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ORIGINAL ARTICLE

Variation in basal heat shock protein 70 is correlated to core temperature in human subjects

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Abstract Heat shock proteins are highly conserved proteins and play an important chaperone role in aiding the folding of nascent proteins within cells. The heat shock protein response to various stressors, both in vitro and in vivo, is well characterised. However, basal levels of heat shock protein 70 (Hsp70) have not previously been investigated. Monocyte-expressed Hsp70 was determined every 4 h, over a 24 h time period, in 17 healthy male subjects (177 ± 6.4 cm, 75.7 ± 10.9 kg, 19.8 ± 4.3 years) within a temperature and activity controlled environment. Core temperature was measured at 5-min intervals during the 24 h period. Hsp70 showed significant diurnal variation ($F = 7.4$; $p < 0.001$), demonstrating peaks at 0900 and 2100 hours, and a nadir at 05.00. Core temperature followed a similar temporal trend (range = 35.96–38.10°C) and was significantly correlated with Hsp70 expression ($r_s = 0.44$; $p < 0.001$). These findings suggest a high responsiveness of Hsp70 expression in monocytes to slight variations in core temperature.

Keywords Hsp70 · Diurnal variation · Core temperature

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Introduction

During both normal and stress situations (for example exercise), cells are constantly in need of molecular chaperones for their survival. Heat shock protein 70 (Hsp70) is known to be expressed in response to a wide variety of stressors (for review see (Kregel 2002), particularly heat (Locke and Noble 1995). A variety of cells and organs express Hsp70 upon heat shock (Leger et al. 2000; Lovell et al. 2006; Staib et al. 2007) as well as peripheral blood cells (Oehler et al. 2001). Hsp70 plays a role in preventing and degrading damaged proteins (Bukau and Horwich 1998), and is also important to the immune system where it acts as a danger signal (Horn et al. 2007) and a cytokine (Asea et al. 2000). It is therefore expected that the levels of Hsp70 will vary dependent on the homeostatic environment within the body. Hsp70 also plays an important role in unstressed cells where, for example, it orchestrates de novo protein synthesis (Beckmann et al. 1990) and has the ability to rescue cells from programmed death by interrupting the apoptotic cascade (Garrido et al. 2001). This would further indicate that there is a constant basal level of Hsp70, albeit a low level. Several studies have shown the upregulation of Hsp70 in vitro by heat shock (Oehler et al. 2001; Sonna et al. 2002), however these studies exposed cells to relatively high temperatures (39–45°C) and do not, therefore, give an indication to the sensitivity of the upregulation of Hsp70 induction that may occur under normal physiological conditions.

Diurnal variations are well known in terms of whole body homeostasis. Circadian variations in leukocyte activation and endothelial function, for example, have been described (Bridges et al. 1991, 1992). Furthermore, a group of 10 healthy male volunteers were studied for 24 h in respect of soluble levels of adhesion molecules, intracellular adhesion

molecule-1 (ICAM-1) and E-selectin (Maple et al. 1998). Results showed a statistically significant circadian variation in these soluble markers ($p < 0.0001$).Soluble ICAM-1 peaked at midday and decreased to a minimum at 0400 hours, whereas soluble E-selectin concentration peaked again at midday but decreased to a minimum at midnight (Maple et al. 1998).

A circadian rhythm also exists in core temperature, produced by vascular tone and is promoted by other factors such as behaviour, i.e. going to sleep, which reduces cardiovascular stress and reduces sympathetic tone whilst in a supine position (Refinetti 1998; Kräuchi and Wirz-Justice 2001). Core temperature rises most rapidly in the morning and decreases most rapidly in the late evening and is accompanied by heat loss from distal limbs. Furthermore, skin temperature and blood flow in these limbs have been shown to peak in late evening and trough in the morning (Waterhouse et al. 2005).

The aims for this study were: (1) to identify any diurnal variation in monocyte expression of Hsp70 and (2) to investigate if the monocyte expression of Hsp70 is correlated to the circadian rhythm observed in core temperature.

Material and method

Subjects

Seventeen non-smoking male subjects participated in the study (177 ± 6.4 cm, 75.7 ± 10.9 kg, 19.8 ± 4.3 years). Smoking may trigger Hsp70 expression through oxidative stress (Anbarasi et al. 2006). Prior to data collection, none of the subjects had been trained excessively or had resided in a hot climate (>30°C) over the preceding 6 weeks. The subjects were moderately trained (running, cycling, sporting participation) with an average training load of 5.9 ± 2.2 h/week. All subjects provided written informed consent in accordance with the departmental and university ethical procedures and following the principles outlined in the Declaration of Helsinki.

Experimental protocol

The subjects abstained from alcohol, caffeine and exercise 48 h prior to testing. Each subject was given a temperature sensor pill (CorTemp™, HQ Inc., Palmetto, FL) to swallow at 0700 hours the morning of the testing to allow adequate time for motility into the small intestine and to minimize the effects of the ingestion of cold liquids on temperature readings (O'Brien et al. 1998). On the day of testing, the subjects reported to a temperature-controlled laboratory (average WBGT 18.1 ± 0.9°C) at 0800 hours for acclimatisation. All subjects were instructed to refrain from any rigorous activities during the study. Furthermore, they were instructed to go to bed at 2300 hours and stay in bed until 0700 hours the morning after.

Dietary intake was equivalent between subjects and food was provided at 0930, 1330 and 1800 hours. Blood samples were taken in the same order every 4 h from 0900 to 0900 hours the following day. Blood samples were drawn by a standard venipuncture technique from the antecubital vein, after 5 min in a supine position, into potassium EDTA Vacuette tubes (Vacuette®, Greiner Bio-one, UK) for subsequent measurements of monocyte Hsp70. All samples were processed immediately.

Heart rate was sampled and recorded every 5 s throughout the 24 h period on eight randomly selected subjects (Team System, Polar Electro, Finland). Core temperature was recorded at 5 min intervals during the 24 h period (CorTemp™ Data Recorder HT150016, HQ Inc, Palmetto, FL).

Flow cytometry

Monocyte Hsp70 assay

Flow cytometry was used to measure intracellular Hsp70 as it has been shown to be more sensitive than western blotting (Bachelet et al. 1998). We measured the expression of monocyte Hsp70 since it is known that these cells produce the greatest amount of Hsp70 within the peripheral blood cells when exposed to heat (Bachelet et al. 1998). Furthermore, we found no increase in Hsp70 in either neutrophils or lymphocytes (data not shown). Whole blood (100 μL) from EDTA tubes was transferred into a 2 mL red blood cell lysing buffer (Erythrolyse, AbD Serotec, UK). The white blood cells were then washed, fixed and permeablised according to the manufacturer's protocol (Leucoperm, AbD Serotec). Either anti-Hsp70:FITC (SPA-810, Stressgen) or isotype matched negative control: FITC (AbD Serotec) antibodies (4 μL) were added together with the permeabilisation buffer and incubated for 30 min. The samples were then washed in PBS and analysed using a BDFACSCalibur (BD Biosciences). A total of 10,000 cells were counted and monocytes subsequently gated and analysed for Hsp70 expression using CELLQuest software (BD Biosciences), taking the increase in mean fluorescence intensity (MFI) from isotype matched negative control.

Statistical analyses

Differences in Hsp70 concentration over time were analysed with a one-way repeated measures analysis of variance using Minitab® version 14.2 (Minitab Inc., State College, PA). Tukey tests were subsequently used to compare the Hsp70 concentration at the first time point

(0900 hours) with each of the other time points. The relationships between Hsp70 expression and core temperature and between Hsp70 expression and heart rate were determined using Spearman rank correlation coefficients using SPSS® for Windows version 16.0 (SPSS Inc., Chicago, IL). Several outliers were observed and since these were checked and verified as valid, the correlations were reported with and without the outliers. Probability significance less than 0.05 were considered statistically significant.

Results

Core temperature

The mean core temperature for the subjects at the time of blood sampling is shown in Fig. 1. One temperature pill was lost due to secretion before the 2100 hours recording. The core temperature was in agreement with data reported previously (Reilly et al. 2007), showing a decrease in the evening with the lowest values during the early morning hours. Core temperature, Hsp70 and to a lesser extent heart rate did not increase to the same temperature at 0900 hours as the previous day. This is probably due to the fact that the subjects had no physical activity the second morning of the trial, being at rest, already at the trial location.

Heart rate

Heart rate data monitored over 24 h is shown in Fig. 2. Our findings correlate with previous studies (Millar-Craig et al. 1978; White 2007). The heart rate data was time-averaged over 5 min intervals and the lowest recoded heart rate from each hour was taken to represent resting heart rate during that hour. A correlation of heart rate and Hsp70 was also observed ($r_s = 0.30$, $p = 0.03$, Fig. 3; $r_s = 0.23$; $p = 0.11$ after the two outliers were removed).

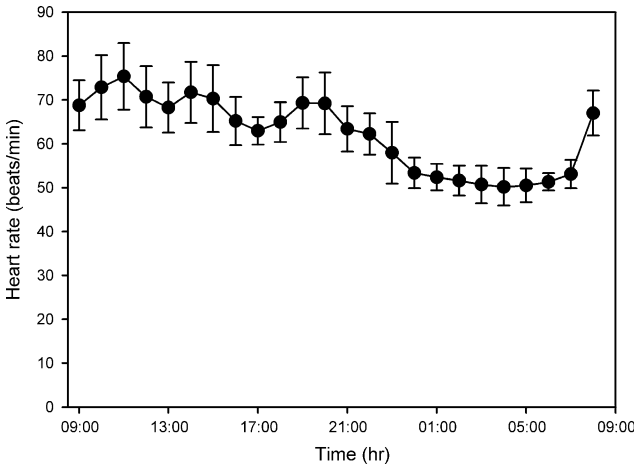

Fig. 2 Mean heart rate over the 24-hr experimental period ($n = 8$). *Error bars* represent the standard deviation of the mean. Outliers in *grey*

Hsp70

There was a statistically significant time-of-day effect for Hsp70 concentration ($F = 7.4$; $p < 0.001$, Table 1 and Fig. 1). Table 1 shows that a paired difference between the Hsp70 concentrations at 0900 hours was statistically significant from all other time points except 2100 hours. Furthermore, there was a significant positive correlation between core temperature and Hsp70 expression ($r_s = 0.44$, $p < 0.001$, Fig. 4; $r_s = 0.41$, $p < 0.001$ after three outliers were removed).

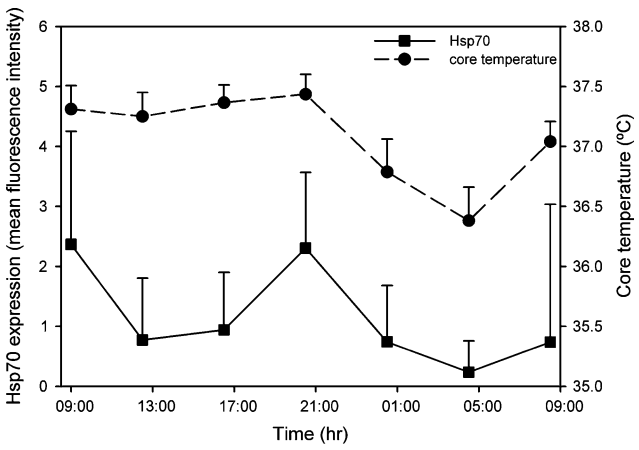

Fig. 1 Mean Hsp70 expression and mean core temperature over the 24-h experimental period ($n = 17$). *Error bars* represent the standard deviation of the mean

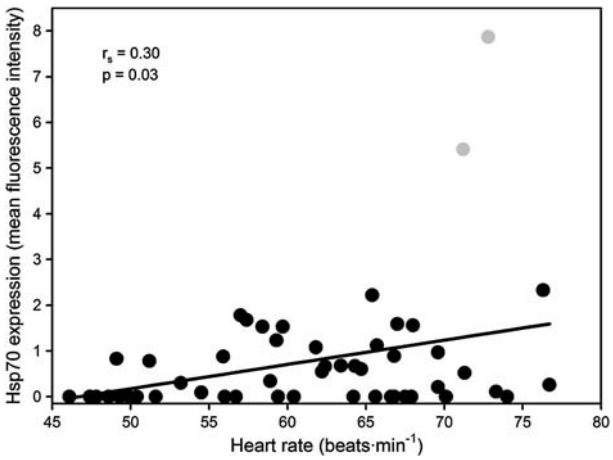

Fig. 3 Scatterplot of Hsp70 expression against heart rate at times 0900, 1300, 1700, 2100, 0100, 0500 and 0900 hours ($n = 8$). The *solid black diagonal line* is the least *squares line* of best fit. r_s = Spearman rank correlation coefficient. Outliers in *grey*

Table 1 Paired comparisons of the mean hsp70 concentration for seven time points during a 24-h period

Comparison (times) (hours)	Mean difference (MFI)	95% confidence interval (MFI)	Adjusted *p* value
0900–1300	−1.57	−2.81, −0.34	0.004
0900–1700	−1.45	−2.70, −0.19	0.01
0900–2100	−0.04	−1.28, 1.19	1.0
0900–0100	−1.54	−2.85, −2.23	0.01
0900–0500	−2.01	−3.30, −0.73	0.0002
0900–0900	−0.56	−2.93, −0.19	0.02

MFI mean fluorescence intensity

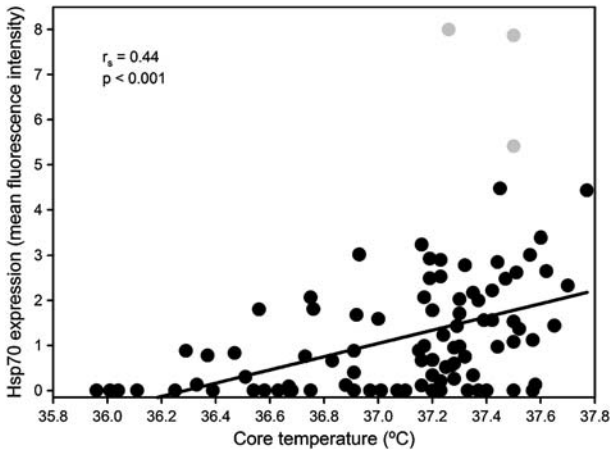

Fig. 4 Scatterplot of Hsp70 expression against core temperature at times 0900, 1300, 1700, 2100, 0100, 0500 and 0900 hours ($n = 17$). The *solid black diagonal line* is the least *squares line* of best fit. r_s = Spearman rank correlation coefficient

Discussion

This study investigated the diurnal variation in monocyte Hsp70 expression and its relationship with core temperature. We found that monocyte Hsp70 expression followed a statistically significant time-of-day variation. We also found that there was a significant positive correlation between core temperature and Hsp70 expression.

Hsp70 is the most inducible and abundant of the heat shock proteins. Its induction by stressors such as heat (Ritossa 1962), exercise (Locke and Noble 1995) and disease (Collins and Hightower 1982) has been investigated in detail. It has also been shown in both rats and humans that higher levels of Hsp70 expression are associated with enhanced tolerance to ischemic stress and cardioprotection (Giannessi et al. 2003; Pantos et al. 2007).

Since the first studies by Ritossa (1962) has been known that Hsp70 is induced by heat. It has since then been studied in many different fields for its ability to protect cells against stress or trauma, however, the basal concentration of Hsp70 in humans have never been investigated. We have, in this study assessed the monocyte expression of Hsp70 over 24 h on male subjects at rest to investigate its basal variation and its correlation to core temperature. We found that, during resting conditions, there is a diurnal variation of Hsp70 and that it strongly correlates with core temperature, suggesting a role in homeostasis (Fig. 4). Similar to blood pressure, Hsp70 shows a diurnal variation (White 2001). Upon waking, a surge in blood pressure, heart rate and a higher core temperature results in increased monocyte Hsp70 expression, demonstrating the sensitivity of Hsp70 induction in vivo. Cellular stress disrupts homeostasis and can lead to rapid Hsp70 expression. This has been widely studied within thermal and exercise preconditioning (Madden et al. 2008). Regular exercise has been shown to lead to decrease basal Hsp70 concentrations within peripheral blood mononuclear cells (Fehrenbach et al. 2000). Human leukocytes isolated following an increase in core temperature to 40°C or above, have also been shown to produce a lower amount of Hsp70 in response to a immediate subsequent thermal challenge (Ryan et al. 1991), suggesting acquisition of thermal tolerance. Data presented here may have implications for athletes in a thermal/exercise preconditioning training regime, as it is known that the degree of Hsp70 expression is linked to basal concentration. Therefore, training at times when the basal concentration is lower may cause an increased Hsp70 response. This could be important in conferring increased cellular protection to subsequent stress via Hsp70.

Aerobically trained athletes have a lower concentration of Hsp70 in PBMC at rest than non-trained individuals, however, Hsp70 is increased in expression following exercise (Fehrenbach et al. 2000). This could be due to the lowering of heart rate shown here (Fig. 3), leading to a shift in the homeostasis balance. However, with sustained exercise core temperature has been shown to equilibrate to a higher baseline temperature (Reilly and Brooks 1986; Davenne and Lagarde 1995). When exercise is performed at times when core temperature is highest (T_c max) and lowest (T_c min), within the circadian rhythm, the further increase due to exercise has been shown to be reduced at T_c max compared to T_c min (Waterhouse et al. 2004). Thermoregulatory changes brought about by exercise may depend upon core temperature prior to the period of exercise and therefore time-of-day may be of importance.

Athletes crossing time zones will also be affected by circadian rhythms, as they may be competing when core temperature is reduced. The reduction in core temperature tends to increase rate of perceived exertion (RPE) during short exercise periods, which may then demand longer recovery times between training sessions. RPE has been shown to increase markedly after 5 min of exercise at

0500 hours compared to 1100, 1700 and 2300 hours (Waterhouse et al. 2004). Furthermore, the same study reported that after 30 min, the lowest RPE was seen when exercise began at 1100 hours. No time-of day difference was observed in heart rate following exercise. Exercise undertaken at 0500 hours (at T_c min), resulted in the most rapid increase in core temperature, albeit to levels still ultimately lower than exercise performed at other times when resting core temperature was higher (Waterhouse et al. 2004). This indicates that thermoregulation may be most effective at T_c max and T_c min to protect from hyperthermia and hypothermia, respectively. As we have shown, Hsp70 is linked to core temperature and therefore the Hsp70 minimum observed at 0500 hours may have an effect on exercise RPE and the coinciding rapid increase in core temperature following exercise at this time. The rapid rise in core temperature could lead to problems in protein synthesis and be responsible for the noted higher rate of Hsp70 expression following exercise from lower basal concentrations. It may be suggested, from the data presented here to exercise at times of the day when lower basal Hsp70 is observed, for example late morning, which may induce increased expression of Hsp70 that could be valuable in conferring tolerance to subsequent bouts of exercise.

Quantification of Hsp70 is not without difficulties. During the course of this study it came to our attention that both the commercial ELISA and the antibody clone used in this and many other studies only recognises so called "bio-available" Hsp70 (Assay Designs, personal communication). That is Hsp70 that is unbound, i.e. not performing chaperonic function. Binding of Hsp70 to mis-folded or nascent proteins thus renders the antibody-binding site blocked and unrecognisable. The antibody clone used (SPA-810) is directed against an amino acid sequence (aa 437–504, worldwide patent WO 01/42423) that is within the peptide-binding domain of Hsp70 (aa 383–508). This has implications in all measurements of Hsp70 in live cells.

In regards to future studies looking at increases in Hsp70 with any intervention it might be important to control for the subjects core temperature pre-testing. Several studies have found that the increase in Hsp70 is correlated to the basal levels (Maloyan et al. 1999; Sandström et al. 2008; McClung et al. 2008). High basal levels could be one reason why very small changes in Hsp70 have been observed (Shastry et al. 2002; Liu et al. 2004; Watkins et al. 2007). The mechanism behind the blunted increase in Hsp70 with higher basal levels has previously been discussed (Sandström et al. 2008). However, there are many factors other than core temperature that affects the up-regulation of Hsp70, which further adds to the difficulty controlling human studies.

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