Isolated hearts treated with skeletal muscle homogenates exhibit altered function

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Abstract Skeletal muscle fiber damage and necrosis can result in the release of intracellular molecules into the extracellular environment. These molecules, termed damage-associated molecular patterns (DAMPs), can act as signals capable of initiating immune and/or inflammatory responses through interactions with pattern recognition receptors. To investigate whether skeletal muscle DAMPs interact with the heart and alter cardiac function, isolated rat hearts were perfused for 75 min with buffer containing 1 $\mu\text{g}/\text{ml}$ of either soleus (slow), white gastrocnemius (WG, fast), or heat-stressed white gastrocnemius (HSWG) skeletal muscle homogenates. Left ventricular developed pressure (LVDP) and rates of pressure increase/decrease ($\pm\text{dP}/\text{dt}$) were measured using the Langendorff technique. Compared to controls, no changes in LVDP or $\pm\text{dP}/\text{dt}$ were observed over the 75-min perfusion when homogenates from the WG muscles were added. In contrast, at 30 min and thereafter, a decreased LVDP and $\pm\text{dP}/\text{dt}$ was observed in the hearts treated with soleus muscle homogenates. The hearts treated with HSWG homogenates also showed a decrease in LVDP from 45 min until the end of perfusion. These results suggest that molecules present in slow muscle and heat-stressed muscle are capable of altering cardiac function. Thus, muscle fiber type and/or heat shock protein content of skeletal muscles may be factors that influence cardiac function following skeletal muscle damage.

Introduction

Cells and tissues, including skeletal muscle fibers, respond to stressors that perturb proteostasis by the rapid yet transient synthesis of proteins known as “stress” or “heat-shock proteins” (HSPs) (Asea 2008; Benjamin and McMillan 1998; Fehrenbach et al. 2005). During episodes of stress, cells elevate HSP content in an attempt to restore proteostasis and promote survival. However, not all cells may survive, and thus, some cells with newly synthesized HSPs would undergo necrosis, conceivably releasing a rich source of HSPs into the extracellular environment. While the intracellular protective effects of HSPs are well documented (Chen et al. 2006; Latchman 2001), when HSPs become extracellular (eHSPs), they appear to promote immune and/or inflammatory responses (Asea et al. 2000; Vabulas et al. 2002). eHSPs, along with many other molecules, have been termed damage-associated molecular patterns (DAMPs) (Rubartelli and Lotze 2007), as they are capable of initiating ligand–receptor-mediated immune and/or inflammatory responses through Toll-like receptors (TLRs) and pattern recognition receptors (PRRs) located on cells and tissues including the heart (Zhang et al. 2010).

Skeletal muscles are known to be comprised of different fiber types that express distinct profiles of proteins which allow them to adequately meet their metabolic and contractile demands (Tiidus 2008). For example, slow-twitch skeletal muscle fibers are known to express a high mitochondrial and HSP content when compared to fast-twitch skeletal muscle fibers (Locke et al. 1991; Staron et al. 1984). While the primary function of skeletal muscle is to generate force, it is well established that conditions such as overuse, unaccustomed exercise, or lengthening muscle contractions can lead to muscle fiber damage and/or necrosis, where muscle-specific molecules gain access to the circulation (Lieber and Fridén 1999; Tiidus 2008; Steensberg et al. 2000, 2002). Given that muscle fiber types differ in the

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profile of proteins expressed, it follows that, depending upon the type of muscle fiber damaged, the proteins and/or cell constituents released will also vary. Interestingly, when HSP60, an HSP normally confined to mitochondria, was added to cultured cardiomyocytes, cell shortening was reduced (Kim et al. 2009). Similarly, when HSP72, the stress-inducible isoform of the HSP70 family, was added to cultured cardiomyocytes, it was shown to cause an inflammatory response and decrease contractility (Mathur et al. 2011). Thus, when skeletal muscle fibers are damaged and their contents released into the extracellular environment, specific molecules such as HSP60 and HSP72 may act as DAMPs and initiate an immune and/or inflammatory response in other cells and tissues, including the heart. In view of this, the objective of the current study was to determine if molecules present in slow, fast, as well as heat-stressed fast-twitch skeletal muscles were capable of altering the function of isolated hearts. In addition, does mitochondrial protein or HSP content play a role? To do this, isolated hearts (Langendorff technique) were treated with skeletal muscle homogenates, and cardiac performance was assessed.

Materials and methods

Animals and heat stress

Male Sprague–Dawley rats (350–474 g; Charles River) were maintained on a 12-h dark/light cycle, housed in pairs at 21 °C, at 50 % relative humidity, and were provided food and water ad libitum. Animals were anesthetized with isoflurane (2–5 % with 1 L O₂/min), and the appropriate tissue (heart or skeletal muscle) was removed. To increase HSP content in the white gastrocnemius muscle, a 15-min 42 °C heat stress was administered to a subset of animals as previously described (Locke 2000). Briefly, animals were placed on a covered heating pad initially set at 50 °C. Rectal temperature was measured throughout heat stress with a lubricated TSD 102CA Thermistor placed in the rectum. The thermistor was connected to a Biopac Data Acquisition System (Santa Barbara, CA USA). Once the rectal temperature reached 41.5 °C, the heating pad temperature was reduced to ~37 °C, and core temperature was maintained at ~42 °C for 15 min. Following heat stress, the animals were immediately removed from anesthesia and returned to their cages. Twenty-four hours later, the animals were anesthetized, and the gastrocnemius muscle was removed and frozen in liquid nitrogen. The soleus and gastrocnemius muscles were removed from unstressed animals and processed in a similar manner.

Muscle homogenates (to be administered to isolated hearts) were prepared by homogenizing the extracted skeletal muscles

in 15 vol of 600 mM NaCl, 15 mM Tris, pH 7.5 at 4 °C. Protein content was determined by the method of Lowry et al. (1951). For each treatment, a specific volume of muscle homogenate was added to create a final concentration of 1 µg/ml in the Krebs–Henseleit buffer that perfused the hearts. Preliminary experiments showed that this concentration of muscle extract has an effect on the heart.

Isolated heart preparation

Animal hearts ($n=5$ for each group) were placed into one of four groups and treated with either (1) no treatment, (2) soleus muscle homogenate, (3) white gastrocnemius muscle homogenate, or (4) white gastrocnemius muscle homogenate (from previously heat-stressed animals).

To assess cardiac function, a 50-mmHg constant pressure, 37 °C non-recirculating retrograde perfusion Langendorff technique was used. The animals were anesthetized using Isoflurane (2–5 % with 1 L O₂/min), and heparin (1,000 units) was injected into the tail vein approximately 5 min prior to heart removal. Once anesthetized, the hearts were rapidly excised and immersed in ice-cold saline (0.85 % NaCl). The aorta was cannulated and secured using 6–0 surgical thread to a three-way valve attached to a syringe. The heart was slowly perfused with 4 ml of cold saline solution, and a drainage tube was inserted through the wall of the left ventricle. The heart was mounted in a 37 °C buffer-filled chamber (Radnoti Glass Technology, Inc., Monrovia, California, USA), and a size 4 latex balloon was inserted into the left ventricle and inflated to 60 µl through tubing connected to a 100-µl Hamilton syringe. The balloon was attached to a pressure transducer (COBE Labs Inc., Lakewood, CO, USA) and connected to a Biopac MP30B-CE Data Acquisition System that allowed for the recording of left ventricular developed pressure (LVDP), as well as rates of pressure increase/decrease ($\pm$ dP/dt). The hearts were perfused at 37 °C with an oxygenated (95 % O₂, 5 % CO₂) Krebs–Henseleit buffer (4.7 mM KCL, 2.0 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM Mg₂SO₄, 118 mM NaCl, 25 mM NaHCO₃, and 11 mM glucose, pH of 7.4) and paced at 320 bpm using two electrodes connected to a BIOPAC BSLSTMA stimulator (306A5029, BIOPAC, Santa Barbara, California). One electrode pierced the right atrium, while a second ground electrode was placed in the buffer. Once the heart was cannulated and normal rhythm clearly established, the hearts were allowed to contract for 15 min (equilibration period). After the 15-min equilibration period, a muscle homogenate was added to the buffer to create a final concentration of 1 µg of muscle homogenate/ml of buffer for all treated hearts. At the end of the treatment/perfusion period (75 min), pacing was stopped, and the electrodes, balloon, and drainage tube were removed. The hearts were removed from the

cannula, trimmed of excess connective tissue, frozen in liquid nitrogen and stored at -70°C for subsequent biochemical analysis.

Protein determination and Western blot analyses

Frozen portions of the left ventricle (40–60 mg) were homogenized in 15 volumes of 600 mM NaCl and 15 mM Tris (pH 7.5) at 4°C using an Ultra-Turrax T8 grinder (IKA Labortechnik, Staufen, Germany). Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed according to the method described by Laemmli (1970). Following electrophoretic separation, proteins were transferred to nitrocellulose membranes (0.22- μm pore size; Bio-Rad Laboratories, Mississauga, Canada) as described by Towbin et al. (1979) and modified to the Bio-Rad mini-protein II gel transfer system as described by Frier et al. (2008). Following protein transfer, the nitrocellulose membranes were blocked in Tris-buffered saline (TBS; 500 mM NaCl, 20 mM Tris HCl, pH 7.5) for 1 h and reacted with a polyclonal antibody specific for HSP72 and HSP60 (SPA-812 and SPA-805; Assay Designs, Plymouth Meeting, PA) diluted 1:1,000 in TTBS (TBS plus 0.05 % Tween 20) with 2 % nonfat dried skim milk powder for 4 h. Blots were washed three times in TTBS for 5 min each time. Blots were incubated for 1 h at room temperature in a 1:1,000 dilution of goat anti-rabbit secondary antibody conjugated to alkaline phosphatase (Bio-Rad Laboratories, Hercules, CA, CAT# 170–6515) in TTBS with 2 % nonfat dried skim milk powder. Blots were washed twice in TTBS, once in TBS, and developed by immersing in a carbonate buffer (100 mM Na_2CO_3 , 1 mM MgCl_2 , pH 9.8) containing 1 ml of 3 % (wt/vol) *p*-nitro-blue tetrazolium chloride *p*-toluidine salt in 70 % dimethylformamide (DMF) and 1 ml of 15 % (wt/vol) 5-bromo-4-chloro-3-indolyl phosphate in 100 % DMF. After development, blots were washed with distilled H_2O and dried and scanned using a Canon Image J scanner.

Statistical analyses

Data acquired by the Biopac for the isolated heart experiments were processed into Microsoft Excel and transferred to SigmaStat (version 3.5) for statistical analyses. A representative 30-s segment of LVDP, $+\text{dP}/\text{dt}$, or $-\text{dP}/\text{dt}$ from each trial was measured at the 0, 15, 30, 45, 60, and 75 min of time points, and data were transferred into Excel where it was converted into a percentage of equilibration for further statistical analysis. A one-way analysis of variance (ANOVA) and ANOVA on ranks (for normalized data) were used to assess

between group differences for cardiac function data. A Tukey's post hoc or Kruskal–Wallis test (for ANOVA on ranks) was used to further determine which groups differed. Statistical significance was set at $p < 0.05$.

Results

HSP expression in the skeletal muscles used as homogenates

To confirm HSP content in White Gastrocnemius (WG), heat-stressed WG and the soleus skeletal muscles, Western blot analyses were performed. As expected, HSP72 was readily detected in soleus muscles (Fig. 1, panel A, lane 1) and in white gastrocnemius muscles from rats subjected to a 15-min, 42°C heat stress 24 h prior (Fig. 1, panel A, lane 3). However, HSP72 was not detected in the WG muscles from unstressed rats (Fig. 1, panel A, lane 2). Similarly, HSP60 was also detected in the soleus and heat-stressed white gastrocnemius (HSWG) muscles (Fig. 1, panel B, lanes 1 and 3, respectively), but not in unstressed WG muscles (Fig. 1, panel B, lane 2). A similar pattern of HSP72 and HSP60 expression was detected in all samples used for treatment (data not shown). Thus, the muscle homogenates used for heart treatments varied in HSP72 and HSP60 content, with the soleus muscle and heat-stressed white gastrocnemius muscle expressing a relatively high amount of HSPs when compared to the unstressed WG. In addition to the known metabolic and contractile differences between these muscles, these data confirm differences in HSP expression between the various muscles used as homogenates.

Skeletal muscle homogenate treatment reduces cardiac function in isolated hearts

The Langendorff isolated heart preparation was used to assess the effect of muscle homogenate treatment on cardiac function. LVDP was used to evaluate the contractile function of the left ventricle. Absolute values for LVDP for each

Fig. 1 Heat stress increases HSP content in the WG muscle. A portion of Western blots for HSP72 (panel A) and HSP60 (panel B) is shown, illustrating the increased content of HSP72 and HSP60 in soleus and HSWG muscle homogenates. Sol, soleus (lane 1); WG, white gastrocnemius (lane 2); HSWG, heat-stressed white gastrocnemius (lane 3)

of the four groups 15 min after an equilibration period showed LVDP to be 94 ± 2 mmHg for the untreated hearts (controls), 101 ± 6 mmHg for the hearts to be treated with soleus muscle homogenates, 99 ± 6 mmHg for the hearts to be treated with WG muscle homogenates, and 94 ± 4 mmHg for the hearts to be treated with HSWG muscle homogenates. No significant differences were detected between groups. Similar results were obtained for the rates of pressure increase (Table 1).

When compared to the untreated (control) hearts, LVDP in the hearts treated with WG muscle homogenate remained unchanged throughout the 75-min perfusion (Fig. 2). In contrast, at 30 min of perfusion and thereafter, the hearts treated with soleus muscle homogenates showed a significant ($p < 0.05$) decrease in LVDP (Fig. 2) when compared to both untreated (control) hearts and WG treated hearts. At the end of perfusion, the hearts treated with soleus homogenates displayed a 27 % ($p < 0.05$) reduction in LVDP. These results suggest that the addition of homogenates from slow skeletal muscles, but not fast skeletal muscles, to isolated hearts results in a reduced cardiac function.

The hearts treated with WG homogenates from heat-stressed animals also showed a significant ($p < 0.05$) decrease when compared to the untreated (control) hearts from the 45-min time point until the end of perfusion, where LVDP remained 25 % ($p < 0.05$) reduced. The reduction in LVDP in the hearts treated with HSWG homogenates, but not WG homogenates, when compared to the untreated (control) hearts suggests that HSPs or other heat-stress-induced molecules play a role in the reduction of cardiac function.

When the rates of pressure increase were examined, no difference was observed between the untreated hearts and hearts treated with WG homogenates. However, a significant ($p < 0.05$) decrease in $+dP/dt$ was observed in the hearts treated with either soleus (28 %) or HSWG (23 %) homogenates compared to the untreated (control) hearts. This trend continued from 30 min until the end of perfusion (Fig. 3). This suggests that molecules released from damaged or

necrotic skeletal muscles with an elevated HSP content may be capable of altering the contractile properties of the heart.

Rate of relaxation, as assessed by $-dP/dt$, was calculated from LVDP and measures the rate at which the left ventricle relaxes during diastole (Fig. 4). Throughout the first 15 min of treatment, the hearts treated with HSWG muscle homogenates displayed a significantly ($p < 0.05$) lower $-dP/dt$ (95 ± 2 % of equilibration) compared to the untreated (control) hearts. By 30 min of perfusion, all hearts treated with muscle homogenates displayed a significantly ($p < 0.05$) reduced $-dP/dt$ compared to the untreated (control) hearts. The hearts treated with soleus homogenates were 86 ± 4 % of equilibration, while the hearts treated with HSWG and WG homogenates were 92 ± 2 and 94 ± 3 % of equilibration, respectively (Fig. 4). This pattern continued at 45 and 60 min of perfusion such that at the end of the 75-min perfusion period, $-dP/dt$ in the hearts treated with soleus, WG, and HSWG muscle homogenates remained significantly ($p < 0.05$) reduced compared to the untreated (control) hearts. When compared to the untreated (control) hearts, the hearts treated with soleus homogenates displayed the greatest reduction of 36 % (62 ± 4 % of equilibration), while the hearts treated with HSWG or WG muscle homogenates displayed a 26 % reduction (72 ± 5 % of equilibration) and a 22 % reduction (76 ± 3 % of equilibration), respectively.

Discussion

The present study assessed the effects of slow, fast, and heat-stressed fast-twitch rat skeletal muscle homogenates on cardiac function. The novel aspect of this work was that hearts treated with muscle extracts demonstrated altered function. First, the hearts treated with any skeletal muscle homogenate showed a reduction in the rate of relaxation ($-dP/dt$) at 30 min and thereafter. Second, and perhaps more importantly, isolated hearts treated with soleus and heat-stressed white gastrocnemius skeletal muscle homogenates displayed a decrease in both LVDP and $+dP/dt$, while the hearts treated with gastrocnemius skeletal muscle homogenate (non-heat-stressed) showed no changes. The soleus and HSWG homogenates contained elevated levels of two known DAMPs (HSP60 and HSP72), suggesting that DAMPs released from specific skeletal muscle fibers may be capable of altering cardiac function. In support of this, eHSPs have been implicated as “alarm proteins” or “alarmins” (Manson et al. 2012; Stoecklein et al. 2012), and both HSP60 and HSP72 have been shown to interact with cardiac TLR4 and depress cardiomyocyte contractility (Kim et al. 2009; Mathur et al. 2011; Vabulas et al. 2002). Therefore, the reduction in LVDP and $+dP/dt$ observed in the hearts treated with soleus and HSWG muscle homogenates as well

Table 1 Absolute values for variables shown at the end of equilibration

Group	LVDP (mmHg) $\pm$ SEM	Rate of pressure increase ($+dP/dt$, mmHg/s) $\pm$ SEM
Control	94 ± 2	$1,527 \pm 86$
Soleus	102 ± 6	$1,646 \pm 169$
White gastrocnemius	99 ± 6	$1,522 \pm 59$
HS white gastrocnemius	94 ± 4	$1,720 \pm 86$

No significant differences were observed. Values are expressed as mean $\pm$ SEM. LVDP left ventricular developed pressure, Con control, Sol soleus, WG white gastrocnemius, HSWG heat shocked white gastrocnemius, SEM standard error of the mean

Fig. 2 Hearts treated with muscle homogenates demonstrate a reduced LVDP. Hearts were placed on the Langendorff, and after a 15-min equilibration period (as described in “Materials and methods”), hearts treated with either white gastrocnemius (*WG*), soleus (*Sol*), or heat-stressed white gastrocnemius (*HSWG*) homogenates and compared to

untreated (*Con*) hearts. Perfusion with the homogenate starts at time zero, and values are expressed as a percentage of the equilibration value. * denotes a significance ($p < 0.05$) vs. *Con*. † denotes a significance ($p < 0.05$) vs. *Con* and *WG* ($n = 5$ for each group). Data are expressed as mean $\pm$ SEM

as the reduced rate of relaxation observed in all treated hearts may be a result of HSPs or other DAMPs directly interacting with the heart.

The profile of proteins expressed by skeletal muscle fibers reflects their specific metabolic and functional requirements. One of the most prominent characteristics of slow-twitch/oxidative (SO) skeletal muscle fibers is a high mitochondrial content relative to the fast-twitch glycolytic (FG) fibers (Tiidus 2008; Staron et al. 1984). Thus, when the soleus, a muscle comprised of primarily SO fibers containing a greater content of mitochondrial DAMPs, is damaged, a greater content of mitochondrial DAMPs would gain access to the extracellular environment and potentially exert their effects, perhaps by the TLR–NF- κ B pathway. In support of this, Zhang et al. (2010) found that mitochondrial damage and the subsequent

release of mitochondrial DAMPs activated inflammatory signaling through TLR1 and 9, resulting in polymorphonuclear neutrophil activation, cytokine up-regulation, systemic inflammation, and organ injury. Thus, the mitochondria-rich SO fibers in the soleus would likely provide a greater source of mitochondrial DAMPs than the predominantly FG fibers in the *WG* and explain why the hearts treated with soleus muscle homogenates, but not the hearts treated with *WG* muscle homogenates, showed alterations in cardiac function.

A second characteristic of (rat) SO skeletal muscle fibers is the relatively high constitutive expression of HSP72, the “inducible” member of the HSP70 family (Locke et al. 1991; Neuffer et al. 1996). Similar to the mitochondrial content, the greater HSP72 DAMP content in the SO fibers of the soleus may have influenced cardiac function. Furthermore, the

Fig. 3 Hearts treated with muscle homogenates demonstrate a reduced +dP/dt. Hearts were placed on the Langendorff, and after a 15-min equilibration period (as described in “Materials and methods”), hearts treated with either white gastrocnemius (*WG*), soleus (*Sol*), or heat-stressed white gastrocnemius (*HSWG*) homogenates and compared to

untreated (*Con*) hearts. Perfusion with the homogenate starts at time zero, and values are expressed as a percentage of the equilibration value. * denotes a significance ($p < 0.05$) vs. *Con* ($n = 5$ for each group). Data are expressed as mean $\pm$ SEM

Fig. 4 Hearts treated with muscle homogenates demonstrate a reduced $-dP/dt$. Hearts were placed on the Langendorff, and after a 15 min equilibration period (as described in “Materials and methods”), hearts treated with either white gastrocnemius (*WG*), soleus (*Sol*), or heat-stressed white gastrocnemius (*HSWG*) homogenates and compared to

untreated (*Con*) hearts. Perfusion with the homogenate starts at time zero, and values are expressed as a percentage of the equilibration value. * denotes a significance ($p < 0.05$) vs. *Con* ($n = 5$ for each group). Data are expressed as mean $\pm$ SEM

hearts treated with the HSWG muscle homogenate also showed a decreased LVDP, while the hearts treated with non-heat-stressed white gastrocnemius muscle showed no alterations. Given that the mitochondrial DAMP content in the WG muscle homogenates would likely be similar with the exception of any HSPs, it suggests that an increased HSP content may be responsible for the observed alteration in cardiac function between these two treatments. The reason for the differences in cardiac function following WG treatment with, and without a prior heat stress, might be due to the increased expression of DAMPs such as HSP60 and/or HSP72. However, heat stress results in many cellular alterations which may also be involved. For example, when HSC70, the constitutively expressed member of the HSP70 family, was added to the buffer of an isolated heart system, myocardial TLR4 was activated and cardiac contractility reduced (Zou et al. 2008). Thus, other HSPs or perhaps other DAMP molecules such as uric acid, which is known to be increased following heat stress, could also play a role (Shi et al. 2003).

While eHSPs appear to be involved in the initiation of the inflammatory response (Asea et al. 2000), their specific function and purpose has yet to be determined. Interestingly, eHSPs are known to be elevated by exercise (Febbraio et al. 2002; Walsh et al. 2001), and immediately following endurance exercise, such as marathon running, reduced cardiac function occurs (Douglas et al. 1987; La Gerche et al. 2008; Middleton et al. 2006; Neilan et al. 2006). Furthermore, even after mild exercise, a phenomenon known as post-exercise hypotension occurs (MacDonald et al. 2000; Halliwill 2001). In view of these findings, and that pathogen-associated molecules such as lipopolysaccharide (LPS) can cause vasodilatation via the TLR–NF- κ B-mediated pathway (Draisma et al. 2009; Hingorani et al. 2000), eHSPs/DAMPs may act in a manner similar to LPS. In the present study, the Langendorff isolated

heart model was used to remove the effect of the immune cells and to allow for a more direct link between skeletal muscle and the heart. Whether these effects occur in vivo, where various DAMPs might be tempered by diverse blood components and cells, remains to be determined.

A number of conditions including unaccustomed exercise or eccentric contractions can cause skeletal muscle fibers to become damaged and necrotic, resulting in the release of muscle fiber contents into the extracellular environment (Komulainen and Vihko 1994). Interestingly, fast-twitch muscle fibers appear to be more susceptible to this type of muscle damage than slow-twitch muscle fibers (Fitts 1994; Komulainen and Vihko 1994), perhaps because of their lower intracellular HSP content (Locke et al. 1991). Thus, a mild stress may selectively damage the fast-twitch muscle fibers containing low HSP/DAMP levels. This might serve as a low intensity or primary alarm signal for other cells and tissues. In contrast, either a continued low-level stress or a more severe stress may damage slow-twitch fibers containing a greater HSP/DAMPs content. This might serve as a more intense or secondary alarm signal to other cells and tissues. Regardless, the release of HSPs/DAMPs occurring from damage to skeletal muscles may be related to a mechanism that allows striated muscles to communicate with other cells and tissues during episodes of stress to alter function accordingly.

In conclusion, the present study shows that molecules from specific skeletal muscles can potentially interact with the heart and alter cardiac function. This interaction may vary according to both fiber type and/or HSP content of the muscle. This damage or stress alarm system may allow striated muscles to communicate systemically. Future studies are required to further elucidate both the molecules involved in this interaction and the exact biological cell signaling pathway.

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