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Effects of gradual coronary artery occlusion and exercise training on gene expression in swine heart

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Abstract

Gradual occlusion (O) of the swine left circumflex coronary artery (LCX) with an ameroid occluder results in complete O within 3 weeks, collateral vessel development, and compensatory hypertrophy. The purpose of this investigation was to determine the independent and combined effects of O and exercise training (E) on gene expression in the swine heart. Adult Yucatan miniature swine were assigned to one of the following groups ($n = 6-9/\text{group}$): sedentary control (S), exercise-trained (E), sedentary swine subjected to LCX occlusion (SO), and exercise-trained swine with LCX occlusion (EO). Exercise consisted of progressive treadmill running conducted 5 d/wk for 16 weeks. Gene expression was studied in myocardium isolated from the collateral-dependent left ventricle free wall (LV) and the collateral-independent septum (SEP) by RNA blotting. E and O each stimulated cardiac hypertrophy independently ($p < 0.001$) with no interaction. O but not E increased atrial natriuretic factor expression in the LV, but not in the SEP. E decreased the expression of β -myosin heavy chain in the LV, but not in the SEP. E retarded the expression of collagen III mRNA in SEP; but not in the LV. Exercise training and coronary artery occlusion each stimulate cardiac hypertrophy independently and induce different patterns of gene expression.

Keywords

exercise; treadmill training; cardiac hypertrophy; coronary artery occlusion

Introduction

Gradual occlusion of the swine left circumflex coronary artery (LCX) with an ameroid occluder results in complete occlusion of the LCX within 3 weeks, leading to collateral vessel development in the region served by the LCX and compensatory hypertrophy of the left ventricle (LV) (1–3). The function of the collateral-dependent region is relatively normal at baseline, but when the demand on the heart is increased by treadmill running, wall thickening is abnormal. The abnormal wall motion caused by LCX occlusion and unmasked by exercise is attenuated by moderate-to-high-intensity exercise training (3). While the regional blood flow, collateral development, and functional consequences of LCX occlusion have been studied

in detail, the cardiac hypertrophy in this region has not been characterized in terms of gene expression or collagen deposition.

Depending on the nature of the stimulus (i.e. exercise vs. disease; periodic vs. constant), cardiac hypertrophy manifests phenotypic correlates that are associated with enhanced, unchanged, or diminished myocardial function. Phenotypic changes that are neutral or beneficial are considered physiological, while changes that presage heart failure or directly impair function are termed pathological. The factors that distinguish physiological from pathological cardiac hypertrophy are only partially understood, and information gleaned from research on rodent models has provided the most mechanistic insights (4–6). Given some of the physiological differences between rodents and larger mammals, however, the generalizability of commonly used phenotypic rodent markers of cardiac hypertrophy and their utility in interpreting the phenotype and evaluating underlying mechanisms in larger mammals remains unclear. In this regard, the swine model has proven useful because its cardiac biochemistry and collateral coronary circulation are remarkably similar to that of the human (1–3). In particular, the slower contracting β -myosin heavy chain is the predominant isoform in the swine heart as it is in the human heart; this contrasts the rodent heart in which the faster contracting α -myosin heavy chain predominates (7). Correspondingly, the kinetics of contraction and relaxation in the swine heart and human heart are much slower than those in the rodent heart.

The miniature swine model of treadmill running produces physiological cardiac hypertrophy with little or no alteration in major biochemical parameters associated with calcium handling, contractile function, or metabolism (8). Indices of swine heart function and blood flow are either maintained, or augmented by exercise training (9,10). Furthermore, exercise training improves blood flow and endothelium-dependent vasorelaxation to collateral-dependent areas in which major coronary arteries are compromised (3,11). Although exercise training does not cause detectable shifts in cardiac biochemical parameters such as myosin ATPase activity, sarcoplasmic reticulum Ca^{2+} -ATPase activity, and enzyme activities of the glycolytic and oxidative pathways (8) that are typically observed in rodents, exercise training does induce significant cardiac hypertrophy of 15–30% that enhances cardiac reserve, improves myocardial blood flow, and augments myocardial function (3,8–11). Whether or not exercise training alters the expression of hypertrophy-associated genes that reflect the physiological phenotype in the porcine heart as it does in the rodent heart has not been reported.

Gradual occlusion of the left circumflex (LCX) coronary artery limits oxygen and nutrient delivery to the left ventricular free wall (LV) and stimulates angiogenesis (2,12). The development of new collaterals in the affected region in response to gradual occlusion of the LCX is sufficient to prevent severe infarction and maintain blood flow at rest, but limits blood flow when the heart is challenged by acute exercise (2). While the consequences of gradual LCX occlusion for local myocardial function and endothelial cell function have been studied extensively (1–3,11), the nature (i.e. physiologic vs. pathologic) and magnitude of the resultant cardiac hypertrophy have not been reported. Abrupt occlusion of a coronary artery in mammals leads to myocardial infarction and hypertrophy of the remaining viable tissue that is phenotypically pathological (13–15). It is not clear, however, whether gradual LCX occlusion stimulates changes in gene expression that are associated with the physiological or pathological phenotype.

Cardiac hypertrophy is typically associated with altered expression of a number of heart-specific genes. Pathological hypertrophy is characterized by reexpression of the so-called “fetal gene program”, while physiological hypertrophy is associated with gene expression changes that are both similar and distinct from those observed in pathological hypertrophy. Ventricular expression of the atrial natriuretic factor gene (ANF) is markedly increased in response to a wide range of pathological insults (16–18), while exercise training often, but not always, elicits

a similar ANF response (19–21). Thus, the expression of ANF in the ventricles is a reliable marker of cardiac hypertrophy; the magnitude of the increase in ANF expression often correlates with the magnitude of cardiac hypertrophy (22). Changes in expression of the reciprocally-regulated myosin heavy chain genes are directionally different in physiological vs. pathological cardiac hypertrophy in rodents (14,23–25) and humans (26–28). The α -isoform of cardiac myosin heavy chain is associated with a faster rate of ATP hydrolysis, faster cardiac muscle contraction, and physiological cardiac hypertrophy, while the β -isoform is associated with slower mechanical and enzymatic properties, greater energetic economy, and pathological cardiac hypertrophy (29–31). During physiological cardiac hypertrophy the extracellular matrix remodels in proportion to myocyte enlargement thereby maintaining a normal matrix-myocyte ratio. In contrast, pathological hypertrophy is associated with a disproportionate increase in the extracellular matrix, termed fibrosis. Because collagen Type III is a major component of the extracellular matrix in the heart, expression of the mRNA encoding it can be a marker of the potential for fibrosis (32,33). Based on the considerations discussed above, it was predicted that exercise training of miniature swine would increase expression of ANF and either decrease or have a neutral effect on the expression of β -myosin heavy chain and collagen genes in the swine left ventricle and septum. In contrast, it was expected that gradual occlusion of the coronary artery in swine would increase the expression of the ANF, β -myosin heavy chain, and collagen genes in the affected (ischemic) portion of the left ventricle free wall.

A number of signal transduction pathways have been defined that link extracellular signals to the transcriptional and translational machinery that produces the hypertrophic accumulation of proteins and the phenotypic characteristics (34–36). The extracellular signal-regulated kinase (ERK) pathway, one of the earliest signal transduction pathways discovered and among the best understood, serves as a prototype for signaling cascades that influence transcription of specific genes and is also involved in growth of cardiac myocytes (35). Two distinct ERK proteins referred to as ERK1 and ERK2 represent 44- and 42-kDa isoforms of ERK, respectively, with identical phosphorylation/activation sites and indistinguishable functional properties (35). Chronic heart-specific activation of MEK1, the kinase immediately upstream of ERK1/2, leads to activation of ERK1/2 and is sufficient to induce cardiac hypertrophy (37). ERK1/2 is transiently activated in the rat heart by an acute bout of exercise, but the activation of ERK is not sustained in the exercise trained heart (38). In contrast, activation of ERK1/2 is sustained for at least 2 months in response to long-term constant pressure overload in guinea pigs (39). Based on these reports, it was predicted that gradual coronary artery occlusion, but not exercise training would lead to a sustained activation of ERK1/2 in the affected area of the left ventricle of swine.

The purpose of this study was to compare the independent and combined effects of gradual occlusion of the LCX coronary artery and exercise training on the phenotype of cardiac hypertrophy in porcine myocardium. It was hypothesized that gradual occlusion of the LCX coronary artery would induce the fetal gene program as indicated by increased expression of ANF and β -MHC mRNAs, and also increase the expression of the collagen type III extracellular matrix gene in the affected (ischemic) region of the LV. It was predicted that exercise training of the non-occluded group would decrease expression of β -MHC, augment expression of ANF, and have no effect on the extracellular matrix gene encoding collagen type III. Finally it was hypothesized that if LCX occlusion increased expression of β -MHC and collagen Type III in the affected (ischemic) region of the LV, then exercise training would attenuate the occlusion-induced changes.

The data presented here show that gradual LCX occlusion and exercise training each stimulated cardiac hypertrophy independently, and when the two stimuli were combined, the hypertrophic growth was twice that of either individual effect. Gradual LCX occlusion increased expression

of ANF, but did not alter that of β -MHC or collagen Type III in the affected portion of the left ventricular free wall. Exercise training decreased expression of β -MHC in the left ventricular free wall and decreased collagen Type III expression in the septum.

Materials and methods

Animals

Adult Yucatan miniature swine of either sex were randomly assigned to one of the following groups ($n = 6-9$ /group): sedentary control (S), exercise-trained (E), sedentary swine subjected to LCX occlusion (SO), and exercise-trained swine with LCX occlusion (EO). The LCX was encircled with an ameroid occluder that provided gradual obstruction of the artery. The exercise training consisted of a progressive treadmill running program conducted 5 d/wk for 16 weeks. Gene expression was studied in the LV and the interventricular septum by RNA blotting.

Animal Instrumentation for Coronary Artery Occlusion

Animals assigned to this group were sedated with ketamine (20 mg/kg im), midazolam (0.5 mg/kg im), and glycopyrrolate (0.004 mg/kg im). Pigs were intubated and maintained on isoflurane anesthesia. The animals were prepared for aseptic surgery, a left lateral thoracotomy was performed, and the pericardium was incised. The LCX was bluntly dissected free of surrounding connective tissue, and the artery was encircled with an ameroid occluder (2.5 to 3.4 mm ID) to provide gradual obstruction of the artery. The ameroid occluder is an internally concave semi-circular metal ring filled with ameroid material that expands as it absorbs moisture. When surgically placed it does not impose any constriction on the artery, but gradually expands until it completely occludes the artery. The occlusion is typically complete by 3 weeks post-surgery, but it typically results in little (usually less than 5%) infarction of the area served by the LCX because of the gradual nature of the ameroid expansion (1-3). Buprenorphine (0.01 mg/kg iv) was administered intraoperatively for pain control. A standard thoracotomy closure was performed in layers. In the immediate postoperative period, intrapleural bupivacaine was instilled for pain relief. Penicillin (30,000 U im) was administered perioperatively, and trimethoprim sulfa (480 mg/50 lb per 0 s) was given for 5 days after surgery. Surgical procedures described were in compliance with the Position of the American Heart Association on Research Animal Use and were approved by the Animal Care and Use Committee of the University of Missouri.

Exercise Training

Eight weeks after surgery, pigs were divided into sedentary and exercise-trained groups. Exercise-trained pigs were acclimated to a low-speed motorized treadmill (Quinton) to initiate an exercise training protocol of progressive intensity adapted from a treadmill training program formulated by Tipton *et al.* (40). During the first week of training, pigs ran on the treadmill at 3 miles/h (mph) and 0% grade for 20-30 min followed by a 15-min sprint at 5 mph. Length of training and treadmill speed were increased over time in accordance with the tolerance of individual pigs. From the fourth week of training, the exercise protocol consisted of an 85-min workout with a 5-min warm-up at 2.5 mph, a 15-min sprint at 5-8 mph, a 60-min endurance run at 4-5 mph, and a 5-min cool-down run at 2 mph. Sedentary pigs remained in pens during the same time period.

Exercise-trained animals were killed 14-16 hours after the last bout of exercise. Sedentary animals were killed at a similar time of day. Samples from the LV free wall and septum were fast frozen in liquid nitrogen and stored at -70°C . The LV free wall samples were taken from an area served by the LCX. The efficacy of training was assessed by comparing heart-to-body weight (HW/BW) ratios, and skeletal muscle oxidative capacity of sedentary and exercise-trained pigs. Skeletal muscle oxidative capacity was assessed by measuring citrate synthase

activity using a spectrophotometer. Coronary and pulmonary arteries of the animals were used in other studies (41,42).

Northern blotting

Northern blotting was performed as described previously with modifications (32). RNA was isolated from left and right ventricles by the method of Chomczynski and Sacchi (43). Ten or twenty micrograms of total RNA was size fractionated by electrophoresis through 1% agarose gels, transferred electrophoretically at 5 V·cm⁻¹ to a nylon (Nytran-SPC) membrane and hybridized with ³²P-radiolabelled probes overnight at 68°C for cDNA probes and 42°C for oligonucleotide probes using PerfectHyb Plus (Sigma). Hybridization intensity was quantified with a Personal Molecular Imager FX (Bio-Rad). Signals visualized on a computer screen were identified by position relative to 18S or 28S rRNA migration, delineated by rectangles and quantified after background subtraction. Each blot was subsequently stripped and reprobed. The signal from each sample was normalized to the signal obtained with a probe for either the 18S rRNA, the 28S rRNA, or glyceraldehydes-3-phosphate dehydrogenase (GAPDH).

Probes

Complementary DNA probes were synthesized from a template by the random prime method as described previously (32). The probe for human pro- α 1(III) collagen was a cDNA kindly provided by M-L. Chu (44). The probes for atrial natriuretic factor (ANF), β -myosin heavy chain (β -MHC), 28S ribosomal RNA, the 18S ribosomal RNA, and GAPDH, were end-labeled synthetic oligonucleotides with the sequences shown in Table 1.

Sample preparation for immunoblotting

Tissue samples taken from both the left ventricle free wall and septum were homogenized in buffer (62.5 mmol/L Tris, pH 6.8, 1 mmol/L PMSF, 10 μ g/ μ l Aprotinin, 10 μ g/ μ l Leupeptin, 100 μ mol/L sodium orthovanadate, 1% Triton X-100). Protein concentrations were determined using the BCA reagent and a Spectrogenesis spectrophotometer. Samples were adjusted to a final concentration of 2 mg/ml in sample buffer (125 mmol/L Tris, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.001% bromphenol blue). A Western blot titration was performed in order to determine the optimal amount of protein to be loaded into each well. 20 μ g of protein per lane provided optimal results for the study of ERK.

Immunoprecipitation of ERK2

After homogenization, a 400 μ g portion of each sample was immunoprecipitated in RIPA buffer (10 mM Tris pH 7.2, 150 mM NaCl, 1% deoxycholic acid, 1% Triton X-100, 0.1% SDS) with 10 μ l of ERK2 rabbit polyclonal antibody (Santa Cruz). 160 μ l of Protein A was added to each sample followed by centrifugation and washing of the pellet with lysis buffer (20 mM Tris pH 8.0, 2 mM EDTA, 50 mM β -glycerol-phosphate, 1 mM sodium orthovanadate, 1% Triton X-100, 10% glycerol, 1 mM PMSF, aprotinin, leupetin). 80 μ l of 2X-sample buffer was added to each pellet and then boiled. 10 μ l (20 μ g) of each sample were loaded into each well for gel electrophoresis.

Immunoblotting of ERK2

All samples underwent SDS-PAGE gel electrophoresis using 10% acrylamide gels. Proteins were then transferred to a PVDF membrane. The antibodies used to study the activity of ERK included the ERK2 rabbit polyclonal antibody (Santa Cruz) followed by the peroxidase-labeled anti-rabbit secondary antibody (Amersham); and the phospho-p44/42 MAPK monoclonal antibody (New England Bio Labs) followed by the peroxidase labeled anti-mouse secondary antibody (Amersham). Antibody binding was detected using Enhanced Chemiluminescence (ECL). Signals were quantified with a laser densitometer.

Statistics

Data are expressed as mean $\pm$ SE. Comparisons between groups were made with two-factor ANOVA (SYSTAT). Outliers were excluded according to the studentized residual method. One outlier in the sedentary group was excluded from the HW/BW data. One outlier in the exercise-trained occlusion group was excluded from the LV ANF data. One outlier in the exercise-trained occlusion group was excluded from the LV β -MHC data. Alpha was set at ($p < 0.05$).

Results

To determine the effects of exercise training and gradual LCX occlusion on the magnitude of cardiac growth, HW/BW ratios were examined (Table 2). Exercise training (13%) and gradual LCX occlusion (13%) each increased the HW/BW ratio independently. There was no significant interaction between the effects of exercise training and LCX occlusion, indicating that the effects of exercise and LCX occlusion were neither synergistic nor interfering, but simply additive.

The efficacy of exercise training was also tested by measuring citrate synthase activity (Table 3). Citrate synthase activity was significantly elevated in the deltoid, the lateral head of the triceps, and the long head of the triceps by exercise training ($p < 0.05$). There were no main effects of LCX occlusion, nor were there any exercise-occlusion interactive effects on citrate synthase activity in any of the three skeletal muscles.

To determine the phenotypic pattern of altered gene expression induced by exercise training and gradual LCX occlusion, the levels of mRNA in the LV free wall and septum were measured by Northern blotting. The relative levels of ANF mRNA were increased by 15% and 47% in the LV of swine that underwent gradual LCX occlusion without and with exercise training, respectively (Figure 1). There was a significant main effect of gradual LCX occlusion on ANF expression in the LV ($p < 0.05$), but the main effect of exercise training was not significant. Although there was no significant interaction between exercise training and LCX occlusion ($p = 0.15$), the ANF mean value was numerically greatest in the EO group suggesting the tendency for a weak interaction. When ANF data from the LV were normalized to GAPDH instead of the 28S rRNA, the results were similar (data not shown). There were no main effects or interactive effects of exercise training and gradual LCX occlusion on ANF expression in the septum.

Exercise training decreased the expression of β -myosin heavy chain in the LV by 25% in non-occluded swine and by 56% in swine with gradual LCX occlusion (Figure 1). There were no significant effects of gradual LCX occlusion on the expression of β -myosin heavy chain in the LV. When β -myosin heavy chain data from the LV were normalized to GAPDH instead of the 28S rRNA, the results were similar (data not shown). There were no differences among any of the 4 groups in the expression of β -myosin heavy chain in the septum.

Expression of collagen III mRNA in the LV was not different among the four groups of swine, but there was a main effect of chronic exercise training to decrease the expression of collagen III mRNA in the septum by approximately 40% (Figure 2). When collagen III data from the LV were normalized to GAPDH instead of the 18S rRNA, the results were similar (data not shown). There were no significant main effects of gradual LCX occlusion on collagen type III expression in the septum, nor were there any interactive effects of occlusion and exercise.

To determine the singular and combined effects of exercise training and LCX occlusion on molecular signaling in the left ventricle, the phosphorylation of ERK1/2 was studied. Tissue samples from the LV and septum were subjected to SDS-PAGE on a 10% gel. ERK1/2

abundance was determined by immunoblot analysis using an ERK2 antibody and a phospho-specific p42/p44 MAPK antibody was used to determine ERK1/2 activation. Then the signal from the phospho-specific pMAPK antibody was normalized to the signal for the polyclonal ERK2 antibody (Figure 3). The relative values for phosphorylation/activation of ERK1/2 expressed in arbitrary units were S, 1.7 ± 0.2 (mean $\pm$ SE); E, 1.6 ± 0.1 ; SO, 1.4 ± 0.1 ; EO, 1.5 ± 0.2 in the LV. In the septum, the values were: S, 4.1 ± 1.1 ; E, 3.1 ± 0.8 ; SO, 2.9 ± 0.3 ; EO, 2.1 ± 0.5 . There were no statistically significant differences in either the abundance or the phosphorylation of ERK1/2 in either the LV or the septum.

Discussion

The aim of this study was to compare the independent and combined effects of gradual occlusion of the LCX coronary artery and exercise training on the phenotype of cardiac hypertrophy in porcine myocardium. The major findings of this study are that 1) gradual LCX occlusion stimulates cardiac hypertrophy that is similar in magnitude to that induced independently by exercise training, 2) the combination of LCX occlusion and exercise training doubles the magnitude of LV hypertrophy, 3) gradual occlusion of the LCX coronary artery induces some features of the fetal gene program as indicated by increased expression of ANF, but not others; the levels of β -MHC, collagen type III were not altered by LCX occlusion, 4) exercise training decreases expression of β -MHC in the LV and decreases expression of collagen Type III in the septum, but does not significantly alter the expression of ANF in either region. The data demonstrate that exercise training and gradual LCX occlusion each stimulate cardiac hypertrophy independently, and that when combined, the effects of occlusion and exercise training are neither synergistic nor interfering, but simply additive.

ANF is a reliable and commonly used mRNA marker of ventricular cardiac hypertrophy. Moreover, its expression in the ventricles is increased in response to both physiological and pathological stimuli (16,19,20). Rapid pacing and pressure overload both increased ANF mRNA expression in the affected swine ventricle (17,18). Thus it is not surprising that gradual occlusion of the LCX coronary artery increased the levels of ANF mRNA in the LV and that there was a weak tendency for the increase in ANF expression to be greatest when exercise training and gradual LCX occlusion were combined. It is noteworthy that the levels of ANF mRNA are elevated at the time of study, 24 weeks after placement of the Ameroid occluder and 16 weeks after the initiation of the exercise training. These findings demonstrate that elevated steady state levels of ANF mRNA are an adaptation to LCX occlusion that persists long after the stimulus is initiated. The data suggest that the hypertrophic response and the elevated expression of ANF are related to ischemia in the collateral-dependent area induced by the limited collateral development after LCX occlusion, ischemia that may be exacerbated by the daily bouts of exercise (2,3).

The functional heterogeneity of myosin provides a mechanism by which the heart can mix isoforms to optimize the coordinated action of sarcomeres from different regions of the ventricular wall that may have different requirements for speed versus economy (45,46). The functional consequences of myosin isoform switching are well understood in rodents and rabbits because the different protein isoforms are separable electrophoretically (47). In contrast, myosin heavy chain protein isoforms from higher mammals have not been successfully separated by electrophoretic methods. Therefore, our understanding of the role of myosin isoforms in higher mammals has been limited to inferences drawn from measurements of mRNA levels. Studies of mRNA levels indicate that both the α - and β -myosin heavy chain genes are expressed in human heart, that they are influenced by thyroid hormone, and that the β -myosin heavy chain isoform predominates (26–28,48). Even though the expression of α -myosin heavy chain is thought to be relatively low in the human heart, decreases in the expression of α -myosin heavy chain mRNA are associated with aging and heart failure in

humans (26–28). At the protein level, exercise training has either neutral effects on myosin isoform expression or tips the balance in favor of α -myosin heavy chain (24,41). In some (but not all) cases, exercise training decreases the expression of β -myosin heavy chain mRNA in the rodent heart (50–52). Previous studies of exercise-trained swine have failed to detect any differences in myosin ATPase activity in LV homogenates, suggesting that there is no significant switching of myosin isoforms in the swine heart in response to exercise training (8). Thus it is surprising that in the present study, exercise training led to a decrease in the expression of β -myosin heavy chain mRNA. The functional significance of this finding is not clear, but it suggests that some of the same exercise training-induced mechanisms that are operative in rodent hearts may be at work in the hearts of higher mammals.

Exercise training attenuates the age-associated increase in crosslinking of collagen in the rodent heart, but increases the expression of collagen type III mRNA in the aged rodent heart (53–55). The effect of exercise training to reduce the steady state levels of collagen Type III mRNA in the septum suggests that collagen regulation may differ by age and species. Reduced activation of the collagen type III gene could be a positive effect of exercise training and could be a mechanism by which exercise stimulates cardiac hypertrophy without fibrosis, but these points are speculative at present. The lack of a similar exercise training effect on collagen gene expression in the LV of non-occluded swine is somewhat puzzling, because the exercise effects on the expression of other genes are most prominent in the LV.

Based on the differential response of ERK1/2 to chronic exercise training and LV pressure overload that was observed in rodents (38,39), it had been predicted that activation of ERK1/2 would be greater in the affected area of the LV in swine with gradual LCX occlusion compared with that of sedentary and exercise-trained swine. Neither LCX occlusion nor chronic exercise increased the steady-state phosphorylation (activation) of ERK1/2 in the left ventricle of miniature swine. The lack of increased activation in the LCX occlusion groups (and the lack of unambiguous activation of any of the markers of pathological hypertrophy employed herein by LCX occlusion) may relate the relatively gradual and modest nature of the occlusion stimulus. Although the gradual LCX occlusion did produce significant cardiac hypertrophy (Table 2), and is typically associated with abnormal wall motion (3), it does not lead to overt heart failure as does the chronic pressure overload model in which ERK1/2 was observed to be chronically activated (39).

The present study has limitations. First, the number of samples studied was sufficient to provide excellent power for main effects, but somewhat less robust power for interactive effects. The power achieved allowed detection of significant interactive effects at an effect size of 1.1 standard deviations (power = 0.8; β = 0.2). Due to greater within-group variability than anticipated, this was less than the 0.5 effect size that was the original goal when sample sizes were planned. Examination of the data and the *p* values for interactive effects suggests that this had little or any effect on the interpretations. The only interaction that is hinted at by *p* value is the interactive effect on ANF expression (pointed out in the Results and Discussion sections above). Secondly, swine of both sexes were used and sex was not balanced evenly across groups. This may be an important consideration, because a number of reports detail important cardiac-specific sex differences in various animal species (56–58). The impact of this limitation on interpretations in the present study is likely to be minimal, however, for the following reasons. 1) Myosin isoform switching in the heart in response to physiological and pathological stimuli has been reported in animals and humans of both sexes (26–28,48,59). 2) LV ANF expression is increased several fold in response to hypertrophic stimuli in animals of either sex (13,16–18,21,22,32,60,61); the increase in ANF expression in response to a hypertrophic stimulus is typically greater in magnitude than the gender differences in ANF expression (62); differences among rat strains in ventricular ANF expression are greater than gender differences (57). 3) Increases in expression of extracellular matrix components are reported

for animals of both sexes with no gender differences in the magnitude of response (62), but not in response to exercise in either sex (53–55,63). 4) Examination of the present data with respect to sex differences did not reveal any within-group anomalies as a function of sex.

In summary, exercise training and gradual LCX occlusion each stimulated cardiac hypertrophy independently; the combined effects of occlusion and training on the magnitude of hypertrophy were additive. Gradual occlusion of the LCX coronary artery induced an increase in the expression of ANF in the affected area of the LV, but not in the levels of β -MHC or collagen type III mRNA. Exercise training decreased expression of β -MHC in the LV and decreased expression of collagen Type III in the septum, but did not significantly alter the expression of ANF. Whereas exercise training induced a pattern of gene expression that was consistent with physiological cardiac hypertrophy, gradual occlusion of the LCX coronary artery caused the expected changes in the expression of ANF in the affected LV, but not in the expression of β -MHC or collagen Type III.

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Fig. 1.

Expression of hypertrophy-associated genes in the swine LV in response to exercise training and/or LCX occlusion. A: Representative Northern blots of mRNA in the LV. B: Bar graph of mean data of ANF mRNA expression in the LV. C: Bar graph of β -MHC mRNA expression in the LV. Values are mean $\pm$ SE for $n = 6-8$ per group. Main effects and interactive effects of exercise training and gradual LCX occlusion are indicated (2-factor ANOVA). ANF, atrial natriuretic factor; β -MHC, β -myosin heavy chain; S, sedentary control non-occluded; E, exercise-trained non-occluded; SO, sedentary with gradual LCX occlusion; EO, exercise-trained with gradual LCX occlusion.

Fig. 2.

Expression of hypertrophy-associated genes in the swine septum in response to exercise training and/or LCX occlusion. A: Representative Northern blots of mRNA in the septum. B: Bar graph of mean data of Collagen Type III mRNA expression in the LV. Values are mean $\pm$ SE for $n = 6-8$ per group. Main effects and interactive effects of exercise training and gradual LCX occlusion are indicated (2-factor ANOVA). ANF, atrial natriuretic factor; β -MHC, β -myosin heavy chain; S, sedentary control non-occluded; E, exercise-trained non-occluded; SO, sedentary with gradual LCX occlusion; EO, exercise-trained with gradual LCX occlusion.

Fig. 3.

Phosphorylation state of ERK1/2 in the LV free wall and septum of swine in response to exercise training and/or LCX occlusion. Representative Western blot of protein samples immunoprecipitated with ERK2 from miniature swine LV free wall and septum. pERK1/2, phosphorylated ERK1/2 detected with an antibody specific for dually phosphorylated ERK1/2; ERK2, p42-ERK detected with an antibody that recognizes p42-ERK regardless of phosphorylation state. There were no significant main effects or interactive effects among the groups (2-factor ANOVA). Dishes of cultured NIH-3T3 fibroblasts were treated with vehicle (–) or fetal bovine serum (+) as negative and positive controls, respectively, for ERK1/2 phosphorylation.

Table 1

Oligonucleotide probes used for Northern blotting.

Target Gene	Sequence	Length	Region	Accession # or Reference
ANF	5'-GGGGGCTGAGAGGGGCCCCACCTTCCTCATTCTGCTCGC-3'	39-mer	Coding	X54669
β -MHC	5'-GCTCCAGGACTGGGAGCTTTGTTGCGCCCTCAGGATGGGG-3'	40-mer	3' UTR	X91846, AB053226 (reference 64)
28S rRNA	5'-TGGCAACAACACATCATCAGTAGGGT 3'	26-mer	Ribosomal	F14557
18S rRNA	5'-ACGGTATCTGATCGTCTTCGAACC-3'	24-mer	Ribosomal	AF102857
GAPDH	5'-CCTCTCTCCTCCTCGCGTGCTCTTGCTGGGGTTGGTGGTCC-3'	41-mer	3' UTR	AF017079

ANF, atrial natriuretic factor; MHC, myosin heavy chain; rRNA, ribosomal RNA; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; UTR, untranslated region.

Table 2

Heart weights and body weights of the four groups of swine.

Dependent Variable	Group				Probabilities (2-factor ANOVA)		
	Sedentary Non-Occluded (S)	Exercise-Trained Non-Occluded (E)	Sedentary Occluded (SO)	Exercise-Trained Occluded (EO)	Occlusion	Exercise	O*E Interaction
Sex (n)	M = 2, F = 6	M = 7, F = 0	M = 1, F = 6	M = 7, F = 1			
Body Weight (kg)	31.0 ± 1.7	32.6 ± 0.9	34.1 ± 1.2	34.6 ± 1.2	NS	NS	NS
Heart Weight (g)	150.5 ± 4.7	172.1 ± 7.9	185.5 ± 8.9	210.1 ± 5.2	<i>P</i> < 0.01	<i>P</i> < 0.001	NS
HW/BW (g/kg)	4.7 ± 0.4	5.3 ± 0.2	5.3 ± 0.1	6.1 ± 0.1	<i>P</i> < 0.001	<i>P</i> < 0.001	NS

Values are mean ± SE. HW, heart weight; BW, body weight; M, male; F, female.

Table 3

Citrate synthase activity in selected skeletal muscles of swine.

Citrate synthase activity ($\mu\text{mol}/\text{min}/\text{g}$)	Group				Probabilities (2-factor ANOVA)		
	Sedentary Non-Occluded (S)	Exercise-Trained Non-Occluded (E)	Sedentary Occluded (SO)	Exercise-Trained Occluded (EO)	Occlusion	Exercise	O*E Interaction
n	6	7	7	8			
Deltoid	15.3 $\pm$ 1.3	21.8 $\pm$ 1.9	13.5 $\pm$ 0.7	22.0 $\pm$ 1.8	NS	$P < 0.05$	NS
Triceps, lateral head	11.2 $\pm$ 1.0	18.2 $\pm$ 1.6	11.0 $\pm$ 1.0	15.9 $\pm$ 1.6	NS	$P < 0.05$	NS
Triceps, long head	10.2 $\pm$ 0.7	15.2 $\pm$ 1.7	10.4 $\pm$ 1.6	11.0 $\pm$ 0.7	NS	$P < 0.05$	NS

Values are mean $\pm$ SE. See reference 65 for methodological details.