after 3 hours, in both trained and sedentary men.[26]

3.3. Circulating gelatinases and regular physical activity

The results described in this section are summarized in Table 3. The studies previously described, focused on the effects of a single bout of exercise on gelatinase levels, did not evaluate the effects of training, with 2 exceptions. Urso et al[23] studied gelatinase levels before and after 2 types of 8-week training: callisthenic (based on body weight exercises and progressive distance running) and resistance (based on machine exercises and march with progressive load carriage). The first type was followed by a reduction in MMP-2 levels with no variation of MMP-9, while the resistance training caused a significant increase in MMP-9. Suhr et al[30] studied a group of elite runners before and after the 6-month training season, without observing variations in the baseline levels of MMP-2 and -9.

A group of veteran marathoners with athlete's heart showed low levels of MMP-2 and -9 in comparison with a group of sedentary healthy controls, without any change in TIMP-1 and -2 concentrations.[37] However, this study did not meet the inclusion criteria for this review because the subjects were not studied before and after a training period, so its results are not shown in Table 3.

The effect of regular physical activity has been explored mainly in patients affected by or at risk for metabolic and cardiovascular diseases.

The effect of training on gelatinase plasma or serum concentrations was studied in old sedentary women,[38] in men with MS factors,[39] in subjects with asymptomatic coronary artery disease or cardiovascular risk factors[40] and in patients with type 2 diabetes mellitus.[41] In these studies training consisted in walking or running, except for elderly women,[38] who performed exercises aiming at increasing synchronization, dexterity, flexibility, strength, and steadiness. The training programs considerably differed in the frequency and intensity of exercise, as well as in the overall duration, ranging from 3 to 24 weeks. Nonetheless, all the programs were effective in lowering the blood concentrations of MMP-9; MMP-2 was evaluated in the group of diabetic patients but showed no variations.[41] In the same study TIMP-2 concentration rose after training whereas TIMP-1 did not change.[41]

Table 3

Summary of the results from studies on the behavior of blood gelatinases and their inhibitors before and after physical training.

	Subjects	Type of training	MMP-9	MMP-2	TIMP
Urso et al[23]	8 recreationally active healthy men	8 wk callisthenic training	↔	↓	
	8 recreationally active healthy men	8 wk resistance training	↑	↔	
Suhr et al[30]	13 male highly trained runners	6-mo training season	↔	↔	
Fiotti et al[38]	17 old sedentary women	24 wk program (1 h twice a week of exercise aiming at the increase in synchronization, dexterity, flexibility, strength and steadiness)	↓		
Roberts et al[39]	31 men with metabolic syndrome factors	3 wk training (daily treadmill walking for 45–60 min)	↓		
Niessner et al[40]	32 subjects with asymptomatic CAD or at risk for CAD	12 wk training (at least 30 min endurance running 3 times per week)	↓		
Kadoglou et al[41]	25 overweight subjects with uncomplicated type 2 diabetes mellitus	16 wk training (self-controlled exercise for at least 150 min per week and 1 additional supervised session per week)	↓	↔	TIMP-1 ↔, TIMP-2 ↑
Büyükyazı et al[42]	24 postmenopausal women	8-wk training (moderate-intensity walking for at least 30 min 5 days per week)	↔		TIMP-1 ↔
Lucotti et al[43]	27 obese type 2 diabetics	3 wk aerobic supervised training (30 min bid 5 days per week)		↓	
	20 obese type 2 diabetics	3 wk aerobic/anaerobic supervised training (45 min bid 5 days per week)		↑	

↑ = increase, ↓ = decrease, ↔ = no significant variation, CAD = coronary artery disease, MMP = matrix metalloproteinase, TIMP = tissue inhibitor of metalloproteinase.

In a group of healthy postmenopausal women a walking program at 60% to 65% of the heart rate reserve 5 days per week for 8 weeks, failed to induce changes in MMP-9 and TIMP-1 levels, maybe because intensity and duration were too low.[42]

In the studies described so far the decrease in MMP-9 concentration was paralleled by a reduction of several inflammation markers, including C-reactive protein,[39–41] fibrinogen,[41] soluble intracellular adhesion molecule-1, soluble P-selectin, and macrophage inflammatory protein-1α.[39] MMP-9 decreased also when body weight did not change significantly during the study.[41]

A study by Lucotti et al[43] compared 2 types of 3-week training, including respectively only aerobic exercise or aerobic exercise with a supplement of resistance exercises, in obese type 2 diabetic patients. Both training programs were associated with diet treatment and followed by reductions in body weight and body mass index. MMP-2 concentration decreased after aerobic training but increased after aerobic/resistance training; the same trend was observed for tumor necrosis factor alpha and monocyte chemoattractant protein-1. The results led the authors to conclude that, unlike aerobic exercise, resistance exercise had an adverse impact on the inflammatory state.

Despite differences in experimental setting, a constant down-regulation of circulating gelatinase levels has emerged as an effect of aerobic training. Most data regarded MMP-9, and were consistent with a general antiinflammatory action of aerobic exercise. The few data available about resistance exercise suggest an opposite effect on gelatinase concentrations.[23,43]

3.4. Gelatinases as mediators of muscle response to exercise

The attempts to acquire information about the response of the gelatinase system to physical exercise from blood testing have not provided a coherent picture until now. In particular, whereas MMP-9 rise seems to be part of a systemic reaction to acute strenuous exercise and is readily detected in blood after acute exercise, MMP-2 expression, which is delayed and more specifically involved in muscular tissue adaptation to work, is more difficult to assess through its measurement in serum or plasma samples. The complex regulation of gelatinase activity,[1] involving pre- and posttranslational steps, imposes additional difficulties.

An aspect of gelatinase response is its dependency on exercise intensity. As regards MMP-9 detection in blood after an acute exercise, some studies suggest that the exercise must have a minimum intensity: cycling at submaximal intensity may not be effective.[28,29] Moreover, when marathon and half-marathon runners were compared,[34] the MMP-9 rise after the race was higher in the former.

As regards MMP-2, useful insights can be found in animal studies, in which the experimental conditions can be more controlled, even though the extrapolation of results to human physiology and pathophysiology must be cautious. In female rats a 2-weeks' training of high-intensity exercise (treadmill running at 70–75% of VO_2max) induced a significantly higher expression of both MMP-2 mRNA and protein in skeletal muscles in comparison with low-intensity exercise (40% of VO_2max).[44] In male rats a high-intensity resistance training (climbing on ladders with an increasing external load) was followed by a significant rise of the active form of MMP-2, while in rats performing the same exercise with no external load only the proform of the enzyme increased.[45] In the same study, MMP-2 activity was directly and strongly correlated to blood lactate increase.[45]

Another key point is the MMP role in angiogenesis induced by exercise. The development of new capillaries within the muscle tissue is stimulated not only by endurance exercise, but also by resistance exercise, whose main effect is muscle fiber hypertrophy.[24] Mechanical overload of muscle tissue, increased shear stress on vessel wall and local ischemia are stimuli able to trigger the outgrowth of new capillaries from the existing vessels. Both MMP-2 and MMP-9 are involved in the processes of angiogenesis,[46] including proteolysis of the capillary basement membrane, ECM degradation allowing the endothelial cell sprouts to migrate into the interstitium, and liberation of proangiogenic and antiangiogenic factors from ECM.

Some of the studies previously described have evaluated angiogenic factors along with MMP expression after acute exercise, in blood[24,25] and muscular tissue[7,13] of healthy men with different levels of training. The influence of mechanical

stimulation[25] and variously provoked hypoxia[7,25] was also investigated. Despite the heterogeneity in experimental conditions, most of the evidence points to a coordinated response of multiple cell types and organs involving MMP synthesis and activation, release of angiogenic factors such as the vascular endothelial growth factor and endostatin, and the mobilization of endothelial progenitor cells from bone marrow.[24]

Besides being crucial to improved muscle performance promoted by training, neoangiogenesis is a beneficial effect induced by regular exercise in patients with arterial hypertension[47] and metabolic diseases.[48] The mechanisms leading to angiogenesis, however, can be impaired by the clinical condition itself. The adaptive response of skeletal muscle to exercise, mediated by MMP-2 expression, was preserved in a group of type 2 diabetics at an early stage of the disease.[15] In another study,[29] when patients with a recent diagnosis of MS and healthy controls performed a cardiopulmonary test, exercise was followed by a rise in serum MMP-9 activity only in MS patients and by a rise in MMP-2 activity only in healthy subjects. Oversimplifying a complex situation, MMP-9 essentially behaves as a marker of inflammation, MMP-2 as the marker of a homeostatic process put in motion.

4. Conclusions

Gelatinase levels in systemic blood reflect the balance between their release from many cell types into the bloodstream and their removal from blood; little is known about the 2 processes, their homeostatic regulation and their derangement in pathological conditions. Muscle tissue is not the only potential source of blood gelatinases after acute exercise, but gelatinase measurement in blood can be all the same useful to detect the systemic response to exercise. A better standardization of exercise conditions and laboratory methods would make interpretation of results much easier.

A single bout of exercise and a regular training appear to have opposite effects on blood MMP-9 levels. On the one hand, acute intense exercise seems to elicit a reaction by inflammatory cells, leading to an increase in circulating MMP-9. On the other hand, regular aerobic training shows an antiinflammatory effect, signaled by a decrease in MMP-9. However, many studies on gelatinases after acute exercise were focused on eccentric exercise, with the aim of testing MMP-9 role as a marker of muscle damage, but the antiinflammatory effect of training was demonstrated only when aerobic exercise was performed regularly. Further research is needed to clarify the chain of events leading from the effects of different types of acute exercise to those of regular training. More attention should be addressed to the role of MMP-2 and TIMPs, and particularly to the balance between each gelatinase and its inhibitor. A better knowledge of the role played by the gelatinase system can be useful to maximize the benefits of exercise in prevention and therapy.

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