Blunted Cardiomyocyte Remodeling Response in Exercise Resistant Rats

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Increasing aerobic exercise capacity with training decreases cardiovascular morbidity and mortality. Of major concern is the key observation that up to 20% of subjects demonstrate little or no change in maximal oxygen consumption (VO_{2max}), with exercise training (1) and can be considered *exercise resistant*. The purpose of this study is to test the hypothesis that genetic variation in training response is associated with cardiomyocyte functional response to training.

Exercise capacity can be divided into two components – an innate capacity operating in the untrained state, followed by an adaptive capacity acquired in response to exercise training. Previously, we developed rat models of low and high innate exercise capacity via two-way artificial selective breeding and showed that high intensity aerobic interval training improved VO_{2max} by increasing cardiomyocyte function in female rats with low innate exercise capacity (2). To improve our understanding of adaptive exercise capacity, we developed a second contrasting rat model system of High Response Trainers (HRT) and Low Response Trainers (LRT) using gain in maximal treadmill running distance with endurance training as the selection criterion. After 7 generations of selection (n=1,371), HRT could improve running distance by 46% with training, whereas the LRT change was equivalent to a 2% loss in capacity; an estimated 10% of the variation for training response was caused by genotypic variance, independent of baseline capacity and body weight (3). Critical to this second model system, we find LRT and HRT demonstrate similar "innate" pre-training running capacities and body weights (3) Here we compared cardiomyocytes isolated from left ventricle (LV) of LRT and HRT female rats in the sedentary (SED) and in rats trained (TR) for 8 weeks using a high intensity aerobic interval training protocol proven superior for increasing VO_{2max} and cardiac function in rats with low exercise tolerance (2)

HRT and LRT were not different for VO_{2max} before training. After training, HRT rats demonstrated a 40% increase in VO_{2max} whereas it remained unchanged in LRT (**Figure 1A**). Cardiomyocytes from the left ventricle were isolated and prepared for confocal microscopy for dynamic cell measurements and stimulated at 7 Hz. In the SED condition, LV cardiomyocytes from the HRT, relative to the LRT, were both significantly longer and narrower; these dimensional differences resulted in a higher length-width ratio and a lower calculated LV cell volume. Training resulted in no adaptive changes in LV cell width or width-length ratio, and maladaptive decreases in LV cell length and cell volume in the LRT rats. In contrast, training uniformly produced positive adaptive morphometric increases in LV cardiomyocyte length, width, and volume in the HRT rats (p-value for interaction of SED-TR difference between LRT-HRT <0.05; morphometric data not shown). In line with VO_{2max}, contractility was not different between LRT and HRT in SED condition but differed in the TR condition. HRT-TR demonstrated a 30% increase in fractional shortening, an 18% increase in speed of shortening, and a 12% increase in re-lengthening in LV cardiomyocytes, whereas no such increases occurred in LRT-TR (**Figure 1B**). Additionally, measures of intracellular Ca^{2+} cycling demonstrated a similar pattern (**Figure 1C**).

An RNA microarray experiment for LV free wall identified 360 differentially expressed genes (DEGs) between HRT-SED relative to LRT-SED and 324 DEGs between HRT-TR relative to LRT-TR (NCBI GEO accession number GSE20997; UniGene IDs were available for ~30% of Applied Biosystems 1700 Rat Genome Survey chip v.1.0, updated probe annotation at http://cord.rutgers.edu/appendix/jbt/Supplemental_Table_1.xls). Of those, osteoglycin (Ogn), an extracellular matrix protein ranked as the greatest DEG and was decreased in HRT relative to LRT in both SED and TR conditions (-2.3 and -4.6 fold, respectively). We also performed a

high-throughput functional annotation analysis (DAVID) to identify enriched biological themes among the DEGs (**Figure 1D**). In the SED condition, a gene set involving 5% of the DEGs mapped to three serine-related activity terms that were enriched 5-fold in HRT relative to LRT. Among these, the gene with the greatest differential (3-fold higher in HRT relative to LRT) was kallikrein-related peptidase 12 (Klk12), a serine protease predicted to be a strong effector of cell growth and response. In the TR condition, 7-12% of the DEGs formed gene sets that identified with terms pertaining to cell adhesion. Genes up-regulated in HRT included the cadherin-associated protein catenin (Ctnnd2) and members of integrin and metalloproteinase-disintegrin (ADAM) families - all critically important in regulating angiogenesis, neurogenesis, and tissue development.

By developing a contrasting rat model system via artificial selection, we show at several levels of organization that: 1) cardiomyocyte remodeling accompanies expansion of VO_{2max} in response to training, and 2) molecular phenotypes involving extracellular matrix genes for growth signaling and cell adhesion are a central feature underlying variation for response to training (4,5)

References

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Figure 1A contrasting rat model system for low (LRT) and high (HRT) response to exercise training. With training LRT fail to alter **(A)** VO_{2max} (p-value for interaction of SED-TR difference between LRT-HRT <0.001), (**B**) LV cardiomyocyte contractility (p-value for interactions: LV cell shortening <0.01, time to 50% shortening and re-lengthening <0.05) and (**C**) intracellular Ca^{2+} cycling (p-value for interactions: twitch and caffeine induced Ca^{2+} apmplitude <0.001, time to 50% Ca^{2+} apmplitude <0.05, and Ca^{2+} decay <0.01). Rats were 9 mo. old females (8 per group), baseline and post-test body weights were 213 ± 19 g and 238 ± 25 g, respectively (group differenes, ns). SED = sedentary condition and TR = trained condition. ns, not significant; *, p <0.05; **,p<0.01 using 2-way ANOVA. (**D**) Enriched functional annotation terms derived from The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (**DAVID**) associated with differentially expressed genes (DEGs) in LV free wall between HRT and LRT. Functional enrichment for 163 UniGene Ids mapped to 360 DEGs in the SED condition and for 130 UniGene Ids mapped to 324 DEGs in the TR condition. A fold enrichment score >2.5 and False Discovery Rate <1% was considered significant. Annotation terms are from The Gene Ontology Term = GO; cellular component (CC), biological process (BP) and molecular function (MF); InterPro = INTERPRO; Simple Modular Architecture Research Tool = SMART; Protein Information Resource = PIR.