Gene Expression Profiling of Exercise-induced Cardiovascular Adaptations: Molecular Insight from Microarray Analyses

Motoyuki Iemitsu, Seiji Maeda, Takashi Miyauchi, Shinya Kuno, Kazuo Murakami and Mitsuo Matsuda

Exercise training causes some physiological cardiovascular adaptations, which act to enhance cardiac and vascular functions at rest and during exercise. However, the molecular mechanisms of these adaptations are unclear. We investigated gene expression profiles of exercise training-induced cardiovascular adaptations. In the experiment, rats exercised on a treadmill for 4 or 8 weeks (4WT and 8WT). The differences in expression levels of 3,800 genes in the heart and abdominal aorta of sedentary control and exercise-trained rats were compared by the microarray analysis. Of the 3,800 genes analyzed in the microarray analyses, in the heart, a total of 45 genes (upregulation of 3 genes and downregulation of 42 genes) in the 4WT and 74 genes (upregulation of 50 genes and downregulation of 24 genes) in the 8WT displayed altered gene expression with exercise training. In the aorta, a total of 57 genes (upregulation of 35 genes and downregulation of 24 genes) in the 4WT and 31 genes (upregulation of 12 genes and downregulation of 19 genes) in the 8WT displayed altered gene expression with exercise training. Thus exercise training caused an alteration of many genes expression in the heart and aorta. The alteration of many genes expression in the heart observed in 8WT, whereas that in the aorta induced in 4WT. The observed difference in the change of the gene expression in the time course between the heart and aorta suggests that there may be a difference in the time course of exercise-induced physiological adaptation in the heart and aorta (e.g., formation of physiological cardiac hypertrophy and enhancement of arterial compliance).

Keywords: exercise training, microarray, heart, abdominal aorta

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production, enhances arterial compliance [Delp and Laughlin, (1997); Maeda, et al., (2001); Maeda, et al., (2003); Maeda, et al., (2004)]. Moreover, exercise training induces enlargement of the lumenal area in the artery [Miyachi, et al., (1998); Miyachi, et al., (2001)]. These exercise training-induced adaptations have preventive and treatment effects for arterial stiffening, atherosclerosis, and a reduction in the risk factor of other cardiovascular diseases. Therefore, exercise training brings beneficial adaptations to the cardiovascular system. However, the molecular mechanisms of these adaptations are unclear.

Essential exercise training periods for these beneficial adaptations differ between the heart and arteries. While long-term (no less than 8 weeks) exercise training developed physiological cardiac hypertrophy [Schaible and Scheuer, (1979); Tharp and Wagner, (1982)], a reduction in arterial stiffness was exhibited through short-term (for 4 weeks) exercise training [Delp and Laughlin, (1997)]. Therefore, we hypothesized that essential exercise training periods for molecular adaptive inductions differ between the heart and arteries.

DNA microarray provides a powerful and efficient tool to compare the differential expression of a large number of genes in a single reaction and enables a systematic analysis of responses of various gene expressions to exercise training. It can be suggested that this powerful and robust technique could help identify the molecular and physiological mechanism underlying the exercise training-induced cardiovascular adaptations. In the present study, the microarray analysis was first used to compare differences in expression levels of 3,800 genes in the left ventricular myocardium and abdominal aorta of sedentary and exercise-trained rats. The time course of exercise training-induced alteration in gene expression manners differs between the heart and abdominal aorta was also investigated.

2. Methods

2.1. Animals and Protocol

The experimental protocols were approved by the Committee on Animal Research at the University of Tsukuba. Male 4-week-old Sprague-Dawley rats (body weight: 68.0-73.5 g) were cared for according to guidelines of the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration. These rats were maintained on a 12:12-h light-dark cycle and received regular food and water ad libitum. Ten rats underwent a 4-week or 8-week treadmill running protocol (4WT; N = 5, and 8WT; N = 5). The rats were trained 5 days/week on a motor-driven treadmill for 15 min/day at 15 m/min for the first 2 days. Duration and intensity of the treadmill training increased daily during the first week until the rats were running for 60 min at 30 m/min, 0% grade. Thereafter, exercise intensity was maintained at this level for the remainder of the training. Another group of 10 rats were confined to their cages for 4 or 8 weeks (4WC; N = 5, and 8WC; N = 5), but were handled daily. All the post-training measurements in the 4WT and 8WT were performed 48h after the last exercise bout in order to avoid acute effects of exercise. After the physiological/hemodynamic measurements, the whole heart and abdominal aorta were rapidly excised and washed thoroughly with cold saline to remove contaminating blood, then the left ventricle was separated from the right ventricle and atria. The left ventricle and abdominal aorta were weighed, frozen in liquid nitrogen, and stored for DNA microarray. 4WC and 8WC rats were killed at the same time point as 4WT and 8WT rats (8 and 12 weeks old).

2.2. Hemodynamic Measurements

On the day of the experiment, the rats were anesthetized with pentobarbital sodium (40 mg/kg body weight i.p.) and transthoracic echocardiography was performed with an echocardiographic system (SSD-900, Aloka, Tokyo, Japan) equipped with a 7.5 MHz convex scan probe as previously described [Iemitsu, et al., (2002)]. The left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), and left ventricular wall thickness at end-diastole were measured. After the echocardiographic measurement, a microtip pressure transducer catheter (SPC-320, Millar Instruments, Houston, TX, USA) was inserted into the left carotid artery, and arterial blood pressure and heart rate were monitored as previously described [Iemitsu, et al., (2001); Sakai, et al., (1996)]. These hemodynamic parameters were recorded using a polygraph system. The body temperature of the rat was maintained at 37°C using a small animal warmer.
2.3. Measurements of aortic pulse wave velocity (PWV)

After the hemodynamic measurement, the aortic PWV was measured as previously outlined in Fitch, et al., (2001). To measure the aortic PWV, two 1.4 Fr. Millar microtip pressure transducer catheter (SPC-320) were implanted, one in the aortic arch via the left carotid artery, and one in the proximal abdominal aortic bifurcation via the left femoral artery. Pulse pressure waves from the two Millar transducers were simultaneously imported to an amplifier and displayed on a data acquisition system (MP100A-CE, Biopack systems, Santa Barbara, CA, USA) at a sampling rate of 1000 Hz. After the experiment was completed, the full length of the aorta was exposed. Propagation time from the aortic arch to abdominal aortic bifurcation in the pressure transducers is the difference in time between the beginning of the upstroke of each pulse wave forms. To measure the pulse wave propagation distance, a silk thread was placed along the aorta and marked at the tips of the two pressure transducers, the thread was removed, and then the straight distance between the two marks were measured. Aortic PWV was calculated by dividing the propagation distance by the propagation time.

2.4. RNA Isolation

Total tissue RNA was isolated by using guanidinium thiocyanate-phenol-chloroform acid extraction with Isogen (Nippon Gene, Toyama, Japan) as previously described in detail [Iemitsu, et al., (2001); Iemitsu, et al., (2003); Iemitsu, et al., (2002)]. Briefly, the tissue was homogenized in Isogen with a Polytron tissue homogenizer. The precipitated RNA was extracted with chloroform, precipitated with isopropanol, and washed with 75% (vol/vol) ethanol. Total tissue RNA was DNase-treated with a Qiagen Rnase-free DNase kit. Then, the samples were purified with a Qiagen Rneasy mini kit to improve the quality of the total RNA. The RNA concentration was determined spectrophotometrically at 260 nm, while the RNA quality was judged based on the ratio of absorbance at 260 nm and 280 nm. Furthermore, RNA samples were analyzed using Agilent 2100 bioanalyzer (Agilent technologies, Palo Alto, CA, USA) and stained to check for integrity of 18 and 28S RNA. For microarray analysis, total RNA of 3 rats/group was analyzed.

2.5. Probe Synthesis and Hybridization

Labeled cDNA probes were synthesized from 20 µg total RNA using the Atlas Glass Fluorescent Labeling kit (BD Biosciences Clontech, Palo Alto, CA, USA) as previously described [Narayanan, et al., (2001); Sergeant, et al., (2002)]. This kit uses indirect, two-step labeling of the target cDNA that incorporates higher levels of labeling than direct, single-step procedures that directly incorporate fluorescently-tagged nucleotides during cDNA synthesis. Aminoallyl-dUTP was incorporated during first-strand cDNA synthesis. Cy3 monofunctional reactive dye (Amersham Biosciences, Piscataway, NJ, USA) was covalently coupled to labeled aminoallyl-dUTP in the first-strand cDNA. The resulting labeled cDNA probe was purified using the Atlas NucleoSpin Extraction kit (BD Biosciences Clontech). The absorbance of labeled cDNA probe (Cy3) was determined spectrophotometrically at 550 nm. The labeled cDNA probe was hybridized with the Atlas glass microarray 3.8.I Rat (BD Biosciences Clontech), and the arrays were incubated overnight at 50 °C. Following hybridization, arrays were washed, dried, and then scanned [Narayanan, et al., (2001); Sergeant, et al., (2002)].

2.6. Microarray Analyses

The fluorescent images of hybridized microarray were scanned with a fluorescence laser confocal slide scanner (GenePix 4000B; Axon Instruments, Union, CA, USA). Images were acquired and analyzed using Array Gauge 1.31 (Fuji Film, Tokyo, Japan) software. Normalization of fluorescence intensities for each glass arrays was rescaled by a normalization coefficient to total fluorescence intensities of glass array in the control rats. This normalization of fluorescence intensities is necessary in order to eliminate the effects of array-to-array variations in hybridization and scanning. Background fluorescence intensities for each spot were subtracted to leave actual fluorescence intensities for each spot on the array. The gene expression was considered positive if the fluorescence intensity was higher than that produced by the respective negative control within each block on the array. Gene expression ratios were calculated by dividing the normalized
fluorescence intensities from trained rats by those from control rats. Differences in gene expression ≥ 3-fold changes were considered significant in this experiment. Accordingly, the expression ratios of greater than 3.0 or less than 0.33 were considered significant in the present study.

2.7. Statistical Analysis

Values are expressed as mean±SE. Statistical analysis was carried out by analysis of variance followed by Scheffé’s F-test for multiple comparisons. Statistical significance was set at *P<0.05 vs. 4WC and **P<0.05 vs. 8WC.

3. Results

There were no significant differences in body weight between 4WC and 4WT rats, while body weight in 8WT was significantly lower than in 8WC (Table 1) rats. There were no significant differences in left ventricular mass index between 4WC and 4WT, while left ventricular mass index in 8WT was significantly higher than that in 8WC. Similarly, there were no significant differences in systolic and diastolic blood pressure and LVESD between 4WC and 4WT, and between 8WC and 8WT. There were also no significant differences in heart rate, LVEDD and left ventricular wall thickness 4WC and 4WT, while the heart rate was lower and LVEDD and left ventricular wall thickness in 8WT were higher than in 8WC (Table 1) rats. PWV was significantly lower in 4WT than in 4WC, and in 8WT than in 8WC (Table 1).

Figure 1 shows the scatter plots (3,800 genes) of sedentary control rats (4WC and 8WC) and exercise trained rats (4WT and 8WT) in the heart. In the aorta, a total of 45 genes (upregulation of 3 genes and downregulation of 42 genes) in the 4WT and 74 genes (upregulation of 50 genes and downregulation of 24 genes) in the 8WT displayed altered gene expression with exercise training (Table 2). Common altered expression in the both 4-week and 8-week exercise training was detected in 1 gene (downregulation) in the heart. Figure 2 shows the scatter plots (3,800 genes) of sedentary control rats (4WC and 8WC) and exercise trained rats (4WT and 8WT) in the abdominal aorta. In the aorta, a total of 57 genes (upregulation of 35 genes and downregulation of 24 genes) in the 4WT and 31 genes (upregulation of 12 genes and downregulation of 19 genes) in the 8WT displayed altered gene expression with exercise training (Table 2). Common altered expression in the both 4-week and 8-week exercise training was detected in 1 gene (downregulation) in the aorta.

### Table 1: Some selected characteristics of both sedentary control and exercise-trained rats

<table>
<thead>
<tr>
<th></th>
<th>4WC</th>
<th>4WT</th>
<th>8WC</th>
<th>8WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>294±4</td>
<td>280±5</td>
<td>436±4</td>
<td>402±8**</td>
</tr>
<tr>
<td>Left ventricle weight, mg</td>
<td>650±20</td>
<td>666±19</td>
<td>782±24</td>
<td>820±29</td>
</tr>
<tr>
<td>Left ventricular index, mg/g</td>
<td>2.14±0.03</td>
<td>2.21±0.03</td>
<td>1.98±0.03</td>
<td>2.20±0.04**</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>106±5</td>
<td>105±4</td>
<td>116±6</td>
<td>116±3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>83±4</td>
<td>85±3</td>
<td>88±8</td>
<td>95±3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>392±5</td>
<td>378±7</td>
<td>380±13</td>
<td>343±13**</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.8±0.1</td>
<td>2.8±0.1</td>
<td>3.3±0.1</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>5.5±0.1</td>
<td>5.6±0.1</td>
<td>6.3±0.2</td>
<td>6.8±0.3**</td>
</tr>
<tr>
<td>Left ventricular wall thickness, mm</td>
<td>0.90±0.04</td>
<td>0.95±0.02</td>
<td>1.05±0.04</td>
<td>1.16±0.02**</td>
</tr>
<tr>
<td>Pulse wave velocity, m/sec</td>
<td>0.67±0.05</td>
<td>0.55±0.03*</td>
<td>0.66±0.04</td>
<td>0.51±0.03**</td>
</tr>
</tbody>
</table>

Values are means ± SE. 4WC = 4-week sedentary control rats, 4WT = 4-week exercise trained rats, 8WC = 8-week sedentary control rats, 8WT = 8-week exercise trained rats, n = number of rats, LVESD = left ventricular end-systolic diameter, LVEDD = left ventricular end-diastolic diameter. * P<0.05 vs. 4WC and ** P<0.05 vs. 8WC.
expression in the both heart and artery was detected in 1 gene (downregulation) in 8-week exercise training.

4. Discussion

In the present study, exercise training performed for 8 weeks induced cardiac hypertrophic adaptations, as shown by the significant increases in LV mass index, LVEDD and LV wall thickness and decreases in resting heart rate. The exercise-trained rats (both exercise trainings for 4 weeks and 8 weeks) demonstrated lower arterial stiffness compared with sedentary controls as evidenced by a reduction in aortic PWV. Therefore, it is suggested that exercise training for 4-8 weeks brings beneficial adaptations for cardiovascular functions. Using DNA microarray technique, a total of 45 relevant genes were identified in the heart and 57 relevant genes in the aorta for 4-week exercise training and a total of 74 relevant genes in the heart and 31 relevant genes in the aorta for 8-week exercise training that displayed greater than 3-fold changes in gene expression. These results suggest that the alteration of various gene expressions participates in a molecular mechanism underlying exercise-training-induced cardiovascular adaptations. Furthermore, the alteration of many genes expression in the heart observed in 8 weeks exercise training, whereas that in the aorta induced in 4 weeks exercise training, and these detected genes change tissue-specifically in heart and aorta. The observed difference in the change of the gene expression in the time course between the heart and aorta suggests that there may be a difference in the time course of exercise-induced physiological adaptation in the heart

and aorta.

Long-term (for 8 weeks) exercise training developed physiological cardiac hypertrophy, while an improvement of arterial stiffness was exhibited by short-term (for 4 weeks) exercise training and this improvement continued through 8-week exercise training. These results suggest that the essential exercise training periods for beneficial adaptations differ between heart and aorta, i.e., exercise training-induced adaptation is more fast in the aorta than that in the heart. In the microarray analysis of the present study, the gene expression in the heart was changed more noticeably in the exercise trained rats for 8 weeks than for 4 weeks. This indicates that the alteration of gene expression in the heart gradually upregulates during 4-8 weeks exercise training. However, the gene expression in the aorta changed more noticeably in the rats that exercise trained for 4 weeks compared with that for 8 weeks. Therefore, it is considered that a difference of the essential exercise training periods for the beneficial adaptation between the heart and aorta is one of the causal factors for the difference in molecular adaptations between the heart and aorta.

We have recently shown that gene expressions of various cardiovascular regulating factors, such as brain natriuretic peptide, angiotensin-converting enzyme, and beta-myosin heavy chain (MHC) and energy metabolic enzymes, such as aco CoA synthase, isocitrate dehydrogenase, lactate dehydrogenase, and phosphofructokinase were not changed, while gene expressions of alpha-MHC, beta1-adrenergic receptor, carnitine palmitoyl transferase-II were increased by exercise training.
in the heart [Iemitsu, et al., (2001); Iemitsu, et al., (2003)]. Furthermore, several studies also have reported that other genes related to cardiac function and hypertrophy, were not changed or up-/down-regulated by exercise training in the heart [Buttrick, et al., (1994); Calderone, et al., (2001); Jin, et al., (2000); Wisloff, et al., (2002)]. In the aorta, Delp and Laughlin (1997) have demonstrated that gene expression of endothelial nitric oxide synthase (eNOS), which implicated in mechanisms underlying the exercise training-induced vasodilation, was increased by exercise training for 4-10 weeks. Thus, previous studies have reported analysis of exercise training-induced alteration in genes, which implicates in function, structure, and energy metabolism, in the heart and aorta at small scales. However, the molecular mechanisms underlying exercise training-induced cardiovascular adaptations are unclear. The present study showed each profile of 3,800 gene expression levels in the heart and aorta for 4 and 8 weeks exercise training, and indicate that exercise training induced alteration of 118 genes (total 4- and 8-week exercise training-induced alteration genes) in the heart and 87 genes in the aorta. These altered genes may be candidate genes in molecular mechanism underlying exercise adaptation.

Figure 1  Scatter plots of 3,800 genes in sedentary control rats (4WC and 8WC) and exercise trained rats (4WT and 8WT) of the heart using DNA microarray analysis. A: Comparison 4WT (Y-axis) to 4WC (X-axis) hearts. B: Comparison 8WT (Y-axis) to 8WC (X-axis) hearts. Differences in gene expression ≥ 3-fold changes in trained rat compared with control rat are considered significant. The two solid lines are a base-line of the expression ratios of greater than 3.0 or less than 0.33 (red square plots). The gray area shows that fluorescence intensity was lower than that produced by the respective negative control within each block on the array.

Note: 4WC=4-week sedentary control rats, 4WT=4-week exercise trained rats, 8WC=8-week sedentary control rats, 8WT=8-week exercise trained rats
training-induced cardiovascular adaptations.

The present study demonstrates that exercise training for 4-8 weeks causes the change of expression of many genes in the heart and the aorta. We demonstrated that aortic distensibility and the production of endothelium-derived vasoactive substances, i.e., nitric oxide and endothelin-1, were improved by exercise training for 8 weeks in humans, but returned to the basal level (pre-exercise training level) in the 8 weeks after the end of exercise training [Kakiyama, et al., (2005); Maeda, et al., (2001)]. Thus, it is possible to suggest that the regulation of gene expression participates in the mechanism underlying the appearance and/or disappearance of exercise training-induced cardiovascular adaptations. It is also suggested that the changed genes through exercise training are able to participate in a critical protein/peptide and/or regulation of its synthesis pathway of exercise training-induced beneficial adaptation in the heart and aorta. Therefore, the candidate genes in the present study would be applicable to a target gene of genetic polymorphism, which is one of the causal factors for individual difference of exercise training-induced cardiovascular adaptations.

Shepherd and Gollnick (1976) reported that the present exercise intensity (30 m/min) of treadmill running in rats is about 75-80% of maximal oxygen consumption. This intensity of running exercise caused cardiac hypertrophy [Schaible and Scheuer, (1979); Tharp and Wagner, (1982)]. Furthermore, it has been reported that an anaerobic threshold (AT) level of a rat is at an exercise intensity during a speed of 25 m/min in treadmill running [Pilis, et al., (1993)]. Therefore, the present exercise intensity (30 m/min) of treadmill running in rats was higher than the AT level of rats. Taken together, it is considered that exercise training in the present study induced left ventricular hypertrophy with the increase in LVEDD. Moreover, the exercise intensity during exercise training in the present study was set at the same intensity of 30 m/min for 8 weeks. There may be difference in the relative exercise intensity between the 4-week exercise training and the 8-week exercise training by the effects of exercise training and growth. Thus, it is possible that the gene expression is influenced by relative exercise intensity and aging. Therefore, exercise training-induced alteration of gene expression in the heart and aorta may differ between the present findings and a result of training using relative exercise intensity. Also, these changes of gene expression may be affected by both growth and aging. The alterations in gene expression by exercise intensity and/or aging in the heart and aorta remain to be elucidated.

The present study shows that exercise training induces an increase or decrease of gene expressions in both the heart and aorta. However, the underlying mechanism between exercise training-induced adaptation and changes in gene expression also remains to be elucidated. The interaction of protein-protein and/or protein-gene is indicated in the regulation of gene expression, therefore, it is possible that exercise training-induced change in various factors results in the up- or down-regulation of gene expression. Furthermore, both the molecular regulation induced by exercise training may result in beneficial adaptations, as both up- or down-regulation of gene expression affects protein/peptide production.

In conclusion, the present results showed each profile of 3,800 gene expression levels in the heart and aorta for 4 and 8 weeks exercise training using DNA microarray technique. This study identified a total of 45 relevant genes in the heart and 57 relevant genes in the artery for 4-week exercise training and a total of 74 relevant genes in the heart and 31 relevant genes in the aorta for 8-week exercise training. These results suggest that the alteration of various gene expressions influences a molecular mechanism underlying the exercise training-induced cardiovascular adaptations. It was also seen that the alteration of many genes expression in the heart was observed in 8 weeks exercise training, whereas alterations in the aorta were induced in 4 weeks exercise training. The observed difference in the change of the gene expression in the time course between the heart and aorta suggests a difference in the time course of exercise-induced physiological adaptation in the heart and aorta (e.g., formation of physiological cardiac hypertrophy and enhancement of arterial compliance).

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References

Iemitsu, M., et al.


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Name: Motoyuki Iemitsu

Affiliation: Institute of Health and Sport Sciences and Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba

Address: 1-1-1 Tennodai, Tsukuba City, Ibaraki 305-8577 Japan

Brief Biographical History:
1992- Department of Health and Sports Sciences, Kawasaki University of Medical Welfare
1996- Master's Program in Department of Medicine and Science in Sports and Exercise, Graduate School of Medicine, Tohoku University
1999- Doctoral Program in Medical Sciences, University of Tsukuba
2004- Research Associate, Institute of Health and Sport Sciences and Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba

Main Works:
• Exercise training improves cardiac function-related gene levels through thyroid hormone receptor signaling in aged rats. Am J Physiol Heart Circ Physiol 286: H1696-H1705, 2004

Membership in Learned Societies:
• Japan Society of Physical Education, Health and Sport Sciences
• Japanese Society of Physical Fitness and Sports Medicine
• Japan Society of Exercise and Sports Physiology
• Japanese Circulation Society