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Research Article

Differential basal synthesis of Hsp70/Hsc70 contributes to interindividual variation in Hsp70/Hsc70 inducibility

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Abstract. The source of intraspecies variation in the expression of heat shock proteins (HSPs) remains unresolved but could shed light on differential stress tolerance and disease susceptibility. This study investigated the influence of variable basal HSP synthesis on differential inducibility of HSP synthesis. Basal and heat-induced synthesis of the major HSP families in peripheral blood monocytes from healthy donors ($n = 42$) were analysed using biometabolic labelling and densitometry. Basal Hsp70/Hsc70 synthesis and percentage induction of Hsp70/Hsc70 synthesis were significantly correlated ($r = -0.57$, $p < 0.0001$), and described most accurately by an exponential decay equation ($R = 0.68$, $R^2 = 0.46$). This regression equation suggests that increasing levels of basal Hsp70/Hsc70 synthesis are accompanied by an exponential decrease in the percentage induction of Hsp70/Hsc70 synthesis. The model fits data from European and non-European population groups independently, although both coefficients in the regression equation were larger for non-Europeans. This implies population group as an additional factor influencing differential HSP expression. The differential inducibility of Hsp70/Hsc70 due to variable basal synthesis of Hsp70/Hsc70 and based upon population group may contribute to differential stress tolerance or disease susceptibility.

Key words. HSP; Hsp70; Hsc70; basal synthesis; induction; population; human monocyte.

All living organisms respond to adverse changes in their environment by increased expression of a class of proteins referred to as heat shock or stress proteins (HSPs) [1]. HSPs are classified into families according to their apparent molecular mass (110, 90, 70, 65 kDa and the small HSPs) [2], of which the Hsp70 family is one of the best characterized, containing both constitutive and inducible members [1, 3–5].

HSP expression is under complex regulatory control accomplished at both the transcriptional and translational level [6, 7]. Transcriptional regulation is exerted via activation of the cytosolic heat shock transcription factor (HSF) from its monomeric inert form, as part of a macromolecular complex with Hsp70, Hsp90 and Hdj1/Hsp40, to an oligomeric DNA-binding form [6]. Besides trimerization of HSF and heat shock element (HSE) binding, activation of *hsp* gene transcription requires inducible hyperphosphorylation of HSF

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trimers at multiple serine residues. An increased demand for Hsp70 and co-chaperones, associated with monomeric HSF, to chaperone stress-denatured proteins causes the release and trimerization of HSF. Conversely, overexpression of Hsp70, Hsp90 or Hdj1/Hsp40 inhibits HSF1 activation and *hsp* gene transcription [8]. Deactivation of trimers is mediated through heat shock factor binding protein 1 (HSBP1) which interacts with both trimeric HSF and Hsp70, thereby facilitating conversion to the monomeric form [6]. Replenishment of the pool of free Hsp70 promotes relocation of inactive HSF monomers to the cytoplasm. Transcription of *hsp* genes is also mediated through a basal transcriptional complex [9] while the constitutive HSE-binding factor (CHBF/Ku) has been implicated in the negative regulation of *hsp* gene expression [10].

Besides transcriptional regulation, the heat shock response is regulated by various post-transcriptional mechanisms [7] including the preferential translation of Hsp70 mRNA during heat shock partly due to features within the 5′ untranslated region (UTR) [11, 12] and increased stabilization of the 3′UTR of HSPs [13, 14]. Translational control is further influenced by the association of Hsp70 with an eIF-2 kinase that, once released, inhibits the activity of a polypeptide chain initiation factor (eIF-2) in order to shut down protein synthesis following heat shock [7].

Hsp70 family members function as components of molecular chaperone complexes located in different intracellular compartments involved in a variety of cellular processes such as protein folding, assembly, transport and translocation across membranes, and prevention of aggregation [3, 15–17]. Besides these normal physiological functions, HSPs have essential roles in cellular protection and adaptation during and following exposure to stressful events. Hsp70, in particular, has been implicated in cellular protection from oxidative stress [18, 19], and mitochondria identified as selective targets of this protection [18]. The partnership between Hsp70 and ATP as proposed by Mallouk et al. [20] has important implications for the decisive role of mitochondrial integrity in cellular fate—survival, apoptosis or necrosis. Overexpression of Hsp70 could function as a two-edged sword—on the one hand promoting survival by rescuing cells from necrosis, while on the other hand promoting the persistence and survival of potentially threatening cells through the prevention of apoptosis [21, 22]. Furthermore, during inflammation, Hsp70-mediated major histocompatability complex (MHC) class I antigen processing and presentation [23, 24] could amplify the immune response to such an extent that it has a deleterious effect on an individual.

Various factors may influence differential HSP expression, such as genetic adaptation to environmental temperature. Organisms inhabiting dry sand deserts of middle Asia and adapted to function at high temperatures are able to synthesize HSP (especially Hsp70) at significantly higher levels at normal physiological temperature than related species from central and northern parts of Russia [25]. Lyashko et al. [26] observed that fibroblasts derived from Turkmen inhabiting hot desert areas of middle Asia are able to synthesize significantly higher levels of the Hsp70 family of proteins following heat shock compared to fibroblasts derived from Russians living in moderate climatic regions of European Russia. Acclimatization may also advance during evolution through variation in the isoforms of expressed HSP. Norris et al. [27] reported that inducibly synthesized Hsp70 was polymorphic compared to the highly conserved constitutive Hsp70 in tropical and desert species of poeciliid fish. Another potential determinant in differential synthesis is the activation/efficiency of transcriptional regulation, comprising the strength of HSF-Hsp70 and HSF-HSE associations, inducible phosphorylation of HSF trimers, and phosphorylation of the C-terminal domain of paused RNA polymerase [28].

Differential expression/inducibility of HSPs could contribute to differential stress tolerance and disease susceptibility and, moreover, has serious implications for the use of HSPs as biomarkers of stress [29–33]. Based upon the significant autoregulatory role of HSPs, in particular Hsp70 and Hsp90, we hypothesized that basal synthesis of HSP influences the degree of HSP induction following stress and thereby contributes to interindividual variation in terms of HSP inducibility. This hypothesis was investigated in peripheral blood monocytes (PBMs) derived from healthy individuals inhabiting South Africa, using biometabolic labelling under control conditions and following heat shock. An inverse exponential relation between basal Hsp70/Hsc70 synthesis and its percentage induction of synthesis following heat shock was observed.

Materials and methods

Human subjects. Healthy volunteers (n = 42) from the South African Blood Transfusion Services participated as donors in the study. The test sample consisted of 25 males and 17 females, with ages ranging between 27 and 35 years. The individuals represented different population groups, namely European (n = 22), African (n = 13), Asian (n = 6) and mixed ancestry (n = 1). All donors resided in Gauteng Province, in and around Johannesburg. The European individuals were mainly descendants of Dutch, French, British and German ancestors who immigrated to South Africa during the 17th–19th centuries. The study was observational in nature, i.e. selection of donors was based upon availability rather than random selection or any specific

characteristics. This explains the unequal distribution of individuals across population groups, gender and age.

Cells and media. Mononuclear cells were isolated from peripheral blood by density gradient centrifugation and seeded in duplicate (5×10^6 cells/ml) in RPMI 1640 medium supplemented with 1% L-glutamine, 10% fetal calf serum and 25 mM HEPES. Media and supplements were purchased from Highveld Biologicals, Kelvin, South Africa. Cell cultures were enriched for PBMs by adherence (37 °C, 45 min, 95% air, 5% CO_2) and non-adherent cells removed by washing with phosphate-buffered saline (PBS). Remaining cells were incubated overnight in RPMI 1640, supplemented as described above. This protocol allowed the preparation of cultures consisting of 70–90% monocytes [34] as determined by flow cytometry based upon forward- and side-angle scatter properties and CD14 cell surface expression.

Heat shock treatment and biometabolic labelling. Cells were heat shocked (44 °C, 20 min) in a circulating water bath, allowed to recover (37 °C, 160 min) and proteins labelled (90 min) with a mixture of 6 μCi ^{35}[S]-methionine and ^{35}[S]-cysteine per ml medium (ICN, Costa Mesa, Calif.). Following harvesting and assessment of number and viability using Trypan blue, cells were lysed by heating for 10 min in 0.1 M Tris buffer containing sodium dodecyl sulphate (SDS, 4%), β-mercaptoethanol (10%) and bromophenol blue (0.1%). Samples corresponding to equal numbers of viable cells were resolved by SDS polyacrylamide gel electrophoresis (SDS-PAGE) [35], and labelled polypeptides were revealed by autoradiography using Cronex Medical X-ray film (Protea Medical Services, Sandton, South Africa).

Western blot analysis. Western blot analysis was done using a mouse monoclonal antibody directed against both the constitutive and inducible isoforms of the human Hsp70 family (SPA-820; Stress-Gen, Victoria, Canada). The primary antibody was revealed by chemiluminescence with a horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (ICN, Costa Mesa, Calif.).

Image analysis. Bands representing labelled polypeptides synthesized following heat shock (molecular masses of 110, 90, 70 and 65 kDa) as well as corresponding bands/areas synthesized under control conditions were quantified by image analysis using Uvpgr32 (UVP, San Gabriel, Calif.) and GeneTools (SynGene, Cambridge, UK) [36]. Relative densities assigned to bands/areas in control and heat shock lanes were calibrated against actin (43 kDa) synthesis in the same sample as reference for control protein synthesis [37]. Synthesis of HSPs under control conditions (37 °C) is referred to as basal synthesis, while synthesis of HSP 160 min after in vitro heat treatment (44 °C, 20 min) is referred to as heat-induced HSP synthesis. Since increased synthesis of HSPs was studied in relation to basal HSP expression, the percentage induction of HSP synthesis was calculated for each HSP using the following equation:

$$\%\ \text{induction of HSP synthesis} = \frac{\text{heat-induced HSP synthesis} - \text{basal HSP synthesis}}{\text{basal HSP synthesis}} \times 100$$

Statistical analysis. Statistical analysis was performed using SPSS 8 (SPSS, Chicago, Ill.), SigmaPlot 4.0 for Windows, Microsoft Excel 97 and CoStat Software (CoHort Software, Berkeley, Calif.).

Results

Interindividual variation in HSP synthesis. HSP synthesis and interindividual variation were first characterized in the test sample. In vitro heat shock of PBMs led to increased synthesis of polypeptides of approximately 110, 90, 70 and 65 kDa (fig. 1A, lane 2). No distinction was made between synthesis of Hsp70 (72 kDa) and Hsc70 (73 kDa) since the relationship between basal and inducible synthesis for the 70 kDa family as a whole was investigated. The 70 kDa band comprised Hsp70 and Hsc70 as determined by Western blot analysis, and is hereafter referred to as Hsp70/Hsc70 (fig. 1B). Polypeptides of 110, 90 and 65 kDa are referred to as Hsp110, Hsp90 and Hsp65, respectively, based on their heat inducibility and apparent molecular mass determined in a comparison with molecular-mass markers.

The distribution of values for basal synthesis and the percentage induction of HSP synthesis in monocytes derived from the 42 donors are shown as box-and-whisker plots in figures 2A and 2B, respectively. In general, Hsp70/Hsc70 was most commonly and abundantly synthesized under control conditions (fig. 2A) and following heat shock (fig. 2B). The presence of outliers (fig. 2) was attributed to the fact that the range of values for HSP synthesis variables in the population was wider than indicated by the test sample [38]. Coefficients of variance for basal HSP synthesis ranged from 41.6 to 46.0% (mean 44.1%), and from 74.9 to 166.7% (mean 124.3%) for percentage induction of synthesis (table 1). Amidst the variation between individuals, a positive, mostly significant, correlation existed within donors between the basal synthesis and between the percentage induction of synthesis of different HSPs (table 2).

Relationship between basal synthesis and percentage induction of HSP synthesis. To investigate whether the percentage induction of HSPs is influenced by the basal

synthesis of HSPs, a correlation analysis was performed, comparing all variables. Basal synthesis of Hsp70/Hsc70 and Hsp90 showed a significant negative correlation with their respective percentages of induction (table 2), while the basal synthesis of Hsp70/Hsc70 also showed a negative correlation with the percentage induction of Hsp65 synthesis. A regression analysis was undertaken comparing all variables to investigate possible relationships (other than linear) between basal synthesis and percentage induction of HSP synthesis. Basal synthesis was used as the independent variable and percentage induction as the dependent variable. Fitting several models revealed a significant relationship between basal synthesis and percentage induction of Hsp70/Hsc70 synthesis described by a two-parameter single exponential decay equation (fig. 3A). One data point was identified as an outlier based on its studentized deleted residual that was located outside the suggested limits of -2.5 to 2.5 and was therefore omitted in the final regression analysis. The equation fitted was:

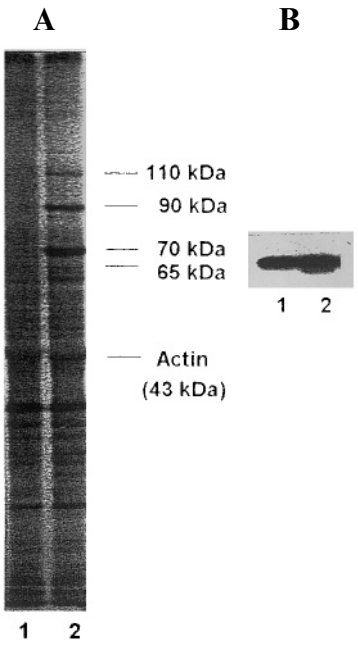


Figure 1. (*A*) A representative autoradiograph illustrating biometabolic labelling in peripheral blood monocytes under control conditions (lane 1) and following heat shock (lane 2). Samples representing equal numbers of viable cells were resolved by SDS-PAGE (9%) and the pattern of radioactive polypeptide bands was captured on X-ray film. Labelled polypeptides, synthesized increasingly following HS (110, 90, 70 and 65 kDa—indicated to the right), and corresponding bands/areas synthesized under control conditions were quantified by densitometry. Actin synthesis was used to standardize polypeptide synthesis among donors under control and heat shock conditions. Polypeptide molecular mass was determined by comparison to molecular-mass markers. (*B*) A representative Western blot illustrating accumulation of Hsp70 (72 kDa) and/or Hsc70 (73 kDa) under control conditions (lane 1) and following heat shock (lane 2) using an antibody directed against the constitutive and inducible isoforms.

$$\%\ \text{induction of Hsp70/Hsc70 synthesis} = 249.4e^{(-1.3\text{basal Hsp70/Hsc70 synthesis})}$$

The values for R and R^2 were 0.68 and 0.46, respectively, while the standard error of the estimate was 37.7. Both coefficients were significantly different from zero ($p = 0.0002$ and $p < 0.0001$, respectively). Figure 3B shows that residuals were normally distributed about the regression line, with 66% of the standardized residuals lying between -1 and $+1$ and 98% between -2 and $+2$, thus supporting a suitable fit of the model. A two-parameter single exponential decay equation also fitted Hsp90 data (% induction of Hsp90 synthesis $= 278.2e^{(-3.2\text{basal Hsp90 synthesis})}$; $R = 0.54$, $R^2 = 0.30$), but standardized residuals were not normally distributed about the regression line and removal of one outlier with a studentized residual of 4.5 obliterated the fit and its significance. Regression analysis showed no significant relationship between basal synthesis and percentage induction of synthesis for either Hsp110 or Hsp65.

The regression model and population group. Population group has previously been suggested as a factor determining differential expression of HSP among individuals [26]. The effect of different population groups within the test sample on the proposed negative exponential relationship between basal synthesis of Hsp70/Hsc70 and the percentage induction of Hsp70/Hsc70 synthesis was therefore evaluated. Because the number of Africans, Asians, Indians and subjects of mixed ancestry was small, these donors were grouped together [$n = 20$, hereafter referred to as non-Europeans (nE)] and compared with Europeans (E, $n = 22$). An independent t test was performed on all variables comparing E and nE data for basal synthesis and percentage induction of synthesis. The mean percentage induction of Hsp70/Hsc70 synthesis was significantly higher in nE compared to E ($p < 0.01$; fig. 4B), while the mean basal synthesis of Hsp70/Hsc70 was lower, although significantly so only at $p = 0.33$ (fig. 4A). Regression lines fitted separately for E and nE Hsp70/Hsc70 data are shown in figure 4C. A steeper exponential decay model appeared to fit nE compared to E data (fig. 4C) and regression equations confirmed that the y intercept and the exponential coefficient were larger (2.0-fold and 3.2-fold, respectively) for nE.

Discussion

This study demonstrated a decreasing exponential relation between the basal synthesis of Hsp70/Hsc70 and the percentage induction of Hsp70/Hsc70 synthesis in PBMs from healthy donors. Almost 50% of the interindividual variation in the induction of Hsp70/Hsc70 synthesis upon heat shock appears to be determined by

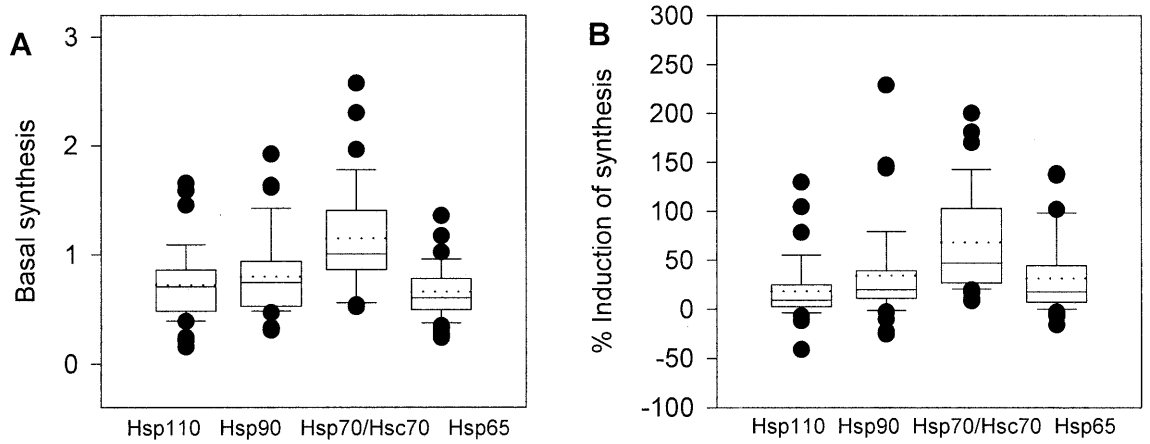


Figure 2. Box-and-whisker plots illustrating the distribution of basal synthesis of HSPs (*A*) and percentage induction of HSP synthesis (*B*) in peripheral blood monocytes from 42 healthy donors. The box boundaries indicate the 25th percentile (closest to zero) and the 75th percentile (farthest from zero), while whiskers indicate the 10th and 90th percentiles. The dotted and solid lines within boxes mark the mean and median values, respectively. Statistically defined outlying data points (outside the 10th and 90th percentiles) are shown as large dots.

Table 1. Coefficients of variance for basal synthesis and percentage induction of Hsp110, Hsp90, Hsp70/Hsc70 and Hsp65 synthesis in monocytes derived from 42 healthy individuals.

Variable	Coefficient of variance (%)				Mean
	Hsp110	Hsp90	Hsp70/Hsc70	Hsp65	
Basal synthesis	45.7	46.0	42.9	41.6	44.1
Percent induction	166.7	137.1	74.9	118.3	124.3

variation in the basal synthesis of Hsp70/Hsc70. The exponential decay regression model fitted data from E and nE populations separately but with larger coefficients in nE.

Prominent interindividual variability in the basal synthesis of Hsp110, Hsp90, Hsp70/Hsc70 and Hsp65 was observed (mean coefficient of variance = 44.1%), which increased almost threefold (mean coefficient of variance = 124.3%) upon heat shock (fig. 2, table 1). This result supports the intraspecies variation in stress protein expression reported in humans [26, 39] and several other organisms [25, 40, 41]. Factors that may contribute to variation in HSP expression under basal conditions and following heat shock may have a genetic and/or environmental origin. One such example is the antioxidant status of individuals, determined by genetic [42] and/or environmental factors [43], which could modulate HSF activation through the level of reactive oxygen species—key signalling molecules in HSP induction [19]. Other factors that might influence HSF activation and HSF-HSE interaction include *Hsf* gene polymorphisms, pre-existing pools of HSF, levels of negative regulators such as Hsp70, and HSF-HSE binding affinity [6]. Differential stability of HSP mRNA [14] and kinetics of HSP synthesis [44] may also contribute to variable HSP expression. Although age influences HSP expression [45, 46], the age range of our test sample was narrow and no significant correlation was found between either age or gender and HSP synthesis (results not shown). The smaller variation in basal HSP synthesis compared to the highly variable induction of HSP synthesis observed in this study (table 1) may implicate differential stress responsiveness, amplified by heat shock, or a larger evolutionary constraint on basal HSP synthesis. For fish, Norris et al. [27] proposed the involvement of an evolutionary constraint in the invariable, constitutively synthesized Hsp70 compared to the induced polymorphic isoforms of Hsp70. The abundant and common synthesis of Hsp70/Hsc70 observed under all circumstances (figs. 1 and 2) supports the well-established key role of the 70 kDa family under normal physiological conditions and in protection and recovery from stress [1, 3, 4]. Whether increased synthesis of HSPs is related to increased survival rates as described by Lyashko et al. [26] could not be verified in the current study because the cells received a relatively mild

Table 2. Correlation analysis illustrating the relationship between HSPs in terms of their basal synthesis and percentage induction of synthesis, as well as between basal synthesis and percentage induction of synthesis, comparing all variables.

Parameters compared	Correlation (r)	Significance (p)
Basal synthesis		
Hsp110: Hsp90	0.76	***
Hsp110: Hsp70/Hsc70	0.51	***
Hsp110: Hsp65	0.55	***
Hsp90: Hsp70/Hsc70	0.67	***
Hsp90: Hsp65	0.51	***
Hsp70/Hsc70: Hsp65	0.46	**
Percentage induction of synthesis		
Hsp110: Hsp90	0.50	***
Hsp110: Hsp70/Hsc70	0.45	**
Hsp110: Hsp65	0.38	*
Hsp90: Hsp70/Hsc70	0.35	*
Hsp90: Hsp65	0.30	ns
Hsp70/Hsc70: Hsp65	0.57	***
Basal synthesis: percentage induction of synthesis		
Hsp90: Hsp90	−0.43	**
Hsp70/Hsc70: Hsp70/Hsc70	−0.57	***
Hsp70/Hsc70: Hsp65	−0.37	*

*** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns, not significant.

heat shock of short duration, not sufficient to induce cell death.

In general, the different HSPs correlated significantly with each other in terms of both their basal synthesis and their percentage induction of synthesis (table 2). This observation suggests co-ordinated regulation of all HSPs by a common denominator under normal and stress conditions, for example the regulation of HSP induction by HSF activation [47]. While *hsp70-2* gene polymorphism in the coding region has been linked to variation in its mRNA expression [48], co-ordinate regulation argues against a role for sequence variation in the coding region of individual *hsp* genes in the differential inducibility observed among donors.

Basal synthesis of Hsp70/Hsc70 correlated negatively with its percentage induction of synthesis by heat shock as well as that of Hsp65, while Hsp90 basal synthesis correlated negatively exclusively with its own percentage induction of synthesis by heat shock (table 2). This inverse relationship between basal and inducible synthesis of Hsp70/Hsc70 and Hsp90 confirms their role as negative feedback regulators in the inducible transcription of *hsp* genes through transient interactions with HSF [44, 49–52]. Besides maintaining HSF in an inactive state, overexpression of Hsp70 inhibits phosphorylation of HSF through activation of protein phosphatase and inhibition of protein kinase C activity [53]. Moreover, Hsp70 has been implicated in the suppression of its own synthesis through its association with Hsp70 mRNA of the elongating nascent polypeptide chain [11]. Upon a chaperoning demand for Hsp70, an eIF kinase is displaced from Hsp70 [7] leading to the suppression of protein synthesis, including that of HSPs [44]. Although basal Hsp70/Hsc70 synthesis did not directly affect the percentage induction of either Hsp110 or Hsp90, the autoregulatory role of Hsp70 in the induction of all HSPs may at least indirectly modulate their expression at a later point in time.

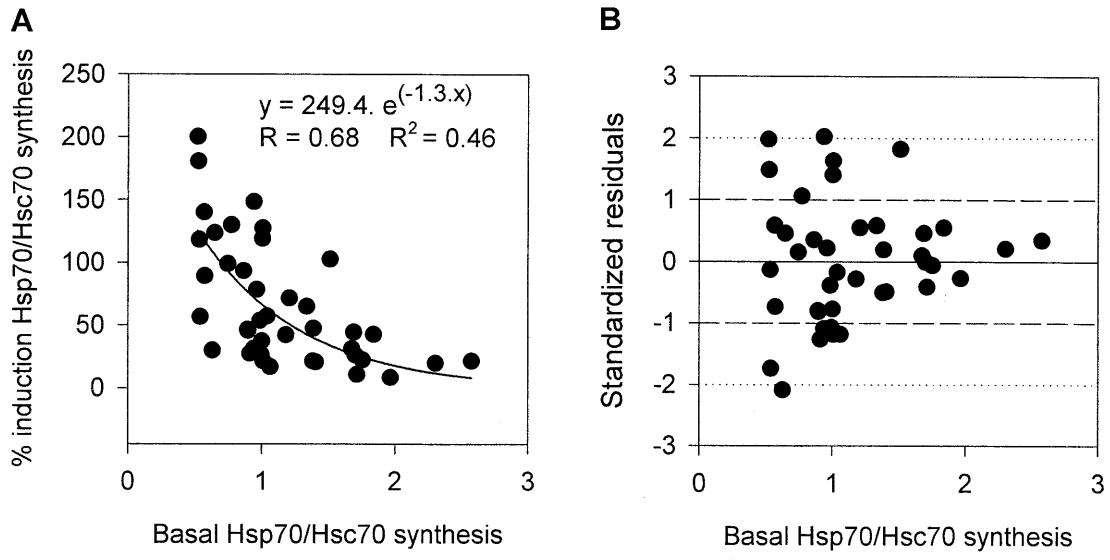
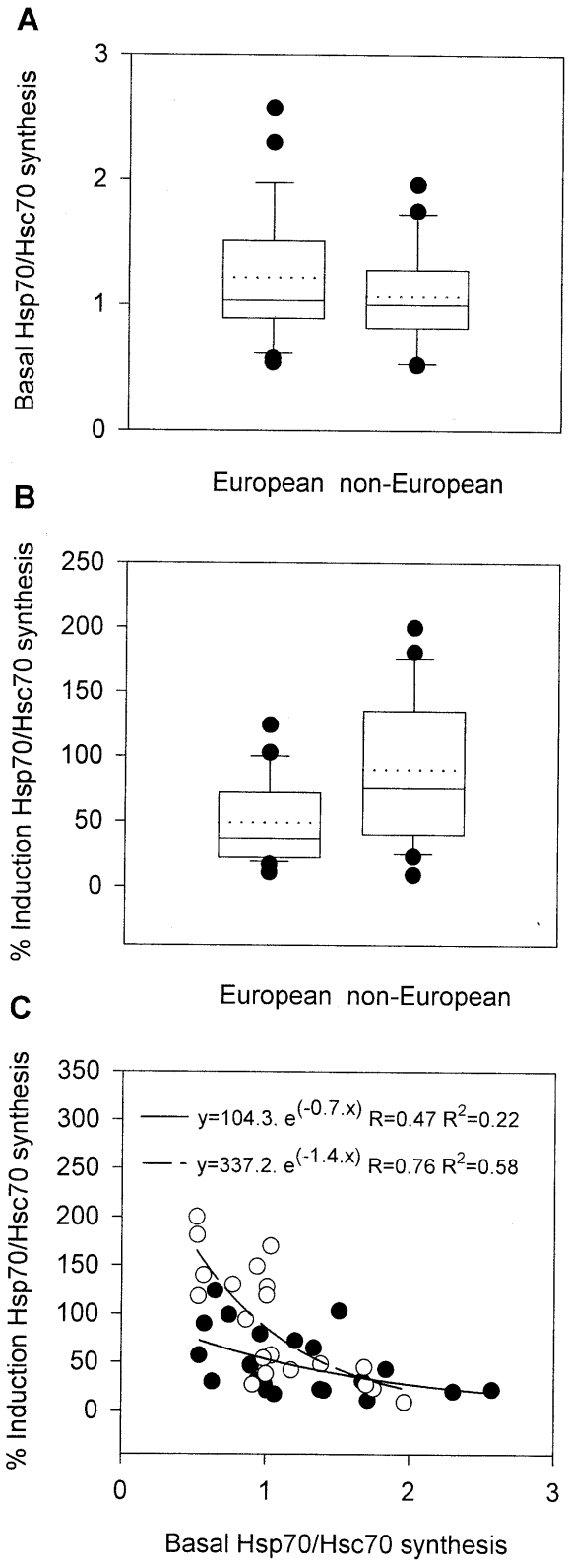


Figure 3. Regression analysis defining the relationship between basal Hsp70/Hsc70 synthesis and percentage induction of Hsp70/Hsc70 synthesis (*A*). A two-parameter single exponential decay curve was found to best fit the data. The even distribution of standardized residuals about the regression line is illustrated in (*B*).



A two-parameter single exponential decay equation was found to describe most accurately the relationship between basal Hsp70/Hsc70 synthesis and percentage induction of Hsp70/Hsc70 synthesis (fig. 3). The role of basal Hsp70/Hsc70 synthesis as predictor of percentage induction of at least Hsp70/Hsc70 synthesis corresponds to the suggested role of Hsp70 as cellular 'thermometer', sensing temperature changes and influencing the induction of HSPs [54]. A slight increase from a minimum level of basal Hsp70/Hsc70 synthesis brings about a significant decrease in the inducibility of Hsp70/Hsc70 upon heat shock (fig. 3A). Differential basal synthesis may relate to an evolutionary compromise between the ability to launch a strong stress response allowed by a relatively low basal synthesis *and* elevated basal production of HSPs at the cost of strong inducibility of a stress response—a phenomenon also described in *Drosophila* [55]. The use of basal Hsp70/Hsc70 synthesis as predictor in the proposed model further relates to a mathematical model proposed by Peper et al. [44]. The significant negative linear relationship (table 2) and the exponential decay relationship, though less significant than with Hsp70/Hsc70, between basal Hsp90 synthesis and percentage induction of Hsp90 synthesis implies a similar role for Hsp90 and may suggest co-operation between Hsp70 and Hsp90 in the management of HSF activity [6].

The proposed regression model suggests that almost 50% ($R^2 = 0.46$) of the variation in the percentage induction of Hsp70/Hsc70 synthesis is based upon differences in basal synthesis. If only approximately 50% of interindividual variation in Hsp70/Hsc70 inducibility can be explained by its differential basal synthesis, what other factors contribute to variable stress responsiveness between individuals? Since population group has been implicated in this regard [26], the influence of this factor on the proposed model was investigated (fig. 4). While the mean basal synthesis of Hsp70/Hsc70 did not differ significantly between E and nE, nE showed a significant, almost twofold higher percentage induction of Hsp70/Hsc70 (fig. 4A, B). This corresponds to the report of Lyashko et al. [26] showing an intensive heat-induced synthesis of HSP in Turkmen compared to

Figure 4. *A*, *B*. Box-and-whisker plots illustrating the distribution of basal Hsp70/Hsc70 synthesis (*A*) and percentage induction of Hsp70/Hsc70 synthesis (*B*) in European ($n = 22$) and non-European ($n = 20$) donors. Independent t tests revealed that the mean values for percentage induction of Hsp70/Hsc70 synthesis were significantly higher in non-Europeans (89.95) compared to Europeans (48.99; $p < 0.01$), while basal Hsp70/Hsc70 synthesis was slightly lower in non-Europeans (1.07) compared to Europeans (1.22; $p < 0.327$). See legend of figure 2 for details on box-and-whisker plots. (*C*) Two-parameter single exponential decay curves fitted separately to European (solid line, ●) and non-European (dashed line, ○) data.

only trace synthesis of HSP in Russians. An exponential decay model applied to both population groups independently (fig. 4C). However, the percentage induction of Hsp70/Hsc70 synthesis at minimal basal Hsp70/Hsc70 synthesis was notably higher in nE than in E and a steeper decrease in percentage induction of Hsp70/Hsc70 synthesis with increasing levels of basal synthesis was evident (fig. 4C). While a strong induction of Hsp70/Hsc70 could benefit survival under extreme climatic conditions, Hsp70/Hsc70 overexpression may not be beneficial under all circumstances. For example, during infection, excessive production of Hsp70/Hsc70 could promote survival of intracellular pathogens [23] believed to be eliminated through apoptosis of infected host cells [20–22]. Whether this phenomenon could contribute to increased susceptibility to infectious diseases such as tuberculosis in nE remains to be determined.

In conclusion, the model described here identified differential basal Hsp70/Hsc70 synthesis as an important determinant in Hsp70/Hsc70 inducibility, while population group has been confirmed as an additional contributing factor. The dissimilarity between individuals in HSP expression in response to an identical stress may shed light on variable stress tolerance and differential disease susceptibility. The differential inducibility of HSPs among individuals in response to an identical stress necessitates particular attention when using induction of HSPs as markers of stress severity in different individuals.

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