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Effects of voluntary exercise and genetic selection for high activity levels on HSP72 expression in house mice

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Belter, Jason G., Hannah V. Carey, and Theodore Garland, Jr. Effects of voluntary exercise and genetic selection for high activity levels on HSP72 expression in house mice. *J Appl Physiol* 96: 1270–1276, 2004. First published December 12, 2003; 10.1152/japplphysiol.00838.2003.—We studied expression of heat shock protein 72 (HSP72) in female mice from four replicate lines that had been selectively bred for high voluntary wheel running (S) and from four random-bred control lines (C). Mice from *generation 23* were sampled after 6 days of wheel access, and those from *generation 14* were sampled after 8 wk of access to wheels either free to rotate or locked. Mice from S lines ran ~2.6 times as many revolutions per day as did those from C lines. Western blotting of tissues from *generation 23* mice indicated that S mice had elevated HSP72 expression in triceps surae muscle, but levels in spleen, kidney, heart, and lung were similar in S and C mice. HSP72 expression in triceps surae from *generation 14* mice was measured by ELISA and analyzed with a two-way analysis of covariance. The interaction between wheel type and line type (S vs. C) was statistically significant, and subsequent analyses indicated that S mice had significantly elevated HSP72 expression only when housed with free wheels. Mice with the previously described mini-muscle phenotype (Houle-Leroy P, Guderley H, Swallow JG, and Garland T Jr. *Am J Physiol Regul Integr Comp Physiol* 284: R433–R443, 2003) occurred in both generations and had elevated HSP72 expression in triceps surae. For the *generation 23* sample, wheel running as a covariate had a significant negative association with HSP72 expression, and the effect of line type was still statistically significant. Therefore, the increased HSP72 expression of S mice is not a simple proximate effect of their increased wheel running.

adaptation; artificial selection; exercise physiology; stress proteins; wheel-running behavior; heat shock protein 72

HEAT SHOCK PROTEINS (HSPs) constitute a ubiquitous class of highly conserved proteins that contribute to cell survival by reducing the accumulation of damaged or abnormal polypeptides within cells (32, 41). Many HSPs, or stress proteins, act as molecular chaperones by aiding in synthesis, transport, and folding of nascent or denatured polypeptides. Some HSPs are produced constitutively and participate in the normal processing of cellular proteins, whereas others are induced in response to specific stimuli and act to maintain cellular viability under stressful conditions (41). A wide variety of stimuli are known to activate the cellular response to stress, including heat and cold stress, heavy metals, hypoxia, oxidative stress, glucose deprivation, and intracellular calcium imbalance (32, 41). In addition, ecological and evolutionary physiologists are giving increasing attention to the cellular stress response as a potential selective force that can shape organismal biology (8).

Physical exercise is one aspect of whole animal physiology that can induce a stress response and may play a role in natural selection on the stress response. Although the mechanisms that lead to induction of the stress response during or after bouts of exercise are not fully understood, they may include such factors as increased body temperature, lactic acid accumulation, myofibrillar damage, oxidative stress, disturbances in calcium homeostasis, and glucose deprivation (4, 17, 34). In various tissues of rats, exercise has been shown to induce expression of HSPs including HSP60, the constitutive (HSC73) and inducible (HSP72) forms of HSP70, HSP90, HSP100, as well as the glucose-regulated proteins GRP75 and GRP78 (3, 6, 14, 23–25, 29, 34, 37, 37).

Patterns of HSP expression vary with the exercise protocol used. Acute exercise protocols typically involve running animals on a treadmill for a set length of time, up to exhaustion (23, 34, 37). In chronic exercise protocols, rats are trained to run on a treadmill for a number of weeks (6, 14, 25, 35, 38). Chronic muscle stimulation has also been achieved via application of electric current (28, 31).

The effect of voluntary exercise on HSP expression in animal models has received less attention. Two studies (3, 29) examined the stress response in rats that were allowed to train voluntarily on running wheels for 8 wk, but in both cases voluntary exercise did not alter expression of the inducible stress protein HSP72 in cardiac or skeletal muscle. However, in rats that ran voluntarily on running wheels for 5 mo, HSP72 expression in quadriceps muscle was significantly higher than in sedentary controls (36).

Previous studies of exercise and HSP expression have focused on the rat as an experimental model, with little use of mice, which are easier to manipulate genetically. In the present study, we examined the impact of exercise on HSP72 expression in a unique collection of mice that have been artificially selected for high voluntary wheel running. These mice consist of eight separate lines, four of which have been selectively bred for high voluntary wheel-running behavior (S lines) and four of which are bred randomly as control (C) lines (10, 39). By *generation 17*, mice from S lines ran ~150% more than C individuals (33), and this differential has been maintained for at least 15 additional generations (10, 39). The increase in running is attributable mainly to an increase in average running speed, rather than an increase in the number of minutes per day ran (10, 25, 33, 39, 40). When active on wheels, S individuals exhibit a small elevation of body temperatures compared with C mice [~0.2–0.3°C (33)] that is statistically explainable by the elevated amount of wheel running.

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The aim of the present work was to determine whether mice selected for voluntary wheel-running propensity had alterations in tissue HSP72 levels compared with nonselected mice. We hypothesized that changes in HSP72 level might be expected on the basis of the purported protective effects of HSP72 against cellular stressors related to chronic exercise (4, 17). Two groups of mice were used in this study. The first group, made up of mice from both S and C lines, consisted of 48 female mice from *generation 23* that were killed after 6 days of wheel access. This experiment was designed to mimic the normal breeding protocol, in which breeders are chosen based on a 6-day exposure to wheels (39). The second group of S and C mice (*generation 14*) had been allowed access to wheels that were either free to rotate or locked for 8 wk [chronic wheel access (40)]. Mixed hindlimb muscles of these mice have been characterized biochemically (15, 16). The two groups of mice allowed us to determine whether differences in chronic voluntary exercise alter the pattern of HSP expression that might be observed between S and C mice.

We also examined the relationship between exercise, HSP expression, and the newly discovered phenotypic trait the "mini-muscle" (11). Mice with this trait have triceps surae and total hindlimb muscle masses that are ~50% lower than expected based on their total body mass, and the phenotype is apparently attributable to a single Mendelian recessive locus (11). Because these muscles show alterations in total and myofibrillar protein content as well as elevated citrate synthase and cytochrome-*c* oxidase activities (16), we hypothesized that they would also show elevated HSP72 expression. This might be expected because treadmill endurance training in rats resulted in parallel increases in both HSPs (specifically, HSP60 and GRP75) and citrate synthase activity in plantaris muscle (25).

METHODS

All procedures used in this study adhered to the guidelines established by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.

Six-day wheel access. Forty-eight female mice (*generation 23*, second litters, 6 chosen randomly from each of 6 families within each of the 8 lines) were given wheel access for 6 days in accordance with the typical selection protocol (39). On the morning after *day 6* (0905–1300 CST), mice were killed by cervical dislocation, and the heart, lungs, triceps surae, spleen, and kidneys were removed immediately and stored at −80°C. Mice were 48–60 days old [58.1 ± 2.65 (SD)] when killed.

Cytosolic protein fractions were extracted by mincing ~100 mg tissue in 500 μl of lysis buffer [10 mM HEPES, 0.1% Triton X-100, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 1× protease inhibitor cocktail (Calbiochem)] followed by incubation for 0.5 h. The slurry was then pulverized with 10 mortar and pestle strokes and centrifuged for 10 min at 10,000 rpm. The supernatant was collected and quantified for total protein concentration by using the Pierce BCA protein assay.

Proteins (25 μg) were resolved by electrophoresis using conventional techniques. After separation, proteins were transferred to nitrocellulose paper and then blocked overnight in 4% nonfat milk in TBST (Tris-buffered saline plus 0.05% Tween 20). After blocking, blots were incubated with antibody raised against the inducible stress protein HSP72 (SPA-810, StressGen, Victoria, Canada) for 1 h. Blots were rinsed three times in TBST and exposed to secondary mouse antibody (mouse IgG-horseradish peroxidase) for 45 min. Antibody detection was carried out by using a chemiluminescent technique, and protein bands were quantified by using scanning densitometry. A standard curve was determined for each gel from HSP72 standards (StressGen) that were loaded in adjacent wells, and all samples on a gel were normalized to that curve. Data are expressed as amount of HSP72 per 25 μg of total protein extract.

Chronic wheel access. Triceps surae muscles from 80 female *generation 14* mice (second litters) were studied. These females were part of a larger group of 160 males and females described by Swallow et al. (40) and Houle-Leroy et al. (15, 16). The mice were housed alone in cages with running wheels for 8 wk, beginning at weaning. Half of the wheels were locked to prevent rotation, and assignment of mice to the free- or locked-wheel group was random within family (see below).

After the mice were killed, muscles were flash-frozen at −80°C, and cytosolic proteins were prepared as described for 6-day wheel-access mice. For this study, we measured HSP72 content using the HSP72 ELISA kit (EKS-700, StressGen), which is a more sensitive assay than Western blotting. Concentration of HSP72 in each sample was estimated by using a standard curve provided by the kit.

Statistical analysis. The MIXED procedure in SAS (SAS Institute) was used to apply nested analysis of covariance (ANCOVA) models to the data from *generations 14* and *23.* In general, our analyses followed the procedures outlined by Houle-Leroy et al. (15). Replicate line ($n = 8$ total), nested within line type, was always considered a random effect, and the effect of line type (S vs. C) was tested over the effect of line. A main effect for the mini-muscle phenotype was also included and was tested over the mean square error.

For the *generation 14* sample [as described by Houle-Leroy et al. (15)], we also tested simultaneously the effects of mini-muscle, line type, and wheel type [sedentary (locked wheels) vs. active (free wheels)]. Effect of mini-muscle was tested over the mean square error. Effect of line type was tested over the mean squares of line, and effect of line was tested over the mean squares of family [as described previously for this sample of mice (15, 16, 40); each of the eight lines was represented by two males and two females chosen at random from each of five families, thus yielding a total of 80 mice]. Effects of wheel type and the wheel type × line type interaction were tested over the mean squares of the wheel type × line interaction. Because ANOVAs (and ANCOVAs) have relatively low power to detect interactions, and following our laboratory's previous biochemical analysis of these mice (15), we considered a wheel type × line type interaction significant if $P < 0.10$. Because the interaction between line type and wheel access was statistically significant ($P < 0.10$) in some cases, we performed additional analyses in which we separated mice by wheel type (i.e., the two different "environments").

Age, time of day of death, and (z-transformed time of death)^2 were included as covariates in all analyses. For the mice with access to free wheels, additional analyses were run with the amount of wheel running (total revolutions) included as another covariate. In all cases, adjusted means were calculated by using the least squares means command in SAS MIXED; all covariates in the model, regardless of statistical significance, were used to calculate adjusted means. Two procedures were used to identify outlying or influential data points that were subsequently removed from ANCOVA models. First, we used the outlier test described by Cook and Weisberg (5), which identified one significant outlier that was subsequently removed before the analysis in Table 1 and Fig. 3. Second, we checked for large effects on P values or parameter estimates, which indicated removal of one data point in Table 4 and Fig. 5. HSP72 concentration was log transformed for all analyses to reduce skewness.

RESULTS

Six-day wheel access (generation 23). Figure 1 presents running across the 6 days of wheel access. On each day, S mice ran significantly more than C mice (all $P < 0.005$), and the fold difference ranged from 2.5 to 2.9. The main effect of mini-

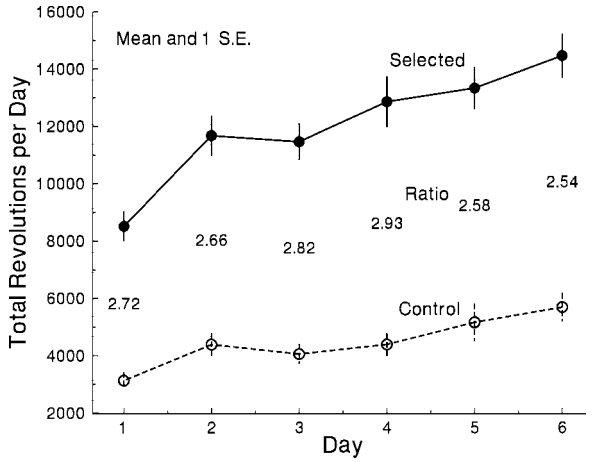

Fig. 1. Mean wheel running across all 6 days for female mice from *generation 23*. The ratio of mean selected revolutions to mean control revolutions is indicated for each day.

Fig. 3. HSP72 concentration in the triceps surae muscle for female mice from *generation 23* (6-day wheel access). *The 7 individuals identified as possessing the "mini-muscle" phenotype (see Fig. 2 and text). Statistical analyses are shown in Table 1 ($n = 45$).

muscle was not statistically significant for any day (all $P \geq 0.4$), nor was the covariate age (all $P > 0.086$).

On the basis of visual inspection and comparison with data from previous generations (11), seven individuals were identified as possessing the mini-muscle phenotype, six of which were in the S lines (Fig. 2; see also Fig. 4). In a nested ANCOVA of log muscle mass ($n = 48$), the effects of log body mass [$P = 0.0001$, partial regression slope $= 1.06 \pm 0.177$ (SE)] and of mini-muscle ($P = 0.0001$) were highly significant, but the effect of line type was not ($P = 0.4179$). Hence, the 41 individuals with normal-sized muscles did not differ significantly in relative muscle mass between S and C lines.

Western analysis revealed that S mice tended to have higher levels of HSP72 in their triceps surae (Fig. 3, Table 1; $P = 0.0561$). In addition, mice that possessed mini-muscles showed significantly higher HSP72 expression ($P = 0.0421$). Finally, amount of wheel running on *day 6* had a significant negative effect ($P = 0.0330$). Results (not shown) were similar when total running over all 6 days was used as the covariate, but the model had a slightly lower log likelihood. None of the other tissues (spleen, kidney, heart, lung; all log transformed) showed a statistically significant effect of line type, mini-muscle, amount of running on *day 6*, or any of the other covariates tested (results not shown).

Chronic wheel access (generation 14). Seven individuals exhibited the mini-muscle phenotype, and all were in the S lines and included both sedentary and wheel-access groups (Fig. 4). In a nested ANCOVA of log muscle mass (Table 2), the effects of log body mass [$P = 0.0001$, partial regression slope $= 0.87 \pm 0.086$ (SE)] and of mini-muscle ($P = 0.0001$) were highly significant, and access to free wheels showed a tendency to increase relative muscle mass ($P = 0.0829$).

The ANCOVA model indicated that mice with the mini-muscle phenotype had elevated HSP72 expression ($P = 0.0001$), as well as statistically significant effects of wheel type and an interaction between line type and wheel type ($P = 0.0604$; Table 3). The interaction means that both wheel type

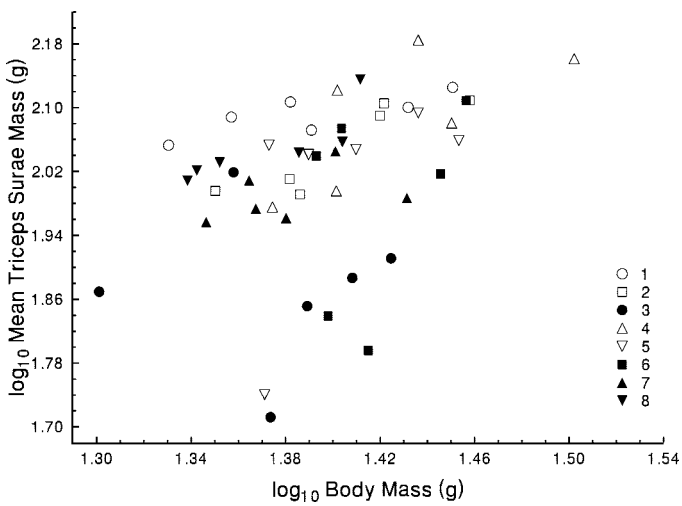

Fig. 2. Mean triceps surae muscle mass in relation to body mass of female mice ($n = 48$) from *generation 23* (6-day wheel access). *Lines 1, 2, 4*, and *5* are controls; *lines 3, 6, 7*, and *8* have been selectively bred for high voluntary wheel running. Seven individuals were identified as possessing the "mini-muscle" phenotype [those with muscles <1.92 on the log scale (4 in *line 3*, 2 in *line 6*, 1 in *line 5*)]; the individual with the smallest body mass (~1.3 on the log scale) was identified as having a normal-size muscle, and no heat shock protein (HSP) data were obtained from this individual.

Table 1. *Nested ANCOVA of log_{10} HSP72 concentration in the triceps surae muscle of generation 23 female mice given 6 days of wheel access*

Source	DF	*F*	*P*
Mini-muscle	1,32	4.48	0.0421
Line type	1,6	5.58	0.0561
Age	1,32	0.22	0.6429
Time of day	1,32	4.23	0.0479
z (time of day)^2	1,32	1.44	0.2391
Revolutions on *day 6*	1,32	4.97	0.0330

DF, degrees of freedom; HSP72, heat shock protein 72; ANCOVA, analysis of covariance. $n = 45$ mice (1 outlier deleted). Concentration is in ng/25 μg total protein. Least squares (adjusted) means ± SE for mini-muscle were 0.978 ± 0.0183 and 1.087 ± 0.0462 for normal and mini-muscles, respectively. For line type, values were 0.953 ± 0.0456 and 1.113 ± 0.0369 for control and selected lines, respectively. Partial regression coefficient (±SE) for revolutions on *day 6* was −0.0000122 ± 0.00000547.

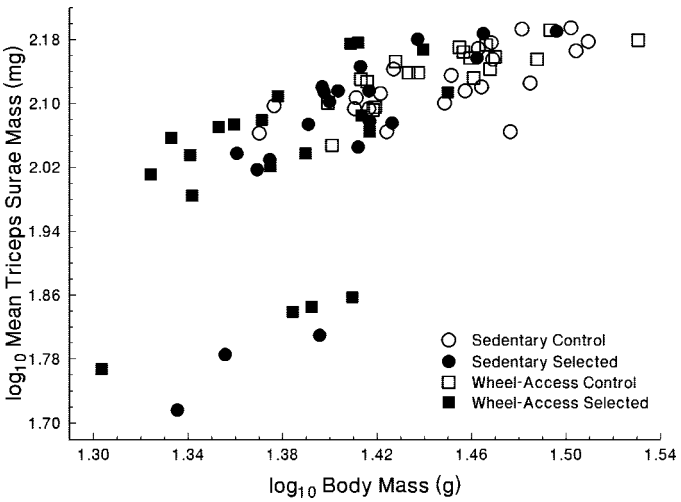

Fig. 4. Mean triceps surae muscle mass in relation to body mass of female mice from *generation 14* (8-wk wheel access). Seven individuals (those with the smallest muscles), all in selected lines, were identified as possessing the "mini-muscle" phenotype. Statistical analyses are shown in Table 2 ($n = 80$).

and line type may be considered to have statistically significant effects, but their effects are contingent on the state of the other factor. Adding either citrate synthase activity or cytochrome-*c* oxidase activity (data from Ref. 15) as an additional covariate to the model presented in Table 3 indicated no statistically significant predictive effect of either variable ($P = 0.2977$ and $P = 0.5794$, respectively). In both cases, the effect of mini-muscle remained highly significant ($P = 0.0043$ and $P = 0.0001$, respectively). Thus the elevated HSP72 expression of the mini-muscles cannot be explained statistically as a simple function of elevated mitochondrial abundance. Inspection of the least squares means (legend of Table 3) indicated that HSP72 expression did not differ between sedentary S and C mice (housed with locked wheels), but it was elevated in S mice compared with C when they were housed with free wheels. Separate analyses of sedentary mice ($n = 39$, line type $P = 0.6369$, mini-muscle $P = 0.0004$) and of active mice ($n = 39$, line type $P = 0.0236$, mini-muscle $P = 0.0001$) confirmed these patterns. Analysis of only the active mice, including amount of wheel running during the 7 days before death as a covariate (Fig. 5, Table 4), supported the effects of mini-muscle and line type on HSP72 expression. [The effect of line type based on the original data set of 39 mice with access to free wheels produced a P value of 0.0994. Deletion of one value from a control line mouse that had the highest residual log HSP72 value reduced this P value to 0.0247, with little effect on significance of any other variable; thus the reduced data set ($n = 38$) is presented in Table 4.]

Table 2. *Nested ANCOVA of mean log_{10} triceps surae mass (mg) for generation 14 female mice with 8 wk of access to wheels that were either free to rotate or locked*

Source	DF	*F*	*P*
Mini-muscle	1,27	366.90	0.0001
Line type	1,6	0.00	0.9783
Wheel type	1,6	4.32	0.0829
Line type × wheel type	1,6	0.00	0.9832
Log_{10} body mass	1,27	102.10	0.0001
Age	1,27	1.10	0.3040
Time of day	1,27	1.86	0.1843
z (time of day)^2	1,27	3.63	0.0676

$n = 80$ Mice. Least squares (adjusted) means ± SE for mini-muscle were 2.110 ± 0.0093 and 1.853 ± 0.0136 for normal and mini-muscles, respectively. Least squares means were 1.975 ± 0.0153 for control mice with locked wheels, 1.976 ± 0.0150 for selected mice with locked wheels, 1.986 ± 0.0155 for control mice with free wheels, and 1.987 ± 0.0153 for selected mice with free wheels.

Table 3. *Nested ANCOVA of log_{10} HSP72 concentration in the triceps surae muscle of generation 14 female mice housed for 8 wk with access to wheels that were either free to rotate or locked*

Source	DF	*F*	*P*
Mini-muscle	1,26	51.86	0.0001
Line type	1,6	1.67	0.2440
Wheel type	1,6	6.85	0.0397
Line type × wheel type	1,6	5.33	0.0604
Age	1,26	4.84	0.0369
Time of day	1,26	7.06	0.0133
z (time of day)^2	1,26	0.06	0.8098

$n = 78$ Mice. Concentration is in ng/0.7 μg total protein. Least squares (adjusted) means ± SE for mini-muscle were 1.578 ± 0.0295 and 2.260 ± 0.0919 for normal and mini-muscles, respectively. Least squares means were 1.871 ± 0.0736 for control mice with locked wheels, 1.817 ± 0.0696 for selected mice with locked wheels, 1.889 ± 0.0743 for control mice with free wheels, and 2.100 ± 0.0642 for selected mice with free wheels.

DISCUSSION

Growing evidence shows that the expression of HSPs in animals exhibits natural variation (20, 21, 27) and is responsive to selection (26). The goal of the present study was to determine whether expression of the inducible stress protein HSP72 differed between lines of mice that had been selectively bred for voluntary wheel running, compared with mice from non-selected control lines (S vs. C lines, respectively, as described in Refs. 10 and 39). Two samples of S and C mice were compared: *generation 23* animals that had acute access to free running wheels for 6 days (as during the normal protocol for choosing breeders) and *generation 14* mice that had chronic access to either free or locked wheels for 8 wk beginning at weaning.

Our results support the hypothesis that selection for high voluntary wheel running is associated with increased HSP72 levels, at least for triceps surae muscle. This was evident from the trend for increased HSP72 in S mice after 6 days of wheel access (*generation 23*, Table 1; $P = 0.0561$) and from the significant elevation of HSP72 in S mice given access to free running wheels for 8 wk (*generation 14*; Table 4, $P = 0.0247$). The difference in significance of the effect in the two data sets may be related to the greater degree of experimental error in the way HSP72 was measured in *generation 23* mice (Western analysis followed by densitometry) compared with the more sensitive ELISA assay for HSP72 that was used for the chronic wheel-access (*generation 14*) study. Alternatively, it may be related to the greater length of time that mice had access to wheels in the *generation 14* sample.

Mice in our study were allowed to run voluntarily, and previous observations suggest that even in the S lines running rarely, if ever, exceeded the maximum aerobic capacity of the

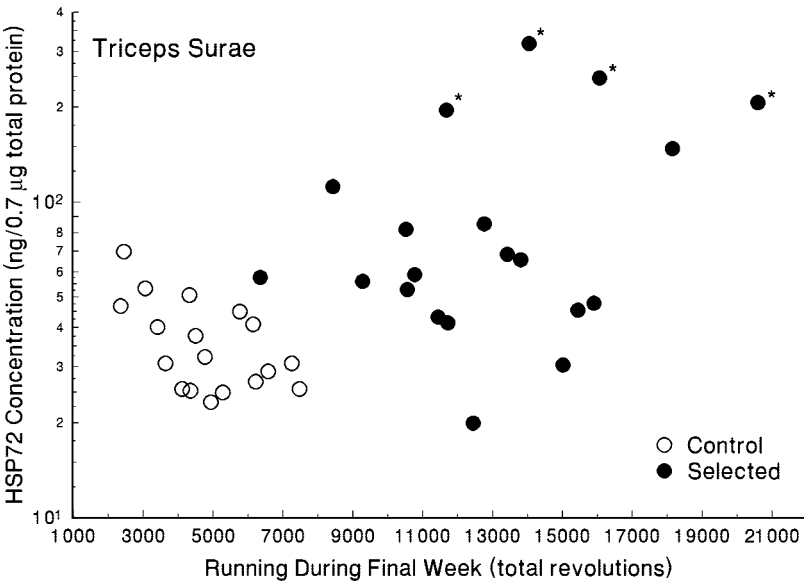

Fig. 5. HSP72 concentration in the triceps surae muscle of female mice from *generation 14* that were housed with access to running wheels free to rotate. *The 4 individuals identified as possessing the "mini-muscle" phenotype (see Fig. 4). Statistical analyses are shown in Table 4 ($n = 38$).

animals (13). Thus this protocol differs from many others used in exercise studies in that the animals were not specifically trained to exercise, and they were never forced to run until exhaustion. Because we were primarily interested in the effect of voluntary exercise on HSP72 expression, the removal of other potentially negative aspects of forced training protocols, such as psychological stress or severe oxidative damage to muscle tissue, was desirable (see also Ref. 12). Our results showed that selection for wheel-running activity did not result in higher HSP72 levels if S mice were not able to run on wheels: S and C mice housed with locked wheels for 8 wk did not differ in HSP72 expression ($P = 0.6369$). Thus elevated HSP72 expression of S mice may be part of the suite of adaptations that promote exercise [see also Mattson et al. (25)], rather than being a response to the stress of a specific training regimen.

Results of several studies have documented the effectiveness of chronic treadmill training protocols in increasing HSP72 expression in rat skeletal muscle (7, 14, 35). Induction of HSP72 in skeletal muscle has also been demonstrated in humans after 4 wk of rowing training (22). Although no comparable data exist for mice, several recent studies have examined the effect of voluntary exercise protocols on HSP72 expression in rats. In contrast to our results, voluntary wheel running for 8 wk did not affect HSP72 expression in triceps muscle in rats that were housed with functional running wheels compared with rats housed with wheels that remained locked (3). Noble et al. (29) also reported that voluntary wheel running in rats had no effect on cardiac HSP72 expression after 8 wk of wheel access, whereas treadmill training over the same time period did. This was true even though the free-wheel-trained rats accumulated greater average weekly running distances than did their treadmill-trained counterparts (29). However, in a recent study using rats that ran voluntarily on running wheels for a much longer period (5 mo), HSP72 expression in quadriceps muscle was significantly higher than in sedentary controls (36). In combination with our observation that mice from C lines did not train with respect to HSP72 expression (see least squares means in Table 3), these results suggest that the amount or intensity of wheel running may be crucial in determining whether it increases HSP72 expression. Importantly, voluntary wheel running can induce training effects in C mice, as demonstrated by significant alterations in some enzyme activities from mixed hindlimb muscle of these same animals (15, 16).

Table 4. *Nested ANCOVA of log_{10} HSP72 concentration in the triceps surae muscle of generation 14 female mice housed for 8 wk with access to free wheels only (see Fig. 5)*

Source	DF	*F*	*P*
Mini-muscle	1,25	37.19	0.0001
Line type	1,6	8.88	0.0247
Age	1,25	1.75	0.1983
Time of day	1,25	1.54	0.2265
z (time of day)^2	1,25	0.87	0.3594
Running in final 7 days	1,25	1.37	0.2523

$n = 38$ Mice (1 outlier deleted). Concentration is in ng/0.7 μg total protein. Least squares (adjusted) means ± SE for mini-muscle were 1.640 ± 0.0357 and 2.314 ± 0.1043 for normal and mini-muscles, respectively. For line type, values were 1.805 ± 0.0799 and 2.148 ± 0.0792 for control and selected lines, respectively. Partial regression coefficient (±SE) for running in final 7 days was −0.00001355 ± 0.00001156 per revolution.

Rats with free access to wheels could be considered comparable to the C mice in our study (i.e., mice not selected for wheel-running propensity), and indeed the C mice showed little or no increase in HSP72 expression after 8 wk of wheel access compared with controls housed with locked wheels (adjusted means reported in the legend of Table 3). We also predicted that S mice would show a response comparable to rats run on treadmills, given that both groups exhibit large biochemical training effects (15, 35). Our results support this hypothesis, because S mice with wheel access exhibited a large increase in HSP72 expression (adjusted means reported in legends of Tables 3 and 4). This large training effect in selected mice combined with no training effect in control mice constitutes a line-type × wheel-type interaction (Table 3), which is similar to other line-type × wheel-type interactions (citrate synthase, cytochrome-*c* oxidase, carnitine palmitoyltrans-

ferase, hexokinase, and glycogen phosphorylase) found by Houle-Leroy et al. (15).

Because mice from the selected lines exhibit a small but significant increase in body temperature (33), it is possible that thermal stress was responsible for elevated HSP72 levels in S compared with C mice. However, comparison of the increase in body temperature in S mice [0.2–0.3°C (33)] to changes induced by exercise in other studies (3–4°C) (23, 24, 34) or by whole limb or whole body heat stress (3–8°C) (18, 30) indicates that the increase in temperature experienced by S mice might not be sufficient to cause elevated HSP expression. This interpretation is supported by data from several other organs (kidney, spleen, lung, heart) that showed no difference between S and C mice for HSP72 expression but would be expected to if thermal stress occurred (9).

An increase in circulating glucocorticoid level is another systemic effect associated with selection for wheel-running propensity that may have influenced HSP72 levels. Our laboratory showed previously that S mice have elevated plasma corticosterone levels compared with C mice, whether or not they are housed with access to running wheels (Ref. 12; T. Garland, unpublished observations). However, we suspect that a causal link between plasma corticosterone levels and HSP72 expression in the mice we studied is unlikely. To our knowledge, direct induction of HSP72 by corticosterone or other glucocorticoids has not been demonstrated, and in fact cortisol administration had no effect on tissue HSP72 levels in fish (1). Furthermore, elevated glucocorticoid levels have been reported to reduce the induction of HSP72 after heat shock in fish tissues and cells (1, 2). Finally, if elevated corticosterone levels in the S mice affected the expression of HSP72, one would expect induction of HSP72 in more tissues than just skeletal muscle.

Our finding that S mice exhibited higher levels of HSP72 expression in skeletal muscle compared with C is, for the most part, consistent with results of other studies that have linked HSP72 expression with physical exercise. However, a surprising observation in the present study was that, at the level of individual variation, the amount of wheel running during the acute (Table 1, *generation 23*) exercise protocol had a statistically significant negative effect on HSP72 expression when other variables were controlled in the statistical model. The functional significance of this effect is unclear, but one possibility is mice that run more develop alternative ways to cope with exercise-induced stress beyond the protective effects of HSP72. For example, changes in other stress proteins such as mitochondrial chaperones (e.g., HSP60, GRP75) (14, 25, 35), or in enzymes involved in aerobic oxidation or antioxidant defense (38), may have occurred in triceps surae muscles of mice that ran more and thereby reduced the need to maintain high HSP72 levels. Results of studies using *Drosophila* larvae suggest that maintenance of high levels of HSPs can be deleterious with regard to growth, development, and survival to adulthood (8, 19, 21). Further research is required to determine whether the reduced expression of HSP72 in individuals that ran more resulted from mechanisms that specifically limit HSP72 levels.

Data from both the acute and chronic wheel-access mice demonstrate that the mini-muscle phenotype is associated with significantly higher expression of HSP72, and the effect was evident in the chronic wheel-access mice, whether they were housed with locked or free wheels. Mini-muscles have reduced myofibrillar and total protein content (mg/g of muscle), although they do not differ in sarcoplasmic protein content (15, 16). Thus the apparent increase in HSP72 content (which is expressed relative to total protein) of mini-muscles might reflect a reduced amount of total protein, rather than an increase in HSP72 per se. However, the magnitude of the difference in total protein content (Fig. 3 in Ref. 16) is much smaller than the difference in HSP72 expression in these muscles (compare adjusted means in Tables 3 and 4), which suggests that elevated HSP72 levels of mini-muscle are physiologically significant and not just a result of changes in total protein content. Mini-muscles also exhibit higher mass-specific activities of the metabolic enzymes citrate synthase and cytochrome-*c* oxidase (16), and thus they resemble muscles after endurance training (7). The elevated HSP72 of mini-muscles remained statistically significant in models that included a covariate of either citrate synthase or cytochrome-*c* oxidase enzyme activity (markers of mitochondrial abundance, located in the matrix and the inner mitochondrial membrane, respectively). It is therefore possible that the elevated HSP72 levels of mini-muscles represent one component of a set of adaptive changes in skeletal muscles that improve aerobic capacity and protect muscle protein from damage during exercise (24).

In conclusion, we found that artificial selection for high voluntary wheel-running activity in mice is associated with higher expression of HSP72 in triceps surae muscle when mice are allowed to run on wheels. Paradoxically, at the level of individual variation, expression of HSP72 was inversely correlated with amount of wheel running in the acute (Table 1) exposure to running wheels. For both S and C mice, HSP72 expression was elevated in individuals that displayed the mini-muscle phenotype, an effect that may promote efficient muscle function in the setting of reduced muscle mass. These results underscore the association between skeletal muscle HSP72 content and physical exercise, and they suggest that the expression of HSP72 in triceps surae of exercising mice is a complex adaptation that is not directly related to amount of exercise per se. Rather, selective breeding for voluntary wheel running behavior in mice may be associated with genetic adaptation for high HSP72 expression that may facilitate muscle function in the high-exercise lifestyle of the animals.

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