Protein Synthesis and Antioxidant Capacity in Aging Mice: Effects of Long-Term Voluntary Exercise

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ABSTRACT

Exercise increases metabolic rate and the production of reactive oxygen species (ROS) but also elevates protein turnover. ROS cause damage to macromolecules (e.g., proteins) and thereby contribute to aging. Protein turnover removes and replaces damaged proteins. The balance between these two responses may underlie beneficial effects of physical activity on aging. Effects of lifelong exercise on antioxidant enzyme activities and fractional synthesis rate of protein (FSRP) were examined at various ages (2–26 mo) in heart, liver, and muscle of mice that had been selectively bred for high wheel-running activity, housed with (S+) or without (S−) a running wheel, and their random-bred controls (C+) housed with running wheels. FSRP decreased with age and increased in muscle of young, but not old, activity-selected mice. Enzyme activity of superoxide dismutase and glutathione peroxidase decreased with age and showed a peak at 10 mo of age in liver. Selection for wheel-running activity did not affect antioxidant enzyme activity. Daily energy expenditure correlated positively with antioxidant levels in liver. This might indicate that oxidative stress (ROS production) increases with metabolic rate, driving upregulation of antioxidant enzymes. Alternatively, the elevated energy expenditure may reflect the energetic cost of elevated protection, consistent with the disposable-soma hypothesis and with other studies showing positive links between energy expenditure and life span. Long-term elevations in voluntary exercise did not result in elevations in antioxidant enzyme activities or protein synthesis rates.

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Introduction

Reactive oxygen species (ROS), such as the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•), are produced as by-products of aerobic metabolism in mitochondria and can cause damage to DNA, lipids, and proteins (Harman 1956; Tyler 1975; Davies et al. 1982; Beckman and Ames 1998; Mecocci et al. 1999). This damage to macromolecules can accumulate with age (Barja 2004) and may contribute to senescence and degenerative diseases associated with aging (e.g., cardiovascular disorders, Parkinson's disease; Melov et al. 1999; McEwen et al. 2005; Wallace 2005). An elaborate defense system consisting of endogenous antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and numerous nonenzymatic antioxidants, including vitamins A, E, and C, glutathione (GSH), ubiquinone, melatonin, and flavonoids, exists to scavenge ROS and thereby prevent deleterious effects (Beckman and Ames 1998). A small amount of the ROS produced escape conversion and can still damage macromolecules. As a second line of defense, damaged macromolecules can be repaired or replaced, as occurs, for instance, by protein turnover, which involves the removal (breakdown) and replacement (synthesis) of inactive or oxidized proteins in the cell. Protein turnover therefore plays a potentially vital role in the prevention of aging (Ryazanov and Nefsky 2002; Sohal 2002; Yarasheski 2003).

Relationships between antioxidant enzyme activity, protein turnover, and energy metabolism have been studied by experimentally increasing metabolic rate, for example, by increasing physical activity. Although some results are contradictory, it is widely accepted that regular physical activity leads to an increase in energy demands, ROS production, and the activities of antioxidant enzymes, especially in muscle (reviewed by Ji [1999]). If the increase in antioxidant defenses in response to exercise is greater than the increase in ROS production, then this would lead to better protection against oxidative damage. Exercise generally also has a stimulatory effect on protein synthesis rate, specifically in skeletal muscle (in rats: Hayase and Yokogoshi 1992; Katzeff et al. 1994; Mosoni et al. 1995; Hernandez et al. 2000; in humans: Rennie et al. 1981; Chesley et al. 1992; Biolo et al. 1995; Phillips et al. 1997; Sheffield-Moore et al. 2004;

Short et al. 2004). Maintaining high levels of antioxidant enzyme activity and high rates of protein synthesis in old age would diminish the accumulation of damaged proteins and increase cell survival. For example, protein turnover and antioxidant enzyme activity have both been shown to increase in calorically restricted animals, a nutritional condition that increases life span in several species, including mice (Weindruch et al. 1986) and rats (McCay et al. 1935); for a recent review, see Masoro (2006). Therefore, aging may be ameliorated by mechanisms, such as exercise, that elevate protein turnover and/or antioxidant enzyme activity.

Mice from lines selectively bred for high wheel-running activity for 31 generations, housed with or without a running wheel, and their random-bred control lines, housed with wheels (Swallow et al. 1998), were used to study effects of lifelong voluntary exercise, as well as genetic selection for high activity, on antioxidant enzyme activities and protein turnover. Although the effects of exercise on antioxidant systems and protein synthesis are often compared between young and old subjects, the influence of exercise throughout life on oxidant systems and the relationship between metabolic rate and antioxidant activity on an individual level have not been well studied. The main aim of this study was to test whether long-term exercise, which increases energy expenditure, induces compensatory changes in antioxidant enzyme activities and protein synthesis rates and whether genetic selection for high wheel-running activity has resulted in mice with enhanced antioxidant protection. In addition, we explored the relationship between energy metabolism and antioxidant enzyme activity at an individual level.

Material and Methods

Animals and Housing

Male laboratory house mice from lines that had been selectively bred for high wheel-running activity for 31 generations and their random-bred controls were used in the experiments (Swallow et al. 1998). Eighty breeding pairs of mice from the lab of T. Garland were used as the basis for a colony at the University of Groningen. In the original selection protocol (Swallow et al. 1998), eight separate lines were created (four control and four selected) by breeding randomly (control) or selecting the highest-running (in revolutions per day) male and female of each family for breeding (selected lines). Mice from each of the eight lines were used in our experiments, although the lines were not equally represented.

Mice were housed with three litter mates from weaning until they were 5 mo old, after which they were housed individually (Macrolon type II long cages, UNO Roestvaststaal, Zevenaar, Netherlands) with wood shavings as bedding material and a running wheel (cages were adapted to fit a plastic running wheel with a 7-cm radius; Vaanholt et al. 2007). Food (standard rodent chow RMH-B [2181], Hope Farms, Woerden, Netherlands) and water were provided ad lib, and animals were on a 12L : 12D cycle. Three experimental groups were created: control mice (C+) and selected mice (S+), both individually housed with a running wheel from the age of 5 mo, and selected mice individually housed without a running wheel from weaning (S−). Mice were killed at four ages after their metabolic rates had been measured over a 24-h period (see below): 2, 10, 18, and 26 mo. At 2 mo of age, animals were not yet housed with a running wheel, and only the comparison between control and selected mice can be made. All procedures concerning animal care treatment were in accordance with the regulations of the ethical committee for the use of experimental animals of the University of Groningen (licenses DEC 2777(-1) and 4184A).

Wheel-running activity of control and selected mice was measured throughout life in a large population of mice (~80) from the same generation of breeding as the mice in this experiment. These mice were housed in the same room and in similar cages.

Tissue Collection

At each age, five to eight mice per group were briefly anesthetized with CO_2 and then killed by decapitation. Animals were dissected, and biopsies of hindlimb muscle (only at 2 and 26 mo), liver, and heart were immediately frozen in liquid nitrogen and stored at −80°C for antioxidant enzyme measurements.

Protein synthesis was assessed only in 2- and 26-mo-old animals. The 26-mo-old individuals were the same animals used for the antioxidant measurements, but for the 2-mo-old group, different animals were used ($n = 8$ per group). For logistic reasons, this group contained only one control (lab designation is line 2) and one selected line (line 7). Food intake and body mass were measured for two consecutive days before the harvesting of tissues for protein synthesis measurements. Protein synthesis was measured using the large-dose method as described by Garlick et al. (1980). Mice were given an intraperitoneal injection of 150 mM $^2H_5$-phenylalanine (1.5 mL per 100-g animal). After 15 min, the mice were anesthetized with CO_2 and then decapitated. Trunk blood was collected in prechilled tubes, with heparin as anticoagulant. Blood samples were centrifuged at 2,600 *g* at 4°C for 15 min, and the plasma was collected and stored at −80°C until analysis. Liver and hindleg muscle were rapidly removed, weighed to the nearest 10^{-4} g, rinsed in ice-cold saline, frozen in liquid nitrogen, and stored at −80°C until analysis. Exact times (nearest second) of injection and freezing of tissues were recorded.

Protein Synthesis

Free and protein-bound enrichments of phenylalanine in liver and muscle tissues were quantified as described by Wester et al. (2004). Approximately 300 mg of frozen tissue was homogenized on ice in 3 mL 7% (w/v) sulphosalicylic acid (SSA). Free phenylalanine was separated from protein-bound phenylalanine by centrifugation at 1,000 *g* at 4°C for 15 min, and the supernatant was retained. The pellet was then washed three

times with 3 mL 7% SSA to remove free phenylalanine. The initial supernatant fraction (free pool) was passed through a 0.4-mL column of Dowex AG 50W-X8 (100–200 mesh), and the resin was rinsed twice with 3.5 mL water before the phenylalanine was eluted with 2 mL 2 M NH_4OH and 1 mL water. The eluate was freeze-dried and stored at −20°C for later analysis. Half of the washed pellet (protein-bound pool) was transferred to an 8-mL screw-topped Pyrex hydrolysis tube and solubilized in 1 mL 0.3 M NaOH for 30 min. A few phenol crystals were added, and the sample was hydrolyzed by adding 7 mL 4 M HCl and heating on a dri-block at 110°C for 18 h. Hydrolysates were dried under vacuum, resuspended in 1 mL 0.5 M sodium citrate (pH 6.2), and stored at −80°C until later analysis.

For the plasma samples, 150 μL was treated with 150 μL 15% SSA and centrifuged at 1,000 *g* at 4°C for 10 min, and 150 μL of the supernatant was passed through a 0.2-mL column of Dowex AG 50W-X8. Elution conditions and subsequent treatments were similar to those for the tissue free-pool samples.

Stable-isotope enrichments of the tissue and plasma free pools were measured by gas chromatography mass spectrometry (GC/MS) after conversion to the tertiary-butyldimethylsilyl (TBDMS) derivatives (Calder and Smith 1988). In the hydrolyzed samples (protein-bound pool, low enrichment), phenylalanine was converted to phenylethylamine by enzymatic decarboxylation before formation of the TBDMS derivative. This was separated by capillary column gas chromatography, and enrichments obtained from electron impact ionization–selective ion monitoring (EI-SIM) mass spectrometry were used to detect the TBDMS derivate of phenylethylamine (Calder et al. 1992; Slater et al. 1995).

The fractional synthesis rate of protein (FSRP, percent per day) was calculated using the equation $FSRP = 100 \times (BP/FP) \times 1{,}440/t$, where BP is the bound pool of phenylalanine in mole percent excess (MPE), FP is the MPE of the free pool of phenylalanine measured in either plasma or tissue, and t is the time (in min) between injection of phenylalanine and freezing of the tissue. The ratio between FPs measured in plasma and liver or muscle was calculated. The ratio was 1.02 ± 0.03 (mean ± SD) in muscle and 1.00 ± 0.03 in liver. Neither ratio was significantly different from unity. From these data, we concluded that the injected phenylalanine effectively equilibrated between tissue and plasma and remained so until time of death. In consequence, the FSRP reported represents the FSRP calculated based on plasma free phenylalanine as representative of the precursor pool.

Antioxidant Enzyme Activities and Protein Content

Before enzyme activity determinations, tissue samples were homogenized by sonication in 20 volumes of ice-cold 50-mM phosphate buffer. After centrifugation (25 min at 3,000 *g*), the supernatant fraction was collected, divided over several tubes, and stored at −80°C for enzyme activity and protein measurements.

Total SOD activity was determined at 25°C by the inhibition of the auto-oxidation of pyrogallol by SOD in the supernatant, following the method of Marklund and Marklund (1974). The reaction was followed spectrophotometrically at 420 nm in the following reaction mixture: 50 mM Tris-DTPA buffer, 15 μL supernatant, and 15 μL pyrogallol in a total volume of 800 μL. Each triplicate measurement was preceded by a blank, containing only pyrogallol in Tris-DTPA buffer. One unit of SOD was defined as the amount of enzyme causing 50% inhibition of pyrogallol auto-oxidation.

GPx activity was determined at 25°C via the oxidation of NADPH in the presence of reduced glutathione (GSH) and H_2O_2 (combining the assays of Paglia and Valentine [1967], and Lawrence and Burk [1976]). The following reaction mixture was used: 4.28 mM sodium azide (to block catalase activity), 1.07 mM EDTA, 4.286 mM GSH, 0.214 mM NADPH, and 1 U mL^{-1} GR in ice-cold 50-mM phosphate buffer. Twenty-five microliters of H_2O_2 and 25 μL of sample were added to the reaction mixture. Reactions were followed spectrophotometrically at 340 nm in a total volume of 700 μL. To correct for spontaneous oxidation reactions independent of GPx, blanks without H_2O_2 were measured and subtracted from the assay values. One unit of GPx was defined as the amount of enzyme that oxidized 1 μmol of NADPH per minute in the presence of reduced glutathione. Protein content of the supernatant fraction was determined using a Bradford assay (Quick Start Bradford protein essay kit 2; Biorad Laboratories, Veenendaal, Netherlands).

Indirect Calorimetry

Before each animal was killed, resting metabolic rates (RMRs) and daily energy expenditure (DEE) were measured by respirometry (Arch et al. 2006), for the same individuals for which antioxidant enzyme activity was measured, in an eight-channel indirect calorimetry system (described previously by Oklejewicz et al. [1997]). The mice were put in flow-through chambers, where oxygen consumption ($\dot{V}o_2$, L h^{-1}) and carbon dioxide production ($\dot{V}co_2$, L h^{-1}) were measured simultaneously with ambient temperature and activity (passive infrared detectors). Oxygen and carbon dioxide concentrations of dried inlet and outlet air (drier: 3-Å molecular sieve, Merck) from each chamber were measured with a paramagnetic oxygen analyzer (Servomex Xentra 4100) and an infrared gas analyzer (Servomex 1440), respectively. The system recorded the differentials in oxygen and carbon dioxide between dried reference air and dried outlet air from the metabolic cages. The flow rate of inlet air was measured with a mass-flow controller (Type 5850 Brooks). Computerized data were collected every 10 min. All mice were measured for 24 h at an ambient temperature of 22°C. Oxygen consumption was calculated according to equation (2) of Hill (1972), to correct for volume changes with a respiratory quotient below 1, and is expressed in standard tem-

perature and pressure. Metabolic rate (MR, kJ h^{-1}) was estimated using the equation $MR = 16.18 \times \dot{V}o_2 + 5.02 \times \dot{V}co_2$ (Romijn and Lokhorst 1961). RMR (kJ h^{-1}) was defined as the lowest value of MR calculated from cumulative means every 30 min (Vaanholt et al. 2007). DEE (kJ h^{-1}) was calculated as the average MR during the entire 24-h measurement period.

Statistical Analysis

Results are reported as mean ± SEM. To test for effects of treatment and/or age, we applied general linear models (GLMs) in SPSS for Windows (ver. 15.0). Group, age, and group × age were fixed factors. Where appropriate, covariates were included in the models (e.g., body mass for MRs and food intake). Because of relatively small sample sizes in some of the eight lines, line was not treated as a random effect nested within the selected or control groups. One significant outlier was identified during testing for protein synthesis in muscle (see Table 2). This data point was removed before final statistical analysis. The significance level was set at $P \leq 0.05$, and all tests were two-tailed.

Results

Wheel-Running Activity

Figure 1 shows average wheel-running activity at 10, 18, and 26 mo of age in a large group of mice ($n \sim 40$ per group) housed under the same circumstances as our experimental mice. Wheel-running activity was higher in selected mice than in control mice at all ages. The difference between the groups diminished with age, and at 26 mo of age, the difference was no longer statistically significant.

Body Mass, Food Intake, and FSRP

At both 2 and 26 mo, C+ mice had greater body mass than S− mice, and body mass increased with age in both groups (see Table 1; post hoc Tukey: $P = 0.025$). At 26 mo of age, S+ mice were also smaller than S− mice ($P = 0.04$). Interestingly, food intake did not significantly differ between groups or vary with age.

FSRP decreased by approximately 15% with age in liver and muscle of C+ and S+ mice. This effect was significant in liver but not in muscle (Table 1). In liver, no significant effect of group or group × age interaction was found. In muscle, overall there was no group effect ($P = 0.09$), but at 2 mo of age, FSRP was significantly higher in muscle of S+ than in that of C+ mice (*t*-test, $P < 0.01$), whereas no difference was found in FSRP between groups at 26 mo of age. Food intake can influence protein synthesis, and therefore we added food intake to the models as a covariate. In none of the models was food intake a significant covariate.

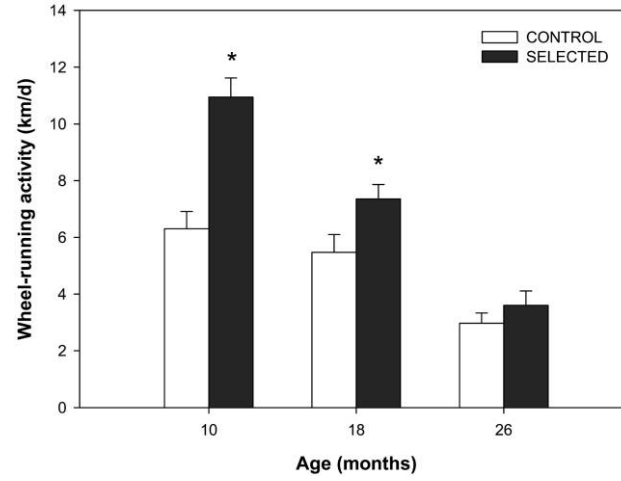

Figure 1. Wheel-running activity in control and activity-selected mice at various ages. An asterisk indicates significant differences between control and selected mice ($P < 0.05$).

Antioxidant Enzyme Activity and MR

The age-related development of antioxidant enzyme activities in the liver (SOD and GPx) and heart (SOD) are shown in Figures 2 and 3, respectively. In the liver, antioxidant enzyme activities (GPx and SOD) varied considerably with age (see Table 2) and were highest at 10 mo of age (post hoc Tukey; at 10 mo, SOD activity was significantly increased compared to that at the other time points; $P < 0.05$). An effect of age on SOD activity in heart tissue was also found (Table 2). A similar pattern in liver, with a peak at 10 mo, can be seen in control mice (see Fig. 3); however, post hoc tests showed a significant difference between SOD activity only at 2 and 26 mo (Tukey; $P < 0.05$), indicating a decrease in SOD activity with age in heart. There was no effect of group, indicating that neither the presence of a wheel (S− vs. S+) nor selection for high wheel-running activity (S+ vs. C+) affected antioxidant activities in liver or heart (Table 2). SOD activity in muscle was measured only at 2 and 26 mo. There was no effect of either age or group (Fig. 4; Table 2).

RMR (kJ g^{-1} d^{-1}) and DEE (kJ g^{-1} d^{-1}) were measured in all animals at all ages. Overall, mass-specific RMR was 1.20 ± 0.26 (mean ± SD), 1.31 ± 0.25, and 1.17 ± 0.15 in C+, S+, and S− mice, respectively. S+ mice thus had a slightly higher RMR (by ~10%) than the other groups, but this difference was not significant ($P > 0.05$; GLM with body mass as covariate). Mass-specific DEE was, on average, 1.53 ± 0.31, 1.82 ± 0.35, and 1.60 ± 0.34 for C+, S+, and S− mice, respectively (~15% higher in S+ mice). Differences in DEE were slightly larger at the young ages, but overall no significant effect of group on DEE was found.

RMR and DEE were added to the models as covariates to test whether they affected antioxidant enzyme activity. In liver, but not heart, DEE significantly predicted both SOD ($P = 0.004$) and GPx ($P = 0.05$) enzyme activity. RMR also signif-

Table 1: Effects of selection for high locomotor activity on body mass, food intake, and fractional synthesis rate of protein (FSRP) in liver and muscle

	2 mo		26 mo			*P*		
Variable	C+	S+	C+	S+	S−	Group	Age	G × A
N	8	8	8	5	7			
Age (d)	73 ± 1	72 ± 2	781 ± 4	781 ± 5	787 ± 6			
Body mass (g)	33.9 ± 1.5	30.0 ± 1.5	41.4 ± 1.5	37.9 ± 1.9	40.4 ± 2.1	.11	.001*	.90
Food intake (g d^{-1})	4.1 ± .4	3.7 ± .3	3.8 ± .5	3.0 ± .2	4.2 ± .2	.14	.31	.53
FSRP liver (% d^{-1})	69.3 ± 2.6	69.6 ± 2.6	56.7 ± 2.6	63.4 ± 3.3	56.4 ± 1.9	.29	.001*	.24
FSRP muscle (% d^{-1})	5.1 ± .5	7.1 ± .5	4.9 ± .5	5.2 ± .6	5.4 ± .6	.09	.064	.14

Note. Results for general linear model with factors of group, age, and group × age (G × A) are given, in addition to means ± SEM for all groups. For analysis of food intake, body mass was included in the model as a covariate. See "Body Mass, Food Intake, and FSRP" regarding analyses of FSRP with food intake as a covariate. Food intake data for two mice were missing. n = sample size.

* Significant results ($P \leq 0.05$).

icantly predicted SOD ($P = 0.029$) activity but not GPx ($P = 0.12$) activity. Including RMR or DEE in the models did not alter the effects of the treatment variables on antioxidant enzyme activities. Figure 5 shows the relationship between DEE and hepatic SOD activity for C+ (*left*) and S+ (*right*) mice. In both groups, a positive relationship between DEE and antioxidant enzyme activity was found at all ages. Similar results were found for the relationships between RMR and SOD activity and between DEE and GPx activity in liver (data not shown). In heart and muscle, MR did not predict SOD activity.

We calculated an index of the potential protection against ROS for each individual mouse by dividing hepatic SOD enzyme activity by DEE. Average protection of C+, S+, and S− mice was 4.6 ± 1.4, 4.5 ± 1.2, and 4.6 ± 1.5 U mg $protein^{-1}$ kJ^{-1} d^{-1}, respectively. These values did not differ significantly.

Discussion

Age had a strong effect on FSRP and antioxidant enzyme activity in both control mice and mice that had been selectively bred for high wheel-running activity. On average, FSRP decreased by approximately 16% between 2 and 26 mo of age in both liver and muscle. The literature on this subject is ambiguous. Most studies have shown an age-related decrease in muscle FSRP (Lewis et al. 1985; Rattan 1996; Dorrens and Rennie 2003), but other reports claim no difference (Volpi et al. 2001; Sheffield-Moore et al. 2005). Discrepancies between studies can have many possible explanations, such as tissue (muscle type) used, sex, diet, and activity level of subjects. In addition to effects on FSRP, age affected antioxidant enzyme activity of SOD and GPx in mouse liver and heart. In liver, peak enzyme activity occurred at 10 mo of age with a subsequent decline, while in heart, SOD enzyme activity declined between 2 and 26 mo. There was no effect of age on SOD activity in muscle, although with the sampling protocol used (measuring only at 2 and 26 mo of age), intermediate changes may have been missed. Conflicting data exist on the effects of age on antioxidant enzyme activities, and comparisons across studies are complicated by the use of different species, ages selected, and organs studied. Most studies have measured antioxidant enzyme activity at only two ages and found increases, decreases, or no differences in antioxidant enzyme activities in various tissues (Rao et al. 1990; Sohal et al. 1990; Gunduz et al. 2004;

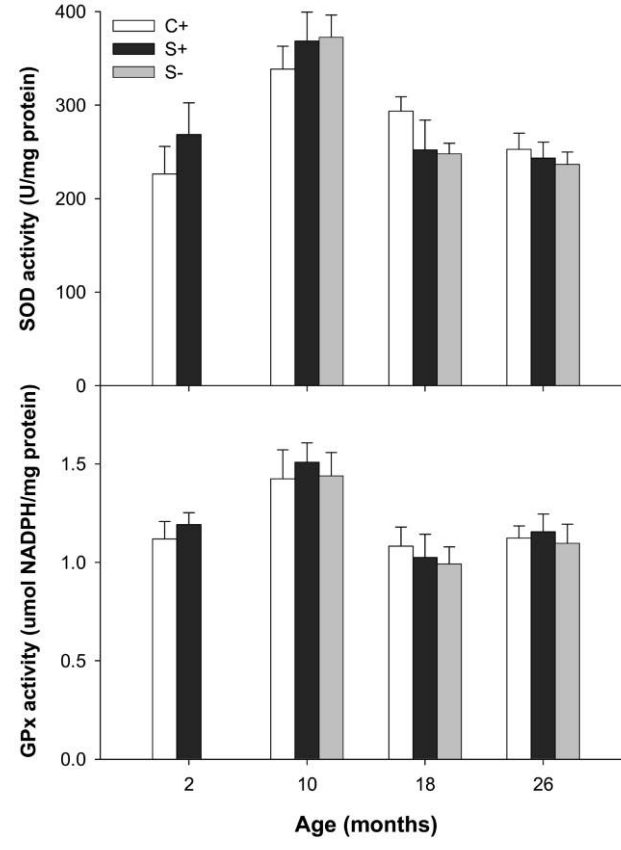

Figure 2. Superoxide dismutase (SOD; *top*) and glutathione peroxidase (GPx; *bottom*) activity in liver of mice selected for high wheel-running activity and their random-bred controls at different ages. One unit of SOD is defined as the amount of enzyme that causes 50% inhibition of pyrogallol auto-oxidation. One unit of GPx is defined as the amount of enzyme that oxidizes 1 μmol NADPH min^{-1} in the presence of reduced glutathione.

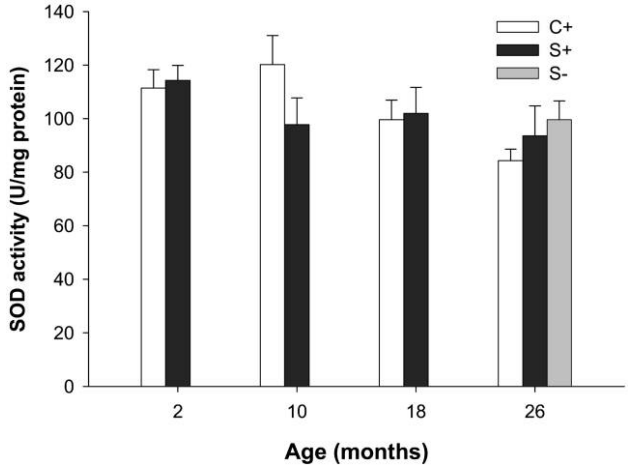

Figure 3. Superoxide dismutase (SOD) enzyme activity in heart of mice selected for high wheel-running activity and their random-bred controls at different ages. One unit of SOD was defined as the amount of enzyme that causes 50% inhibition of pyrogallol auto-oxidation.

Kakarla et al. 2005). Antioxidant enzyme activity has been measured in C57BL/6J mice at 3, 11, 19, and 27 mo of age, and a similar peak in SOD activity in heart and liver was found at 11 mo of age (L. M. Vaanholt, personal observation). In rats, SOD and CAT enzyme activity in the brain at five ages also followed a pattern in antioxidant enzyme activity similar to that shown here, with a peak in activity at 12 mo of age (Tsay et al. 2000). These results highlight the importance of measuring antioxidant enzyme activities at various ages. Doing this might resolve discrepancies in existing data.

Numerous studies have shown oxidative stress and oxidative damage to macromolecules to increase with age (for a review, see Beckman and Ames 1998). The decrease in antioxidant enzyme activity and protein synthesis rates we observed here may explain these effects. What causes the decrease in antioxidant enzyme activity and FSRP is unclear. It may be related to the impairment of mitochondrial function with age. Dysfunctional mitochondria have decreased rates of electron transfer by the selectively diminished activities of complexes I and IV, and this causes increases in oxidative stress (for a review, see Navarro and Boveris 2007). If antioxidants and enzymes necessary for protein synthesis become damaged (and nonfunctional) by oxidants with age, this will lower their activity and subsequently the defense against ROS, which will then further increase oxidative stress and could result in a vicious circle (Ryazanov and Nefsky 2002).

In agreement with numerous other studies (Chesley et al. 1992; Hayase and Yokogoshi 1992; Hernandez et al. 2000; Short et al. 2004) that showed increased muscle FSRP in response to exercise, we found an increase in muscle FSRP in 2-mo-old mice that had been selectively bred (S+), compared to randomly bred control mice (C+). At this age, the activity of the mice was not measured because both groups were still housed without a running wheel. In a similar study, however, where animals were housed in a cage with a locked running wheel, overall activity recorded by passive infrared sensors was found to be increased by 130% in selected mice at 2 mo of age (L. M. Vaanholt, T. Garland Jr., and G. H. Visser, unpublished data). Therefore, we can assume that activity levels were also increased in the 2-mo-old mice in our study. This is supported by the observed increase seen in muscle FSRP. Several studies have shown that exercising at old ages still induces an increase in FSRP (Yarasheski et al. 1993; Sheffield-Moore et al. 2004; Short et al. 2004). We did not find a difference in muscle FSRP between 26-mo-old C+, S+, and S− mice. As shown in Figure 1, the difference in activity between control and activity-selected mice decreased with age (see also Morgan et al. 2003; Bronikowski et al. 2006), and in old animals (26 mo), wheel-running activity no longer differed significantly between C+ and S+ mice. This would explain why protein synthesis was not different between these groups at that age. FSRPs also did not differ between sedentary and active selected mice (S− vs. S+); a more intensive workout at old age may thus be necessary to evoke increases in protein synthesis rates. These findings also suggest that prior exercise history has no long-term effect on muscle FSRP. In liver, selection for activity did not affect FSRP. Previous studies have reported both increased (Mosoni et al. 1995) and decreased (Hayase and Yokogoshi 1992) hepatic protein synthesis in response to exercise, and the effects remain unclear.

Selective breeding for high wheel-running activity has previously been shown to result in a shortened life span in male mice of the sixteenth generation of breeding (Bronikowski et al. 2006). In our mice of the thirty-first generation of breeding, similar results were found: median life span was 826 ± 24 d in C+ mice, compared to 735 ± 28 d in S+ mice (L. M. Vaanholt, S. Daan, T. Garland Jr., and G. H. Visser, unpublished

Table 2: General linear model (GLM) on effects of group and age on antioxidant enzyme activities

		Group			Age			G × A	
Trait	*N*	df	*F*	*P*	df	*F*	*P*	*F*	*P*
SOD liver	74	2, 63	.08	.92	3, 63	12.2	.001*	.1	.88
GPx liver	71	2, 60	.3	.78	3, 60	9.1	.001*	.1	.98
SOD heart	62	2, 53	.5	.62	3, 53	3.3	.029*	1.3	.27
SOD muscle	34	2, 29	.5	.85	3, 29	.9	.36	1.2	.29

Note. GLMs included group, age, and group × age (G × A) as fixed factors. To test for relationships between resting metabolic rate (RMR, kJ d^{-1}) or daily energy expenditure (DEE, kJ d^{-1}) and enzyme activity, both were added to the model as covariates separately (data not shown; see "Antioxidant Enzyme Activity and MR"). Sample sizes were $n = 8$ for 2 mo; $n = 8$, 5, and 5 for C+, S+, and S− mice, respectively, at 10 mo; $n = 8$, 7, and 7 for the three groups at 18 mo; and $n = 8$, 5, and 5 for the three groups at 26 mo. In three mice, sample volume of liver was too small to measure both superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activity, reducing the sample sizes for these measurements. Also, in heart, SOD activity in S− mice was measured only at 26 mo of age. *N* represents the total sample size and df the degrees of freedom.

* Significant effects ($P \leq 0.05$).

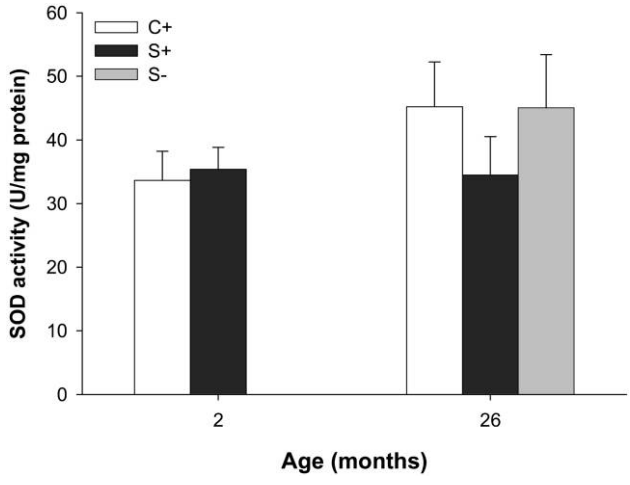

Figure 4. Superoxide dismutase (SOD) enzyme activity in muscle of mice selected for high wheel-running activity and their random-bred controls at different ages. One unit of SOD was defined as the amount of enzyme that causes 50% inhibition of pyrogallol auto-oxidation.

manuscript). MR or protection against oxidative stress, as measured in this study, did not differ significantly between both groups of mice and thus cannot explain these differences in life span. Other factors (i.e., effects of selection) must be involved.

Exercise (S+ vs. S−) or selection for activity (S+ vs. C+) did not affect antioxidant enzyme activities in liver, heart, or muscle. This is in contrast to a study by Gunduz et al. (2004) comparing antioxidant enzyme activity in rats that underwent 1 yr of forced swimming exercise with that in sedentary controls. In that study, exercising rats had increased antioxidant enzyme activity in heart and liver relative to sedentary controls (Gunduz et al. 2004). Animals in our study exercised voluntarily, and the difference in activity between the groups may not have been large enough to observe effects on antioxidant enzyme activities. In agreement with this, Selman et al. (2002) compared antioxidant enzyme activity in heart, liver, and muscle of sedentary and voluntarily exercising voles and also observed no differences. Another study on voluntarily exercising male rats has shown that GPx and CAT activities were not altered, while SOD activity increased in response to exercise (Yamamoto et al. 2002). In voluntarily exercising female rats, increases in both SOD and CAT in liver of young (3 mo) and old (12 mo) animals were found (Kakarla et al. 2005). Female rats voluntarily undergo up to 10-fold more wheel-running activity than males (Yamamoto et al. 2002), which might explain the discrepancy between studies. In agreement with this, in the activity-selected lines used here, females ran approximately 25% more than males (Morgan et al. 2003), and hepatic antioxidant mRNA expression (SOD2 and CAT) at 20 mo of age was different for the females selected for activity compared with controls but not for males (Bronikowski et al. 2002).

MR measured by indirect calorimetry was slightly, but not significantly, increased in S+ mice compared to C+ and S− mice. A previous study did show a significant increase in DEE in activity-selected mice compared to controls (Vaanholt et al. 2007). In our experiment, animals did not have access to running wheels in the respirometer, which may have resulted in an underestimation of the DEE in the home cage. On an individual level, both RMR and DEE positively correlated with liver antioxidant activity at all ages (see Fig. 5). One interpre-

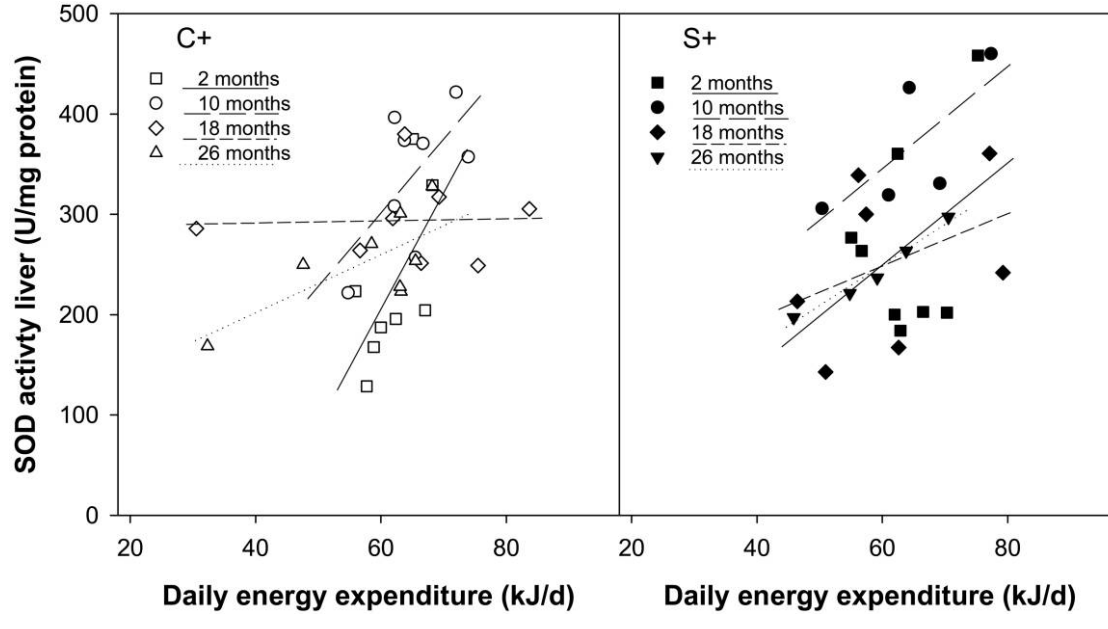

Figure 5. Relationship between daily energy expenditure and superoxide dismutase (SOD) activity in liver of C+ (*left*) and S+ mice (*right*) at various ages.

tation of these data is that individual mice with a higher MR protected themselves against increased ROS production due to the elevated metabolism by increasing their antioxidant enzyme activity. However, this interpretation assumes that the production of ROS is directly proportional to energy expenditure. More recent studies have suggested that the links between energy metabolism, ROS production, and aging are substantially more complex than this simple interpretation allows (Brand 2000; Speakman et al. 2004; Speakman 2005). In particular, individuals with higher MR within mammalian species have been shown to live longer in both mice (Speakman et al. 2004) and dogs (Speakman et al. 2003), while no link has been observed in *Drosophila* (Hulbert et al. 2004; Van Voorhies et al. 2004). Moreover, during caloric restriction, mass-specific levels of energy expenditure are either unchanged (McCarter et al. 1985; Greenberg and Boozer 2000) or elevated (Selman et al. 2005) at the same time that ROS production is reduced. The positive relationship between antioxidant enzyme activity and MR observed here could be explained by the disposable-soma theory (Kirkwood 1992, 2005). This theory suggests that protecting the soma is costly and hence that animals must trade off this cost against other demands, such as reproduction. The elevated MR in mice with high levels of antioxidant enzymes might then reflect the direct energetic costs of increased protection.

In conclusion, protein synthesis and antioxidant enzyme activity decreased with age in both control and activity-selected male mice. This may explain increases in oxidative stress and damage to macromolecules that are generally observed with age. Several studies have shown that exercise has a beneficial effect on aging (i.e., it increases median life span; Holloszy 1988; Navarro et al. 2004; Bronikowski et al. 2006), and beneficial effects of exercise on protein turnover rates and/or antioxidant enzyme activities could underlie this effect. Upregulation of these systems would diminish the damage caused by ROS (which are produced in parallel with MR under certain conditions) and could thereby increase median life span. In practice, we did not show any major effects of long-term voluntary exercise or of selective breeding for high activity on either trait in our mice. We did show a positive relationship between energy metabolism and protection against oxygen radicals at the individual level, possibly reflecting the "costs" of protection predicted by the disposable-soma theory.

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