

Research article

## Differential Response of Heat Shock Proteins to Uphill and Downhill Exercise in Heart, Skeletal Muscle, Lung and Kidney Tissues

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### Abstract

Running on a horizontal plane is known to increase the concentration of the stress biomarker heat-shock protein (HSP), but no comparison of the expression of HSP70 has yet been established between the uphill (predominantly concentric) and downhill (predominantly eccentric) muscle contractions exercise. The objective of the study was to investigate the relationships between eccentric and concentric contractions on the HSP70 response of the lung, kidney, gastrocnemius, soleus and heart. Twenty-four male Wistar weanling rats were divided into four groups: non-exercised and three different grades of treadmill exercise groups: horizontal, uphill (+7%) and downhill (-7% of inclination). At the optimal time-point of six hours after the exercise, serum uric acid, creatine kinase (CK) and lactate dehydrogenase (LDH) were determined by standard methods and HSP70 by the Western blot analysis. HSP70 responds differently to different types of running. For kidney, heart, soleus and gastrocnemius, the HSP70 expression increased, 230, 180, 150 and 120% respectively of the reference (horizontal). When the contraction was concentric (uphill) and compared to downhill the increase in response of HSP70 was greater in 80% for kidney, 75% for gastrocnemius, 60% for soleus and 280% for the heart. Uric acid was about 50% higher ( $0.64 \pm 0.03 \text{ mg}\cdot\text{dL}^{-1}$ ) in the uphill group as compared to the horizontal or downhill groups. Similarly, the activities of serum CK and LDH were both 100% greater for both the uphill and downhill groups as compared to the horizontal group ( $2383 \pm 253$  and  $647.00 \pm 73$  U/L, respectively). The responsiveness of HSP70 appeared to be quite different depending on the type of tissue, suggesting that the impact of exercise was not restricted to the muscles, but extended to the kidney tissue. The uphill exercise increases HSP70 beyond the eccentric type and the horizontal running was a lower HSP70 responsive stimulus.

**Key words:** HSP70, stress, concentric exercise, eccentric exercise, metabolic stress.

### Introduction

The stress proteins belong to a highly conserved group present in practically all animal tissues. These, including the heat shock protein 70 (HSP70), are responsible for part of the natural endogenous defence system against potential damage caused by overexertion and many other types of stress, in all the tissues in which they are present. They are expressed in specific tissues within a relatively short period of time following alterations in homeostasis related to physical exercise and the resulting physiological alterations, such as an increase in body temperature, oxidative stress, ischemia and reperfusion, osmotic stress, hypoxia, glucose depletion and inflammation (Belter et

al., 2004; Jang, 2008; Morimoto, 1998; Staib et al., 2009). Although it is known that the exercise may stimulate HSP70 response, at present, it is not known how the different types of muscle contraction affect the response of HSP70 in different tissues.

In different tissues, HSP70 has been indicated as a protein that aids the antioxidant system, protecting other protein tissues from possible damage and even from denaturation. For example, in the heart, the HSPs are known to be very active (Starnes, 2002), and it is well accepted that chronic physical activity can reduce the risk of cardiovascular diseases. Physical exercise, particularly horizontal treadmill running, has been widely documented as inducing an elevation of the stress proteins in the skeletal muscle and myocardium, this being dose-responsive, that is, more exercise will cause a greater increase in the HSP70 of these tissues (Atalay et al., 2004; Smolka et al., 2000; Staib et al., 2009), showing that exercise can stimulate responses of the HSPs which are not limited to the muscle, but so far, no studies comparing the effect of different types of muscle contraction (concentric and eccentric exercise) on the response of HSP70 in different tissues have been found, so this approach is the novelty of the present investigation.

Different types of muscle contraction cause different physiological alterations that are mostly caused by mechanical and metabolic stress. Eccentric contraction, here stimulated by the downhill treadmill exercise, causes great tension on cellular structures resulting in microscopic muscle lesions of varying magnitude (Macpherson et al., 1997; Prasartwuth et al., 2005) in addition to the appearance of cytosolic enzymes in the serum (Cannon et al., 1991; Nosaka and Clarkson, 1994). Muscle biopsies have revealed that eccentric exercise causes more damage to the Z-bands in sarcomeres and myofibrils, followed by a major inflammatory reaction, loss of muscle functionality and delayed pain as compared to concentric exercise (Gibala et al., 1995; Jones et al., 1989; Larsen et al., 2007; Lynn et al., 1998; Rodenburg et al., 1994; Stauber et al., 1989). On the other hand, while concentric contractions, here stimulated by the uphill treadmill exercise, show lower power outputs and less micro-lesions than eccentric contractions, it has been known since the early nineteen fifty's (Abbot et al., 1952; Asmussen, 1953) that concentric contractions increase the metabolic demands, pH, temperature and oxygen pressure more than eccentric muscle contractions.

Therefore, due to: a) different alterations in microscopic muscle lesions, inflammatory reaction, metabolic demand, pH, temperature, hypoxia, oxidative and osmotic

stress that different types of exercise can cause; and b) lack of literature data about the effect of different types of muscle contractions in HSP70 of different tissues; the aim of this paper was to investigate the relationship between the eccentric-concentric cycle (horizontal 0 degree [Hor]), eccentric (downhill  $-7^\circ$  [DH]), and concentric (uphill  $+7^\circ$  [UH]) contractions, in the HSP70 response of rat lung, kidney, gastrocnemius, soleus and heart. The authors hypothesize that different types of muscle contraction could generate different responses in HSP70.

## Methods

Male Wistar (21-day old, specific-pathogen free) rats, bred at the Multidisciplinary Centre for Biological Research, University of Campinas, SP, Brazil, were housed ( $\sim 22^\circ\text{C}$ , 55% RH, inverted 12-hour light cycle) in individual growth cages, with free access to commercial chow (Labina, Purina, Brazil [aminogram and proximate composition analyses in Table 1]) and water at all times, until they reached  $445 \pm 27$  g. The research methodology was approved by the Ethics Committee on Animal Experimentation (CEEA-UNICAMP, protocol 2297-1). The animals were randomly assigned to any of four groups according to the type of exercise selected: a) control non-exercised, b) uphill (predominantly concentric contraction) inclination ( $+7^\circ$ ), c) downhill (predominantly eccentric contraction) inclination ( $-7^\circ$ ), d) control – horizontal motion, or no inclination.

**Table 1. Proximal composition and amino acids analysis (g/100g) of the diet.**

<b>Macronutrients (percent composition)</b>	<b>Lipids</b>	5.1%
	<b>Ashes</b>	8.3%
	<b>Moisture</b>	9.0%
	<b>Protein</b>	21.9%
	<b>Carbohydrate</b>	55.8%
<b>Amino acids (g / 100g)</b>	<b>ASP</b>	2.00
	<b>GLU</b>	4.03
	<b>SER</b>	1.08
	<b>GLN</b>	.97
	<b>HIS</b>	.54
	<b>ARG</b>	1.61
	<b>TER</b>	.78
	<b>ALA</b>	1.04
	<b>PRO</b>	1.28
	<b>TYR</b>	.78
	<b>MET</b>	.45
	<b>CYS</b>	.28
	<b>VAL</b>	1.00
	<b>ILE</b>	.89
	<b>LEU</b>	1.76
	<b>PHE</b>	1.07
	<b>LYS</b>	1.15
<b>TOTAL</b>	20.71	

## Protocol

The animals in each of the three exercised groups ran on a programmable treadmill for 35 minutes at a constant speed of  $15 \text{ m}\cdot\text{min}^{-1}$ , thus establishing a time-distance reference point for all the groups. This was the time that took the animals in the uphill running group to the point

of exhaustion, and was taken as a reference for the other groups. The animals were sacrificed by decapitation following a 6-hour recovery period, as recommended by Jing, Wu and Wang (2007) and Wischmeyer et al. (2001). Immediately after sacrifice, the gastrocnemius, soleus, lung, kidney and heart were collected and stored in liquid nitrogen until the time of analysis.

## Protein extraction and immunoblotting

The protein content of the supernatants was determined by the Lowry method (1951). For immunoblotting, the middle third of tissues were homogenates ( $\sim 70\text{mg}$ ) were subjected to SDS-PAGE and transferred onto a PVDF membrane as described previously (Lollo et al., 2012). The loading control was tubulin (Abcam, Cambridge, catalogue number ab44928, diluted 1:3000) and beta-actin (Abcam, Cambridge, catalogue number ab8226, diluted 1:2000 1:1000). The blots were probed with the appropriate antibodies (Stressgen, Victoria, B.C., Canada Ref SPA810) to assess the protein level of the HSP70. The appropriate secondary antibody, conjugated to peroxidase and the BM chemiluminescence blotting system (Abcam), was used for detection. The bands were visualized by chemiluminescence (GE, ImageQuant LAS 4000), and the band intensities quantified by scanning and processing with the ImageJ program (v. 1.44 for Windows).

## Biochemical parameters

Kits for blood sampling: Six hours after the training session, blood samples were collected in Vacutainers, maintained at  $4^\circ\text{C}$ , and centrifuged at  $3000\times g$  ( $4^\circ\text{C}$ , 15 min) to obtain the serum. The sera were assessed for uric acid (catalogue number 0017, Laborlab kits, São Paulo, Brazil), for CK (catalogue number 0028, Laborlab kits, São Paulo, Brazil) and LDH (catalogue number 0040, Laborlab kits, São Paulo, Brazil), all the three analysis were carried out in Epoch microplate reader (BioTek, Instruments, Inc., Winooski, VT).

## Statistical analyses

The statistical treatment of the data was carried out by ANOVA and the means ranked by Duncan, adopting the standard values of  $p < 0.05$  as a criterion for significance.

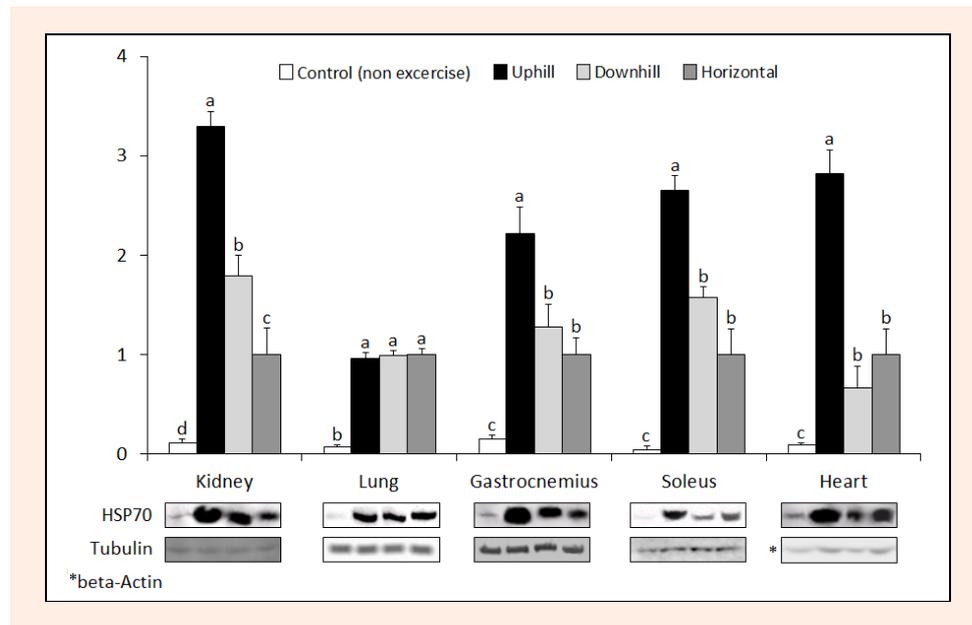
## Results

### HSP70

The results showed that the concentrations of HSP70 in heart, kidney, gastrocnemius, soleus and lung (Figure 1) were always very low or non-detectable in the sedentary animals, exercise being efficient in raising the HSP70 concentrations at least 8 times in all these tissues ( $p < 0.001$ ). The type of exercise also influenced the response of HSP70 except for the lung. In the other tissues the uphill exercise induced more HSP70, followed by the downhill and horizontal exercises.

### Biochemical parameters

The results for the serum enzymes CK and LDH (Figure 2) are indicators of cell micro-lesions, and show that the exercise protocol used was effective in generating muscle



**Figure 1.** Effect of the different exercise regimes in HSP70 concentration at 6 hours post-exercise (means  $\pm$  SEM [standard error of the mean]). Different letters denote significant differences between different activity groups ( $p < 0.05$ ).

micro-lesions, and hence in increasing the levels of these enzymes in all the exercised groups as compared to the sedentary group ( $p < 0.001$ ), the horizontal exercise apparently generating less micro-lesions.

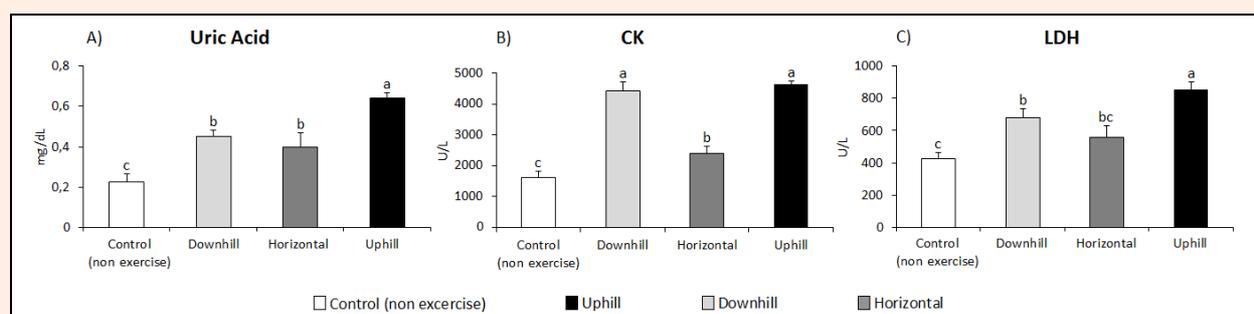
## Discussion

We carried out a comparison of the HSP70 expression between the uphill (predominantly concentric) and downhill (predominantly eccentric) contraction exercises using horizontal running as the control. The acute exercise based on concentric contractions was the type that resulted in the highest expressions of HSP70 than horizontal plane (Figure 1) in heart (~180%), kidney (~230%), gastrocnemius (~120%) and soleus (~150%), but not in the lung, suggesting that metabolic stress in the former tissues was more intense at the selected time of six hours post-exercise, the optimal time-point after stress (Jing et al., 2007). HSP70 may increase after the exercise because a number of stressful stimuli, such as hyperthermia, hypoxia and ischemia (Khalil et al., 2011; Rohde et al., 2005). The concentric contraction would be more efficient

in generating that stimulus. Regarding the sedentary group (normal homeostasis) HSP70 was expressed at very low or undetectable levels (Figure 1), the samples were taken at rest.

Although the predominantly eccentric-contraction exercise is the type of physical exercise that causes the greatest number of micro-lesions, it was found that the tissues responded differently to the type of exercise, with different levels of expression of this biomarker. The acute exercise based on concentric contractions was the type that resulted in the highest concentrations of HSP70 in heart, kidney, gastrocnemius and soleus, but not in the lung, suggesting that metabolic stress in the former tissues was more intense at the selected time of six hours post-exercise, the optimal time-point after stress (Jing et al., 2007).

According to Oishi et al. (2002), the response of the soleus muscle occurs between 4 and 8 hours post-heat stress in contrast to the gastrocnemius muscle, which peaks at about 48 hours. It should be pointed out that, although the time point selected for the gastrocnemius was not that for the maximal expression of the biomarker,



**Figure 2.** Effect of the different exercise regimes in serum biochemical parameters at 6 hours post-exercise. (means  $\pm$  SEM [standard error of the mean]). Different letters denote significant differences between different activity groups ( $p < 0.05$ ). Uric acid in (mg/dL); CK and LDH in (U/L).

the results showed a clear-cut differential response of this muscle with the type of exercise. In addition it is not known if there is any difference between the soleus and gastrocnemius in terms of the speed of response to concentric and eccentric muscle contractions, but the similarity between the patterns of these two muscles shown in Figure 1 suggests that in the rat, whatever the difference, it would be negligible.

With regard to the extent of the response, Samelman (2000) has stated that the HSP70 is distributed in different concentrations, depending on the type of muscle fibre. Oishi et al. (2002) also addressed this issue, explaining that whereas fast fibres have a rather anaerobic metabolism and make use of less oxygen than slow fibres, they are less responsive to oxidative stress and other factors that may lead to HSP70 expression. Therefore, the HSPs have a tendency to appear in greater concentrations in the soleus than in the gastrocnemius muscles during the uphill exercise, in agreement with that observed in the present data.

The HSPs are known to be very active in heart tissue (Starnes, 2002), and it is well accepted that chronic physical activity can reduce the risk of cardiovascular diseases. Although the mechanisms for such an effect are not yet well understood, one hypothesis is that the higher concentrations of HSPs in the myocardium can be taken as a sign of protection, while Qian et al., (1998) proposed that the HSPs were capable of restoring or renaturing the denatured proteins and key enzymes produced during the stressing process. The present data showed that the heart tissue was substantially more responsive to the concentric exercise, as compared to the eccentric and the horizontal types, as can be seen in Figure 1.

Physical exercise is capable of raising the respiratory rate producing hyperventilation, mucosal dryness of the upper airways and exposure to foreign particles, thus increasing the risk of infections (Moreira et al., 2009), in addition to the elevation of body temperature, all of which are sources of pulmonary stress. However, the present data revealed that under such conditions of time and type of exercise, no significant variations in responsiveness of the lung tissues were detected, and therefore it is suggested that the inclination does not generally have any bearing on the lung.

Of the three grades (uphill, horizontal and downhill), uphill is classically known to be a more intense exercise with greater caloric demand (Abbot et al., 1952; Asmunssen, 1953), followed by horizontal and downhill (Prasartwuth et al., 2005), but the response was not the same for HSP70 (Figure 1). Notably horizontal running was the least responsible for change in HSP70 in the kidney, while downhill running was clearly the exercise with less metabolic demand, indicating that the HSP70 kidney response to exercise was not dose-responsive to metabolic demand. Maybe the eccentric contractions could stimulate the HSP70 response by mechanisms dependent on muscle damage, which are greater in this type of exercise and are modulated by an increase in inflammation markers (Lavie et al., 2011). It was concluded that the type of muscle contraction can influence HSP70 kid-

ney response more than metabolic demand.

With regard to kidney tissue, the considerable responsiveness to concentric exercise (Figure 1) was indeed expected, if one considers there was an increase in blood pressure and glomerular pressure, resulting in turn, from a variety of factors such as dehydration, hyperfiltration of damaged enzymes and other proteins originated in the micro-lesions (Bassit et al., 2008; Egermann et al., 2003; Fallon et al., 2001; Noakes, 1987).

Uric acid is a metabolite that can function in the plasma as an antioxidant due to its capacity to neutralise the peroxy radical. The conversion of hypoxanthine into xanthine and uric acid, catalysed by xanthine oxidase, is associated with the production of superoxide anions contributed by ubiquinone from the electron transport chain (Chevion et al., 2003; Landray et al., 1998; Santos et al., 2004), and according to Speranza et al. (2007), uric acid is a good indicator of the stress caused by physical exercise. In this study, the serum uric acid concentrations rose in the animals after the uphill exercise as compared to the animals running downhill or horizontally for the same period of time (Figure 2), this is consistent with the expression of HSP70. The uric acid data may help here as it is also an indicator of AMP deamination, which increases with exercise intensity as the ATP resynthesis rates increase, xanthin oxidase suggesting that uphill exercise was the one most demanding ATP resynthesis, followed by the horizontal and downhill exercise, which did not differ between them, the lowest levels being found in the sedentary animals.

The animals undergoing concentric exercise were also those that showed the greatest increases in creatine kinase and lactate dehydrogenase activities, consistent with the highest concentrations of HSP70 observed in most of the tissues analysed. It should be pointed out, however, that this increase referred to the time course of the first 6 hours post-exercise and not the situation after 24 or 48 hours. As normally accepted, these two indicators reach their maximum levels at times of at least 24 hours post-exercise (Brancaccio et al., 2008).

In addition, it was interesting to observe that the metabolic stress, more associated with concentric exercise than with downhill exercise, seemed to elicit a greater response from the mechanism that produces the HSPs than the physical stress known to produce tissue injury, which typically occurs in the eccentric exercise. Finally, as limitation of this study we point the lack of mRNA response and histological analysis of the samples of the tissues here studied.

## Conclusion

The data suggest that HSP70 may respond differently to different types of muscle contraction independently of the workout, particularly considering the concentric and eccentric types at a time point of 6 hours after exercise. The concentric exercise appeared to be more HSP70 responsive than eccentric or concentric-eccentric exercise. The responsiveness did not remain restricted to the muscle, but that was extended to kidney. It was also clear that the

energy demand was not the only factor influencing the response of HSP70, since the soleus and the kidney of the animals in the downhill exercise group showed higher levels of HSP70 than the animals exercised horizontally.

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### Key points

- Exercise can induce increases in HSP70 in the lung, kidney and heart, and in the soleus and gastrocnemius muscles, probably due to systemic alterations such as hypoxia, increase in temperature and the production of free radicals.
- Predominantly concentric contractions (running uphill), seem to be the most efficient way of increasing the HSP70 concentrations in the different tissues, followed by eccentric contraction (downhill) and lastly the concentric-eccentric cycle (horizontal).
- The energy demand, already known to influence HSP70, appears not to be the only factor responsible for the response of these proteins, considering that for the kidney and the soleus muscle, downhill running was more efficient in raising the HSP70 response than horizontal running.
- Future research should explore the mechanisms by which the eccentric, concentric and eccentric-concentric contractions are capable of influencing the responses of the heat shock proteins, opening possibilities for increasing the levels of these proteins in desirable situations, such as to protect against excess free radicals or injuries.

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