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Access to exercise and its relation to cardiovascular health and gene expression in laboratory animals

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Abstract

The interaction between genes and environment can influence cardiovascular disease (CVD). This 16 month study investigated if genes associated with cardiovascular (CV) regulation were expressed differently in animals having: 1) no access to physical activity or exercise (SED), 2) access to hour-long, twice weekly activity (PA), and 3) access every-other-day to a running wheel (EX). Out of 31,000 genes, a CV subset comprising 44 genes was investigated. Ten genes from this subset were expressed differently in EX compared with SED, and 34 genes were expressed differently in PA compared with SED ($p<0.05$). Total cholesterol (70 ± 8 vs. 101 ± 9 mg dl^{-1}), triglycerides (104 ± 8 vs. 127 ± 4 mg dl^{-1}), resting systolic blood pressure (130 ± 3 vs. 141 ± 3 mmHg), mean arterial pressure (110 ± 2 vs. 120 ± 2 mmHg) and heart rate (380 ± 6 vs. 405 ± 9 beats min^{-1}) were lower in EX compared with SED ($p<0.05$), but intracellular adhesion molecule levels did not differ among groups. Mean gene expressions for *Gja1, Fdft1, Edn1, Cd36,* and *Hmgb2* differed in animals according to access to physical activity. These genes play roles in heart rate, cholesterol biosynthesis, blood pressure, cell adhesion, and transcription and neurogenesis regulation, respectively. In

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conclusion, a total of 44 CV genes were expressed differently in SED compared to PA and EX; and SED showed more physiological evidence of CVD.
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Keywords: Oxidative stress; Physical activity; Hypokinesis; Cardiovascular; DNA microarray; *Edn1*; *Hmgb2*; *Cd36*; *Gja1*; *Fdft1*

Introduction

Given the important interaction between genetics and environment, animal models pose an interesting paradox. Most animal studies use animals from the same genetic line, so genetics is controlled for in a way that most human studies usually are not. The environment in which most laboratory animals reside is also controlled in terms of type and amount of food and access to physical activity. A controlled environment may actually have some drawbacks, especially related to physical activity. That is because in natural settings, animals usually engage in physical activity such as exploration, foraging for food, or play (Sallis, 2000; McCarter et al., 1997; Ingram, 2000). In laboratory settings, animals are often sedentary and are fed ad libitum (AL). The combination of sedentary behavior and AL food places laboratory animals at risk for an early onset of adverse physiological events related to cardiovascular disease (CVD) (Keenan et al., 1999; O'Connor and Eikelboom, 2000; Bortz, 1982).

CVD is not a major cause of death in most rodent models. Nevertheless, laboratory rats do show signs and symptoms of CVD such as high blood pressure, hyperlipidemia, and diabetes (Bolego et al., 1999). It is well known that regular exercise improves CV function (Brown, 2003), in part by activating genes that encode components of contractile elements, metabolic pathways, and protein synthesis (Chen, 2001). Clusters of genes associated with CV energy metabolism and ischemia-reperfusion have been shown to change in an intensity-dependent manner (Boluyt et al., 2003; Iemitsu et al., 2003). Highly trained animals demonstrate healthier CV systems compared to less-trained animals. Nevertheless, even low-intensity running wheel exercise, if performed regularly, has been reported to have significant beneficial effects on blood lipid profile and resting blood pressure (Suzuki and Machida, 1995). Both acute and chronic exercise can modify gene expression of key components of cardiac function, including endothelin-1 (*Edn1*) (Iemitsu et al., 2003), atrial natriuretic peptide (*ANP*), and atrial myosin light chain (*aMLC*) (Diffee et al., 2003) which are associated with contractile function of the heart and vascular tone. Similarly, running wheel exercise increased levels of heat shock proteins (HSP) in cardiac tissue (Noble et al., 1999).

The benefits of both low and moderate intensity exercise on CV health and longevity have been reported in several large-scale human studies (Lee and Paffenbarger, 2000; Hakim et al., 1998; Paffenbarger et al., 1993; Blair et al., 1989). We used laboratory rats to investigate whether access to physical activity in a large box for 1 h, twice weekly would be adequate to elevate a sedentary status to a moderately active one, and thereby affect gene expression and/or physiological markers associated with CVD risk. We chose twice weekly activity in a large box to determine whether a minimal level of physical activity, similar to weekend activity among a human population, would mitigate the deleterious effects of a sedentary environment. We investigated the gene-environment interaction in rodents to learn whether some genes associated with CV regulation may be expressed differently in animals that resided

solely in a standard cage compared with animals that had access to physical activity in a large box or access to exercise on a running wheel.

Methods

Seventy-two weanling male Sprague Dawley rats (Charles River) were weighed at 3 weeks old and randomly divided into three equal groups: 1) SED: sedentary control, 2) PA: twice weekly 1-h physical activity in a large box, and 3) EX: every-other-day voluntary wheel running access. One rat from SED and EX died part way through the experiment. Necropsies showed the deaths were isolated and unrelated to treatment. Rats were housed in pairs in climate-controlled rooms with 12-h light/dark cycle with light between 0700 and 1900. The study was performed in accordance with ethical procedures and policies approved by the Institutional Animal Care and Use Committee.

Paired animals resided in standard plastic cages (0.454×0.238 m) following the housing protocol in Alessio et al. (2005). SED animals remained housed in standard cages throughout the experiment. PA animals were removed from their cages twice per week for 1-h periods of physical activity in a large plastic box (0.9×0.6 m) that contained plastic tubes for exploration and movement. During each session, 6 animals were placed in each box and were monitored to ensure that all animals were active but not aggressive. Separate video recordings of animals in the PA group during activity in a large box were analyzed to estimate the distance covered in 1 h. Video recordings of a sample of animals in the SED group were made and analyzed to estimate the distance covered in a standard cage in 24 h. EX animals were rotated between being housed alone in cages equipped with running wheels and being housed in pairs in standard plastic cages. The exercise wheels (Nalgene, Rochester, New York) recorded the number of revolutions per day, translated to meters per day. Animals were similarly handled for weekly weighing and cage changes and biweekly blood pressures and heart rate measurements (Alessio et al., 2005).

Monthly systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) measurements were collected using a non-invasive tail cuff method. Animals were placed in a restrainer with the base of the tail placed through an IITC NIBP Sensor (IITC Life Sciences, Woodland Hills, CA). A heat lamp was used to warm the animal for approximately 2–3 min. Then, the sphygmomanometer and pressure cuff were inflated to approximately 200 mmHg and the IITC Model 31 NIBP amplifier and software (IITC Life Sciences, Woodland Hills, CA) measured pulse detection with a photoelectric sensor in a tail cuff. Once the initial threshold was reached, SBP was determined and then heart rate was measured by the sensing cuff and amplifier, which measured the amplitude and time between pulses in the rat's tail.

At age 16 months, half of the animals in each treatment group were sacrificed at rest and half following exhaustive exercise. Only animals at rest were investigated in this study. All animals were sacrificed by decapitation between 0900 and 1200 h, in the first part of the light/dark cycle. Within 1 min, whole blood was collected into small tubes, chilled and centrifuged to recover serum. Serum was deep frozen (-80 °C) until assayed for total cholesterol (TC), triglycerides (TG) and intracellular adhesion molecule (ICAM). Organs and tissues were immediately removed and snap frozen in liquid nitrogen. Left ventricles from the frozen hearts were used for RNA extraction and gene analyses.

Blood lipids

Fresh whole blood samples (35 μl) were analyzed using solid-phase and enzymatic methods to measure TG on a Cholestech LDX Analyzer (Cholestech Corporation, Model # 10-004, Hayward, CA) (Scholz-Issa et al., 1996).

ICAM

ICAM was measured in serum with an ELISA kit (Quantikine for rat sICAM, R&D Systems, Minneapolis MN).

Cholesterol

Serum cholesterol was measured in serum with an enzymatic method utilizing cholesterol esterase, cholesterol oxidase and peroxidase (Sigma Diagnostics Procedure 352, Sigma-Aldrich, St. Louis MO).

RNA extraction and gene microarray analyses

Total RNA was isolated from 36 heart tissue samples (SED, $n=12$; PA, $n=12$; EX, $n=12$) using the RNeasy Kit (Qiagen, Valencia CA). Approximately 100 mg from the left ventricle of heart tissues yielded 6–20 μg of total RNA. Homogenization of the heart tissue was done in a three-step process using a plastic pestle to disrupt the tissue in a microcentrifuge tube followed by a tissue homogenizer (Omni International, Inc., Warrenton VA). The homogenate was then pressed through a 20-gauge syringe in Buffer RLT (Qiagen, Valencia CA). RNA isolation followed the manufacture's protocol (Qiagen, Valencia CA).

The absorbance ratio (A_{260}/A_{280}) obtained from a ND-1000 UV/VIS spectrophotometer (Nanodrop Technologies, Inc., Montchanin DE) and the bands obtained with agarose gel electrophoresis indicated that the RNA was intact. RNA samples were additionally analyzed with the Agilent 2100 Bioanalyzer (Agilent Technologies) to evaluate the integrity of the RNA.

All samples had high integrity RNA and were hybridized to rat genome arrays for gene screening and tag expression. Samples were pooled within each treatment group to yield 4 gene chips per treatment group, with RNA from 3 animals on each chip, to ensure correct quantity of total RNA. The GeneChip® Rat Genome 230 2.0 Array was used for comprehensive screening of the transcribed rat genome on a single array (Roy et al., 2003). Microarray hybridization targets were arranged in reference to previous protocols (Roy et al., 2003). The arrays were washed and stained with streptavidin–phycoerythrin and scanned using the GeneArray scanner (Agilent Technologies). Raw data were analyzed with the Affymetrix Microarray Suite, version 5.0. Genes that significantly ($p<0.05$) changed expression (either up-regulated or down-regulated compared with the SED group) and had 75% concordance (genes were present in 3 of the 4 chips for each group) were identified as legal genes. The fold change and detection call (present, marginal, or absent) were additionally used to identify and filter legal genes. Expression queries were performed on the NetAffx™ Analysis Center to identify genes by functional topic from the rat genome using the keywords: cardiovascular, blood pressure, heart rate, and cholesterol.

Analysis of variance with repeated measures (ANOVA-RM) was used to compare physiological data collected over time in each group. A 3×2 ANOVA test was used to compare mean biomarkers among the three different experimental groups at 6 and 16 months. Sphericity was tested by the Greenhouse–

Geisser method, and the Bonferroni correction within each response–variable comparison was used to decrease the chance of a Type I error. Post hoc comparisons were made by Bonferroni and comparison–contrast tests. Physiological markers were reported as mean ± S.E.M. A statistical *t* test was used to select genes with differing expression. A probability level of 0.05 was set for significance.

Results

Body mass

We have previously reported that the following 2 months of regular exercise on the running wheel, male rats in the EX group consistently weighed less than PA and SED animals at ages 3–16 months (Alessio et al., 2005).

Distances covered in voluntary wheel running, physical activity, and a standard cage

Mean distance covered in the running wheel by animals in the EX group peaked at age 2 months (5642 ± 1773 m day^{-1}) and gradually declined, ranging between 1612 and 4836 m day^{-1} throughout most of the study. A separate analysis of locomotion showed that the mean distance covered in a large box by animals in the PA group ranged between 161 and 322 m day^{-1} and the mean distance covered by SED animals that resided solely in a standard cage averaged only 129 m day^{-1} (Alessio et al., 2005).

The substantial difference between distance covered by the EX compared to either the PA or SED groups indicates a dramatically different total amount of daily energy expenditure. Locomotion in a large box twice weekly for 1 h does not add substantially to the minimal distance covered in a standard cage. Both represent less than 10% of the mean distance covered in 24 h in the EX group.

Blood lipids

Mean serum TC at 16 months values are shown in Fig. 1. ANOVA indicated a significant difference ($F_{2,31}$=4.68) with Bonferroni post hoc analyses showing the EX<SED ($F_{1,17}$)=9.3,

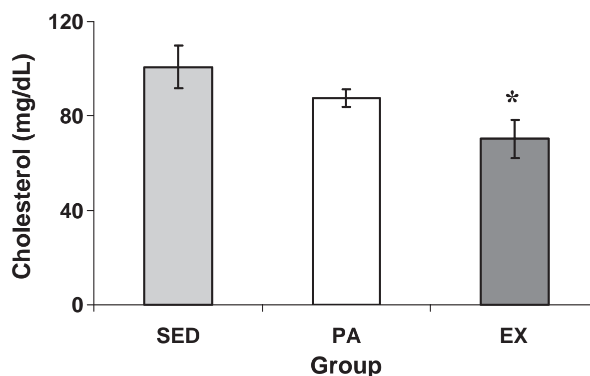

Fig. 1. Mean total cholesterol at 16 months in SED, PA, and EX (*EX<SED, $p<0.05$).

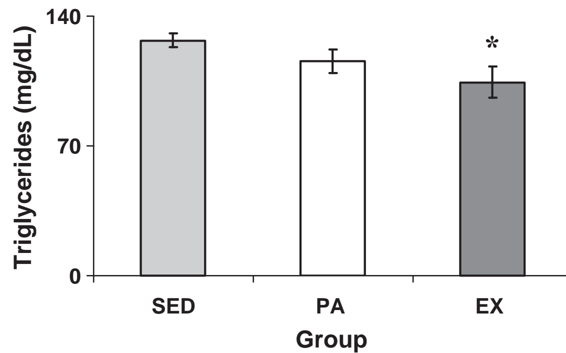

Fig. 2. Mean total triglycerides at 16 months in SED, PA, and EX (*EX < SED, $p < 0.05$).

$p < 0.05$. Mean TG at 16 month values (Fig. 2), showed similar results with EX < SED, $F_{1,19} = 5.74$, $p < 0.05$.

ICAM

Mean ICAM values did not differ among the three groups with SED = 17289 ± 2925 pg/dL, PA = 13689 ± 799 pg/dL, EX = 18059 ± 1372 pg/dL ($F_{2,26} = 2.36$, $p = 0.11$) (Fig. 3).

Resting blood pressure

Resting blood pressure changed over time in all three groups. Initially, all groups had mean SBP above the usual resting level of 120 mmHg and above 140 mmHg, the threshold for hypertension in Sprague Dawley rats (Johns et al., 1996; Bohlender et al., 1997). This was likely due to stress associated with the procedure, which included a restrainer for up to 10 min. After 5 months, however, most of the animals acclimated, and eventually resting SBP declined to levels that were lower than the first several month means (Fig. 4). SBP showed similar trends over time whereas

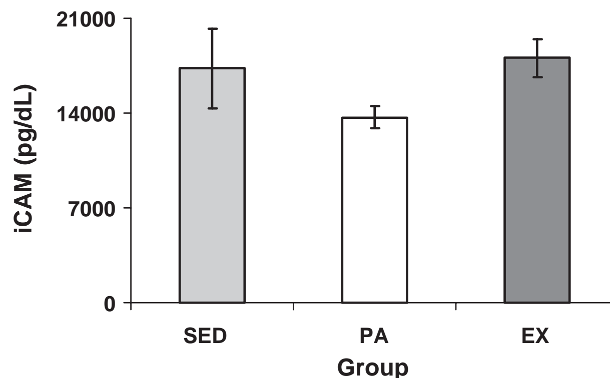

Fig. 3. Mean intracellular adhesion molecule levels at 16 months in SED, PA, and EX.

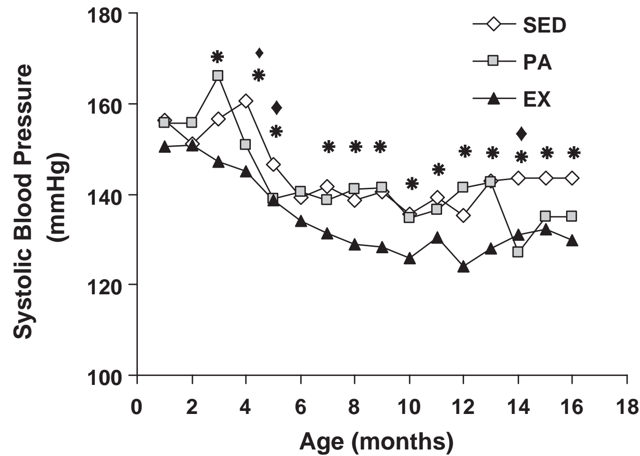

Fig. 4. Systolic blood pressure over time in SED, PA, and EX (♦SED > PA, *SED > EX, $p < 0.05$).

resting MAP (Fig. 5) and DBP did not (Fig. 6). A significant time effect for SBP ($F_{15,890} = 24.50$, $p < 0.05$) was observed, however, a dramatic decrease occurred in SBP after the fifth month, probably owing to acclimation rather than age. When comparing SBP among the three groups over time, after month 5, there was no group × time interaction effect, but there was a significant group effect ($F_{2,20} = 10.82$, $p < 0.05$). Bonferroni post hoc analyses indicated that SBP was higher in SED compared with EX (difference = 10.51, $p < 0.05$) and higher in SED compared with PA (difference = 5.74, $p < 0.05$).

Resting heart rate

At 16 months, mean HR values were 381 ± 7, 400 ± 10, and 405 ± 10 beats min^{-1} in the EX, PA, and SED groups, respectively, with EX < SED ($F_{1,28} = 4.5$, $p < 0.05$). Fig. 7 shows mean HR over 16 months

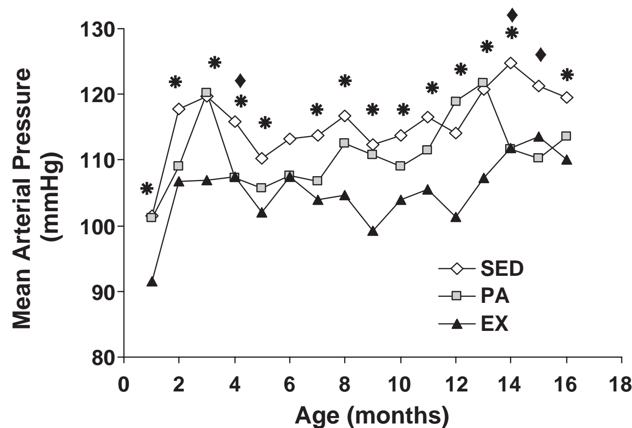

Fig. 5. Mean arterial pressure over time in SED, PA, and EX (♦SED > PA, *SED > EX, p < 0.05).

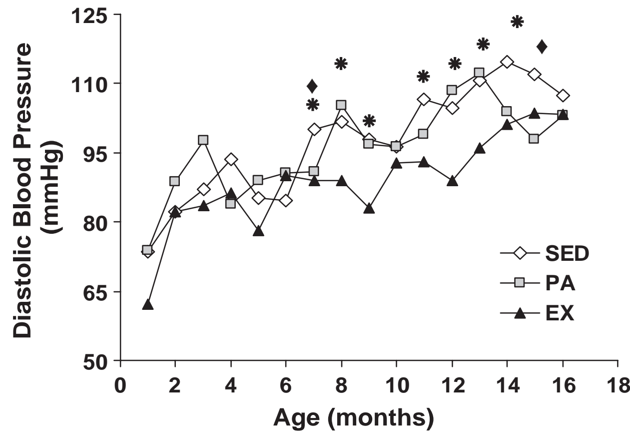

Fig. 6. Diastolic blood pressure over time in SED, PA, and EX (♦SED > PA, *SED > EX, $p < 0.05$).

with EX often, but not always, significantly lower than SED, and PA sometimes significantly lower than SED.

RNA and microarray results

For this study, we compared only the 120 genes identified as being important in cardiovascular regulation by the NetAffx™ Analysis Center software. Table 1 includes all PA and EX legal genes involved with CV regulation that were determined to be significantly different from SED animals ($p < 0.05$). Ten genes from the EX and 34 genes from the PA groups had different expression signals compared with SED. Nine genes were uniquely expressed by EX compared with either PA or SED. Three genes: *Edn1*, *Hmgb2*, and *Cd36* were identified in both PA and EX as being expressed differently compared with SED. *Edn1*, *Cd36*, and *Hmgb2* are involved in blood pressure, cell adhesion, and transcription and neurogenesis regulation, respectively. Two genes were only expressed in EX: *Gja1*,

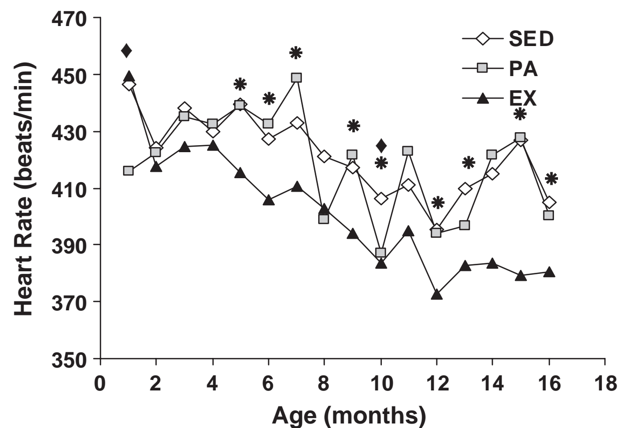

Fig. 7. Resting heart rate over time in SED, PA, and EX (♦SED > PA, *SED > EX, $p < 0.05$).

Table 1
PA and EX legal genes that were expressed differently from SED ($p<0.05$)

Gene symbol	Biological function	Gene expression value		
		SED	PA	EX
Edn1	Regulation of pH, G-protein coupled receptor protein signaling pathway, protein kinase cascade, respiratory gaseous exchange, regulation of blood pressure, positive regulation of cell proliferation, pathogenesis, glucose transport	926 ± 25	672 ± 52	655 ± 46
Hmgb2	Regulation of transcription, DNA-dependent, neurogenesis	2138 ± 47	2372 ± 93	2418 ± 86
Cd36	Fatty acid metabolism, transport, cell adhesion, long-chain fatty acid transport	47 763 ± 840	51 804 ± 1529	43 386 ± 758
Gja1	Cell communication, cell–cell signaling, regulation of heart rate	10 889 ± 100	–	8512 ± 705
Fdft1	Cholesterol biosynthesis, isoprenoid biosynthesis, lipid biosynthesis	5714 ± 102	–	5097 ± 146
Atp5g1	Transport, ion transport, proton transport, response to ethanol	73 691 ± 8123	–	83 167 ± 6801
Prkch	Protein amino acid phosphorylation, intracellular signaling cascade	753 ± 55	–	895 ± 56
Edg5	G-protein coupled receptor protein signaling pathway	2574 ± 236	–	1999 ± 248
B4galt6	Carbohydrate metabolism, glycosphingolipid biosynthesis	4811 ± 214	–	4954 ± 246
Yy1	Regulation of transcription, DNA-dependent, negative regulation of steroid metabolism	2337 ± 77	–	2709 ± 71
Hmgcl	–	4100 ± 42	3285 ± 144	–
Hmgcs1	Acetyl-CoA metabolism, cholesterol biosynthesis	2561 ± 154	3033 ± 143	–
Cyp11a	–	2420 ± 120	2022 ± 123	–
Cyp27b1	Electron transport, vitamin D metabolism	486 ± 44	316 ± 45	–
Lss	Steroid biosynthesis, metabolism	1632 ± 134	2197 ± 71	–
Abca1	–	273 ± 12	215 ± 18	–
Srebf1	Regulation of transcription, DNA-dependent, lipid metabolism, cholesterol metabolism, lipid biosynthesis, regulation of transcription	1443 ± 45	1212 ± 76	–
Vldlr	Lipid transport, endocytosis, cholesterol metabolism	6899 ± 144	7782 ± 333	–
Fads1	Fatty acid desaturation	1000 ± 24.6	1325 ± 74	–
Prdx6	Lipid catabolism	20 439 ± 1137	17 100 ± 355	–
Sc4mol	Metabolism, sterol biosynthesis	1504 ± 102	2053 ± 116	–
Ptgds2	Prostaglandin biosynthesis	535 ± 41	387 ± 36	–
Pggt1b	Protein amino acid geranylgeranylation	361 ± 20	607 ± 64	–
Gpd2	Glycerol-3-phosphate metabolism, electron transport	1461 ± 58	1988 ± 175	–
Hmox1	Phospholipid metabolism, heme oxidation, intracellular signaling cascade, small GTPase mediated signal transduction, DNA damage response, signal transduction resulting in induction of apoptosis	744 ± 49	947 ± 43	–
Pik3r1	Intracellular signaling cascade, B-cell differentiation	2862 ± 233	2462 ± 330	–
Atp5g2	Proton transport	34 019 ± 2417	23 766 ± 696	–
Dbi	Acyl-CoA metabolism, steroid biosynthesis, transport	16 931 ± 872	12 071 ± 658	–

Table 1 (*continued*)

Gene symbol	Biological function	Gene expression value		
		SED	PA	EX
Pik3r1	Intracellular signaling cascade, B-cell differentiation	5800 ± 771	9662 ± 1460	–
Fdps	Cholesterol biosynthesis, isoprenoid biosynthesis	1632 ± 134	2197 ± 71	–
–	Electron transport, fatty acid metabolism, fatty acid beta-oxidation	799 ± 44	615 ± 26	–
Acox3	Protein amino acid phosphorylation, signal transduction	1694 ± 115	2211 ± 108	–
Prkcl1	Fatty acid metabolism, metabolism	1566 ± 303	1186 ± 84	–
Facl5	Lipid metabolism, signal transduction, intracellular signaling cascade, phototransduction	2056 ± 81	2523 ± 137	–
Plcb4	Fatty acid metabolism, metabolism, regulation of fatty acid metabolism	8164 ± 381	9612 ± 335	–
Facl4	Response to superoxide, fatty acid metabolism, transport, mitochondrial transport	1394 ± 42	1603 ± 11	–
Ucp3	Xenobiotic metabolism, lipid transport	2175 ± 297	1650 ± 217	–
Lbp	Lipid metabolism, fatty acid metabolism, metabolism	583 ± 43	677 ± 54	–
Facl2	Regulation of transcription, DNA-dependent, lipid metabolism, cholesterol metabolism, lipid biosynthesis, regulation of transcription	21 034 ± 915	23 964 ± 165	–
Srebf1	Lipid transport, endocytosis, cholesterol metabolism	1443 ± 45	1712 ± 45	–
Lipe	Endocytosis	1142 ± 91	709 ± 43	–
Cav	Regulation of transcription, DNA-dependent, neurogenesis	17 743 ± 86	21 526 ± 817	–

which regulates heart rate, blood pressure, and cholesterol, and *Fdft1*, which regulates lipid and cholesterol biosynthesis. Fig. 8a–e shows mean gene expression of SED, PA, and EX and chromosome location for genes: *Gja1, Fdft1, Edn1, Cd36* and *Hmgb2*.

Discussion

Exercise is one of many factors that can influence specific gene expression (Diffee et al., 2003). It is not known which genes are down-regulated or up-regulated in response to regular exercise or what impact modified gene expression has on physiological markers of disease, such as CVD. We chose DNA microarray analysis to obtain data about genes that might serve as useful indicators of biological changes associated with CVD. The GeneChip Rat Genome 230 2.0 Array provided over 30,000 transcripts and variants from the rat genome. Physiological measurements provided various indices of CV health and disease.

We realize that there are shortcomings to using DNA microarray analysis to identify genes associated with physiological markers. For example, it is difficult to identify cause-and-effect relations between select gene expressions and physiological function because up-regulated gene expression does not always mean more protein due to negative feedback loops. Furthermore, sensitivity changes in target cell receptors may affect the end result of an elevated gene expression. Most importantly, the physiological function of many genes and proteins is not well understood, so linking altered gene regulation with physiological changes is not simple. However, by comparing large numbers of genes, well-established

physiological markers, and several levels of exercise we have obtained valuable information from the DNA microarray approach and are able to hypothesize about the roles that sedentary and exercise behavior play in the gene–environment influence on CV health.

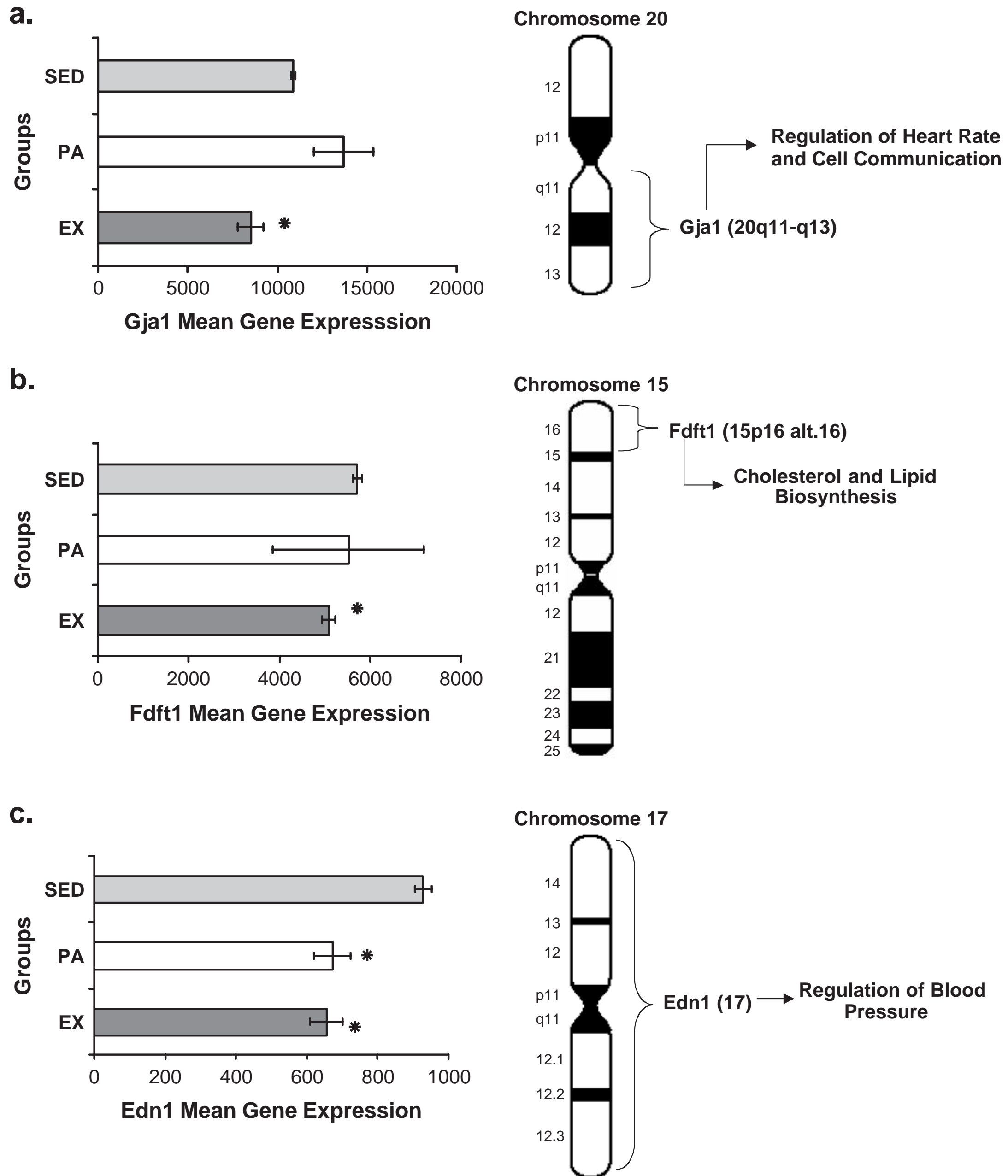

Fig. 8. Mean gene expression for SED, PA and EX with predicted chromosome location for (a) *Gja1*, (b) *Fdft1*, and (c) *Edn1* (d) *Cd36*, and (e) *Hmgb2* (*SED significantly different from PA and/or EX, $p < 0.05$). Figures are modification from The Rat Genome Database.

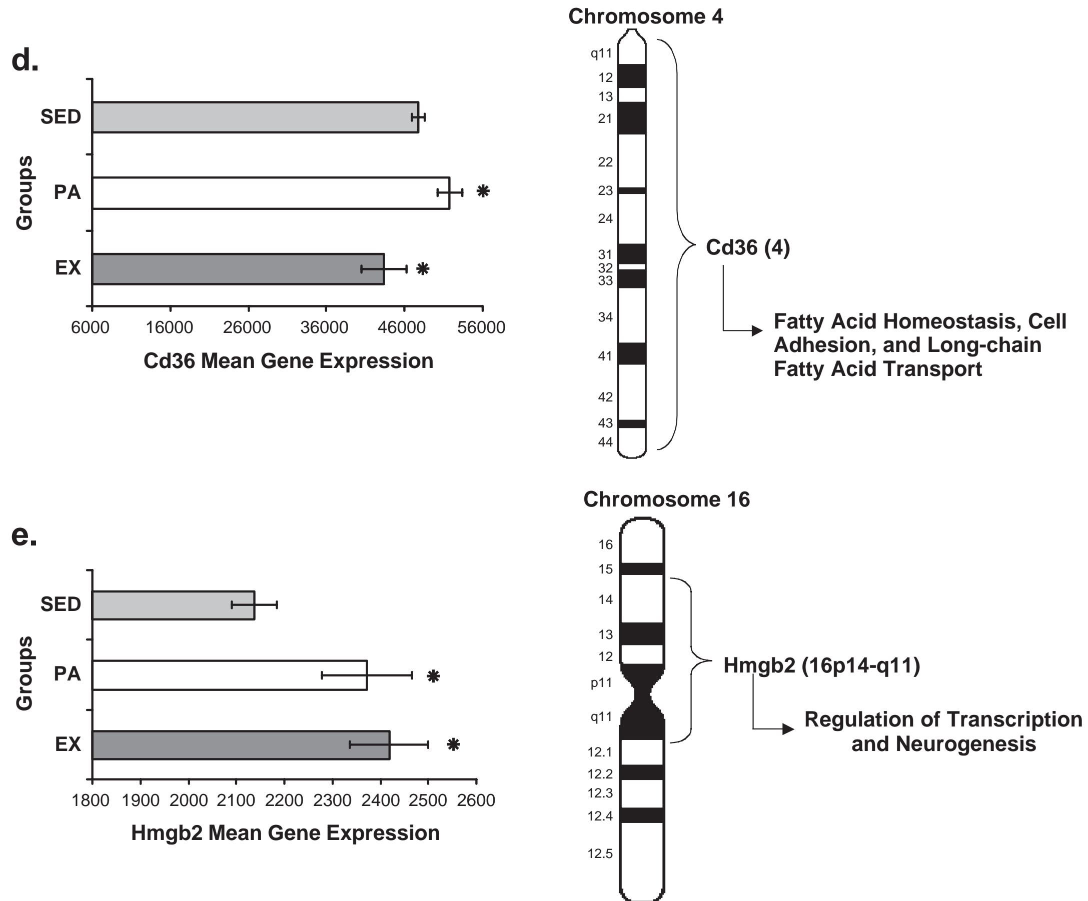

Fig. 8 (*continued*).

Most studies that investigate exercise-induced changes on a variety of health variables use a motorized treadmill, group swimming, or individual running wheels for exercise intervention. The control animals for such studies are often sedentary animals with no access to exercise. CVD risk may be increased in animals where access to physical activity outside a standard cage is limited. Mueller et al. (1997) have stated that the sedentary control condition, which is often used as a basis for comparison in animal studies, may not really represent a normal baseline. It is well known that providing access to running wheels for exercise results in significant improvements to many key biomarkers of health, including body weight and body composition (Narath et al., 2001). We tested a compromise physical activity intervention that was designed to change the activity level from sedentary to at least moderately active in animals that resided solely in a cage. Twice weekly, six animals were placed in a large box and allowed to move about freely for 1-h sessions. The majority of activity occurred within the first 30 min of each session, but activity was still observed during the remaining 30 min. We hypothesized that the PA group would achieve a level of activity that was higher than a SED group, which resided primarily in a standard animal cage, but lower than an EX group which had 24-h access to a running wheel every-other-day.

We were interested in determining if regular exercise and/or twice weekly 1-h PA sessions resulted in changes in CV gene expression and physiological biomarkers. It has been well accepted among researchers that physical activity provides protection against CVD. For example, a study by Seip et al. (1995) suggested that exercise increased gene expression for proteins known to play a role in lipoprotein metabolism. Environmental factors in addition to the genetic contribution of the WNK1 and WNK4 genes have been found to influence CVD, particularly hypertension (Garcia et al., 2003). In the current study, thirty-four genes were expressed differently between SED and PA. Ten were expressed differently between SED and EX, including *Fdft1* which is located on chromosome 15p16alt.16 and *Gja1* which is located on chromosome 20q11–q13. EX and PA shared three genes that were significantly different from SED: *Edn1*, *Hmbg2*, and *Cd36*. *Edn1*, located on chromosome 17, codes for a potent vasoconstrictor peptide produced by vascular endothelial cells that regulate blood pressure. *Hmbg2*, located on chromosome 16p14–q11, is involved in regulation of transcription and neurogenesis. *Cd36*, located on chromosome 4, is associated with fatty acid homeostasis, cell adhesion, and long-chain fatty acid (palmitate) transport.

Edn1 transcript level was highest in the SED (926 ± 25), compared with PA (672 ± 52) and EX (655 ± 46). A lower expression of *Edn1* corresponds with a more relaxed, dilated vasotone and is associated with low systolic blood pressure. Higher levels of *Edn1* have been shown to parallel a type of calcium channel, T-type Ca^{2+} current, that appears in cardiac failure (Izumi et al., 2003). Other studies that have measured *Edn1* in either the aorta or heart muscle have reported contradictory results. Maeda et al. (2002) reported that *Edn1* mRNA in the aorta was markedly lower in a sedentary aged group compared with a sedentary young group, whereas it was significantly higher in a trained aged group compared with a sedentary aged group. Their data indicated that *Edn1* mRNA in the aorta decreases with age and increases with exercise training. In contrast, Iemitsu et al. (2003) reported that mRNA expression of *Edn1* in the heart increased with age. Similar to Maeda et al. (2002), Iemitsu et al. (2003) found *Edn1* expression was significantly higher in an exercise-trained aged group compared with a sedentary aged group. *Edn1* was associated with exercise-induced cardiac hypertrophy. The present *Edn1* results show that the SED group had elevated *Edn1* expression and higher SBP compared with EX. In contrast, EX had the lowest *Edn1* expression and lowest SBP.

Hmgb2 (High mobility group box 2) was expressed at the lowest level in SED (2137 ± 47) compared with PA (2372 ± 93) and EX (2418 ± 85). HMBG2 proteins are small and relatively abundant chromatin-associated proteins. They act primarily as architectural facilitators in the regulation of nucleoprotein complexes specifically in the assembly of complexes involved in recombination and transcription (Travers, 2003). Recent genetic and biochemical evidence suggests that these proteins can facilitate nucleosome remodeling. It is not clear if the higher *Hmgb2* transcript levels in the EX group would directly influence development of CVD.

Cd36 transcript levels were highest in PA (51803 ± 1528) and lower in SED (47763 ± 840) and EX (43385 ± 758). A human study reported that *Cd36* transcript abundance increased following exercise training (Turnstall et al., 2002), suggesting greater fatty acid uptake across the plasma membrane and subsequent fat oxidation. Several studies have examined the role of *Cd36* in animal models as a possible mediator of blood pressure regulation. Although some have reported that *Cd36* has no influence on blood pressure (Kadlevoca et al., 2004), Greenwalt et al. (1995) found an up-regulation of Cd36 protein in hypertensive animals.

Blood lipids measured in this study followed a stepwise pattern with EX < PA < SED, but mean TC and TG were significantly different only between the EX and SED group. The percent difference in TG

between the EX and SED (−22%) was not as large as the 55% decrease reported by Suzuki and Machida (1995), although a different strain of rat was used in the study. However, our TC (40%) difference between EX and SED approached this level. ICAM did not show a similar pattern as TC and TG and no significant differences were reported in ICAM among the groups. HR, SBP, and MAP showed a similar relation among the three groups throughout most of the 16 months, with EX < PA < SED. Our SBP results showed a consistent difference between SED and EX in the range of 10%, ending with an 8.5% difference at 16 months which was similar to Suzuki and Machida (1995), in which SBP was lowered 7% by running wheel exercise.

Access to regular running wheel exercise appears to minimize some of the consequences associated with an AL diet by reducing TC, TG, HR, SBP, and MAP. Providing access to 1 h of twice weekly PA was a compromise intervention between the usual sedentary housing and voluntary wheel running. Based upon the CV variables measured in this study, the PA intervention offered minimal benefits over the SED animals, consistent with the minimal increase in exercise we noted for the PA animals compared to the SED animals. A separate analysis of distance covered in the large box with the lights on compared with the lights off, indicated that the total distance covered was twice as high during the dark. Nevertheless, the total distance covered in the large box during 1 h in the darkness still averaged less than 0.3 miles. We did note that mean PA values for most of the CV variables measured were in between EX and SED, suggesting that more frequent sessions in activity boxes may result in substantial differences between SED and PA animals in future experiments. Although there were more differences in gene expression between PA and SED compared to EX and SED, a close inspection of the data showed that in several instances genes with similar biological functions were expressed in opposite directions in the PA animals. It is possible that in the PA treatment, simultaneous induction and repression of closely related genes canceled out any expression that affected biological outcome such as cholesterol or blood pressure.

Gene expressions of *Gja1, Fdft1, Cd36*, and *Edn1* showed a trend where greater expression of each gene was associated with higher blood pressure. DNA microarray data identifies genes that may be expressed differently in animals from different treatments. More robust, quantitative data can be obtained by real time PCR analysis of the genes that were identified in this paper. Additional insights into the effects of sedentary, limited activity, and exercise behaviors will be obtained when we analyze our gene array data for genes involved in other exercise-related physiological conditions including the metabolic syndrome and cognitive abilities. In this study, a comparison of gene expression of *Gja1, Fdft1, Cd36, Hmgb2* and *Edn1* within the treatment groups demonstrated evidence that two distinct activity levels: 1. sedentary and 2. regular voluntary running exercise, did notably affect gene expression.

In conclusion, levels of expression of 44 CV regulatory genes were significantly different between SED and PA (34 gene differences) and EX (10 gene differences) with three, *Edn1*, *Hmgb2*, and *Cd36* shared between PA and EX. Physiological outcome differences were reported between SED and EX in TC, TG, HR, SBP, and MAP measurements. Animals with regular access to running wheel exercise expressed nine unique CV regulatory genes and, compared with PA and SED, demonstrated the healthiest CV profiles. Compared with PA and EX, animals with no access to physical activity or exercise displayed the most evidence of CVD.

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