Chapter 3: Expression of Heat Shock Proteins is Altered in HIV Infection

3.1 Introduction

Heat shock proteins (hsps) are a group of highly conserved proteins that represent between 2% and 15% of total cellular protein and are expressed by every living organism. The main functions of hsps are to regulate apoptosis and to act as intracellular molecular chaperones that facilitate protein folding and assembly (Morimoto *et al.,* 1994). Some hsps are highly immunogenic and elicit humoral, cytotoxic T-lymphocyte (CTL) and natural killer (NK) cell responses against viruses, tumors and infectious diseases (Brenner & Wainberg, 1999). Previous studies conducted during my Honours candidature reported altered leukocyte expression of hsp70 in HIV-positive individuals, compared with age-matched, uninfected controls. The results of this work are summarized below.

Baseline levels of lymphocyte hsp70 expression (37°C) were increased following heat shock (at 42.5°C) in both controls and patients with HIV-disease. Significant augmentation in lymphocyte hsp70 expression following heat shock was demonstrated by Western immunoblots. Representative Western immunoblots from HIV-positive and HIV–negative samples (Figure 3.1a,) together with corresponding β-actin controls (Figure 3.1b). Fold-increases in lymphocyte hsp70 expression (Figure 3.1c,d) following heat shock were 4.52 ± 2.97 in HIV-positive individuals compared with 2.60 ± 1.29 for HIV-negative controls ($p=0.001$). The increase in hsp70 expression in lymphocytes from HIV-positive individuals was primarily due to lower baseline levels, rather than absolute amounts. Similar trends were demonstrated using ELISA (results not shown). Using this assay, fold-increases in lymphocyte hsp70 expression following heat shock were 4.88 ± 3.78 in HIV-positive individuals compared with 2.80 ± 1.21 in HIV-negative controls ($p=0.065$).

The aims of the current study were to further investigate the mechanisms of altered hsp expression in HIV-positive individuals by examining the *de novo* synthesis of hsps and the expression of another of the major stress proteins, hsp90 in protein samples extracted from the lymphocytes of the above cohort of HIV-positive individuals and HIV-negative control subjects.

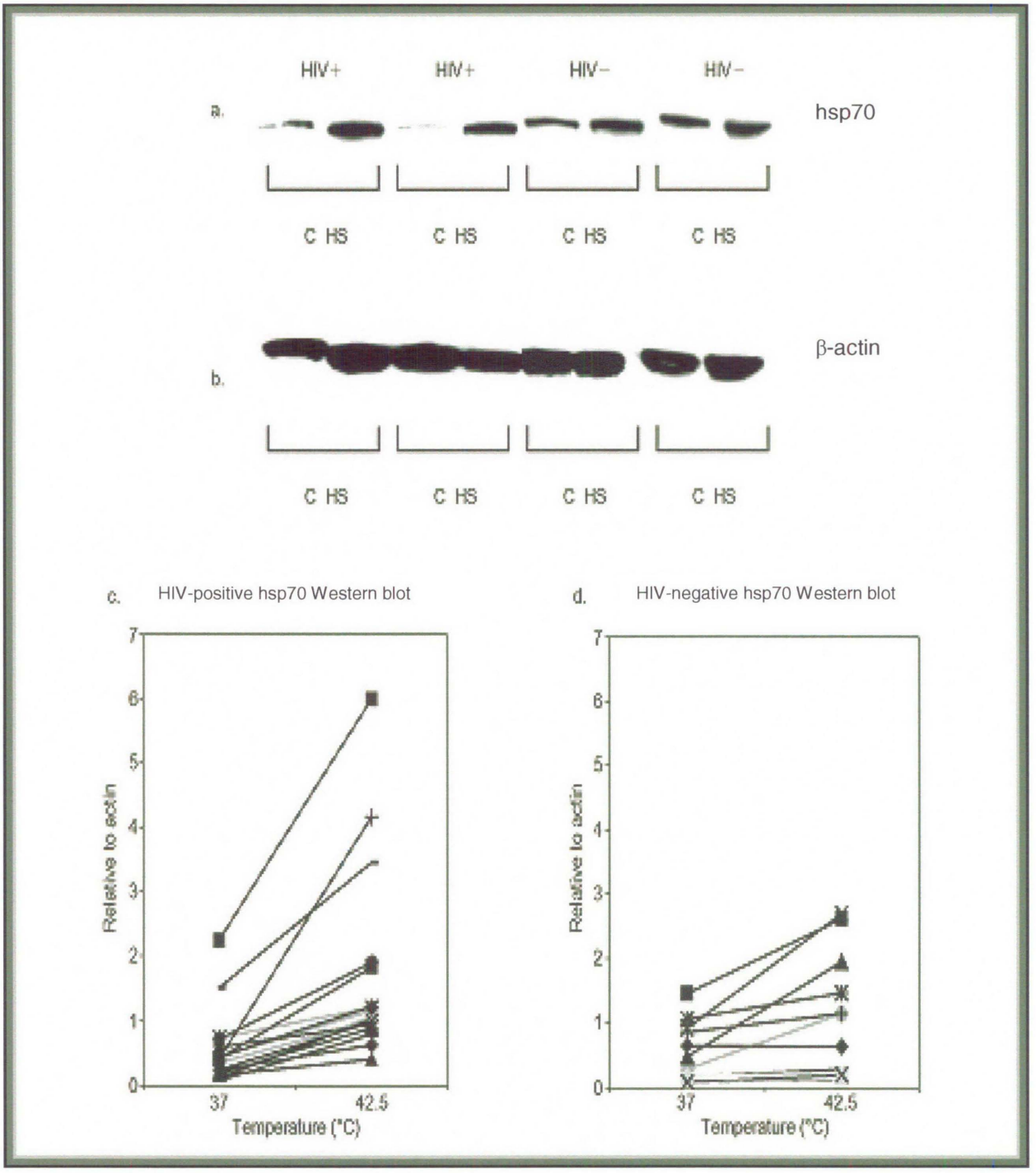

Figure 3.1: Western blots and fold-increase of hsp70 in lymphocytes from HIV-positive and HIV-negative individuals. Lymphocyte hsp70 expression following heat shock (HS) is increased in patients with HIV disease relative to baseline values (C). Representative samples for HIV-positive subjects and HIV-negative controls (Fig 3.1a) are shown. Each patient is represented by one bracket. The corresponding β-actin internal controls are shown in Fig 3.1b. Fold-increase in hsp70 (Western blot) is significantly increased in HIV-positive patients (Fig. 3.1c) as compared with HIV-negative controls (Fig. 3.1d).

3.2 Study Design

3.2.1 Study participants

Twenty HIV-positive males aged 32 – 64 years (mean age = 47.4 ± 8.4) were recruited through the Albion Street Centre, Surry Hills, Sydney. Fifteen HIV-negative males aged 33 – 60 years (mean age = 45.4 ± 9.2) recruited through the Sydney Children's Hospital, Randwick, Sydney (9 individuals) and the University of New England, Armidale (6 individuals) volunteered as control subjects. Patient profile data from HIV-positive participants was collected at the Albion Street Centre and consisted of treatment status, immunological status, alcohol and cigarette use as well as dietary supplement intake. Immunological status was defined as viral load which was determined by the South Eastern Area Laboratory Services (SEALS), Prince of Wales Hospital, Sydney; and CD4+, CD8+ and CD38+ cell counts which were performed by the Immunology Department, Sutherland Hospital, Sydney. Percentages CD4(+) T-cell subsets and CD8(+) T cells were determined by flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Fluorescent monoclonal antibodies (Becton Dickinson) and tri-level CD4 Whole Blood Control-FluroTrol-CD4 (BioErgonomics, St Paul, MN) were also used. Absolute values of subsets were determined by extrapolation from an absolute lymphocyte count. Plasma HIV-1 RNA levels were determined using the Amplicor HIV-1 Monitor Ultra Sensitive Specimen Preparation Protocol Ultra Direct Assay (Roche Molecular Systems Inc., Somerville, NJ) with a limit of quantification of 50 copies/ml.

3.2.2 Blood Collection

Human peripheral blood was collected in 9 ml lithium heparin Vacuette® blood collection tubes (Greiner Bio-One). At each of the collection locations (Sydney and Armidale), blood was collected by trained personnel. All blood samples were processed within 24 h of collection.

3.2.3 Patient Profile Data

To maintain the integrity of the trial, patient profile data collected from the HIV-positive cohort at the Albion Street Centre (ASC) was withheld until all experimental work had been completed. This data is tabulated in the Appendix and included treatment status, immunological status, alcohol and cigarette use as well as dietary supplement intake. A summary appears below in Table 3.1. Data collected from the

HIV-negative cohort also appears in the Appendix, and is summarized in Table 3.2. This data was used to determine any within cohort effects for each of the parameters measured.

Table 3.1: Baseline Demographics of HIV-Positive Subjects

Number	20 Males
Age	mean 47.4 ± 8.4 years (range 32-64 years)
Treatment status	14 (70%) On treatment 6 (30%) Off treatment
Viral Load	Log 1.7 ± 1.4 copies/ml (range log < 1.7-4.78 copies/ml) Undetectable = 12 (60%) Detectable = 8 (40%)
CD4+	500 ± 304 cells/mm^3 (range 120-1360)
Smoking	10 (50%) Smokers 10 (50%) Non-smokers
Dietary Supplements	9 (45%) Use supplements 11 (55%) No supplements

Table 3.2: Baseline Demographics of HIV-Negative Subjects

Number	15 Males
Age	Mean 45.4 ± 9.2 (range 33-60 years)
Origin	9 (60%) WRL* - Sydney 6 (40%) UNE# - Armidale
Smoking	2 (13%) Smokers 13 (87%) Non-smokers
Dietary Supplements	0 (0%) Use supplements

*Westfield Research Laboratories, #University of New England

3.3 Results

3.3.1 Autoradiograms

^{35}S labeled proteins (10 μg) extracted from lymphocytes, were run on 1D-SDS-PAGE to determine the effect of heat shock on induction of hsps in lymphocytes. Control (37°C) and heat shock (42.5°C) samples from each subject were run side by side in each gel (Figures 3.2 – 3.7). Expression of hsps was calculated by densitometric analyses of peak intensity of bands relative to β-actin, the internal control (43 kDa). The position of bands was determined by using a molecular weight marker for each gel.

Following heat shock of lymphocytes, expression of hsp110, hsp90 and hsp70 were upregulated in all individuals regardless of their HIV-status (Figures 3.8 – 3.10). The *de novo* synthesis of hsp70 (relative to β-actin) measured at 37°C in the HIV-positive cohort was 0.57 ± 0.04 and this did not differ significantly from the HIV-negative cohort (0.76 ± 0.13) (Figure 3.8). The expression of hsp70 (relative to β-actin) after heat shock at 42.5°C was also not significantly different at 2.82 ± 0.26 in the HIV-positive cohort and 3.00 ± 0.67 in the HIV-negative cohort. The *de novo* synthesis of hsp90 (relative to β-actin) measured at 37°C in the HIV-positive cohort was 1.09 ± 0.10 and this did not differ significantly from the HIV-negative cohort (1.20 ± 0.24) (Figure 3.9). The expression of hsp90 (relative to β-actin) after heat shock at 42.5°C was also not significantly different with 2.34 ± 0.47 in the HIV-positive cohort and 2.81 ± 0.27 in the HIV-negative cohort.The *de novo* synthesis of hsp110 (relative to β-actin) measured at 37°C in the HIV-positive cohort was 0.47 ± 0.11 and this did not differ significantly from the HIV-negative cohort (0.33 ± 0.05) (Figure 3.10). The expression of hsp110 (relative to β-actin) after heat shock at 42.5°C was also not significantly different at 1.26 ± 0.15 in the HIV-positive cohort and 0.92 ± 0.17 in the HIV-negative cohort.

The ratio of control (37°C) versus heat shock (42.5°C) hsp70, hsp90 and hsp110 expression was subsequently examined as a fold-increase (Figure 3.11). The mean fold increase in hsp70 for the HIV-positive individuals was 5.52 ± 0.89 which was not different ($p > 0.05$) to the HIV-negative individuals with 4.62 ± 0.71. The mean fold increase in hsp90 for the HIV-positive individuals was 2.82 ± 0.34 and the HIV-negative individuals was 2.06 ± 0.24, which was approaching significance ($p = 0.07$).

There was a significant difference in fold increase in hsp110 ($p = 0.03$) with the HIV-positive individuals 4.46 ± 0.58 and the HIV-negative individuals with 2.33 ± 0.65.

Two HIV-positive subjects had patterns of hsp expression that were noteworthy. These patterns are illustrated in Figure 3.2 in lanes 1 and 2 (control and heat shock) for the first individual, and lanes 3 and 4 (control and heat shock) for the second individual. The patterns of expression were quite unlike any other individuals in this study, whether HIV-positive or HIV-negative. There were very strong bands at approximately 65 kDa, 41 kDa and 25 kDa. Curiously, the second individual also had extremely low expression of β-actin (43 kDa). However, from the information available, these two individuals did not appear to have any defining clinical features.

The five individuals (3 HIV-positive and 2 HIV-negative) who were represented in Figure 3.7 had relatively low levels of protein extracted from their lymphocytes, hence accurate quantification of hsp synthesis in these subjects was not possible.

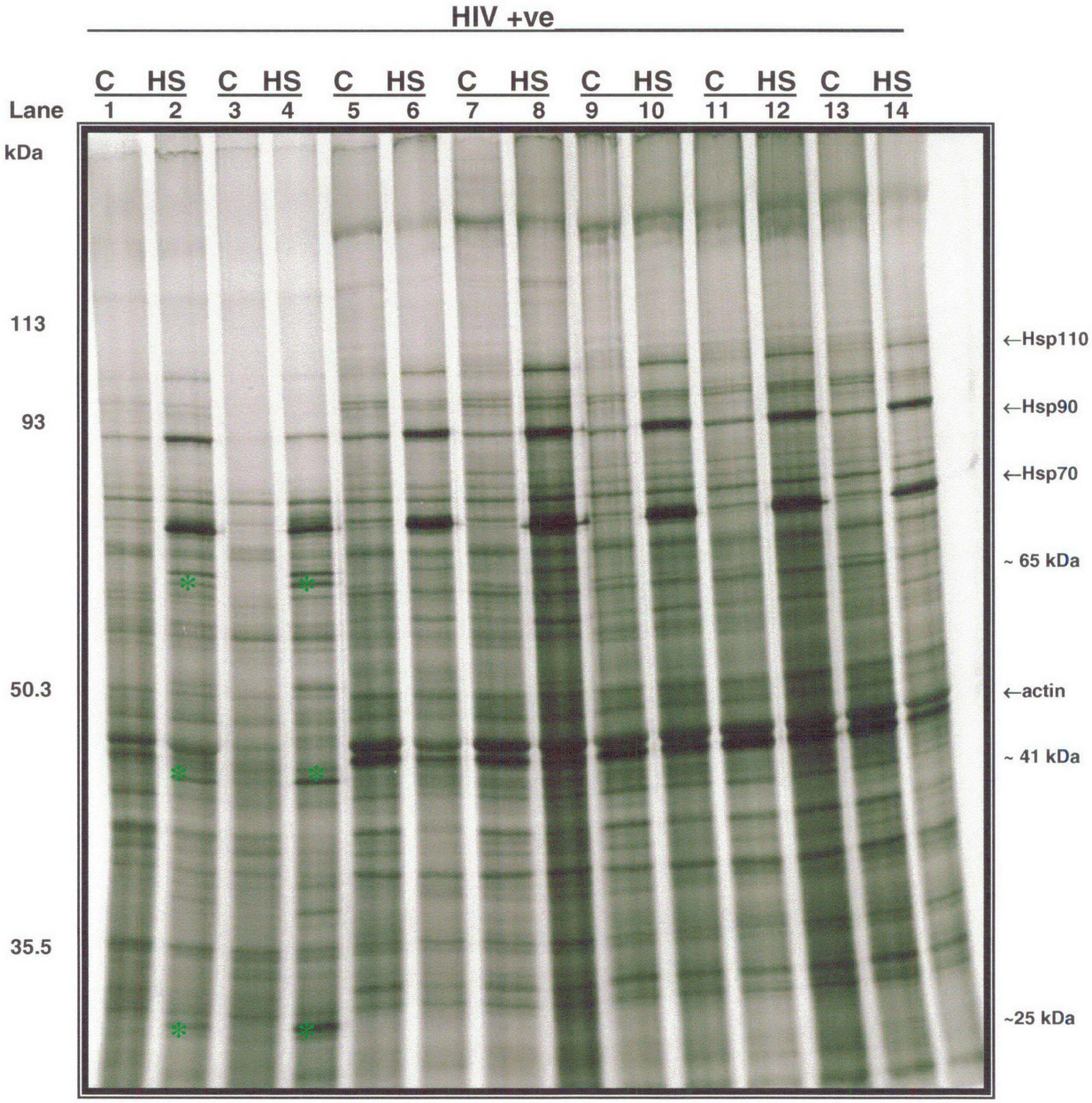

Figure 3.2: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from control and heat shocked lymphocytes. **HIV +ve** = samples from HIV-positive individuals. **C** = control samples incubated at 37°C for 4 h. **HS** = heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h. * denotes unusual protein expression.

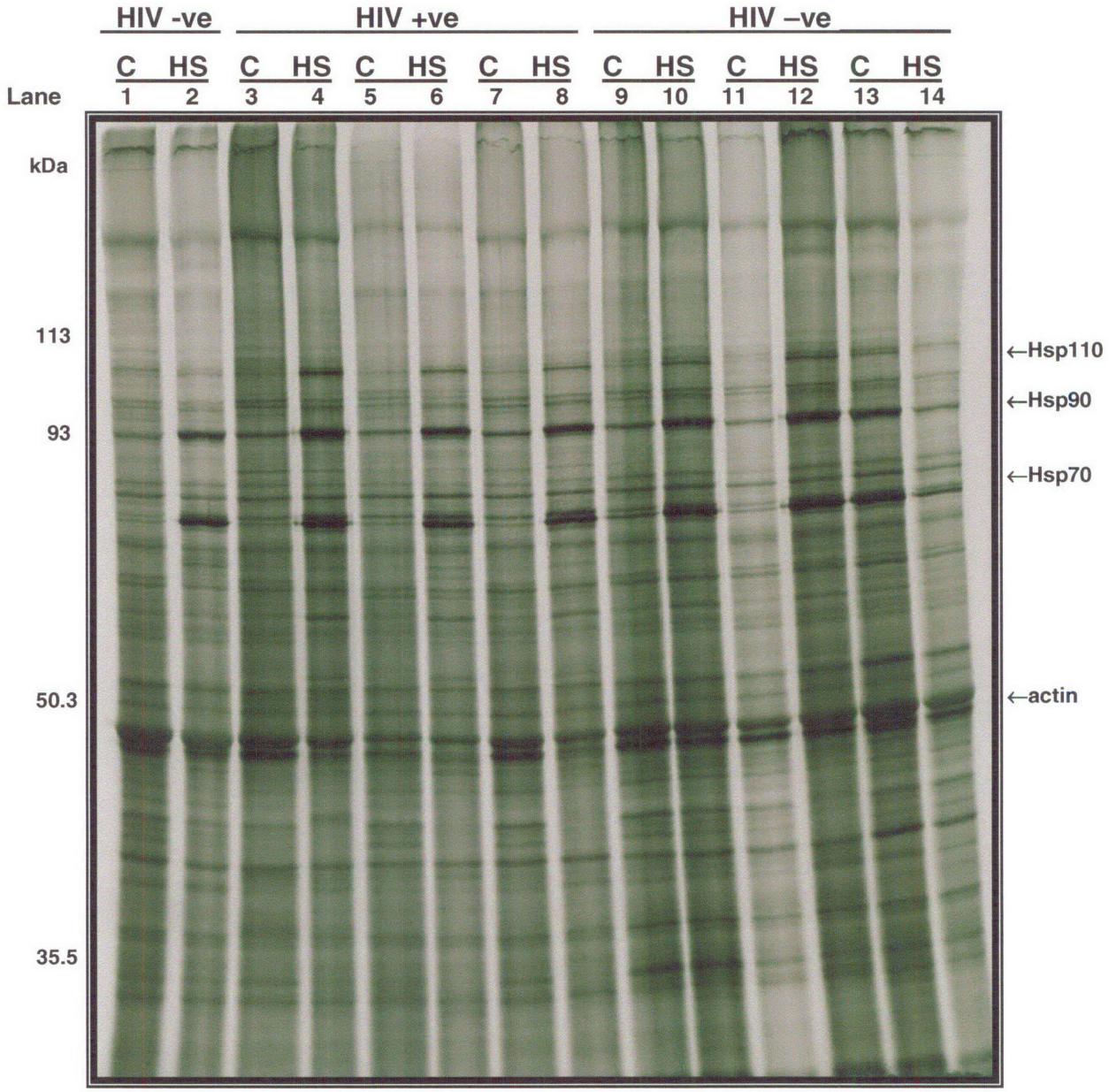

Figure 3.3: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from control and heat shocked lymphocytes. **HIV –ve** = Samples from HIV-negative individuals. **HIV +ve** = samples from HIV-positive individuals. **C** = control samples incubated at 37°C for 4 h. **HS** = heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

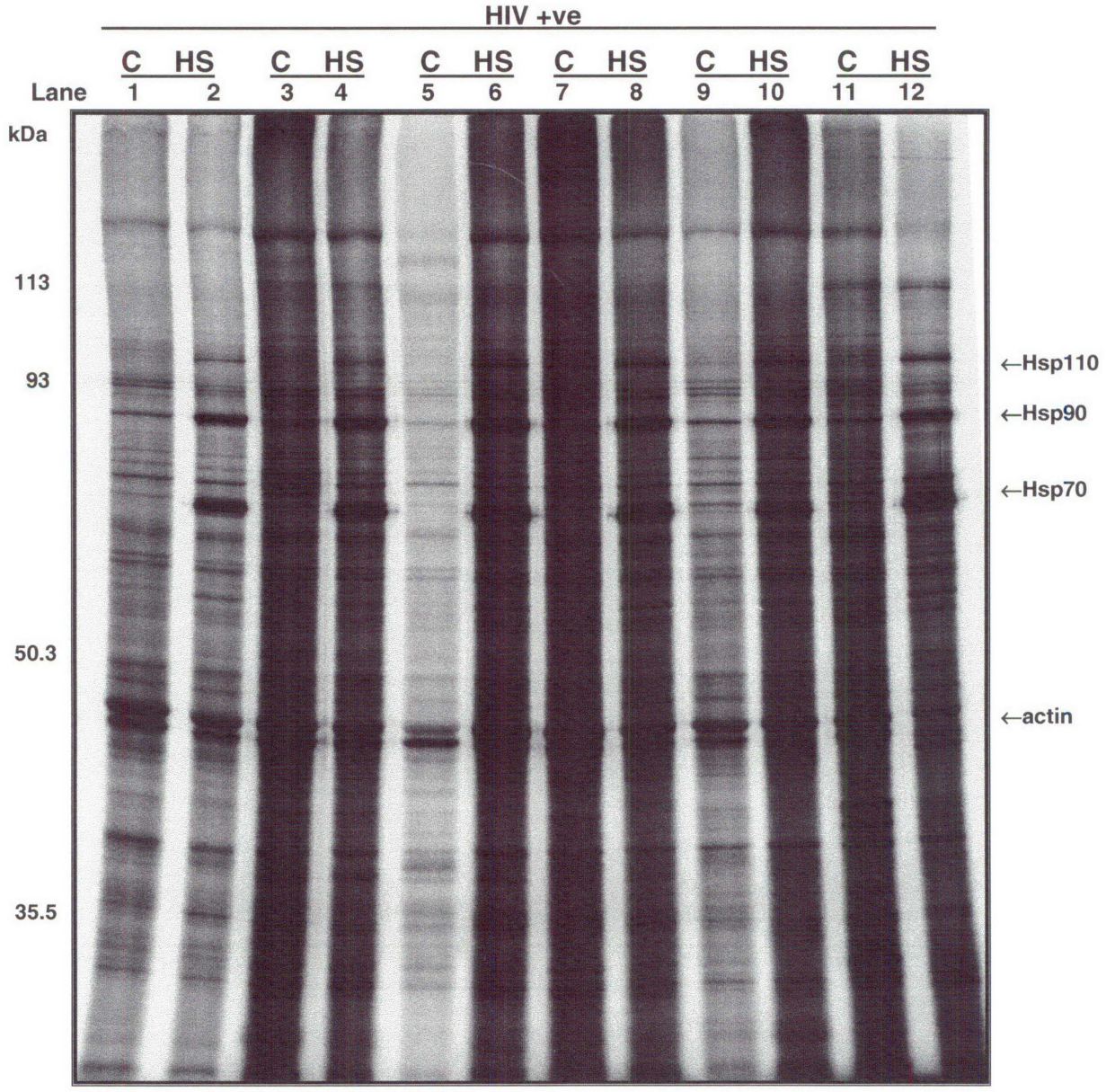

Figure 3.4: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from control and heat shocked lymphocytes. **HIV +ve** = samples from HIV-positive individuals. **C** = control samples incubated at 37°C for 4 h. **HS** = heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

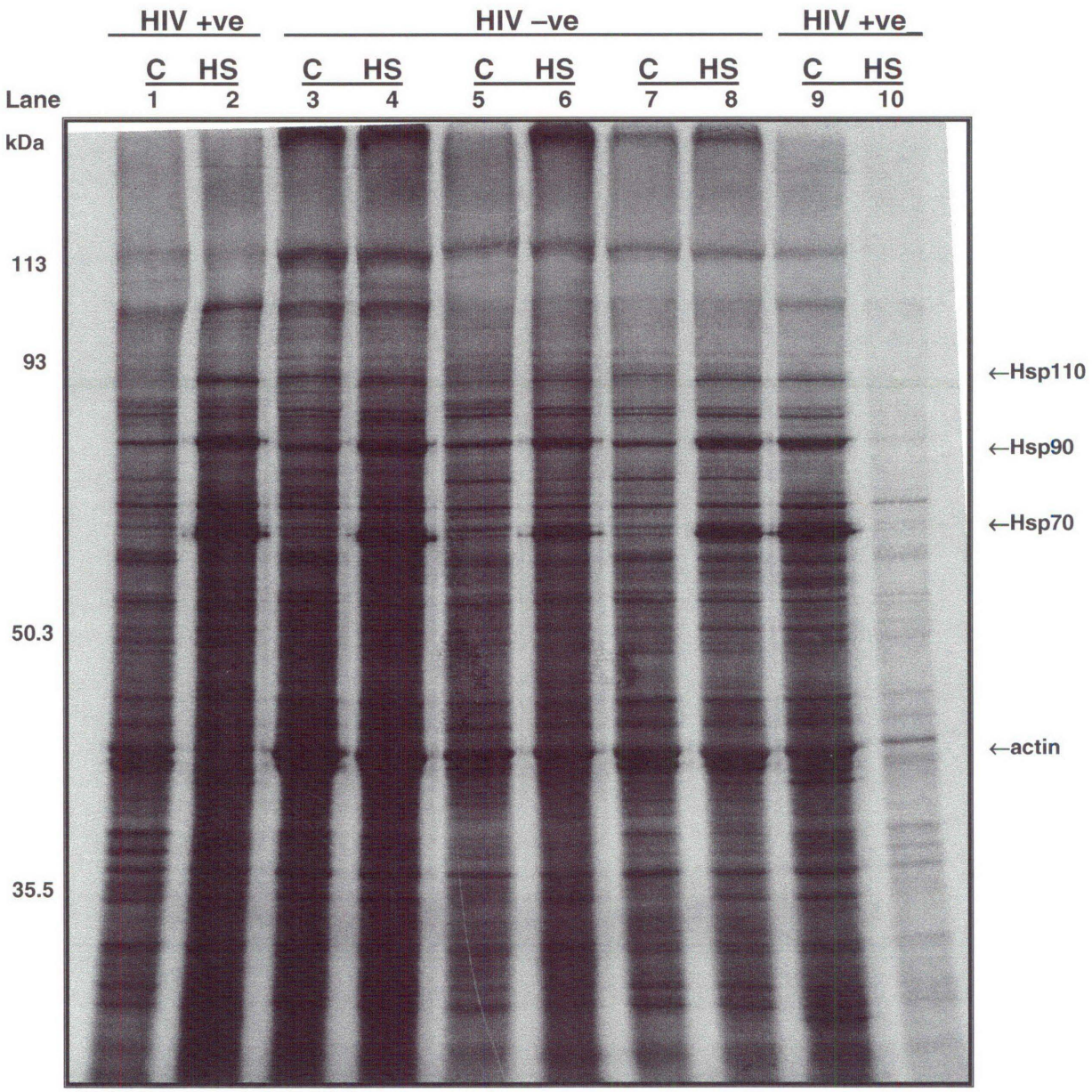

Figure 3.5: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from control and heat shocked lymphocytes. **HIV –ve** = Samples from HIV-negative individuals. **HIV +ve** = samples from HIV-positive individuals. **C** = control samples incubated at 37°C for 4 h. **HS** = heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

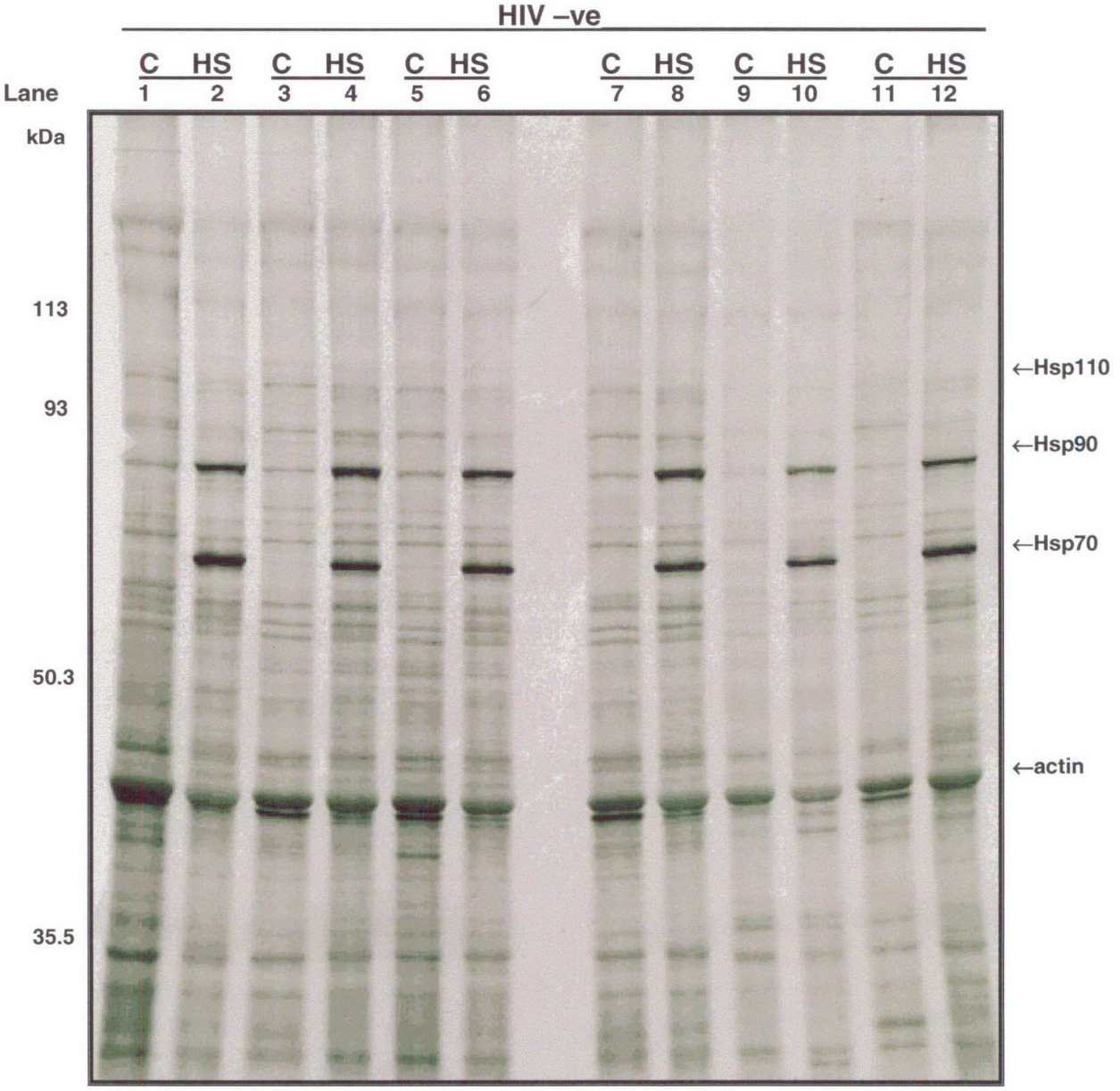

Figure 3.6: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from control and heat shocked lymphocytes. **HIV –ve =** Samples from HIV-negative individuals. **C =** control samples incubated at 37°C for 4 h. **HS =** heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

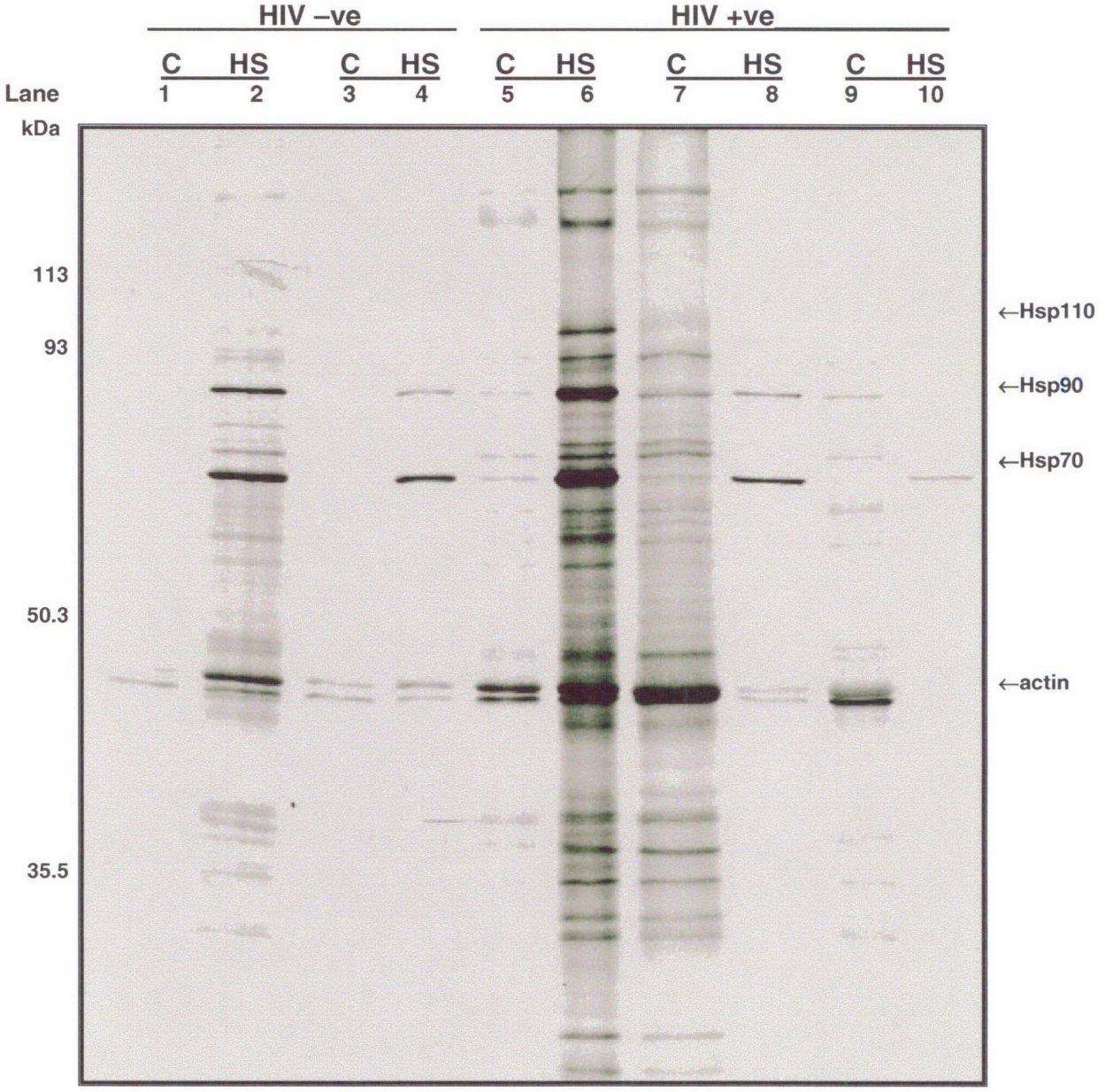

Figure 3.7: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from control and heat shocked lymphocytes. **HIV –ve** = Samples from HIV-negative individuals. **HIV +ve** = samples from HIV-positive individuals. **C** = control samples incubated at 37°C for 4 h. **HS** = heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

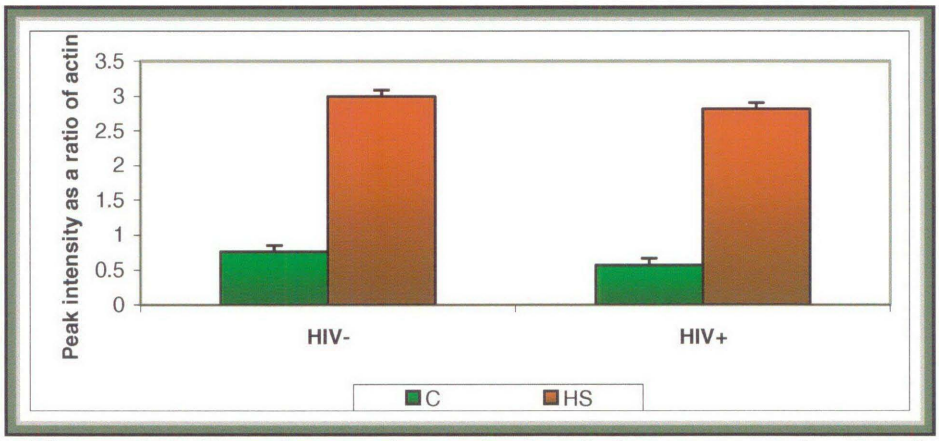

Figure 3.8: Densitometric analysis of *de novo* synthesis of lymphocyte hsp70. C = control samples incubated at 37°C for 4 h. HS = heat shock samples incubated at 42.5°C for 1 h then 37°C for 3 h. Values are means ± SEM (HIV- n = 8) (HIV+ n =18).

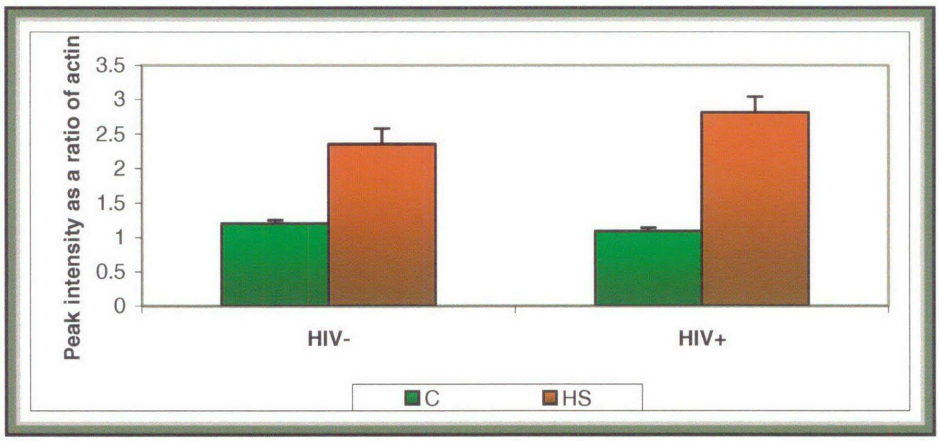

Figure 3.9: Densitometric analysis of *de novo* synthesis of lymphocyte hsp90. C = control samples incubated at 37°C for 4 h. HS = heat shock samples incubated at 42.5°C for 1 h then 37°C for 3 h. Values are means ± SEM (HIV- n = 8) (HIV+ n =18).

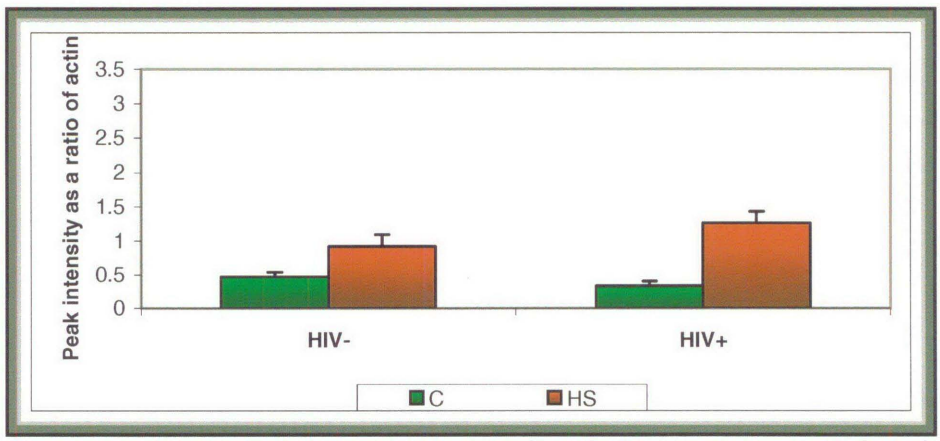

Figure 3.10: Densitometric analysis of *de novo* synthesis of lymphocyte hsp110. C = control samples incubated at 37°C for 4 h. HS = heat shock samples incubated at 42.5°C for 1 h then 37°C for 3 h. Values are means ± SEM (HIV- n = 8) (HIV+ n =18).

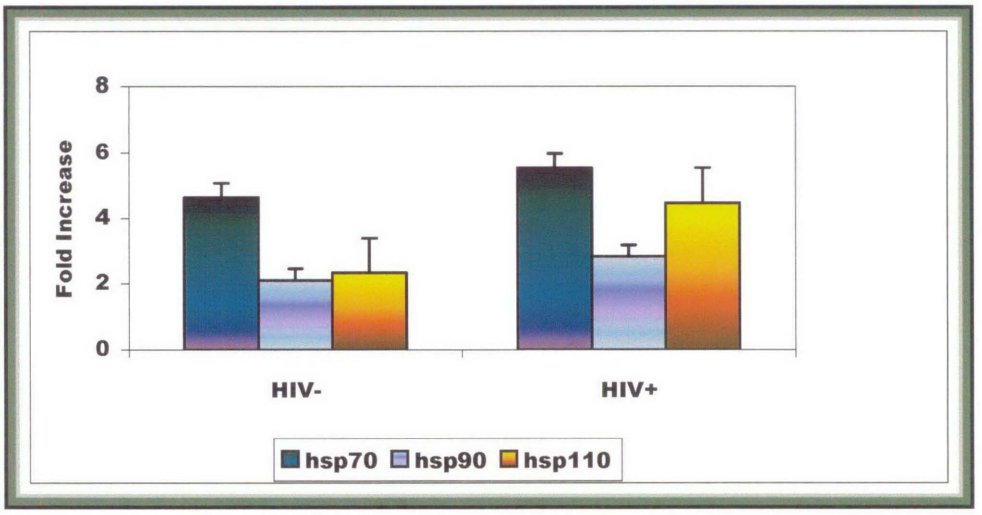

Figure 3.11: Densitometric analysis of fold-increase in hsps after heat shock. Values are means ± SEM (HIV+ n =18) (HIV- n = 8)

3.3.2 Western immunoblots for hsp90

Expression of hsp90 in leukocytes was measured by Western immunoblot (Figures 3.12A and 3.12B). Comparison of densitometric analysis of Western immunoblots measuring hsp90 expression in lymphocytes from HIV-positive and HIV-negative individuals showed no significant differences. The mean constitutive hsp90 expression (relative to β-actin) for the HIV-positive cohort was 0.36 ± 0.03 and for the HIV-negative cohort 0.28 ± 0.04 ($p > 0.05$). The mean expression of hsp90 after a mild heat shock (42.5°C) was 0.44 ± 0.03 for the HIV-positive cohort and 0.36 ± 0.03 for the HIV-negative group ($p > 0.05$). Individual results are displayed graphically in Figure 3.13 and Figure 3.14. The fold-increase in hsp90 was subsequently measured as a ratio of expression at 42.5°C compared with the expression at 37°C. As shown in Figure 3.15 the fold-increase in the HIV-positive samples was 1.27 ± 0.12 compared with the HIV-negative samples at 1.36 ± 0.17 ($p > 0.05$).

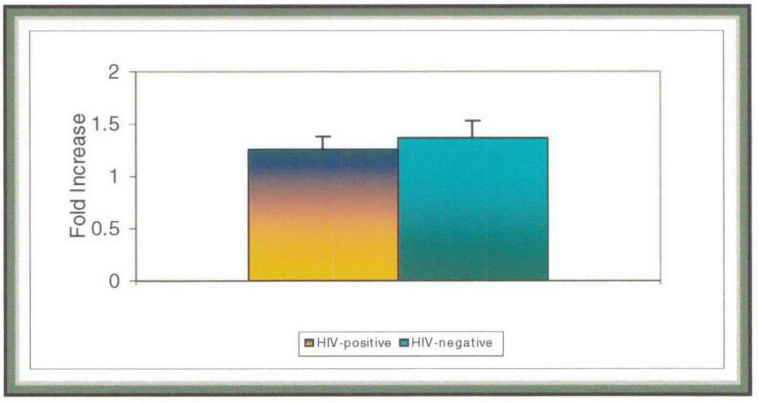

Figure 3.12: Fold-increase in hsp90 expression. Values are means ± SEM. (HIV+ n = 6; HIV- n = 4) $p > 0.05$

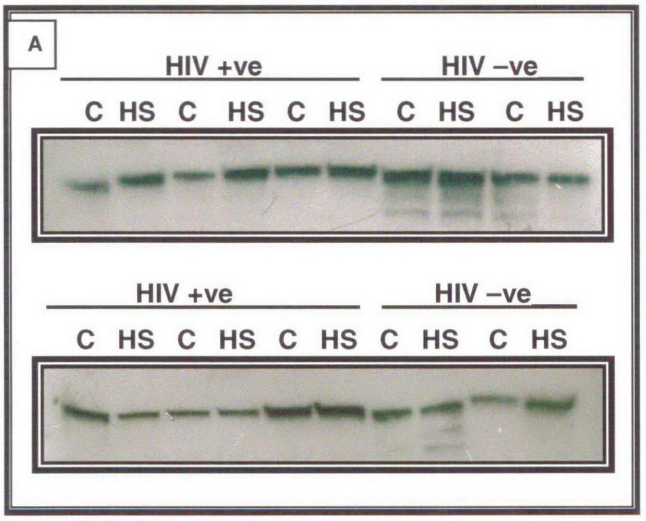

Figure 3.13A: Western immunoblots measuring hsp90 expression. HIV +ve = Samples from HIV-positive individuals. **HIV –ve** = Samples from HIV-negative individuals. **C** = expression measured under control conditions at 37°C. **HS** = expression measured under heat shock conditions at 42.5°C.

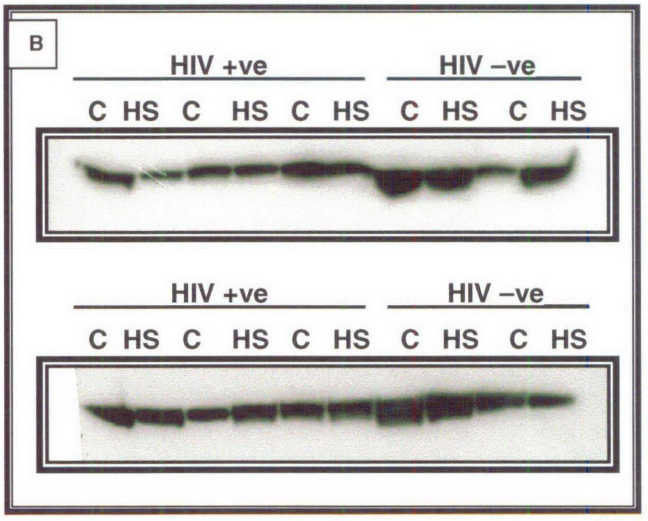

Figure 3.13B: Corresponding Western immunoblots measuring β-actin expression. HIV +ve = Samples from HIV-positive individuals. **HIV –ve** = Samples from HIV-negative individuals. **C** = expression measured under control conditions at 37°C. **HS** = expression measured under heat shock conditions at 42.5°C.

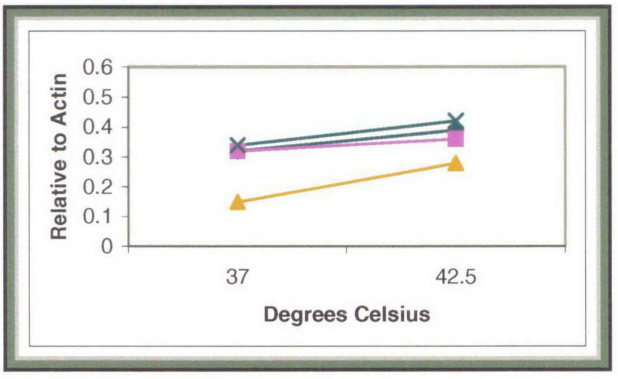

Figure 3.14: Western immunoblots of hsp90 from HIV-negative individuals (n = 4).

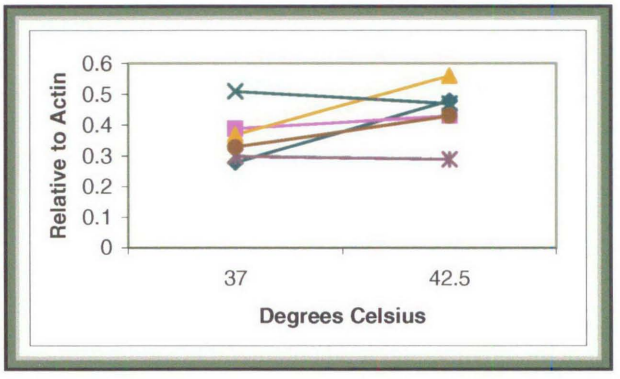

Figure 3.15: Western immunoblots of hsp90 from HIV-positive individuals (n = 6).

3.3.3 Hsp70 plasma ELISA

Concentrations of hsp70 in plasma were measured by ELISA. There was no difference in plasma hsp70 levels in the HIV-positive or HIV-negative individuals examined in this study (Figure 3.16). The mean plasma hsp70 concentration in the HIV-positive cohort was 10.24 ± 2.63 ng/ml plasma and in the HIV-negative cohort 10.83 ± 1.84 ng/ml plasma ($p > 0.05$).

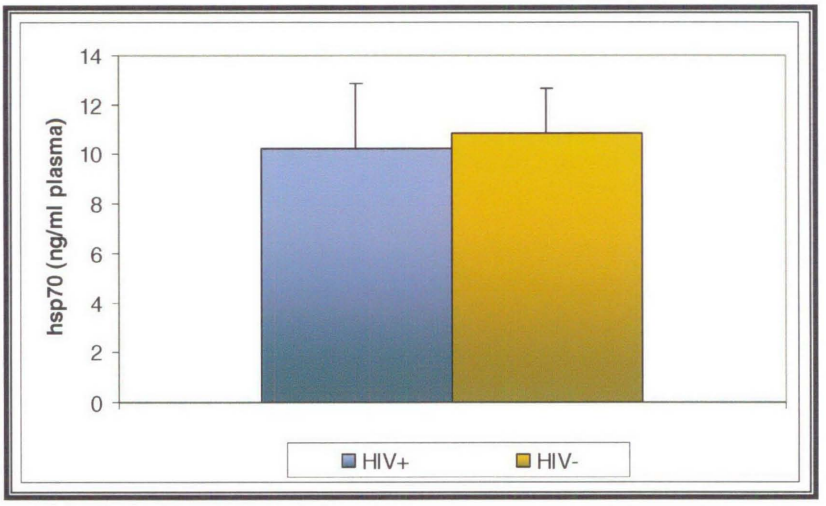

Figure 3.16: Plasma hsp70 in HIV-positive and HIV-negative individuals as measured by ELISA. Values are means ± SEM (HIV+ n = 11, HIV- n = 10).

3.4 Discussion

The current studies examined the expression of newly synthesized proteins in leukocytes by 1D-SDS-PAGE, the expression of relative amounts of constitutively expressed and heat shock induced hsps in leukocytes by Western immunoblot and the level of hsps in the plasma of HIV-positive individuals and uninfected control subjects. Comparison of patterns of hsp expression in HIV-positive and HIV-negative individuals revealed that patients with HIV-disease displayed altered expression of several of the heat shock proteins. There were apparent differences in levels of expression of newly synthesized hsp110 and hsp90 between the HIV-positive and HIV-negative groups as measured by autoradiography. Significant differences were also apparent in absolute amounts of hsp70 as measured by Western immunoblot. Specifically, constitutive expression of hsp70 was down regulated in HIV-positive individuals with a resultant augmented fold-increase in heat shock induced hsp70 relative to constitutively expressed hsp70.

There are reports of altered hsp expression in other disease states, and in particular, studies in experimental animal models have demonstrated evidence of the participation of hsp in autoimmune diseases. Hsp60 was discovered in the joints of mice with pristane-induced arthritis (Barker *et al.,* 1996). The β-cells of non-obese diabetic mice also showed increased expression of hsp60 (Brudzynski *et al.,* 1992). There is evidence of increased hsp synthesis in human host cells following microbial infection and in inflammatory diseases such as rheumatoid arthritis, multiple sclerosis (MS), Alzheimer's disease, systemic lupus erythematosus (SLE), Graves disease, atherosclerosis, insulin-dependent diabetes mellitus, psoriasis and chronic gastritis (reviewed in Kaufmann and Schoel, 1994). Specifically, raised expression of hsp60 has been reported in inflamed synovial tissue (Boog *et al.,* 1992) and lymphocytes (Sato *et al.,* 1996) in juveniles with chronic rheumatoid arthritis. More recently, in human studies, hsp70 was dramatically increased in the synovial fluid of patients with rheumatoid arthritis (Martin *et al.,* 2003). In addition, the expression of hsp70 on the surface of myeloid dendritic cells (DC) was upregulated in these patients implicating a role for hsp70 in chaperoning autologous antigen into synovial fluid DC via the CD14/CD91 receptors (Martin *et al.,* 2003). Over-expression of hsp90 was reported in B and T cells of patients with SLE, which correlated with active central nervous system (CNS) and cardio-respiratory disorders (Erkeller-Yueksel *et al.,* 1992). An induction of hsp60 was also found in the oligodendrocytes of patients with

MS (Selmaj *et al.,* 1991; Freedman *et al.,* 1992). Progressive decreases in brain levels of several chaperones, including hsp70, were observed in a mouse model of Huntington's disease (HD), indicating a possible role for hsps in the molecular mechanisms of HD (Hay *et al.,* 2004). It has been suggested that altered expression of hsp60 and hsp70 may play a role in the development of diabetic metabolic disturbances and complications in both type I and type II diabetes mellitus (Reviewed in Koranyi *et al.,* 2004). In patients with Hashimoto's thyroiditis, high expression of hsp60 was reported in follicular cells (Kotani *et al.,* 1996). Conversely, expression of hsps in human lymphocytes is attenuated in ageing. It has been reported that hsp90, hsp70 and hsp60 expression are all reduced, and the heat induced response of hsps 105, 56, 47, 40, 27 and 16 are all diminished with age (Rao *et al.,* 1999; Njemini *et al.,* 2004).

A report by Wainberg *et al.* (1997), indicating that hsp27 and hsp70 expression was upregulated in a HIV-infected lymphocyte cell line, initially inspired our hypothesis that hsp expression may be altered in patients with HIV-disease. We subsequently reported that lymphocyte hsp70 expression is altered in HIV-positive individuals (Agnew *et al.,* 2003). This was a highly significant publication given that it was the first clinical report on hsp expression in HIV-positive patients and to date there have been no other studies of this nature. The research presented in this Chapter therefore included novel, cutting-edge findings that extend the current understanding of altered hsp expression in patients with HIV-disease.

After a mild heat shock (from 37°C to 42.5°C for 1 h) lymphocytes displayed an augmented *de novo* synthesis of hsp110, hsp90 and hsp70, relative to β-actin, in all individuals regardless of HIV-status. The results of the current studies indicate that although absolute amounts of hsp70, as measured by Western immunoblot, were augmented in HIV-positive individuals, the amount of newly synthesized hsp70 did not differ and there were no differences in the stress response in the newly synthesized hsp70. The finding that plasma hsp70 levels were no different in the HIV-positive cohort as compared with the HIV-negative cohort suggests that the lower basal levels of hsp70 in the leukocytes of HIV-positive individuals (Agnew *et al.,* 2003) is not a result of release of more hsp70 into the circulation in these individuals.

A number of reports have been published describing elevated serum hsp60 and/or hsp70 in disease states such as infection (Giraldo *et al.,* 1999; Njemini *et al.,* 2003), pre-eclampsia (Jirecek *et al.,* 2002), cardiovascular disease (Pockley *et al.,* 2000; Xu *et al.,* 2000; Genth-Zotz *et al.,* 2004), and renal disease (Wright *et al.,* 2000), and reduced levels of serum hsp60 and hsp70 in ageing (Rea *et al.,* 2001). Other researchers, however, have demonstrated evidence that high levels of human hsp70 are associated with a low risk of coronary artery disease, probably through its multiple protective effects on a cell's response to stress (Zhu *et al.,* 2003). Furthermore, it has been reported that circulating hsp70 levels predict the development of atherosclerosis in subjects with established hypertension, and it has been proposed that hsp70 protects against or modifies the progression of atherosclerosis in this subject group (Pockley *et al.,* 2003).

The role of elevated serum hsp60 is believed to be via activation of pro-inflammatory process that lead to blood vessel pathology (Xiao *et al.,* 2005), presumably via the induction of TNF-α and matrix metalloproteinase in macrophages (Chen *et al.,* 1999) as well as induced expression of E-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and increased production of IL-6 in endothelial cells (Kol *et al.,* 1999). However, the clinical significance of elevated serum hsp70 is yet to be elucidated.

A limiting factor of the current studies was the high degree of inter-individual variability observed in the concentrations of hsp70 measured in this assay. This phenomenon was also observed by Njemini *et al.* (2003) indicating that the determinants of hsp70 release from leukocytes may be highly individual, and until the mechanisms of this release are elucidated, caution is urged in the use of this ELISA for clinical purposes. It is likely, however, that the results of the current study, indicating no difference in circulating hsp70 levels in HIV-positive individuals, were due to the recruitment of a cohort of clinically stable patients suggesting that hsp70 release from viable cells may be a result of active infection. Future studies of this nature, therefore, should compare hsp70 levels in the plasma of HIV-positive individuals at differing stages of disease progression.

The hsp90 isoforms are essential cellular components having cytoprotective, hormone signaling and cell cycle control functions (Zhao *et al.,* 2002). Expression of the major isoform, hsp90α, was examined in the current studies. The results of this

small-scale study demonstrated that there was no difference in the expression of hsp90α, as measured by Western immunoblot, in either constitutive (37°C) or induced (42.5°C) expression ($P > 0.05$). There was no difference in the measured fold-increase (measured as heat shock induced expression relative to control expression). It was interesting to note, however that two HIV-positive individuals displayed unusual patterns of expression of hsp90α. These individuals did not display augmented expression after heat shock of their cells (Figure 3.14, individuals x and ✳). It should be noted that these individuals were not the same two individuals displaying unusual patterns of hsp expression in Figure 3.2. There were also no defining clinical features in the information currently available about these participants that might suggest a possible explanation for these observations.

The difference in stress response of newly synthesized hsp90 approached significance ($p = 0.07$) in the cohort examined in this study, it may be that further studies using a larger cohort would reveal an enhanced stress response in newly synthesized hsp90.

There is noteworthy research being conducted on the expression patterns of hsp90 in various tumors with the view to treating these cancers with substances that inhibit the expression of hsp90. This research has far reaching benefits given that the range of tumors currently being examined that appear to be suitable candidates include acute and chronic pancreatic carcinomas (Gress *et al.,* 1994; Ogata *et al.,* 2000), malignant melanomas (Becker *et al.* 2004), breast cancer (Jameel *et al.,* 1992) and leukemia (Yufu *et al.,* 1992). The relevance of altered hsp90 expression in a HIV-positive cohort could potentially lead to the identification of a suitable, alternate therapeutic target.

In the current studies, newly synthesized hsp110 displayed an enhanced stress response in proteins extracted from leukocytes from HIV-positive individuals. Whilst many of the heat shock protein families, such as hsp60, hsp70 and hsp90 have been widely studied for many years, there has been very little published research on hsp110. Given the involvement of the other major heat shock protein families in disease states, it is conceivable that altered hsp110 expression could be a factor in some clinical conditions. A very recent report by Manjili *et al.* (2005) demonstrated that, hsp110 induced dendritic cells to upregulate the expression of MHC class II ,

CD40 and CD86 molecules, and to secrete the pro-inflammatory cytokines IL-6, IL-12 and TNF-α, implying that hsp110 acts as a danger signal through its interaction with antigen presenting cells (APC) and tumor cells . These findings may be related to the structural and functional similarities between hsp110 and hsp70 (Easton *et al.,* 2000) and the evidence that hsp70 activates APC through specific receptors to stimulate secretion of pro-inflammatory cytokines, up-regulation of co-stimulatory molecules and activation of dendritic cells (Asea *et al.,* 2000; Singh-Jasuja *et al.,* 2001).

The upregulation of stress proteins following a mild, non-lethal heat shock is an adaptive response that is thought to complement the constitutive antioxidant defense system found in mammalian cells (Rodenhiser *et al.,* 1985). We have previously demonstrated increased plasma protein carbonyl formation ($p < 0.05$) and decreased plasma antioxidant status ($p < 0.05$), both measures of oxidative stress, in the HIV-infected cohort (Agnew *et al.,* 2003). It is possible that increased oxidative stress parameters may be a factor involved in the alteration of hsp expression in this cohort as it has previously been demonstrated that dietary supplements of mixed antioxidants modulate hsp synthesis (Peng *et al.,* 2000).

Although the mechanisms of altered hsp expression in HIV-positive individuals are yet to be elucidated, the findings of the current studies are highly significant considering the growing body of evidence highlighting the interactions between hsps and immune function following viral infection. A recent report that expression of the hsp receptor, CD91, is increased in monocytes from patients with long term non-progressive HIV disease (Stebbing *et al.,* 2003) is of particular importance given that key components of the soluble factor, termed CAF, that suppresses HIV-replication, and which is secreted from stimulated CD8 T lymphocytes in high amounts from such individuals have been identified as α-defensins (Zhang *et al,* 2002). The latter in turn have been demonstrated to be associated with CD91 which mediates internalization of α-defensins (Nassar *et al.,* 2002). *In vitro* studies have shown that expression of hsps is upregulated in a range of DNA- and RNA-virally infected cells (Sedger & Ruby, 1994). The activation of hsps is virus and cell-type specific and has been reported to occur following infection with Newcastle disease virus (Collins & Hightower, 1982), herpes simplex virus (Notariamni & Preston, 1982; La Thangue & Latchman, 1988), several paramyxoviruses (Pelluso *et al.,* 1978), measles virus (Vasconcelos *et al.,* 1998), Epstein-Barr virus (Kotsiopriftis *et al.,* 2005), Hantavirus

(Yu *et al,* 2005), SV40 (Sainis *et al.,* 2000), varicella-zoster virus (Ohgitani *et al.,* 1998), rotavirus (Xu *et al.,* 1998), and cytomegalovirus (Santomenna *et al.,* 1990). Hsp induction as a response to viral infection is a noteworthy finding, since unlike many bacteria and parasites, viruses are not known to encode hsp genes, and consequently hsp expression in virally infected cells represents hsp entirely of cellular origin (Sedger & Ruby, 1994). There is increasing evidence that viruses utilize host hsps for replication. Specifically, HIV-1 binds cyclophilin A, a cytosolic binding protein that acts as a molecular chaperone, via the HIV Gag protein, p24 (Franke & Luban, 1996). Hsp70 was also found to be incorporated into HIV-1 virions via Gag (Gurer *et al.,* 2002). Other hsps incorporated into primate lentiviruses include hsp60 and hsp90 into HIV-1, hsp70 into HIV-2 and the simian immunodeficiency viruses SIV(MAC) and SIV(AGM) (Gurer *et al.,* 2002). Furthermore, it has been discovered that hsp70, facilitates nuclear import of HIV-1 pre-integration complexes (PICs) and hsp70 can replace viral protein R of HIV-1 during nuclear import of the pre-integration complex (Agostini *et al.,* 2000).

In summary, the results of the studies presented in this Chapter suggest that the mechanisms of altered hsp expression in HIV-positive individuals are likely to be quite complex given the nature of chaperone-virus interactions. The significance of altered hsp expression in HIV-positive individuals remains to be determined, however, given the recent reports on the role of these proteins in cross-presentation of antigens (Millar *et al.,* 2003), α-defensin internalization (Nassar *et al.,* 2002; Zhang *et al,* 2002) and pro-inflammatory cytokine production (Asea *et al.,* 2000), further investigation is merited. In this relatively small cohort no correlation between fold increases in lymphocyte hsp70 expression and viral load, CD4 T cell count or antiretroviral treatment status could be determined. Studies involving much larger cohorts will be required to examine such correlations.