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Aerobic capacity-dependent differences in cardiac gene expression

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Departments of [1]Circulation and Medical Imaging and [2]Mathematical Sciences, Norwegian University of Science and Technology, Trondheim, Norway; [3]Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, United Kingdom; [4]Department of Pharmacology, Cardiovascular Biology, and Metabolic Diseases, University of Toledo, Toledo, Ohio; and [5]Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, Michigan

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Bye A, Langaas M, Høydal MA, Kemi OJ, Heinrich G, Koch LG, Britton SL, Najjar SM, Ellingsen Ø, Wisløff U. Aerobic capacity-dependent differences in cardiac gene expression. *Physiol Genomics* 33: 100–109, 2008. First published January 2, 2008; doi:10.1152/physiolgenomics.00269.2007.—Aerobic capacity is a strong predictor of cardiovascular mortality. To determine the relationship between inborn aerobic capacity and cardiac gene expression we examined genome-wide gene expression in hearts of rats artificially selected for high and low running capacity (HCR and LCR, respectively) over 16 generations. The artificial selection of LCR caused accumulation of risk factors of cardiovascular disease similar to the metabolic syndrome seen in human, whereas HCR had markedly better cardiac function. We also studied alterations in gene expression in response to exercise training in these animals. Left ventricle gene expression of both sedentary and exercise-trained HCR and LCR was characterized by microarray and gene ontology analysis. Out of 28,000 screened genes, 1,540 were differentially expressed between sedentary HCR and LCR. Only one gene was found differentially expressed by exercise training, but this gene had unknown name and function. Sedentary HCR expressed higher amounts of genes involved in lipid metabolism, whereas sedentary LCR expressed higher amounts of the genes involved in glucose metabolism. This suggests a switch in cardiac energy substrate utilization from normal mitochondrial fatty acid β-oxidation in HCR to carbohydrate metabolism in LCR, an event that often occurs in diseased hearts. LCR were also associated with pathological growth signaling and cellular stress. Hypoxic conditions seemed to be a common source for several of these observations, triggering hypoxia-induced alterations of transcription. In conclusion, inborn high vs. low aerobic capacity was associated with differences in cardiac energy substrate, growth signaling, and cellular stress.

metabolic syndrome; metabolism; hypoxia; $\dot{V}O_{2max}$; hypertrophy

ALTHOUGH MAXIMAL OXYGEN UPTAKE ($\dot{V}O_{2max}$) is statistically linked with cardiovascular mortality (23, 36), the mechanistic nature of this association is unknown and difficult to explore in humans. Specifically, it is well defined that the continuum of heart function is linearly related with the level of $\dot{V}O_{2max}$ (39). Within the gene-environment interactions, inheritance may account for as much as 70% of the variation in aerobic capacity in human (7). Hence, genetic predisposition and inborn aerobic capacity are likely to contribute toward cardiovascular disease and mortality.

Rats with different inborn running capacities have been artificially selected over generations to generate strains with genetically determined high or low intrinsic capacity (25). The evolved strains of high capacity runners (HCR) and low capacity runners (LCR) have a 30% difference in $\dot{V}O_{2max}$ (17). Selecting for low running capacity also resulted in accumulation of risk factors that predispose to cardiovascular disease. That is, LCR have features of the metabolic syndrome, whereas HCR show an athletic phenotype with markedly better cardiac and vascular function relative to LCR (17, 52). These models were generated expressly for efficient and invasive evaluation of cardiometabolic disease that can lead to highly focused, mechanistic-based, studies in humans.

Here we performed whole genome microarray analysis to screen for differences in cardiac gene expression between LCR and HCR rats in sedentary and exercise-trained conditions. We hypothesized that genes differentially expressed between the sedentary LCR and HCR that regressed with aerobic capacity would include a set at least partly responsible for the differences in aerobic capacity between the strains. The cardiac gene set expressed in response to training was not different between the LCR and HCR. For the sedentary rats, 1,540 cardiac genes were differentially expressed for pathways including cardiac energy substrate, growth signaling, contractility, and cellular stress.

MATERIALS AND METHODS

Animals. We used rats artificially selected for high and low aerobic capacity, starting from the N: NIH stock obtained from the National Institutes of Health (NIH) (US). The model is described elsewhere (25, 52). Briefly, the rats in each generation were tested for exercise capacity by treadmill running at 11 wk of age. The individuals with the highest and lowest running capacity were selected, and each group served as the mating population for the next generation. Female rats from *generation 16* were used in this study. The study includes four groups: LCR trained ($n = 4$), LCR sedentary ($n = 4$), HCR trained ($n = 4$), and HCR sedentary ($n = 4$). Experimental protocols were approved by the respective Institutional Animal Research Ethics Councils.

Endurance training. We trained the rats with an aerobic interval training program that was previously described by Høydal et al. (17). In brief, after 10 min of warm-up, rats ran uphill (25°) on a treadmill for 1.5 h, alternating between 8 min at an exercise intensity corresponding to 85–90% of $\dot{V}O_{2max}$ and 2 min of active recovery at 50–60%. Exercise was performed 5 days per week over 8 wk; controls were age-matched rats that remained sedentary. We measured $\dot{V}O_{2max}$ every week in exercising rats to adjust speed to maintain the intended intensity throughout the experimental period. The $\dot{V}O_{2max}$ test protocol consisted of 20 min warm-up at 50–60% of $\dot{V}O_{2max}$, whereupon treadmill velocity was increased by 0.03 m/s every 2 min until VO_2 plateau despite increased workload. The apparatus and method have been previously described and validated (17). The animals in the sedentary groups were treated similarly to the exercise

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groups, except for exposure to exercise training and weekly $\dot{V}O_{2max}$ tests.

Tissue collection. At ~7 mo of age and 48 h after the last exercise session all the animals were killed. One section of the left ventricle was formalin fixated for immunohistochemistry and morphological studies, whereas another section was snap-frozen in liquid nitrogen and stored at −80°C for later genetic screening and protein analysis.

Cardiomyocyte dimension measurements. Single cardiomyocytes from the left ventricle were dissociated by a previously described protocol (51). In brief, the heart was removed immediately after animals were killed, kept for 1 min in ice-cold perfusion buffer, and connected to a standard Langendorff retrograde perfusion system for cell isolation. Cell length and midpoint width were measured in 50 morphologically intact myocytes from four sedentary HCR and four sedentary LCR.

Ribonuclease (RNA) isolation. Tissue samples (20 mg) were homogenized in 100 μl TRIzol (Life Technologies, Gaithersburg, MD) using a Mixer Mill MM301 (Geneq, Montreal, Canada) at 20–25 Hz. RNA clean-up was performed using RNA Mini kit (Qiagen, Germantown, MD). RNA isolation and clean-up were performed according to the manufacturer's instructions.

RNA integrity, purity, and quantity were assessed by Bioanalyzer (Agilent Technologies, Santa Clara, CA) and Nanodrop (NanoDrop Technologies, Baltimore, MD). The concentration of total RNA was measured by Nanodrop with ultraviolet spectrophotometry at 260/280 nm. RNA quality was assessed by electrophoresis on Bioanalyzer chips (Agilent Technologies). High-quality RNA was classified as a 260/280 ratio >1.8. Only samples with a 260/280 ratio between 1.8 and 2.2 and no signs of degradation were used for analysis.

Processing of Affymetrix data. Labeled cRNA was prepared and hybridized to the RAE 230 2.0 chip from Affymetrix GeneChip (Affymetrix, Santa Clara, CA) comprising 31,042 probe sets. On the Affymetrix GeneChip arrays, each gene was represented by a set of 11–20 probe pairs consisting of a perfect match (PM) and a mismatch probe. The statistical analysis is based on summary expression measures for each probe set.

Computing summary measures. The summary measure for each probe set is computed based on a linear statistical model for background-corrected, normalized, and log-transformed PM values for each probe pair by use of the robust multiarray average (RMA) method (21). The PM values are normalized by the quantile normalization method (5); the arrays are normalized such that the empirical distribution of the expression measures is equal across arrays.

Statistical analysis for finding differentially expressed genes. For each gene (probe set), a linear regression model, including parameters representing the effect of aerobe capacity, is specified. Based on the estimated effects, tests for significant differential expression are performed by moderated *t*-tests (44).

To account for multiple testing, we calculated adjusted *P* values controlling the false discovery rate (FDR), with the use of the Benjamini-Hochberg step-up procedure (4). Consequently, selecting differentially expressed genes based on a threshold of 0.05 on the adjusted FDR *P* values means that the expected proportion of genes falsely classified as differential expressed should be <0.05.

All statistical analyses on the gene expression data are performed using the R language (R Development Core Team, 2004) and packages affy, affyPLM, and limma from the Bioconductor project (12).

Database submission. The microarray data were prepared according to Minimum Information About Microarray Experiment (MIAME) recommendations and deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE9445.

Functional clustering according to gene ontology annotations. To obtain information about gene functions, we used the *e*GOn web tool described by Beisvag et al. (3). Lists of differentially expressed genes between HCR and LCR ($P < 0.05$) were submitted into *e*GOn, which automatically associates Gene Ontology (GO) terms from public databases to the submitted gene reporters. Annotations according to GO were obtained from the Norwegian Microarray Consortium annotation database (www.genetools.no), which was based on UniGene build no. 157 from November 2006.

We applied the master-target test from *e*GOn to assess the relative numbers of GO annotations linked to differentially expressed genes, compared with the relative numbers of the same GO annotations linked to all the genes on the microarray. We also applied the mutually exclusive target-target test to compare the genes that were significantly more expressed in HCR with the genes that were significantly more expressed in LCR.

The differentially expressed genes were also imported into the Ingenuity Pathway Analysis Application Tool to identifying gene networks that significantly describes the differences between HCR and LCR.

Validation of microarray results. Left ventricle protein levels of adenosine triphosphate (ATP) synthase (mitochondrial F1 complex), the major histocompatibility complex class I (MHC Cl. I), tubulin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were mea-

Table 1. *Physiological parameters of the same animals as in this study, previously reported by Høydal et al. (17)*

	LCR-SED	HCR-SED	LCR-TR	HCR-TR
		In vivo exercise capacity		
$\dot{V}O_{2max}$ (ml·kg$^{-0.75}$·min^{-1}) pretest values	39.1±2.3	50.6±4.2*	38.8±2.2	50.9±3.9
$\dot{V}O_{2max}$ (ml·kg$^{-0.75}$·min^{-1}) end-point values	38.0±2.3	49.6±4.3*	57.0±4.6†	70.4±4.1‡
		Systolic cell function		
Cell shortening, %	8.2±0.8	11.7±1.5*	11.5±1.5†	17.1±1.1‡
Relative time to peak shortening shortening, ms	7.6±0.9	5.7±1.0*	5.6±1.0†	4.0±0.3‡
Systolic [Ca^{2+}], nM	420±12	441±17*	456±17†	547±47‡
Amplitude of the [Ca^{2+}], nM	181±19	249±15*	258±14†	397±43‡
Relative time to peak [Ca^{2+}], ms	0.21±0.03	0.15±0.03*	0.15±0.02†	0.10±0.02‡
		Diastolic cell function		
Time to 50% relengthening, ms	67.0±3.7	58.7±2.3*	60.8±3.0†	43.5±7.7‡
Diastolic [Ca^{2+}], nM	239±11	192±3*	199±8†	150±5‡
Time to 50% decay of [Ca^{2+}] transient, ms	77.8±2.9	66.3±2.4*	66.3±2.4†	55.7±3.4‡

Exercise capacity and isolated left ventricular cell variables from low capacity runners (LCR) and high capacity runners (HCR), separated in groups of sedentary control (SED) and exercise-trained (TR). Before exercise, the LCR-SED and HCR-SED rats differed significantly (*$P < 0.01$) for all variables. LCR-TR had significantly improved function compared to LCR-SED (†$P < 0.01$), and the HCR-TR had improved function compared to HCR-SED (‡$P < 0.01$). More details on the functional data are published in Høydal et al. (17).

sured to confirm that single genes and pathways were the same at protein level, as on gene level.

Homogenized left ventricular samples ($n = 4$ per group) were loaded onto a 4–12% NuPAGE Bis-Tris Gel (Invitrogen, Carlsbad, CA), separated by electrophoresis, and transferred to a Protran 85 nitrocellulose membrane (Whatman, Kent, UK). The membrane was incubated with ATP synthase (mitochondrial F1 complex) (Santa Cruz Biotechnology, Santa Cruz, CA), tubulin (Sigma-Aldrich, St. Louis, MO), and GAPDH (Santa Cruz Biotechnology) primary antibodies. Horseradish peroxidase-conjugate secondary antibodies and enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) were used for protein detection. All protein levels were normalized to total actin (Sigma-Aldrich) and quantitated using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

The protein levels of MHC Cl. I (Abcam, Cambridge, UK) were analyzed on formalin-fixed, paraffin-embedded left ventricular sections (4 μm) by a standard immunohistochemistry protocol. Results were visualized by Envision + TM detection system (DakoCytomation, Glostrup, Denmark). The degree of positive-staining was determined by semiquantitative microscopy, arranging all the stained tissue specimens from the highest to the lowest degree of staining in two separate staining experiments.

Statistics for protein levels. Data are presented as means ± SE. To analyze statistical difference within groups we applied two-tailed Student *t*-test using Prism software (GraphPad Software). $P < 0.05$ was considered statistically significant.

RESULTS

Physiological data. Previous studies of the same strains of animals reported that LCR were born with a higher cardiovas-

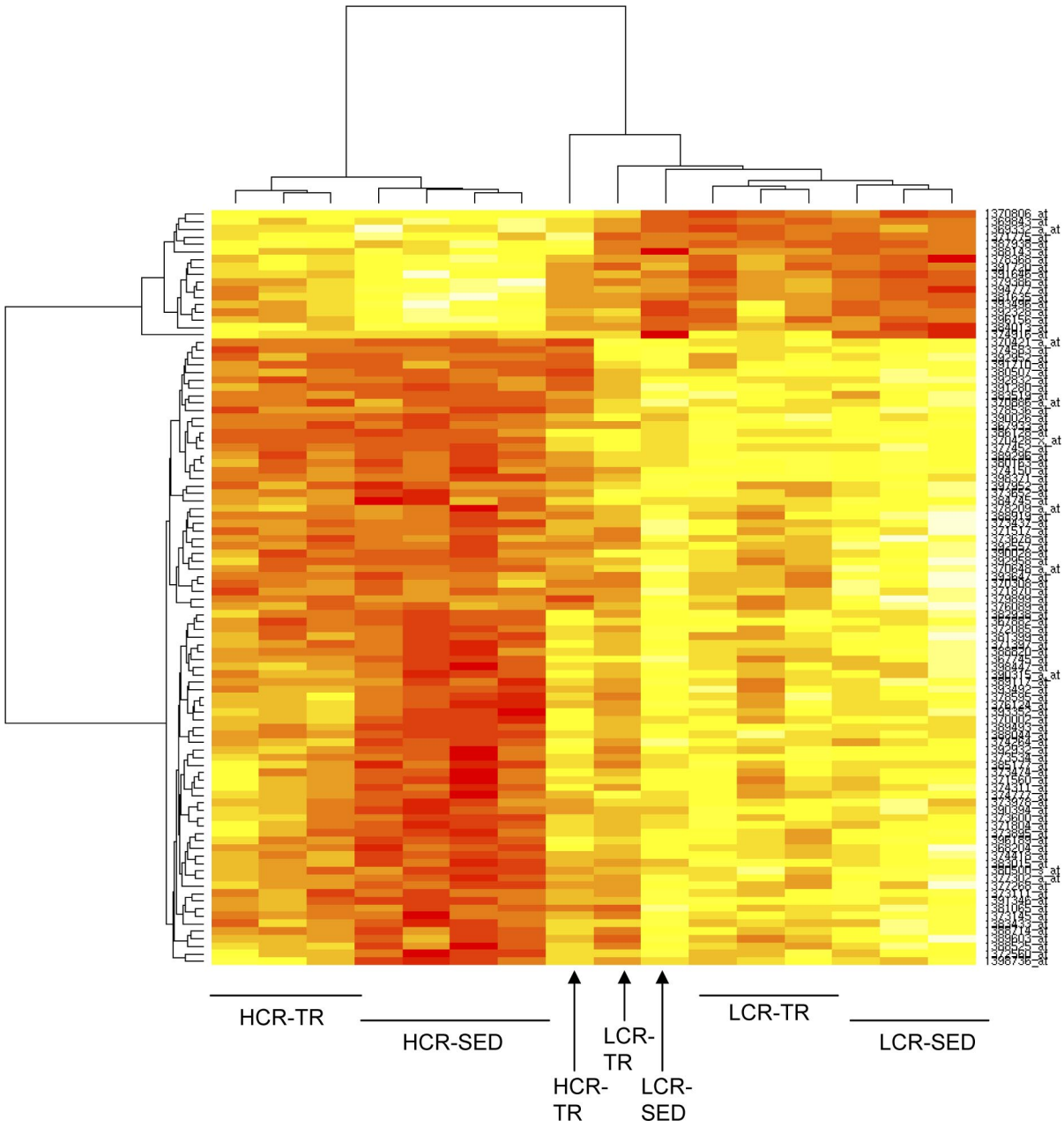

Fig. 1. Heat map of the 100 most significant probe sets (comparing HCR sedentary and LCR sedentary). Probe sets for which the abundance was above the mean are shown in red, below the mean are shown in yellow, and equivalent to the mean are in white. HCR-TR, exercise-trained high capacity runners; HCR-SED, sedentary high capacity runners; LCR-TR, exercise-trained low capacity runners; LCR-SED, sedentary low capacity runners.

cular risk profile than HCR. LCR were reported to be insulin-resistant, with high glucose and lipid content in blood (52). Compared with HCR, LCR have more abdominal fat, high blood pressure, vascular and cardiac dysfunction, and a lower $\dot{V}O_{2max}$ (52).

Initial exercise capacity, postexercise period $\dot{V}O_{2max}$, cardiomyocyte contractility, and calcium (Ca^{2+}) handling of the animals in this study have previously been reported by Høydal et al. (17) (Table 1). Before exercise, the sedentary LCR and HCR rats differed significantly for all variables. Exercise-trained LCR had significantly improved function compared with sedentary LCR, and the trained HCR had improved function compared with sedentary HCR.

Isolated cardiomyocytes from sedentary LCR were significantly wider (32 ± 3 μm) than the cardiomyocytes from sedentary HCR (24 ± 4 μm) ($P < 0.01$). The mean length of the isolated myocytes was 123 ± 3 and 124 ± 4 μm in HCR and LCR, respectively.

Gene expression data. Of the 28,000 screened genes in the microarray analyses, a total of 1,540 genes were differentially expressed between the sedentary HCR and LCR ($P \leq 0.05$); 618 of the genes were more expressed in HCR vs. LCR, and 922 were more expressed in LCR vs. HCR. Only one gene was found differentially expressed by exercise training in this study. This gene (probe set: 1374916_at) was found upregulated by exercise in the LCR group but had unknown name and function. Differences between the four groups are illustrated in a heat map (Fig. 1).

Gene clusters and cellular pathways. Biological processes, molecular functions, and cellular components describing the differences between HCR and LCR are listed in Table 2. Molecular functions and biological processes significantly more represented in HCR vs. LCR are listed in Table 3.

Ingenuity Pathway Analysis revealed a significant cardiotoxicity in LCR, whereas HCR were characterized by increased Ca^{2+} signaling (Table 4).

The most differentially expressed genes. The genes with the greatest fold-change of increase in HCR compared with LCR are displayed in Table 5, whereas the genes with the greatest fold-change of increase in LCR compared with HCR are displayed in Table 6. These genes are also among the most significant genes in the study. Genes identified as "transcribed loci" or that were not in the UniGene database were not included in Tables 5 and 6.

Verification of gene expression results on protein level. Left ventricle protein levels of ATP synthase (mitochondrial F1 complex) and MHC Cl. I were measured to confirm single genes that were found among the most-differentially expressed genes (Table 6). Both proteins were more expressed in LCR than HCR, as in line with the gene expression data (Figs. 2 and 3).

Protein levels of tubulin and the microtubule binding protein, GAPDH were measured to support the results showing that microtubule-based processes were more pronounced in LCR than HCR (Table 3). Both proteins were more expressed in LCR than HCR, as in line with the gene expression data (Fig. 2).

DISCUSSION

Genes differentially expressed between the sedentary HCR and LCR that also regress with aerobic capacity are considered to include a set of genes at least partly responsible for the differences in aerobic capacity between the strains. Because of the strong linkage between aerobic function and disease, this set of differentially expressed genes is also postulated to

Table 2. *GO categories overrepresented among the differentially expressed genes between HCR and LCR, compared with the total number of genes on the array ($P < 0.05$)*

GO Number	GO Category	# on Array	# Genes Differentially Expressed HCR vs. LCR	*P*
GO:0003674	**Molecular function**	**8,882**	**417**	
GO:0003682	chromatin binding	75	11	0.001
GO:0030228	lipoprotein receptor activity	9	3	0.007
GO:0042975	peroxisome proliferator activated receptor binding	10	3	0.010
GO:0008168	methyltransferase activity	65	8	0.011
GO:0004708	MAP kinase kinase activity	12	3	0.016
GO:0030331	estrogen receptor binding	5	2	0.020
GO:0008150	**Biological process**	**8,586**	**404**	
GO:0001701	embryonic development	60	10	0.000
GO:0043283	biopolymer metabolism	1,664	106	0.001
GO:0006259	DNA metabolism	359	30	0.002
GO:0000070	mitotic sister chromatid segregation	12	4	0.002
GO:0006974	response to DNA damage stimulus	173	17	0.003
GO:0006512	ubiquitin cycle	191	19	0.003
GO:0006260	DNA replication	105	12	0.004
GO:0007020	microtubule nucleation	3	2	0.006
GO:0007059	chromosome segregation	26	5	0.007
GO:0006281	DNA repair	129	13	0.009
GO:0007017	microtubule-based process	146	14	0.010
GO:0001666	response to hypoxia	78	9	0.011
GO:0042770	DNA damage response, signal transduction	31	5	0.014
GO:0005575	**Cellular component**	**8,333**	**378**	
GO:0030938	collagen type XVIII	5	3	0.001
GO:0005874	microtubule	124	14	0.001

GO, gene ontology.

Table 3. *GO categories overrepresented among the differentially expressed genes between HCR and LCR in left ventricle ($P < 0.05$)*

GO Number	GO Category	HCR > LCR	HCR < LCR	*P*
GO:0003674	**Molecular Function**	**190**	**236**	
GO:0004930	G protein-coupled receptor activity	11	0	0.000
GO:0003676	nucleic acid binding	25	65	0.000
GO:0004872	receptor activity	37	11	0.000
GO:0003723	RNA binding	1	25	0.000
GO:0004871	signal transducer activity	46	21	0.000
GO:0004888	transmembrane receptor activity	25	5	0.000
GO:0005102	receptor binding	17	4	0.001
GO:0001584	rhodopsin-like receptor activity	8	0	0.001
GO:0015276	ligand-gated ion channel activity	10	1	0.003
GO:0030594	neurotransmitter receptor activity	7	0	0.003
GO:0008150	**Biological Process**	**181**	**227**	
GO:0043283	biopolymer metabolism	32	76	0.000
GO:0007154	cell communication	70	40	0.000
GO:0016477	cell migration	14	1	0.000
GO:0006928	cell motility	16	2	0.000
GO:0007166	cell surface receptor linked signal transduction	28	8	0.000
GO:0007267	cell-cell signaling	24	6	0.000
GO:0007186	G protein-coupled receptor protein signaling pathway	18	3	0.000
GO:0040011	locomotion	16	2	0.000
GO:0009653	morphogenesis	30	12	0.000
GO:0050877	neurophysiological process	29	6	0.000
GO:0006139	nucleobase, nucleoside, nucleotide, and nucleic acid metabolism	31	77	0.000
GO:0044238	primary metabolism	80	141	0.000
GO:0016070	RNA metabolism	1	25	0.000
GO:0007165	signal transduction	59	38	0.000
GO:0019226	transmission of nerve impulse	18	2	0.000
GO:0030154	cell differentiation	24	9	0.001
GO:0044237	cellular metabolism	84	144	0.001
GO:0043170	macromolecule metabolism	53	104	0.001
GO:0016071	mRNA metabolism	1	16	0.001
GO:0006397	mRNA processing	1	15	0.001
GO:0008152	metabolism	93	151	0.002
GO:0007017	microtubule-based process	0	12	0.002
GO:0007399	nervous system development	24	10	0.002
GO:0051869	physiological response to stimulus	25	11	0.002
GO:0008380	RNA splicing	0	12	0.002
GO:0007507	heart development	7	0	0.003
GO:0030097	hemopoiesis	7	0	0.003
GO:0002520	immune system development	7	0	0.003
GO:0002217	physiological defence response	7	0	0.003
GO:0048468	cell development	15	5	0.005
GO:0046907	intracellular transport	7	26	0.006
GO:0002376	Immune system process	15	6	0.013
GO:0009888	tissue development	9	2	0.014
GO:0030099	myeloid cell differentiation	5	0	0.017
GO:0007218	neuropeptide signaling pathway	5	0	0.017
GO:0006817	phosphate transport	5	0	0.017
GO:0048771	tissue remodeling	5	0	0.017
GO:0042127	regulation of cell proliferation	12	4	0.018
GO:0044248	cellular catabolism	3	14	0.025
GO:0001525	angiogenesis	8	2	0.026

HCR > LCR, the number of genes significantly more expressed in HCR than LCR that belong to a certain molecular function or biological process. HCR < LCR, the number of genes significantly more expressed in LCR than HCR that belong to a certain molecular function or biological process.

contain candidate's causative of the divide for health risks between the LCR and HCR.

We have previously reported that both LCR and HCR improved their $\dot{V}O_{2max}$, cardiomyocyte contractility, and Ca^{2+} handling in response to exercise training (17). Exercise training did in fact restore the inherited disadvantages of LCR in terms of $\dot{V}O_{2max}$, cardiomyocyte contractility, and Ca^{2+} handling (17). Although previous studies in rats have reported significant changes in cardiac gene expression in response to training (20, 22, 34), we only detected one gene in this study. Due to clear left ventricular alterations in response to exercise, the lack of significant findings at the mRNA level might be explained by strict statistical demands under-reporting differentially expressed genes. As in this study, several genes have high differential expression, so minor changes, although significantly in vivo, might not make the statistical cut-off.

Cardiac metabolism. The most prominent difference between sedentary HCR and LCR was the cardiac energy metabolism. HCR expressed higher amounts of genes involved in lipid metabolism, whereas LCR expressed higher amounts of

Table 4. *The most significant toxicological function and pathways in HCR and LCR*

	HCR	LCR
Top toxicological functions		cardiotoxicity – cardiac proliferation ($P = 0.036$) hepatoxicity – hyperproliferation ($P = 0.027$)
Top pathways	chemokine signaling ($P < 0.00$) axonal guidance signaling ($P < 0.00$) Ca^{2+} signaling ($P = 0.00$)	protein ubiquitination pathway ($P < 0.00$) riboflavin metabolism ($P < 0.00$) estrogen receptor signaling ($P < 0.00$)

the genes involved in glucose metabolism and transport. By simply selecting for running capacity, we have created a difference in cardiac energy substrate utilization from the normal mitochondrial fatty acid β-oxidation (FAO) in HCR to carbohydrate metabolism in LCR. In a diseased heart the main energy substrate often switches from FAO to carbohydrate metabolism, with a downregulation of enzymes involved in FAO (38). Initially, this improves the efficiency of the heart, since the amount of ATP produced per O_2 consumed is higher in glucose oxidation than FAO. However, as the condition progresses towards an uncompensated state, the capacity of utilizing glucose decreases (28).

Running capacity is related to the ability to deliver and utilize oxygen (O_2) (49). It has previously been shown that LCR have impaired O_2 supply, extraction ratio, and tissue diffusion capacity, compared with HCR (15, 16). In line with impaired O_2 availability in LCR, the biological process response to hypoxia was found to separate HCR from LCR. Since hypoxia reduces FAO (19) and increases glucose metabolism (27), the lower tissue O_2 in LCR may explain the changes in cardiac energy metabolism. Reduced O_2 supply to peripheral tissue in LCR (15) are supported by the lower expression of genes associated with hemopoiesis, such as the hemoglobin β-chain complex. In our experimental situation, these genes were probably detected from remains of coagulated blood in the tissue samples.

Differences in metabolism may be linked to uncoupling protein 4 (UCP4). Uncoupling proteins are potential regulators of FAO, and high expression is associated with high resting metabolism, low fasting plasma glucose and insulin levels, and an increased glucose clearance rate (6, 8). Different expression of cardiac UCP4 might be involved in determining cardiovascular risk, and might be a target for pharmacological interventions.

Energy, whether originating from glucose metabolism or FAO, is mainly generated in the respiratory chain. mRNA encoding two respiratory chain enzymes, ATP synthase (mitochondrial F1 complex) and nucleoside diphosphate kinase, was more abundant in LCR compared with HCR. This might be a compensatory mechanism to increase the energy production and has previously been reported in failing human hearts (31).

Blood lipids. We have previously reported high levels of plasma triglycerides and free fatty acids in LCR (52), which partly may be explained by low expression of cholesterol-lowering proteins as very low density lipoprotein receptor (VLDLr) and colony stimulating factor 1 (CSF1), as reported here and elsewhere (18, 37, 38, 53). Administration of CSF1 has been tested as a potential therapy for hypercholesterolemia and atherosclerosis, and favorable results have been reported (50). Low expression of CSF1 and VLDLr, in addition to a general lower FAO, might explain the accumulation of serum triglycerides and free fatty acids in LCR.

Table 5. *Genes with significantly stronger expression in HCR compared with LCR ($P < 0.05$)*

Category	ID	Gene	Abbreviation	Fold-change
Contractility/Ca^{2+} signaling	1369843_at	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	Chrna1	1.49
	1388292_at	potassium inwardly-rectifying channel, subfamily J, member 3	Kcnj3	1.34
	1370367_at	solute carrier family 1-glutamate transport	Slc1a1	1.32
	1368976_at	CD38		1.29
	1370857_at	smooth muscle alpha-actin		1.28
	1387433_a_at	solute carrier family 25 – mitochondrial uncoupling protein 4	UCP4	1.23
Growth	1387938_at	brain and acute leukemia, cytoplasmic	BAALC	2.16
	1367571_a_at	insulin-like growth factor 2	IGF2	1.47
	1388143_at	collagen, type XVIII, α1		1.33
	1369825_at	matrix metallopepdidase 2	MMP2	1.28
	1380582_at	colony stimulating factor 1	CSF1	1.25
Neurotramsmitter transport	1369332_a_at	sygnaptotagmin 1	Rims1	1.69
Lipid metabolism	1371775_at	acyl-coenzyme A dehydrogenase (short chain)	Acadsb	1.44
	1368840_at	very low density lipoprotein receptor	VLDLr	1.38
	1367836_at	carnitine palmitoyltransferase 1	CPT-1A	1.29
	1388210_at	mitochondrial acyl-CoA thioesterase 1	MTE-1	1.26
	1369098_at	very low density lipoprotein receptor	VLDLr	1.24
	1387455_a_at	very low density lipoprotein receptor	VLDLr	1.24
	1387245_at	lipase, gastric	Lipf	1.22
O_2 transport	1371245_a_at	hemoglobin β-chain complex	Hbb	4.81
Electron transport	1370806_at	retinol saturase	Rmt7	4.30
Signal transduction	1373847_at	transmembrane 4 superfamily (predicted)	Tm4 sf1	1.38
	1368097_a_at	reticulon 1	Rtn1	1.24

Table 6. *Genes with significantly stronger expression in LCR compared with HCR ($P < 0.05$)*

Category	ID	Gene	Abbreviation	Fold-change
Contractility	1395350_at	tropomyosin 1 α	tpm1	1.54
	1390471_at	tropomyosin 1 α	tpm1	1.52
	1379936_at	tropomyosin 1 α	tpm1	1.52
Growth	1367933_at	S-adenosylmethionine decarboxylase	amd1	2.08
	1379157_at	Ca^{2+} channel, L-type, voltage-dependent (α1C subunit)	cacna1c	1.82
	1378403_at	Ca^{2+} channel, L-type, voltage-dependent (α1C subunit)	cacna1c	1.82
	1375677_at	transducer of ErbB-2	tob2	1.54
	1391431_at	transducer of ErbB-2	tob2	1.52
	1387301_at	fibroblast growth factor 1	fgf1	1.43
	1370421_a_at	TBP-interacting protein		1.43
	1370427_at	platelet-derived growth factor, alpha	PDGFa	1.35
	1367859_at	transforming growth factor, beta 3	tgfb3	1.28
Glucose metabolism	1388044_at	6-phosphofructo-2-kinase	PFKfb2	1.96
	1383519_at	hexokinase 2	HK2	1.56
Ca^{2+} handling	1392965_a_at	smooth muscle-associated protein 2	smap2	1.75
	1370932_at	LDL receptor-related protein 4	lrp4	1.54
Cellular stress	1370428_x_at	RT1 class Ib major histocompatibility complex I	RT1Aw2	50.00
	1374583_at	mRNA decapping enzyme		2.50
	1395190_at	aldose reductase	akr1b10	1.89
	1368025_at	DNA-damage-inducible transcript 4	ddit4	1.79
Microtubule	1367882_at	microtubule-associated protein 1 A	mtap1a	2.33
	1370886_a_at	kinesin 2		2.22
	1382938_at	microtubule-associated protein 1A	mtap1a	2.04
Protein binding	1376124_at	Wiskott-Aldrich syndrome-like (human)	was1	1.67
	1380163_at	SEC23 interacting protein		1.61
Respiratory chain	1380070_at	ATP synthase (mitochondrial F1 complex)	atp5b	1.72
	1391710_at	nucleoside diphosphate kinase	nme2	1.82
Catalytic activity	1371776_at	PI3K (regulatory subunit 1) (p85 a)	pik3r1	5.55
	1368188_at	4-hydroxyphenylpyruvic acid dioxygenase	hpd	1.79
	1367745_at	diacylglycerol kinase ζ	DAG-kζ	1.59
	1374066_at	cyclin-dependent kinase 11	CDK11	1.56
Signal transduction	1370648_a_at	SH3 domain binding protein CR16		2.01
	1378392_at	guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1	gnb2l1	1.79
Glucose transport	1368249_at	Kruppel-like factor 15	klf15	1.52

LDL, Low density lipoprotein; PI3K, phosphatidylinositol 3-kinase.

Ca^{2+} signaling and contractility. We have previously reported impaired contractility and Ca^{2+} handling in LCR cardiomyocytes (17, 52). One the mRNA level, Ca^{2+} signaling was found to be significantly more pronounced in HCR than LCR, suggesting that impaired Ca^{2+} signaling may be involved in the depressed contractility observed in LCR. In cardiac muscle, Ca^{2+} signaling also play a central role in regulating gene expression, hypertrophy, and apoptosis (54).

Both cholinergic receptor (nicotinic, alpha polypeptide 1) and inward rectifying potassium (K^+) channel (subfamily J, member number 3) were less expressed in LCR than HCR. These ion channels regulate the heart rate and contractility. Less inward K^+ channels in LCR will potentially lower the myocyte membrane potential, hence making the myocytes from LCR more susceptible to delayed after-depolarization and ventricular tachyarrhythmia, which was recently reported in this animal model (30). Reduced density of inward rectifying K^+ channels and a lower resting membrane potential have been reported in failing human hearts (26).

LCR also expressed less CD38 than HCR. CD38 is responsible for most of the synthesis of cyclic-ADP-ribose in the myocardium, which in turn controls the Ca^{2+} homeostasis in cardiac myocytes. Cyclic-ADP-ribose enhances the sensitivity of Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum, and thus the contraction (46). This coincides with the previously reported lower Ca^{2+} transient and contractility in LCR myocytes (17, 52).

Physiological versus pathological growth signaling. The cardiomyocyte shape represents an interesting phenotypic characterization of cardiac hypertrophy. Cardiomyocyte volume correlates with cardiac mass in a variety of conditions, including pressure and volume overload, hyperthyroidism, and postmyocardial infarction (14, 24, 55). In physiological conditions, the width/length ratio is tightly regulated and remains unchanged in normal body growth (41); however, this may change under certain conditions. In this study, we found a large cardiomyocyte width in LCR. An increase in width is most often associated with pressure overload (13). This is in consensus with our previous findings of significantly elevated mean blood pressure in LCR compared with HCR (52).

Increased cardiomyocyte size in LCR was consistent with the gene expression data reporting cardiac proliferation as the most significant pathological function detected in LCR. The presence of hypertrophic signaling in LCR was further emphasized by the high expression of glycolytic enzymes, as glucose is a more efficient substrate for ATP production than free fatty acids (48). Pathological hypertrophy is also linked to upregulation of genes associated with embryonic growth, as mRNAs encoding s-adenosylmethionine decarboxylase, transducer of ErbB-2 (1), fibroblast growth factor 1, transforming growth factor, beta 3, and TBP-interacting protein 120B (2) were significantly more abundant in LCR than in HCR. In addition, LCR expressed more of the voltage-dependent L-type Ca^{2+} channel α1C pore subunit than HCR, whose upregulation has

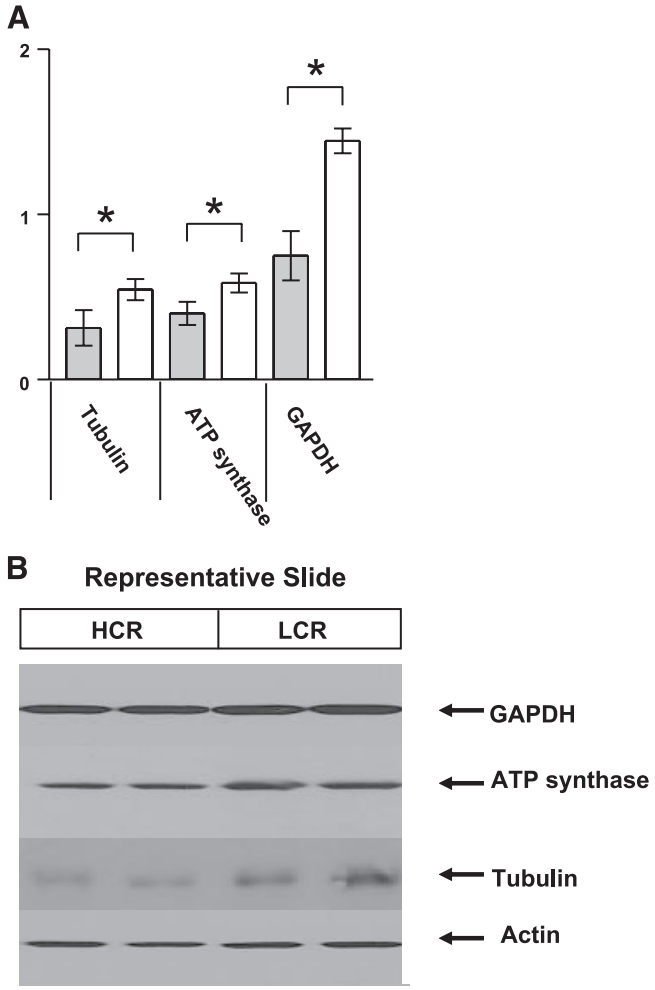

Fig. 2. *A*: semiquantitative protein level of tubulin, ATP synthase (mitochondrial F1 complex), and GAPDH. HCR, gray bars; LCR, white bars. Density ratio to total actin (arbitrary units) $*P < 0.05$. *B*: representative slides from the Western analysis. ATP, adenosine triphosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCR, high capacity runners; LCR, low capacity runners.

been associated with maladaptive growth and heart failure in mice (35).

Microtubule (MT)-based processes were more pronounced in LCR than HCR. This can be related to our findings of elevated blood pressure (52) and pathological cardiac growth in LCR, since increased MT density and increased level of MT binding proteins are associated with pressure overload cardiac hypertrophy (9, 40, 45). MTs are also involved in hypoxic signaling, which was found to be different in HCR and LCR. MTs have been suggested to regulate translation of the hypoxia-inducible factor 1α, but the mechanism is not yet elucidated (11). Even so, drugs destroying MTs are used to disrupt hypoxic signaling in cancer (32). To support the importance of MT-based processes in LCR, we analyzed the protein levels of tubulin and the MT-binding protein, GAPDH and found higher levels of both proteins in LCR compared with HCR.

In contrast to LCR, HCR seem to have a healthier cardiac growth signaling pattern, based upon a higher expression of genes associated with tissue development and remodeling. HCR expressed more collagen type XVIII α1 and the collagen-breakdown protein matrix metalloproteinase 2 than LCR. This suggests a higher tissue turnover rate in HCR than LCR, with continuous breakdown and construction of the connective tissue in the heart. Interestingly, angiogenesis was significantly more pronounced in HCR compared with LCR. Angiogenesis is normally enhanced during the acute phase of adaptive cardiac growth, but as the heart enters the chronic phase of pathological remodeling, angiogenesis is normally impaired. In fact, disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure (42). This suggests that the cardiac pathological growth in LCR might be past the acute phase and is now accompanied by impaired angiogenesis. The pathological cardiac hypertrophy with reduced contractility as in our animal model (17) has also previously been accompanied by impaired coronary angiogenesis (42).

Cellular stress. Clusters of differentially expressed genes associated with DNA damage and hypoxia suggest a cardiac stress response in LCR. This is further emphasized by the

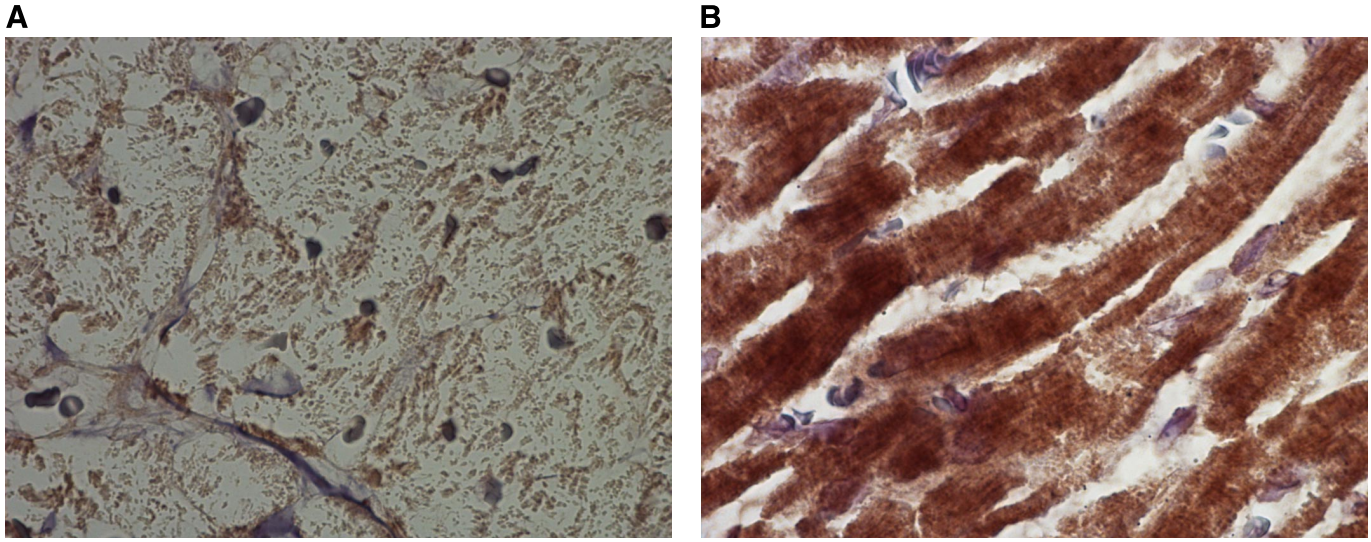

Fig. 3. Representative slides from: *A*: immunohistochemical staining of MHC Cl. I in HCR left ventricle. *B*: immunohistochemical staining of MHC Cl. I in LCR left ventricle. Positive staining is shown in dark-brown. MHC Cl. I, major histocompatibility complex class I.

2.5 times higher expression of the mRNA decapping enzyme in LCR. Increased expression of the *Saccharomyces cerevisiae* homolog is observed under osmotic, oxidative, heat, and metabolic stress conditions (33). Since decapping activity is likely to be conserved in other species (47), oxidative and metabolic stress conditions might occur in LCR hearts. In addition, LCR expressed high amounts of DNA damage-inducible transcript 4, which is particularly upregulated following hypoxia (10, 43).

LCR expressed 50 times more of the inflammatory regulator RT1 class 1 locus A1 than HCR, which also was confirmed at the protein level. Upregulation of the human analog, MHC Cl.I has been found in the myocardium of inflammatory cardiomyopathy patients (29). This suggests a prevailing inflammatory process in the myocardium of LCR rats.

Study limitations. This study could not determine whether the identified differential gene expression patterns represent the cause or the consequence of the inborn differences in aerobic capacity. Further follow-up studies with modification of particular genes are needed for this purpose. Since the expression of mRNA does not necessarily reflect the expression of the protein, we chose to validate some of our microarray results on protein levels rather than the gene level. By this approach, pathogenic mechanisms of disease that involve protein modifications are accounted for.

Conclusion

This study has demonstrated that differences in inborn aerobic capacity are associated with differences in gene expression patterns in the heart. It has also generated novel hypotheses on the association between inborn aerobic capacity and the risk for developing cardiovascular disease.

The cardiac gene expression pattern of inborn low aerobic capacity involves activation of survival mechanisms to meet the body's demands. First, the low aerobic capacity is associated with a metabolic switch from oxidation of fatty acids to glucose, thus improving the energy efficiency of the heart, e.g., in early stages of heart failure. Second, upregulation of embryonic growth factors and increased cardiomyocyte size suggest a growth pattern with elements of pathological signaling. Hypoxic conditions might be the common source for all these observations, triggering hypoxia-induced alterations of transcription toward compensatory mechanisms for an insufficient heart.

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