Human resting extracellular heat shock protein 72 concentration decreases during the initial adaptation to exercise in a hot, humid environment

Helen C. Marshall, Richard A. Ferguson, and Myra A. Nimmo

Department of Applied Physiology, Faculty of Science, University of Strathclyde, Glasgow, G4 0QU, UK

Abstract Heat shock protein (Hsp) 72 is a cytosolic protein that also is present in the circulation. Extracellular Hsp72 (eHsp72) is inducible by exercise and is suggested to act as a danger signal to the immune system. The adaptive response of eHsp72 to repeated exercise-heat exposures in humans remains to be determined. An intracellular animal study found a reduced Hsp72 response, with no change in resting levels, during heat stress after a single day of passive heat acclimation. The current study therefore tested whether adaptations in human eHsp72 levels would similarly occur 24 hours after a single exercise-heat exposure. Seven males completed cycle exercise ($42.5\% \dot{V}_{O_{2peak}}$ for 2 hours) in a hot, humid environment (38°C , 60% relative humidity) on each of 2 consecutive days. Blood samples were obtained from an antecubital vein before exercise and 0 hours and 22 hours postexercise for the analysis of eHsp72. Exercise-heat stress resulted in enhanced eHsp72, with a similar absolute increase found on both days (day 1: 1.26 ng/mL [0.80 ng/mL]; day 2: 1.29 ng/mL [1.60 ng/mL]). Resting eHsp72 decreased from rest on day 1 to day 2's 22-hour postexercise sample ($P < 0.05$). It is suggested that the reduction in resting eHsp72 after 2 consecutive exercise-heat exposures is possibly due to an enhanced removal from the circulation, for either immunoregulatory functions, or for improved cellular stress tolerance in this initial, most stressful period of acclimation.

INTRODUCTION

Heat shock proteins (Hsps) are produced in response to a wide variety of stressors, including oxidative stress (Salo et al 1991; Smolka et al 2000), glucose deprivation (Febbraio et al 2002b), hyperthermia (Skidmore et al 1995; Kregel and Moseley 1996; Fehrenbach et al 2001), and exercise (Skidmore et al 1995; Liu et al 1999; Noble et al 1999; Fehrenbach et al 2000a, 2000b). The most thermosensitive and highly inducible Hsp belongs to the 70 kDa family (Mizzen and Welch 1988) and is commonly known as Hsp72. Primarily thought of as an intracellular protein with cytoprotective functions, it also has been suggested that Hsp72 has a functional role when released by a variety of cells (Febbraio et al 2002a; Broquet et al 2003;

Hunter-Lavin et al 2004; Lancaster et al 2004) into the circulation (eHsp72) (Pockley et al 1998). eHsp72 has been suggested to have an immunological function (Moseley 1998; Asea et al 2000; Bethke et al 2002; Campisi and Fleshner 2003; Fleshner et al 2003) with the in vitro attachment of eHsp72 to the surface of monocytes being found to stimulate the production of several cytokines (Asea et al 2000). Any change to the eHsp72 concentration may therefore have a subsequent effect on the cytokine cascade and resulting inflammatory response. In addition, elevated levels of eHsp72 have been found in patients with pulmonary and renal vascular disease (Wright et al 2000), and have been linked with a reduced risk of coronary artery disease (Zhu et al 2003). It appears, therefore, that eHsp72 is an important molecular marker about which little is known in conjunction with exercise-heat stress in humans.

Prolonged exercise in a temperate environment has

Correspondence to: Myra A. Nimmo, Tel: +44 (141) 548 5791; Fax: +44 (141) 548 5792; E-mail: m.a.nimmo@strath.ac.uk.

Received 19 August 2005; Revised 20 November 2005; Accepted 28 November 2005.

been shown to be an adequate stressor to enhance the concentration of eHsp72 (Walsh et al 2001; Lancaster et al 2004; Fehrenbach et al 2005), the magnitude of which is both exercise-intensity and duration dependent (Fehrenbach et al 2005). After acute exercise, the concentration of eHsp72 has been shown to return to resting levels within 24 hours (Walsh et al 2001; Fehrenbach et al 2005). An enhanced level of stress, as measured by intracellular Hsp72 levels in human monocytes, has been found after prolonged exercise in a high ambient temperature (Fehrenbach et al 2001); however, the effect of exercise-heat stress on eHsp72 has yet to be shown.

Repeated exposures to exercise-heat stress result in many adaptations, the combination of which ultimately improves thermal tolerance and athletic performance. During acute heat stress, before the physiological adaptations of heat acclimation, the strain imposed on the human body is reduced through enhanced Hsp72 synthesis (Moseley 1997). An *in vitro* study on mammalian cell lines found an initial heat shock (43°C, 1.5 hours) to result in an overall reduction in the amount of Hsp72 synthesis during a subsequent heat shock (45°C, 30 minutes) (Mizzen and Welch 1988). This is supported by an *in vivo* study in rats that showed a single day of passive heat acclimation to result in a blunted rat heart Hsp72 increase after heat stress (41°C, 2 hours) compared with no prior acclimation (Maloyan et al 1999). However, the resting Hsp72 level was found to remain unchanged in the initial stage (1 or 2 days) of passive heat acclimation, in contrast to the enhanced resting concentration found after long-term heat acclimation (30 days) (Maloyan et al 1999). To our knowledge, however, no literature is available on the adaptation of resting eHsp72 or the eHsp72 response to the early stages of heat acclimation in humans.

It is therefore hypothesized that eHsp72 will increase after an acute exercise-heat stress with a blunted response during a second consecutive, identical exposure. However, no change in resting eHsp72 is expected.

MATERIALS AND METHODS

Subjects

Seven male subjects (mean [SD]; age, height, body mass, and peak oxygen uptake [$\dot{V}_{O_{2peak}}$]: 26 years [6 years]; 1.78 m [0.11 m]; 83.1 kg [13.5 kg]; 4.03 L/min [0.51 L/min]) volunteered for this study, which was carried out over the winter period (average daily temperature of 7°C). The subjects had undertaken no formal acclimation or spent any time in a hot environment over the preceding 2 months. The study was conducted in accordance with the Declaration of Helsinki, was approved by the Local Ethics Committee, and subjects provided written informed con-

sent. Subjects visited the laboratory at a consistent time of day for each visit.

Experimental protocol

Preliminary tests

A submaximal and a maximal exercise test were carried out 4 days before the familiarization to determine the workload required to elicit 38% $\dot{V}_{O_{2peak}}$. The submaximal test involved four 5-minute bouts of exercise (49, 63, 77, and 91 watts [W]) on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) with 5-minute rest between each workload. Pedal cadence was maintained at 70 revolutions/min and expired gases were measured continuously using an automated gas analysis system (Oxycon Gamma, Mijnhardt, The Netherlands). After a 15-minute rest, a continuous, incremental cycling test to volitional exhaustion was performed. The initial workload was set at 70 W, with work rate increasing by 35 W every 2 minutes until 6 minutes, and every minute thereafter until exhaustion. Expired gases were measured continuously to derive $\dot{V}_{O_{2peak}}$.

Trial preparation

Subjects abstained from caffeine, tobacco, and alcohol ingestion and refrained from strenuous physical activity for 48 hours before testing. They recorded their dietary intake and physical activity for 48 hours before the familiarization trial and were then asked to replicate these before the remaining trials. The evening before experimental testing, subjects consumed water at 10 mL/kg body mass. Subjects consumed their last meal at least 6 hours before testing, followed by a meal-replacement drink (Complan, H.J. Heinz Co. Ltd., Hayes, UK) 2 hours before reporting to the laboratory. The drink was reconstituted by adding semiskimmed milk at 2.86 mL/kg body mass to powder at 0.81 g/kg body mass. Constituents of the drink based on the amount ingested by a 70-kg subject were—energy: 1057 kJ; protein: 8.8 g; carbohydrates: 34.8 g (of which sugars: 26.3 g); fat: 8.5 g (of which saturates: 3.8 g); and sodium: 0.13 g. Along with the drink the subjects consumed water at 10 mL/kg body mass minus the volume of milk in the meal-replacement drink.

Familiarization

At least 4 days after the preliminary test and 1 week before the main trials, an initial trial was undertaken to familiarize subjects with the investigative procedures, verify $\dot{V}_{O_2}$, and establish sweat loss, which was to be replaced during the subsequent euhydrated exercise trials. Because eHsp72 levels have been shown to fall back to

resting levels 24 hours after exercise stress (Walsh et al 2001; Fehrenbach et al 2005), it was believed that even after exercise combined with heat stress, 7 days would be sufficient for levels to return to resting values with no carryover from familiarization to main experimental trial.

On arrival at the laboratory subjects voided and euhydration status was confirmed by the measurement of urine specific gravity (USG < 1.020, Multistix SG, Bayer Diagnostics, Newburg, UK) (Armstrong et al 1994; Hoffman et al 1994). Nude body mass (NBM) was recorded and a rectal thermistor was inserted. Subjects entered a thermoneutral environment (mean [SD]; 26.7°C [0.5°C]; 24.3% [4.0%] relative humidity [RH]) where they remained seated for 30 minutes. Resting rectal temperature (T_{re}) was recorded before entering the heat chamber (mean [SD]; 38.0°C [0.1°C]; 60.0% [0.3%] RH; wind 1.18 m/s [0.02 m/s]), where exercise commenced immediately. This involved 2 hours of cycling at a workload initially corresponding to 38% $\dot{V}_{O_{2peak}}$, which equated to cycling, on average, at 42.5% (3.6%) of $\dot{V}_{O_{2peak}}$ over the course of the 2 hours, because of $\dot{V}_{O_{2peak}}$ drift in the heat. Subjects were given a 2-minute break every 30 minutes in order to walk or stretch. On completion of exercise subjects towel dried and NBM was remeasured, allowing the estimation of whole-body sweat loss.

Experimental trial

Subjects visited the laboratory on 3 consecutive days. The first 2 visits (days 1 and 2) involved exercise-heat stress and a resting blood sample was taken on the third visit. The protocol and the environmental conditions were identical to those of the familiarization trial. In addition, before the 30-minute rest period in the thermoneutral environment, a cannula was inserted into an antecubital vein while the subject was in the supine position. Arterialized venous blood samples were taken pre-exercise and immediately post- and 22 hours postexercise. The 22-hour postexercise sample of day 2 was taken on the third visit. Day 1's 22-hour postexercise sample also served as the resting sample on day 2. During exercise, sweat loss, as calculated from the familiarization trial, was replaced with a 20 mmol/L sodium chloride solution (Gisolfi et al 1990) every 15 minutes (Gisolfi and Duchman 1992). Provision was weighted toward the first hour of exercise; 20% (0 minutes), 20% (15 minutes), 15% (30 minutes), 15% (45 minutes), 15% (60 minutes), 5% (75 minutes), 5% (90 minutes), and 5% (105 minutes). The initial 20% consumed in the present study equated to 400–450 mL, after which it was attempted to maintain this volume in the stomach with regular fluid ingestion to promote gastric emptying (Costill and Saltin 1974).

Table 1 Urine specific gravity (USG) and nude body mass (NBM) on arrival at the laboratory

Parameter	Day		
	1	2	3
USG	1.014 (0.006)	1.011 (0.005)	1.012 (0.005)
NBM (kg)	83.2 (12.9)	83.7 (12.9)	83.4 (13.1)

Values are mean (SD).

Measurements

Core temperature

A rectal thermistor (Edale Instruments Ltd., Roedoure, UK), inserted 10 cm beyond the anal sphincter, was used for the measurement of T_{re} . Temperature was monitored via a 1000 series 8-bit Squirrel data logger (Type 1002, Grants, Cambridge, UK).

Blood sampling and analysis

Blood was collected from the indwelling catheter (Insyte 20 G, Becton Dickinson, Madrid, Spain) via vacutainers. Hematocrit (Hct) was determined immediately from whole-blood in triplicate, using the microcapillary technique. Serum was obtained from an SST vacutainer (BD Vacutainer Systems, Plymouth, UK) by centrifugation and frozen (−20°C) for later eHsp72 analysis. An enzyme-linked immunosorbent assay was used to determine serum Hsp72 concentration in duplicate, following the instructions of the manufacturer (StressGen Biotechnologies [EKS-700], Victoria, BC, Canada).

Statistical analyses

Data are presented as mean (standard deviation). A 2-factor (trial by time) repeated-measures analysis of variance (ANOVA) was performed to ascertain any treatment or exercise effects on T_{re} and eHsp72. A repeated-measures 1-way ANOVA was used to analyze resting eHsp72, Hct, NBM, and USG to determine any change across the 3 days. The change in eHsp72 on both days was analyzed by paired-sample Student's *t*-test. Tukey's honestly significant difference post hoc analysis was used to locate differences when the ANOVA revealed a significant difference. The significance level was set at $P < 0.05$.

RESULTS

Subjects were euhydrated with no difference in NBM or USG upon arrival at the laboratory on all 3 days (Table 1).

Exercise in the heat resulted in an increased T_{re} on both days (day 1: rest 37.0°C [0.2°C], exercise 38.2°C [0.3°C]; day 2: rest 36.9°C [0.2°C], exercise 38.2°C [0.3°C]; $P <$

Fig 1. Extracellular heat shock protein 72 (eHsp72) levels (mean [standard deviation]) during the initial stage of exercise-heat acclimation. +, significant difference from rest; *, significant difference from day 1 rest.

0.001), with no difference in rest or exercise values between days.

Exercise-heat stress increased Hct by the same amount on both days (day 1: 1.7% [1.1%]; day 2: 1.7% [1.4%]). Over the 3 days, resting Hct decreased by 5.0% (1.4%) ($P < 0.05$) (day 1: 0.42 [0.03] ratio; day 3: 0.40 [0.02] ratio).

During exercise, eHsp72 increased by a similar amount on both days (day 1: 1.26 ng/mL [0.80 ng/mL]; day 2: 1.29 ng/mL [1.60 ng/mL]). Resting eHsp72 levels decreased progressively in 6 of 7 subjects (Fig 1), becoming significant ($P < 0.05$) by day 3 (Fig 2).

In order to determine the effect of a single passive-heat stress on eHsp72 in the experimental environment, a sub-study was conducted on 5 subjects (mean [SD]; age, height, and body mass: 25 years [5 years]; 1.85 m [0.11 m]; 79.2 kg [15.6 kg]). Two hours of passive heating (38.0 °C [0.1°C], 60.0% [0.2%] RH) was found to have no effect on the eHsp72 concentration (baseline: 1.12 ng/mL [0.98 ng/mL]; 2 hours of passive heating: 1.16 ng/mL [0.75 ng/mL]).

Fig 2. Individual subjects' resting extracellular heat shock protein 72 (eHsp72) levels during 2 consecutive exercise-heat exposures. Day 3 value is day 2's 22-hour postexercise sample.

DISCUSSION

The effect of both acute and repeated exercise-heat stress on human eHsp72 levels has been studied for the first time. A reduced resting eHsp72 concentration was an early adaptation to repeated exercise-heat exposures. The stress imposed during each exercise-heat bout resulted in an enhanced eHsp72 concentration; however, this elevation in eHsp72 was not altered by an identical exposure 24 hours previously.

The possibility that the decline in eHsp72 is due to consumption of large volumes of fluid is unlikely because the volume consumed was matched to the loss during exercise, and resting USG did not differ between days. However, resting Hct decreased (5%) after 2 repeated exercise-heat exposures because of a possible plasma volume expansion. No plasma volume data are available from this study and comparison with the literature is difficult because plasma volume expansion is dependent on both the sodium content of the diet (Armstrong et al 1993) and the fluid consumed during exercise (Costill et al 1975). However, previous work in our laboratory found a 6% expansion by the third day of exercise in humid heat (H.C. Marshall and M.A. Nimmo, unpublished data). The 36% reduction in eHsp72 over the same period suggests that the plasma volume expansion was not the sole factor determining the eHsp72 decrease. This reduction in eHsp72 was found despite an increased level in a single outlying subject (Fig 1).

The concentration of eHsp72 is a result of the balance between its release into, and removal from, the circulation. A contribution to the reduction could have arisen from a greater proportion being attached to the surface of monocytes, eliciting an immunological or inflammatory function (Asea et al 2000). A further function for eHsp72, and a potential reason for its reduction, is that cells less capable of synthesizing their own Hsp72, such as neuroblastoma cells, can absorb and utilize Hsps in their surrounding media (Guzhova et al 2001). A mechanism for receptor-facilitated transmembrane transport of Hsp or Hsp-peptide complexes from the plasma membrane into the cytosol has previously been suggested (Castellino et al 2000; Basu et al 2001). During the initial adaptation to exercise-heat stress, it is possible that certain cells are taking up eHsp72 to improve their stress tolerance, without a concurrent increase in cellular release.

The reduction in resting eHsp72 levels, in addition to the increased utilization described above, could be attributed to a reduced intracellular production or a reduced cellular release. However, this seems less likely because Fehrenbach et al (2001) found human monocyte and granulocyte Hsp72 protein levels to peak 24 hours after an acute exercise-heat stress (60-minute run at 90% indi-

vidual anaerobic threshold; 28°C, 50% RH) and remain elevated above baseline 48 hours postexercise. Examination of these data suggests that the intracellular level of Hsp72, at least in these cell types, is not a limiting factor in its release into the circulation. In addition, an in vitro study has indicated that the release of Hsp72 from epithelial cells is manifested through a system of lipid rafts and when heat-stimulated, translocation of Hsp70 to these rafts is enhanced (Broquet et al 2003). More recently, it has been shown that the release of Hsp70 from peripheral blood mononuclear cells is not via a lipid-raft-dependent pathway, but via exosomes, with an elevated exosome Hsp70 content upon heat shock (Lancaster and Febbraio 2005). Therefore, it appears that a reduced intracellular production or cellular release are unlikely to be responsible for the lowered resting eHsp72 levels in the present study.

The enhanced eHsp72 concentration after exercise-heat stress is supported by previous findings in less stressful temperate environments (Walsh et al 2001; Lancaster et al 2004; Fehrenbach et al 2005). Although an enhanced eHsp72 concentration was found after both stress exposures, a blunted response during the second exposure was not found, contrasting with the literature. This may be due to the differing sample site (Mizzen and Welch 1988; Maloyan et al 1999), a lower initial heat stress (Mizzen and Welch 1988), or differing modes of heat acclimation (Maloyan et al 1999). Animal studies have suggested that the release of endogenous Hsp72 during exposure to stress is mediated in part by catecholamines via stimulation of an α_1 -adrenergic receptor-mediated pathway (Fleshner et al 2004; Johnson et al 2005). Norepinephrine is likely the primary catecholamine responsible for Hsp72 release because, compared with epinephrine, it has a higher affinity for α_1 -adrenergic receptors (Pocock and Richards 2004) and the depletion of epinephrine (95–99%) was found to have no effect on the release of Hsp72 (Johnson et al 2005). Norepinephrine is well known to increase during prolonged exercise in the heat (Melin et al 1988; Hoffman et al 1994; Brenner et al 1997) and may be linked with the enhanced eHsp72 concentration during exercise-heat stress in the present study. Acclimation has been found to have no effect on norepinephrine at rest or during exercise (Febbraio et al 1994; Nielsen et al 1997), providing a possible reason for the similar eHsp72 response during both days. However, it suggests that the reduction in resting eHsp72 found in the present study is due to factors other than norepinephrine.

In conclusion, 2 consecutive exercise-heat exposures resulted in a decreased resting concentration of eHsp72 in humans. This was possibly due to an enhanced role in the immune response or an enhanced cellular uptake by cells unable to produce Hsps, providing an improved

stress tolerance in this initial, most stressful period of acclimation. The design of the experiment does not allow us to determine whether the responsible factor for the eHsp72 decline was heat, exercise, or a combination of both. Further research is required to determine the exact cause of the lowered resting eHsp72.

ACKNOWLEDGMENTS

We are grateful to Glasgow City Council, Glasgow School of Sport, and the West of Scotland Institute of Sport for financial support for this study.

REFERENCES

- Armstrong LE, Hubbard RW, Askew EW, De Luca JP, O'Brien C, Pasqualicchio A, Francesconi RP. 1993. Responses to moderate and low sodium diets during exercise-heat acclimation. *Int J Sport Nutr* 3: 207–221.
- Armstrong LE, Maresh CM, Castellani JW, Bergeron MF, Kenefick RW, LaGasse KE, Riebe D. 1994. Urinary indices of hydration status. *Int J Sport Nutr* 4: 265–279.
- Asea A, Kraeft S-K, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. 2000. Hsp70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 6: 435–442.
- Basu S, Binder RJ, Ramalingam T, Srivastava PK. 2001. CD91 is a common receptor for heat shock proteins gp96, Hsp90, Hsp70, and calreticulin. *Immunity* 14: 303–313.
- Bethke K, Staib F, Distler M, Schmitt U, Jonuleit H, Enk AH, Galle PR, Heike M. 2002. Different efficiency of heat shock proteins (Hsp) to activate human monocytes and dendritic cells: superiority of Hsp60. *J Immunol* 169: 6141–6148.
- Brenner IKM, Zamecnik J, Shek PN, Shephard RJ. 1997. The impact of heat exposure and repeated exercise on circulating stress hormones. *Eur J Appl Physiol* 76: 445–454.
- Broquet AH, Thomas G, Masliah J, Trugnan G, Bachelet M. 2003. Expression of the molecular chaperone Hsp72 in detergent-resistant microdomains correlates with its membrane delivery and release. *J Biol Chem* 278: 21601–21606.
- Campisi J, Fleshner M. 2003. Role of extracellular Hsp72 in acute stress-induced potentiation of innate immunity in active rats. *J Appl Physiol* 94: 43–52.
- Castellino F, Boucher PE, Eichelberg K, Mayhew M, Rothman JE, Houghton AN, Germain RN. 2000. Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. *J Exp Med* 191: 1957–1964.
- Costill DL, Coté R, Miller E, Miller T, Wynder S. 1975. Water and electrolyte replacement during repeated days of work in the heat. *Aviat Space Environ Med* 46: 795–800.
- Costill DL, Saltin B. 1974. Factors limiting gastric emptying during rest and exercise. *J Appl Physiol* 37: 679–683.
- Febbraio M, Ott P, Nielsen HB, Steensberg A, Keller C, Krstrup P, Secher NH, Pedersen BK. 2002a. Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. *J Physiol* 544: 957–962.
- Febbraio M, Snow RJ, Hargreaves M, Stathis CG, Martin IK, Carey MF. 1994. Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. *J Appl Physiol* 76: 589–597.
- Febbraio M, Steensberg A, Walsh R, Koukoulas I, van Hall G, Saltin B, Pedersen BK. 2002b. Reduced glycogen availability is asso-

- ciated with an elevation in Hsp72 in contracting human skeletal muscle. *J Physiol* 538: 911–917.
- Fehrenbach E, Niess AM, Veith R, Dickhuth H-H, Northoff H. 2001. Changes of Hsp72-expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. *J Leuk Biol* 69: 747–754.
- Fehrenbach E, Niess AM, Schlotz E, Passek F, Dickhuth H-H, Northoff H. 2000a. Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *J Appl Physiol* 89: 704–710.
- Fehrenbach E, Niess AM, Voelker K, Northoff H, Mooren FC. 2005. Exercise intensity and duration affect blood soluble Hsp72. *Int J Sports Med* 26: 552–557.
- Fehrenbach E, Passek F, Niess AM, Pohla H, Weinstock C, Dickhuth H-H, Northoff H. 2000b. Hsp expression in human leukocytes is modulated by endurance exercise. *Med Sci Sports Exerc* 32: 592–600.
- Fleshner M, Campisi J, Amiri L, Diamond DM. 2004. Cat exposure induces both intra- and extracellular Hsp72: the role of adrenal hormones. *Psychoneuroendocrinology* 29: 1142–1152.
- Fleshner M, Campisi J, Johnson JD. 2003. Can exercise stress facilitate innate immunity? A functional role for stress-induced extracellular Hsp72. *Exerc Immunol Rev* 9: 6–24.
- Gisolfi CV, Duchman SM. 1992. Guidelines for optimal replacement beverages for different athletic events. *Med Sci Sports Exerc* 24: 679–687.
- Gisolfi CV, Summers RW, Schedl HP. 1990. Intestinal absorption of fluids during rest and exercise. In: *Fluid Homeostasis During Exercise, Perspectives in Exercise Science and Sports Medicine*, ed Gisolfi CV, Lamb DR. WBC Brown & Benchmark, Dubuque, IA, 129–180.
- Guzhova I, Kislyakova K, Moskaliyova O, Fridlanskaya I, Tytell M, Cheetham M, Margulis B. 2001. In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. *Brain Res* 914: 66–73.
- Hoffman JR, Maresh CM, Armstrong LE, et al. 1994. Effects of hydration state on plasma testosterone, cortisol and catecholamine concentrations before and during mild exercise at elevated temperature. *Eur J Appl Physiol* 69: 294–300.
- Hunter-Lavin C, Davies EL, Bacelar MMFVG, Marshall MJ, Andrew SM, Williams JHH. 2004. Hsp70 release from peripheral blood mononuclear cells. *Biochem Biophys Res Commun* 324: 511–517.
- Johnson JD, Campisi J, Sharkey CM, Kennedy S, Nickerson M, Fleshner M. 2005. Adrenergic receptors mediate stress-induced elevations in extracellular Hsp72. *J Appl Physiol* 99: 1789–1795.
- Kregel KC, Moseley PL. 1996. Differential effects of exercise and heat stress on liver Hsp70 accumulation with aging. *J Appl Physiol* 80: 547–551.
- Lancaster GI, Febbraio MA. 2005. Exosome-dependent trafficking of Hsp70: a novel secretory pathway for cellular stress proteins. *J Biol Chem* 280: 23349–23355.
- Lancaster GI, Moller K, Nielsen B, Secher NH, Febbraio MA, Nybo L. 2004. Exercise induces the release of heat shock protein 72 from the human brain in vivo. *Cell Stress Chaperones* 9: 276–280.
- Liu Y, Mayr S, Opitz-Gress A, Zeller C, Lormes W, Baur S, Lehmann M, Steinacker JM. 1999. Human skeletal muscle Hsp70 response to training in highly trained rowers. *J Appl Physiol* 86: 101–104.
- Maloyan A, Palmon A, Horowitz M. 1999. Heat acclimation increases the basal Hsp72 level and alters its production dynamics during heat stress. *Am J Physiol* 276: R1506–R1515.
- Melin B, Cure M, Pequignot JM, Bittel J. 1988. Body temperature and plasma prolactin and norepinephrine relationships during exercise in a warm environment: effect of dehydration. *Eur J Appl Physiol* 58: 146–151.
- Mizzen LA, Welch WJ. 1988. Characterization of thermotolerant cell. I. Effects of protein synthesis activity and the regulation of heat-shock protein 70 expression. *J Cell Biol* 106: 1105–1116.
- Moseley PL. 1997. Heat shock proteins and heat adaptation of the whole organism. *J Appl Physiol* 83: 1413–1417.
- Moseley PL. 1998. Heat shock proteins and the inflammatory response. *Ann N Y Acad Sci* 29: 206–213.
- Nielsen B, Strange S, Christensen NJ, Warberg J, Saltin B. 1997. Acute and adaptive responses in humans to exercise in a warm, humid environment. *Pflugers Arch* 434: 49–56.
- Noble EG, Moraska A, Mazzeo RS, Roth DA, Olsson MC, Moore RL, Fleshner M. 1999. Differential expression of stress proteins in rat myocardium after free wheel or treadmill run training. *J Appl Physiol* 86: 1696–1701.
- Pockley AG, Shepherd J, Corton JM. 1998. Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. *Immunol Invest* 27: 367–377.
- Pocock G, Richards CD. 2004. The hormonal regulation of the body. In: *Human Physiology—The Basis of Medicine*. Oxford University Press, New York, NY, 211–250.
- Salo DC, Donovan CM, Davies KJA. 1991. Hsp70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. *Free Radic Biol Med* 11: 239–246.
- Skidmore R, Gutierrez JA, Guerriero V Jr, Kregel KC. 1995. Hsp70 induction during exercise and heat stress in rats: role of internal temperature. *Am J Physiol* 268: R92–R97.
- Smolka MB, Zoppi CC, Alves AA, Silveira LR, Marangoni S, Pereira-da-silva L, Novello JC, Macedo DV. 2000. Hsp72 as a complementary protection against oxidative stress induced by exercise in the soleus muscle of rats. *Am J Physiol* 279: R1539–R1545.
- Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio M. 2001. Exercise increases serum Hsp72 in humans. *Cell Stress Chaperones* 6: 386–393.
- Wright BH, Corton JM, El-Nahas AM, Wood RFM, Pockley AG. 2000. Elevated levels of circulating heat shock protein 70 (Hsp70) in peripheral and renal vascular disease. *Heart Vessels* 15: 18–22.
- Zhu J, Quyyumi AA, Wu H, et al. 2003. Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease. *Arterioscler Thromb Vasc Biol* 23: 1055–1059.