INTRODUCTION

The prevalence of cardiovascular disease highlights the necessity of developing effective treatments to improve heart function.1,2 Aerobic exercise leads to marked structural and mechanical cardiac adaptations that result in improvements in the overall cardiac function. Structurally, aerobic exercise has been shown to result in increased ventricular volume and proportional thickening of the muscular walls3,4,5 and greater left ventricular mass in rats6. Mechanically, aerobic exercise results in increased shortening velocity7,8 and calcium sensitivity9–11 in isolated rat heart muscles. Boldt et al.12 demonstrated that after 12 weeks of aerobic treadmill running, trained rats had significantly greater left ventricular volume and wall thickness, and isolated skinned cardiac fiber bundles had greater maximal shortening velocities and increased calcium sensitivity than that of sedentary controls. Therefore, exercise is thought to be an effective means of improving cardiac function by stimulating both structural and mechanical adaptations.

In skeletal muscles, whey protein supplementation has been shown to enhance anabolic adaptations in response to resistance exercise13,14. This has been shown to be due to greater rates of muscle protein synthesis (MPS) in response to protein ingestion following exercise compared to that of exercise alone15. Whey protein, in comparison with other protein sources, is a potent stimulator of MPS as it is a complete protein that is quickly digested and is high in branched-chain amino acids such as leucine15. With aerobic training, chronic post-exercise protein supplementation has been shown to enhance adaptations in VO2max16 by increasing blood volume17, skeletal muscle mitochondrial biogenesis18, and skeletal muscle regeneration through increased MPS19. The rate of muscle protein turnover, which is a combination of muscle protein breakdown and synthesis, is 83% greater in rat cardiac muscle than that in type I (soleus) and type II (gastrocnemius) muscle fibers20. Therefore, the cardiac muscle may be a receptive target for protein supplement intervention. Protein supply has also been shown to result in acute increases in MPS in isolated cardiac preparations21. Morgan et al.22 used a Langendorff isolated rat heart preparation and measured a 40% increase in MPS following the infusion of serum with high amino acid concentrations compared to that...
of baseline. Similarly, Garlick and Grant23 administered rat serum amino acid injections and observed an increase in cardiac MPS in vivo. Given the evidence for the ergogenic effects of whey protein on skeletal muscle following resistance and aerobic exercise,19,24–26 and the observed effects on cardiac MPS22,23, ingestion of a high-protein diet may also have an ergogenic effect on cardiac muscle and its adaptation to aerobic exercise. Therefore, this study aimed to determine the effects of a high-whey-protein diet on cardiac structural and mechanical properties and cardiac function in response to aerobic training. We hypothesized that whey supplementation enhances the structural and mechanical adaptation of the heart in response to chronic aerobic exercise.

METHODS

Exercise and dietary intervention

Twelve-week-old male Sprague–Dawley rats were randomly assigned to four groups: (i) aerobic exercise with a standard diet (Ex+Standard, n = 6), (ii) aerobic exercise with a high-protein diet (Ex+Pro, n = 5), (iii) sedentary with a standard diet (Sed+Standard, n = 6), and (iv) sedentary with a high-protein diet (Sed+Pro, n = 5). One animal from each of the Sed+Pro and Standard+Pro groups died during the intervention and were excluded. The standard diet (AIN93-M) consisted of 13% protein, derived entirely from casein. The protein diet contained whey protein that was incorporated directly into the diet by the manufacturer. It had the same composition as the standard diet, except for an additional 13% protein from the whey concentrate (substituted by mass for corn starch) (5Whey, TestDiet, St. Louis, MO, USA), thereby doubling the dietary protein of the conventional standard diet (Table 1). Rats in the exercise groups participated in a progressive treadmill program12,27, which consisted of 60 min of a progressive treadmill running program 5 days/week up to a speed of 25 m/min. The sedentary group was placed on a treadmill (Columbus Instruments Exer-3R treadmill, Columbus, OH, USA) and walked for 15 min at 10 m/min once/week. All training sessions lasted 12 weeks. The animals were housed individually at 21°C on a 12:12 light–dark cycle and had access to food and water ad libitum. The study protocol (AC16-130) was approved by the University of Calgary Animal Care Committee and conformed to the guidelines for the Care and Use of Laboratory Animals.

Fitness indices

At the end of the 12-week intervention, aerobic fitness was determined for all rats by measuring the time to exhaustion using a graded treadmill test27,28. Each animal began walking on a treadmill at 12 m/min. The speed was increased by 1 m/min every 2 min for the first 16 min and then by 2 m/min every 3 min until failure. A shocker located on the back of the treadmill delivered mild electrical stimulation when the animals touched it. Exhaustion was defined when the animal touched the shocker five times within 1 min, or remained on the shocker28. Body mass was recorded weekly, and body fat composition was measured at the end of 12 weeks using dual-energy X-ray absorptiometry.

Cardiac structural and functional adaptations

Echocardiographic evaluation was performed using an Esaote MyLab™30Gold Cardiovascular Ultrasound System (Canadian Veterinary Imaging, Georgetown, Ontario, Canada). Rats were anesthetized with isoflurane and placed in the dorsal decubitus position, and the ventral thoracic area was shaved. Two-dimensional images were obtained from orthogonal long-axis four- and two-chamber views29. The end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction were calculated from three consecutive cardiac cycles by tracing the endocardial border of the left ventricle (LV) in end-diastole and end-systole using the Simpson biplane method29. The LV internal diameter (LVID) and anterior and posterior wall thicknesses (LVAW and LVPW, respectively) were determined using M-mode

Table 1. Composition of the standard and experimental diets used in this study.

| AINM standard diet | High-protein diet |
|--------------------|-------------------|
| **Caloric composition (% based on energy)** | **Caloric composition (% based on energy)** |
| Protein | 13 | 20.0 |
| Carbohydrate | 50.9 | 50.0 |
| Fat | 4.1 | 20.0 |

| AINM standard diet | High-protein diet |
|--------------------|-------------------|
| **Diet ingredients (% based on mass)** | **Diet ingredients (% based on mass)** |
| Corn Starch | 46.5 | 30.06 |
| Maltodextrin | 15.5 | 15.5 |
| Casein | 14.0 | 14.0 |
| Whey protein concentrate | 0.0 | 16.51 |
| Sucrose | 10.0 | 10.0 |
| Powdered cellulose | 5.0 | 5.0 |
| Soybean oil | 4.0 | 4.0 |
| AIN93M mineral mix | 3.5 | 3.5 |
| AIN93M vitamin mix | 1.0 | 1.0 |
| Choline bitartrate | 0.25 | 0.25 |
| L-Cystine | 0.18 | 0.18 |
| t-Butylhydroquinone | 0.008 | 0.008 |
imaging during systole (LVIDs, LVAWs, LVPWs) and dia-
tole (LVIDd, LVAWd, LVPWd). LV mass was determined
according to Watson et al. (2004). Measurements were
performed at baseline and after 12 weeks of training.

Tissue isolation
Rats were anesthetized with isoflurane, the chest cavities
were opened, and the aorta and vena cava were severed.
The hearts were immediately flushed with rigor solution, the
external vessels were dissected away, and the heart mass was
recorded. The LV was cut open and 3–4 thin strips of tra-
beculae were sliced along the LV wall and placed in a rigor
solution on ice. Following 2 h in rigor solution, the samples
were transferred to a 50/50 rigor/glycerol solution and left
overnight at 4°C. The following morning, the muscle strips
were transferred to a fresh 50/50 rigor/glycerol solution and
chemically skinned at −20°C for 3 weeks before mechani-
cal testing. All samples were tested between 3 and 4 weeks
postharvest. An additional strip of trabeculae was dissected,
immEDIATELY flash frozen in liquid nitrogen, and stored at
−80°C for biochemical analysis.

Preparation for mechanical testing
All mechanical tests were performed in accordance with
previous work (Boldt et al., 2020a, 2021). Once skinned,
a strip of muscle was removed and placed in a relaxing solu-
tion on ice. Following 2 h in rigor solution, the samples
were transferred to a 50/50 rigor/glycerol solution and left
overnight at 4°C. The following morning, the muscle strips
were transferred to a fresh 50/50 rigor/glycerol solution and
chemically skinned at −20°C for 3 weeks before mechani-
cal testing. All samples were tested between 3 and 4 weeks
postharvest. An additional strip of trabeculae was dissected,
immEDIATELY flash frozen in liquid nitrogen, and stored at
−80°C for biochemical analysis.

Maximal active stress
The maximal active isometric force, normalized to the
cross-sectional area to obtain stress, was determined using a
maximal activating solution (pCa = −log([Ca²⁺]) = 4.2). Once
the peak stress was reached, the sample was returned to the
relaxation solution for deactivation. Maximal active stress
was calculated as the difference between the total (measured)
stress and resting passive stress immediately preceding the
contraction.

Passive stress
Samples were stretched passively from a resting aver-
age sarcomere length of 2.2 μm to 2.42 μm (10% of the
sample’s total resting length) at a rate of 5% fiber length/s.
Once the sample had been stretched to 2.42 μm, the length
was held constant for 20 s to allow for stress relaxation
before being returned to the initial length (2.2 μm). The
peak passive force was taken as the maximum value at the
end of the stretch, whereas the steady-state passive force
was determined as the mean value of the last second after
the stress relaxation was complete. Peak and steady-state
passive forces were also measured at an average sarcomere
length of 2.53 μm (15% stretch from the initial fiber length)
at the same rate of stretch, normalizing forces to each sam-
ple’s cross-sectional area to obtain stress. Following the
passive stretches, the samples were reactivated maximally
to ensure that they did not sustain damage. If the maximum
active stress decreased by more than 15% from the initial
contraction before stretching, the data from that fiber were
excluded.

Unloaded shortening velocity
The maximal unloaded shortening velocity was deter-
mined at an initial sarcomere length of 2.2 μm using the
slack test. The samples were maximally activated and
rapidly shortened (in 2 ms) by 10% (ΔL) of the sample’s
length. Thus, the samples became slack and the measured
force dropped to zero. The time from the onset of rapid
shortening until the force was redeveloped (Δt) was mea-
sured. The samples were then re-lengthened, relaxed, and
allowed to rest for 3 min. This rapid shortening test was re-
peated for ΔL values of 11%, 12%, and 13% of the sample’s
length. The slope of the linear relationship between ΔL and
Δt is used to determine the unloaded (maximal) shortening
velocity.

Calcium sensitivity
The sensitivity of the muscle to calcium was determined
by establishing a force–pCa curve between pCa 7.0–4.2.
The baseline resting force was measured before transferr-
ing the sample to the first solution (pCa 7.0). Once the
force stabilized, the sample was moved to the next solution,
which contained a higher calcium concentration (pCa 6.8).
The samples were exposed to solutions of continuously
increasing calcium concentrations of pCa 6.6, 6.4, 6.2, 6.0,
5.8, and 5.4, until reaching pCa 4.2 (maximal activating
solution). The relative force at each calcium concentration
was calculated by dividing the difference between the max-
imum force at each concentration and the baseline force by
the difference between the maximum force produced at pCa
4.2 and the baseline force. The force–pCa relationship was
calculated by approximating the data using least-squares re-
gression for the Hill equation in SigmaPlot 13. Calcium sen-
sitivity was defined and quantitatively compared between
samples by determining the pCa value that yielded half of
the maximal tension (pCa₅₀) and the corresponding slope of
the force–pCa relationship: crossbridge cooperativity (nH).
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Myosin heavy chain composition

Myosin heavy chain (MHC) composition was determined using SDS-PAGE gel electrophoresis on 4.5% and 7.5% acrylamide stacking and separating gels, respectively. Myofibrillar protein was extracted from muscle powder and mixed with solubilization buffer (62.5 mM Tris HCl, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.02% bromophenol blue, pH 6.8) to a final concentration of 2.2 mg/1 µL. The samples were boiled for 3 min and immediately centrifuged at 5000 RPM for 15 min at 4°C. The solubilized samples were loaded (0.5 µL/well) into 0.75-mm-thick acrylamide gels, and the gels were run in a Bio-Rad Mini-Protean® III unit at 4°C for 10 h at a constant voltage of 72 V and 25 h at a constant current of 1 mA/gel. Following electrophoresis, the gels were stained with Coomassie blue for 60 min and then destained with a 50% ethanol, 7% acetic acid solution for 5 min and a 5% ethanol, 7% acetic acid solution for at least 60 min. After destaining, the gels were scanned using a Bio-Rad scanner and analyzed with ImageJ for optical density (OD) to determine the relative composition of MHCs, α-MHC, and β-MHC. Results were expressed as the composition of α-MHC relative to total MHC (i.e., OD of α-MHC/[OD of α-MHC + OD of β-MHC]).

Solutions

The relaxing solution contained the following (in mmol/L): 170 potassium propionate, 2.5 magnesium acetate, 20 MOPS (3-[N-morpholino]propane sulfonic acid), 5 K2EGTA, 2.5 adenosine triphosphate (ATP), and 14.5 creatine phosphate, at pH 7.0. The activating solution contained the following (in mmol/L): 170 potassium propionate, 2.5 magnesium acetate, 10 MOPS, 2.5 ATP, and CaEGTA and K2EGTA mixed at different proportions to obtain a pCa of 4.2, at pH 7.0. The washing solution contained the following (in mmol/L): 185 potassium propionate, 2.5 magnesium acetate, 10 MOPS, and 2.5 ATP, at pH 7.0. All solutions contained one tablet of protease inhibitors (Complete; Roche Diagnostics, Quebec, Canada) per 100 mL of solution.

Results

Fitness indices

After 12 weeks of intervention, animals in the exercise groups (Ex+Standard and Ex+Pro) had significantly lower body mass than that in sedentary animals (Sed+Standard and Sed+Pro) regardless of diet (p = 0.007–0.012), but there were no differences between groups for body fat percentage (p = 0.910) (Table 2).

Animals from the Ex+Standard and Ex+Pro groups had significantly longer exhaustion times on the graded treadmill test than that in the Sed+Standard (p = 0.013 and p = 0.002, respectively) and Sed+Pro groups (p = 0.010 and p = 0.002, respectively) (Figure 1). There were no differences in the time to exhaustion between the two sedentary groups (p

![Figure 1. Time to exhaustion on graded exercise test for each group. * indicates a significant difference compared to that of Sed+Standard group. † indicates a significant difference from the Sed+Pro group. Significance was set at p < 0.05.](image-url)
Figure 2. Cardiac structural data from echocardiography. Closed data points indicate baseline value, and open data point indicates end point data. ‡ indicates a significant difference between baseline and end point values. Significance was set at p < 0.05.
Cardiac structural and functional adaptations

There were no statistical differences between the groups in terms of absolute heart mass (p = 0.757) and heart mass normalized to tibial length (p = 0.108) (Table 2). When normalized to body mass, hearts from the Ex+Standard and Ex+Pro groups were significantly greater than those from the Sed+Standard (p = 0.043 and p = 0.009, respectively) and Sed+Pro groups (p = 0.046 and p = 0.010, respectively), but were not different between the animals of the two exercise groups (p = 0.500).

There were no differences between the groups in any structural variables measured by echocardiography at baseline or at the end of the exercise intervention period (Figure 2). Compared with that of baseline, there were no significant changes in ESV (p = 0.498–0.753), LVAWs (p = 0.140–0.893), LVPWd (p = 0.062–0.336), or LVPWs (p = 0.344–0.686) for any of the groups. EDV increased by 29% in animals from the Ex+Pro group (p = 0.043) and 19% in animals from the Ex+Standard group (p = 0.028) but remained the same for the Sed+Standard (p = 0.463) and Sed+Pro (p = 0.344) groups. LVIDd significantly increased in Ex+Pro (p = 0.043) and Ex+Standard (p = 0.046) groups, tended to be higher (p = 0.068) in the Sed+Pro group, but did not change in the Sed+Standard group (p = 0.752). LVIDs increased in the Ex+Standard (p = 0.026) and Ex+Pro (p = 0.042) groups but were not different in the Sed+Standard or Sed+Pro groups (p = 0.173, p = 0.078). LVAWd was unchanged in the Sed+Standard (p = 0.136), Sed+Pro (p = 0.593), and Ex+Pro (p = 0.197) groups, but increased in the Ex+Standard group (p = 0.044). LV mass significantly increased from baseline to end point in the Sed+Pro (p = 0.043), Ex+Standard (p = 0.028), and Ex+Pro (p = 0.043) groups, but remained unchanged in the Sed+Standard group (p = 0.249).

Mechanical testing

Active and passive stress

There were no differences in the maximal active stress production between groups (p = 0.535) (Figure 3A). Similarly, there were no differences between any groups for peak passive stresses or passive stress following stress relaxation for stretch magnitudes of 10% (p = 0.338) or 15% (p = 0.176) of the initial length (2.2 µm) (Table 3).

Unloaded shortening velocity

The maximum unloaded shortening velocity was significantly greater in hearts from Ex+Standard and Ex+Pro groups than that in both Sed+Standard (p = 0.001 and 0.001, respectively) and Sed+Pro (p = 0.001 and 0.001, respectively) groups (Figure 3B). However, there were no differences between the Sed+Standard and Sed+Pro (p = 0.497) or between the Ex+Standard and Ex+Pro (p = 0.479) groups.

Calcium sensitivity

Calcium sensitivity, measured using pCa50, was significantly greater in hearts from Ex+Standard and Ex+Pro...
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Table 3. Passive stress data from skinned trabeculae preparations.

|                | Passive 10% Peak Stress (kN/m²) | Passive 10% Steady State Stress (kN/m²) | Passive 15% Peak Stress (kN/m²) | Passive 15% Steady State Stress (kN/m²) |
|----------------|---------------------------------|----------------------------------------|---------------------------------|----------------------------------------|
| Sed+Standard   | 11.9 (9.6–17.7)                 | 7.8 (6.6–11.1)                         | 22.2 (21.2–25.0)                | 13.8 (12.3–15.7)                       |
| Sed+Pro        | 14.9 (14.1–17.6)                | 10.4 (9.1–11.0)                        | 28.5 (28.2–29.7)                | 18.9 (18.5–19.1)                       |
| Ex+Standard    | 17.6 (13.4–27.9)                | 10.9 (8.2–17.7)                        | 36.04 (28.7–58.9)               | 22.6 (16.9–36.6)                       |
| Ex+Pro         | 22.3 (20.7–25.6)                | 14.6 (12.1–16.9)                       | 45.0 (38.7–45.4)                | 24.8 (23.9–28.5)                       |

Data displayed are medians(IQR). There were no significant differences for passive stress between groups.

Figure 4. (A) Representative MHC gel distribution and densitometric scans. (B) α-MHC composition. (C) Correlation between maximum shortening velocity (Vmax) and α-MHC composition relative to total MHC for each animal. † indicates a significant difference compared to that of the Sed+Standard group. ‡ indicates a significant difference from the Sed+Pro group. Significance was set at p < 0.05.

Biochemical testing

Ex+Standard and Ex+Pro groups had significantly greater α-MHC composition (relative to total MHC) than that in both Sed+Standard (p = 0.004 and 0.008, respectively) and Sed+Pro groups (p = 0.008 and 0.013, respectively) (Figure 4A and 4B). Differences based on diet were not observed between the sedentary (p = 0.953) and exercised groups (p = 0.953). A significant correlation (r = 0.73; p = 0.001) between the α-MHC composition, total MHC, and maximum shortening velocity was noted (Figure 4C).

DISCUSSION

This study aimed to investigate the effects of dietary whey protein supplementation on the structural and mechanical properties of the heart in response to aerobic training in rats. The main findings were that animals in the aerobic exercise groups had increased heart volumes, greater unloaded fascicle shortening velocities, and increased calcium sensitivities compared with that in the non-exercise control group animals. The high-protein diet intervention did not produce any adaptations in the structure or function of the heart in the exercise-trained or untrained animals compared to that in animals fed a regular, low-protein, control diet.

Fitness indices

The training protocol used in this study has previously been shown to increase VO₂max and time to exhaustion measured on graded exercise tests. Exercise has been shown to improve body composition and reduce overall body mass. In a previous study, using the same training protocol and the same strain of rats, Boldt et al. observed significantly lower body fat in aerobically trained rats than that in sedentary control animals. However, there was no additional benefit from the addition of dietary protein.

Exercise has been shown to improve body composition and reduce overall body mass. In a previous study, using the same training protocol and the same strain of rats, Boldt et al. observed significantly lower body fat in aerobically trained rats than that in sedentary control animals. In the present study, we did not observe differences in body composition, although the trained animals did, on average, have lower body mass. The AIN93-M control standard diet was
used in this study for its excellent control of protein sources, but it has a higher caloric content than that of other rat chow diets used in previous studies\(^4,5,35\). This greater caloric content may explain the lack of difference in body composition observed in the present study, as the energy cost of exercise did not overcome the high caloric density of the AIN93-M diet.

**Cardiac structural adaptations to the enriched protein diet**

Structural adaptations of the LV in response to exercise have been characterized\(^4,5,35\). Previous studies on swimmers and runners identified larger EDV and ventricular masses in athletes than that in age- and sex-matched nonathletes\(^3\). Spence et al.\(^4\) found proportional increases in EDV and wall thickness after 6 months of aerobic running training in initially untrained subjects when compared to that of baseline values. Similarly, Baggish et al.\(^36\) observed increased LV mass and volume in men and women, university teams, and long-distance rowers after 90 days of rowing-specific, intense aerobic training. The findings of the present study agree with the literature. While the LV dimensions did not change from baseline for sedentary animals, chamber volume indicated by EDV, LVIDd, and LVIDs significantly increased from baseline in animals from the Ex+Standard and Ex+Pro groups. Although there were no statistical differences between the exercise groups, all cardiac-specific increases were greater in Ex+Pro groups than that in Ex+Standard groups (EDV, 29% vs. 19%; LVIDd, 20% vs. 13%; LVIDs, 56% vs. 50%, for the Ex+Pro and Ex+Standard groups, respectively), suggesting that there may be small adaptations with the high-protein diet that were missed because of the relatively small number of animals used.

In skeletal muscles, the beneficial effects of protein ingestion are typically associated with an increase in MPS. Ingestion of a whey protein supplement following a resistance exercise training bout has been shown to result in a 125% increase in MPS, leading to greater muscle mass when continued chronically\(^13,37\). Hulmi et al.\(^38\) observed significantly greater increases in muscle cross-sectional area and strength following a 21-week resistance training program in individuals consuming a protein supplement than in those receiving a placebo.

Under normal resting conditions, the heart replaces all its proteins over 30 days\(^39\). The turnover rate of muscle protein is approximately 83% greater in cardiac muscle than that in primarily slow-twitch type I fibered (soleus) and primarily fast-twitch type II fibered (gastrocnemius) muscles in rats\(^20\). These high rates of MPS potentially present a prime target for improving cardiac function through interventions, such as protein supplementation and/or exercise. Lollo et al.\(^40\) evaluated the activity of the mammalian target of rapamycin (mTOR) anabolic pathway following 4 weeks of aerobic exercise and whey protein supplementation in rats and observed dose-dependent activation of these pathways in response to whey protein following treadmill exercise. To evaluate the potential role of protein supplementation in the rate of cardiac MPS, isolated heart preparations were fused with amino acid solutions\(^24,25\). Supplying leucine and other amino acid cocktails to isolated rat hearts resulted in a 25%–40% greater MPS compared to that of control conditions with normal amino acid levels\(^21,22\). Garlick and Grant\(^25\) observed increased rates of MPS following intravenous injection of amino acids in rats, with the greatest effects observed in response to essential amino acid injections. These studies demonstrate the potential of the heart to respond to the supply of proteins.

We found that a high-protein diet had no effect on cardiac structure in the sedentary group. Lollo et al.\(^30\) compared cardiac anabolic activity and total cardiac protein content following 4 weeks of leucine supplementation in untrained and sedentary rats. They observed minimal increases in mTOR phosphorylation with leucine in sedentary animals but did not detect differences in total muscle protein content. However, in combination with exercise, leucine led to significant activation of the mTOR pathway and increased protein content in the heart. Therefore, it is not surprising that in the present study, protein alone did not affect structural adaptation.

**Cardiac mechanical adaptations to protein supplement**

In rats, cardiac contractility is reduced by obesity\(^41,42\) and aging\(^43\) and improved by exercise\(^8,9,44\). These alterations in contractility have been attributed to changes in maximal isometric stress production\(^7\), calcium sensitivity\(^9,10\), and maximal unloaded shortening velocity\(^7,8\).

In the present study, the maximal active stress did not differ between groups. This is consistent with previous literature\(^7,8,10,27\). Although active stress production of the myocardium has been shown to be greater following resistance exercise training compared to that of sedentary controls\(^12\), it has been consistently shown to be unchanged after 11 weeks of aerobic exercise in rats trained 5–7 days per week for 30–240 min per day\(^7,8,10,27\).

In contrast to maximum active stress production, the sensitivity of the myocardium to calcium activation has been shown to increase following aerobic exercise\(^8,10,12\). Diffee et al.\(^9\) compared calcium sensitivity in rat myocardium following a treadmill protocol similar to that used in the present study. They observed significantly greater pCa\(_{50}\) values (increased calcium sensitivity) in the myocardium from aerobically trained rats compared to that of the untrained control animals, but they did not observe differences in nH. Boldt et al.\(^12\) compared calcium sensitivity following aerobic only, resistance only, or a combination of aerobic and resistance training and observed increases in pCa\(_{50}\) for the aerobic only and resistance only groups, but not for the combined aerobic and resistance training group. This lack of change in calcium sensitivity in the combination trained group was compensated, in part, by a significant increase in nH. In the present study, we observed an increase in pCa\(_{50}\) and an increase in nH for Ex+Standard and Ex+Pro groups. Increased calcium sensitivity with cardiac muscle stretching is thought to be a key factor for increased contractility with increasing length, as described by the Frank–Starling law.
Ejection power is a function of active stress production and the rate of shortening. Since maximum active isometric stress has been found to consistently remain unchanged with aerobic exercise, ejection power has been shown to be modulated primarily by shortening the velocity of the myocardium. In line with our findings, Diffee and Chung\(^8\) compared the isotonic force–velocity relationship following 11 weeks of aerobic exercise training in rats. They observed no differences in maximum isometric stress, but they observed greater rates of shortening at the corresponding relative force outputs and, consequently, a greater power output at corresponding forces and a greater peak power output. In contrast to Diffee and Chung\(^8\), who did not find a statistical difference in the unloaded shortening velocity in single permeabilized myocytes, Boldt et al.\(^12\) observed greater unloaded shortening velocities in the hearts after 12 weeks of resistance and 12 weeks of aerobic exercise training. However, the results by Diffee and Chung are likely due to issues of power in their statistical analysis, as the mean differences for the unloaded shortening velocity, reported by them in their Table 2, were substantial (trained \([\text{mean} \pm 1 \text{ SE}] 1.41 \pm 0.06 \text{ muscle length/s vs. untrained} 0.87 \pm 0.07 \text{ muscle length/s; Table 2}]\(^9\). In contrast to Diffee and Chung\(^8\), who did not find changes in MHC composition between their trained and untrained animals, we observed a significant correlation \((r = 0.73)\) between \(\alpha\)-MHC composition and shortening velocity, consistent with findings that exercise leads to a greater proportion of \(\alpha\)- vs. \(\beta\)-MHC. Increases in \(\alpha\)-MHC composition are correlated with increases in cardiac tissue-unloaded shortening velocity.\(^{47-49}\)

We did not observe differences between groups for maximum active stress production or passive stress measured at an average sarcomere length of 2.42 and 2.53 \(\mu\)m. However, animals in the exercise groups had higher maximal rates of shortening, greater calcium sensitivity, and greater crossbridge cooperativity compared to that of the sedentary group. The magnitude of these differences was similar between the Ex+Standard and Ex+Pro groups, suggesting no additional benefit of the high-protein diet on the mechanical properties of the myocardium. Similarly, the high-protein diet did not produce differences in the mechanical properties of the sedentary groups (Sed+Standard and Sed+Pro).

**Ergogenic effect of protein with exercise**

There may be several reasons why we did not observe enhanced cardiac structural or mechanical adaptation with the addition of a high-protein diet in combination with aerobic exercise. First, protein supplementation may have no effect on cardiac adaptation to exercise. The heart is a critical organ, and unlike the skeletal muscle, the body would likely prioritize the delivery of amino acids to the heart for recovery. Protein-restricted diets (6% protein) have been shown to result in negative cardiac structural and contractile remodeling,\(^{50,51}\) but the standard diet in this study had a 13% protein content, which was likely sufficient for proper adaptation of the heart to external stimuli and increased demand.

The change in heart volume did not differ statistically between the two exercise groups, but was consistently larger for EDV and LVID in the Ex+Pro group (29% and 20%, respectively) compared to that of the corresponding values in the Ex+Standard group (19% and 13%, respectively). These small differences, with an observed power of 65%, might have been lost owing to a lack of statistical power. In the present study, echocardiography was used to measure the overall cardiac structure. However, since microscopic cardiomyocyte hypertrophy was measured, differences in cardiac muscle hypertrophy that were not captured by echocardiography at the whole heart level in the selected planes may have been detected.

There is strong evidence that skeletal muscles can optimize anabolic response following resistance training.\(^{52,53}\) We chose whey protein in this study because it has been shown to be an effective supplement for skeletal muscle adaptations. It is a complete protein, has a high rate of uptake from partial hydrolysis, and has a high leucine content.\(^{54,55}\) Our protein-supplemented diet had twice the protein content than that of the standard diet. The effects of whey protein on skeletal muscle adaptations have been shown to depend on the dose\(^{54,55}\) and timing of ingestion relative to the resistance training bout\(^{56-58}\). We supplied protein to the animals’ food; therefore, the timing of ingestion relative to the training bouts was not controlled, and food intake was likely distributed throughout the day. Thus, the single-dose load was probably small and not optimally timed relative to the so-called anabolic window that opens following a training session.\(^{58}\) It is possible that single-dose supplements administered after daily exercise training sessions would have been more effective than generic supplementation through food.

There was potentially a time effect of the whey protein diet that was not captured in our study design. For example, if the cardiac tissue adaptations in the Ex+Pro group occurred at a greater rate than that in the Ex+Standard group and then plateaued, the Ex+Standard group caught up with these adaptations in the 12-week intervention period. Similarly, cardiac structure in the Ex+Pro and Ex+Standard groups adapted from baseline, but it remains unknown how they might have continued to adapt if the intervention period was longer than 12 weeks. Cardiac tissue adaptations in the Ex+Standard group would have eventually plateaued, whereas they may have continued to adapt in the Ex+Pro group. However, these are all speculations, and further work should be done to confirm whether there is an ergogenic effect of protein supplementation on cardiac adaptation with aerobic exercise training. Similarly, further work should include direct measures of blood pressure, adaptations of the renin–angiotensin system, cardiac MPS, and molecular measures to evaluate glucose and fatty acid metabolism of the heart, along with measures of mitochondrial content and function.

Based on the results of this study, we conclude that 12
weeks of aerobic exercise training leads to both structural and mechanical adaptations of the heart. However, the addition of a high-whey-protein diet did not enhance these adaptations in comparison to a regular protein control standard diet.

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REFERENCES

1. Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007-2008 to 2015-2016. Jama. 2018;319:1723-5.
2. McAloon CJ, Boylan LM, Hamborg T, Stallard N, Osman F, Lim PB, et al. The changing face of cardiovascular disease 2000-2012: an analysis of the world health organisation global health estimates data. Int J Cardiol. 2016;224:256-64.
3. Morganroth J, Maron BJ, Henry WL, Epstein SE. Comparative left ventricular dimensions in trained athletes. Ann Intern Med. 1975;82:521-4.
4. Spence AL, Naylor LH, Carter HH, Buck CL, Dembo L, Murray CP, et al. A prospective randomised longitudinal MRI study of left ventricular adaptation to endurance and resistance exercise training in humans. J Physiol. 2011;589:5443-52.
5. Utomi V, Oxborough D, Whyte GP, Somarouco J, Sharma S, Shave R, et al. Systematic review and meta-analysis of training mode, imaging modality and body size influences on the morphology and function of the male athlete’s heart. Heart. 2013;99(23):1727-33.
6. Radovits T, Oláh A, Lux Á, Németh BT, Hidi L, Birtalan E, et al. Rat model of exercise-induced cardiac hypertrophy: hemodynamic characterization using left ventricular pressure-volume analysis. Am J Physiol Heart Circ Physiol. 2013;305:H124-34.
7. Chung E, Diffee GM. Moderate intensity, but not high intensity, treadmill exercise training alters power output properties in myocardium from aged rats. J Gerontol A Biol Sci Med Sci. 2012;67:1178-87.
8. Diffee GM, Chung E. Altered single cell force-velocity and power properties in exercise-trained rat myocardium. J Appl Physiol. 2003;94:1941-8.
9. Diffee GM, Seversen EA,Titus MM. Exercise training increases the Ca2+ sensitivity of tension in rat cardiac myocytes. J Appl Physiol. 2001;91:309-15.
10. Diffee GM, Nagle DF. Exercise training alters length dependence of contractile properties in rat myocardium. J Appl Physiol. 2003;94:1137-44.
11. Kemi OJ, Ellingsen O, Ceci M, Grimvald S, Smith GL, Condorelli G, et al. Aerobic interval training enhances cardiomyocyte contractility and Ca2+ cycling by phosphorylation of CaMKII and Thr-17 of phospholamban. J Mol Cell Cardiol. 2007;43:354-61.
12. Boldt K, Jomaa V, Turnbull J, Fedak PWM, Herzog W. Mechan-
Cardiac adaptation to aerobic exercise and protein consumption in animal models. Med Sci Sports Exerc. 2009;41:1155-63.
29. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. J Am Soc Echocardiogr. 1989;2:358-67.
30. Boldt K, MacDonald G, Joumaa V, Herzog W. Mechanical adaptations of skinned cardiac muscle in response to dietary-induced obesity during adolescence in rats. Appl Physiol Nutr Metab. 2020; 45:893-901.
31. Edman KA. The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. J Physiol. 1979;291:143-59.
32. Danieli-Betto D, Betto R, Midrio M. Calcium sensitivity and myofibrillar protein isoforms of rat skinned skeletal muscle fibres. Pflugers Arch. 1990;417:303-8.
33. Poirier P, Després JP. Exercise in weight management of obesity. Clin Cardiol. 2001;19:459-70.
34. Hallam M, Reimer R. Impact of diet composition in adult offspring is dependent on maternal diet during pregnancy and lactation in rats. Nutrients. 2016;8:46.
35. Pluim BM, Zwiderman AH, van der Laarse A, van der Wall EE. The athlete’s heart: a meta-analysis of cardiac structure and function. Circulation. 2000;101:336-44.
36. Baggish AL, Wang F, Weiner RB, Elinoff JM, Tournoux F, Boland A, et al. Training-specific changes in cardiac structure and function: a prospective and longitudinal assessment of competitive athletes. J Appl Physiol. 2008;104:1121-8.
37. Burd NA, Gorissen SH, Van Vliet S, Snijders T, Van Loon LJ. Differences in postprandial protein handling after beef compared with milk ingestion during postexercise recovery: a randomized controlled trial. Am J Clin Nutr. 2015;102:828-36.
38. Hulmi JJ, Kovanen V, Selänne H, Kraemer WJ, Häkkinen K, Mero AA. Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression. Am J Physiol. 2009;37:297-308.
39. Razeghi P, Taegtmeyer H. Cardiac remodeling: UPS lost in transit. Circ Res. 2005;97:964-6.
40. Lollo PCB, Batista TM, Moura CS, Morato PN, Cruz AG, Faria JAF, et al. l-Leucine supplemented whey protein. Dose–response controlled trial. J Nutr. 2011;141:568-73.
41. Boldt K, MacDonald GZ, Joumaa V, Herzog W. Mechanical adaptations of skinned cardiac muscle in response to dietary-induced obesity during adolescence in rats. Appl Physiol Nutr Metab. 2020; 45:893-901.
42. Relling DP, Esberg LB, Fang CX, Johnson WT, Murphy EJ, Carlson EC, et al. High-fat diet-induced juvenile obesity leads to cardiomyocyte dysfunction and upregulation of Foxo3a transcription factor independent of lipotoxicity and apoptosis. J Hypertens. 2006;24:549-61.
43. Chung E, Diffee GM. Effect of aging on power output properties in rat skinned cardiac myocytes. J Gerontol A Biol Sci Med Sci. 2011; 66:1267-73.
44. Diffee GM. Adaptation of cardiac myocyte contractile properties to exercise training. Exerc Sport Sci Rev. 2004;32:112-9.
45. Fuchs F, Smith SH. Calcium, cross-bridges, and the Frank-Starling relationship. News Physiol Sci. 2001;16:5-10.
46. Shiels HA, White E. The Frank–Starling mechanism in vertebrate cardiac myocytes. J Exp Biol. 2008;211:2005-13.
47. Fernandes T, Baraúna VG, Negrão CE, Phillips MI, Oliveira EM. Aerobic exercise training promotes physiological cardiac remodelling involving a set of microRNAs. Am J Physiol Heart Circ Physiol. 2015;309:H543-52.
48. Hashimoto T, Kambara N, Nohara R, Yazawa M, Taguchi S. Expression of MHC-β and MCT1 in cardiac muscle after exercise training in myocardial-infarcted rats. J Appl Physiol. 2004;97:843-51.
49. Herron TJ, Korte FS, McDonald KS. Loaded shortening and power output in cardiac myocytes are dependent on myosin heavy chain isoform expression. Am J Physiol Heart Circ Physiol. 2001;281: H1217-22.
50. Martins CD, Chianca Jr DA, Fernandes LG. Cardiac autonomic balance in rats submitted to protein restriction after weaning. Clin Exp Pharmacol Physiol. 2011;38:89-93.
51. Penitente AR, Novaes RD, Chianca Jr DA, Da Silva MF, Silva ME, Souza AM, et al. Protein restriction after weaning modifies the calcium kinetics and induces cardiomyocyte contractile dysfunction in rats. Cells Tissues Organs. 2013;198:311-7.
52. Gorissen SH, Wittard OC. Characterising the muscle anabolic potential of dairy, meat and plant-based protein sources in older adults. Proc Nutr Soc. 2018;77:20-31.
53. Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmann M, Helms E, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. Br J Sports Med. 2018;52:376-84.
54. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. J Physiol. 2013;591:2319-31.
55. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2008;89:161-8.
56. Burd NA, West DW, Moore DR, Atherton PJ, Staples AW, Prior T, et al. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. J Nutr. 2011;141:568-73.
57. Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, Flakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am J Physiol Endocrinol Metab. 2001;280:E882-93.
58. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol Endocrinol Metab. 1997;273:E99-107.