Sex difference in the heat shock response to high external load resistance training in older humans

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Key words

Heat shock protein70, Sex, Aging, Resistance training, External training load.

ABSTRACT

BACKGROUND

Literature reports on the effects of resistance training on heat shock protein70 (Hsp70) adaptation in older subjects are scarce. Moreover, the optimum training load required to obtain a beneficial adaptation profile is lacking. Therefore, the aim of this study was to determine the effects of resistance training at various external loads on extracellular Hsp70 (eHsp70) resting levels in older humans.

METHODS

Fifty-six community-dwelling older (68 ± 5 years) volunteers were randomized to 12 weeks of resistance training (3x/week) at either high-resistance (HIGH, 8 males, 10 females, 2x10-15 repetitions at 80%1RM), low resistance (LOW, 9 Males, 10 Females, 1x80-100 repetitions at 20%1RM), or mixed low resistance (LOW+, 9 Males, 10 Females, 1x60 repetitions at 20%1RM followed by 1x10-20 repetitions at 40%1RM). Serum was available from 48 out of the 56 participants at baseline and after 12 weeks for determination of eHsp70. Mid-thigh muscle volume (computed tomography), muscle strength (1RM & Biodex dynamometer) and physical functioning (including 6 minutes walk distance [6MWD]) were assessed.

RESULTS

There was a sex-related dichotomy in the heat shock response to high external load training. We observed a significant decrease in eHsp70 concentration in the HIGH group for female, but not male, subjects. **At baseline, men had a larger muscle volume, leg press and leg extension 1RM compared to women (all $p < 0.001$). Also, the 6MWD was significantly higher in men compared to women at baseline. However, this difference disappeared when correcting for height. Moreover, the overall functional performance and physical activity scores were similar in men and women. None of the participants' characteristics nor any of the outcome variables differed between groups at baseline. There was a significant increase in the strength and physical performance parameters in both men and women post-exercise (all $p < 0.05$). Females in the HIGH group clearly demonstrated a larger gain in leg press 1RM and the isometric knee extensor strength compared to females in the LOW group ($p = 0.036$ and $p = 0.044$, respectively). More so, we found an inverse association between the change in eHsp70 levels and improvement in isometric knee extensor strength and 6MWD ($r = -0.443$, $p = 0.002$ and $r = -0.428$, $p = 0.002$; respectively) post exercise.**

CONCLUSIONS

Our results show that resistance training at high external load decreases the resting levels of eHsp70 in older females. Whether this reflects a better health status requires further investigation.

1. INTRODUCTION

Heat shock proteins (Hsp) are intracellular proteins that are ubiquitously synthesized in the cells of virtually all organisms in response to stressful and disease conditions such as inflammation (1-9). Though basically intracellular proteins, a great variety of stimuli have been reported to prompt Hsp70 release from living cells, including exercise and inflammatory cytokines (10, 11). Wu and colleagues showed that extracellular Hsp70 (eHsp70) can promote tumor cell proliferation and resistance to apoptosis (12). Moreover, the presence of Hsp70 in the extracellular milieu has often been associated with a poor prognosis of disease (13). Therefore, it has been widely accepted that eHsp70 serves as a danger signal that promotes immune responses and enhances host defense against insults (14, 15).

Physical activity has been reported to induce Hsp70 expression both intracellular and extracellular in humans and rodents (10, 16, 17). Although the underlying mechanisms of exercise-induced stress response have not been completely elucidated, it has been demonstrated that the metabolic changes caused by exercise including elevation in body temperature, up-regulation of reactive oxygen intermediates, and modulation of inflammatory mediators (18) can potentially induce Hsp synthesis in cells. Moreover, studies have portrayed that cells release Hsp into the systemic circulation during exercise through exocytotic mechanisms (19). In this light, distinction should be made between the exercise-induced acute changes (sudden, temporary changes during or immediately following an exercise bout) and the long term adaptation effects of exercise (changes in basal levels, when the acute exercise-induced effects have been washed out) on the heat shock response. It is also noteworthy that exercise-induced changes in Hsp70 response might be different according to the location of the protein, type of physical exercise, fitness level, as well as training load (17, 20). Many groups have demonstrated an acute exercise-induced increase in the levels of eHsp70 using different exercise protocols (10, 16, 21). However, the increase in eHsp70 was transient in all the studies because measures taken various hours after exercise were either similar to pre-exercise levels (10, 21) or lower (16, 22).

Sex differences in exercise-induced Hsp70 response have been reported in several tissues using mostly animal models. Paroo and coworkers reported a sex difference in exercise-induced Hsp production in skeletal muscles in rodents (23), with males demonstrating stronger responses than females. In a parallel study, the same group reported a similar increase in post-exercise Hsp70 in cardiac muscles of male

compared to female rats (24). Also, Nickerson et al. (25) found an attenuated Hsp72 response in the liver, pituitary gland, and mesenteric lymph in female compared with male Fischer 344 rats after stress. Scant evidence suggests sex differences in Hsp response in humans as well. Morton and colleagues (26) reported a diminished stress response after exercise in human skeletal muscle from female compared to male subjects. These sex differences have been attributed to sex-specific differences in hormonal, muscular and/or inflammatory responses following exercise (23, 27, 28).

Aging is characterized by a low grade chronic inflammation marked by elevated levels of inflammatory mediators (29). In line with current observations, it can be expected that persistent inflammatory challenges will augment the levels of eHsp70 in older subjects, with a consequent poor quality of life. Thus, processes that can reduce the resting levels of eHsp70 might be of relevance in the geriatric population. To date, reports on the effects of resistance training on eHsp70 adaptation in older subjects are scarce. Moreover, the optimum training load required to obtain a beneficial adaptation profile is lacking. Therefore, we sought to characterize the long term effects of resistance training at various external loads on eHsp70 resting levels in older humans.

2. MATERIALS AND METHODS

2.1 Participants and study design

In the present study, advertisements were used to recruit community dwelling elderly subjects for a 12 week resistance training exercise. The recruitment strategies and study approaches are reported in detail elsewhere (30, 31). Initially, 89 persons responded to the advertisement, but 33 subjects did not match the inclusion criteria. Participants were selected based on the following exclusion criteria: current participation in structured endurance exercise, participation in resistance exercise in the last 6 months prior to the study, unstable cardiovascular disease, neuromuscular disease, acute hernia, and hip/knee problems. The remaining 56 subjects (68±5 years) were randomly allocated to three intervention groups with different training loads - high external load resistance training (HIGH), mixed low external load resistance training (LOW+) and low external load resistance training (LOW) - for 12 weeks. Eight subjects could not be taken into consideration (2 from the HIGH, 3 from the LOW+ and 3 from the LOW intervention groups) for statistical analyses due to insufficient samples for eHsp70 determination either at baseline or at 12 weeks. Worth noting, there were no statistically significant difference in their general characteristics compared with those of the participants that were included in the analyses. Measurements of height

and weight were taken and body mass index (weight (kg)/ height² (m²)) was calculated. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki and each participant gave a written informed consent.

2.2 Resistance training protocol

Resistance training took place at the local fitness and health center and participants were supervised by a fitness instructor. Training was performed as reported previously (30, 31). Briefly, after an initial familiarization session in which training techniques were explained and demonstrated, participants exercised 3 times weekly on nonconsecutive days for 12 weeks (total of 36 sessions). The exercises (leg press, leg extension and seated row) were performed on Technogym™ (Technogym, Gambettola, Italy) devices, designed for resistance training. Each exercise session started with a brief warm-up (10 minutes) on a cycle ergometer (Technogym Bike Excite, Gambettola, Italy) or on a treadmill (Technogym Run Excite, Gambettola, Italy). Exercises were performed at a moderate speed with rest periods of 2 minutes in between and the training sessions were closely monitored by qualified fitness instructors. All participants were verbally encouraged to continue the exercises until failure (i.e. inability to perform more repetitions due to local muscle fatigue). Immediately after each individual exercise, participants graded their level of perceived exertion on the OMNI-Resistance Exercise Scale of Perceived Exertion (scale from 0 to 10) (32). As described previously (30, 31), the one repetition maximum (1RM) was evaluated every 4 weeks (at baseline, before the first training session in week 5 and week 9, and after 12 weeks of training) and training loads were adapted accordingly.

The exercise protocols were designed to be approximately equal in volume (% 1RM x number of repetitions). The HIGH resistance protocol (2 sets separated by 1 min interval of 10-15 repetitions at 80% of 1RM) was based on ACSM's guidelines for resistance training (33). These guidelines recommend performing at least one set to the point of failure for healthy individuals. In the HIGH group, the external resistance was initially set at 80% of 1RM. To ensure that maximal effort would be reached at the end of each set, participants were instructed to perform at least 10 to 15 repetitions. Two sets were performed with one minute of rest between sets. In the LOW+ group, participants were instructed to complete first 60 repetitions at 20% of 1RM, and immediately afterwards (no rest), the external load was increased to 40% of 1RM and participants were instructed to perform 10-20 additional repetitions. Participants in the LOW group were instructed to complete 1 set of 80-100 repetitions at 20% of 1RM.

During every exercise session, participants were asked to perform a maximum number of repetitions. If they could perform more repetitions than the targeted range, the external load was increased. As described previously, adherence (number of training sessions attended as a percentage of the total number of training sessions) to the program was 95.7% in the HIGH, 95.8% in the LOW and 95.3% in the LOW+ groups, with no significant differences between groups (31).

2.3 Muscle volume measurement

The muscle volume was measured as described previously by Van Roie et al. (30). Briefly, four 2.5 mm-thick axial slices were measured in the middle of the right upper leg by a computed tomography scan (Siemens SOMATOM Definition Flash, Forchheim, Germany). The axial images were obtained at the midpoint of the distance between the medial edge of the greater trochanter and the intercondyloid fossa of the femur. The four slices were then put together as one 10 mm-thick slice and the software program Volume (Siemens) was used to calculate the overall muscle volume (in cm³) for the 10 mm-thick slice. Standard Hounsfield Unit ranges for skeletal muscle (0–100) were used to segment muscle tissue area. Corrections were made for bone marrow. All measurements were performed by the same expert radiologist in the university hospital.

2.4 One repetition maximum

The assessment of leg press 1RM started with a warm-up set of 8 repetitions at approximately 50% of the estimated 1RM, followed by another set of 3 repetitions at 70% of the estimated 1RM (30). Subsequent lifts (ranging from 3 to 5) were single repetitions with progressively heavier resistances until failure. A rest period of 1 to 5 min was allotted between each attempt to ensure recovery. The heaviest successful lift was determined as 1RM.

Leg extension 1RM was estimated through a logarithmic regression formula (30). In this testing procedure, participants had to complete a maximum number of repetitions at a resistance that was selected by the supervisor (5 to 15 repetitions were targeted). Using the formula, the percentage of 1RM was estimated based on the number of repetitions, and subsequently, the 1RM was derived. This method was chosen over a direct method, as it was considered less time consuming. Strength characteristics of the knee extensors were measured on a Biodex Medical System 3[®] dynamometer (Biodex Medical Systems, Shirley, NY) as reported elsewhere(30).

2.5 Functional performance

The modified physical performance test (mPPT) (34) was executed at baseline in order to document the overall functional performance status of the participants. The mPPT consists of nine functional items: (i) Romberg test for balance, (ii) chair sit-to-stand test, (iii) lifting a book from waist height to a shelf at shoulder level, (iv) putting on and taking off a coat, (v) picking up a penny from the floor, (vi) turning 360°, (vii) walking 15 m, (viii) ascending one flight of stairs, and (ix) climbing four flights of stairs. The score of each item ranged from 0 (the inability to complete the task) to 4 (the highest level of performance), with a summary performance score (mPPT score) of maximum 36 points.

To further document the functional status of the participants at baseline and to analyze the effects of the intervention on functional performance, the following tests were performed at baseline and post intervention: 6-minute walk test, 30-second chair sit-to-stand test, timed up-and-go test and 5-repetition chair sit-to-stand test. The 6-minute walk test was performed over a walking course of 20 m (35). Participants walked up and down the course at a fast but comfortable pace, and the 6-minute walk distance (6MWD, in meters) was reported. The 5-repetition and 30-second chair sit-to-stand tests were performed using a standard chair without arm rests (36). Participants crossed both arms against the chest, started from a seated position (upper back against seat), stood up to full extension and sat down again (upper back against seat). The time required (in seconds) to perform 5 chair stands was evaluated in the 5-repetition chair sit-to-stand test. This test was performed twice, using the best result in further analyses. In the 30-second chair sit-to-stand test, the number of successful repetitions was counted over a 30-second period. Due to the exhausting nature of this test, it was performed only once. In the timed up-and-go test, the time (in seconds) required for the participant to stand up from a standard armchair, walk a distance of 3 m, turn, walk back and sit down again was measured (37). The test was performed as quickly as possible, however, without running. The best result from two trials was used.

2.6 Physical activity

Self-reported PA was assessed with the Godin Leisure-Time Exercise Questionnaire (38). Participants were asked to report the number of times that they were engaged in light, moderate and strenuous intensity PA for at least 20 minutes in a typical week. An overall measure of PA was obtained using the following formula:

total PA = (9 x number of times of strenuous activity) + (6 x number of times of moderate activity) + (3 x number of times of light activity). However, it is worth mentioning that the questionnaire was administered after training, where participants were asked to recall their engagement in PA in a typical week before the start of the intervention and post training.

2.7 Hsp70 determination

Venous serum samples were collected from all participants at baseline (before the start of the exercise) and at the end of the 12 weeks resistance training program (24h - 48h after the last training), each time at the same moment of the day, and stored at -80°C until eHsp70 determination. The time of blood sampling was equally distributed between the groups. eHsp70 was analyzed using a commercially available ELISA kit – Amp’d Hsp70 high sensitivity ELISA kit (Enzo Life sciences, NY, USA) – following the manufacturer’s instructions. The ELISA kit had a detection range from 0.039 to 5 ng/mL. The baseline and 12 weeks samples from the same participant were run on the same plate to avoid differences in results due to plate-to-plate variability.

2.8 Statistics

Data were tested for normality using the Kolmogorov-Smirnov goodness of fit test. Data that were not normally distributed were log-transformed and the transformed data were further checked for normality. eHsp70 was not normally distributed even after log-transformation and as such, nonparametric tests were applied for eHsp70 analysis. Independent samples t-test or Mann-Whitney U test was used to check sex-related differences. One-way ANOVA was used to test for difference between the intervention groups. When a significant difference was detected, Bonferroni post-hoc tests were performed. Between groups differences in changes over time were analyzed with repeated measures ANOVA using time as within subjects’ factor and group as between subjects’ factor. To detect changes within groups (baseline vs. 12 weeks) paired sample t-test was employed. For parameters that were not normally distributed, the Wilcoxon’s Signed Rank test was applied for within group differences, and Kruskal-Wallis and Mann-Whitney U tests for between group differences. Statistical analysis was performed using IBM SPSS version 22.0. Differences were considered to be significant for two-sided $p < 0.05$.

3. RESULTS

The baseline characteristics of the participants are shown in table 1. As expected, men had a larger muscle volume, **leg press and leg extension 1RM compared to women** ($p < 0.001$). Although, BMI trended to be higher in male subjects compared to females, there were no relationship between BMI and eHsp70. No sex difference was found for age, or eHsp70 concentration at baseline, even when corrected for BMI. **None of the participants' characteristics nor any of the outcome variables differed between groups at baseline.**

To have an overall idea of the functional performance status of the participants at baseline, we did the mPPT functional test. The mPPT score ranged from 31 to 36, indicating a good functional status at baseline for the participants. There was no sex- or group-related difference in the mPPT score at baseline (see Table 1).

We also used individual functional performance tests including the 6-minute walk test, 30-second chair sit-to-stand test, timed up-and-go test and the 5-repetition chair sit-to-stand test. No significant between groups difference was noticed - for all the functional performance measures- among females or males (see Table 1). When the entire cohort was taken into consideration, the timed up-and-go was significantly shorter for male compared to female subjects. On the other hand, the 6MWD was significantly longer for male compared to female. However, this difference disappeared when correcting for height. No sex-related difference was found for the other functional parameters. Similarly, there was no significant sex- or group-related difference for PA scores at baseline (see Table 1).

Table 1. Characteristics of the study participants at baseline according to sex and intervention group

Parameter	Male (n=23)	Female (n=25)	P*	HIGH (n=16: 8F, 8M)	LOW+ (n=16: 8F, 8M)	LOW (n=16: 9F, 7M)	P
Age (years)	68.0±5.2	67.8±5.3	0.917	67.8±4.5	67.0±5.8	68.9±5.3	0.618 [§]
BMI (kg/m²)	28.2±3.2	26.3±3.6	0.060	25.8±2.9	28.1±3.1	27.8±4.3	0.135 [§]
eHsp70 (ng/mL)^a	0.13 (0.18)	0.18 (0.18)	0.092	0.16 (0.15)	0.16 (0.19)	0.14 (0.29)	0.914 [†]
mPPT-score^a	36.0 (1.0)	36.0 (1.5)	0.338	36.0 (0.8)	36.0 (1.8)	36.0 (1.0)	0.536 [†]
6MWD (m)	613±79	541±65	0.001	602±67	577±94	549±73	0.173 [§]
30-s STS (reps)	15.4±2.2	15.8±2.4	0.585	16.0±2.6	15.1±1.9	15.6±2.4	0.570 [§]
TUG (s)	5.69±0.86	6.32±1.00	0.024	5.80±0.89	6.20±0.91	6.07±1.13	0.457 [§]
5x STS (s)	9.14±1.58	9.55±1.71	0.389	9.06±1.81	9.59±1.16	9.41±1.93	0.663 [§]
PA score^a	18.0 (29.0)	14.0 (11.0)	0.150	16.0 (10.0)	12.0 (23.5)	15.0 (12.0)	0.859 [†]
MV left (cm³)	185±26	125±17	<0.001	151±37	156±40	154±37	0.928 [§]
MV right (cm³)	189±24	127±15	<0.001	158±36	158±40	154±36	0.930 [§]
Log leg press 1RM	2.14±0.14	1.8±0.13	<0.001	1.96±0.24	1.97±0.17	1.98±0.24	0.942 [§]
Leg extension 1RM	35.5±4.9	26.4±5.8	<0.001	31.0±5.4	30.3±6.7	31.0±8.9	0.946 [§]
IKES (NM)	0.93±0.14	0.88±0.19	0.317	0.92±0.15	0.91±0.17	0.89±0.18	0.884 [§]

The values denote mean (SD), unless otherwise stated. ^a Median (IQR); BMI = Body mass index; eHsp70 = extracellular heat shock protein 70; mPPT = modified physical performance test; 6MWD = 6-minute walk distance; 30-s STS = 30-second chair sit-to-stand; TUG = timed up-and-go; 5x STS = 5-repetitions chair sit-to-stand; PA = physical activity; MV = muscle volume; 1RM = one repetition maximum; IKES = isometric knee extensor strength (at 120° knee angle); HIGH = high external load resistance training; LOW+ = mixed low external load resistance training; LOW = low external load resistance training; [§]Results of one-way analysis of variance between the intervention groups; [†]Result of Kruskal-Wallis test between intervention groups; *Difference between men and women.

Figure 1 shows the change in eHsp70 concentration between baseline and after 12 weeks resistance training for the different intervention groups. There was no significant change in eHsp70 concentration between the different intervention groups **at 12 weeks compared to baseline** when the entire population was taken into consideration. Similarly, we observed no significant difference in eHsp70 concentration between the intervention groups **at 12 weeks compared to baseline** for male subjects. However, a trend towards a time by group interaction effect was found for female subjects. More so, the levels of eHsp70 for females in the HIGH intervention group decreased significantly after training.

Figure 1. Change in extracellular Hsp70 concentration at 12 weeks compared to baseline in the various intervention groups. High = high external load resistance training; Low+ = mixed low external load resistance training; Low = low external load resistance training. The p values above the plots indicate results of Kruskal-Wallis test between the intervention groups. * Decreased significantly after exercise compared to baseline ($p < 0.05$).

To evaluate the effectiveness of the interventions on muscle and strength gains, we analyzed pre-post changes in muscle volume, leg press 1RM, leg extension 1RM and the isometric knee extensor strength (see Table 2). There was a significant increase in muscle volume ($p < 0.01$) after exercise in both male and female subjects and no significant time by group interaction effect was noticed for muscle volume from baseline to post exercise in females or males separately. The muscle volume remained significantly **larger** in male subjects ($p < 0.001$) compared to female subjects post-exercise, and there was a positive correlation between the change in the muscle volume and eHsp70 in males ($r = 0.557$, $p = 0.006$; data not shown).

The results of repeated measures ANOVA indicated a significant time effect for strength parameters ($p < 0.01$ for all, see Table 2). When male and female subjects were analyzed separately, all intervention groups showed a significant increase from baseline to post exercise in both leg press 1RM and leg extension 1RM (1RMLE) (all $p < 0.05$, see Table 2). Regarding leg press 1RM, a significant time by group interaction effect was observed from baseline to post exercise in the female subjects ($p = 0.029$), with post-hoc analyses portraying a significant increase in both the HIGH and the LOW+ intervention groups compared to the LOW intervention group ($p = 0.036$ and $p = 0.012$, respectively). On the other hand, a trend towards a time by group interaction effect was observed for leg extension 1RM in females ($p = 0.099$).

For the isometric knee extensor strength, there was a significant increase from baseline to post exercise in the HIGH group for both male and female subjects, and in the LOW+ intervention group for male participants only. More so, we observed a time by group interaction for the isometric knee extensor strength in female subjects ($p = 0.039$), with the HIGH group improving significantly more than the LOW+ and LOW groups ($p = 0.005$ and $p = 0.044$, respectively). There was no significant time by group interaction effect for any of the strength parameters in male subjects. Analysis of the exercise-induced changes in eHsp70 and strength parameters revealed an inverse association between changes in eHsp70 levels and improvement in isometric knee extensor strength ($r = -0.443$, $p = 0.002$) as well as the 6MWD ($r = -0.428$, $p = 0.002$, data not shown).

Table 2. Mean and SD at baseline and 12 weeks for muscle volume, leg press and leg extension one repetition maximum, and the knee extensor strength for the three intervention groups according to sex

Parameter	HIGH (n=16: 8F, 8M)	LOW+ (n=16: 8F, 8M)	LOW (n=16: 9F, 7M)	Time effect	Time*group effect
Male					
MV left (cm³)					
Baseline	182.3±22.9	188.2±29	185.3±27.8	0.009	0.155
12 weeks	187.2±21.8**	188.3±26.1	191.4±27.4*		
MV right (cm³)					
Baseline	188.0±20.7	192.2±25.6	185.0±28.4	0.007	0.229
12 weeks	192.2±21.2*	193.1±26.6	191.3±28.0*		
Log leg press 1RM					
Baseline	2.14±0.17	2.10±0.13	2.19±0.13	<0.001	0.439
12 weeks	2.26±0.16**	2.19 ±0.15**	2.27±0.15*		
Leg extension 1RM					
Baseline	34.69±3.08	33.07±5.48	39.27±3.90	<0.001	0.157
12 weeks	45.54±4.23**	41.02±4.80**	47.16±5.15**		
IKES					
Baseline	0.93±0.09	0.90±0.17	0.96±0.14	0.001	0.595
12 weeks	1.01±0.08*	0.98±0.11*	1.01±0.12		
Female					
MV left (cm³)					
Baseline	120.2±13.6	124.5±13.9	130.4±21.1	<0.001	0.352
12 weeks	125.4±13.7**	129.2±15.1*	132.6±19.8		
MV right (cm³)					
Baseline	127.4±13.4	124.1±13.1	129.1±18.2	<0.001	0.240
12 weeks	132.5±13.4*	128.8±14.8*	130.9±17.0		
Log leg press 1RM					
Baseline	1.78±0.14	1.84±0.06	1.83±0.18	<0.001	0.029
12 weeks	1.97±0.06**	2.01±0.06**	1.92±0.13**		
Leg extension 1RM					
Baseline	27.36±4.81	27.50±6.96	24.59±5.58	<0.001	0.099
12 weeks	34.75 ±3.82**	35.43 ±7.15**	29.06±6.34**		
IKES					
Baseline	0.90±0.20	0.92±0.18	0.83±0.18	0.003	0.039
12 weeks	1.01±0.18**	0.91±0.18	0.86±0.18		

MV = muscle volume; 1RM = one repetition maximum; IKES = isometric knee extensor strength (at 120° knee angle); HIGH = high external load resistance training; LOW+ = mixed low external load resistance training; LOW = low external load resistance training; **p<0.01, significantly different from baseline; *p<0.05, significantly different from baseline.

To evaluate the effectiveness of the interventions on functional performance, we analyzed pre-post changes in the functional performance measures in the study participants (see Tables 3). Results showed a significant increase in the 6MWD, number of chair stands in 30sec, and time to complete 5 chair stands from pre to post exercise in both male and female subjects (all $p < 0.05$). For the timed up-and-go a significant improvement from baseline to post exercise was seen only in male subjects ($p = 0.011$).

For female subjects, the HIGH intervention group showed a significant improvement for the number of chair stands in 30sec ($p = 0.007$), and time to complete 5 chair stands ($p = 0.040$). More so a trend to improvement for 6MWD ($p = 0.069$) and timed up-and-go ($p = 0.053$) was seen in female subjects in the HIGH group. LOW+ females showed a significant improvement for the number of chair stands in 30sec ($p = 0.028$) and time to complete 5 chair stands ($p = 0.030$, see Table 3). For females in the LOW group, we found a significant improvement only for time to complete 5 chair stands ($p = 0.010$).

For male subjects, the HIGH intervention group showed a significant improvement for time to complete 5 chair stands ($p = 0.007$), and there was a trend to improvement for the number of chair stands in 30sec ($p = 0.051$). LOW+ showed a significant improvement for number of chair stands in 30sec ($p = 0.008$) and time to complete 5 chair stands ($p = 0.005$). For males in the LOW group, we found a significant improvement for the number of chair stands in 30sec ($p = 0.047$), and a trend for improvement for timed up-and-go ($p = 0.061$).

Table 3. Pre-post changes for functional parameters for the three intervention groups according to sex

Parameter	HIGH (n=16: 8F, 8M)	LOW+ (n=16: 8F, 8M)	LOW (n=16: 9F, 7M)	Time effect	Time*group effect
Male					
6MWD (M)					
Baseline	639±61	613±110	584±54	0.032	0.483
12 weeks	680±66	621±117	614±36		
30-s STS (repetitions)					
Baseline	16.5±2.9	15.0±1.5	14.6±1.6	<0.001	0.902
12 weeks	17.6±3.1	16.4±1.5**	15.7±1.8*		
TUG (s)					
Baseline	5.30±0.88	5.90±0.81	5.90±0.86	0.011	0.518
12 weeks	5.08±0.61	5.69±0.69	5.41±0.44		
Log 5x STS (s)					
Baseline	0.93±0.10	0.98±0.05	0.96±0.06	<0.001	0.120
12 weeks	0.88±0.07**	0.92±0.04**	0.93±0.04		
Female					
6MWD (M)					
Baseline	565±53	540±63	521±76	0.044	0.698
12 weeks	597±44	571±61	532±80		
30-s STS (repetitions)					
Baseline	15.5±2.3	15.3±2.4	16.4±2.7	0.004	0.214
12 weeks	16.5±2.3**	16.6±1.8*	16.7±2.6		
TUG (s)					
Baseline	6.26±0.63	6.51±0.95	6.21±1.34	0.313	0.405
12 weeks	5.99±0.59	6.22±0.79	6.33±1.54		
5x STS (s)					
Baseline	0.97±0.06	0.97±0.06	0.97±0.10	<0.001	0.872
12 weeks	0.93±0.03*	0.92±0.06*	0.92±0.07*		

The values denote mean (SD). 6MWD = 6-minute walk distance; 30-s STS = 30-second chair sit-to-stand; TUG = timed up-and-go; 5x STS = 5-repetitions chair sit-to-stand; HIGH = high external load resistance training; LOW+ = mixed low external load resistance training; LOW = low external load resistance training; **p<0.01, significantly different from baseline; *p<0.05, significantly different from baseline.

4. DISCUSSION

In the present study, 12 weeks exercise at high external load significantly decreased eHsp70 resting levels in older female subjects. These results corroborate with reports from other studies in the literature (16, 22, 39). Ogawa and colleagues (40) reported a significant reduction in eHsp72 after 12 weeks of resistance training in elderly women, some of whom had diseases associated with chronic low-grade inflammation. Accordingly, Periard and colleagues (16) demonstrated a down-regulation of basal eHsp70 expression 24h post-exercise on subjects who cycled to exhaustion at 60% and 75% of maximal oxygen uptake. To date, we are aware of only one study who investigated the eHsp70 response to resistance exercise in post-menopausal females (40). These results are in agreement with their findings that portrayed diminished eHsp70 levels after 12 weeks of resistance training in post-menopausal women. However, our study is unique in the sense that we used a homogeneous sample rather than theirs, where some participants had inflammation, a potent modifier of the Hsp response. Our results indicate that the high external load protocol used in this set up may offer a beneficial effect for Hsp adaptations in females.

Our results provide evidence towards sex-related differences in eHsp70 response following high external load exercise in older subjects. These sex differences have been attributed to sex-specific differences in hormonal, muscular and/or inflammatory responses following exercise. In fact, sex differences in inflammatory responses following exercise have been reported, with women demonstrating an attenuated inflammatory response post-exercise compared to men (27). Though we found previously evidence for an overall anti-inflammatory effect of resistance training in the same cohort (31), there were no significant relationships between changes in inflammatory biomarkers and changes in eHsp70 (data not shown).

Sex dichotomy in eHsp70 response following resistance training at high external load might also be due to differences in muscular responses during fatigue induced by high intensity exercise (28). Jammes and colleagues (41) reported that the activation of skeletal muscles by fatigue triggered a widespread activation of Hsp70 in other resting (i.e. not exercising) muscles as well as various organs including the kidney and the brain. Further, Hunter et al. (42) observed that sex differences in the fatigability of arm muscles was dependent on contraction intensity and absolute force during contractions, which is directly related to muscle size. In the current study the muscle volume was significantly bigger in male compared to female subjects, and there was a positive correlation between

the change in the muscle volume and eHsp70 in males ($r=0.557$, $p=0.006$). Further research in this direction is required to elucidate the possible role played by the muscles in eHsp70 resting levels following resistance exercise.

Finally, exercise may modulate Hsp response differently in males and females by influencing hormonal activity, particularly estrogen (23, 43). Moghadasi and colleagues (44) reported that resistance training -consisting of 50–60 min of circuit weight training per day, 3 days a week, for 12 weeks - resulted in a significant increase in estrogen in young women. Similarly, Lu and colleagues showed that 2 months treadmill exercise increased serum levels of estrogen in the female rat hippocampus (45). As reported for other steroid hormones, increased estrogen levels may be attributable to decreased metabolic clearance rate caused by the reduction in hepatic blood flow during exercise (46). In accordance, Keizer and colleagues found a significant decrease in the degradation rate of estradiol in young women after 10 minutes of cycling at high intensity exercise of 70% VO₂max (47), which was not confirmed in a later study by Montagnani et al. (48) using a less intensive treadmill protocol in healthy untrained women. Consistent with this observation, Consitt et al. (49) observed that 40 minutes of cycling (75% of maximal heart rate) was capable of increasing serum levels of estradiol. However, they did not find any significant difference in estrogen levels after 40 min of resistance-exercise session at a low external load including three sets of eight exercises at 10% of 1RM. More so, Jurkowski et al. (50) tested healthy, premenopausal women during 20 minutes of cycling - at light (30 to 35% VO₂max), heavy (60 to 66% VO₂max) and exhaustive intensity (85 to 95% VO₂max) - and noted a significant increase in estradiol only after heavy and exhaustive exercise. Therefore, it has been acknowledged that training load (high training load, high hormone response) may potentially influence hormone responses to exercise (51).

With aging, many anabolic and sex-specific hormones are well known to decline (52). To address the effects of age on hormone responses, Rudman and colleagues (53) used exogenous supplementation of anabolic hormones and reported that the body could still respond to anabolic hormones even though endogenous levels may be very low. More importantly, Tissandier and colleagues (54) demonstrated that age-related decline in the levels of other hormones including IGF-I and DHEA, was attenuated in men who engaged in long-term endurance training. In accordance, Copeland et al. (55) reported an increase in estradiol in response to resistance and endurance exercise in women of various age categories including postmenopausal women. Taken together, these findings indicate that

older persons - if offered a high training load program - have the ability to up-regulate the levels of sex hormones in response to exercise.

The mechanism by which estrogen can induce diminished Hsp70 response may be multifactorial. To begin with, the chemical structure of estradiol indicates a phenol ring containing a free hydroxyl group that can be donated to other molecules (56). This confers estrogen the property of an antioxidant. In this regard, estrogen has been shown to protect various cell types during acute stress by increasing the anti-oxidant properties of the cells (57, 58). Further, Wiseman (59) found that the administration of estrogen inhibited lipid peroxidation in microsomal and liposomal systems. In this light, estrogen can dampen exercise-induced oxidative stress signals necessary for the initiation of stress responses. Also, estrogen may influence Hsp70 expression in response to exercise via its temperature-regulating properties (60) or by inhibiting the cyclic AMP-mediated pathway, which is known to be an important regulator of exercise-induced Hsp70 expression (61).

In the present study, we found significant improvements in functional performance parameters and significant strength gains in leg press 1RM, leg extension 1RM and isokinetic knee extensor strength from pre to post exercise. These strength gains are comparable to gains reported in other studies (62). No time by group interaction effect was observed for physical performance and strength parameters in men, however, women in the HIGH group clearly demonstrated a larger gain in leg press 1RM and the isometric knee extensor strength compared to women in the LOW group. These differences might be linked to the supplementary mechanical stimulus, created by increasing resistance in the HIGH group. However, it might as well be due to the type of muscle fatigue triggered during training. Neyroud et al. and Yoon et al. (63, 64) reported that sustained contractions at low resistance tend to cause more central fatigue, while protocols at higher resistances tend to induce more peripheral fatigue. More so, we found that differences in 1RM gains between LOW and HIGH were already apparent after 4 weeks of training. Suggesting that, they were probably due to coordinated adaptations specific to the movement that was trained. More interestingly, we found an inverse association between the change in eHsp70 levels and improvement in isometric knee extensor strength and 6MWD ($r=-0.443$, $p=0.002$ and $r=-0.428$, $p=0.002$; respectively) following exercise. Therefore, the diminished eHsp70 after high external load training seen in this study may be evidence of a long-term adaptation to exercise that has been reported to be associated with successful aging (65).

5. Limitations

A limitation of the present study could be the sample size. Given the small sample size, it is possible that our study was not sufficiently powered to detect small differences. Another factor that may weaken our study is the fact that we did not have enough samples to investigate the changes in the levels of estrogen that may have occurred with exercise; without this information we can only speculate on the influence of estrogen on the differential heat shock response seen in the participants. **More so, it would have been interesting to include a young control group in whom the levels of sex hormones are generally high and well known to increase after exercise.** The authors also acknowledge the limitation of not having a non-exercise control group, which might have masked supplementary benefits resulting from training. Although this study has some limitations, it adds a highly needed element in the exercise-induced Hsp70 adaptation in older subjects. Thus far, the scant data in this field on older subjects have not addressed the impact of training load. Our attempt to use different training loads in a randomized study design offers insight into the load dependent adaptability of eHsp70 in the geriatric population and makes our study complementary to previous studies. We believe that this profile of the eHsp70 response in older subjects will act as a guide for future experiments concerning eHsp70 and their association with exercise in older subjects. Another strong point is that this study was performed in an elderly population with a distinctly different physiologic profile compared to the young study participants in most of the available reports. Further research is highly needed to fully elucidate the heat shock response following exercise and the precise relevance for well-being.

6. Concluding remarks

In conclusion, our results show that resistance training at high external load leads to decreased resting levels of eHsp70 in older females. **We found an inverse association between the change in eHsp70 levels and improvements in functional performance and strength parameters following exercise.** More so, in a previous study we demonstrated that 6 weeks of intensive strength training induced a significant decrease in the resting levels of Hsp70 in peripheral blood cells, which resulted in a better potential to respond to physical stress (3). Whether the decrease in eHsp70 following high external load training in the present study might guarantee a better cellular protection when subsequently challenged with pathological stressful conditions like inflammation requires further investigation.

ACKNOWLEDGEMENTS

This work was supported by the Research Foundation Flanders, Belgium (FWO-Vlaanderen, grant n°11B8913N), as E. Van Roie was a Ph.D fellow of FWO. Also, the authors are very grateful to Sports and Health Center Respiro (Rotselaar, Belgium) for the use of the training equipment. The sponsors were not involved in the study design; in the collection, analysis and interpretation of data; in the writing of the report; neither in the decision to submit the article for publication. The intervention comply with the current laws of the country in which they were performed. All authors certify that they comply with the ethical guidelines for publishing in Experimental Gerontology. None of the authors have any conflict of interest with any entity with regard to this study.

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Highlights

- Training at high external load decreases the basal level of eHsp70 in older females
- **Exercise-induced decrease in eHsp70 is associated with functional improvement**
- Muscle size and perhaps estrogen may shape eHsp70 response to high load training