Plasma adenosine triphosphate and heat shock protein 72 concentrations after aerobic and eccentric exercise.

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ABSTRACT

The endolysosome pathway has been proposed for secretion of heat shock protein (Hsp)72 with a regulatory role for extracellular adenosine triphosphate (ATP). Here, we tested the hypothesis that extracellular ATP mediates the increase in plasma Hsp72 after exercise. We measured plasma ATP, Hsp72, cathepsin D, norepinephrine, free fatty acid, glucose, and myoglobin in 8 healthy young males (mean±SE: age, 22.3±0.3 years; height, 171.4±0.8 cm; weight, 68.8±3.1 kg; body mass index, 23.5±1.1 kg/cm2; VO^2 max, 44.1±3.8 mL/kg/min) before and at 0, 10, 30, and 60 min after aerobic exercise (cycling) and elbow flexor eccentric exercise. Subjects cycled for 60 min at 70-75% VO_2 max (mean±SE; 157.4±6.9 W). Eccentric strength exercise consisted of flexing the elbow joint to 90° with motion speed set at 30°/sec at extension and 10°/sec at flexion. Subjects performed 7 sets of 10 eccentric actions with a set interval of 60 sec. The motion range of the elbow joint was 90°-180°. Compared with the levels of Hsp72 and ATP in plasma after bicycle exercise, those after eccentric exercise did not change. A significant group × time interaction was not observed for Hsp72 or ATP in plasma. A significant correlation was found between Hsp72 and ATP in plasma (r=0.79, P<0.05), but not between Hsp72 and norepinephrine (r=0.64, P=0.09) after bicycle exercise. A significant correlation between ATP and norepinephrine in plasma was found (r=0.89 P<0.01). We used stepwise multiple-regression analysis to determine independent predictors of exercise-induced elevation of eHsp72. Candidate predictor variables for the stepwise multiple-regression analysis were time (Pre, Post, Post10, Post30, Post60), exercise type (aerobic, eccentric), ATP, cathe-

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psin D, norepinephrine, epinephrine, glucose, and FFA. In the regression model for Hsp72 in plasma, increased ATP and glucose were the strongest predictors of increased Hsp72 (ATP: R^2=0.213, β=0.473, P=0.000; ATP and glucose: R^2=0.263, β=0.534, P=0.000). Collectively, these results imply that ATP in plasma is a trigger of Hsp72 release after exercise.

Key words: Endolysosome, ABC-family transporter, Cathepsin D

INTRODUCTION

Heat shock proteins (Hsp) are highly conserved proteins that are expressed both constitutively and under stressful conditions. In particular, those in the 70-kDa family are released from various cell types, including glia cells (22), human peripheral blood mononuclear cells (14), and cancer cells (33), after *in vitro* challenge with cytokines or heat stress; and also from human brain (29), leukocytes (15), and hepatosplanchnic tissue (13) during and/or after exercise. Walsh et al. first described an exercise-induced increase of extracellular Hsp (eHsp)72 (50). Subsequently, other exercise-related studies have shown that the concentration of Hsp72 in serum or plasma (i.e. eHsp72) is dependent on the duration and intensity of exercise (17), that eHsp72 elevation is accompanied by parallel increases in cytokine levels (51) and in biomarkers for oxidative stress (16), and that a specific vitamin E isoform attenuates the exercise-induced increase of eHsp72 (18, 39). It is clear that extracellular Hsps can play a role as pro-inflammatory immune effectors (10, 36). However, it is unclear whether eHsp72 plays a role as a pro-inflammatory mediator or for chaperoning proteins to prevent aggregation or proteolysis of damaged proteins due to exercise.

The mechanism of excretion out of possible intracellular storage sites is controversial. Recent work from several groups has suggested that Hsps are released by both passive (necrotic) and active mechanisms (3, 43). During exercise, the release of Hsp72 from damaged cells only partially contributes to circulating eHsp72. A comparative study between endurance exercise of different intensities and durations revealed that bouts of running with the highest eHsp72 levels in plasma were associated with the most pronounced creatine kinase concentrations, a prominent marker of tissue damage (24). On the other hand, release from injured tissue can largely be excluded because eHsp72 increases after exercise even in the absence of enhanced plasma creatine kinase levels (30). Moreover, despite missing signs of liver cell damage, hepatosplanchnic release of Hsp72 has been measured after exercise (13). At present, active secretory processes, rather than passive release due to cell damage, are considered to be responsible for Hsp72 release during exercise (30).

The classical pathway can be excluded because Hsp72 lacks a peptide leader sequence that targets the protein for secretion (8). Active secretion via exosomes and lipid rafts may be an alternative secretory mechanism (6, 8). Inhibition of Hsp72 release from peripheral blood mononuclear cells (PBMCs) by monensin (Na^+ ionophore), methyl-β-cyclodextrin (disrupts membrane rafts), or methylamine (inhibits endocytosis) suggests that Hsp72 is transported via the Golgi region into lysosomal lipid rafts prior to exocytosis (6). In the non-classical protein

transport pathway, lipid rafts are specialized membrane microdomains that are formed within the exoplasmic leaflet of the Golgi membrane, and may play a role in Hsp72 exocytosis (6). However, the effect is controversial due to cytotoxicity.

Exosome-mediated Hsp72 secretion is also a potential mechanism in the exercise-induced eHsp72 response. Accumulated intracellular Hsp72 in the leukocytes or other tissues due to exercise may be actively secreted through exosomes into circulation. Hsp72 release from whole blood cells and isolated PBMCs (31) and an increase of exosomal Hsp72 content in PBMCs after experimental heat shock (31) have been found. Exosomes, which are small membrane vesicles secreted by various cell types, including B cells (9), T cells (5), dendritic cells (44), mast cells (46), epithelial cells (49), and PBMCs (31), may provide a secretory pathway allowing cells to actively release specific Hsps. Lancaster *et al.* demonstrated that exosomes gradually increase in both culture medium (RPMI 1640, 0% fetal bovine serum) and PBMC cell cultures under basal incubation (37°C) in a time-dependent manner, and concomitantly the Hsp70 content of exosomes increases, but not significantly (31). Bausero *et al.* suggested that Hsp72 is released within the exosomes via a non-classical protein transport pathway in an intracellular calcium-dependent fashion (4), but not due to extracellular calcium.

Recent studies have implicated the endolysosome pathway for secretion of Hsp72 (35). Hsp72 secretion involves the entry of Hsp72 into endolysosomes through adenosine triphosphate (ATP)-binding cassette (ABC)-family transporters, where they co-localize with intravesicular cathepsin D. These organelles are then transported to the cell surface. Subsequent fusion of Hsp72 containing endolysosomes with the cell surface results in the localization of the lysosomal marker, i.e., lysosomal-associated membrane protein (LAMP) 1 in the plasma membrane and release of Hsp72 along with other protein such as cathepsin D. Although the cell signals involved in triggering stress-induced Hsp72 release through this lysosomal pathway are unknown, recent data suggests a regulatory role for extracellular ATP (34).

The type of exercise strongly influences the increase in Hsp72 in blood. For instance, in aerobic exercises, such as treadmill running, serum Hsp72 increases several fold both during and after the exercise (50). In contrast, eccentric exercises such as elbow flexion, do not induce an increase in eHsp72 (24). However, downhill running has been shown to increase eHsp72 (42). This difference in Hsp72 levels is seen despite both aerobic and eccentric exercises inducing muscle damages. This may be explained by the lysosome mechanism. Extracellular ATP regulates Hsp72 release from ABC-family transporters, and, thus, muscle damage does not contribute to increased eHsp72; instead, eHsp72 increases with extracellular ATP. Therefore, we presently tested the hypothesis that extracellular ATP mediates the increase in plasma Hsp72 after exercise.

METHODS

Subjects

Eight healthy untrained male subjects (mean±SE: age, 22.3±0.3 years; height, 171.4±0.8 cm; weight, 68.8±3.1 kg; body mass index, 23.5±1.1 kg/cm^2; VO_2 max, 44.1±3.8 mL/kg/min) participated in the study. None of the subjects per-

formed strenuous exercise for at least one week before the experiment. All subjects were informed of the purpose and risks of the study before giving written informed consent. This study was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by the Ethics Committee at Tokyo Metropolitan Institute of Gerontology.

Experimental protocol

Preliminary tests

Maximal oxygen uptake (VO_2 max) test was carried out one week before to determine the workload required to elicit 70% VO_2 max. The graded maximal exercise test involved four 5-min bouts of exercise on an electronically braked cycle ergometer (Lode Excalibur, Gronigen, Netherlands). Pedal cadence was maintained at 60 revolutions/min and expired gasses were measured continuously using an automated mass spectrometer for respiratory analysis system (Arco systems, Chiba, Japan). A continuous, incremental cycling test to volitional exhaustion was performed. The initial workload was set at 50 W, with work rate increasing by 50 W every 4 min until 200 W, and by 10 W every 1 min until exhaustion. Expired gases were measured continuously to derive VO_2 max.

Aerobic exercise tests

All subjects cycled for 60 min at 70% VO_2 max (mean±SE: 157.4±6.9 W) in warm conditions (ambient temperature, 24-25°C; relative humidity, 45%). The subjects reported to the laboratory, then they rested in a sitting position for 30 min, and had blood samples taken (Pre). Subjects were then moved to the cycle ergometer and commenced exercise. There was a 3- to 5-min warm-up period of cycling at 30-45% of VO_2 max, immediately followed by 60 min at 70-75% of VO_2 max in warm conditions. The subjects then had a 60-min rest recovery phase in warm conditions after exercise. Blood samples were obtained immediately after the exercise (post) and at 10, 30, and 60 min after exercise. The subjects were permitted to drink a maximum of 400 mL of commercial bottled water during exercise testing.

Eccentric exercise

All subjects participated in a second trial. On arrival at the laboratory, the subjects rested in a sitting position for 30 min, and had blood samples taken (Pre). Subjects were then moved and placed on an isokinetic machine (Biodex Multi-Joint System 3, Biodex Medical Systems; Shirley, NY, USA). The elbow joint angle was flexed to 90° and compulsory eccentric strength was loaded, with motion speed set at 30°/sec at extension and 10°/sec at flexion. Subjects performed 7 sets of 10 eccentric actions with a set interval of 60 sec. The motion range of the elbow joint was 90°-180°. The subjects then had a 60-min recovery phase in warm conditions after exercise. Blood samples were obtained immediately after the exercise (post) and at 10, 30, and 60 min after exercise.

All exercise bouts including preliminary testing were performed between 09:00 and 15:00. The trials were separated by at least 1 week to ensure complete recovery between trials. Except for the last 48 h before each trial, when exercise was regulated by the study protocol, the subjects completed their regular training program and usual daily activities during the study period. During the study peri-

od, the subjects maintained their normal diet, but their food intake was limited for 2 h before exercise testing. All subjects wore similar uniforms during exercise testing.

Blood sampling and analysis

For analysis of eHsp72, cathepsin D, and ATP, whole blood was placed in a tube containing 30 μl of EDTA and spun at 1000 × *g* at 4°C for 10 min, and the supernatant was stored at -80°C until analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the plasma concentrations of Hsp72 (Stressgen Biotechnologies Co.; Victoria, BC, Canada), cortisol (Immuno-Biological Laboratories Co. Ltd.; Tokyo, Japan), and IL-6 (R&D Systems; Minneapolis, MN, USA). Cathepsin D activity was quantified with a Cathepsin D Assay kit (Fluorimetric) (AnaSpec; San Jose, CA, USA). Briefly, after 5-FAM fluorescence reference standards and samples were simultaneously incubated at 37°C for 10 min, 50 μL of the fluorogenic peptide 5-FAM/QXL^{TM} 520 was added as a substrate. After mixing the reagents completely by shaking the plate gently for 30 sec, measurements of lysis (unquenched MCA peptide) were obtained with a microtiter plate fluorometer (SpectraMax Gemini XS; Molecular Devices, Sunnyvale, CA, USA; excitation: 490 nm; emission: 520 nm). Activity values were expressed in relative fluorescence units. ATP in plasma samples was determined using the luciferin-luciferase technique. Briefly, plasma was diluted 1 part in 100 in sterile, doubly distilled water. Diluted plasma was then assayed immediately using a commercially available firefly luminescent assay kit (BA100, Toyo Bnet, Tokyo, Japan) using an internal standard procedure. All samples were assayed in duplicate. The coefficient of variation of 9 duplicate resting plasma samples was 7%. Norepinephrine was measured using high-performance liquid chromatography. The plasma concentrations of free fatty acid (FFA) and glucose were measured using an immunoenzyme technique and UV hexokinase technique, respectively, and the serum concentration of myoglobin was measured using a radio immunoassay technique (SRL Co.; Tokyo, Japan).

Statistics

A statistics software package was used for all statistical calculations (SPSS ver.17; Tokyo, Japan). We compared the plasma concentrations of eHsp72, ATP, cathepsin D, and norepinephrine between cycling and elbow flexor exercise using a two-way ANOVA (time × groups) with repeated measures. When the analyses indicated a significant difference, Tukey's post-hoc test was used to locate the difference. Pearson correlation analysis was used to identify the association among eHsp72, ATP, cathepsin D, norepinephrine, and myoglobin. We used stepwise multiple-regression analysis to determine independent predictors of exercise-induced elevation of eHsp72. Candidate predictor variables for the stepwise multiple-regression analysis were time (Pre, Post, Post10, Post30, Post60), exercise type (aerobic, eccentric), ATP, cathepsin D, norepinephrine, epinephrine, glucose, and FFA. The level of probability to reject the null hypothesis was set at $P<0.05$ (two-tailed). All comparative data are expressed as means±SE.

RESULTS

Changes in plasma levels of Hsp72, ATP, cathepsin D, and norepinephrine after aerobic and eccentric exercise.

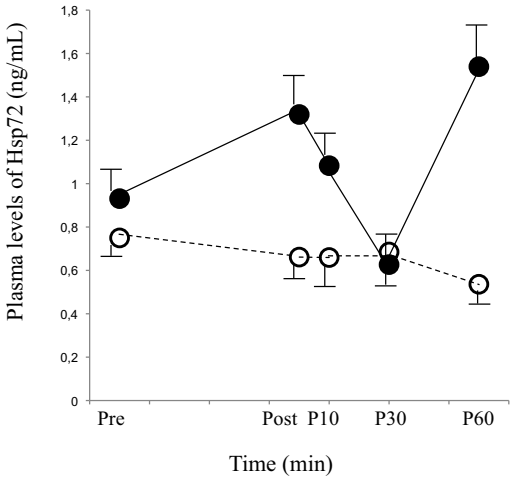

Fig. 1A. Changes in eHsp72 during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

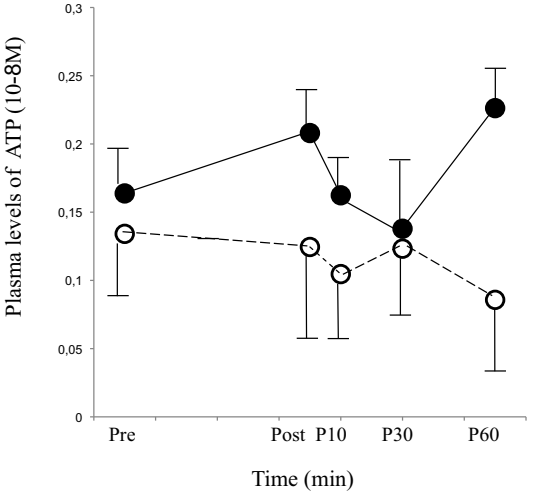

Fig. 1B. Changes in ATP during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

Cycling aerobic exercise, but not eccentric exercise, resulted in an increase of circulating Hsp72 (Fig. 1A). However, a significant group × time interaction was not observed for Hsp72 in plasma.

It has been proposed that extracellular ATP contributes to the induction of eHsp72 during and after stress exposure by an endolysosome mechanism (34). To examine the possible role of extracellular ATP in mediating the elevation of plasma Hsp72 after exercise, subjects underwent both bicycle ergometer exercise and elbow flexor exercise. Compared with the levels of ATP in plasma after bicycle exercise, those after eccentric exercise did not change. A significant group × time interaction was not observed for ATP in plasma (Fig. 1B).

In the lysosomal pathway, if ABC-family transporter co-localization with intravesicular cathepsin D involves the release of Hsp72 into the extracellular space, then cathepsin D may also be released (35). To determine whether cathepsin D mediates the elevation of plasma Hsp72 after exercise, plasma levels of cathepsin D after both aerobic and eccentric exercise were measured. Cathepsin D increased after aerobic exercise, but not after eccentric exercise. A significant group × time interaction

was observed for cathepsin D in plasma after both types of exercise (Fig. 1C; P<0.05).

Norepinephrine has often been demonstrated to induce eHsp72 during stressor exposure; Jonson *et al.* proposed that increases in norepinephrine acting upon α1 adrenergic receptors results in a calcium flux within the cell and a subsequent release of Hsp72 within exosomes (27). To examine the effect of norepinephrine on the exercise-induced increase in eHsp72, the changes in norepinephrine after the two types of exercise were analyzed by 2-way ANOVA with repeated measures. A significant group × time interaction was observed for norepinephrine in plasma after both types of exercise (Fig. 1D; *P*<0.01).

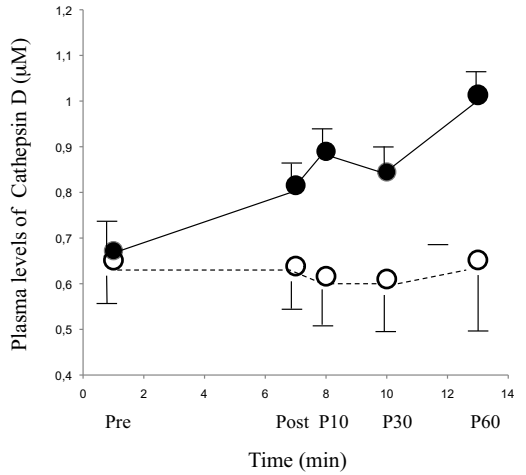

Fig. 1C. Changes in cathepsin D during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

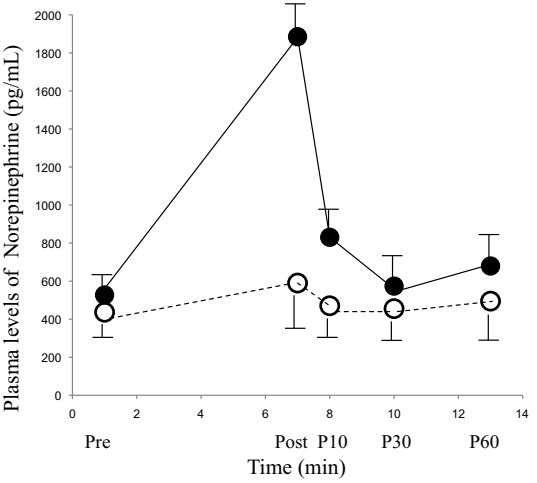

Fig. 1D. Changes in norepinephrine during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

Correlation analyses

The cycling exercise

A significant correlation was found between Hsp72 and ATP in plasma immediately after and 10 min after bicycle exercise (Table 1; r=0.79 and r=0.78 *P*<0.05, respectively), but not between eHsp72 and norepinephrine (Table 1). Significant correlations between ATP and norepinephrine in plasma were found immediately after exercise (r=0.89, *P*<0.01). There were no significant correlations between cathepsin D and other variables after exercises.

The eccentric exercise

After the elbow flexor lengthening contraction, significant negative correlations were found between eHsp72 and cathepsin D immediately after the exercise (r=–0.77, *P*<0.05). Significant correla-

ATP

	Post		Post 10		Post 30		Post 60	
	r	*P*	r	*P*	r	*P*	r	*P*
eHsp72								
Post	0,792	0,019 *						
Post 10	0,776	0,024 *	0,621	0,100				
Post 30	0,367	0,371	0,432	0,285	0,502	0,205		
Post 60	-0,251	0,548	-0,313	0,450	-0,340	0,410	0,434	0,282

Norepinephrine

	Post		Post 10		Post 30		Post 60	
	r	*P*	r	*P*	r	*P*	r	*P*
eHsp72								
Post	0,636	0,090						
Post 10	0,543	0,165	0,238	0,571				
Post 30	0,018	0,967	-0,279	0,503	-0,209	0,620		
Post 60	-0,453	0,259	-0,451	0,262	-0,291	0,485	0,029	0,946

Table 1. Correlation between eHsp72 and ATP or norepinephrine in plasma after aerobic exercise. *; significant differences $P<0.05$ by Pearson correlations. Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

tion between ATP and norepinephrine in plasma was found immediately after the exercise ($r=0.71$, $P<0.05$).

Multiple-regression analysis

To further determine potential associations of the elevation of eHsp72 after exercise, we used a stepwise multiple-regression analysis to determine independent predictors of exercise-induced elevation of eHsp72. Candidate predictor variables for the stepwise multiple-regression analysis were time (Pre, Post, Post10, Post30, Post60), exercise type (aerobic, eccentric), ATP, cathepsin D, norepinephrine, epinephrine, glucose, and FFA. In the regression model for eHsp72 in plasma, increased ATP and glucose were the strongest predictors of increased eHsp72 (ATP: $R^2=0.213$, $\beta=0.473$, $P<0.001$; ATP and Glucose: $R^2=0.263$, $\beta=0.534$, $P<0.001$).

DISCUSSION

The present study demonstrated that circulating levels of ATP are associated with plasma levels of Hsp72. It has been proposed that lysosome exocytosis is a possible mechanism of Hsp release from cells (34); a schematic model involves the activity of ABC-family transmembrane transporters and the participation of purinergic receptors. Extracellular ATP binding causes the opening of purinergic receptor channels, and the entry of Hsp72 into the secretory compartment of lysosomes through ABC-family transporters. The lysosomes are then transported to the cell surface. Subsequent fusion of Hsp72 containing lysosomes with the cell surface results in release of Hsp72 (35). We postulated that circulating levels of ATP stimulated during exercise lead to lysosome exocytosis with the release of Hsp72. In the present study, the plasma levels of ATP were associated with the elevation of eHsp72 after bicycle exercise, which, at least in part, supports our hypothesis on the mechanism of Hsp release—that circulating ATP is a necessary

factor to induce secretion of Hsp72 during aerobic exercise. This may be the reason that both marathon running (15) and downhill running (42) induce increases in eHsp72, whereas eHsp72 does not increase after elbow flexion (24). This shows that exercise-induced elevation of eHsp72 is not caused only by muscle damage, and also raises the possibility that circulating ATP plays a role in the elevation of eHsp72 during exercise.

It is well known that erythrocytes function as O_2 sensors, contributing to the regulation of skeletal muscle blood flow and O_2 delivery. This is caused by the release of ATP during exercise depending on the number of unoccupied O_2 binding sites in the hemoglobin molecule (20). It is also known that muscle contraction-derived ATP can affect adrenergic transmission by acting on purinergic receptors on sympathetic nerve endings, in order that elevated peripheral sympathetic nervous activity and the resultant increased neurovascular levels of norepinephrine evoke vasoconstriction and serve to maintain blood pressure and perfusion to vital organs (32). ATP-sensitive P2X purinoceptors have been shown to enhance norepinephrine exocytosis in cultured cervical ganglion neurons and cardiac synaptosomes (47, 45). Recent evidence suggests that the vasodilatory and sympatholytic functions of intraluminal ATP are mediated via endothelial P2 receptors (38). The source of ATP in plasma remains unclear, but skeletal muscle may release ATP during contractions (19, 38). Endothelial (7) and skeletal muscle cells (23) may release ATP in response to mechanical stress. The present study demonstrated that ATP in plasma was positively and strongly associated with plasma norepinephrine levels after both types of exercise, results that accord well with previous investigations regarding the relationship of ATP and norepinephrine in plasma. However, it has been suggested that human skeletal muscle does not release Hsp72 into the blood during exercise, since the increase in eHsp72 in serum precedes the increase of Hsp72 mRNA and protein in muscle (50), and also because eHsp72 can be found in arterial, but not venous, blood flow in the contracting leg (13).

The P2X receptor is ubiquitously expressed and belongs to a family of ligand-gated channels that are activated by extracellular ATP (11). When activated by ATP, the ionotropic P2X receptors ($P2X_1$-$P2X_7$) form nonselective ion channels permeable to Na^+, K^+ and, primarily, to Ca^{2+} (40). Among the P2X receptors, $P2X_7$ receptors are expressed in humans, including in glia cells (41), macrophages (26), and lymphocytes (21), but not in skeletal muscle (11). The human $P2X_6$ receptor, however, is heavily expressed in skeletal muscle (40). As previous studies have shown, exercise induces increases in the circulating levels of eHsp72 from human hepatosplanchnic tissue (13), from human brain (29), and from leukocytes (25). These results lead us to speculate that $P2X_7$ receptors (and not other P2X receptors) are related to the mechanism of release, and that cells or tissues where the receptors are expressed are the source for Hsp72 release into circulation in response to exercise.

Although secretion mechanisms may vary between cell types, it has been demonstrated that in human LPS-activated monocytes, secretory lysosomes are the site of ATP-induced IL-1β processing; ATP also triggers exocytosis of these organelles with secretion of IL-1β and caspase-1 (2). Calderwood *et al.* suggested that Hsp70 release is a form of leaderless secretion, and its mechanisms of release resemble IL-1β in that they require the activity of ABC-family transmembrane

transporters and the likely participation of $P2X_7$ receptors (8, 34). Regarding IL-1β secretion through lysosome-related vesicles, Andrei *et al.* demonstrated that IL-1β is contained in part within organelles co-fractionating with Rab-7-positive structures and displaying ultrastructural features of late endosomes and dense vesicles; a fraction of IL-1β-containing organelles contains the endolysosomal protein cathepsin D or the lysosomal marker LAMP-1 (1). We were particularly interested in the mechanism of release of eHsp72. We therefore hypothesized that the plasma concentration of cathepsin D should be positively correlated with eHsp72 in plasma if endolysosomes are associated with the mechanism of release of eHsp72 during exercise. However, the present results show that cathepsin D was not associated with eHsp72 in plasma after aerobic exercise, although the concentration of cathepsin D in plasma gradually increased after aerobic exercise but not after eccentric exercise. Thus, we could not confirm that the endolysosome is involved in the mechanism of eHsp72 release. In general, IL-1β does not increase after exercise, whereas eHsp72 increases after exercise. Even though both mechanisms of release are similar, there should be some differences. Mambula *et al.* also observed that IL-1β does not increase in cultured prostate cancer cell (LNCaP) medium after heat shock, whereas eHsp72 in the same medium increases (33).

Cathepsin D takes part in the digestion of exhausted and denatured cellular proteins or proteins showing abnormal structures, and those which enter the cell via endocytosis (37). Dohm *et al.* observed that the proportion of free cathepsin D activity is increased in exercised rats, and suggested that lysosomal enzymes may be involved in increased muscle protein degradation (12). After the eccentric exercise, we did not observe a relationship between cathepsin D and myoglobin. However, both cathepsin D and myoglobin were negatively correlated with eHsp72 respectively after the elbow flexor lengthening contraction. Increased Hsp70 mRNA and Hsp70 expression in human skeletal muscle 2 h after a single bout of treadmill running and 48 h after lengthening resistance exercise have been observed, respectively (48, 50). Previous studies indicate that Hsp72, myoglobin (24, 28), and cathepsin D (37) are independently involved in muscle damage after exercise, but it is not clear what the significance of the relationship among cathepsin D, myoglobin, and Hsp72 is, especially in regards to plasma levels. Further investigations are needed.

In conclusion, we demonstrated that ATP in plasma is associated with eHsp72 in plasma after aerobic exercise, suggesting that extracellular ATP may be a trigger of Hsp72 release. In terms of the endolysosomal mechanism, we measured cathepsin D as a lysosomal enzyme. However, cathepsin D was not associated with eHsp72 in plasma after aerobic exercise, although the concentration of cathepsin D in plasma gradually increased after aerobic exercise but not after eccentric exercise. Exercise thus results in an increase of extracellular ATP, which is a signal for modulating sympathetic nerve activity, and may be a trigger for releasing Hsp72.

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