CHAPTER 21

BIOCHEMICAL CHANGES IN RESPONSE TO INTENSIVE RESISTANCE EXERCISE TRAINING IN THE ELDERLY

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Abstract: Sarcopenia, the age-related loss of muscle mass and muscle strength, is closely related to inflammatory processes and seems to be aggravated by concomitant age-related alterations in cell-protecting mechanisms involving heat shock proteins (HSP). The most effective modality to counter sarcopenia is intensive resistance exercise, which also induces an inflammatory reaction and influences Hsp70 expression in peripheral blood mononuclear cells (PBMC) and several tissues. In this review we focus on the influence of physical exercise on the HSP-expression during aging. Following a systematic literature search it can be concluded that there is very limited information available at this moment. Animal studies have described a blunted exercise-induced HSP response in the skeletal muscle, heart and liver of older rodents compared to younger ones. The human studies have shown that physical exercise lowers the basal Hsp70 expression in PBMC, probably by reducing the low-grade inflammation and the oxidative stress. A 6-weeks intensive resistance training program in elderly persons improves the heat-induced Hsp70 expression in PBMC, a phenomenon which might be related to a better cellular protection during stressful situations. More research is warranted in this domain, especially involving elderly persons in different clinical conditions and exploring the effects of different exercise schedules

Keywords: Sarcopenia; cytokines; heat shock proteins; physical exercise; elderly inflammation

Abbreviations: AGE, advanced glycation end products; HSF1, heat shock factor 1; HSP, heat shock protein; IGF-1, insulin-like growth factor-1; IL-6, interleukin; NO, nitric oxide; PBMC, peripheral blood mononuclear cell; RAGE, receptor for AGE; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha

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INTRODUCTION

During ageing significant alterations occur in the skeletal muscle. A well known phenomenon is the age-related muscle atrophy and strength loss, defined as sarcopenia (Rosenberg, 1997). The process starts at the age of 30–40 years and progresses insidiously at an average rate of 1% loss of strength per year (Marcell, 2003). Above the age of 70 years the loss rate increases up to more than 3% per year (Aniansson et al., 1992). Due to sarcopenia, activities of daily living in elderly persons necessitate efforts close to their maximal strength and loss of independency can occur (Hortobagyi et al., 2003). Sarcopenia-induced muscle weakness is, therefore, a typical characteristic of frailty, one of the major geriatric syndromes (Fried et al., 2001). Sarcopenia is extensively documented in the literature and belongs to the hot topics within the geriatric and gerontological research domains (Morley, 2004). The total cost of health care directly induced by sarcopenia in the United States of America for the year 2000 was estimated at 18.5 billion dollar (Janssen et al., 2004). It is to be expected that, given the worldwide ageing of the population, the cost will further increase, not only in industrialized, but also in developing countries.

FACTORS CONTRIBUTING TO SARCOPENIA

The underlying mechanism of sarcopenia is not yet completely understood. Given the high variability of sarcopenia, several factors will contribute to the process. As shown in Table 1, the factors identified can be subdivided into decreased anabolic and increased catabolic processes, for both of which endogenous and exogenous factors can be recognized. Remarkably, several factors are tightly related to inflammatory processes.

At the level of the muscle itself, sarcopenia is characterized by loss of muscle fibers and atrophy of the remaining muscle cells (Lexell et al., 1988). This phenomenon is strongly related to increased levels of inflammatory markers in the blood circulation (Visser et al., 2002; Cesari et al., 2004), especially interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α), which are known to have cytotoxic and proteolytic properties (Mackinnon, 1992; Gabay and Kushner, 1999). In fact, during normal aging circulating levels of inflammatory cytokines such as IL-6 and TNF-α become slightly elevated (Hager et al., 1994), a phenomenon corresponding to a chronic low-grade inflammatory profile (Krabbe et al., 2004). The exact mechanisms by which inflammatory cytokines promote myofibrillar proteolysis are not yet completely understood. Recent insights revealed an up-regulation of the ubiquitin-proteasome pathway and calcium-activated pathway of calpains induced by TNF-α, and probably also other cytokines (IL-1 or IL-6), and thus inducing muscle protein breakdown (Mitch and Goldberg, 1996; Argiles et al., 2000; Muscat and Dressel, 2000; Zoico and Roubenoff, 2002).

Muscle weakness due to sarcopenia is more important than can be explained by atrophy alone. The atrophy of type-2 (fast twitch) muscle fibers seems to be more

Table 1. Factors contributing to sarcopenia

Metabolic pathway	Type	Factor	References*
↓ Anabolism	Endogenous	↓ Hormonal stimulation (growth hormone, IGF-1, testosterone, oestrogen)	Carter et al. (2002), Grounds, (2002), and Payette et al. (2003)
		Loss of motorneurones, denervation of muscle fibres	Doherty et al. (1993) and Vandervoort (2002)
		↑ Non-contractile tissue in muscle	Overend et al. (1992) and Kent-Braun et al. (2000)
	Exogenous	↓ Physical activity	Doherty (2003) and Roubenoff (2003)
		Bed rest, immobilisation	Bloomfield (1997)
		Malnutrition	Morley (2001)
↑ Catabolism	Endogenous	↑ Basal inflammatory profile (IL-6, TNF-α)	Hager et al. (1994) and Ershler and Keller (2000)
	Exogenous	Stress-induced inflammation: life events, depression	Lutgendorf et al. (1999) and Sutherland et al. (2003)
		Disease	Ershler and Keller (2000), Giordano et al. (2003) and Jagoe and Engelen (2003)

*Non-exhaustive references.

important than the loss of type-1 (slow twitch) fibers, which might explain the important loss of muscle strength and explosivity (muscle power, the capacity to generate a high force in a short time) due to sarcopenia (Lexell et al., 1988). In fact, the absolute loss of explosivity with ageing is even more important than the loss of maximal strength (Lauretani et al., 2003), indicating that contraction-speed is also impaired due to sarcopenia. Interestingly, when normalized to cell size, the contractile strength and velocity of isolated muscle fibers are not significantly affected by ageing (Trappe et al., 2003). Supplementary loss of muscle contractile properties might be due to age-related alterations in the connective tissues surrounding the muscle fibers (endomysium, perimysium and epimysium). Less described in the context of sarcopenia is the age-related augmentation of the proportion of non-contractile tissue in the muscle (Kent-Braun et al., 2000). Besides proliferation of intra-muscular fat-tissue (Overend et al., 1992; Kent-Braun et al., 2000), the formation of cross-links between collagen molecules leads to profound changes in composition of the muscle-tendon complex as well as its mechanical properties (Avery and Bailey, 2005). Non-enzymatic alterations of the extracellular matrix, among which accumulation of Advanced Glycation End products (AGE, mediated by condensation of a reducing sugar with an amino group) (Monnier et al., 2005) are related to ageing and lead to permanent cross-links. These processes are responsible for an increasing proportion of insoluble extracellular matrix and thickening of the tissues, as well as increasing mechanical stiffness and loss of elasticity (Kjaer, 2004; Avery and Bailey, 2005; Monnier et al., 2005). Interaction of AGE with the receptor for AGE (RAGE) causes activation of intracellular signaling, gene expression, and production of IL-6, TNF-α and free radicals (Ahmed and Thornalley, 2007). The proteolytic activity of these inflammatory processes makes the collagen more vulnerable and increases sarcopenia. Moreover, inflammatory processes involving TNF-α can, by increasing the cellular production of reactive oxygen species (ROS) and nitric oxide (NO), depress muscle contractibility, thus inducing supplementary weakness (for review Zoico and Roubenoff, 2002).

SARCOPENIA AND ALTERED CYTOPROTECTIVE MECHANISMS

The aforementioned inflammatory processes related to sarcopenia (both systemic and at the tissue level) are thought to be intensified by a concomitant age-related decline in cellular protection mechanisms, i.e. heat shock protein (HSP) expression. In fact, HSP are expressed during stress-situations (e.g. hyperthermia, oxidative stress, infection) and protect the cellular integrity by acting as “chaperones” for intracellular proteins. Several HSP families are identified, which can be classified according to their molecular weight. Recently, a comprehensive classification based upon the HUGO Gene Nomenclature has been proposed, including HspA, HspB, HspC, HspD, HspH and DNAJ (Hightower et al., 2008). Especially the “Hsp70 chaperoning machine” (HspA-family) is the most inducible by stress (Vos et al., 2008). Alterations in Hsp70 expression are thought to be involved in age-related

dysfunctions such as thermoregulation and sarcopenia (Horowitz and Robinson, 2007; Lee et al., 2007).

An established method to study intracellular stress-induced HSP-expression is by exposing the cell to a heat shock (exposure to temperatures above 40°C). Under these conditions HSP transcription is up regulated via heat shock factor 1 (HSF1). It has been shown that heat-induced Hsp70 expression is significantly attenuated in peripheral blood mononuclear cells of older individuals (Rao et al., 1999; Njemini et al., 2002; Singh et al., 2006). Moreover, several animal studies demonstrated lower levels of Hsp60 and Hsp70 in skeletal muscle (Doran et al., 2007) as well as a lower stress-induced expression of Hsp70 in various tissues (for review (Lee et al., 2007)). Also, it is assumed that the promoter for the *hsp* gene has a responsive element both for HSF1 and for the signal transducers of inflammatory cytokines. Several reports have shown that during inflammation, the expression of Hsp70, both intracellular (Njemini et al., 2003b) and in serum (Njemini et al., 2003a; 2004), is related with circulating IL-6 and TNF-α in elderly persons. Also in the skeletal muscle itself, the expression of chaperones belonging to the Hsp70-family is up regulated when exposed to elevated levels of IL-6 (Febbraio et al., 2002b).

In fact, when applied separately, both heat-shock and inflammatory cytokines have a stimulating effect on HSP production. But when applied together, they appear to decrease each other's stimulating effect. Thus, it can be supposed that a constant, low-grade inflammatory profile in the elderly, characterized by slight elevations in circulating IL-6 and TNF-α, might negatively interfere with the induction of HSP during stressful conditions. Under these circumstances the cellular protection might be reduced and thus precipitate loss of muscle mass and muscle strength, a phenomenon seen during acute infections (Bautmans et al., 2005a), surgery and trauma in elderly patients.

The role of extracellular Hsp70 is not yet completely understood. It has been shown that the presence of extracellular Hsp70 can have a protective effect against necrotic cell death of smooth muscle cells (Johnson and Tytell, 1993) and against apoptosis of motor neurons (Robinson et al., 2005). From these insights in can be assumed that circulating Hsp70 plays a protective role against conditions related to inflammation. Recently, we have described the interference between circulating levels of IL-6 and Hsp70 on muscle endurance in elderly nursing home residents (Bautmans et al., 2008). In fact, subjects with both high serum levels of IL-6 and Hsp70 showed significantly worse muscle endurance compared to those with high IL-6 and low Hsp70. It has been reported that in the absence of serious inflammatory conditions, low levels of serum Hsp70 are associated to successful biological aging (Terry et al., 2006) and might reflect a strong anti-inflammatory status of the individuals' immune system (Franceschi et al., 2007). In our study, residents with acute conditions were excluded. In such situation, the signaling function of IL-6 is probably less inflammation-related. In fact, cytokines are also involved in the healing process and the down regulation of the acute phase in the resolution of inflammation (Kushner, 1998) and it has been suggested that IL-6 can exert both inflammatory and anti-inflammatory signaling (Tilg et al., 1997). High levels of serum Hsp70,

on the other hand, can be seen as reflecting higher and less controlled low grade inflammatory activity (Njemini et al., 2004), with detrimental effects on muscle cells.

RESISTANCE EXERCISE TO COUNTER SARCOPENIA

There exists a large body of evidence that physical exercise, particularly intensive strength training, can improve muscle strength in elderly persons, thus countering sarcopenia-related muscle weakness (Latham et al., 2003). Important strength gains (up to >100% in 6–9 weeks) can be obtained following strength training in the elderly, even in subjects aged 90 years and older (Fiatarone et al., 1990). The rapid strength gains (already in the first 4–6 weeks) are explained by increases in voluntary muscle activation; after prolonged training muscle hypertrophy becomes measurable (Hakkinen et al., 2000; Macaluso and Vito, 2004; Reeves et al., 2004).

Acute biochemical changes occur following intensive physical exercise, characterized by the release of signaling molecules and proteins, and a cascade of cellular responses (for review Pedersen and Hoffman-Goetz, 2000 and Coffey and Hawley, 2007), from which several are similar to the processes related to sarcopenia. In fact, physical exercise provokes an inflammatory reaction with the release of inflammatory cytokines, especially IL-6, into the blood circulation (Pedersen et al., 2001). In this context, IL-6 is mainly released from the contracting muscles (Ostrowski et al., 1998) and would exert a different function from that seen during e.g. acute infections, rather acting as a "myokine" and signaling the need for endogenous glucose production (Pedersen et al., 2001). Also enhanced HSP expression following physical exercise has been described in the muscle itself (Liu et al., 2000), as well as in peripheral blood mononuclear cells (Fehrenbach et al., 2000b; Whitham et al., 2004) and in the serum (Walsh et al., 2001; Febbraio et al., 2002a) of young individuals. These acute phase responses seem to play an essential role in the adaptation of the muscle following exercise. Possibly, the release of cytokines activates satellite cells in the muscle (Kadi et al., 2005) and the accumulation of HSP protects the muscle cells against the exercise-induced elevation of oxidative stress (McArdle et al., 2002). Moreover, it is assumed that Hsp70 can play a role in muscle hypertrophy following strength training (Kilgore et al., 1998). The activators for the HSP-response to exercise are probably multifactorial (for review Kregel, 2002). In fact, already within five minutes of a warm-up exercise of moderate intensity, the muscular temperature rises with 2°C (Saltin et al. 1968) and when exercising for prolonged periods, temperatures inside large muscles above 39°C have been described for humans and up to 44°C in rats (Brooks et al., 1971; Febbraio, 1996). Such elevated temperatures are known to be a powerful stimulus for Hsp70 (Njemini et al., 2002). On the other hand, since in vivo infusion of IL-6 activates intramuscular *hsp72* gene expression (Febbraio et al., 2002b), it can be assumed that exercise-induced accumulation of IL-6 is also a major contributor. Although studies are less abundant, elderly persons equally seem to be able to respond to physical stress by a significant increase of IL-6 in the blood circulation (Bruunsgaard and Pedersen, 2000; Toft et al., 2002; Bautmans et al., 2005b). It has been described, however, that acute eccentric exercise

induces a smaller systemic response in the aged (Toft et al., 2002) and did not elicit significant accumulation of IL-6 transcripts in the muscle of older subjects contrary to young ones (Hamada et al., 2005).

HSP-RESPONSE TO PHYSICAL EXERCISE IN THE ELDERLY

In this section, we will focus on experimental trials targeting the HSP-response to physical exercise in the elderly. Therefore, the bibliographic databases PubMed and Web of Science were systematically screened (Appendix) and the relevant articles found are reported and discussed.

ANIMAL STUDIES

Twelve animal-studies were identified and are summarized in Table 2. All investigations were performed on mice or rats and reported exercise-induced HSP-expression either in the skeletal muscle, the heart or the liver. The exercise stimuli applied consisted in treadmill running (endurance exercise) (Kregel and Moseley, 1996; Naito et al., 2001; Demirel et al., 2003; Starnes et al., 2003, 2005; Rinaldi et al., 2006; Huffman et al., 2008; Kayani et al., 2008) or electrical muscle stimulation (resistance exercise) (Vasilaki et al., 2002, 2003; Murlasits et al., 2006; Vasilaki et al., 2006).

Overall, a blunted HSP content, both at rest and following exercise, is reported in the tissues of the older animals compared to the young or middle-aged. In several studies, a significant exercise-induced increase in HSP-levels was detected in the older animals, although in a lower proportional change compared to young ones. Interestingly, Naito et al. (2001) demonstrated that in old rats the exercise-induced Hsp72 expression was similar to that seen in middle-aged animals for the high-oxidative (slow-twitch fibers) muscles, but lower for the muscles containing mainly fast-twitch fibers. Also, muscles containing many slow-twitch fibers showed higher resting HSP-levels compared to fast-twitch muscles, a phenomenon also reported by other investigators (Locke et al., 1991). Moreover, oestrogen plays a role in the expression of HSP, especially in heart. In fact, female animals show higher levels of Hsp72 in the heart compared to male, and this level decreases after ovariectomy (Voss et al., 2003). On the other hand, oestrogen seems to inhibit exercise-induced Hsp70 expression in rat skeletal muscle (Paroo et al., 2002), and stress-induced Hsp72 in rat pituitary gland, mesenteric lymph nodes and liver, but not in adrenal gland, spleen or heart (Paroo et al., 2002). Only 2 of the 12 studies we have found used female older animals (Naito et al., 2001; Vasilaki et al., 2002). At this moment, it remains unclear whether the gender ratio might have influenced the results of the animal studies. Interestingly, no age-differences were observed for exercise-induced responses of HSF1 protein and activation and HSP-mRNA, suggesting that the blunted HSP-response following exercise is not caused by reduced HSF1 availability or capacity to make mRNA in response to exercise.

Table 2. Studies describing the influence of physical exercise on HSP-expression in elderly animals

References	Population	Type of exercise	Main outcome	Results
Demirel et al. (2003)	Male Fischer-344 rats, 6 (Y) or 24 (O) mo randomized to control, exercise, hyperthermia	–2 d treadmill running (1 h/d, 75% VO2Max). –Hyperthermia, 15 min at 41°C (colonic temperature), using a heating blanket.	Myocardial –Hsp72 expression –HSF1 protein and activation –Hsp72-mRNA	–Lower ↑ myocardial Hsp72 after exercise and hyperthermia in O compared to Y –No difference between Y and O for HSF-1 protein, HSF-1 activation, and Hsp72-mRNA
Huffman et al. (2008)	82 Male C57BL/6 mice, aged 9 weeks, divided in 6 groups (young control, sedentary, exercise, exercise, 9%CR, exercise+9%CR, 18%CR)	24 wk treadmill running 5 d/wk (max 1 h)	–Hsp25, Hsp70 and Hsp70i in liver and skeletal muscle	–↑Hsp25 and Hsp70i in muscle of runners –↑Hsp25 in liver of runners+9%CR compared to sedentary
Kayani et al. (2008)	12–14 mo old (Y) and old 24 mo old (O) male C57BL6/J mice (*N*=96) divided in 5 groups (Y, O, Y training 10 wk, O training 10 wk, Y training 12 mo)	Treadmill running 15 m/min for 15 min 3 d/wk	–Whole M. Quadriceps Hsp70 content –Whole M. Gastrocnemius Hsp70-mRNA content	–↑Hsp70 and ↓Hsp70m-RNA in untrained O compared to untrained Y, differences not observed in trained O
Kregel and Moseley (1996)	12mo (A) and 24mo (O) old male Fischer 344 rats	3 groups: –Control –Passive heating (until corporal T=41°C) –Exertional heating (treadmill run until corporal T=41°C)	–Hsp72 in liver	–↑ Hsp72 in liver following –Heat (+192%l) in A, not in O –Exercise in A (+292%) and in O (+232%)
Murlasits et al. (2006)	Male (*N*=19) male Fisher344XBN F1 rats, 10 Y (3 mo) and 9 O(30 mo)	Eccentric contractions during full electrical stimulation of dorsiflexor muscles, 8x 10 rep, 3/wk during 4,5 wk	–Protein levels of Hsp72, Hsc70 and Hsp25 in muscle –mRNA levels of Hsp72 genes (Hsp70-1, Hsp70-2, Hsp70-3)	–Exercise ↑ Hsp72 and Hsp27 in O, which was 40% lower compared to ↑ in Y –No change in mRNA levels of *hsp72* genes

Table 2. (continued)

References	Population	Type of exercise	Main outcome	Results
Naito et al. (2001)	3 mo (Y) and 23 mo (O) old female Fisher 344 rats, assigned to either a sedentary control or an endurance exercise trained group (N=6 per group)	10 wk running 1 h/d, 5 d/wk on a treadmill at ~77%VO2peak	–Hsp72 in M. Soleus (SOL), M. Plantaris (PL), and the red (RG) and white portions (WG) of the M. Gastrocnemius	–↑ Hsp72 following exercise compared to controls in Y (SOL +22%, PL +94%, RG +44%, WG +243%) and O (SOL +15%, PL +73%, RG +38%, WG +150%) –↑ Hsp72 similar for Y and O in high oxidative muscle, but ↓ in O fast twitch muscle compared to Y
Rinaldi et al. (2006)	6 mo (Y, *N*=6) and 24 mo (O, *N*=18) old male Wistar rats, divided in 3 groups: sedentary young (SY, $n = 6$), sedentary old (SO, n = 8) and trained old (TO, $n = 10$).	6 wk running on treadmill at 30 m/min, 45 min/d 5 d/wk	–Hsp70 and Hsp27 in heart	–↓Hsp70 and ↑Hsp27 in SO compared to SY –↑Hsp70 in TO compared to SO –↑Hsp27 in TO compared to SY and SO
Starnes et al. (2003)	4 mo (Y), 12 mo (A) and 21 mo (O) old male Fischer 344 rats (*N*=60) assigned to control (*N*=10 at each age) or exercise (*N*=10 at each age)	1 h running on treadmill at 70–75% of maximum oxygen consumption	Hsp70 in heart 24 h post exercise	–↑ Hsp70 following exercise: 105% in Y, 27% in A and 24% in O

Table 2. (continued)

References	Population	Type of exercise	Main outcome	Results
Starnes et al. (2005)	40 male Fischer 344 rats, 20 Y (3 mo old) and 20 O (22 mo old), divided in 4 groups (Y and O sedentary, Y & O exercise)	10 weeks on a treadmill 15° incline, 15 m/min for up to 1 h, 5 d/wk	–Hsp70 in heart, liver and M Vastus Medialis –Hsp70-mRNA in heart	–No differences in Hsp70 between sedentary Y & O –Exercise ↑ Hsp70 in muscle of Y (+125%) and O (+70%) –Exercise ↑ Hsp70 in heart (+45%) and liver (+233%) of Y –No change in Hsp70-mRNA in heart
Vasilaki et al. (2002)	6 mo (A, *N*=5) and 28 mo (O, *N*=5) old female Wistar rats	15 min of electrically evoked isometric contractions (0.5s every 5s at 100 Hz/60 V pulse width of 0.1 ms)	Hsp70 & Hsc70 in M. Gastrocnemius 24 h post exercise	–No difference in resting levels between O & A –No change in Hsp70 or Hsc70 in O following exercise –↑ Hsp70 in A following exercise
Vasilaki et al. (2003)	12mo (A) and 30mo (O) old B6XSJL mice	15 min of electrically evoked isometric contractions (0.5 s every 5 s at 100 Hz/60 V pulse width of 0.1 ms)	Hsp25, Hsp25mRNA content and HSF1 binding in M Tibialis Anterior 4, 12 and 24 h post exercise	–No change in Hsp25 and Hsp25mRNA in O, contrary to A (↑) –Similar HSF-1 binding in O & A

Table 2. (continued)

References	Population	Type of exercise	Main outcome	Results
Vasilaki et al. (2006)	12 mo (A) and 30 mo (O) old male B6XSJL mice (*N*=60) allocated to exercise or control	15 min of electrically evoked isometric contractions (0.5 s every 5 s at 100 Hz/60 V pulse width of 0.1 ms)	Hsp70, Hsc70, Hsp25, Hsp25mRNA content and HSF1 binding in M Tibialis Anterior and M.Gastrocnemius immediately, 4, 12 and 24 h post exercise	–No age-difference for Hsp25 in controls –↑Hsc70 in O controls compared to A controls –↑Hsp25 in A following exercise from 4 to 24 h postexercise, no change in O –↑Hsp25mRNA in A immediately following exercise –Hsc70 ↑ in A and ↓ in O 4 h post exercise –↑Hsp70 in A 12 and 24 h post exercise

CR=calorie restriction, Hsp70i=inducible Hsp70, Hsc70=Hsp70 cognate, HSF-1=Heat shock factor-1, d=day, wk=week, mo=month, rep=repetition.

HUMAN STUDIES

Only a limited number of trials in humans were found (N=3), all describing the effect of physical exercise on Hsp70 expression in peripheral mononuclear blood cells (Table 3). Two studies performed by the same group (Simar et al., 2004, 2007) investigated the acute effects of a maximal incremental treadmill test on Hsp72 expression. In the first study, three age-groups were compared: G25 (N=8), G65 (N=12) and G85 (N=8) (mean age 25±1, 66±1 and 82±1 years respectively), all performing regularly physical activity (walking, gymnastics) in an organized setting (Simar et al., 2004). At rest, the percentage of Hsp72-positive lymphocytes was significantly higher in G25 (compared to G85), as well as intracellular Hsp72 expression in monocytes (compared to G65 and G85) and granulocytes (compared to G85). The maximal exercise test induced a significant increase in positive lymphocytes only in G85. Overall, intracellular Hsp72 decreased in granulocytes following the exercise test, which was explained by the authors by possible nuclear translocation of Hsp72 in these cells and post-exercise granulocytosis, thus increasing the proportion of granulocytes less exposed to the exercise stress in the analysis.

The second study compares older subjects (mean age 73±7 years) who were either highly physically active (exercising 10 h/wk at least 9 mo/year, N=16) or were inactive (not engaged in any sports activity, N=16) (Simar et al., 2007). At baseline, the highly active subjects showed a significantly lower number of leukocytes positive for Hsp72 (<50%) and a significantly lower intracellular Hsp72 content (<50%) compared to inactive subjects. Moreover, the highly active group showed higher antioxidant capacity and lower levels of oxidative damage, both significantly related to lower intracellular Hsp72 content. No significant changes in Hsp72 expression were found following the exercise test.

The paper by Bautmans et al. (2005b) was the only study found describing the influence of an intensive strength training program on intracellular Hsp70 expression in older humans. Elderly volunteers (mean age 68±5 years, N=31) performed during 6 weeks 3 times a week intensive resistance exercises (3 series of 10 repetitions at 70– 85% of the maximal strength) for the hip adductors and abductors, knee & hip extensors, shoulder abductors and large trunk muscles. At baseline and following 6 weeks training, resting levels of Hsp70 in monocytes (M) and lymphocytes (L) were determined both without and after application of a heat shock (incubation at 42°C). The amount of Hsp70 expression in M and L determined without heat shock decreased significantly after the training program. In contrast, heat shocked M and L (at 42°C) produced significantly higher amounts of Hsp70 at the end of the six-week strength training compared to baseline.

Both the crossectional study by Simar et al. (2007) and the prospective intervention study by Bautmans et al. (2005b) describe an attenuation of basal Hsp70 expression in PBMC of elderly persons following regular physical exercise, which is in agreement with findings in young human subjects (Fehrenbach et al., 2000a, b; Shastry et al., 2002). Possibly, this phenomenon reflects immunologic adaptations. In fact, it is assumed that regular physical exercise has a strong regulating effect

Table 3. Studies describing the influence of physical exercise on HSP-expression in elderly humans

References	Population	Type of exercise	Outcome	Results
Bautmans et al. (2005b)	10 male, 21 female, age 68±5 years	6 weeks IST	Hsp70 in PBMC – (IAS) – After heat shock (42°C)	– ↓ Hsp70 IAS after IST – ↑ Hsp70 42°C after IST
Simar et al. (2004)	3 age groups: – G25: age 25±1 years, 4 male, 4 female – G65: age 66±1 years, 8 male, 4 female – G85: age 82±1 years, 3 male, 5 female	MITT	– PBMC positive for Hsp72 – Hsp72 in PBMC	– ↑ Hsp72 positive Leukocytes in G85 after MITT – ↓ Hsp72 in granulocytes in all groups after MITT
Simar et al. (2007)	– 16 highly active (8 male and 8 female) – 16 low active (8 male and 8 female) – age 73±7 years	MITT	– Leukocytes positive for Hsp72 – Hsp72 in PBMC	– >50% lower Hsp72 in PBMC of highly active compared to low active subjects – no influence of MITT

PBMC=Peripheral blood mononuclear cells, IST= intensive strength training, IAS= immediately after sampling, MITT= maximal incremental treadmill test.

on systemic inflammatory processes such as seen in cardiovascular disease and the basal low-grade inflammatory profile in elderly (Pedersen and Bruunsgaard, 2003; Petersen and Pedersen, 2005). In this context, the exercise-induced bouts of IL-6 elevations in the circulation may play an important signaling function. The exercise schedules as described in the study of Bautmans et al. (2005b) were sufficiently intensive to elicit significant increases of IL-6 serum levels. As well, they observed a trend of resting IL-6 levels to decrease after the 6 weeks training program. Thus, the lower basal intra-cellular Hsp70 expression in trained elderly persons might reflect less systemic inflammatory activity. In fact, the exposure to (repetitive) mild stress has been shown to improve survival and longevity both at the cellular and organism level and Hsp-expression might play an important role (for review Minois, 2000; Horowitz and Robinson, 2007).

The optimal schedule (type of exercise, intensity, duration, frequency) for physical exercise in the elderly remains speculative. In fact, also in the elderly there exists a strong dose-response relationship following exercise (Williams et al., 2007). When targeting the age-related muscle weakness, it is now clear that intensive resistance exercise is the most efficient modality. Based on the studies in young subjects, it appears that the HSP-response is dependent on exercise intensity, as can be demonstrated both at the level of the muscle itself (Liu et al., 2000) as in PBMC (Shastry et al., 2002; Whitham et al., 2004). Resistance training resulted in larger increases in intramuscular Hsp70 compared to endurance training (Liu et al., 2004). Possibly, the lack of acute increases in intracellular Hsp70 expression following the maximal exercise test in the studies by Simar et al. (2004, 2007) might, besides a too low power given the low sample size, have been due to a combination of an age-related decrease in the capacity to respond to physical stress and a too low intensity of the treadmill exercise. In analogy, Shastry et al. (2002) also conclude, after comparing HSP-responses following physical exercise in young persons, that "exercise at an intensity that is within normal limits for moderately trained individuals is not a sufficient stimulus of Hsp70 production in leukocytes". Thus, it seems that more intensive exercises are necessary to elicit acute HSP-responses in PBMC and muscle cells and that intensive resistance exercises might be a preferential choice in the aged. It has to be noted that, when prescribing intensive training in older persons, it is important to increase the exercise intensity progressively and to introduce a sufficient recovery period between the training sessions, in order to allow recovery from the exercise-induced oxidative stress. In fact, it is well known that intensive muscle contractions produce oxidative stress in the tissues (Coffey and Hawley, 2007), one of the mechanisms triggering the HSP-response. Due to an accumulation of non-enzymatic cross-links (AGE) in the aged muscles and their interaction with RAGE, the oxidative stress is already increased in the tissues, thus potentially impairing the resistance against supplementary exercise-induced oxidative stress. Too abrupt and unbalanced training programs in untrained elderly, who show impaired stress-induced cellular defense mechanisms (HSP-response), might lead (initially) to increased cell damage and higher risks for injury. Given the higher accumulation of AGE's in diabetic persons, elderly persons with diabetes mellitus and insufficiently regulated

hyperglycemia might therefore be at higher risk (as recently suggested in animal models (Stoppa et al., 2006)).

The improvement of heat-induced Hsp70 expression in PBMC as seen following 6 weeks resistance training in the study of Bautmans et al. (2005b) has also been described in young trained athletes (Fehrenbach et al., 2000a) and might reflect a better cellular protection during stressful situations. In this context, although not investigated yet, it can be hypothesized that training-related adaptations of intracellular Hsp70 expression might play a role in the improved wound healing by physical training, as recently described in old mice (Keylock et al., 2008) and in elderly humans (Emery et al., 2005).

CONCLUSION

In summary, ageing is related to significant loss of muscle mass and muscle strength, called sarcopenia. The underlying mechanisms are closely related to inflammatory processes and it is assumed that these are aggravated by concomitant age-related alterations in cell-protecting mechanisms involving HSP, especially the Hsp70-family. The most effective modality to counter sarcopenia is intensive resistance exercise. Physical exercise induces, in a dose-response relationship, an acute phase reaction and influences Hsp70 expression in PBMC and several tissues. In this review we focused on the influence of physical exercise on the HSP expression during aging. Following a systematic literature search it can be concluded that there is very limited information available at this moment. Animal studies describe a blunted exercise-induced HSP response in the skeletal muscle, heart and liver of older rodents compared to younger ones. The exact origin of this age-related reduction is not yet identified. Based on the studies that were included in our review it seems that this phenomenon is not caused by reduced HSF1 availability or capacity to make mRNA in response to exercise. Only three reports involving elderly human subjects were identified in the literature search, from which two crossectional and one prospective intervention study. From these studies we can conclude that physical exercise lowers the basal Hsp70 expression in human PBMC, probably by reducing the low-grade inflammation and the oxidative stress. A 6-weeks intensive resistance training program at higher age improves the heat-induced Hsp70 expression in PBMC, a phenomenon which might be related to a better cellular protection during stressful situations and an accelerated recovery process following injury or disease in trained versus untrained elderly persons. Comparisons between exercise-induced HSP-responses in elderly animal and human subjects are difficult to perform given the different substrates used in these experiments (tissues of muscle, heart, liver in animals versus PBMC in humans). As a final conclusion it appears that physical exercise in elderly can have an important regulating effect on inflammatory processes and cellular protection mechanisms involving HSP-expression. Since this area has been incompletely studied at this moment, more research is warranted, especially

involving elderly persons in different clinical conditions and exploring the effects of different exercise schedules.

APPENDIX

The bibliographic databases PubMed and Web of Science were screened (last search performed on August 7th 2008) systematically for *experimental trials* targeting the HSP-response to physical exercise in the elderly. For the search the following search keys were used:

PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi):

(Aged OR Aged, 80 and over OR Ageing OR Aging OR Elderly) AND (Exercise OR Exercise Therapy OR Exercise Movement Techniques) AND (Heat-Shock Proteins OR Heat-Shock Response OR Chaperones OR Stress proteins) Keywords in this search were used both as Medical Subject Heading (MeSH, the U.S. National Library of Medicine controlled vocabulary thesaurus used for indexing MEDLINE articles) and as free keywords (as text).

Total hits=183, from which relevant trials N=15 (3 on humans, 12 on animals)

Web of Science (http://apps.isiknowledge.com):

(Aged OR Ageing OR Aging OR Elderly) AND (Exercise OR Exercise Therapy OR Exercise Movement Techniques) AND (Heat-Shock Proteins OR Heat-Shock Response OR Chaperones OR Stress proteins)

Total hits=92, from which relevant trials N=12 (3 on humans, 9 on animals)

Papers were selected when they corresponded to all of the following criteria:

– full article, reporting a trial (reviews and conference abstracts were excluded),
– article involving older humans (age≥60 years) or older animals
– investigating the influence of physical exercise on HSP-expression

Fifteen trials corresponded to these criteria, from which three on humans and 12 on animals.

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