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Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart

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Bronikowski, A. M., P. A. Carter, T. J. Morgan, T. Garland, Jr., N. Ung, T. D. Pugh, R. Weindruch, and T. A. Prolla. Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart. *Physiol Genomics* 12: 129–138, 2003. First published November 12, 2002; 10.1152/physiolgenomics.00082.2002.—We present the first quantitative gene expression analysis of cardiac aging under conditions of sedentary and active lifestyles using high-density oligonucleotide arrays representing 11,904 cDNAs and expressed sequence tags (ESTs). With these data, we test the hypothesis that exercise attenuates the gene expression changes that normally occur in the aging heart. Male mice (*Mus domesticus*) were sampled from the 16th generation of selective breeding for high voluntary exercise. For the selective breeding protocol, breeders were chosen based on the maximum number of wheel revolutions run on *days 5* and *6* of a test at 8 wk of age. For the colony sampled herein, mice were housed individually over their entire lifetimes (from weaning) either with or without access to running wheels. The hearts of these two treatment groups (active and sedentary) were assayed at middle age (20 mo) and old age (33 mo). Genes significantly affected by age in the hearts of the sedentary population by at least a 50% expression change (n = 137) were distributed across several major categories, including inflammatory response, stress response, signal transduction, and energy metabolism. Genes significantly affected by age in the active population were fewer (n = 62). Of the 42 changes in gene expression that were common to both treatment groups, 32 (72%) displayed smaller fold changes as a result of exercise. Thus exercise offset many age-related gene expression changes observed in the hearts of the sedentary animals. These results suggest that adaptive physiological mechanisms that are induced by exercise can retard many effects of aging on heart muscle at the transcriptional level.

aging; artificial selection; exercise; microarray; *Mus domesticus*; stress/inflammation response

EXERCISE HAS BEEN SHOWN to improve overall health (e.g., increased cardiovascular performance; Ref. 5) and to offset the risk of age-related disease (2). However, in rodents, the impact of exercise on lifespan is primarily on median, not maximum lifespan (15, reviewed in Ref. 27). This observation suggests that although exercise is beneficial, it may not retard aging at the molecular level. In fact, there is a potential cost of exercise in terms of cellular oxidative damage, particularly in the heart (17). Recently developed techniques for high-throughput microarray analyses of transcripts have advanced the mechanistic study of aging and are promising tools for evolutionary studies of aging and rate-of-senescence interventions. Experiments have begun to reveal global patterns of age-related gene expression changes in thousands of genes in multiple tissues and the effects of genetic and nutritional interventions (e.g., 23). Exercise has been unequivocally associated with a slowing of age-specific mortality increases in rats, and thus with increased median lifespan (14, 15). However, because of increased oxygen consumption and metabolism during exercise, exercise is associated with an increase in the production of reactive oxygen species (ROS) and upregulation of antioxidant defenses during bouts of exercise in rodent skeletal muscle, heart, and liver (e.g., 17, 18). If this results in an increase in oxidative damage to cellular components, then exercise would be detrimental to health and lifespan. Alternatively, exercise may retard the aging process through assorted beneficial physiological consequences or by inducing an adaptive response that results in a net decrease in oxidative damage. In any case, the lack of molecular markers of the aging process has prevented an evaluation of the impact of this intervention at the molecular level of target tissues.

Here we present heart gene expression profiles from both active and sedentary middle- and old-aged house mice that have been selectively bred for 16 generations for high, early age voluntary exercise on running wheels. Because these mice run at relatively high levels for most of their lifespan, they represent a novel opportunity to study the effects of voluntary exercise. We ask: *1*) What changes in gene expression occur late in life relative to mid-life in a sedentary lifestyle? *2*)

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How does lifelong exercise affect these expression changes? and *3*) What are the genes affected by age in the population of mice that have voluntarily exercised their entire lifetimes? We discuss our findings in relation to recent reports implicating insulin signaling and the stress and inflammation responses in the aging process and in relation to the survival patterns and exercise levels in these unique lines of mice.

MATERIALS AND METHODS

Mouse colony. Mice in this report were members of a colony derived from a laboratory selective-breeding population designed to study the evolutionary correlates of exercise behavior (41). The selection colony was begun from outbred genetically heterogeneous Institute for Cancer Research mice (*Mus domesticus*) purchased from Harlan Sprague Dawley (Indianapolis, IN; Building 202, Barrier A). To study the health effects of voluntary wheel-running on lifetime measures of exercise, health, and survival, the population in this report was produced from second litters of the *generation 15* breeders for *generation 16* of the selection colony. At *generation 15*, the divergence between selection and control males in running behavior was 150% at 2 mo of age (i.e., the selection test age); control males averaged 4.5 km/day, whereas artificial selection males averaged 11.3 km/day (7). The artificial selection experiment maintains a replicated design. There are four closed selection lines and four closed control lines (i.e., randomly bred with respect to wheel running). Ten families are used to propagate each line. Five breeding pairs from each of the four selectively bred lines and four randomly bred lines were mated to produce 20 families from each selection history; details of aging colony design may be found in Refs. 8 and 32. Four experimental groups were initiated with four males and four females from each of these 40 families: selectively bred active (with wheels) and sedentary (without wheels), and control active and sedentary. Thus each of these four treatment groups contained 40 males and 40 females. In this report, we sampled male (nonsibling) mice from the selectively bred treatment groups (active and sedentary) at two ages.

In the colony sampled herein, housing with and without access to running wheels was begun when mice were 28 ± 3 days old in the mouse facility at Washington State University (WSU); mice were housed under the following conditions from weaning until natural death. Mice in the active group were placed individually (at weaning) in cages with a 10-cm radius running wheel and electronic wheel-revolution counter built into the cage top. The mouse thus had the option of voluntarily getting into the wheel and running. On the same day, mice designated to be in the sedentary group were placed individually in standard rodent cages of the same dimensions. Thus each "sedentary" mouse was free to move about its cage, but did not have access to a running wheel, whereas each "active" mouse was housed from weaning through death with access to a running wheel. Body mass and number of running revolutions were recorded weekly, the latter by an automated magnetized counter. Mice were checked daily; water and food (Harland Teklad rodent diet W 8604) were available ad libitum. Apparent food consumption was determined weekly by subtracting the amount eaten from the amount offered. Extra siblings from all 40 families were maintained as sentinel mice for specific pathogen screens. All screens were negative until an exposure to mouse hepatitis virus (MHV) at 24 mo post start date (through a barrier breakdown). No treatment was initiated for the virus. However, no necropsies indicated either MHV or liver damage as cause of death (performed by the veterinarians of Laboratory Animal Resource Center at WSU). Necropsies also included the examination of all other organs. Cage bottoms were cleaned once every 2 wk, wheels were cleaned once every 4 wk, and clean wheels were randomly assigned to active mice; this ensured no consistent effect on individual mice of any differences in mechanical resistance among wheels. Mean and maximum lifespan are reported in RESULTS.

Animals and tissues. At 20 and 33 mo of age, eight male selectively bred mice were randomly (conditional on surviving to the sample age) chosen for death by decapitation; four sedentary males and four active males, one from each of the four replicate lines. Mice were decapitated, exsanguinated, and dissected; tissues and organs were weighed and placed immediately into a −80°C freezer (all protocols approved by WSU Animal Care and Use Committee). All organs were examined for overt disease; if an animal expressed tumors or other abnormalities, another animal was chosen. For the active old group, only three mice were used in this study based on this disease-free criterion. Both ventricles (left and right) were weighed and subjected to total RNA extraction using the guanidinium isothiocyanate method (TRIzol Reagent; Life Technologies, Grand Island, NY).

High-density oligonucleotide array hybridization. Each mouse heart transcriptome was assayed individually, one array per mouse heart. Ten micrograms of total RNA was assayed from each mouse heart; thus the amount of heart tissue used per mouse varied among individuals (see Table 1). All mRNAs present in 10 μg of total RNA per individual were converted to double-stranded cDNA (Superscript Choice System, Life Technologies) and used as templates to synthesize biotin-labeled cRNA (T7 Megascript kit; Ambion, Austin, TX). Biotin-labeled cRNA was purified using RNeasy affinity columns (Qiagen, Valencia, CA). We hybridized cRNA to high-density mouse oligonucleotide arrays (Mouse Genome Array MU74Av2; Affymetrix, La Jolla, CA) as described (23). After hybridization, the gene arrays were washed and stained in a fluidic station (model 800101, Affymetrix) and scanned at a resolution of 6 μm with a Hewlett-Packard GeneArray scanner (model 900154, Affymetrix).

Preliminary data analysis by Affymetrix algorithms. The Affymetrix Mouse Genome Array MU74Av2 was based on the cDNA sequences from the UniGene (8/96 and Build 4.0) and TIGR (Build 1.0 beta) databases. This mouse array contains 12,422 probe sets representing 11,904 known or putative genes. Approximately 16 probe pairs of oligonucleotide probes in a probe set (16 perfect match and 16 mismatch probes) are used to measure the transcript level of a gene.

Table 1. *Descriptive statistics for study subjects*

	Active	Sedentary
Middle age		
n	4	4
Body mass, g	35.5 ± 4.6	40.4 ± 4.8
Age, days	596 ± 1.8	595 ± 1.7
Heart mass, mg	196 ± 28	200 ± 26
Total RNA, μg	213 ± 35	276 ± 41
Old age		
n	3	4
Body mass, g	31.8 ± 4.4	31.9 ± 4.8
Age, days	974 ± 0	974 ± 0.5
Heart mass, mg	179 ± 22	194 ± 42
Total RNA, μg	153 ± 55	224 ± 110

Values are means ± SD; *n* = no. of individuals.

Each probe pair consists of a perfect match (PM) probe and a mismatch probe (MM), which allows direct subtraction of cross-hybridization signals after background subtraction. GeneChip Analysis Suite 3.3 was used to quantify the image data. Affymetrix software determines the presence of mRNA in samples and computes the signals of probe sets. The software calculates differences and ratios between perfect match and mismatch signals, which are representative of the hybridization levels of their targets in each probe set. The average of the differences between perfect match and mismatch signals (after removing the outliers beyond 3 standard deviations) is used to estimate the relative mRNA level of each transcript. Signals in each image are normalized by global scaling, in which the average signals of all probe sets in an image are scaled to the target average intensity by multiplying a scaling factor. We used the resulting variable, an individual's probe set signal intensity ("signal intensity") for each gene, as the dependent variable in all statistical analyses. Additionally, we report the fold change (FC) for genes determined to change significantly with age. As the probe set signal intensity is directly related to its expression level, the ratio of intensities between two images becomes the FC. To calculate FC between young and old mice, the following formula is used by the software

$$\mathrm{FC} = (\mathrm{Signal}_{\mathrm{Old1}} - \mathrm{Signal}_{\mathrm{Young1}}) / \max\ [\mathrm{Q}_{\mathrm{factor}}, (\min(\mathrm{Signal}_{\mathrm{Old1}}, \mathrm{Signal}_{\mathrm{Young1}}))]$$

$$\text{Either } +1 \text{ if } \mathrm{Signal}_{\mathrm{Old1}} \geq \mathrm{Signal}_{\mathrm{Young1}}, \text{ or } -1 \text{ if } \mathrm{Signal}_{\mathrm{Old1}} < \mathrm{Signal}_{\mathrm{Young1}}$$

where $\mathrm{Signal}_{\mathrm{Old1}}$ is the signal of a probe set for *gene 1* from a mouse that was either an old animal in the sedentary or exercised groups, and $\mathrm{Signal}_{\mathrm{Young1}}$ is the signal of the same probe set, for the same *gene 1* from a mouse in the corresponding middle-aged group, and $\mathrm{Q}_{\mathrm{factor}}$ is a measure of the nonspecific fluorescent intensity background. Sixteen possible pairwise comparisons were performed (i.e., four middle-aged compared with four old-aged individuals) and their average was computed as a final FC for each treatment group.

Statistical analysis. In this report, we focus on two comparisons: "sedentary aging," which comprises the gene expression changes with age in selection males housed without running wheels; and "exercise aging," which is the same but for selection males housed with access to running wheels. Statistical analyses were performed for each gene on each individual's calculated signal intensity. We used two approaches to ascertain probable signal in our data while attending to concerns about experiment-wise error rates for the analysis of thousands of expression variables. First, we based all of our analyses on an adjusted Student's *t* statistic calculated for each gene. Adjusted posterior probabilities (in the Bayesian tradition) adjust the significance level based on regularized expressions for the variance of each gene (3). These post hoc *t* statistic adjustments of the log-transformed data were implemented in the software Cyber-T (25); the Bayesian prior was calculated with the 101 genes whose signal intensities were most similar to the gene in question. For example, if a gene's signal intensity was 1,000, then the nearest 101 genes were used to obtain an expected variance for that gene. Genes that met the criterion of $P < 0.01$ were considered significant. However, to warrant consideration, a gene had to be expressed in all individuals in at least one treatment group. This limited the number of genes considered to $n = 8{,}300$.

Second, because some of these genes may have represented false-positive results, we queried our data for the false discovery rate (FDR) for different numbers of significant genes. This rate is experiment-specific and depends on the observed variability of each gene. FDR was calculated using the SAM Microsoft Excel add-in (45). By allowing a 10% FDR, an almost complete overlap was observed between this method and the conservative Bayesian method in the genes that were identified. Thus we report those genes implicated by the Bayesian *t*-test at $P < 0.01$ and note that based on the separate FDR analysis, the global FDR of these lists is 10%.

For the genes significantly affected by age in the sedentary population, we report the proportion by which exercise prevented this change in expression

$$\text{Exercise prevention} = (\text{FC sedentary} - \text{FC active}) \times (\text{FC sedentary})^{-1}$$

where FC sedentary is the average fold change in old relative to young sedentary mice, and FC active is the same value in active mice. Values greater than one occurred when the FC in the active population was of opposite sign than in the sedentary population (e.g., induced in sedentary animals with age and downregulated in active animals with age). Values less than one occurred when exercise exacerbated the gene expression change (e.g., induced in sedentary animals with age, greater induction in active animals with age).

RESULTS

Survival and running phenotypes. We present summary results for male selection mice: median (50% survival) and maximum (10% survival) lifespan, kilometers per day at ages 2 mo (i.e., the selective-breeding test age), 20 mo, and 33 mo. Selection males housed with running wheels ($n = 40$) had greater median lifespan than did those not housed with running wheels ($n = 40$) (50% survival, SD, SE = 698 days, 4.0 days, 2.2 days; and 599 days, 7 days, 2.1 days for active and sedentary selection males, respectively), but little difference in maximum lifespan (10% survival, SD, SE = 921 days, 21 days, 2 days; and 902 days, 11 days, 1.6 days for active and sedentary selection males, respectively) (Fig. 1). For selected male mice housed with running wheels, mean kilometers per day decreased over the lifespan from an average 6.2 to 4.8 to 0.6 km/day at 2 mo, 20 mo, and 33 mo of age. We note that selection males ran more than control males across the lifespan (control-to-selection differentials: 2 mo, 77%; 20 mo, 92%; 33 mo, 100%).

In an analysis of variance of body mass testing for the effects of age, exercise, and their interaction, only age was significant; body mass was smaller in older than in younger animals ($F_{1,11} = 6.29$, $P = 0.03$). When heart mass was tested for the same effects and included body mass as a covariate, only body mass was a significant predictor of heart mass (larger animals had smaller hearts, $F_{1,11} = 6.16$, $P = 0.03$). Finally, in an analysis of variance testing the same effects and including heart mass as a covariate, only heart mass was a significant predictor of amount of total RNA; larger hearts yielded less RNA than smaller hearts ($F_{1,11} = 7.11$, $P = 0.02$). It therefore follows that younger animals yielded more total RNA than older animals. Note however, that regardless of total yield, 10 μg total RNA was used from each animal.

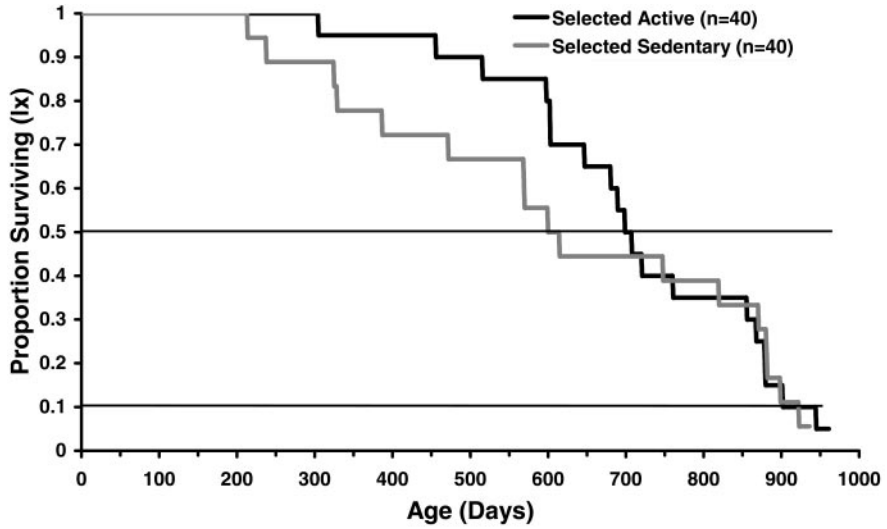

Fig. 1. Age-specific survival (l_x) for males of the selected genetic background in two exercise treatments; active mice ($n = 40$) are indicated with a black line, sedentary mice ($n = 40$) are indicated with a gray line. The l_x values were computed with the actuarial method in Program SAS, Procedure Lifetest. Median lifespan (50% survival) and maximum lifespan (10%) survival are indicated.

General patterns of gene expression in the aging hearts of sedentary mice. Of the 8,300 genes that were expressed in all members of at least one of the four experimental groups, 137 (1.6%) were significantly changed in the old relative to young mice in the sedentary population ($P < 0.01$) and were up- or downregulated by at least 50% (i.e., $\pm$ |1.5|-fold change). Most of these 137 genes could be classified into broad functional groups, although 36 code putative proteins and are as yet unnamed and of unknown function. Genes classified under the inflammatory, signal transduction, stress, and energy metabolism categories are listed in Tables 2 (increased expression with age) and 3 (decreased expression with age). For the complete list of significantly altered genes by at least 50% with age, see Supplemental Tables 7 and 8 (Supplemental Tables 7–10 are available online at the *Physiological Genomics* web site).[1]

Lifelong voluntary exercise prevents many age-related changes in gene expression. Of the 137 genes whose expression was significantly different in old vs. young sedentary mice, 70 were attenuated by exercise by at least 50%, i.e., had exercise prevention proportions of at least 0.50. In addition, six expression changes were exacerbated by exercise, and 28 were reversed in the active population (see Supplemental Tables 7 and 8). Furthermore, in the active population, fewer genes were significantly affected by age by at least a 50% change in expression level ($n = 62$) than in the sedentary population. The effect of exercise on large alterations in gene expression was particularly striking given that we observed only one gene, atrial natriuretic factor (ANF), displaying a greater than 10-fold increase in expression in the exercised animals compared with six genes displaying such changes in the sedentary animals. Most of the 62 genes could be classified into several functional groups, but 13 were of unknown function. Genes classified under inflammatory, stress, signal transduction, and energy metabolism categories are listed in Tables 4 and 5. For the complete list of all genes significantly up- or downregulated by at least 50% with age in the active population, see Supplemental Tables 9 and 10.

As another measure of the effect of exercise, we investigated genes that were either upregulated or downregulated as a result of aging in both the exercised and sedentary populations. We included in this analysis all genes that had an age change in expression that was statistically significant at $P < 0.05$ (Bayesian adjusted) in both the exercised and sedentary populations and which also displayed a similar overall trend with respect to induction or repression. These genes represent the most statistically robust alterations in gene expression as a function of age because they were identified as significant in two independent gene expression analyses. Therefore, they represent a useful set to evaluate the differential impact of aging on the sedentary and exercised populations. We identified 42 genes that met these criteria, 27 representing upregulations in mRNA level and 15 representing downregulation in mRNA levels (Table 6). We then conducted a slope heterogeneity test to determine whether the interaction between age and exercise was significant. In practice, this test measures differences in the signal-intensity changes in the two activity treatments (active vs. sedentary). Of the 42 genes examined, only three showed a statistical difference at $P < 0.05$. However, three-factor analysis of variance, which tests for the interaction of age-by-gene-by-exercise, suggested that gene expression changes in relation to aging are different in the exercised and sedentary groups when the population of genes is analyzed as a whole ($F_{41,462} = 1.36$, $P < 0.07$). This is supported by the fact that the overall effect of exercise on age-related gene expression changes was 60%, i.e., a global exercise prevention proportion of 0.60. Additionally, of the 42 changes in gene expression that were common to both activity groups, 32 (72%) displayed smaller FCs as a result of

[1] The Supplemental Material (Tables 7–10) to this article is available online at **http://physiolgenomics.physiology.org/cgi/content/full/12/2/129/DC1**.

Table 2. *Genes significantly increased ($P < 0.01$) in expression with age in the sedentary population (inflammatory response, stress response, signal transduction, and energy metabolism)*

ORF	Fold	*P*	Gene	Function	Exercise Prevention
			Inflammatory Response		
M64086	38.7	0.0026	Spi-2 proteinase inhibitor	Induced by acute inflammation	0.93
X51547	32.2	0.0030	Lysozyme P	Mediates inflammation	0.94
X66295	13.4	0.0030	Complement component 1, q sub, c peptide	Complement cascade	0.84
M83219	11.7	0.0004	Macrophage migration inhibitory factor	Calcium binding protein	0.99
M22531	9.2	0.0030	Complement C1q B chain	Complement cascade	0.89
U96684	8.7	0.0025	Paired-Ig-like receptor A3	Humoral immune response	0.93
AI848825	6.8	0.0001	Maternal antigen that embryos require	Unknown in heart	0.80
AW061307	5.3	0.0003	Tumor necrosis factor	Mediates signaling in B cells	0.61
K01238	4.9	0.0051	MuIFN-alpha-2 interferon-alpha-2	Inflammation modulation	1.13
U59488	4.4	0.0060	Neutrophil cytosolic factor 4	Superoxide formation during B cell phagocytosis	0.98
X58861	3.9	0.0059	Complement component 1, q sub, a peptide	Complement cascade	0.33
D84655	3.3	0.0078	Tumor necrosis factor receptor 6	Mediates signaling in B cells	1.17
U57524	2.8	0.0059	I-kappa B alpha chain	Inhibits transcription of NF-kappaB	0.93
L12120	2.8	0.0078	Interleukin 10 (IL-10) receptor alpha chain	Proliferation of B and T cells	0.37
X06454	2.5	0.0038	Sex-limited protein Slp(w7) alpha-gamma chain	Complement component	0.72
AB016424	2.3	0.0052	RNA binding motif 3 protein	Auto-antigens	0.71
M58156	2.2	0.0019	MHC Class I antigen	MHC antigen	0.77
U28280	2.0	0.0090	Orphan receptor	Immune response regulation	0.84
M27034	1.8	0.0093	MHC class 1 D-region cell surface antigen (D2d)	MHC antigen	1.17
X67809	1.8	0.0032	Cyclophilin C-associated protein	In vivo, binds cyclosporin A (CsA)	0.92
M62541	1.7	0.0034	CD20 Antigen	B cell differentiation antigen	−0.06
			Stress Response		
U20735	5.8	0.0090	Transcription factor junB	Immediate stress response transcription factor	0.62
AJ223208	4.1	0.0029	Cathepsin S	Lysosomal protease	0.64
X82648	4.0	0.0011	Apolipoprotein D	Induced by denervation	0.79
K02781	3.8	0.0002	Atrial Natriuretic Factor (ANF)	Cardiovascular homeostasis.	−3.19
AW048883	3.6	0.0029	Heat shock protein (HSPB2)	Chaperone	0.73
X53081	3.0	0.0012	Erythropoietin receptor (EPO-R)	Cytokine family of receptors	0.56
D00466	2.4	0.0003	Apolipoprotein E	Induced by denervation	0.28
V00835	2.1	0.0087	Metallothionein I	Induced by oxidative stress	0.43
X59846	1.8	0.0028	Growth arrest specific (gas6).	Induced by low serum levels	0.36
U13705	1.5	0.0068	Glutathione peroxidase 3	H_2O_2 breakdown	−0.48
U38261	1.5	0.0087	Extracellular superoxide dismutase (EC-SOD)	Extracellular antioxidant enzyme.	0.91
			Signal Transduction		
X04480	3.7	0.0023	IGF-I	Insulin signaling	0.96
L42115	3.0	0.0025	Insulin activated AAAT	Insulin signaling	1.16
U19520	3.0	0.0074	Munc 18, AKA nSec1 or rbSec1	Syntaxin binding protein	1.00
AB007135	2.7	0.0097	Insulin related receptor (IRR)	Insulin signaling	0.95
X06368	1.8	0.0028	Colony stimulating factor 1 receptor	Transmembrane receptor	0.00
X78874	1.6	0.0033	Chloride channel protein 3 (CLCN3)	Chloride channel signaling	0.77
			Energy Metabolism		
M68902	1.6	0.0058	Protein tyrosine phosphatase	Protein phosphorylation	0.70
U08439	1.5	0.0016	COX ViaH	Mitochondrial electron transport	0.96
X15963	1.5	0.0099	COX	Mitochondrial electron transport	1.43

GenBank accession numbers are listed under "ORF" (open reading frame); "Fold" refers to fold change; *P* is the probability value associated with the Bayes' regularized *t*-test.

exercise. When taken as a whole, our data support the conclusion that exercise retards the transcriptional alterations associated with age at two levels: a reduction in the number of genes displaying statistically significant changes and a reduction in fold changes for those genes.

DISCUSSION

We undertook this study to ask: *1*) How does aging affect gene expression profiles in sedentary and exercising populations?; and ultimately to ask *2*) Can exercise attenuate age-related phenotypes at the transcrip-

Table 3. *Genes significantly decreased ($P < 0.01$) in expression with age in the sedentary population (inflammatory response, stress response, signal transduction, and energy metabolism)*

ORF	Fold	*P*	Gene	Function	Exercise Prevention
			Inflammatory Response		
X17069	−3.1	0.0032	Immunophilin FK binding protein (FKBP)-52	Immunosuppressant binding protein	0.44
AI848851	−1.6	0.0045	Polydomain protein	Extracellular multi-domain protein	1.31
			Stress Response		
L06047	−3.6	0.0070	Glutathione-S-transferase, alpha 1 (Ya)	Reactive sulfyhdryl transfer	0.95
L40406	−2.2	0.0020	Heat shock protein (HSP105)	Chaperone	0.50
X51942	−1.7	0.0078	Phenylalanine hydroxylase (PAH)	Catabolism of phenylalanine	0.11
U09659	−1.5	0.0072	Chaperonin 10	Heat-shock 10 kDa	0.23
			Signal Transduction		
AI854793	−5.1	0.0006	Guanine nucleotide binding (G-protein) 11	Regulates ion channels	0.94
AF093259	−2.5	0.0047	Homer-2a	At excitatory synapses	0.96
AV319920	−2.4	0.0079	Protein kinase WNK1	MAP signal-regulated protein kinase	1.00
AF039833	−2.3	0.0028	Neurexin IV	Nerve cell signaling	0.99
Y17852	−2.1	0.0098	GDAP1-GDAP10	Sialytransferase signal transduction pathway	0.41
X95518	−1.6	0.0088	Neuronal tyrosine threonine phosphatase 1	Induced by nerve growth factor and insulin	0.19
X70842	−1.6	0.0091	Tyrosine kinase Flk-1	Ligand in vasculogenesis and angiogenesis	0.94
			Energy Metabolism		
AW048431	−3.1	0.0022	PACAP receptor 46-5	Glucagon receptor	1.21
U89906	−2.0	0.0007	Alpha methylacyl CoA racemase	Fatty acid breakdown	0.58
AW124122	−1.8	0.0002	Uncoupling protein 3 (UCP-3)	Uncouples oxidative phosphorylation	0.40
X14961	−1.6	0.0037	Heart fatty-acid binding protein	Fatty acid breakdown	−0.03
U59282	−1.5	0.0068	ATP synthase E chain	ATPase pathway	0.26
AI844043	−1.5	0.0105	ETF-ubiquinone oxidoreductase	Mitochondrial electron transport	−0.10

tional level? Using lines of genetically heterogeneous mice, selectively bred for high voluntary exercise, we found the majority of genes affected by age in our sedentary population to belong to the general categories of inflammation and stress response, and cell signaling (with 10% FDR). We further found that many of these transcription-level changes were offset by exercise in our active population. Additionally, the active population was characterized by fewer age-related expression changes overall. These results suggest that lifelong exercise can retard cardiac aging at the transcriptional level. We note that posttranscriptional events may negate subsequent effects on protein levels, and the reactions they catalyze, and caution is required in interpreting the biological implications of changes in gene expression. We first discuss our results relative to the survival and running phenotypes of these mice, and second, relative to recent reports implicating each of inflammatory response, stress response, and cell signaling in the aging process.

Median lifespan was lengthened as a result of lifelong exercise. Our data provide further support to the previous observations that exercise increases mean, but not maximum, lifespan in rodents. Median lifespan was increased by 17% (~100 days) in our wheel-access selectively bred male mice, but maximum lifespan was unaffected (Fig. 1). These results are in agreement with work by Holloszy (14, 15; see also Ref. 31) and McCarter and colleagues (27, 28) who have also demonstrated extension of median, but not maximum, lifespan in rodents with access to running wheels.

Why does exercise not extend maximum lifespan if it appears to retard aging at the molecular level as indicated by the gene expression analysis? Most likely, aging retardation at the molecular level by exercise is not observed in all tissues, including some that may limit lifespan. For example, if exercise does not reduce aging rates in replicative tissues, then it will not retard age-related tumor onset, which tends to limit maximum lifespan. Another possibility relates to the observation that wheel running decreased to an average 680 m/day at 33 mo of age vs. >6,000 m/day at 2 mo of age. This minimal level of late-age running may explain the lack of maximum lifespan extension if the benefits of exercise are short-term and thus require habitual high exercise. That more genes were affected with age in the sedentary population relative to the active population suggests that exercise affords protection in the heart muscle against normal age-related expression changes. Sedentary mice, while able to move about and conduct normal home-cage activity, presumably were unable to obtain cardiovascular conditioning, which has been shown to prolong average lifespan and lead to physiological adaptations in antioxidant defenses (18) and to decreased oxidative stress in heart and liver (22).

Aging is associated with inflammatory and stress responses in the heart. The immune/inflammation response has been shown to become less effective in

Table 4. *Genes significantly increased ($P < 0.01$) in expression with age in the exercised population (inflammatory response, stress response, signal transduction, and energy metabolism)*

ORF	Fold	*P*	Gene	Function
			Inflammatory Response	
M64086	2.8	0.0012	Spi-2 proteinase inhibitor	Induced by acute inflammation
X58861	2.6	0.0093	Complement component 1, q sub, a peptide	Complement cascade
L31958	2.5	0.0002	Mammary transforming protein (MATI)	Neoplastic transformation of NIH 3T3 cells
U35323	2.5	0.0012	MHC class II antigen	Collagen induced arthritis
M80206	2.3	0.0035	Poliovirus receptor (Pvr) homolog	Immunoglobulin superfamily
X66295	2.2	0.0019	Complement component 1, q sub, c peptide	Complement cascade
U88328	1.7	0.0044	Suppressor of cytokine signalling-3 (SOCS-3)	Cytokine-mediated signal transduction
U29678	1.6	0.0097	C-C chemokine receptor type 1 (CCR-I)	Stem cell proliferation
AB031386	1.5	0.0010	LR8 or Clast1	CD40-activated genes
			Stress Response	
K02781	15.9	0.0001	Atrial natriuretic factor precursor (ANF)	Cardiovascular homeostasis
Y11091	3.3	0.0005	Map kinase interacting kinase (Mnk)	Stress-activated protein kinase cascades
U13705	2.2	0.0006	Glutathione peroxidase (GPx) 3	H_2O_2 breakdown
U20735	2.2	0.0085	Transcription factor junB	Immediate stress response transcription factor
D00466	1.7	0.0068	Apolipoprotein E (APO-E)	Induced by excitoxic stress
			Signal Transduction	
D13664	2.6	0.0100	Osteoblast specific factor 2 (OSF-2)	Cell adhesion molecule
AI849565	2.5	0.0035	Neuropeptide Y5 (NPY) receptor	Appetite and energy balance
M70642	2.4	0.0002	Connective tissue growth factor (Fisp12)	Insulin like growth factor binding protein family
U58882	1.9	0.0026	LIM and SH3 protein 1	SH3 domain found in signaling molecules
U29056	1.9	0.0031	Src-like adapter protein (SLAP)	Downstream signaling intermediate
Y17566	1.8	0.0025	Pheromone receptor M21	Elicits social behaviors/neuroendocrine responses
U92477	1.6	0.0081	AblSH3 binding protein	SH3 domain found in signaling molecules
AW227647	1.6	0.0096	Lag protein	Opening of cation-selective channels
			Energy Metabolism	
AI838015	2.3	0.0008	LONP	Mitochondrial ATP dependent protease
D86177	1.7	0.0052	P14P5K	Insulin regulation

fighting infectious disease and injury with increasing age (reviewed in Ref. 30). Furthermore, long-term exercise training may counteract age-related declines in immune function (e.g., 33, 46). The major transcriptional class induced as a result of the aging process in sedentary mice was the inflammatory response (21 genes). This class included a concerted induction of complement genes, which are involved in innate immunity, including the genes encoding the component 1, q subunits, *C1qb* and *C1qc*, and *Complement C4*. The low level of induction of this system in our exercising population suggests less inflammation to heart muscle with exercise. Lee et al. (23) reported an induction of the complement cascade in the aging brain of mice, which, similar to exercise in our experiment, was offset by caloric restriction, an intervention that retards the aging process and extends lifespan in rodents (49). Previously, McGeer and colleagues (50) demonstrated that myocardial tissue locally expresses complement and that this expression significantly increases in response to ischemia and reperfusion. Other inflammatory response genes induced in the sedentary popula-

Table 5. *Genes significantly decreased ($P < 0.01$) in expression with age in the exercised population [inflammatory response, stress response (none), signal transduction, and energy metabolism]*

ORF	Fold	*P*	Gene	Function
			Inflammatory Response	
M94349	−2.6	0.0054	Immunoglobulin lambda chain	Immunoglobulin family
AF039839	−1.7	0.0095	Adriamycin-resistant related (arr) protein	Adriamycin-resistant phenotype
Y15003	−1.6	0.0068	Alpha 2,3-sialyltransferase (mST3Gal V)	GM3 synthetic activity
			Signal Transduction	
AI838337	−2.0	0.0052	PDZ-RGS3	Mediates signaling from the ephrin-B cytoplasmic tail
AW049642	−1.9	0.0033	Secreted frizzled related sequence protein 5	Modulates WNT signal transduction
AF033195	−1.6	0.0022	9-*cis*-retinol dehydrogenase	Ligand for TFs: retinoic acid / retinoid receptors
AI843959	−1.6	0.0229	Gamma-aminobutyric acid B receptor	Neurotransmission
X63473	−1.5	0.0021	Muscarinic actylcholine receptor m4	Inhibition of adenylate cyclase
			Energy Metabolism	
AU018994	−1.8	0.0062	F1-ATP synthase g subunit	Proton channel component

Table 6. *Genes significantly affected by age ($P < 0.05$) in both the active and sedentary environment*

ORF	Gene	FC(sed)	FC(act)	P (for $F_{1,11}$)
M64086	Spi proteinase inhibitor	38.7	2.8	0.05
X51547	Lysozyme P	32.2	1.9	0.23
X79214	U1 small ribonucleoprotein 1C	21.4	2.3	0.05
X52643	MHC class 2 antigen	14.1	1.4	0.97
X66295	Complement component 1, q sub, c	13.4	2.2	0.61
U09816	GM2 activator protein	8.9	1.8	0.49
U20735	Transcription factor junB	5.8	2.2	0.46
AW061307	Tumor necrosis factor	5.3	2.1	0.10
AI851160	Transmembrane Protein 9	4.0	1.7	0.31
U88328	Suppressor of cytokine signaling 3	4.0	1.7	0.59
X58861	Complement component 1, q sub, a	3.9	2.6	0.75
K02781	Atrial natriuretic factor (ANF)	3.8	15.9	0.002
X54511	Myc basic motif homolog	3.2	1.9	0.44
D00466	Apolipoprotein E	2.4	1.7	0.17
V00835	Metallothionein 1	2.1	1.2	0.82
X61800	CCAAT enhancer BP	2.1	1.2	0.76
X00496	1a associated invariant chain	2.0	1.8	0.91
X12761	Jun oncogene	1.8	1.2	0.66
X06368	Colony stimulating factor 1 receptor	1.8	1.8	0.95
M62541	CD20 antigen	1.7	1.8	0.58
L48687	Voltage gated sodium channel polypeptide	1.6	1.1	0.51
X70853	Fibulin	1.6	1.2	0.25
U13705	Glutathione peroxidase 3	1.5	2.2	0.23
AI846720	Unknown	1.4	1.9	0.64
D63423	Annexin V	1.3	1.1	0.52
AI849180	Unknown	1.2	1.8	0.57
U19597	Cyclin dependent kinase 4	1.1	1.7	0.35
M17818	Unknown	−11.9	6.9	0.28
M16360	Major urinary protein 5	−8.2	−6.4	0.21
AV355798	Unknown	−5.5	−6.4	0.21
AW226939	Carboxylesterase 3	−4.4	−1.8	0.65
AW045632	Unknown	−2.4	−2.1	0.09
AA666635	Ribosomal protein subunit L13	−2.0	−2.1	0.78
U79024	Coiled coil-like protein 1	−1.9	−1.1	0.07
X17069	Immunophilin FKBP 52	−1.7	−1.6	0.60
AF033350	Cell division control related protein 1	−1.6	−1.3	0.23
AI841415	Unknown	−1.6	−1.2	0.58
AF109905	MHC Hsc70t	−1.5	−1.5	0.32
Z14050	Dodecenoyl CoA isomerase	−1.4	−1.7	0.75
AF030343	Peroxisomal/mt dienoyl-CoA isomerase	−1.2	−1.6	0.69
AW049326	Nucleolar protein family A, member 3, homolog to Nop10p	−1.2	−1.6	0.59
AF039839	Adriamycin-resistant related protein	−1.1	−1.7	0.58

FC(sed) and FC(act) are the average fold changes between old and middle-aged mice in the sedentary and active environments, respectively. P is the unadjusted probability value associated with the F test for slope-heterogeneity, i.e., the test of the interaction between age and environment. A significant P value indicates that the fold changes were significantly different for active and sedentary mice.

tion, but not in the active population, include genes involved in B and T cell functioning and genes involved in the modulation of inflammation (Table 2). Although these same categories were induced in the active population with age, the greatest induction was by 180% in active mice (a total of six inflammatory response genes were upregulated 100% or more) vs. an almost 3,800% increase in the sedentary population (where 18 inflammatory response genes were upregulated by 100% or more).

Empirical evidence has been rapidly accumulating in several model systems that specifically implicates stress resistance as a factor in aging either through decreased efficiency of the stress response with age or from altered stress resistance at particular ages [e.g., rhesus monkey (20), mouse (23, 29), nematode (19), and fruit fly (24, 34)]. In our study, aging was associated with the induction of a stress response, including the expression of the antioxidant enzymes glutathione peroxidase, extracellular superoxide dismutase (EC-SOD), and metallothionein I. Also induced were two genes previously associated with oxidative stress responses, the apolipoproteins ApoD and ApoE. ApoE induction is associated with Alzheimer's disease, and ApoE-deficient animals display increased tissue levels of isoprostanes (38) and 3-nitrotyrosine (26), both of which are markers of oxidative stress. ApoD is constitutively expressed in multiple tissues (39), whereas both ApoE and ApoD are dramatically induced in response to denervation injury (6). The induction of these genes in the heart has not been previously reported, but our results suggest that they may be involved in the cardiac response to aging, perhaps in association with damage to the sympathetic neurons that innervate the heart or because of oxidative stress to cardiomyocytes. Taken as a whole, our observations suggest that the senescent heart is under a proinflammatory state associated with oxidative stress.

Aging in the heart is associated with alterations in expression of genes involved in hypertrophy and bioenergetics. In general, the aged heart displays increases in extracellular matrix (ECM) protein deposition (9), fibrosis (48), and cardiomyocyte hypertrophy (35). Although heart mass did not differ between active and sedentary mice, sedentary aged mice exhibited a 3.8-fold increase in the expression of the gene encoding atrial natriuretic factor (ANF), a peptide hormone of cardiac origin that participates in the homeostatic control of intravascular volume and vascular tone, and that is elevated in congestive heart failure (CHF) (37). Aging also resulted in the induction of myosin light chain type 2 (MLC2V), a gene previously identified as induced in idiopathic dilated cardiomyopathy (IDCM) (13). These observations suggest commonality of pathways involved in CHF, IDCM, and the aging process. Possibly, these alterations result from age-associated alterations in cardiomyocyte bioenergetics, as suggested by upregulation in the mitochondrial electron transport system (ETS) genes cytochrome oxidase (COX) Va and COX VIa, and lactate dehydrogenase. Eukaryotic COX is a multicomponent enzyme consisting of 13 polypeptides in mammals and it is believed that subunits VIII and VIa may function to modulate enzyme activity in response to changes in metabolic conditions (47). We also observed decreases in expression of genes involved in fatty acid metabolism, such as α-methylacyl-CoA racemase, involved in peroxisomal fatty acid α-oxidation, and heart fatty-acid binding protein (HFABP), which functions as a vehicle of cytosolic fatty acid transport. Interestingly, we also observed a decrease in expression of the F_1F_0-ATP synthase E chain, which is regulated in response to fatty acid intake (42). These gene expression results are in agreement with the previous observation of impaired cardiac mitochondrial fatty acid oxidation with aging (36).

A reduction in levels of homologues of insulin, insulin-like growth factors (IGFs), and receptors in the insulin-signaling pathway has been shown to confer greater longevity in yeast (12, 16), nematodes (21, 44), fruit flies (10, 43), mutant long-lived mice (4, 11), and caloric-restricted mice (40). Therefore, the as-yet unidentified mechanism of insulin signaling on lifespan may be evolutionarily conserved. A primary mammalian insulin pathway ligand, IGF-I, as well as insulin-related receptor (IRR) and insulin-activated AAAT (IaAAAT) were upregulated in sedentary old mice relative to young mice. IGF-I and IRR inductions were almost completely offset in the active population (96% and 95%, respectively), whereas IaAAAT was reversed, which is consistent with the observation of increased median lifespan in active mice. That genes involved in insulin signaling were not significantly reduced in expression in old active mice correlates with the observation that exercise training eliminates age-related differences in skeletal muscle insulin receptor and insulin receptor substrate 1 (IRS-1) abundance in rats (1).

We have presented the first large-scale assay of transcriptional changes in aging mouse hearts and their modulation by exercise. We found more genes to be significantly affected by age in our sedentary population of mice than in our active population of mice. Moreover, many alterations in gene expression affected by age in the sedentary population were attenuated by exercise. We also observed a striking effect of exercise on large alterations in gene expression, as we observed only one gene displaying greater than a 10-fold increase in expression in the exercised animals compared with six genes displaying such changes in the sedentary animals. Our results indicate a global, overall decrease in the induction of inflammatory and stress responses in the presence of a lifetime of active exercise, despite decreased exercise levels in the very old exercising mice. Further studies specifically targeting the genes implicated in this study as well as genes involved in insulin signaling will further our understanding of how habitual physical activity promotes extended life expectancy at the transcriptional level. Additionally, investigations of other tissues in the exercising and sedentary populations should reveal whether the benefits of exercise in aging retardation affect multiple tissues, a finding that would be consistent with the increase in median lifespan induced by exercise.

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Table 7. Significantly increased (P<0.01) genes by at least 50% with age in the sedentary population in all categories. Genbank Accession numbers are listed under ORF, Fold refers to fold change, and *P* is the probability value associated with the Bayes' regularized t-test.

ORF	Fold	*P*	Gene	Function	Exercise Prevention
INFLAMMATORY RESPONSE					
M64086	38.7	0.0026	Spi-2 proteinase inhibitor	Induced by acute inflammation	0.93
X51547	32.2	0.0030	Lysozyme P	Mediates inflammation	0.94
X66295	13.4	0.0030	Complement component 1, q sub, c peptide	Complement Cascade	0.84
M83219	11.7	0.0004	Macrophage migration inhibitory factor	Calcium binding protein	0.99
M22531	9.2	0.0030	Complement C1q B chain	Complement Cascade	0.89
U96684	8.7	0.0025	Paired-Ig-like receptor A3	Humoral immune response	0.93
AI848825	6.8	0.0001	Maternal antigen that embryos require	Unknown in heart	0.80
AW061307	5.3	0.0003	Tumor necrosis factor	Mediates signaling in B cells	0.61
K01238	4.9	0.0051	MuIFN-alpha-2 interferon-alpha-2	Inflammation modulation	1.13
U59488	4.4	0.0060	Neutrophil cytosolic factor 4	Superoxide formation during B cell phagocytosis	0.98
X58861	3.9	0.0059	Complement component 1, q sub, a peptide	Complement cascade	0.33
D84655	3.3	0.0078	Tumor necrosis factor receptor 6	Mediates signaling in B cells	1.17
U57524	2.8	0.0059	I-kappa B alpha chain	Inhibits transcription of NF-kappaB	0.93
L12120	2.8	0.0078	Interleukin 10 (IL-10) receptor alpha chain	Proliferation B and T cells	0.37
X06454	2.5	0.0038	Sex-limited protein Slp(w7) alpha-gamma chain	Complement component	0.72
AB016424	2.3	0.0052	RNA binding motif 3 protein	Auto-antigens	0.71
M58156	2.2	0.0019	MHC Class I antigen	MHC antigen	0.77
U28280	2.0	0.0090	Orphan receptor	Immune response regulation	0.84
M27034	1.8	0.0093	MHC class I D-region cell surface antigen (D2d)	MHC antigen	1.17
X67809	1.8	0.0032	Cyclophilin C-associated protein	in vivo, binds cyclosporin A (CsA)	0.92
M62541	1.7	0.0034	CD20 Antigen		-0.06
STRESS RESPONSE					
U20735	5.8	0.0090	Transcription factor junB	Immediate stress response transcription factor	0.62
AJ223208	4.1	0.0029	Cathepsin S	Lysosomal protease	0.64
X82648	4.0	0.0011	Apolipoprotein D	Induced by excitotoxic stress	0.79
K02781	3.8	0.0002	Atrial Natriuretic Factor (ANF)	Cardiovascular homeostasis.	-3.19
AW048883	3.6	0.0029	Heat shock protein (HSPB2)	Chaperone	0.73
X53081	3.0	0.0012	Erythropoietin receptor (EPO-R)	Cytokine family of receptors	0.56
D00466	2.4	0.0003	Apolipoprotein E	Induced by excitotoxic stress	0.28
V00835	2.1	0.0087	Metallothionein 1	Induced by oxidative stress	0.43
X59846	1.8	0.0028	Growth arrest specific (gas).	Blood coagulation cascade	0.36
U13705	1.5	0.0068	Glutathione peroxidase 3	H2O2 breakdown	-0.48
U38261	1.5	0.0087	Extracellular superoxide dismutase (EC-SOD)	Extracellular antioxidant enzyme.	0.91
SIGNAL TRANSDUCTION					
X04480	3.7	0.0023	IGF-1	Insulin signaling	0.96
L42115	3.0	0.0025	Insulin activated AAAT	Insulin signaling	1.16
U19520	3.0	0.0074	Munc 18, AKA nSec1 or rbSec1	Syntaxin binding protein	1.00
AB007135	2.7	0.0097	Insulin related receptor (IRR)	Insulin signaling	0.95
X06368	1.8	0.0028	Colony stimulating factor 1 receptor	Transmembrane receptor.	0.00
X78874	1.6	0.0033	Chloride channel protein 3 (CLCN3)	Chloride channel signaling	0.77
ENERGY METABOLISM					
M68902	1.6	0.0058	Protein tyrosine phosphatase	Protein phosphorylation	0.70
U08439	1.5	0.0016	COX ViaH	Mitochondrial electron transport	0.96
X15963	1.5	0.0099	COX	Mitochondrial electron transport	1.43
CELLULAR REGULATION					
X54511	3.2	0.0059	Myc basic motif homologue1 (Mbh1)	Cellular architecture	0.41
AF015309	2.9	0.0056	Nucleolar microspherular (MSP58)	Activated in G1 phase	0.36
X67209	2.2	0.0016	NPDC-1	Suppresses cell proliferation	0.56
X12761	1.8	0.0024	Jun oncogene	Activated in G0/G1 transition	0.32
AF015881	1.5	0.0035	Nuclear factor erythroid related factor (NF-E1)	Leucine zipper	0.31
DNA METABOLISM					
Y09688	3.0	0.0040	Endonuclease III	DNA biosynthesis	1.63
Z30939	2.7	0.0091	Histone gene complex 1	DNA structure	0.66
TROPHIC FACTORS					
X79214	21.4	0.0090	U1 small nuclear ribonucleoprotein 1C (Snrp1c)	Zinc finger motif	0.89
U09816	8.9	0.0010	GM2 activator protein	Ganglioside activation	0.80

AJ243964	5.6	0.0024	Dickkopf3	Expressed by heart induction	0.76
Y17709	3.4	0.0029	Frizzled Receptor 9	Membrane protein for WNT ligands	0.86
U29539	2.7	0.0045	Inducible E3 protein	Nerve cell propagation	0.52
L10244	2.5	0.0059	SSAT	Polyamine pool maintenance	0.34
X16995	2.2	0.0066	Orphan nuclear receptor N10	hormone receptor	0.38
AI839708	2.1	0.0012	HOX-A10	Cell positional identity	1.14
AF080090	1.7	0.0014	Semaphorin IV	Remodeling of nerve/muscle cell connections	0.05
X70853	1.6	0.0017	Fibulin (BM90)	Extracellular matrix structure	0.55
D87902	1.5	0.0080	ADP ribosylation factor 5	Involved in transport vesicle formation	0.69
MUSCLE SPECIFIC					
M19436	6.3	0.0013	Myosin light chain, cardiac atrial muscle	Muscle contraction	1.41
AI648850	4.7	0.0023	Myosin light chain	Muscle contraction	1.31
AA839903	3.4	0.0040	Myosin light chain 2, ventricular	Muscle contraction	1.62
M91602	1.8	0.0004	Myosin light chain 2	Muscle contraction	0.60
X04405	1.7	0.0036	Myoglobin	Muscle contraction	0.98
PROTEIN TURNOVER					
X60980	5.8	0.0000	Thymidine kinase	Involved in transcription	0.73
AI853035	3.5	0.0039	Fbox protein 9	Protein degradation	0.91
AI843063	1.9	0.0046	Muc5AC	O-glycan chain support	1.17
M17878	1.6	0.0011	Elongation factor Tu	aminoacyl-tRNA binding to ribosome	0.16
UNKNOWN					
AW124470	6.6	0.0010			0.85
AI324061	6.5	0.0001			1.22
AI842277	4.5	0.0026			1.09
AA790307	4.3	0.0028			0.68
AV268095	3.7	0.0056			2.38
AV317524	2.9	0.0077			0.70
AW123801	2.9	0.0017			1.14
AI846233	2.8	0.0069			0.74
AA590358	2.8	0.0009			0.84
M32486	2.7	0.0062			0.83
AW046627	2.3	0.0021			1.20
AV097950	2.3	0.0018			0.55
AW122725	2.2	0.0094			1.29
C85523	1.9	0.0003			0.68
AI842828	1.8	0.0080			0.26
AV356071	1.7	0.0002			1.17
AV298880	1.6	0.0090			1.07
AW123953	1.5	0.0061			0.28

Table 8. Significantly decreased (P<0.01) genes by at least 50% with age in the sedentary population in all categories. Genbank Accession numbers are listed under ORF, Fold refers to fold change, and *P* is the probability value associated with the Bayes' regularized t-test.

ORF	Fold	*P*	Gene	Function	Exercise Prevention
INFLAMMATORY RESPONSE					
X17069	-3.1	0.0032	Immunophilin FK binding protein (FKBP) -52	Immunosuppressant binding protein	0.44
AI848851	-1.6	0.0045	Polydomain protein	Extracellular multi-domain protein	1.31
STRESS RESPONSE					
L06047	-3.6	0.0070	Glutathione-S-transferase, alpha 1 (Ya)	Reactive sulfhydryl transfer	0.95
L40406	-2.2	0.0020	Heat shock protein (HSP105)	Chaperone	0.50
X51942	-1.7	0.0078	Phenylalanine hydroxylase (PAH)	Catabolism of phenylalanine	0.11
U09659	-1.5	0.0072	Chaperonin 10	Heat-shock 10kDa	0.23
SIGNAL TRANSDUCTION					
AI854793	-5.1	0.0006	Guanine nucleotide binding (G-protein) 11	Regulates ion channels.	0.94
AF093259	-2.5	0.0047	Homer-2a	At excitatory synapses	0.96
AV319920	-2.4	0.0079	Protein kinase WNK1	MAP signal-regulated protein kinase	1.00
AF039833	-2.3	0.0028	Neurexin IV	Nerve cell signaling	0.99
Y17852	-2.1	0.0098	GDAP1-GDAP10	Sialytransferase signal transduction pathway	0.41
X95518	-1.6	0.0088	Neuronal tyrosine threonine phosphatase 1	Tyrosine/threonine phosphatase	0.19
X70842	-1.6	0.0091	Tyrosine kinase Flk-1	Ligand in vasculogenesis and angiogenesis	0.94
ENERGY METABOLISM					
AW048431	-3.1	0.0022	PACAP receptor 46-5	Glucagon receptor	1.21
U89906	-2.0	0.0007	Alpha methylacyl CoA racemase	Fatty acid breakdown	0.58
AW124122	-1.8	0.0002	Uncoupling protein 3 (UCP-3)	Uncouples oxidative phosphorylation	0.40
X14961	-1.6	0.0037	Heart fatty-acid binding protein	Fatty acid breakdown	-0.03
U59282	-1.5	0.0068	ATP synthase E chain	ATPase pathway	0.26
AI844043	-1.5	0.0105	ETF-ubiquinone oxidoreductase	Mitochondrial electron transport	-0.10
CELLULAR REGULATION					
AW125272	-1.9	0.0090	Breast Cancer Susceptibility 2	Cellular Division Rate	1.14
DNA METABOLISM					
X05862	-2.1	0.0030	Histone genes H2B and H2A	DNA structure	0.70
U51866	-1.6	0.0032	Casein kinase II (CK2)	DNA replication	0.73
TROPHIC FACTORS					
M16360	-8.2	0.0002	Major Urinary Protein 5	Pheromone binding	0.22
M16358	-4.1	0.0094	Major Urinary Protein 4	Pheromone binding	0.26
AA543502	-2.0	0.0036	Muscle specific enolase beta subunit	Muscle determination	0.60
AI530375	-1.9	0.0086	Sepiapterin reductase (SPR)	Controls neurotransmitter levels	1.11
M95200	-1.6	0.0087	VEGF	Blood vessel growth	0.30
AI842068	-1.5	0.0021	MSAL	Zinc finger protein	0.34
MUSCLE SPECIFIC					
U79024	-1.9	0.0048	Coiled coil like protein 1	Protein tyrosine phosphatase	0.46
PROTEIN BIOSYNTHESIS					
AW049185	-3.4	0.0077	Nuclear protein SR25	RNA splicing	1.22
AI875598	-2.1	0.0012	Neprilysin-like peptidase gamma	Amyloid degradation	0.29
AW125562	-1.7	0.0012	Mitochondrial ribosomal protein L34	DNA translation	0.54
TRANSCRIPTION REGULATION					
AI527477	-4.5	0.0001	Proline rich protein	induced by camp	1.13
UNKNOWN					
M17818	-11.9	0.0000			0.42
AI835060	-9.0	0.0017			0.95
AV355798	-5.5	0.0000			-0.17
AW123983	-4.2	0.0041			0.61
AW060819	-3.4	0.0029			0.80
AA833077	-3.2	0.0031			0.86
AW121931	-3.0	0.0098			1.53
AV372577	-2.6	0.0037			0.61
AW045632	-2.4	0.0017			0.45

AW125453	-1.9	0.0006	1.27
AI837302	-1.8	0.0035	0.24
D89902	-1.6	0.0075	0.93
AV363907	-1.6	0.0020	0.97
AI853772	-1.6	0.0096	0.83
AI847069	-1.6	0.0059	0.68
AV221100	-1.5	0.0001	0.33
AW208630	-1.5	0.0012	1.02
AW124487	-1.5	0.0022	0.36

Table 9. Significantly increased (P<0.01) genes by at least 50% with age in the exercised population in all categories. Genbank Accession numbers are listed under ORF, Fold refers to fold change, and *P* is the probability value associated with the Bayes' regularized t-test.

ORF	Fold	*P*	Gene	Function
INFLAMMATORY RESPONSE				
M64086	2.8	0.0012	Spi-2 proteinase inhibitor	Induced by acute inflammation
X58861	2.6	0.0093	Complement component 1, q sub, a peptide	Complement Cascade
L31958	2.5	0.0002	Mammary transforming protein (MAT1)	Neoplastic transformation of NIH 3T3 cells
U35323	2.5	0.0012	MHC class II antigen	Collagen induced arthritis
M80206	2.3	0.0035	Poliovirus receptor (Pvr) homologue	Immunoglobulin superfamily
X66295	2.2	0.0019	Complement component 1, q sub, c peptide	Complement Cascade
U88328	1.7	0.0044	Suppressor of cytokine signalling-3 (SOCS-3)	Cytokine-mediated signal transduction
U29678	1.6	0.0097	C-C chemokine receptor type I (CCR-I)	Stem cell proliferation
AB031386	1.5	0.0010	LR8 or Clast1	CD40-activated genes
STRESS RESPONSE				
K02781	15.9	0.0001	Atrial natriuretic factor precursor (ANF)	Cardiovascular homeostasis
Y11091	3.3	0.0005	Map kinase interacting kinase (Mnk)	Stress-activated protein kinase cascades
U13705	2.2	0.0006	Glutathione peroxidase (GPx) 3	H2O2 breakdown
U20735	2.2	0.0085	Transcription factor junB	Immediate stress response transcription factor
D00466	1.7	0.0068	Apolipoprotein E (APO-E).	Induced by excitotoxic stress
SIGNAL TRANSDUCTION				
D13664	2.6	0.0100	Osteoblast specific factor 2 (OSF-2)	Cell adhesion molecule
AI849565	2.5	0.0035	Neuropeptide Y5 (NPY) receptor	Appetite and energy balance
M70642	2.4	0.0002	Connective tissue growth factor (Fisp12)	Insulin like growth factor binding protein family.
U58882	1.9	0.0026	LIM and SH3 protein 1	SH3 domain found in signaling molecules
U29056	1.9	0.0031	Src-like adapter protein (SLAP)	Downstream signaling intermediate
Y17566	1.8	0.0025	Pheromone receptor M21	Elicits social behaviors/ neuroendocrine responses
U92477	1.6	0.0081	AblSH3 binding protein	SH3 domain found in signaling molecules
AW227647	1.6	0.0096	Lag protein	Opening of cation-selective channels.
ENERGY METABOLISM				
AI838015	2.3	0.0008	LON	ATP dependent protease
D86177	1.7	0.0052	PI4P5K	Insulin regulation
TROPHIC FACTORS				
U09816	1.8	0.0080	GM2 activator protein	Ganglioside activation
AV232600	1.7	0.0025	17beta hydroxysteroid dehydrogenase	Modulates androgen activity
MUSCLE SPECIFIC				
AF035643	2.6	0.0005	Vesicle associated membrane protein 5	Trafficking associated with mygenesis
AJ223855	2.0	0.0073	Telethonin	Muscle assembly regulation factor
PROTEIN METABOLISM				
L31609	2.6	0.0001	S29 ribosomal protein	Protein synthesis
M69196	1.5	0.0099	PC1 precursor	Hormone and neuropeptide synthesis
CELLULAR REGULATION				
M83749	2.8	0.0006	Cyclin D2	G1/S transition
U19597	1.7	0.0081	Cyclin dependent kinase 4	Inhibits progression of G1 phase
TRANSCRIPTION REGULATION				
Z36885	1.7	0.0039	ELK4	Mediates RAS signaling
UNKNOWN				
AV313633	2.4	0.0093		
M27347	2.0	0.0032	Phosphorylcholine specific T suppressor	
AV351758	2.0	0.0044		
X81059	2.0	0.0046	Testis expressed gene 271	

Table 10. Significantly decreased (P<0.01) genes by at least 50% with age in the exercised population in all categories. Genbank Accession numbers are listed under ORF, Fold refers to fold change, and *P* is the probability value associated with the Bayes' regularized t-test.

ORF	Fold	*P*	Gene	Function
INFLAMMATORY RESPONSE				
M94349	-2.6	0.0054	Immunoglobulin lambda chain	Immunoglobulin family
AF039839	-1.7	0.0095	Adriamycin-resistant related (arr) protein	Adriamycin-resistant phenotype
Y15003	-1.6	0.0068	Alpha 2,3-sialyltransferase (mST3Gal V)	GM3 synthetic activity
SIGNAL TRANSDUCTION				
AI838337	-2.0	0.0052	PDZ-RGS3	Mediates signaling from the ephrin-B cytoplasmic tail
AW049642	-1.9	0.0033	Secreted frizzled related sequence protein 5	Modulates WNT signal transduction
AF033195	-1.6	0.0022	9-cis-retinol dehydrogenase	Ligand for TFs: retinoic acid / retinoid receptors
AI843959	-1.6	0.0229	Gamma-aminobutyric acid B receptor	Neurotransmission
X63473	-1.5	0.0021	Muscarinic actylcholine receptor	inhibition of ATP synthesis
ENERGY METABOLISM				
AU018994	-1.8	0.0062	F1-ATP synthase g subunit	proton channel component
DNA METABOLISM				
D44464	-2.0	0.0062	Uridine phosphorylase	Nucleotide catabolism
TROPHIC FACTORS				
M16360	-6.4	0.0092	Major urinary protein 5	pheromone binding
AW260482	-1.5	0.0068	Acetyltransferase tubedown 1	vascular and physiological angiogenesis
MUSCLE SPECIFIC				
M19436	-2.6	0.0037	Myosin light chain, cardiac atria	Muscle contraction
AW045665	-2.4	0.0079	Microtubule associated protein 1A	Stabilizes microtubules
CELLULAR REGULATION				
U42383	-2.1	0.0023	Fibroblast growth factor inducible 13	Inhibits cell cycle progression
TRANSCRIPTION REGULATION				
M23236	-2.0	0.0004	Proline rich protein	Induced by cAMP
UNKNOWN				
M17818	-6.9	0.0068		
AV355798	-6.4	0.0091		
AV268095	-5.1	0.0044		
AW045632	-2.1	0.0010		
AA644978	-1.9	0.0010		
AI849490	-1.9	0.0073		
AV296797	-1.8	0.0024		
AW121847	-1.7	0.0013		
AI851309	-1.7	0.0028		

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editorial focus

In for the long run: Focus on "Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart"

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WHILE IT IS KNOWN that exercise has an effect upon aging and lifespan in rodents, what is not yet well understood is how exercise ameliorates the deleterious effects of aging at the cellular level. Microarray analysis makes it possible for researchers to assess changes in mRNA transcript levels in particular tissues which may translate into significant changes in protein levels. Thus a better understanding of the functional genetics of exercise can provide insight into the physiological genomics of the aging process.

In this release of *Physiological Genomics* (Ref. 2; see page 129 in this release), Bronikowski et al. (2) employ expression profiling of mouse hearts to examine age- and exercise-related changes in gene expression. Their goal was to assess the changes in gene expression that occur in the hearts of both sedentary and active mice and to determine what effect exercise had upon genes demonstrated to be differentially regulated in the sedentary animals. They used male mice drawn from an ongoing breeding program, now past its 16th generation, that selects individuals with a predilection for voluntary exercise. In the experimental group, the mice were given free access to a running wheel (the "active" group); control animals (the "sedentary" group) were housed in cages without one. Subjects' hearts were harvested and assayed at midlife (20 mo) and old age (33 mo) by hybridization to an Affymetrix mouse cDNA array. The only previous study of age-related changes of gene expression profiles in the heart involved left ventricular cardiomyocytes extracted from 4-mo-old and 20-mo-old C57BL/6 mice (1).

In keeping with previous findings, the authors demonstrated that exercise increased the median, although not the maximum, lifespan of the active mice (by 17%). The expression of 137 genes changed by at least 50% between old and younger mice in the sedentary group. Changes in 70 of these same genes were significantly attenuated in the exercise group. In addition, fewer genes were significantly affected by age in the active vs. sedentary mice. In the sedentary population, the major classes of differentially expressed genes were associated with inflammation and stress response, leading the authors to conclude that the aging heart experiences oxidative stress leading to a pro-inflammatory state. Interestingly, these changes were attenuated even though very old active mice exercised much less than they had in their youth.

This study is encouraging for those of us who try to exercise on a regular basis to ward off the functional decline associated with aging. Bronikowski et al. studied only the heart, but previous expression profiling studies suggested that aging also increases oxidative stress and inflammation in skeletal muscle and brain (3, 4). Whether the exercise produced systemic effects that would inhibit this aspect of aging in all organs, or whether it only produced local effects caused by the increased workload of the heart and exercising muscles, is an important question that will require additional research.

The conclusions based on any exploratory analysis need to be confirmed prospectively before they can be fully accepted. One of the pitfalls in interpreting studies of exercise, caloric restriction, or other anti-aging interventions is the problem of regression to the mean. Whenever thousands of variables (mRNA levels in this case) collected from 3–4 animals are compared with data from 3–4 other animals, there are many statistically significant differences by chance alone (due to real heterogeneity among animals, not necessarily measurement error). When two other independent groups are compared, the differences between the first two groups that were caused by sampling error are absent or attenuated. If the first set of animals is used to define ordinary aging, and the second set undergoes an anti-aging intervention, then the anti-aging treatment may get undue credit. When specific genes are

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hypothesized to behave this way a priori, there is very strong evidence that the anti-aging intervention is responsible. Post hoc conclusions from exploratory studies are more tentative. In this particular study, however, there were some reassuring data to suggest that regression to the mean cannot explain the apparent effect of exercise on gene transcription. First, statistical methods suggested that no more than 10% of the age-related differences should be attributable to sampling error, whereas many more of them were reversed by exercise. Second, in exercising animals only half as many genes were affected by aging, an effect that cannot be explained by regression to the mean.

The "middle-aged" mice would be comparable to 75-yr-old humans in terms of the age at which half of the population has died. The old animals must be considered extremely old for this line of mice, since only 5% of them survive to 31 mo of age. Thus Bronikowski et al. examined a very select group of mice at the oldest age. Moreover, all mice in the study were selected for their high volume of voluntary treadmill running. These issues raise several questions that warrant further investigation. Do the age-related changes in gene expression observed in this study occur only near the end of life? Would access to treadmills have a similar effect in ordinary mice? Would forced exercise have the same effect as voluntary exercise, or would it have the opposite effect? How much exercise is needed to retard aging at the level of gene expression?

Microarray data provide only a starting point for expanding our knowledge of the molecular basis of factors that influence the rate of aging. Additional studies are needed to determine which cells within the tissue are affected, whether effects are focal or diffuse, and which of the changes in gene expression are physiologically significant. Questions about the physiology of aging have sent many investigators to the microarray laboratory, and now tables full of microarray data should be sending investigators back to the physiology laboratory with new ideas.

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