Effects of Acute Exercise on Heat Shock Protein 72 Expression and Delayed Onset Muscle Soreness in Skeletal Muscle of Young and Older Men

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Abstract

The purpose of this study was to investigate the effects of acute eccentric exercise on heat shock protein (Hsp) 72 expression, and the relationships between Hsp72 levels with age, DOMS, and type I myosin heavy chain (MHC) protein levels in skeletal muscle of young and older men. Seven older (60.9 ± 0.8 years) (mean ± SE) and nine young (23.1 ± 1.1 years) healthy, moderately active males performed five sets of one legged eccentric exercise. The intensity of the first four sets were 60, 66, 72, and 84% of the subjects' pre-determined concentric10-RM (repetition maximum) with 10 repetitions in each set. The subjects exercised to fatigue at 120% of the 10-RM conc for the last set. Muscle biopsies were obtained from the vastus lateralis muscle one week prior to the exercise from the contralateral leg, and 30 minutes, 24 hours and 72 hours after the exercise from the exercised leg. Subjects reported DOMS (delayed-onset muscle soreness) at the time of each muscle biopsy from a questionnaire. Muscle tissues were analysed by SDS-PAGE and Western blotting.

There was no difference in Hsp72 levels before and after exercise, nor was there difference in the two subject groups at any of the time points. There was significant difference in DOMS before and after exercise ($p < 0.001$) but no difference between the young and the older subjects. There was no correlation between Hsp72 content and age, DOMS, or type I MHC contents. Based on these findings, it seems that the nature and the volume of exercise may be partly responsible for Hsp72 expression in human skeletal muscle. More studies are needed in examining the relationship between Hsp72 content and DOMS and type I MHC levels in human skeletal muscle.

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Introduction

Heat shock proteins (Hsp) 72 are a type of stress protein with a molecular weight of 72 kD (kilo Daltons) which are induced by heat and certain stressors associated with exercise, such as changes in pH level, accumulation of lactic acid, hypoxia (Locke, Noble & Atkinson, 1990), and oxidative stress (Locke, 1997). Hsp72 can be induced in various cells including brain and heart cells (Beck, Paidas, Tan, Yang & De Maio, 1995), liver (Kregal & Moseley, 1996), spleen (Pahlavani, Harris, Moore & Richardson, 1996), renal (Wang & Borkan, 1996), and skeletal muscle cells (Locke, Noble & Atkinson, 1990). Although the exact mechanism is still not known, Hsp help in repairing damaged proteins and protecting proteins from future attacks by various stressors mentioned above. Hsp also serve as molecular chaperones in protein folding and degradation, removing degraded proteins from cells to avoid high toxicity that may otherwise cause cell death.

Hsp72 protein expression is found to be closely related to type I myosin heavy chain (MHC) content in rat skeletal muscle (Locke, Noble & Atkinson, 1991a, 1991b). Type I MHC are proteins that code for the slow twitch or type I muscle fibre. There seems to be a larger proportion of type I MHC present in the aged than the young muscle (Klitgaard, Mantoni, et al., 1990). The relationship between Hsp72 and type I MHC has never been investigated in human tissue.

Aging has been associated with a decrease in Hsp expression in numerous animal studies and some *in vitro* studies in human subjects. To date, studies on Hsp72 expression in human subjects *in vivo* have focused on younger population (Knowlton et al., 1998; Liu, Mayr, et al., 1999; McGrath, Locke, Cane, Chen & Ianuzzo, 1995; Puntschart, Vogt, Widmer, Hoppeler &

Billeter, 1996). No one has yet examined specifically how aging might affect heat shock protein expression *in vivo* in the human tissue.

Delayed onset muscle soreness (DOMS) is a sensation of pain that results from unaccustomed exercise due to damages to the muscle fibre. Many of the stress inducers for DOMS and Hsp72 are similar, yet it is not known whether a relationship exists between the two.

In general, past research in the field of heat shock proteins has focused on animal studies. Those that examined human subjects employed mostly younger adults. The implications for Hsp studies on older individuals may be of great significance, since the ability of aging human cells to express Hsp upon a proteotoxic event is not known. Once the extent of the ability to express Hsp in aging human cells due to various stresses is established, the results can be used to examine the possibility of enhancing the protection and recovery of aging cells from future proteotoxic damages. Therefore, further research in human subjects, particularly *in vivo* studies on older individuals are needed to advance the knowledge with regard to Hsp.

Purpose

The purpose of this study was three-fold: to compare Hsp72 protein levels in skeletal muscle of young and older men after acute exhaustive exercise, to examine the relationship between Hsp72 levels and DOMS, and to examine the relationship between the pre-exercising levels of type I MHC and Hsp72 proteins.

Hypotheses

1. Hsp72 levels will increase as a result of exhaustive eccentric exercise stress in men.
2. Hsp72 levels will increase to a lesser degree in older men following exhaustive eccentric exercise compared to young men.
3. Hsp72 levels will correlate positively with DOMS ratings after eccentric exercise.
4. Hsp72 and type I MHC levels will correlate positively in skeletal muscle of young and older men.

Review of Literature

Stress Proteins

Stress proteins (SP) are highly conserved proteins that are found in all organisms from bacteria to higher plants and humans (Schlesinger, 1990). Several types of SP have been discovered since the first report of the stress response in *Drosophila* cells (Ritossa, 1962). As the name suggests, SP can be induced by a variety of stresses, such as increased temperature, ischemia, reactive oxygen species, the presence and synthesis of abnormal proteins in the cell, uncoupling of oxidative phosphorylation, glucose deprivation, and alterations in pH and calcium levels (Locke, 1997).

Role as Molecular Chaperones

Some SP may be involved in normal cellular processes during unstressed conditions (Locke, 1997). These proteins are known as the "molecular chaperones" because they facilitate the folding of the polypeptides into their mature, tertiary structure, but are not part of the complex themselves (Locke, 1997). In addition, SP induce newly synthesized proteins to fold into conformations that facilitate movement through cellular membranes (Wolfe, 1993).

Heat Shock Proteins

The SP that are induced by heat are called heat shock proteins (Hsp). Besides heat and high cellular temperature (Moseley & Gisolfi, 1993), Hsp can be induced by exposure to heavy metals, ethyl alcohol and oxygen deprivation (Locke, 1997; Benjamin, Kroger & Williams, 1990; Hortobagyi et al., 1996). Other stressors that are capable of inducing the expression of Hsp include the accumulation of excess amounts of oxygen radicals (Locke, 1997), decreases in

glycogen content and pH levels, and intracellular calcium concentrations (Locke, Noble & Atkinson, 1990).

Hsp are classified according to their molecular weight in kD. There are several families of Hsp: Hsp70, Hsp90, Hsp27, Hsp47, Hsp110, Hsp100 and Hsp60 proteins (Schlesinger, 1990). The focus of this review is on the Hsp70s since they are particularly relevant to the study of exercise (Locke, Noble & Atkinson, 1990) and aging (Heydari, Wu, Takahashi, Strong & Richardson, 1993).

There are four isoforms of Hsp70 found in most organisms, ranging from yeast to humans (Schlesinger, 1990). The glucose regulated proteins (GRP) are induced when the cells experience conditions such as glucose starvation and anoxia (Mizzen, Chang, Garrels & Welch, 1989), calcium ionophores, (Mizzen et al., 1989), and other agents that perturb the N-linked glycosylation of nascent proteins (Munro & Pelham, 1986). The two isoforms of GRP are GRP 78 and GRP 75, located in the sarcoplasmic/endoplasmic reticulum and mitochondria, respectively (Pelham, 1982).

Another isoform, the heat shock cognate 73 (Hsc73) is constitutively synthesized (which means it is present under control or unstressed conditions) and is only slightly stress inducible in most cells (Locke, Noble & Atkinson, 1990). The fourth isoform, Hsp72, is the highly inducible form of the Hsp70 family. Both Hsc73 and Hsp72 can also act as molecular chaperones, and are involved in protein synthesis, folding, transport, disassembly and degradation (Locke, 1997; Schlesinger, 1990). They are also able to restore the function of proteins that are damaged during stress or prevent protein aggregation and denaturation (Locke, 1997) which can be toxic to a cell if not prevented.

Induction of Hsp70

The precise procedure for the induction of Hsp and their protective functions are still not known. Although some researchers have detected the absence of HSF (heat shock transcription factor) binding in some instances where Hsp72 were induced (Locke, Tanguay & Ianuzzo, 1996), other researchers have proposed a mechanism for Hsp induction. It involves the binding of HSFs, a regulatory protein (Locke & Tanguay, 1996b), to the heat shock elements (HSEs) that are found on the promoter regions (about 80 to 150 nucleotides upstream from the start point for transcription) of many Hsp (Pelham, 1982; Bienz & Pelham, 1986; Wolfe, 1993). HSF1, one of the heat shock transcription factors, is activated by the presence of heat, denatured proteins and other related stresses (Sarge, Murphy & Morimoto, 1993).

In the absence of stress, the Hsp70 are bound to HSF1 in the cytoplasm. However, in the presence of stress, the affinity of the stress-damaged or incorrectly folded proteins for Hsp70 are higher than the HSF1 for Hsp70. This leads to the formation of the Hsp70-damaged protein complex, and the free HSF1 forms a trimer. This trimer enters the nucleus and binds to the HSE, activating the transcription and subsequently the translation of Hsp70. As more Hsp70 are released by the ribosomes, there is an increased amount of free Hsp70. In the process of the complex formation, the damaged proteins are restored through refolding (Wang & Borkan, 1996), although the exact mechanism is not known. The Hsp also enhance cell recovery by increasing the delivery of precursor proteins to important organelles such as the mitochondria (Wang & Borkan, 1996).

Once the free Hsp70 level increases, the damaged proteins lose their affinity for Hsp70 and are released from the complex. The free Hsp70 bind to the activated HSF1 and thus inhibit

further activation (Locke, 1997). In addition, Liu, Kim, Yang and Li (1993) suggested that the over expression of the Hsp70 protein may decrease the level of HSF1:HSE-binding activity, allowing the HSF1 to bind to the Hsp70, and no new Hsp70 will be synthesized.

Studies on Heat Shock Proteins70

There is a vast amount of literature on Hsp70. This review of literature will cover only those that pertain to our study.

Animal Studies

Some researchers have investigated the content of Hsp in different animal tissues. Blake, Gershon, Fargnoli and Holbrook (1990) examined the effect of *in vivo* heat shock on the Hsp70 mRNA expression in the brain, liver, lung and skin of rats. Compared to previous *in vitro* studies, they found maximum Hsp70 expression in these tissues at one hour after heat shock, but not in the liver (Blake, Gershon, et al., 1990). Three hours after removal of the heat stress, the Hsp70 content had returned to baseline levels. In the liver, maximum Hsp70 expression occurred six hours after heat stress. It was proposed that there are physiological factors which influence the cellular stress response *in vivo* that cannot be studied *in vitro*.

Beck et al. (1995) examined the Hsp72 expression in brain, liver, colon and heart after *in vivo* heat shock in rats. They found that Hsp72 content was highest in the liver and colon, and lowest in the heart and brain. These investigators concluded that this discrepancy of expression is due to intrinsic cellular characteristics rather than to physiological or environmental conditions, as their result differed from past *in vitro* studies (Beck et al., 1995).

Locke, Noble and Atkinson (1991a, 1991b) demonstrated that Hsp72 is expressed in unstressed rat skeletal muscles. In particular, its constitutive expression is proportional to the type I muscle fibre composition. For instance, the soleus muscle which is primarily comprised of type I fibres, has a higher level of Hsp72 than the white gastrocnemius, which consists predominately of type IIb fibres.

In a stress-related study, increased Hsp72 content was observed in hypertrophied muscle in the blue-winged teal (Kilgore, Timson, et al., 1994). The researchers indicated that stressors eliciting changes in muscle protein expression, including mechanically excising muscle tissue resulting in the loss of muscle mass may elicit Hsp72 synthesis.

Liu, Kim, et al. (1993) observed in the rodent fibroblast cells that a high level of HSF-DNA binding activity is insufficient for the induction of Hsp70 mRNA synthesis. These researchers noted that the regulation of the heat shock response involves the heat-inducible HSF (HSF 1) and a constitutive HSE-binding factor (CHBF).

In contrast to the above finding, HSF1 activation was observed in *in vivo* rat skeletal muscle cells (Locke & Tanguay, 1996b). Previous studies on the HSF activation have always been based on *in vitro* studies. Locke and Tanguay (1996b) found that increased level of Hsp in muscle from a previous heat treatment actually increases the level of HSF activation, contrary to the simple prediction from cell culture experiments in which HSF activation was found to be inversely related to Hsp70 content. Locke and Tanguay (1996b) attributed this discrepancy to different relative stress levels as well as the amount of Hsp already present when the second heat shock was applied. They suggested that it may be the differences in HSF1 protein content, which remains to be determined, that is responsible for the observed differences. In addition, Locke and

Tanguay (1996b) postulated that the amount of "free" Hsp70, which cannot be distinguished from the bound form of Hsp70, may be the factor that causes HSF activation.

High amounts of Hsp72 were detected in unstressed swine heart cells (Locke, Tanguay & Ianuzzo, 1996). However, no HSF activation was detected in these cells. It was concluded that hearts of large mammals (swines as to rodents) express Hsp72 constitutively without stress, and that the Hsp72 expression may be activated by a different transcription factor other than HSF in the swine heart (Locke, Tanguay & Ianuzzo, 1996).

Human Studies

To determine if Hsp72 is an inducible protein in humans, McGrath et al. (1995) obtained human lymphocytes from blood samples and subjected the cells to heat shock at 37°C for 3 hours, or 45°C for 30 minutes then 37°C for 2.5 hours. Although the subjects were described only as "normal individuals", the results revealed that Hsp72 is an inducible protein in humans (McGrath et al., 1995).

The same researchers conducted an *in vivo* study on Hsp72 expression in the human heart (McGrath et al., 1995). The subjects were patients undergoing cardiac operations; their age ranged from 4 to 70.6 years. Three samples of the heart muscle were obtained from the right atria before bypass, after reperfusion, and after bypass. Hsp72 protein content in the human heart was 168% before bypass, 164% after reperfusion, and 143% after bypass of the Hsp72 levels in the swine heart (McGrath et al., 1995). There was no observable increase in Hsp72 content after reperfusion and bypass, compared to the before bypass level. The researchers postulated that the already high levels of Hsp72 may have been sufficient to render myocardial protection for the ischemic episode that followed. Furthermore, they also suggested that this high Hsp72 level

before bypass may have inhibited Hsp72 transcription, since Hsp72 has been shown to inhibit additional production by binding to the heat shock transcription factor. Another reason suggested for the lack of increase in Hsp72 was that the duration for the surgical procedure was too short, that the time for detecting Hsp72 accumulation may have been insufficient.

Recently, another group of scientists investigated the expression of Hsp72 in normal and failing human hearts (Knowlton et al., 1998). The human heart samples were obtained from normal, unused donor hearts and hearts of patients with dilated and ischemic cardiomyopathy. Considerable individual variation was found in Hsp72 content among both the normal and failing hearts. However, it was noted that no Hsp72 protein was increased significantly in either diseased hearts compared to the normal hearts. Knowlton and coworkers (1998) suggested that the failure to increase Hsp72 may represent a loss of responsiveness (or desensitization), and that the continual stress stimuli in patients with failing heart may blunt or block the stress response.

In summary, studies of the effect of heat shock on animal cells have shown 1) *in vivo* physiological factors influence the expression of Hsp that cannot be detected by *in vitro* studies; 2) discrepancy of Hsp72 expression may be due to cellular characteristics and not the environmental conditions, and 3) there is a discrepancy involving the role HSF activation plays in Hsp studies. In human cells, researchers have demonstrated that 1) Hsp72 is an inducible protein in human lymphocytes; 2) Hsp72 content is inherently high in patients undergoing cardiac operation and there is no further increase in Hsp72 content in these patients after the surgery, compared to the normal, unused donor hearts.

Hsp and Exercise

Animal Studies

Several investigators have examined the effect of exercise on the expression of Hsp. With respect to the duration of exercise required to elicit the heat shock response, the findings are somewhat inconsistent. Also, employing different types of exercise (ie. short term vs. long term) and the effects of exercise on the muscle type can also lead to different conclusions. Sim, Locke and Noble (1991) demonstrated that short-term (four weeks) exercise training provided a sufficient stimulus to increase Hsp72 content in rat hindlimb muscles. It was discovered that acute exercise (21 days) elicited a marked increase in stress protein synthesis in untrained rats while this response was not apparent in trained rats (Brickman et al., 1996). Contrary to Sim et al. (1991)'s finding, Brickman et al. (1996) found that chronic (63 days) exercise was required to increase Hsp70 content in trained rat skeletal muscle instead of acute exercise.

Locke, Noble and Atkinson (1990) demonstrated that exhaustive exercise with treadmill running is a sufficient physiological stimulus to induce the synthesis of Hsp72 in rat soleus muscles, peripheral lymphocytes and spleen cells, when compared to non-exercised untrained control cells.

Samelman & Alway (1996) showed a significantly greater expression of Hsp72 and Hsc73 in rat plantaris muscles that underwent 16-20 weeks of endurance training as compared to the untrained controls. It was concluded that increased expression of Hsp72/73 is a general adaptive mechanism to exercise stress in fast muscles.

Investigating the heat shock response in different type of muscle undergoing exercise in rats, Hernando and Manso (1997) found a higher level of Hsp72 in the soleus, which was the slow

twitch, active muscle, than the extensor digitorum longus (EDL), the fast twitch, less active muscle before exercise. Two hours after exercise, Hsp72 level increased appreciably in both muscles. However, by 4 hours post exercise, Hsp72 level in the soleus continued to increase linearly, but in the EDL, Hsp72 level declined following the increase at two hours post exercise. Based on observing Hsp72 increase in both the active and less active muscle post exercise, Hernando and Manso (1997) substantiated that the signal which initiates the stress signal is produced by and affects the whole organism. Due to the different patterns of synthesis, the investigators further proposed that the stress response experienced by both skeletal muscle was different, and that there may be a specific function for Hsp72 that is only prevalent in slow-twitch fibres (Hernando & Manso, 1997).

Human studies

Puntschart et al. (1996) demonstrated a significant Hsp70 mRNA increase in the medius vastus lateralis muscle following acute exercise. Four young males and one female subjects exercised on a treadmill at their individual anaerobic threshold for 30 minutes and the muscle biopsies were obtained at 4 minutes, 30 minutes, and 3 hours after the exercise. In spite of an increase in Hsp70 mRNA expression, the levels of Hsp70 proteins did not increase even at 3 hours after exercise. Puntschart et al. (1996) suggested two reasons for the lack of increase in Hsp70 protein levels: the increase was delayed, or the amount of pre-exercising protein level was too high that any newly synthesized protein was too small to produce a significant difference.

Ten highly trained, 18 year-old rowers underwent a four-week intense training program for the World Championships (Liu, Mayr, et al., 1999). Muscle biopsies were performed before, and at the end of each week of training. The amount of training peaked in the second week and

dwindled in the third and fourth weeks. The training program was so intense that blood lactate levels frequently reached 4 - 8 mmol/L. Liu, Mayr, et al. (1999) found a significant increase in Hsp70 content after the first week of training. It is not known if Hsp70 content from the four subsequent biopsies were statistically different from each other, although the Hsp70 content appeared highest after the third week of training. Liu, Mayr, and colleagues (1999) also found Hsp70 protein increased greatest following the second week of training in which the amount of training was highest, and reduced following the cessation of training. With regard to the mechanisms of Hsp70 protein increase in rowers, Liu, Mayr, et al. (1999) contend that it was not so much the "heat-shock" effect of exercise which promoted induction, but likely the lowering of muscle pH as a result of high lactate levels, the increased levels of oxidative free radicals, and glycogen depletion from substrate oxidation (Liu, Mayr et al., 1999).

In summary, most studies on Hsp and exercise were performed on animals. The findings suggest that: 1) researchers are in conflict as to whether acute or chronic exercise will stimulate Hsp72 expression in trained rat hindlimb muscles, although acute exercise increases the stress protein synthesis in untrained rats; 2) exhaustive exercise is sufficient to induce or enhance Hsp72 in rat soleus, peripheral lymphocytes and spleen cells; 3) there is an increase in Hsp72/73 expression with response to exercise in fast twitch muscles in animals; 4) the stress signal may affect the whole organism, and the active and less active muscle experience different patterns of Hsp72 synthesis; 5) Hsp70 mRNA and protein levels can be increased in young human skeletal muscles, and 6) the volume of training may affect the amount of Hsp70 expressed.

Heat Stress and Exercise on Hsp Expression

Animal Studies

Some investigators have examined the effects of both exercise and heat stress on the expression of heat shock proteins. Locke, Noble, Tanguay, et al. (1995) showed that exercise is an adequate stimulus for HSF activation and the resultant accumulation of Hsp70 mRNA in rat hearts compared to hearts from heat treated rats.

In another heart study (Locke, Tanguay, Klabunde & Ianuzzo, 1995), rats underrwent one bout of exercise, three bouts of exercise, heat shock, or no heat shock. It was found that hearts from heat shocked rats and rats that underwent three bouts of exercise showed a significant higher amount of Hsp72 content than the control rats and the rats that underwent one bout of exercise. There was no Hsp72 protein difference in hearts of the control rats and rats that underwent one bout of exercise, nor in the hearts of the heat shocked rats and rats that underwent three bouts of exercise. In regard to exercise, Locke, Tanguay, Klabunde, et al. (1995) suggested that one bout of exercise was not sufficient to elevate Hsp72 content, and that since the half-life of Hsp72 is ~ 48 hours, second and third bouts of exercise were necessary to maintain the elevated levels of Hsp72 (Locke, Tanguay, Klabunde, et al., 1995). This result was confirmed in a subsequent work by Brickman et al. (1996) that a longer period of training is needed for the expression of Hsp70.

Skidmore, Gutierrez, Guerriero and Kregel (1995), on the other hand, contended that it is not heat shock but the exercise itself that signals Hsp production. These researchers demonstrated Hsp72 induction in rats exercising under cool environment while maintaining the colon temperature of these rats at the baseline level.

Human Studies

Ryan, Gisolfi and Moseley (1991) examined the synthesis of Hsp70 from human leukocytes. Two subjects exercised in the heat and maintained their rectal temperature above 40°C for 60-75 minutes. Five subjects exercised in a cool environment and their rectal temperature was below 40°C. Leukocyte samples were obtained before and post exercise, and incubated in either 37 or 41°C. There was an increase of 1.4 to 2.5-fold in Hsp70 synthesis in pre-exercised leukocytes incubated in 41°C. Hsp70 level was reduced when the leukocytes obtained from subjects who exercised in the heat were further incubated in 41°C. Ryan and coworkers (1991) suggested that the increase of Hsp70 levels is independent of core body temperature, that factors other than heat stress may contribute to the induction of stress proteins during exercise. Furthermore, it was postulated that the reduction of 70K stress protein synthesis is a result of the presence of the previously synthesized 70K stress protein (from exercise) regulating its own synthesis (Ryan et al., 1991).

In summary, most studies on the effects of exercise and heat stress on Hsp expression were performed on rat hearts and skeletal muscles and few were based on humans. The findings suggest that 1) exhaustive exercise is sufficient in eliciting HSF activation and mRNA accumulation in rat hearts; 2) heat stress and exercise improved postischemic recovery in rat hearts; 3) Hsp70 synthesis in human leukocytes can be regulated by the presence of previously synthesized Hsp from prior heat stress, and 4) factors other than increases in temperature may be responsible for Hsp induction during or following exercise.

Hsp and Aging

Animal Studies

The effect of ischemia on Hsp72 and Hsp73 mRNA induction was examined in young and old rat hearts (Nitta, Abe, Aoki, Ohno & Isoyama, 1994). A brief period of ischemia decreased but did not delay the induction of Hsp72 and Hsp73 mRNAs in old rat hearts. In addition, a longer period of ischemia induced the mRNAs of both Hsp and Hsp72 protein expression in the old hearts, although the levels of the mRNAs were comparable to that of the young heart. Nitta et al. (1994) concluded that there may exist a defective sensing mechanism for stress in the old hearts. Besides ischemic stress, hypertension is also a stress which aging heart cells undergo. A decrease in Hsp72 expression due to hyperthermic stress was observed in older rat hearts that were spontaneously hypertensive and normotensive (Bongrazio, Comini, Gaia, Bachetti & Ferrari, 1994).

In another study, Locke and Tanguay (1996a) demonstrated reductions in HSF1 activation, Hsp72 mRNA and Hsp72 protein content compared to adult rat hearts following heat stress. It was proposed that the increased susceptibility of aged hearts to physiological stresses may be explained in part by the decreased ability to activate HSF1 leading to a decreased Hsp expression and subsequent loss of myocardial protection (Locke & Tanguay, 1996a).

Similar studies were also conducted on tissues other than rat hearts. Fargnoli, Kunisada, Fornace, Jr., Schneider and Holbrook (1990) examined the expression of Hsp70 mRNA and proteins in cultures of lung- or skin-derived fibroblast cells in young and old rats subjected to heat shock. They observed lower levels of Hsp70 mRNA and protein induction in the lung and skin fibroblasts of the aged rats. Blake, Fargnoli, et al. (1991) also examined the expression of Hsp70

on the brain, lung and skin cells of both young and old rats that underwent heat stress. They found that the expression of the Hsp70 mRNA was lowest in the brain and highest in the lung of old rats. Compared to the young rats, however, the aged rats had a decrease in Hsp70 mRNA expression. Blake, Fargnoli, et al., (1991) concluded that this decrease was due to the inability of old rats to generate a rise in core temperature compared to the young rats following heat stress.

Blake, Udelsman, Feulner, Norton and Holbrook (1991) studied the stress induced Hsp70 expression and found that Hsp70 can be induced in the adrenal cortex of aging rats by restraint stress. This expression was eliminated in hypophysectomized rats, although it was restored when an adrenocorticotropic hormone was given to these young, hypophysectomized rats. However, the induction of Hsp70 declined when adrenocorticotropic hormone was administered to aged, hypophysectomized rats.

In a study on the synthesis and induction of Hsp70 and Hsc73 in rat hepatocytes (Wu, Gu, Heydari & Richardson, 1993), it was discovered that Hsp70 synthesis decreased by 37% in the old rat hepatocytes as compared to the young rats when they were both subjected to temperatures of 40 to 45°C. However, the basal level and the induced level of the Hsc73 synthesis did not change significantly with age. The investigators attributed the difference to the fact that the Hsc73 is not as inducible as Hsp70 protein, since Hsc73 is a constitutively expressed protein.

Wheeler, Bieschke and Tower (1995) investigated the expression of Hsp70 in young and old *Drosophila* fly cultures. Young flies, which were 5 - 7 days posteclosion, underwent heat shock at 37°C for 2 hours, whereas the old flies, 36 - 38 days posteclosion, were not heat shocked. The Hsp70 protein was found to be induced 7 to 10 times in the old flies, relative to the young flies, and was specific for flight muscle and leg muscle. Wheeler et al. (1995) concluded

that the flight muscle and leg muscle expression of Hsp70 during normal aging was a consequence of oxidative damage. It was suggested that this specificity of Hsp70 expression may result from the preferential susceptibility to some type of damage or alteration, or that it may result from the relevant damage taking place throughout the organism, but that Hsp70 expression is a tissue-specific response to this damage, for which a pathway exists only in the flight and leg muscle.

Human Studies

Liu, Lin, Choi, Sorhage and Li (1989) reported a decrease of heat shock protein expression in several types of aged cells with various types of stress. They reported a reduced inducibility of Hsp72 in old, cultured human diploid fibroblast cells under heat shock (42-43 °C). Old cells were defined as having a population doubling levels of > 40. Reasons for this reduced inducibility of Hsp72 remains unknown.

Although aging was not one of the objectives in studying Hsp72 in human hearts, some researchers observed that there is no correlation between Hsp72 and the age of the subjects. McGrath et al. (1995) found that there was no significant correlation among their subjects for Hsp72 levels according to age, ranging from 4 to 70.6 years. Similarly, Knowlton et al. (1998) noted that there was no correlation between the age and Hsp72 levels, even though the age range of the subjects was less (21 to 44 years).

In summary, most of the studies of Hsp and aging were based on rat studies. Some researchers revealed a decrease in Hsp induction or Hsp mRNA expression in the aged animal and human cells as compared to the young. The exact cause for this decrease is inconclusive: one group of researchers suggested a defective sensing mechanism for stress. Another group suggested an inability to generate a rise in core temperature to induce Hsp expression. Yet other

scientists found that oxidative damage induces the expression of Hsp70 proteins in aged cells. Researchers conducting human studies conveyed that there seems to be no relationship between age and the expression of Hsp70.

Hsp, Aging and Exercise

Few studies have examined the effect of exercise and heat shock on Hsp72 expression in aging cells. Kregel and Moseley (1996) investigated the liver cells of young and old rats. Both groups were subdivided into three groups: a control group exposed to an ambient temperature of 25°C, a passive heating group (the heat shocked group) and an exertional heating group (the exercised group). Hsp72 induction in the exercising old rats increased by 232% compared with the control and heat groups, while no significant increase in Hsp72 protein of the heat shocked old rats was observed. It was concluded that aging is not the sole cause for the inability to produce Hsp72, since the aging rats were able to produce Hsp72 when they underwent exertional hyperthermia (Kregel & Moseley, 1996).

Some studies examined specifically the effect of heat shock, aging and caloric/food restriction on the expression of Hsp70 (Heydari et al., 1993; Pahlavani et al., 1996). The methodologies employed in both studies were relatively similar: three groups of male rats (young and fed ad libitum, old and fed ad libitum and old fed with restriction) underwent heat stress and the effect on Hsp70 protein and mRNA expression were examined. The differences in the methods were that Heydari et al. (1993) restricted the *caloric* intake and examined the *hepatocytes* whereas Pahlavani et al. (1996) restricted the *amount* of food intake and examined the *lymphocytes*. In both studies, Hsp70 induction by heat shock declined 40-50 % with age.

However, caloric restriction was found to completely reverse the age-related decrease in Hsp70 induction in hepatocytes. In other words, Hsp70 protein and mRNA levels were significantly higher in caloric restricted than the regular fed ad libitum rat hepatocytes. On the contrary, no effect was observed in the spleen lymphocytes. The discrepancy may be attributed to the different tissue types, or the dissimilar diets (Pahlavani et al., 1996).

In summary, animal studies have shown that reductions in caloric or food intake and exercise may influence the expression of Hsp72 protein and mRNA levels in aging rats. Studies of the effects of aging, reduced energy levels through either exercise or food/caloric restriction on human Hsp72 protein and mRNA levels have yet to be investigated.

Hsp, DOMS and Eccentric Exercises

DOMS, or delayed-onset muscle soreness is the sensation of discomfort or pain in the skeletal muscle (Armstrong, 1984). It has been observed in human subjects a few days following unaccustomed exercise (Armstrong, 1984; Gleeson et al., 1995), or following large increases in the volume of exercise (Smith et al., 1994). The duration for DOMS is inconclusive. Muscle discomfort is first felt between 8-12 (Fitzgerald, Rothstein, Mayhew & Lamb, 1991) or 8-24 hours after exercise (Armstrong, 1984; Smith et al., 1994), peaks between 24-72 hours (Armstrong, 1984; Clarkson & Dedrick, 1988; Smith et al., 1994), and disappears in about 5 (Smith et al., 1994) or 5-7 days post-exercise (Armstrong, 1984). Muscle damage often accompanies DOMS (Jones, Newham & Torgan, 1989; O'Reilly et al., 1987), and is indicated by increases in the level of serum creatine kinase (Jones et al., 1989; O'Reilly et al., 1987; Smith et al., 1994), lactic acid, and disruption of the muscle fibre and connective tissue (MacIntyre, Reid &

McKenzie, 1995). Exertional rhabdomyolysis, the extreme of DOMS, occurs after unaccustomed, exhaustive exercise, particularly in the heat (Armstrong, 1984).

Eccentric exercise is the contraction type in which the active muscle is lengthened while producing force (Gleeson et al., 1995). An example of eccentric exercise is the action of the quadriceps muscles in performing descent down a flight of stairs (Fielding et al., 1991). Eccentric exercise has been reported to cause most soreness and damage to the exercising muscle (Armstrong, 1984; O'Reilly et al., 1987). Fewer muscle fibres are recruited during the exercise for a given workload, leading to a greater tension per active cross-sectional area, which could cause mechanical and biochemical disruptions to the structural elements in the muscle fibre (Armstrong, 1984; Schwane, Johnson, Vandenakker & Armstrong, 1983; MacIntyre et al., 1995; Dean, 1988).

The mechanical damage of eccentric exercise arise from the high tension formed in the cross bridges which result in broadening, streaming, and total disruption of the Z-lines (MacIntyre et al., 1995; Schwane et al., 1983). Damage to the intermediate filaments or the strain during active lengthening which exceeds the limits of the extrasarcomeric cytoskeletal framework also result in cytoskeletal damage (MacIntyre et al., 1995). These mechanical injuries lead to acute inflammation, which is a generalized response of the body to any kind of tissue injury (Smith, 1991). It has been suggested that acute inflammation responses are associated with DOMS (MacIntyre et al., 1995). Symptoms of acute inflammation, such as pain, swelling, loss of function, heat, redness, histological changes, biochemical markers, and cellular infiltrates, are similar to those of DOMS (Smith, 1991).

In addition to mechanical injury due to high tension development in the muscle, elevated muscle temperature resulting from eccentric exercise also lead to muscle damage (Armstrong, 1984). Other biochemical accumulation of toxic waste from eccentric exercise result in DOMS (Armstrong, 1984). Since waste products such as lactate and the associated H^+ that are toxic to the cells, and increases in temperature have been shown to induce the expression of Hsp (Locke, Noble & Atkinson, 1990; Moseley & Gisolfi, 1993), it is possible that eccentric exercise may also induce Hsp expression. In addition, a review on studies of reactive oxygen species (Essig & Nosek, 1997) revealed that a bout of oxidative stress can lead to rapid increases in Hsp synthesis, although the source of the oxidative stress was not mentioned.

In conclusion, some researchers have shown that the muscle damage from eccentric exercise can result in DOMS, and others have demonstrated that some of the inducers of DOMS are the same stressors that induce the expression of heat shock proteins. However, no literature to date has examined the relationship between DOMS and heat shock protein expression.

Hsp72 and Type I MHC

Myosin heavy chains are a set of proteins which encode for the contractile element, the myosin head, in the muscle structure. The myosin molecule is made up of three different types of polypeptide chain: heavy chains (approximately 200 kDa), and two different types of light chains (each of approximately 20 kDa) (reviewed in Staron & Johnson, 1993). A single myosin molecule is a hexamer that contains two identical heavy chains, two identical P light chains, and either two identical or non-identical alkali light chains (Staron & Johnson, 1993). Due to this complexity of subunit choice, muscles can express isomyosin forms which have different

compositions. In the human skeletal muscle, slow-twitch, or type I fibre expresses type I MHC, and the two fast-twitch fibre types IIa and IIb express type IIa MHC and type IIb MHC, respectively. Selective expression of the different isomyosin forms appears to be related to muscle function and adaptation to physiological demands (Staron & Johnson, 1993). In response to changes in neural input, hormonal levels and functional demands such as increased amount of exercise due to training, or decreased amount of exercise such as detraining or disuse, skeletal muscle fibre can alter its phenotypic expression by a sequential transformation of the MHC proteins (Staron & Johnson, 1993).

Past research have demonstrated that the aging muscle contain a larger proportion of type I MHC proteins than younger muscle (Larsson, Sjodin, & Karlsson, 1978; Melichna et al., 1990). Klitgaard, Ausoni and Damiani (1989) and Klitgaard, Zhou, et al. (1990) observed a significantly greater amount of type I MHC proteins in old control group compared to the young control group, although the amount of type I MHC protein in the old control group was comparable to that of a group of trained, old runners. Klitgaard, Ausoni, et al. (1989) and Klitgaard, Mantoni, et al. (1990) concluded that relative content of type I MHC increased with aging, and attributed this increase to the selective atrophy of type II fibres. Furthermore, in a cross-sectional study involving subjects aged 23 to 77 years, Balagopal, Rooyackers, Adey, Ades and Nair (1997) found that the decline of the rate of MHC protein synthesis is related to age. However, the type of MHC protein examined was unknown.

In contrast to these findings, Klitgaard, Mantoni, et al. (1990) observed a higher proportion of fibres with coexistence of MHC types IIa and IIb in the vastus lateralis of the older

sedentary subjects, compared to the young sedentary subjects. Reason for this discrepancy is unknown (Klitgaard, Mantoni, et al, 1990).

Some researchers examined the relationship between Hsp72 and type I MHC. To investigate the effect of rat plantaris compensatory hypertrophy on Hsp72 and amount of type I muscle fibre and MHC, Locke, Atkinson, Tanguay and Noble (1994) removed the gastrocnemius muscle from the rat hindlimb. Significant increases in all three variables in the plantaris compared to the contra lateral muscle was found, although only increases in MHC significantly correlated with increases in Hsp72 proteins. It was concluded that compensatory hypertrophy induced the coexpression of several myosin isoforms which complicates the fibre identification. This explains the reason why increases in Hsp72 protein level did not correlate with increases in type I fibres. In addition, changes in Hsp72 content in rat diaphragm closely followed the increase in type I MHC expression (Noble and Dzialoszynski, 1995).

Contrary to the above findings, Ornatsky, Connor, and Hood (1995) found that MHC and Hsp70 gene expression were independently regulated. The rat tibialis anterior muscle was chronically stimulated for 10 days at 10 Hz. Ornatsky et al. (1995) observed that there was a 9-fold increase in Hsp70 level, but type I MHC amounts remained unchanged. The investigators attributed the discrepancy of finding to the lack of understanding on the functions during chronic stimulation and different tissue distributions of the HSFs (Ornatsky et al., 1995). Furthermore, large amount of Hsp72 protein was discovered in unstressed swine heart cells (Locke, Tanguay & Ianuzzo, 1996). In contrast to Locke, Noble and Atkinson (1991b) and Locke, Atkinson et al. (1994)'s findings, this high amount of Hsp72 was not related to the amount of type I MHC protein in the swine heart.

In summary, researchers generally agree that older individuals have a higher proportion of type I MHC protein in their skeletal muscle. Hsp72 protein expression has been shown to correlate highly with the expression of type I MHC in rats. However, it is unknown whether this relationship exists in human muscle. Due to the functional role that the type I MHC proteins play in determining the muscle fibre type and the widely occurrence of sarcopenia in old age, as well as the protective role that Hsp72 renders to the muscle proteins, examining the relationship between type I MHC and Hsp72 protein expression in young and aged humans would be beneficial in the furtherance of studies in heat shock proteins and aging.

Methods

Subjects

This study consisted of ten young and nine elderly, healthy, moderately active men from the City of Thunder Bay, Ontario. One young subject and two senior subjects dropped out of the study after the first muscle biopsy, therefore the final sample size was nine younger and seven older subjects. All subjects were asked not to engage in any vigorous exercise program or activity that would lead to muscle fatigue or damage 72 hours prior to testing (Smith et al., 1994).

This study was approved by the Ethics Committee for Research on Human Subjects at Lakehead University. The elderly subjects responded to public addresses at the 55 Plus Centre, and/or local newspaper, radio advertisement in the City of Thunder Bay, Ontario. The young subjects responded to in-class announcements by the investigator at Lakehead University. All subjects signed an informed consent letter, and the elderly subjects were medically approved to engage in the acute exercise bout and undergo the muscle biopsy sessions (see Appendix 1). Subjects with hip or knee replacements, previous hip fracture, and/or significant arthritis (requiring a cane for ambulation) without medical approval were excluded from the study.

Instrumentation

Exercise

The exercises were performed on the Renaissance leg extension machine in the weight room of the Fieldhouse at Lakehead University. Additional dumbbells of 0.5, 1, and/or 2 kg were placed on the weight plates as necessary to approximate the percentages of exercise intensity.

A music keyboard (Yamaha Portatone PSR-500) with built-in metronome was used to establish the rate of exercise.

DOMS

A 7-point Likert scale of muscle soreness (High, Howley & Franks, 1989) was used to assess muscle soreness (see Appendix 2).

Software

Northern Eclipse (Empix Imaging Inc. Mississauga, ON) and Scion Image Analyzer (National Institue of Health) were used to analyze the band intensity of the immunoblots.

Procedures

The subjects' one leg knee extensor 10-RM (repetition maximum) was first determined at least seven days in advance of testing. One leg was randomly assigned for the 10-RM determination by coin toss. Subjects began exercising with a light weight (30 lbs load). Depending on the ease with which this was completed, increasing load was progressively added (at increments of 10 lbs) until the subject can no longer perform 10 repetitions. The final weight was determined to be the subjects' 10-RM. The subjects were told to relax and rest between trials

for as long as they needed to ensure adequate recuperation during the 10-RM determination (Wathen, 1994).

Approximately one week following the 10-RM determination, muscle biopsy from the non-exercised leg was obtained to serve as control. The muscle samples were obtained from the vastus lateralis. A local anaesthetic (Xylocaine, 1%) (~ 3 cc; amount varied depending on the subjects) was injected in the thigh area prior to surgery. The muscle tissue sample (approximately 20-70 mg) was immediately immersed in liquid nitrogen, and stored at - 80°C for further analysis (see below). Prior to each muscle biopsy, the subjects indicated the intensity of their muscle soreness.

Eccentric exercises took place one week following the 10-RM determination. All subjects performed eccentric exercise on the 10-RM-determined leg. The exercise was performed from full extension of the knee to the resting position of the lever arm. Subjects were instructed to perform the exercises as smoothly as possible, not dropping the load and let the gravity do the work for them.

Subjects performed four sets of 10 repetitions each, and one fifth set in which subjects exercised until exhaustion. The intensity for each set was 50%, 55%, 60%, 70%, and 100% of the eccentric equivalence of the 10-RM for concentric contractions (i.e. 60%, 66%, 72%, 84% and 120% of the concentric 10-RM). The frequency of the exercise was one contraction every 2 seconds. After each contraction, a research assistant lifted the lever arm for the next contraction, in order to ensure that the damage was due to the eccentric phase of exercise alone.

There was a two-second rest between each repetition, allowing the research assistant to lift the load. A 10-second rest period between each set was given, allowing the experimenter to

change the weights for the next set. For the final set at 120% of the 10-RM, the point of fatigue was defined as failure to complete two repetitions consecutively.

In addition to the muscle biopsy obtained prior to the exercise, three more muscle biopsies were obtained from the exercised leg at 30 minutes, 24-hours, and 72 hours following the exercise.

Analysis of Muscle Biopsy Samples

The muscle biopsy samples were analyzed for the amount of Hsp72 and type I myosin heavy chain protein relative to a known amount of rat soleus sample.

Hsp72 and Type I MHC Protein Level Determination

Polyacrylamide Gel Electrophoresis. The muscle sample was added to 19 volumes of 15 mM Tris buffer, 600 mM of NaCl containing 1/200th volume each of 0.2 M phenylmethylsulfonyl fluoride in ethanol, 0.2 mg/ml Pepstatin A in ethanol and 0.1 mg/ml leupeptin in ddH_2O as protease inhibitors. Samples were homogenized with a Tekmar homogenizer for three bursts of 10 seconds separated by 5 seconds, with the Tekmar TR-10 controller set at 70. During homogenization, samples were kept on ice and then stored at -70°C until further analysis.

Proteins were quantified using the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951), using known amounts of purified bovine serum albumin (BSA, Fraction V, Sigma) to generate a standard curve. Two, 5, 10, 20, 30, 40, 50, 60, 80, and 100 µg of BSA and unknown protein samples were brought to a volume of 500 µl with double distilled water. Five millilitres of the Lowry reagent solution, prepared by adding 5 ml of 4% w/v Na-tartrate and 5 ml of 2% w/v $CuSO_4 \cdot 5H_2O$ to 240 ml of 3% w/v Na_2CO_3 in 0.1 NaOH were added; the tubes vortexed and allowed to stand at room temperature. After a 10 minute incubation, 500 µl of 20% (v/v) Folin

phenol reagent (BDH) was added with vortexing, and tubes were allowed to stand for 30 minutes. Absorbency of the solution was measured at 660 nm and protein concentration was determined by using a linear regression equation generated from the standard curve data.

One dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE, Laemmli, 1970) was performed on the muscle homogenate using the BioRad Mini-Protean II gel apparatus, as described previously (Locke, Atkinson, Tanguay & Noble, 1994). Muscle homogenates (100 μg per lane) was separated on a 12% polyacrylamide separating gel for both Hsp72 and type I MHC immunoblots. The gels were ran at a constant voltage of 110V for 2.5 hours at room temperature. Hsp72 protein and type I MHC protein conent were determined using a known amount of rat soleus muscle homogenate as internal control.

For each blot, 5 μl of molecular marker (Kaleidoscope Prestained Standards, BioRad) was loaded in lane 1. Lanes 2, 4, and 6 contained the pre-exercise, 24 hour, and 72 hour post-exercise samples of the young subject, respectively, and lanes 3, 5, and 7 contained the pre-exercise, 24 hour, and 72 hour post-exercise samples of the aged subject, respectively. Lanes 8 and 9 contained the control rat soleus muscle homogenate.

The concentrations of the proteins loaded were 200 μg of total protein per lane for Hsp72 immunoblots and 40 μg of total protein per lane for Type I MHC immunoblots. The concentration for the control rat soleus was 40 μg of total protein for all immunoblots.

Immunoblotting. Following 1-D electrophoresis, proteins were electrophoretically transferred to 0.2 μm pore size nitrocellulose (Towbin, Staehelin & Gordon, 1979) in a BioRad Mini-Protean trans blot apparatus, as described previously (Locke, Atkinson, Tanguay & Noble, 1994). Two sandwiches consisting of two pieces of filter paper, a gel, a piece of nitrocellulose and two more

pieces of filter paper were placed in the blotting folder of the apparatus between two brillo pads. Transfer occurred overnight for a total of 200 Volt·hours at 4°C, which maintained the buffer temperature at below 15°C.

Blots were blocked in Tris buffered saline (TBS; 20 mM Tris HCl pH 7.5, 500 mM NaCl, pH 7.5) containing 5% dry milk powder (DMP) for about 6 hours at room temperature. The membranes were washed twice for 5 minutes each in TTBS (TBS containing 0.05% Tween-20) and transferred to an antibody solution consisting of Hsp72 (1:2500) and type I MHC (1:200) antibodies in TTBS with 2% DMP and 0.02% sodium azide as a preservative. Blots were flipped several times over the first two hours of the incubation and then left in the solution for a total of 14 hours at room temperature on a rotary shaker.

Following the primary antibody incubation, blots were washed twice in TTBS for 5 minutes each with shaking and transferred to a second antibody solution. This solution contained 2% DMP in TTBS with a goat anti-mouse or a goat anti-rabbit alkaline phosphatase conjugated secondary antibody (BioRad, 1:1000) for the type I MHC blots and Hsp72 blots, respectively. The membranes were washed twice in TTBS and once in TBS, five minutes each with shaking, then transferred to a bicarbonate buffer (100 mM $NaCO_3$, 1 mM $MgCl_2$) containing 1 ml each of 3% w/v p-nitro blue tetrazolium chloride p-toluidine salt in 70% w/v N,N-dimethylformamide (DMF) and 1.5% w/v 5-bromo-4-chloro-3-indolyl phosphate in 100% DMF.

After colour development (2-10 minutes), blots were rinsed in two changes of a large volume of distilled water and placed between filter paper to dry. The blots were scanned on a Hewlett Packard ScanJet (6100C/T) using the Northern Eclipse scanning software program

(Mississauga, Ontario, Canada). The bands were analyzed for intensity using the Scion Image Analyzer (National Health Institute).

Antibodies. The Hsp72 levels in muscle were assessed using a monoclonal antibody to Hsp72 (StressGen SPA-810). The monoclonal antibody to type I MHC was generously donated by Dr. Peter Merrifield (Dept. Of Anatomy, University of Western Ontario). The secondary antibody for Hsp72 was goat anti-rabbit (Bio-Rad) and for type I MHC was goat anti-mouse (Bio-Rad).

Data Analysis

Descriptive statistics were used to describe the central tendency and variability for ensuring that the homogeneity of variance had not been not violated.

Two 2x3 repeated measures mixed factorial ANOVAs were used to analyze 1) DOMS and 2) Hsp72 content relative to the known rat soleus standard. The independent variables were group (young / old), and time (pre, 24 hours, and 72 hours after the exercise bout). The results from the 30-minutes-post-exercise samples were not used for the purpose of this study. A Student's t-test was used to compare the pre-exercising Type I MHC protein levels relative to the rat soleus known standard between the young and the aged groups.

Bivariate correlations were used to examine the relationship between Hsp72 and age, DOMS, and pre-exercise levels of type I MHC. Results were considered significant at a value of $p < 0.05$.

Results

Subjects

Ten young and nine aged males volunteered to participate in the study. However, after the first muscle biopsy, one young subject and two seniors withdrew from the study. The final sample size included nine young men (23.1 ± 1.1 years) (mean ± SE) and seven older men (60.9 ± 0.8 years). The subjects completed an activity inventory questionnaire (Appendix 2) and there was no difference between the activity levels of the two groups ($p = 0.404$) (see Table 1).

Table1: Subject Age and Activity Inventory

		n	Age (yrs)	Activity Inventory [a]
Group	Young	9	23.1 ± 1.1	3.11 ± 0.70
	Old	7	60.9 ± 0.8 *	2.14 ± 0.91

Note. [a] The values for the lifestyle inventory indicate number of times per week the subjects experienced heavy sweating and rapid heart rate due to the activity performed. Values are in means ± SE. *: $p < 0.001$.

DOMS

The results of the self-evaluated delayed-onset muscle soreness are presented in Figure 1 (see also Table 2 in Appendix 3). Significant increases in DOMS was found before and after the exercise ($p < 0.001$; $F = 16.54$). No age difference was found between the two subject groups at each time period (pre-exercise, 24 hours and 72 hours post-exercise) ($p = 0.439$; $F = 0.636$) (see Figure 1). Also, no interaction effect of age and time factors was found.

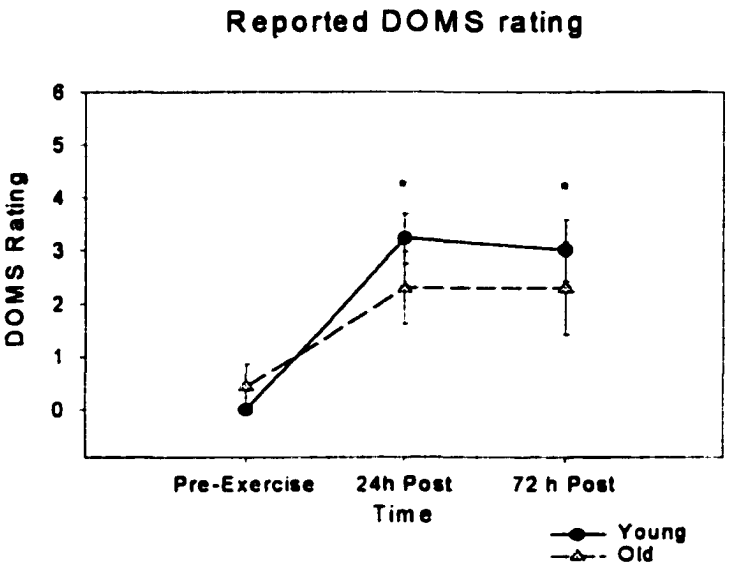

Figure 1. Reported DOMS at time of muscle biopsies. The DOMS questionnaire is taken from High et al., 1989 (see Appendix 2).

Type I Myosin Heavy Chain

Type I MHC level in the young subjects was 100.00 ± 6.19 and 93.85 ± 12.42 in the older subjects, mean ± SE. There was no difference in the baseline (pre-exercise) levels of Type I myosin heavy chain between the young and the aged groups (see Figure 2).

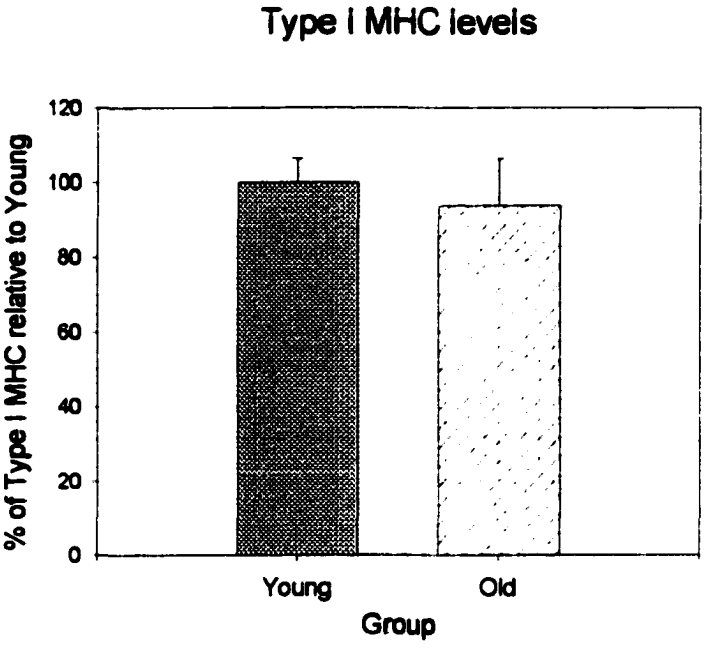

Figure 2. Pre-exercising levels of type I MHC. Values are expressed in % relative to the type I MHC present in rat soleus standard, scaled such that the mean level in young subjects is 100%.

Hsp72 Protein

The percent of Hsp72 protein content relative to a known sample of rat soleus standard, expressed relative to the pre-exercise levels of young subjects, is presented in Appendix 3. There was no difference in Hsp72 content before and after exercise ($p = 0.622$; $F = 0.486$). Similarly, there was no difference in Hsp72 content between the young and the older subjects ($p = 0.550$; $F = 0.384$). In addition, there was no interaction effect of group and time on the levels of Hsp72 protein ($p = 0.802$; $F = 0.223$) (see Figure 3).

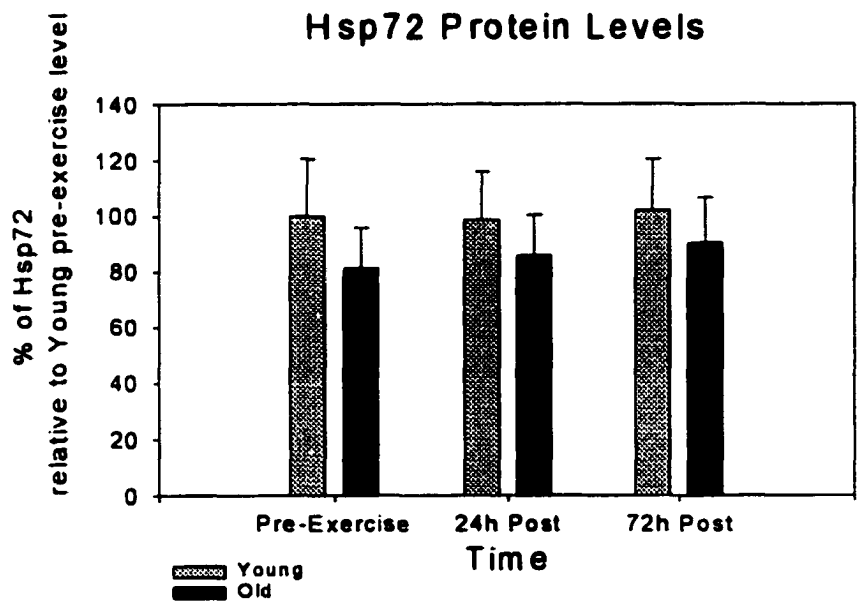

Figure 3. Hsp72 levels before and after exercise.

Correlations

There was no correlation between age and Hsp72 protein level before exercise (r = -0.241), and 24 hours (r = -0.161) and 72 hours (r = -0.130) after exercise. There was no correlation between the DOMS rating and Hsp72 protein expression before (r = -0.201), 24 hours (r = -0.0453) or 72 hours (r = 0.00801) after exercise. There was no correlation between the resting levels of Hsp72 protein and Type I myosin heavy chain (r = 0.315) (see Table 2).

	Hsp72 Pre-exercise	**Hsp72** 24h-Post Ex	**Hsp72** 72h-Post Ex
Age	-0.241	-0.161	-0.130
Type I MHC	0.315	–	–
DOMS Pre-Exercise	-0.201	–	–
DOMS 24h-Post Ex	–	-0.0453	–
DOMS 72h-Post Ex	–	–	0.00801

Table 2. Hsp72 Contents and Age, Type I MHC, and DOMS Correlation. The values indicate correlation coefficient.

Discussion

Rationale for Hypotheses

The purpose of this study was to compare the extent of Hsp72 expression in young and aged subjects following a single bout of exhaustive exercise. Some investigators demonstrated attenuation in Hsp72 protein expression in the aged human diploid fibroblast cells (Liu, Lin, et al., 1989). Other investigators revealed that aged rat hearts (Bongrazio et al., 1994; Nitta et al., 1994; Locke & Tanguay, 1996a), aged rat skin- or lung-derived fibroblast cells (Fargnoli et al., 1990), and cells from aged rat adrenal cortex (Blake, Udelsman, et al., 1991) displayed a decreased Hsp70 expression compared to the young rats. However, Kregel and Moseley (1996) reported an increase of Hsp72 protein expression in liver cells of exercised old rats. It was therefore hypothesized that, the increase in Hsp72 expression after exercise would be greater in the young than in the aged skeletal muscle of men, using the pre-exercising levels of Hsp72 as the control.

Moreover, a high correlation between Hsp72 and type I MHC content has been observed in rat skeletal muscle (Locke, Atkinson, et al., 1994; Locke, Noble & Atkinson, 1991a, 1991b). Aging human skeletal muscle has been reported to contain a greater proportion of type I myosin heavy chain than the young skeletal muscle due to a shift in the fibre type composition (Klitgaard, Ausoni, et al., 1989; Klitgaard, Mantoni et al., 1990; Larsson et al., 1978; Melichna et al., 1990). Based on these observations, it was also hypothesized that the muscle Hsp72 protein and type I MHC correlation, and the pre-exercising levels would be elevated in aged versus young individuals.

The perceived soreness generally associated with unaccustomed exercise is commonly referred to as DOMS. The etiology of DOMS includes a rise in local temperature, increases in lactic acid, mechanical injury due to damage to the intermediate filaments or disruption of the muscle fibre and connective tissue (MacIntyre et al., 1995). Hsp72 can also be induced by the same factors (reviewed by Kilgore, Musch, et al., 1996; Locke, 1997). Therefore, the third hypothesis was that the DOMS experienced by the subjects would be accompanied by an increase in Hsp72 expression, although the time course of the relationship was not predicted.

Exercise Protocol

In the present study, eccentric exercise on a knee extension machine to exhaustion was employed to create the stress stimuli to induce the expression of Hsp72. Eccentric contraction was used because it has been reported to result in greatest soreness and injury relative to concentric or isometric contractions (Fitzgerald et al., 1991; MacIntyre et al., 1995; Smith, 1991). To reduce concentric strengthening of the muscle, the lever arm of the knee extension apparatus was returned to the starting position by a research assistant.

The goal of the exercise in the present study was to cause muscle damage, therefore limiting the injury to the exercising muscle was a primary issue. It was important, on the other hand, to prevent unnecessary injury, especially with the senior subjects involved. A concern arose that the use of a warm-up and the low level of intensity in the early stages of exercise may mitigate the stress to the muscle and reduce the amount of muscle damage that otherwise would have occurred. However, the warm-up should not have become a confounding variable, since researchers have repeatedly demonstrated that significant difference in DOMS was observed

following exercise, with or without warm-up and stretching exercises (High et al., 1989; Rodenburg, Steenbeek, Schiereck & Bär, 1994; Smith, Brunetz, et al., 1993).

The work rate was two seconds per contraction followed by approximately two seconds of rest to prepare for the next contraction. The resting period between each set was 20 seconds. In a study with higher exercise intensity (Mair et al., 1995), subjects performed seven sets of 10 contractions at 150% of the 1-RM conc. Each contraction lasted one to two seconds, with 15 seconds rest between each contraction, and two to three minutes rest between each set. Since the level of intensity was different, 15 seconds rest between each contraction and two minutes rest between each set was too long. In another study (Brown, Child, Day & Donnelly, 1997), subjects performed 50 repetitions of eccentric contractions at 100% of the 1-RM conc. Each contraction lasted ~ 1.6 seconds, with a 10 seconds rest between each contraction. Since there were five sets in the present study instead of one set, as it was in Brown et al. (1997), and the intensity of the first four sets were low enough that they served as a warm-up (Wathen, 1994), there is sufficient reason to believe that two seconds of rest between each contraction and 20 seconds of rest between each set was appropriate to induce DOMS.

In the final set of exercise, subjects were requested to render their maximum effort until they were unable to keep up with the work rate. Fitzgerald et al. (1991) compared muscle soreness from exercising at 90% of maximum concentric power and exercising with maximal effort without a specified exercise intensity. The results showed that the subjects who exercised with maximal effort experienced significant differences of DOMS whereas those who exercised at 90% of maximum concentric power did not (Fitzgerald et al., 1991). The final set of our exercise protocol reflected both scenarios: high intensity and maximum effort.

It is possible that, when examining DOMS using either a visual analogue scale (e.g. Fitzgerald et al., 1991) or a rating scale based on a given number with a corresponding description of the soreness (e.g. our study), these scales may suffer from scaling error when used for repeated testing (reviewed in MacIntyre et al., 1995). Nevertheless, the Likert scale of DOMS rating remains the best non-invasive tool for describing and quantifying the pain associated with DOMS (Armstrong, 1986; MacIntyre et al., 1995).

DOMS Rating Scale

Our results showed a significant difference in DOMS rating before and after eccentric exercise in both subject groups. This was expected, as eccentric exercise has been reported to cause DOMS in subjects (Armstrong, 1984; Smith, Fulmer, et al., 1994). Similar results were reported in other studies which employed different exercise intensity and exercise machines. Croisier et al. (1996) used a similar exercise volume of eccentric contractions as in the present investigation and reported a significant increase in the DOMS rating at 24 hours and 48 hours post exercise. In another study, subjects performed 7 sets of 10 concentric or eccentric contractions at 90% of 1-RM on a Kin-Com dynamometer (Fitzgerald et al., 1991); the exercise intensity was slightly greater than ours, nevertheless, the results were similar: DOMS rating significantly differed after subjects performed eccentric exercise with maximum effort. In addition, the exercise protocol for a low intensity stepping test (High et al., 1989) provided a stress similar to the early stages of our exercise protocol. Subjects used the left leg to step down (eccentric) and the right leg to step up (concentric). Significant muscle soreness 24 hours post exercise was present only in the left leg and was similar to our finding.

Although significant differences were found in the subjective muscle soreness rating before and after the exercise in the present study, there was no difference in muscle soreness at 24 and 72 hours post exercise. Previous research has revealed that the peak time of DOMS varies and as such makes it difficult to determine a time course. Some investigators reported that peak DOMS occurred at 24 hours post exercise (Fitzgerald et al., 1991; High et al., 1989; Smith, Brunetz, et al, 1993); others found it occurred at 24 *and* 48 hours (Pyne, Baker, Telford & Weidermann, 1997; Schawne et al., 1983), 48 hours (Smith, Fulmer, et al., 1994), or 48 *and* 72 hours (Rodenburg et al., 1994) post exercise. The time course for DOMS varied between studies and consequently investigators can only make conclusions based on the time course for DOMS employed in their studies. In the present study, DOMS may have peaked at 48 hours, and gradually decreased, thus no difference in soreness would be observed at 24 and 72 hours post exercise. It would be helpful in future studies to determine the peak time of DOMS, as the peak may mark the time when the stress signal is most intense and Hsp72 expression may be highest.

Hsp72 Expression: effect of aging

Pre-exercising Hsp72 levels in the young subjects were no different than that of the old subjects in the present study. Furthermore, there was no correlation between age and Hsp72 levels. In support of our finding, Knowlton et al. (1998) revealed that, regardless of the age of the patients with normal hearts, there was no difference in Hsp72 protein levels. McGrath et al. (1995) also found no correlation between age and Hsp72 levels in patients (ranging from 4 to 70.6 years) undergoing cardiac operation (McGrath et al., 1995). Similarly, in animal studies, the basal levels of Hsp70 protein were no different in young rat hepatocyte and old rat hepatocyte (Wu et al., 1993). On the contrary, observations from in vitro studies on human and animals

showed that aged cells have a reduced inducibility to mount the heat shock response than young cells even though the resting levels of the Hsp may have been the same (Blake, Fargnoli, et al., 1991; Blake, Udelsman, et al., 1991; Liu, Lin, et al., 1989). The different conclusions may have arisen from the fact that the older subjects in the present study have maintained an active lifestyle, and thus aging had not affected them as it would have in those who are not as active. It is also possible that the reduced inducibility in aged cells were due to environmental conditions in the in vitro studies and not the cellular intrinsic characteristics (Beck et al., 1995), since the result of in vivo studies demonstrated that there was no difference in the Hsp72 expression in young and aged human tissue.

Hsp72 Expression: effect of exercise

There was no before and after difference in the Hsp72 protein content in either the aged or the young group. This was a surprising result, as the question of a delayed Hsp72 protein synthesis (Puntschart et al., 1996) was addressed by obtaining the muscle samples at a prolonged time frame than the three hours post exercise (Puntschart et al., 1996). The other reason suggested by Puntschart et al. (1996) for the lack of Hsp72 increase was that the pre-exercising levels were so high that any subsequent increase was not sufficient to result in any statistical significance.

The Hsp72 protein content in human samples is a lot higher (ranging from 185% to 947%; see Appendix 5) than the standard rat soleus control. In support of our finding, Hsp72 protein content in the myocytes of patients before undergoing bypass surgery was found to be 168% of the amount in unstressed swine heart (McGrath et al., 1995). In addition, Hsp72 content in the unstressed swine heart, was almost 400 % of that of the rat soleus standard (Locke, Tanguay &

Ianuzzo, 1996). Therefore, the Hsp72 levels may have been high enough before the exercise bout, as was proposed by Puntschart et al. (1996), that the observed increases at 24 hours post exercise for both groups and at 72 hours post exercise in the older subjects were too small to be significant. In addition to Puntschart et al. (1996)'s postulation, the constitutive high levels of Hsp72 may have inhibited Hsp72 transcription, because Hsp72 has been shown to inhibit additional Hsp72 transcription by binding to the heat shock transcription factor (Locke, 1997; McGrath et al., 1995).

To date, few studies have examined Hsp72 in exercising human subjects in vivo. A summary of the research literature seems to suggest that the type (Liu, Mayr, et al., 1999) and intensity (Puntschart et al., 1996) of exercise training may be partly responsible for Hsp72 induction in human skeletal muscle. Muscle Hsp70 from highly trained 18 year-old rowers was examined after the rowers underwent a 4-week intense training program for the World Championships (Liu, Mayr, et al., 1999). The duration of the exercise training was much more extended than in previous studies on Hsp70 involving human subjects. The amount of Hsp70 was increased significantly following the first week of training, and the Hsp70 level peaked after the third week of training (Liu, Mayr, et al., 1999). Moreover, a maximum increase of Hsp70 was observed at the end of the second week, which coincided with the maximum amount of exercise workload. Liu, Mayr, and coworkers (1999) concluded that the volume of exercise is related to the amount of Hsp70 increase. A previous study involving exercising rats supports this notion: Brickman et al. (1996) detected Hsp70 increase in rats that underwent 63 days of treadmill run while it was unfounded in rats that trained for only 21 days.

It has been further suggested that failure to increase Hsp72 protein levels in some cases may be due to desensitization or a loss of responsiveness (Knowlton et al., 1998). This loss of responsiveness may be the result of continual stress stimuli which may blunt or block the stress response. Since all the subjects in our study were moderately active, it may be possible that they were more desensitized to the stress signal. However, this would not explain why there was a significant increase in Hsp72 in elite rowers who continually trained for four weeks (Liu, Mayr, et al., 1999).

Temperature increase has been shown to be one of the major stressors that induce the stress response in non-exercised animals (Locke, 1997). Significant increases in Hsp70 protein content was observed in studies of young rat (Locke, Noble & Atkinson, 1990) and aged rat skeletal muscle tissues (Bongrazio et al., 1994), and other animal tissues (Fargnoli et al., 1990; Locke, Noble & Atkinson, 1990) in which rectal temperature increased to above. In recent years, however, researchers have suspected that Hsp can be induced by factors other than a rise in temperature from exercise (Ryan et al., 1991; Locke, 1997). Skidmore et al. (1995) demonstrated that maintaining colon temperature at the baseline by having rats exercise in a cool environment can still induce the stress response. Although temperature was not measured in the present study, it seems unlikely that the intensity and duration of exercise would be sufficient to produce a rise in rectal temperature as high as 41°C (see Ryan et al., 1991). Nevertheless, lack of increase in core temperature should not have been a variable in preventing a significant increase in Hsp72 expression, as Puntschart et al. (1996) had observed no increase in Hsp72 expression, yet the core temperature in the subjects of their study would have elevated as a result of the exercise intensity (Puntschart et al., 1996).

On the other hand, the presence of increased DOMS may suggest that the muscle temperature in the exercised limb may have increased in the present study. This speculation of increased muscle temperature due to exercise, but resulting in no increases in Hsp72 synthesis further supports the notion that factors other than increases in temperature may be responsible for the induction of Hsp72 protein during exercise (Locke, 1997; Ryan et al., 1991).

An earlier study comparing the thermal responses of human and rodent cells revealed that human cells were more heat-resistant than rodent cells in the range of 41 - 45°C (Hahn, Ning, Elizaga, Kapp & Anderson, 1989). This may partly explain the reason why there was no Hsp72 increase in the present study or in Ryan et al. (1991)'s study, as subjects may have been heat-resistant even at core temperatures between 40 and 41°C.

Another factor which may have accounted for a lack of Hsp72 response may be related to recent evidence suggesting that the signal which initiates the stress response may affect the whole organism (Hernando & Manso, 1997). Hernando & Manso (1997) found that significant amount of Hsp72 protein was expressed in both the active and inactive exercised muscle, with different patterns of response. The stress response declined following an increase in Hsp72 expression in the inactive muscle, whereas Hsp72 level continued to increase in the active muscle (Hernando & Manso, 1997). It was further suggested that, since the site of muscle damage is the musculotendinous junctions, and the size of the muscle sample was only a portion of the muscle, and not the entire muscle fibre itself as in rat studies, it is quite possible that the muscle biopsy sample obtained from the belly of the muscle may not have encountered the most damage (Armstrong, 1984; Smith, 1991). As previous researchers have noted, it is also possible that in

humans, the stress signal will affect the whole body (Hernando & Manso, 1997), with a different response in different sites.

The exercise in the present study was of acute nature, instead of chronic endurance exercise, which was the type employed in the study on rowers where significant Hsp70 increase was observed (Liu, Mayr, et al., 1999). Wheeler et al. (1995) noted that Hsp70 protein expression during normal aging is a result of oxidative damage. Oxidative damage may arise from excess production of ROS (reactive oxygen species), which results from increased contractile activity associated with fatiguing exercise (Essig & Nosek, 1997). Many researchers have speculated that the impact of different kinds of exercise on specific muscle fibre types may result in differing amounts of Hsp70 protein induction (Puntschart et al., 1996; Liu, Mayr, et al., 1999). However, the relationship between type I muscle fibre and ROS has yet to be investigated (Essig & Nosek, 1997).

It has been suggested that strenuous exercise can produce so much ROS that the normal anti-oxidant defense system becomes exhausted (Essig & Nosek, 1997). As a consequence, the superabundance of these oxidative free radicals induce the Hsp70 production (Liu, Mayr, et al., 1999). Based on the results from the present study, and those of Puntschart et al. (1996) and Liu, Mayr, et al. (1999), it seems likely that Hsp72 expression in human skeletal muscle is partially dependent on 1) the resting levels of Hsp72 prior to exercise; 2) the amount of exercise (one week of training instead of one or two hours); 3) the location of the muscle injury and the muscle biopsy site, and 4) the type of exercise that can result in increased levels of oxidative stress (endurance vs. strength training).

Hsp72 and DOMS

No correlation between DOMS and Hsp72 protein levels was found in our study. This was most likely due to the fact that no difference in Hsp72 content before and after exercise was found, whereas there was significant difference in the before and after exercise DOMS rating. Unfortunately, in order to avoid iatrogenic complications for the subjects, neither muscle biopsy nor DOMS rating were obtained at 48 hours post exercise. Highest protein regeneration was observed at 72 hours after stimulation (Smith, 1991). Therefore, the possibility exists that a correlation existed between DOMS rating at 48 hours post exercise and Hsp72 protein content at 72 hours post exercise.

Past research have demonstrated that the vastus lateralis muscle of older individuals contain a significantly higher proportion of type I fibre (Larsson et al., 1978; Melichna et al., 1990) when compared to younger individuals. In addition, a higher content of type I MHC in vastus lateralis was found in the aged than in the young (Klitgaard, Mantoni, et al., 1990a). Unlike the findings from previous studies, there was no difference in the type I MHC content in the two age groups. This could be due to differences in the activity level of our subjects as compared to those from other studies: the results of the activity inventory showed that there were no differences in the activity levels of the young and the old subjects. In support to this view, Klitgaard, Mantoni, et al. (1990a) found that the type I MHC content was no different in the young control subjects than in the old swimmers nor in old strength-trained individuals (Klitgaard, Mantoni, et al., 1990a). The senior subjects in the present study maintained an active lifestyle similar to their young counterparts, and as such, the effect of sarcopenia, or the loss of muscle mass may have reduced.

Locke et al. (1991a, 1994) reported that Hsp72 expression appear to be muscle specific, as a high correlation of Hsp72 protein and type I muscle fibre was found in rat skeletal muscle (Locke, Atkinson, et al., 1994; Locke, Noble & Atkinson, 1991a, 1991b). Single fibre analysis had shown that type I muscle fibre expresses only type I MHC (Staron & Johnson, 1993). The result of the correlation analysis showed that the pre-exercising type I MHC content did not significantly correlate with pre-exercising Hsp72 in either the young ($p = 0.261$) or aged ($p = 0.753$) group. This was not originally predicted, but was later anticipated, as there was no difference in either the type I MHC or Hsp72 protein in both subject groups. Therefore, whether a relationship exists between type I MHC and Hsp72 in human skeletal muscle remains to be determined.

Limitations and Recommendations

In conclusion, although the methodologies varied, the findings of the present study were supported by previous studies. From our investigation it is unclear as to how or if Hsp72 relates to DOMS, and type I MHC content in human skeletal muscle. One major concern in the present study remains the intensity prescription of the exercise. In order to study the expression of Hsp72, it was necessary that subjects undergo a severe bout of exhaustive eccentric exercise. However, the difficult dilemma is to design the same exercise prescription with equal amount of work or stress such that both the older and young subjects will experience sufficient stress signal to mount the heat shock response without unnecessary injury. Therefore, to have some control over the exercise intensity, relative workload such that the exercise intensities were determined according to the subjects' 10-RM. The 10-RM was probably not accurately determined, as was reflected by the fact that the subjects were able to perform more than 10 repetitions (ranging from

23 to 123) at 120% of the 10-RM eccentrically following exercising four sets of 10 repetitions each at much lesser intensity. This problem, nevertheless, should not influence the results, as each subject exercised until fatigue.

Avoiding iatrogenic injury was also another consideration. It would have been ideal to obtain a 48 hour and one week post exercise muscle sample in addition to the 24 hour and 72 hour muscle samples, since results from Liu, Mayr, et al.'s (1999) study suggest that it would seem possible that Hsp72 expression may be delayed until one week following exercise, in addition to the possible relationship between peak time for DOMS and maximum amount of Hsp72 expressed at 48 hours post exercise. However, to expect the subjects to undergo that many muscle biopsy samples is unrealistic and may result in additional subject withdrawal. It would help in future studies to examine the relationship between DOMS and Hsp72 protein content by investigating whether there was structural damage as a result of the eccentric exercise through immunohistochemistry.

Researchers studying exercise-induced muscle damage had often examined the serum creatine kinase level, as it is a good indication of muscle fibre damage (Gleeson et al., 1995; Mair et al., 1995). It would have further clarified whether or not the exercise protocol in the present study caused muscle fibre damage by obtaining serum samples from the subjects and analyze for the creatine kinase content, in addition to examining the Hsp72 protein levels.

Further suggestions for future studies include controlling for or measure the amount of food or caloric intake by the subjects, as previous studies have shown that Hsp72 levels were increased in aged rats when their caloric intake was restricted (Heydari et al., 1993; Pahlavani et al., 1996). Also, as adrenocorticotropic hormone had been shown to play a role in enhancing

Hsp72 protein synthesis in aged rats (Blake, Udelsman, et al., 1991), examining how this hormone might affect Hsp72 protein synthesis in humans in future studies may shed valuable insight to Hsp and its functions in protein repair and protection.

Conclusion

This study demonstrated a few findings in the area of Hsp. First, there was no Hsp72 increase after exhaustive eccentric exercise in men. Secondly, no difference in Hsp72 levels was found between young and older humans. This was a novel finding in Hsp research. Thirdly, there was no correlation between the Hsp72 level and DOMS. And lastly, there was no correlation between the pre-exercising levels of Hsp72 and type I MHC in either young or older human skeletal muscle. Based on this study, it is still not known whether aging plays a role in attenuating heat shock protein expression upon sufficient exercise stress. As well, more research is needed to investigate the relationship between aging, muscle fibre typing, muscle damage, and heat shock proteins.

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Appendix 1

Cover Letter

March 9, 1998

Dear Sir:

I am a graduate student in the School of Kinesiology, Lakehead University. I have successfully completed an Honours Bachelor of Science degree at the University of Toronto specializing in physiology. I am conducting a research under the direction and supervision of Dr. Robert Thayer of Lakehead University on the subject of aging and exercise stress, which has been approved by the Ethics Advisory Committee of Lakehead University. The successful completion of this research will partially fulfill the requirements of the Masters of Science program, Specialization in Gerontology in which I am currently enrolled. With the knowledge and experience I learn from this research as well as the completion of my degree, I hope to serve the seniors through further research, or working with seniors in a one-to-one or small group basis.

What is this study about?

I am studying the heat shock proteins (Hsp72), which are stress proteins that are induced by heat and/or exercise stresses. The function of the Hsp72 is to help cells to recover from stressful events by repairing damaged proteins. These proteins also protect other proteins from damages in future stressful conditions. Furthermore, Hsp72 serve as molecular chaperones by keeping the newly formed proteins in their correct configuration, so that they can be utilized readily. Finally, as the molecular chaperones, Hsp72 escort the irreparable proteins to be excreted quickly so that these proteins will not accumulate and become toxic to the cell.

I am mostly interested in finding out whether Hsp72 are produced in the aging human muscle when the cells undergo exercise stress, and if they are, how much, and when are they produced compared to the young muscle cells. In order to examine the amount of Hsp72 in the cells, four (4) muscle biopsies (described later) will be an essential component in this study. Each muscle sample will be approximately 80~90 mg.

Why do the study?

Most studies that examined heat shock proteins were done on animals. The results of studies involving older animals are inconclusive: some researchers showed a decrease, yet others showed an increase in the Hsp expression compared to the younger animals.

Few studies have been performed on human skeletal muscle in the detection of Hsp expression. Researches on the expression of Hsp72 in aging human skeletal muscle are probably ongoing but have yet to be published.

Since so little is known about its presence and amount in the elderly, this study will advance the knowledge in this area. Furthermore, the results of this study may be used by other researchers to look into possible relationship between cardiovascular diseases such as myocardial infarction and the expression of this protein. The induction and expression of Hsp72 from this study will also help protect cells from damages in the immediate future.

In addition, the muscle samples will be used to investigate muscle fibre typing and muscle damage in future studies which will be conducted by new student(s), my advisor, Dr. Robert Thayer, and Dr. Earl Noble from the University of Western Ontario.

What do the participants have to do?

I hope to have 10 healthy older and 10 healthy young men to participate in this study. All testing will be done in the Fieldhouse at Lakehead University. The subjects' 10-RM (the maximum amount of weight that can be lifted 10 times) will be determined as soon as it is possible.

As mentioned above, muscle biopsies, which are commonly used in a variety of medical research settings will be performed for analyzing Hsp72. One biopsy (the "before" sample) will be taken on or around Monday, March 30, 1998, depending on the number of subjects. Ten days following the "before" sample, the subject will perform leg extension exercises until fatigue. There will be 5 sets of eccentric exercises at the intensity of 50, 55, 60, 70, and 100% of the 10-RM conc equivalence. The research director will be present during the entire implementation of the exercise.

Three more biopsies (the "after" samples) will be taken at 30 minutes, 24 hours and 72 hours after the exercise. Subjects will receive an injection of local anaesthetic and a small incision (~1cm) in the skin prior to the biopsies in the thigh area. All muscle biopsies will be performed by a qualified, experienced physician. For a brief period of time following the procedure, the possibility of infection exists. Proper care and hygiene offered by the laboratory setting will greatly reduce this possibility.

Besides the exercise and the muscle biopsies, the participants will be asked to fill out a questionnaire and report the level of muscle soreness they experience. During the 10 days prior to the exercise testing, subjects are asked to refrain from performing activities with high intensities that may lead to muscle soreness and/or fatigue.

In addition, the senior participants will require medical approval and all participants will sign a letter of informed consent prior to participating. Subjects have the right to withdraw from the study at any time. Results of the study may appear in a publication; however, the identity of the participants will remain confidential at all times. After the completion of the research the results will be made available to the participants upon request. In addition, the data from the research will be stored by Dr. Thayer for a period of seven years.

I thank you in advance for your interest in this research. Should any concern arise, please do not hesitate to contact myself using the information provided below.

Sincerely yours,

Renee Ho
Hon. B.Sc., M.Sc. Candidate
School of Kinesiology
Lakehead University
343-8544 (B) or 343-0478 (H)

Letter of Informed Consent

For participation in the following research study:

Effects of acute eccentric exercise on Hsp72 expression
in young and aged human skeletal muscle

Principal Investigators: Dr. R. Thayer, Ph. D. & R. Ho, B. Sc., M. Sc. candidate
School of Kinesiology, Lakehead University
Thunder Bay, Ontario P7B 5E1
807-343-8544

I, _____ consent to take part in a study which will examine the effects of a bout of acute exercise regimen on the muscles of the thigh. I will require medical approval prior to participating. The principal investigators have explained to me what I will be doing in this study.

I understand that two undergraduate kinesiology students will provide assistance during the exercise regimen. I understand the creation and implementation of this exercise regimen will take place under the supervision of the research director. I understand that care will be taken to avoid physical harm and injury during the program and testing by the investigators. The risks and benefits of engaging in this study have been clearly outlined.

Each subject will perform eccentric exercise to exhaustion. The exercise itself will require 20 minutes of my time. The procedure is perfectly harmless but may result in minor soreness and stiffness which may persist for a brief period following the exercise.

I am willing to undergo four needle biopsies of muscle tissue. Each sample will be approximately 80-90 mg. One biopsy from one thigh will be taken 10 days before the commencement of the exercise, and three biopsies will be taken at 30 minutes, 24 hours, , and 72 hours after the exercise. I am aware that biopsies will be performed by a qualified and experienced physician at the C.J. Sanders Fieldhouse of Lakehead University, Thunder Bay, Ontario. I will be given a small incision on the skin and injected with a local anaesthetic in the thigh area prior to surgery. For a brief period of time following the biopsy procedure, I may

experience muscle soreness. Since muscle biopsies involve minor surgery, I understand that the possibility of infection exists. I also understand that proper care and hygiene will be used during the biopsy procedures to greatly reduce the likelihood of biopsy-related infection. I understand that needle biopsies are a routine medical practice and commonly used in a variety of medical research settings.

I have the right to withdraw from the study at anytime. Results of the study may appear in a publication; however, my identity will remain confidential at all times. After the completion of the research, the results will be made available to me upon request. I understand that the Ethics Review Board of Lakehead University has approved this research.

Signature of Participant Date

Phone # _____

Signature of Witness Date

Medical approval for participation.

Signature of Physician Date

I have explained the nature of the study to the participant and believe she/he has understood it.

Signature of Researcher Date

Appendix 2

Likert scale for Assessing Muscle Soreness

Please check the sentence below that best describes your level of muscle soreness over the past hour.

[ ] A complete absence of soreness
[ ] A light pain felt only when touched / a vague ache
[ ] A moderate pain felt only when touched / a slight persistent pain
[ ] A light pain when walking up or down stairs
[ ] A light pain when walking on a flat surface / painful
[ ] A moderate pain, stiffness or weakness when walking / very painful
[ ] A severe pain that limits my ability to move

From High, Howley & Frank, 1989.

Appendix 3

Table 3. Self Reported DOMS Rating

DOMS		Time		
		Pre-Exercise	24h-Post-Exercise	72h-Post-Exercise
Age	Young	0 ± 0	3.222 ± 0.465 *	3.000 ± 0.577 *
	Old	0.429 ± 0.429	2.286 ± 0.680*	2.286 ± 0.865*

Note. Values are mean ± SE. The values range from 0 to 6, 0 being having a complete absence of soreness and 6 being having a severe pain that limits ability to move. * : $p < 0.001$ compared to Pre-Exercise values.

Table 4. Hsp72 Protein levels

Hsp72		Time		
		Pre-Exercise	24h-Post-Exercise	72h-Post-Exercise
Group	Young	558.93 ± 114.28	550.31 ± 97.01	569.74 ± 103.31
	Old	453.60 ± 82.12	479.09 ± 82.04	505.21 ± 90.45

Note. The values are expressed in % relative to the levels in the control rat soleus sample.

The values are mean ± SE.

Appendix 4

Name: _____
Date: _____
Age: _____

Activity Inventory

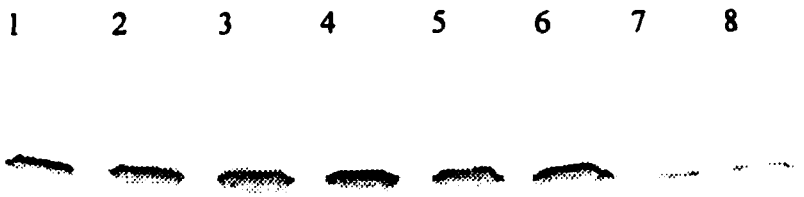
Over a typical seven-day period (one week), how many times do you engage inphysical activity that is sufficiently prolonged and intense to cause sweating and a rapid heart beat?

3 At least three times
2 Normally once or twice
1 Rarely or never

Have done	Currently doing		Have done	Currently doing	
—	—	Aerobics/exercise-to-music	—	—	Martial arts
—	—	Archery	—	—	Orienteering
—	—	Badminton	—	—	Racquetball
—	—	Baseball/softball	—	—	Ringette
—	—	Basketball	—	—	Roller skating
—	—	Bicycling (utility or pleasure)	—	—	Rowing
—	—	Bowling	—	—	Running/jogging
—	—	Broomball	—	—	Sailing
—	—	Calisthenics	—	—	Skateboarding
—	—	Camping	—	—	Skiing/cross-country
—	—	Canoeing/kayaking	—	—	Skiing/downhill
—	—	Climbing	—	—	Snowshoeing
—	—	Coaching	—	—	Soccer
—	—	Curling	—	—	Squash
—	—	Dancing	—	—	Stair climbing
—	—	Fencing	—	—	Swimming
—	—	Floor hockey	—	—	Tai chi
—	—	Football	—	—	Table tennis
—	—	Gardening, yard work	—	—	Tennis
—	—	Golf	—	—	Volleyball
—	—	Handball	—	—	Walking
—	—	Hiking	—	—	Weight training
—	—	Hockey	—	—	Wind surfing
—	—	Horseback riding	—	—	Yoga
—	—	Household chores	—	—	_____
—	—	Ice skating	—	—	_____
—	—	Inline skating	—	—	_____

Appendix 5

Sample Hsp72 Blot



From Left to Right:

Lane 1: Young, Pre-exercise

Lane 2: Old, Pre-exercise

Lane 3: Young, 24 hour Post-exercise

Lane 4: Old, 24 hour Post-exercise

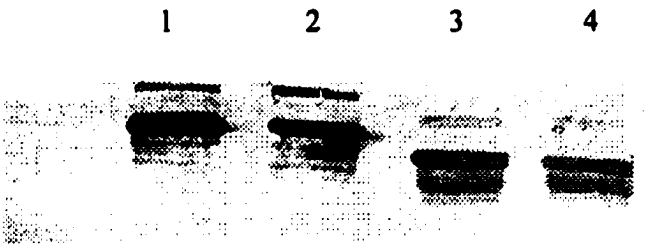
Lane 5: Young, 72 hour Post-exercise

Lane 6: Old, 72 hour Post-exercise

Lane 7: Standard rat soleus control

Lane 8: Standard rat soleus control

Sample Type I MHC Blot



From Left to Right:

Lane 1: Young

Lane 2: Old

Lane 3: Standard rat soleus control

Lane 4: Standard rat soleus control