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Effects of activity levels on aortic calcification in hyperlipidemic mice as measured by microPET/microCT

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Abstract

Background and aims: Cardiovascular disease risk is associated with coronary artery calcification and is mitigated by regular exercise. Paradoxically, elite endurance athletes, who have low risk, are likely to have more coronary calcification, raising questions about the optimal level of activity.

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Credit Author Statement

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Declaration of interests

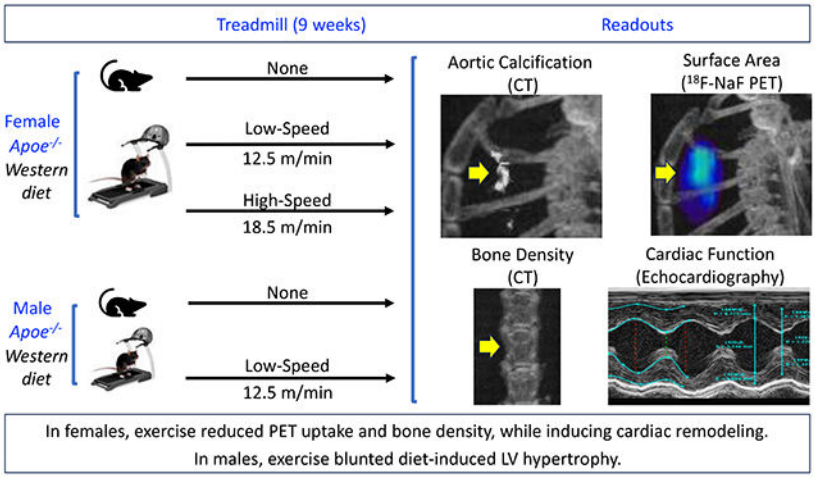
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Methods: Female hyperlipidemic (*Apoe*$^{-/-}$) mice with baseline aortic calcification were subjected to highspeed (18.5 m/min), low-speed (12.5 m/min), or no treadmill exercise for 9 weeks. ^{18}F-NaF microPET/CT images were acquired at weeks 0 and 9, and echocardiography was performed at week 9.

Results: In controls, aortic calcium content and density increased significantly. Exercise regimens did not alter the time-dependent increase in content, but the increase in mean density was blunted. Interestingly, the low-speed regimen significantly reduced ^{18}F-NaF uptake, a marker of surface area. Left ventricular (LV) systolic function was lower while LV diameter was greater in the low-speed group compared with controls or the high-speed group. In the low-speed group, vertebral bone density by CT decreased significantly, contrary to expectations. Male hyperlipidemic (*Apoe*$^{-/-}$) mice were fed a Western diet and also subjected to low-speed or no exercise followed by imaging at weeks 0 and 9. In males, exercise also did not alter the time-dependent increase in aortic calcification. Exercise did not affect ^{18}F-NaF uptake or bone mineral density, but it blunted the diet-induced LV hypertrophy seen in controls.

Conclusions: These results suggest that, in mice, exercise has differential effects on aortic calcification, cardiac function, and skeletal bone mineral density.

Graphical Abstract



Keywords

Calcification; exercise; imaging; PET; CT; hyperlipidemia

1. Introduction

Cardiovascular calcification, especially coronary artery calcification (CAC), is associated with risk for adverse cardiovascular events and mortality [1, 2], and often coexists with osteoporosis, whereas physical activity is associated with lower risk for cardiovascular events and mortality [3]. It is, thus, a conundrum that the prevalence of CAC is significantly greater in elite endurance athletes compared with less active controls [4]. Both prevalence and severity are greater in veteran male elite athletes than in controls matched for

conventional risk factors [5–7]. Interestingly, this greater prevalence of CAC in elite athletes is not associated with greater cardiovascular risk, and cardiovascular outcomes of elite athletes appear to be better than the general population [8].

The optimal activity level for benefitting cardiovascular and bone health is not clear. A U-shaped association has been reported for the physical activity level and cardiovascular events [9]; although the confidence intervals are larger at the higher levels of physical activity, it suggests that there may be little or no additional benefit at those levels. A recent study shows that exercise intensity, but not exercise volume, is associated with changes in CAC [10]. Prevalence of cardiovascular disease and risk factors were lower with low and moderate volumes of exercise but not further benefited by higher doses of exercise [11]. Thus, in this study, we compared the effects of low- and high-speed exercise regimens on quantitative cardiovascular and skeletal bone parameters in mice with underlying calcific atherosclerosis.

2. Materials and methods

2.1 Animals

Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles. Both male and female *Apoe*-null mice were acquired from the Jackson Laboratory. The number of animals ordered for each group (n = 20) was calculated to detect a 25% change with 80% power assuming a standard deviation of 25% with a significance of 0.05, including the allowance for a loss of 3 mice/group to attrition. Retired male and female breeders [apolipoprotein E null ($Apoe^{-/-}$) mice on C57BL/6 background, > 7 month-old, Jackson Laboratory, Bar Harbor, ME] were placed on a “Western” diet (21% fat and 0.2% cholesterol, Envigo) for 9 weeks to induce baseline aortic calcification. For female mice, after 9 weeks on the Western diet, the diet was switched to normal chow prior to the start of the exercise regimen to reduce the confounding effects of the diet. However, since male mice are known to have less aortic calcification than female mice [12], they were continued on the Western diet throughout the exercise regimen to ensure development of adequate aortic calcification.

2.2 Treadmill exercise regimen

2.2.1 Female mice.—The 60 mice that were ordered were administered a Western diet for 9 weeks by the Jackson Laboratory, but, due to diet-induced health issues, only 41 were deemed well enough to tolerate shipping. All the mice were acclimated on the treadmill (Columbus Instruments Exer-3/6 Animal Treadmill Rodent 6-Lane) for 3-4 days and underwent a one-time, 10-minute, test session for running capacity at 12.5 (low-speed) and 18.5 (high-speed) meters/min. All mice tolerated the low-speed test run. Five mice were not able to complete the high-speed test run, and they were removed from the subsequent experiments. The remaining mice were then randomly divided into 3 groups: (1) a control group (no treadmill, n = 7), (2) a low-speed group (n = 13), and (3) a high-speed group (n = 16). The exercise groups underwent a 9-week-long exercise regimen (0° slope, no electric shock stimulation). The treadmill speed for each group was kept constant at 0, 12.5 m/min or 18.5 m/min, and duration was also kept constant at 30 minutes (5 days/week). At week

2, all mice in the third group were excluded because some failed to complete the run. A new group 3 was constituted from six randomly selected mice from group 2. The new group 3 underwent high-speed exercise for the remaining 7 of the 9 week-study. Exclusions, for illness and inability to tolerate the exercise regimen, were based on statistical consultations to preserve the randomization and prevent selection bias, including removal of an entire group from analysis and creation of a new grouping by random selection.

2.2.2 Male mice.—Male mice (n = 40) were acclimated and tested as described above. All tolerated the low-speed test run. Several mice did not tolerate the high-speed test run, and they were excluded. The remaining 26 mice were assigned to 2 groups: (1) a control group (no treadmill, n = 13) and (2) a low-speed group (12.5 m/min, n = 13). Both were subjected to a 9-week-long exercise regimen as mentioned above. Three mice from the control group and one mouse from the exercise group had to be euthanized due to health issues during the study.

2.3 Serial in vivo ^{18}F-NaF μPET/μCT imaging

We used μCT imaging to detect total mineral content, and ^{18}F-NaF μPET imaging to detect calcium mineral surface area, based on the known binding of fluoride ions at the surface of calcium hydroxyapatite mineral (the mineral in human vascular calcification) to form fluoroapatite [13]. Irkle and colleagues elegantly showed that ^{18}F-NaF uptake occurs at the surface of calcium deposits in human carotid arteries [14]. Fluoride ions can also exchange with hydroxyl groups in deeper levels of mineral deposits over time. However, the time course of this deeper penetration is slower than the radioactive decay of the ^{18}F (half-life ~ 110 minutes). Thus, tracer uptake represents mineral surface area.

Fused ^{18}F-NaF μPET/μCT imaging was performed prior to the start of the exercise regimen and again at week 9 at the Preclinical Imaging Facility of the Crump Institute for Molecular Imaging at the California NanoSystems Institute at UCLA. The imaging and analysis were performed as described previously [15]. Briefly, mice were injected with ~90 μCi ^{18}F-NaF via the tail vein. One hour post-injection, mice were anesthetized and imaged in the μPET scanner (GNEXT) for 10 min and, subsequently, in the μCT scanner (CrumpCAT, UCLA) for 2 min in the same gantry.

2.4 Enumeration of aortic calcium deposits

The μCT data sets were exported to NIFTI file format and segmented using 3D Slicer image computing platform (https://www.slicer.org; version 5.3.0) [16], as previously described [17]. A threshold value of 350 HU was chosen for isocontour automated edge detection for optimal signal-to-noise ratio. Digital subtraction of skeletal bone was based on anatomic considerations, and identification of individual deposits (to the extent allowed by resolution) by adjustments of the threshold. A threshold of 20 microns for size was chosen to optimally distinguish discrete calcium deposits from background noise, based on the signal from the intrathoracic space.

2.5 MicroCT and μPET image analysis

Images were analyzed using AMIDE software.[18] For female mice, CT quantification for aortic calcification was performed by three-dimensional isocontour (automated edge detection) regions of interest (ROIs) with a minimum threshold of 300 Hounsfield units (HU). Since male mice had less advanced calcification than females, quantification was assessed using geometric ellipsoidal ROIs over the cardiac and aortic silhouettes with a threshold of 400 HU, which we found adequate to reduce background noise from cardiac tissue. The volumetric HU (vHU) was calculated by multiplying the density (HU) with the volume of the ROIs. For both sexes, PET quantification for aortic calcification was performed from an isolated volumetric ROI encompassing parts of cardiac and aortic regions with a minimum ^{18}F-NaF isocontour threshold of 2% injected dose per cubic centimeter (%ID/cc). The mean threshold of background ^{18}F-NaF uptake, measured at the cardiac silhouette of four mice, was 0.8 %ID/cc. The CT mineral content and the total PET uptake were each determined from values of mean density and size. CT quantification for the lumbar bone density was performed by geometric box ROI analysis encompassing the L3 vertebra with a threshold of 1000 HU, and the mean density (HU) was used for the comparative analysis.

2.6 Echocardiography

Mice were anesthetized (3.0% isoflurane for initiation and 1.5–2.0% isoflurane for maintenance delivered via nose cone), and M-Mode and Tissue Doppler echocardiography of the left ventricle were performed using a VisualSonics Vevo 3100 equipped with a 30-MHz linear transducer.

2.7 Serum lipid levels

Mice were fasted, and blood was collected at euthanasia. Serum lipid panels were tested by IDEXX Bioanalytics (Sacramento, CA).

2.8 Statistical analysis

Values are expressed as mean ± SEM. Statistical analysis was performed with Prism software (GraphPad, v. 9.4.1). Data were first analyzed for normal distribution, and, for two group comparisons, Student's *t*-test or the Mann-Whitney test was used, as appropriate. For three group comparisons, ANOVA, Kruskal-Wallis, or Dunn's tests were used, as appropriate. A paired *t*-test was used for comparison of data of the same mouse between different time points. For comparisons among multiple groups with repeated measures, two-way ANOVA followed by Holm-Sidak post-hoc analysis was employed. All tests were 2-sided, and a p value ≤ 0.05 was considered statistically significant.

3. Results

3.1 Effects of activity levels on progression of aortic calcium by μCT/μPET imaging

Aortic calcium content and mineral surface area were assessed by μCT/μPET imaging. All mice were scanned at 2 time points: once prior to the start of the treadmill regimen and another after the 9-week exercise regimen.

3.1.1 Female mice.—In female mice, all 3 groups showed significant progression in their aortic calcium content over the 9-week period (Fig. 1A). However, both low (12.5 m/min)- and high (18.5 m/min)-speed treadmill regimens did not further affect the progression (Fig 1B). The mean density of the calcium deposits in the control group showed a significant increase in density but not in the exercise groups (Fig. 1C). The numbers of aortic calcium deposits by segmentation of CT images using 3D Slicer showed that the low-speed group had a significant increase in the number of deposits (Fig. 1D).

In humans, the mineral surface area in atherosclerotic plaques may impact plaque vulnerability due to compliance mismatch of edges with surrounding distensible tissue resulting in debonding. Therefore, in this study, we determined the effects of exercise regimens on the calcium mineral surface area by ^{18}F-NaF PET imaging in mice. Since fluoride ions adsorb onto the surface of hydroxyapatite mineral, the amount of ^{18}F-NaF tracer uptake reflects the mineral surface area. Results showed that aortic ^{18}F-tracer uptake was significantly reduced only in the low-speed exercise group (Fig. 2A–C).

3.1.2 Male mice.—We repeated the experiment in male mice. We used only controls and the low-speed regimen since ^{18}F-tracer uptake showed a significant reduction in the low-speed female group. As male *Apoe*$^{-/-}$ mice are known to have less aortic calcification [12], they were continued on the Western diet during the study period to ensure adequate calcification. As seen in the female mice, the aortic calcium content progressed significantly in both control and low-speed groups over the 9-week period (Fig. 3A), and the degree of progression was not affected by the exercise regimen (Fig 3B). The mean density of the calcium deposits in both the control and low-speed groups increased significantly over time (Fig. 3C). Unlike in female mice, the ^{18}F-tracer uptake in the low-speed group did not change significantly over the study period (Fig. 3D). As for the number of aortic calcium deposits by CT segmentation, individual deposits are difficult to reliably resolve or discern due to the more diffuse nature of the calcification in hyperlipidemic male mice.

3.2 Effects of activity levels on cardiac function

3.2.1 Female mice.—Echocardiography was performed at week 9. As shown in Table 1, female mice in the low-speed group had significantly lower values of left ventricular ejection fraction (LVEF) and percent fractional shortening (%FS), and increased LV diameter compared with control and high-speed exercise groups after the study period. To determine whether low LVEF in the low-speed group was attributable to cardiac fibrosis, we performed histochemical staining with Sirius red. Fibrosis, was quantified by Image J (NIH) as % stain normalized to total area. Results showed that mean cardiac fibrosis was less than 1% in all groups and not significantly greater in the low-speed group (data not shown).

3.2.2 Male mice.—Echocardiography was performed at 2 time points: once prior to the start of the treadmill regimen and another after the 9-week exercise regimen. Male mice showed no significant changes in LVEF, %FS, and LV diameter over time and no significant differences between the groups (Table 2). However, in the control group, structural parameters (LV mass and anterior wall thickness) significantly increased over the 9-week period (Table 2).

3.3 Effects of Western diet on serum lipid levels in both males and females

Serum was collected at euthanasia. Results confirmed adequacy of the hyperlipidemia achieved with the Western diet superimposed on the genetic modification. Serum lipid levels were not significantly different between controls and treadmill groups, except for HDL-cholesterol in male mice, which was, unexpectedly, significantly lower in the treadmill vs. controls (Table 3).

3.4 Effects of activity levels on skeletal bone mineral density

Bone density was assessed by microCT imaging. As shown in Fig. 4A, in female mice only, vertebral bone mineral density was significantly reduced in the low-speed group, and there was a strong trend toward reduced bone density in the high-speed group ($p = 0.06$; Fig. 4A). Unlike in females, exercise did not affect vertebral bone mineral density in male mice (Fig. 4B).

3.5 Reanalysis of combined treadmill groups

In female mice, when the 2 exercise groups were merged and compared with the controls, there were two significant outcomes that are similar to those found in the 3-group analysis include: 1) an increase in aortic calcium content with time in both control and combined exercise groups, and 2) a decrease in bone density in the combined exercise group but not controls (Fig. 5A and B). As for PET and echocardiographic results, the significant differences that had been found in the low-speed group were not present in the combined exercise group, except for a trend toward lower LVEF/FS ($p = 0.06$). No new significant findings arose with the 2-group analysis.

4. Discussion

Cardiovascular risk is reduced with regular physical activity [9]. Interestingly, prevalence of cardiovascular disease is reported to be lower with mild and moderate doses of lifelong exercise but not further benefited by high doses of exercise [11]. In this study, we compared the effects of no, low- and high-speed treadmill regimens on calcification and quantitative cardiovascular measures in hyperlipidemic mice with underlying calcific atherosclerosis (Fig. 6). The treadmill speeds were chosen on the basis of a previous study that measured cardiorespiratory indices in C57BL6 mice [19], and the use of treadmill speeds, in lieu of cardiorespiratory indices, to create reproducible, graded levels of activity was also consistent with prior studies [20–22]. Results from CT analysis show that, over a 9-week period, aortic calcification progressed significantly in all mice, and the treadmill regimens did not significantly affect progression over that of controls. Interestingly, in females only, exercise attenuated a normal age-dependent increase in calcium mineral density.

The pattern of calcium deposit distribution, in addition to the quantity of calcium mineral, is a key factor in the risk of plaque rupture in humans [23–26]. Thus, we also measured mineral surface area by ^{18}F-NaF uptake. The surface area was significantly reduced in the female mice in the low-speed treadmill group, but not in the control or high-speed groups. Such a change in the distribution of mineral would be expected to reduce the risk of plaque rupture in humans[25]. From these findings, one may infer and speculate on possible growth

mechanisms for calcium deposits in each group based on values of the four parameters (mineral content, density, surface area, and numbers). In both male groups and the female control group, mineral growth likely occurred at internal sites inaccessible to fluoride tracer binding and, in the high-speed group, the growth likely occurred by symmetric expansion of the mineral [27]. In the low-speed group, since it also had increased deposit numbers, we speculate that the decrease in surface area was due to mineralization of intertrabecular gaps inside porous deposits as well as formation of nacent deposits.

Echocardiographic findings show that, in the female mice, the low-speed exercise group had lower values of ejection parameters and greater LV chamber size compared with the control and high-speed exercise group. These changes likely represent physiological adaptation to exercise. In humans, higher levels of physical activity are associated with greater LV chamber size [28]. This is physiologic and not due to fibrosis as evidenced by our histological analysis, showing that cardiac fibrosis in the low-speed group was not greater than in the other two groups. The high-speed group may have undergone changes similar to a combination of concentric and eccentric hypertrophy, the known physiological effects of a combination of increased cardiac output and blood pressure/afterload where the higher LV chamber diameter (due to increased cardiac output) is counteracted by the greater anterior wall thickness, which reverses the increase in chamber size.

Naresh and colleagues have shown that mice on a high fat diet develop about a 30% increase in LV mass, possibly a pathological form of hypertrophy because it was associated with ischemia [29]. In our study, we also found about a 30% increase in LV mass, but only in control male mice, which were also on a high fat diet. We speculate that this may be due to an increase in aortic stiffness that is expected to accompany aortic calcification. Aortic stiffening is known to cause pathological LV hypertrophy [30, 31]. This effect was attenuated in the male mice in the exercise group, suggesting a protective effect. This increase in LV mass was not seen in the female mice, presumably because they were not on a high fat diet during the study period.

With respect to the serum lipid levels, total, LDL-, and HDL-cholesterol and triglyceride levels were in the range expected for *Apoe*$^{-/-}$ mice. Of note, HDL-cholesterol was significantly lower in the male exercise group compared with male controls. Although we cannot exclude the possibility that the exercise group started with a lower HDL at baseline, this finding is interesting given that the opposite effect is consistently seen in human subjects [32].

With respect to exercise effects on the skeleton, exercise is generally viewed as beneficial for bone health, largely due to the effects of weight-bearing and high-impact exercise. Thus, our finding that low-speed treadmill group had lower lumbar vertebral bone density than controls in only female mice was unexpected. One potential mechanism would be endocrinological effects of exercise. Interestingly, in women, cross-country running is associated with amenorrhea and low bone mass [33].

Limitations include small numbers of mice in female groups, resulting in insufficient power to exclude a false negative result and reducing the likelihood that the discovered effect

is genuinely true [34]. Another potential limitation would be that the echocardiographic results in females were cross-sectional only. Notably echocardiography was performed under anesthesia. Although anesthesia is known to depress cardiac function, performing the exam in conscious animals would introduce variation among the animals [35] and especially among the groups because of varying degrees of excitement, which would increase cardiac contractility and heart rate due to sympathetic stimulation, potentially in a group-dependent manner since exercise history can affect sympathetic tone. We felt that the effects of anesthesia would be more uniform among the groups if we used isoflurane anesthesia, which has less inotropic and chronotropic effects than most other agents [36]. Results of aortic calcification cannot be directly compared between male and female due to differences in pattern of calcification requiring different methods of quantitative assessment.

Overall, these results suggest that exercise differentially influences cardiovascular and skeletal health. While aortic calcium content increased significantly in all mice (both males and females), only the low-speed exercise group in the females had a significant decrease in mineral surface area. Such a change in the distribution of mineral would be expected to reduce plaque vulnerability in humans. These findings may provide valuable context for interpretation of calcium scans in athletic individuals.

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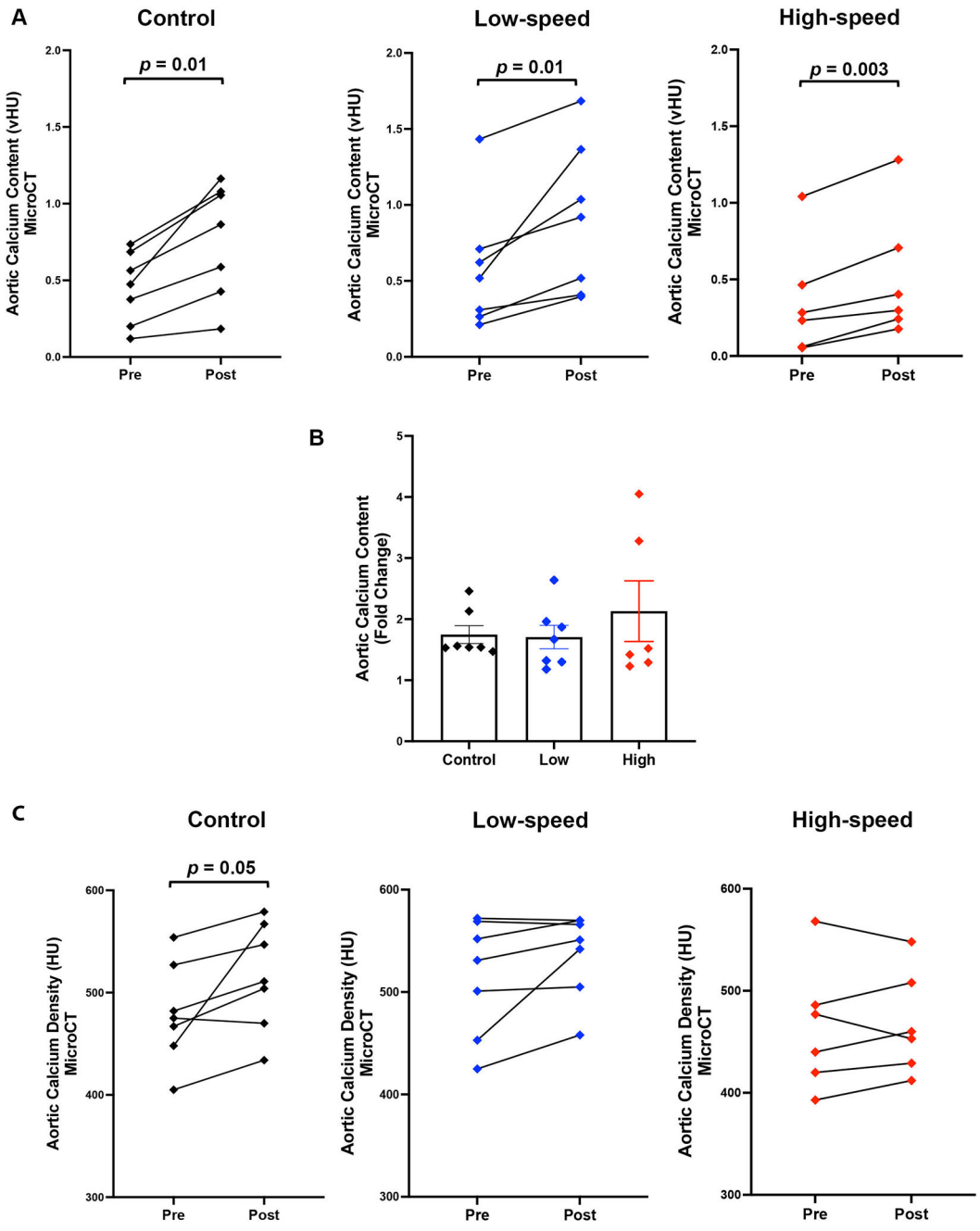
Highlights

- Aortic calcium content as measured by microCT increased significantly over the 9-week period, in both control and treadmill groups.
- Progression of aortic calcification in the activity groups was not significantly different from that of the control groups.
- In female mice, a low-speed exercise regimen reduced aortic mineral surface area as measured by microPET and vertebral bone mineral density as measured by microCT.
- In male mice, a low-speed exercise regimen blunted the Western diet-induced left ventricular hypertrophy observed in controls.



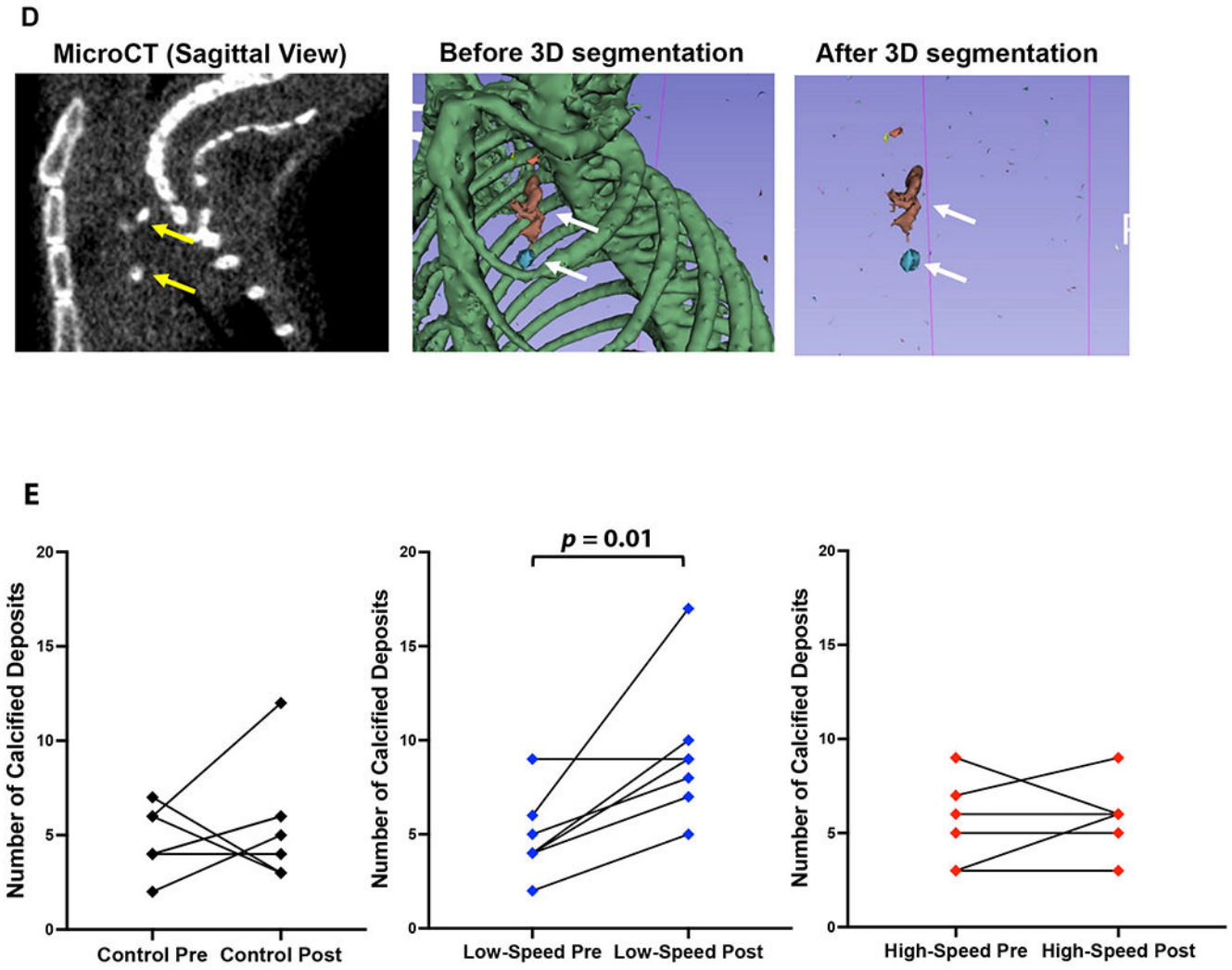


Figure 1.

Effects of treadmill regimen on aortic calcification in female mice. (A) Aortic calcium content by microCT in Control, Low-speed, and High-speed groups of mice (zero, 12.5, and 18.5 m/min, respectively) before (Pre) and after (Post) the 9-week treadmill regimen. (B) Fold change in aortic calcium content by microCT in the 3 groups. (C) Mean aortic calcium density in Control, Low-speed, and High-speed groups of mice before (Pre) and after (Post) the 9-week treadmill regimen. (D) Representative images of steps in segmentation and enumeration of calcium deposits in the aortic root and ascending aorta of a mouse. The middle and right panels are approximately on the same scale. (E) Number of discrete deposits Pre- and Post-9-week protocol in Control, Low-Speed-, and High-speed groups in the female mice.

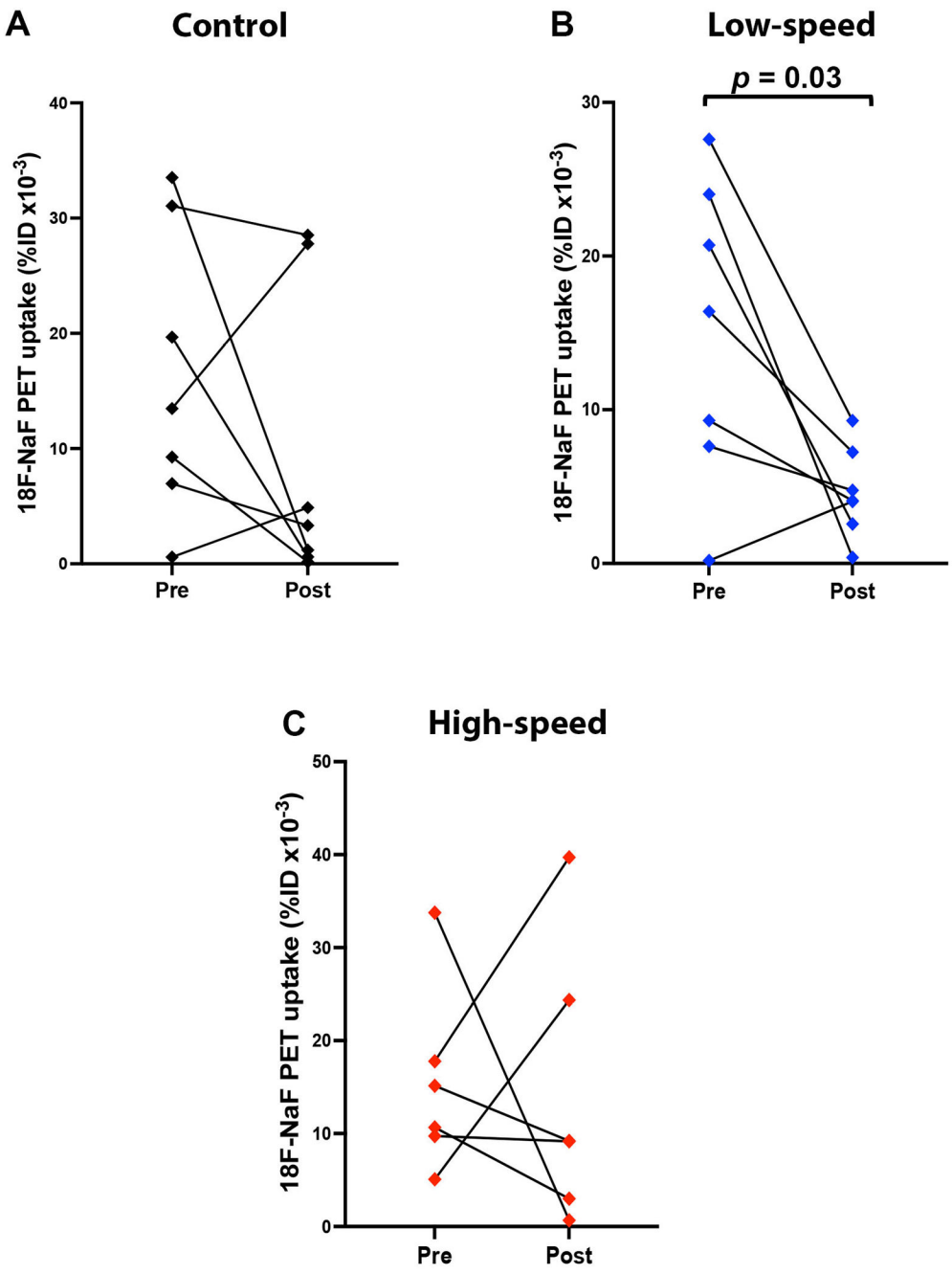


Figure 2.

Effects of treadmill regimen on PET tracer uptake in female mice. ^{18}F-NaF tracer uptake before (Pre) and after (Post) the 9 week treadmill regimen in (A) Control, (B) Low-, and (C) High-speed groups of mice (zero, 12.5, and 18.5 m/min, respectively).

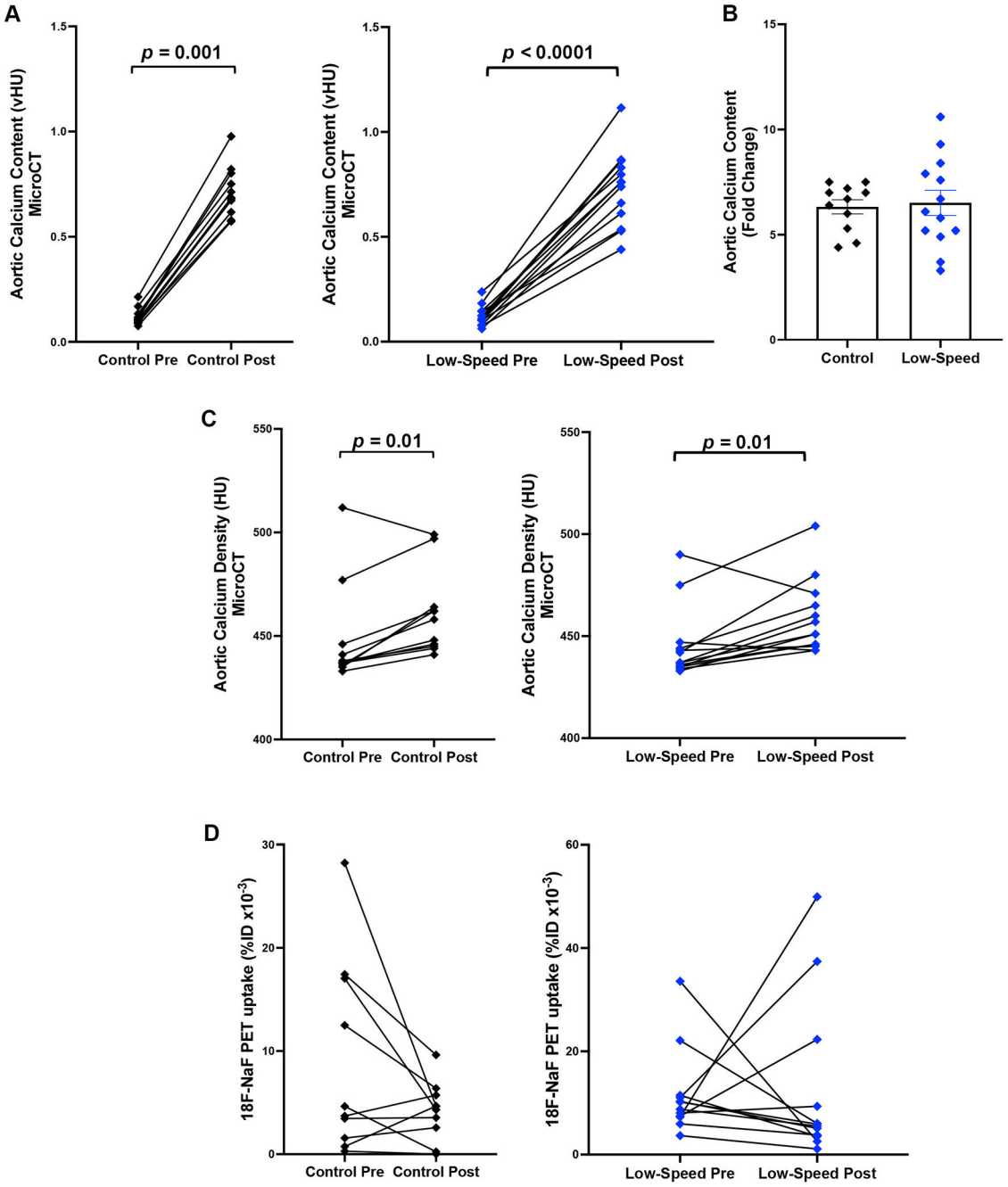


Figure 3.

Effects of treadmill regimen in male mice. (A) Aortic calcium content by microCT in Control and Low-speed groups of mice (zero and 12.5 m/min, respectively) before (Pre) and after (Post) the 9-week treadmill regimen. (B) Fold change in aortic calcium content by microCT. (C) Mean aortic calcium density in Control and Low-speed groups of mice before (Pre) and after (Post) the 9-week treadmill regimen. (D) ^{18}F-NaF tracer uptake before (Pre) and after (Post) the 9 week treadmill regimen in Control, and Low-speed groups of mice (zero and 12.5 m/min, respectively).

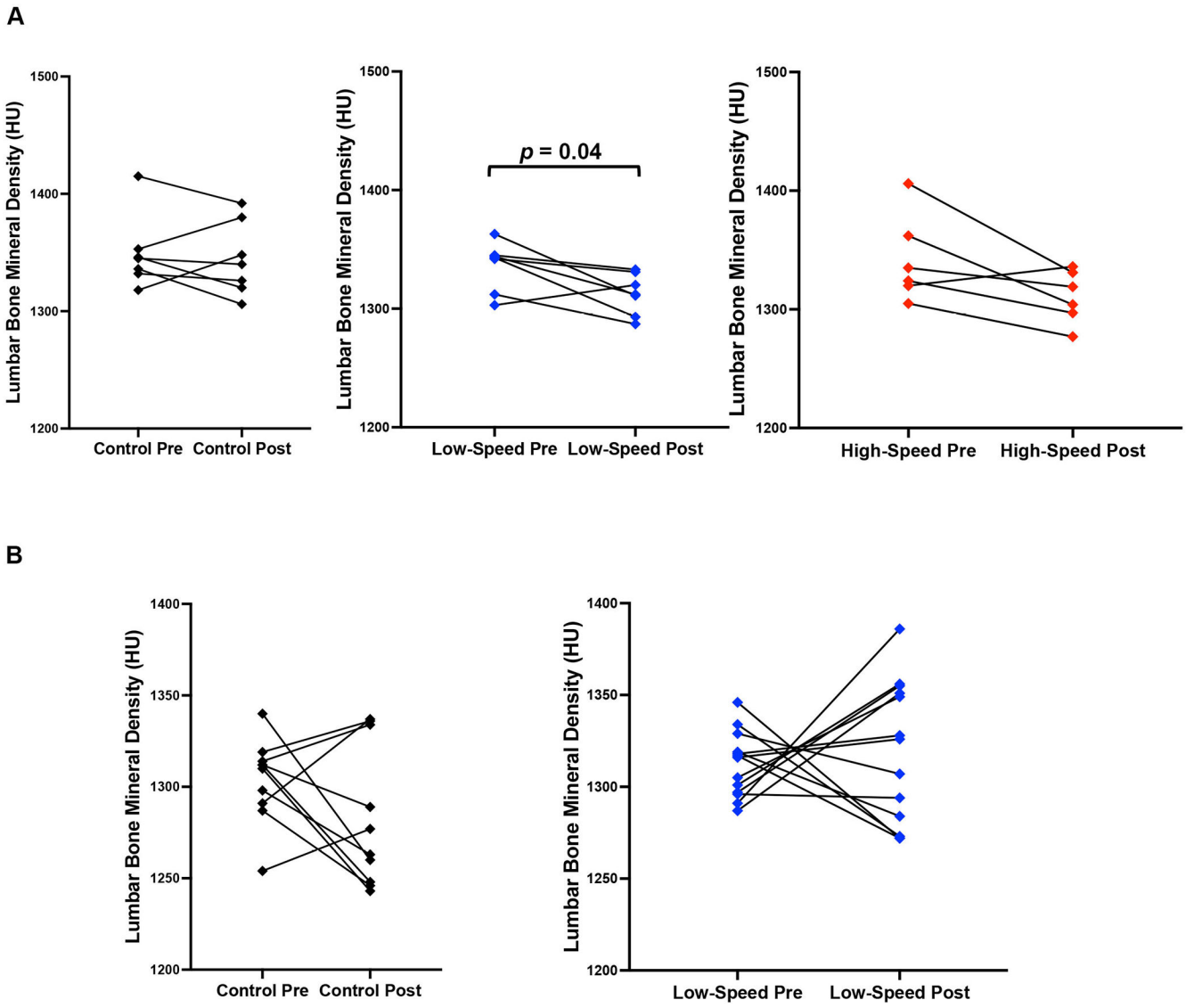


Figure 4.

Effects of treadmill regimen on skeletal bone mineral density in male and female mice. Lumbar vertebral (L3) bone mineral density by microCT Pre- and Post-9-week protocol in (A) female mice, and (B) male mice.

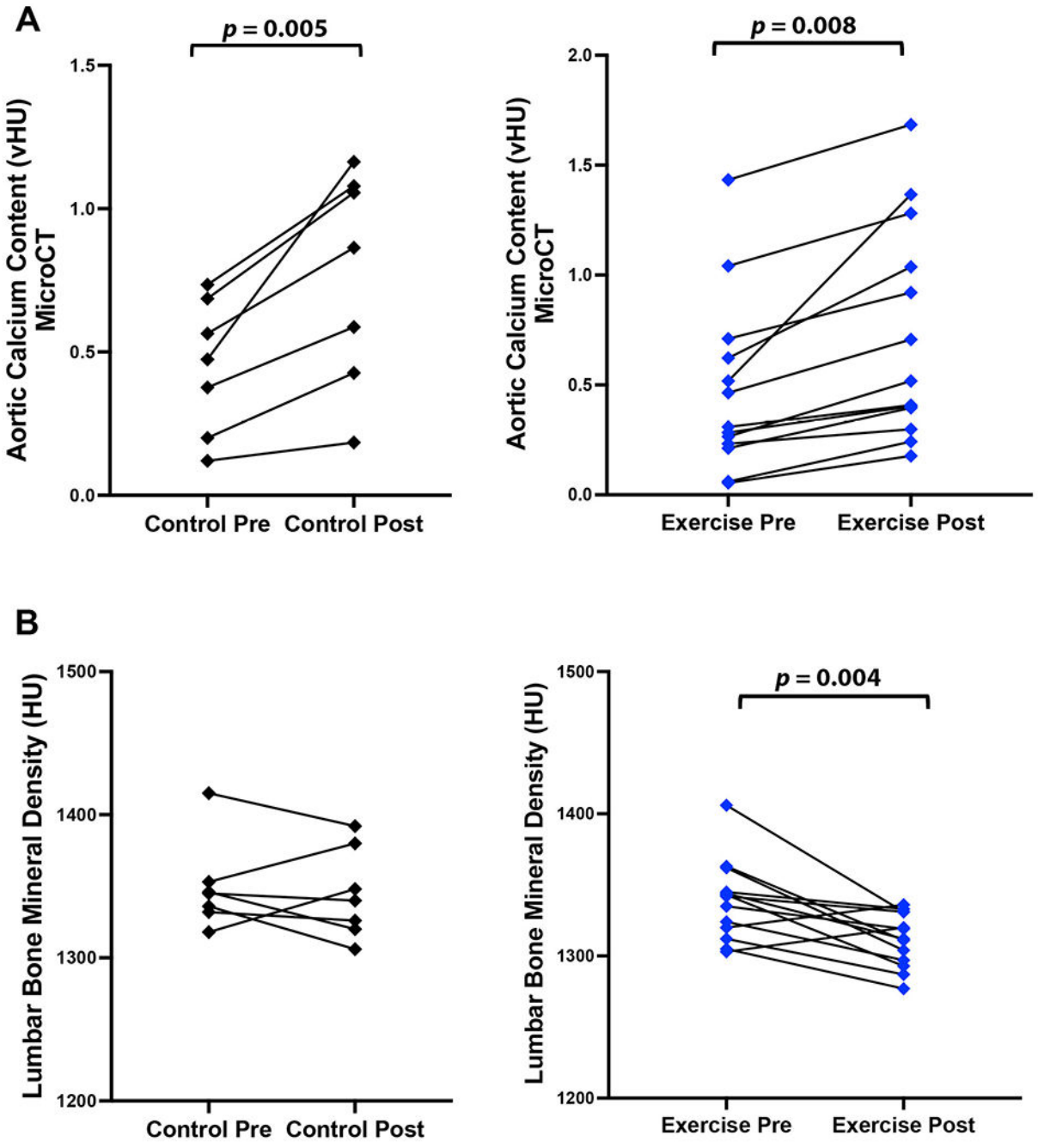


Figure 5.

Reanalysis using combined treadmill groups in female mice. Aortic calcium content by microCT in control and combined treadmill groups before (Pre) and after (Post) the 9-week treadmill regimen. (B) Lumbar vertebral (L3) bone mineral density by microCT Pre- and Post-9-week protocol in female mice.

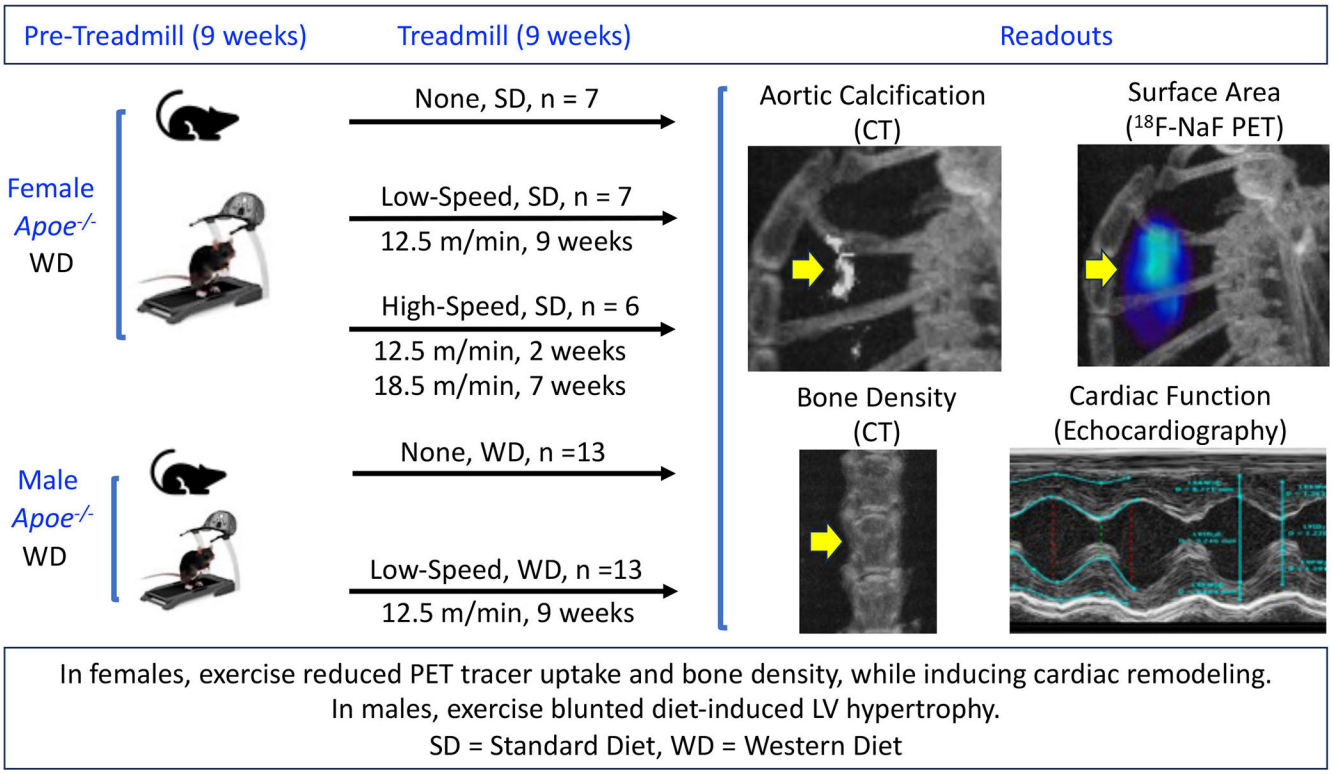


Figure 6.

Schematic of experimental design. During the pre-treadmill period, male and female mice were put on Western diet (WD) to induce baseline atherosclerotic calcification. During the treadmill period, female mice were switched to a standard diet while male mice were continued on WD for continued development of atheroslcerotic calcificaiton. In female mice, the high-speed group started at the low speed (12.5 m/min) for 2 weeks prior to running at the high speed (18.5 m/min) for 7 additional weeks to complete the study. Based on the findings from the female group, only control and low-speed groups were tested in male mice. Aortic calcification content and bone mineral density were quantified from microCT scans; surface area of the aortic calcium deposits was quantified from ^{18}F-NaF microPET scans; and cardiac structure and function were quantified from echocardiography.

Table I.

Effects of Exercise on Echocardiographic Parameters Female *Apoe*$^{-/-}$ Mice

	(A) Control Mean ± SEM	(B) Low-Speed Mean ± SEM	(C) High-Speed Mean ± SEM
Stroke volume (uL)	25 ± 2	31 ± 2	27 ± 2
LV ejection fraction (%)	69 ± 4	54 ± 4[a,b]	70 ± 3
Fractional shortening (%)	38 ± 3	28 ± 2[a,c]	39 ± 3
Cardiac output (mL/min)	13 ± 1	17 ± 1	15 ± 1
LV mass (corrected, mg)	69 ± 4	94 ± 11	81 ± 6
LVAW;s (mm)	1.3 ± 0.1	1.3 ± 0.2	1.5 ± 0.1
LVAW;d (mm)	0.9 ± 0.1	0.9 ± 0.2	1.1 ± 0.1
LVPW;s (mm)	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
LVPW;d (mm)	0.85 ± 0.05	0.81 ± 0.04	0.78 ± 0.04
LV diameter;s (mm)	1.9 ± 0.2	2.7 ± 0.2[d,e]	1.9 ± 0.1
LV diameter;d (mm)	3.1 ± 0.1	3.7 ± 0.2[d,f]	3.1 ± 0.1
Heart rate (bpm)	531 ± 13	550 ± 10	546 ± 7

[a] p =0.04 *vs.* Control;

[b] p = 0.04 *vs.* High-speed;

[c] p = 0.03 *vs.* High-speed;

[d] p = 0.01 *vs.* Control;

[e] p = 0.01 *vs.* High-speed;

[f] p = 0.02 *vs.* High-speed

Left ventricular mass (LV Mass); left ventricle anterior wall, systole (LVAW;s); left ventricle anterior wall, diastole (LVAW;d); left ventricle posterior wall, systole (LVPW;s); left ventricle posterior wall, diastole (LVPW;d)

Table II.

Effects of Exercise on Echocardiographic Parameters Male *Apoe*$^{-/-}$ Mice

	(A) Control		(B) Low-Speed	
	Pre Mean ± SEM	**Post Mean ± SEM**	**Pre Mean ± SEM**	**Post Mean ± SEM**
Stroke Volume (uL)	33 ± 2	38 ± 3	32 ± 2	35 ± 3
Ejection Fraction (%)	61 ± 3	68 ± 3	63 ± 3	68 ± 2
Fractional Shortening (%)	32 ± 2	38 ± 2	34 ± 2	37 ± 2
Cardiac Output (mL/min)	17 ± 1	17 ± 1	16 ± 1	16 ± 1
LV Mass (Corrected, mg)	105 ± 5	134 ± 10[a]	99 ± 5	100 ± 5[b]
LVAW;s (mm)	1.4 ± 0.1	1.7 ± 0.1[c]	1.4 ± 0.1	1.5 ± 0.1[d]
LVAW;d (mm)	1.0 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
LVPW;s (mm)	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
LVPW;d (mm)	0.96 ± 0.06	1.08 ± 0.08	1.00 ± 0.07	0.92 ± 0.04
LV diameter;s (mm)	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1
LV diameter;d (mm)	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1
Heart Rate (bpm)	524 ± 18	445 ± 16[e]	493 ± 11	463 ± 14

[a] $p = 0.001$ *vs.* Control Pre;

[b] $p = 0.001$ *vs.* Control Post;

[c] $p = 0.01$ *vs.* Control Pre;

[d] $p = 0.04$ *vs.* Control Post;

[e] $p = 0.002$ *vs.* Control Pre.

Left ventricular mass (LV Mass); left ventricle anterior wall, systole (LVAW;s); left ventricle anterior wall, diastole (LVAW;d); left ventricle posterior wall, systole (LVPW;s); left ventricle posterior wall, diastole (LVPW;d)

Table III.

Serum Lipid Levels

	(A) Female			(B) Male	
	Control Mean ± SEM	**Low-Speed Mean ± SEM**	**High-Speed Mean ± SEM**	**Control Mean ± SEM**	**Low-Speed Mean ± SEM**
Cholesterol (mg/dL)	393 ± 13	363 ± 29	422 ± 51	1103 ± 74	1076 ± 26
Triglycerides (mg/dL)	108 ± 14	139 ± 11	108 ± 10	95 ± 8	97 ± 4
HDL Cholesterol (mg/dL)	13 ± 2	15 ± 1	14 ± 2	28 ± 5	16 ± 2[a]
LDL Cholesterol (mg/dL)	115 ± 6	123 ± 9	110 ± 22	364 ± 36	344 ± 16

[a] $p = 0.02$ *vs.* control male; high-density lipoprotein (HDL); low-density lipoprotein (LDL)