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ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 584 – 591
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Physical activity, coronary artery calcium, and bone mineral density in elderly men and women: a preliminary investigation

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Received 15 March 2007; accepted 14 November 2007

Abstract

The aim of this study was to examine the relationship between cardiorespiratory fitness, coronary artery calcification (CAC), and bone mineral density (BMD) in older adults. Thirteen highly active, endurance-trained "master athletes" (7 male and 6 female) and 12 sedentary (6 male and 6 female) older adults (age 60-78 years) were recruited for this study. All subjects had CAC measured by electron beam computed tomography; BMD assessed by dual-energy X-ray absorptiometry; and plasma mineral regulatory proteins, including matrix Gla protein, fetuin-A, osteocalcin, osteopontin, and osteoprotegerin, measured by enzyme-linked immunosorbent assay. Furthermore, maximal oxygen consumption (VO_2max) was measured in each subject to provide an objective measure of cardiorespiratory fitness. As expected, whole-body BMD was elevated in master athletes compared with sedentary adults (1.17 ± 0.02 vs 1.09 ± 0.02 g/cm^2, $P < .05$). CAC score did not differ between activity groups, but was 8-fold lower in women compared with men ($P < .05$). The CAC score was not correlated with BMD, and there was no correlation between CAC and VO_2max when both sexes were included in the analysis. When the sexes were analyzed separately, several relationships were evident in men only. There was a significant inverse correlation between VO_2max and the number of calcified coronary artery lesions ($r = -0.596$, $P < .05$), and the correlation between VO_2max and logCAC score approached significance ($r = -.493$, $P = .08$). Furthermore, fetuin-A, a systemic inhibitor of vascular calcification, was positively correlated with VO_2max in men ($r = 0.679$, $P < .05$). These data provide preliminary evidence that chronic exercise may simultaneously inhibit CAC and increase BMD. The positive correlation between VO_2max and plasma fetuin-A levels in men indicates a potential mechanism by which exercise may correlate negatively with CAC. Additional studies with larger sample sizes will be needed to determine if the protective effects of chronic exercise on CAC and BMD are sex specific or mediated through common mechanisms such as changes in circulating levels of mineral regulatory proteins.

1. Introduction

Osteoporosis and cardiovascular disease (CVD) are commonly considered to be 2 independent disorders that occur with aging. However, bone tissue and calcified arterial plaques have some similar morphologic characteristics, leading to speculation that these 2 diseases may be mechanistically linked. Furthermore, vascular calcification (VC), which was previously thought to be a benign, passive precipitation of calcium phosphate, is now understood to be a highly regulated disorder with many properties similar to bone formation [1]. In the presence of a variety of CVD risk factors, including elevated plasma glucose, advanced glycation end products, and oxidized low-density lipoprotein (LDL), vascular smooth muscle cells can differentiate into cells with an osteoblast-like phenotype [1]. These cells are then capable of producing a bone-like matrix and mineralizing in the presence of calcium and phosphorus. This process appears to be regulated in part by proteins secreted from vascular smooth muscle cells that are normally associated with bone mineralization or resorption, including alkaline phosphatase, osteoprotegerin (OPG), fetuin-A, osteopontin, matrix Gla protein (MGP), and bone matrix protein-2a [1]. However, the precise role that

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doi:10.1016/j.metabol.2007.11.024

these mineral regulatory proteins have on VC or the relationship between VC and bone mineral density (BMD) has not been fully elucidated.

Vascular calcification also is no longer considered a benign process because recent research has shown that it has deleterious effects on vascular structure and function. Vascular calcification has been associated with endothelial dysfunction [2], increased artery wall intima-media thickness, and arterial stiffness [3] and may also affect the stability of atherosclerotic plaques [4]. Possibly because of these effects, VC has been *independently* associated with a variety of clinical end points, including amputation, stroke, myocardial infarction, poor surgical outcomes, and CVD mortality [5,6].

Despite the apparent similarities between VC and bone metabolism, mineral *deposition* often occurs in the vasculature under the same conditions as mineral *loss* in bone [7]. The reasons for this paradoxical relationship are unclear, but several potential mechanisms have been described. For example, oxidized lipids have been shown in vitro to promote mineralization in vascular cells but inhibit it in osteoblasts [8]. In addition, OPG-deficient mice develop early-onset osteoporosis and VC [9], indicating a potential link between specific mineral regulatory proteins and these disorders. However, it is uncertain whether VC and osteoporosis result from similar metabolic disturbances or if they are independent processes that sometimes coexist for other reasons [1].

Physical *inactivity* is well established as a risk factor for both osteoporosis and CVD, but little is known about the relationship between physical activity and VC or the altered mineral metabolism that appears to manifest its development. Desai et al [10] found a modest inverse relationship between self-reported leisure time physical activity and coronary artery calcification (CAC) levels in subjects with multiple CVD risk factors, whereas Taylor et al [11] found no such relationship in a group of younger, healthier subjects. However, no studies to date have assessed the relationship between objective measures of cardiorespiratory fitness (eg, maximal oxygen consumption [VO_2max]) and VC and the potential interactions with BMD and circulating levels of mineral regulatory proteins.

The primary aim of this study was to examine the relationship between cardiorespiratory fitness, CAC, and BMD in a group of sedentary older adults (SEDs) and a group of highly trained "master athletes" (MAs). Master athletes were compared with age-matched sedentary individuals included in this study to try to maximize the differences in cardiorespiratory fitness levels between the groups. We sought to determine if high levels of cardiorespiratory fitness can simultaneously inhibit CAC and increase BMD and to examine potential mechanisms for these effects. Consequently, we also measured circulating levels of mineral regulatory proteins to determine if differences in these proteins are related to differences in BMD and/or CAC between our subjects.

2. Methods

2.1. Subjects

Thirteen endurance-trained MAs (7 male and 6 female) and 12 SED (6 male and 6 female) older adults (age 60-78 years) were recruited for this study. Eligibility requirements included the following: age 60 to 80 years; body mass index (BMI) <27 kg/m^2; no prior evidence of CVD, renal disease, or diabetes; nonsmoking for >20 years; and no other medical conditions that would preclude subjects from participating in a VO_2max test. Subjects also were excluded if they had previously diagnosed coronary heart disease, diabetes, or renal disease or were on common medications for osteoporosis, including bisphosphonates, raloxifene, calcitonin, and parathyroid hormone. Women on hormone replacement therapy (HRT) were allowed to participate, but were required to maintain their current HRT regimen (on or not on HRT) throughout the duration of the testing. In total, 2 women in the SED group and 1 in the MA group were taking HRT. The MAs were recruited from local running clubs. To be included in the MA group, subjects must have been actively training and have competed in middle- or long-distance races at the local or national level for at least 2 years. For inclusion in the SED group, subjects must not have participated in *regular exercise training*, defined as >15 minutes of exercise 3 times per week at a continuously elevated heart rate, for at least 1 year. All subjects signed an informed consent document, and all procedures were approved by the Institutional Review Board at the University of Illinois.

2.2. Aerobic fitness (vo_{2max})

A graded treadmill exercise test was conducted to determine maximal aerobic capacity and the heart rate, blood pressure, and electrocardiographic responses to exercise. Oxygen uptake was measured continuously and recorded every 30 seconds using open-circuit spirometry (Parvomedics True Max 2400, Sandy, UT). Subjects walked or jogged on a treadmill at the fastest comfortable pace at 0% grade for 3 to 4 minutes, after which the grade increased by 1% to 2% every 1 or 2 minutes. No abnormal electrocardiographic responses were noted during any of the tests, so each test was performed until the subjects were unable to continue because of volitional exhaustion. The VO_2max was considered to have been achieved when at least 2 of the following criteria were met: (1) a plateau in oxygen uptake with an increase in work rate (<150 mL/min); (2) a heart rate >95% of the age-predicted maximum (ie, 220 − age); or (3) a respiratory exchange ratio >1.15.

2.3. Coronary artery calcification

Coronary artery calcification was measured by electron beam computed tomography at the Heart Check America Center in Bloomington, IL. During the scan, the subjects were placed in the supine position on a table and were fixed with 4 electrodes to their abdominal region to measure heart

rate. The subjects held their breath for approximately 20 to 30 seconds during the scan. The total number of calcified lesions in the coronary arteries was counted by a radiology technician, and the size and extent of CAC were quantified by the method of Agatston [12]. The same technician scored each scan to reduce variability and was blinded to the subject's activity group to prevent scoring bias.

2.4. Dual-energy x-ray absorptiometry

Bone mineral density and whole-body soft tissue composition were measured by dual-energy x-ray absorptiometry (DXA) using a Hologic QDR 4500A bone densitometer (software version 11.2; Bedford, MA) from scans of the whole body, lumbar spine, and proximal femur. Short- and long-term accuracy of the densitometer was verified by scanning a manufacturer's hydroxyapatite spine phantom of a known density. All DXA scans were performed by an Illinois state–licensed x-ray technologist and analyzed by the same investigator. Precision for DXA measurements of interest is between 1% and 1.5% in our laboratory.

2.5. Plasma lipids, mineral regulatory proteins, and c-reactive protein

Blood was drawn from all subjects after an overnight fast, and plasma was collected by centrifugation and stored at −80°C until analyzed. Plasma total cholesterol and triglycerides were measured enzymatically using reagent kits from Infinity (Melbourne, Australia). High-density lipoprotein cholesterol (HDL-C) was measured enzymatically after dextran sulfate–$MgCl_2$ precipitation [13]. Low-density lipoprotein cholesterol (LDL-C) was calculated using the equation of Friedewald et al [14]. Circulating levels of C-reactive protein (CRP) and several mineral regulatory proteins were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits or matched antibody pairs. The following ELISA kits were used: CRP (Diagnostic Automation, Calabasas, CA), MGP (Alpco Diagnostics, Windham, NH), fetuin-A (Biovendor Laboratory Medicine, Chandler, NC), and osteocalcin and osteopontin (Alpco Diagnostics, Windham, NH).

An OPG ELISA was developed using an OPG capture antibody and a biotinylated OPG detection antibody (R & D Systems, Minneapolis, MN). Briefly, the capture antibody was diluted to 6.17 μg/mL in coating buffer (phosphate-buffered saline [PBS]); and 100 μL per well was added to the ELISA plates and incubated at 4°C overnight followed by washing. The plates were blocked with 300 μL of PBS containing 1% bovine serum albumin, 5% sucrose, and 0.05% NaN_3 at room temperature for 1 hour to reduce nonspecific binding. Serial dilutions of OPG standards (0.03-15.28 ng/mL) and plasma were added to the appropriate wells and incubated for 2 hours at room temperature. One hundred microliters of 0.25-μg/mL detection antibody was then added to each well, and plates were incubated at room temperature for 2 hours. After this incubation, 100 μL of streptavidin–horseradish peroxidase (dilution to 1:200 in PBS containing 1% bovine serum albumin) was added; and the plates were incubated at room temperature for 20 minutes. Finally, 100 μL of substrate (TMB; BD Biosciences, San Diego, CA) was added to each well and incubated for 30 minutes. The reaction was stopped by adding 50 μL of stop solution (1 mol/L H_3PO_4) to each well, and the color change was quantified by measuring the absorbance at 450 nm. The intra- and interassay coefficient of variation for each of the ELISAs we performed was less than 5% and 10%, respectively, in our laboratory. Each of the above assays was performed with duplicate samples on a single microplate to eliminate interassay variation.

2.6. Statistics

All data were analyzed using SPSS version 12.0 (SPSS, Chicago, IL). Distribution statistics for the residuals were calculated to determine whether assumptions of normality were met (ie, skewness and kurtosis <2.0). The residuals for CAC score were skewed, so this variable was log-transformed for statistical analyses; the raw data are presented in the text and tables for meaningful comparisons. Initially, we used 2 × 2 (activity × sex) univariate analysis of variance to test for an interaction and for main effects of activity and sex on all major outcomes. Main effects were only considered when interactions were not significant because a significant interaction indicates that the effect of one independent variable depends on the value of the other. Because CAC levels were so low in women and because there were significant interactions between sex and physical activity level for several variables, we also evaluated within-sex differences in selected variables between activity groups using Student *t* tests. Correlation analysis was used to identify relationships between selected variables of interest. All data are expressed as mean ± SEM. An α level of .05 was considered statistically significant. Although a more conservative α may have been justified to account for the multiple variables that were analyzed, we felt that the standard α level of .05 was appropriate in this preliminary analysis where the goal is to identify potential hypotheses that can be definitively addressed in larger follow-up studies.

3. Results

3.1. Subject characteristics

A total of 12 women (6 SEDs and 6 endurance-trained MAs) and 13 men (6 SEDs and 7 endurance-trained MAs) participated in this study. Subject characteristics within each sex are shown in Table 1. The average age of the male and female participants was 66.5 ± 1.4 and 68.0 ± 1.7 years, respectively, and did not differ significantly between activity groups in either sex. As expected, VO_2max was greater in MAs compared with SEDs in both men (P = .008) and women (P = .01). In men, BMI did not differ between SEDs and MAs (P = .52); but the percentage of body fat was significantly lower in MAs than in SEDs (P = .009). Because

Table 1
Subject characteristics

	Men			Women		
	MA (n = 7)	SED (n = 6)	*P*	MA (n = 6)	SED (n = 6)	*P*
Age (y)	66.3 ± 4.4	66.7 ± 6.1	.90	66.8 ± 6.5	69.2 ± 4.5	.52
BMI (kg/m^2)	23.6 ± 2.1	24.5 ± 2.6	.52	22.1 ± 1.5	24.6 ± 1.1	<.01
% Body fat	17.2 ± 5.0	25.5 ± 3.9	<.01	23.0 ± 5.0	36.1 ± 2.7	<.01
VO_2max (mL/[kg min])	36.5 ± 6.5	25.4 ± 5.6	<.01	29.4 ± 5.9	20.3 ± 3.1	<.01

Data presented are means ± SEM.

of the difficulty in recruiting inactive women with low BMI, female MAs had significantly lower BMI (P = .009) and percentage of body fat (P = .001) than female SEDs.

3.2. Coronary artery calcium and bone density

The CAC and BMD measurements are shown in Table 2. No interaction between physical activity and sex was detected for CAC score (P = .14). There was no main effect of physical activity on CAC score (P = .12), but there was a main effect of sex (P = .02) because the average CAC score was 8-fold higher in men than women. When men and women were analyzed separately, CAC levels were moderately higher in SED men than MA men (Fig. 1), although this difference was not statistically significant (P = .12). In women, there was no difference in CAC between SEDs and MAs.

There was no interaction between physical activity and sex on the number of calcified lesions detected (P = .14). However, there was a main effect of sex, with men having 5-fold more lesions than women (P = .02). In addition, the main effect of physical activity approached significance (P = .08), with SEDs having 3.2-fold more lesions than MAs. When the men were analyzed separately, lesion number was 4-fold higher in SEDs than MAs, although this also was not statistically significant (16.2 ± 6.6 vs 4.1 ± 1.2, P = .08).

There was no interaction between physical activity and sex on whole-body (wb) BMD (P = .30). There was a significant main effect for physical activity because wb-BMD was 7% higher in MAs than SEDs (P = .02), but there was no main effect for sex (P = .19). There was a significant interaction between physical activity and sex for lumbar spine (Ls) BMD (P = .02) because Ls-BMD was 12% lower in female MAs compared with SEDs (0.91 ± 0.04 vs 1.02 ± 0.07, P = .02), but was 21% higher in male MAs compared with SEDs (1.11 ± 0.19 vs 0.92 ± 0.04, P = .03). For hip BMD, there were no interactive or main effects of physical activity and sex.

3.3. Plasma variables

There were no significant interactive or main effects of physical training and sex on plasma total cholesterol, LDL–C, triglycerides, CRP, osteopontin, osteocalcin, OPG, or fetuin-A (Table 2). However, there was a significant main effect of physical activity on HDL-C levels, with MAs having significantly higher HDL-C than SEDs (P = .04). Furthermore, there was a significant interaction (P = .008) between physical activity and sex for MGP levels. In men, MGP levels

Table 2
Effects of physical activity and sex on CAC, BMD, and plasma variables

	Main effect of physical activity			Main effect of sex			Interaction effect
	MA	SED	*P*	Male	Female	*P*	*P*
CAC (Agatston score)	70 ± 26.0	211 ± 101.4	.12	239 ± 90.7	29 ± 14.8	.02	.14
No. of calcified coronary artery lesions	2.9 ± 2.3	9.2 ± 2.4	.08	10.2 ± 2.3	1.9 ± 2.4	.02	.10
Wb-BMD (g/cm^2)	1.17 ± 0.02	1.09 ± 0.02	.02	1.15 ± 0.02	1.11 ± 0.02	.19	.31
Hip BMD (g/cm^2)	0.92 ± 0.03	0.88 ± 0.03	.35	0.91 ± 0.03	0.88 ± 0.03	.54	.36
Ls-BMD (g/cm^2)	1.01 ± 0.03	0.97 ± 0.03	.36	1.01 ± 0.03	0.97 ± 0.03	.31	<.01
CRP (mg/L)	3.2 ± 0.9	3.6 ± 0.9	.79	3.6 ± 0.9	3.2 ± 0.9	.73	.12
OPG (ng/mL)	1.2 ± 0.2	1.3 ± 0.2	.74	1.2 ± 0.2	1.3 ± 0.2	.92	.83
MGP (ng/mL)	22.4 ± 4.2	22.4 ± 4.4	.99	23.9 ± 4.4	20.9 ± 4.2	.64	<.01
OC (ng/mL)	9.3 ± 1.4	9.6 ± 1.4	.87	8.9 ± 1.3	10.1 ± 8.8	.54	.46
OP (ng/mL)	65.9 ± 5.1	63.6 ± 5.3	.75	59.7 ± 5.1	69.8 ± 5.3	.18	.18
Fetuin-A (μg/mL)	55.2 ± 7.0	46.8 ± 7.3	.42	51.1 ± 7.1	50.8 ± 7.3	.98	.71
Total cholesterol (mg/dL)	193 ± 12	198 ± 12	.77	190 ± 12	201 ± 12	.51	.53
HDL-C (mg/dL)	56.9 ± 3.5	45.7 ± 3.7	.04	48.8 ± 3.5	53.9 ± 3.7	.33	.76
LDL-C (mg/dL)	123 ± 9.5	133 ± 9.9	.48	126 ± 9.5	130 ± 9.9	.78	.47
Triglyceride (mg/dL)	66 ± 11	98 ± 11	.06	76 ± 11	88 ± 11	.46	.74

Data presented are means ± SEM. OC indicates osteocalcin; OP, osteopontin.

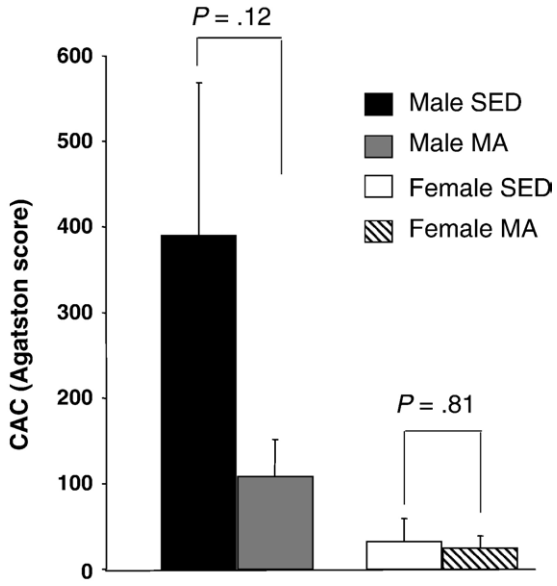

Fig. 1. Comparison of CAC score within physical activity and sex groups. Data presented are means ± SEM.

were 2.5-fold higher in SEDs than MAs (29.8 ± 9.0 vs 12.1 ± 1.6, $P = .06$), whereas in women, MGP was 2.2-fold higher in MAs than SEDs (32.7 ± 7.8 vs 15.0 ± 3.7, $P = .07$). In addition, when the data for men and women were analyzed separately, CRP levels were 2.3-fold lower in MA women compared with SED women (2.0 ± 1.6 vs 4.4 ± 1.7, $P = .03$).

3.4. Correlation analysis

The relationships between CAC, BMI, VO_2max, and plasma variables are shown in Table 3. Neither logCAC nor the number of calcified lesions was significantly correlated with VO_2max or any measure of BMD; however, both were inversely correlated with plasma osteocalcin. Whole-body BMD was positively correlated with OPG, whereas hip BMD was inversely associated with osteocalcin; and the correlation between hip BMD and plasma fetuin-A approached significance ($P = .054$). The VO_2max was positively correlated with wb-BMD and inversely with percentage of body fat, an effect that remained after controlling for sex ($r = 0.43$, $P = .057$ and $r = 0.45$, $P = .04$, respectively). There was also a trend for a positive correlation between LDL-C levels and logCAC score ($r = 0.393$, $P = .052$) and a trend for an inverse correlation between LDL-C and wb-BMD ($r = -.411$, $P = .057$).

When the sexes were analyzed separately, several relationships were evident in men only. There was a significant inverse correlation between VO_2max and the number of calcified coronary artery lesions ($r = -0.596$, $P_{¡} = .03$), and the correlation between VO_2max and logCAC score approached significance ($r = -0.493$, $P = .08$). There also was a positive correlation between VO_2max and plasma fetuin-A in men ($r = 0.679$, $P = .01$), but not women ($r = -0.003$, $P = .992$) (Fig. 2).

4. Discussion

To our knowledge, this is the first study to simultaneously investigate the effect of chronic exercise on both CAC and BMD. Exercise has well-established beneficial effects on BMD [15], but little is known regarding its effects on CAC. Because of the underlying similarities between bone and VC, exercise may influence both processes through similar mechanisms.

Table 3
Pearson correlation coefficients for the associations between selected variables

	logCAC	Wb-BMD	Hip BMD	Ls-BMD	VO_2max	No. of calcified lesions
LogCAC	1	.047	.024	−.184	−.061	.722 *
Wb-BMD	.047	1	.553 **	.346	.495 *	−.132
Hip BMD	.024	.553 **	1	.338	.294	.007
Ls-BMD	−.184	.346	.338	1	.107	−.249
VO_2max	−.061	.495 *	.294	.107	1	−.256
OPG	−.044	.449 *	.237	−.022	.056	−.009
MGP	.239	−.210	−.213	−.272	−.113	.087
OC	−.456 *	−.396	−.480 *	−117	.014	−.404 *
OP	−.138	−.396	.032	.277	−.051	−.212
Fetuin-A	.114	.204	.416	.061	.323	−.120
CRP	−.151	−.270	−.005	.356	−.311	−.233
TC	.310	−.366	.139	.011	−.162	.238
HDL-C	−.024	.022	.173	.172	.131	−.082
LDL-C	.393	−.411	.086	−.060	−.162	.359
TG	−.013	−.123	.044	.031	−.368	−.130
BMI	.352	−.262	−.343	.016	−.363	.396
% Body fat	−.118	−.516 *	−.193	−.154	−.761 **	−.101
Age	.135	−.061	.109	.378	−.400	.258

* $P < .05$.
** $P < .01$.

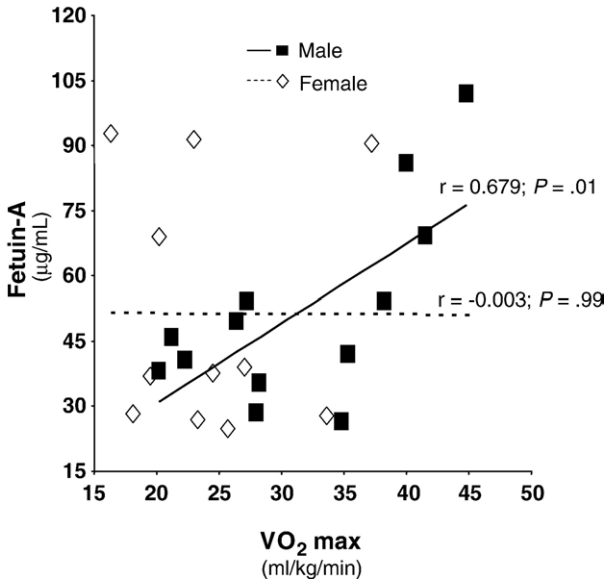

Fig. 2. Correlation between plasma fetuin-A and VO_2max in male and female participants.

In this study, when all subjects were included in the analysis, we did not find a statistically significant difference in CAC levels or the number of calcified lesions between MAs and SEDs (Table 2), or a correlation between VO_2max and either CAC or the number of calcified lesions (Table 3). However, when the sexes were analyzed separately, a moderate relationship between CAC and aerobic fitness was evident in men, as indicated by a significant inverse correlation between VO_2max and the number of calcified lesions ($r = -0.596$) and a trend for an inverse correlation between VO_2max and logCAC score. Furthermore, CAC score and the number of calcified lesions were moderately, but not statistically significantly, lower in MA men compared with SED men. These data provide preliminary evidence that chronic exercise may lower the risk of developing CAC in men, although additional studies with larger sample sizes will be needed to confirm these preliminary findings.

The lack of an association between physical activity or fitness and CAC in the women in this study was not surprising given the low average CAC levels in this group and the fact that 6 of the 12 women in this study (3 in each activity group) had a CAC score of zero. Other studies confirm that women tend to have much lower CAC levels than men of a comparable age [16,17]. For example, a recent epidemiologic study by Iribarren et al [16] found that 59% of the women between the ages of 60 and 69 years had CAC scores of 0, as opposed to just 25% of men; and the median CAC score was 110 among men and just 2 among women.

Very few studies have attempted to address the effectiveness of exercise in preventing CAC, and the data to date are rather equivocal [10,11]. Taylor et al [11] found no relation between physical activity levels and CAC scores in a group of healthy middle-aged subjects, whereas Desai et al [10] found a modest inverse relationship between CAC and physical activity level in asymptomatic individuals with multiple CVD risk factors. However, these were both observational studies that used questionnaires to estimate physical activity levels. Objective measures of fitness (eg, VO_2max) were not obtained in either study, so the relationship between fitness and CAC was not determined. As a result, the inverse relationship we found between VO_2max and the number of calcified lesions in men is a novel finding that can contribute to future research regarding the relationship between aerobic fitness and CAC.

Chronic physical activity may inhibit bone loss and VC by distinct mechanisms, although some common factors also may play a role. It is generally accepted that the positive correlation between physical activity or VO_2max and BMD is mediated primarily by the overloading forces on the bone that occur during exercise training, whereas metabolic and cardiovascular adaptations associated with chronic exercise do not significantly influence BMD [15]. However, recent evidence suggests that metabolic factors that are influenced by exercise, including circulating lipid levels and nitric oxide synthesis, also may have a significant effect on bone metabolism. For example, oxidized LDL-C has been shown to inhibit osteoblast differentiation of bone- and marrow-derived preosteoblasts in vitro [8]; and lipid lowering agents such as statins stimulate bone formation in rodents [18] and increase bone density in postmenopausal women [19]. Furthermore, recent evidence suggests that elevated nitric oxide production may promote bone formation by increasing osteoblast activity [20]. As a result, exercise training–induced reductions in plasma oxidative stress and plasma lipids [21] and increases in endothelial nitric oxide synthase activity [22] also may help explain the positive relationship between VO_2max and BMD.

Similar mechanisms may contribute to exercise-induced inhibition of VC. For example, exercise training in mice induces the expression of antioxidants in the arterial wall, including catalase [23] and superoxide dismutase [24], both of which inhibit LDL oxidation in the vasculature. Although oxidized-LDL *inhibits* osteoblastic differentiation of preosteoblasts, it paradoxically *promotes* osteoblastic differentiation of vascular cells in vitro [8]. As a result, the exercise-induced increase in antioxidant defenses in the vascular wall may simultaneously increase BMD and inhibit VC.

In addition, endurance exercise training has beneficial effects on many other putative risk factors for VC and osteoporosis, including elevated glucose, LDL-C, and CRP, as well as low HDL-C; so it is reasonable to assume that exercise may inhibit these disorders via risk factor reduction. However, there are no published exercise training interventions in humans or animal models that have examined these questions. In this study, HDL-C levels differed between physical activity groups (Table 2); but there was no correlation between HDL-C and either BMD or CAC (Table 3). By contrast, there was no difference in LDL-C

levels between physical activity groups; but there was a trend for a positive correlation between LDL-C and logCAC (P = .052) and for a negative correlation between LDL-C and wb-BMD (P = .057). This suggests that reducing LDL-C levels may simultaneously protect against osteoporosis and VC.

The finding that OPG-deficient mice have accelerated aortic calcification and osteoporosis [25] led to speculation that mineral regulatory proteins may be implicated in both osteoporosis and VC. One purpose of this study was to examine if exercise may mediate its protective effects on these disorders by altering the expression of these proteins. Although we did not find any differences in mineral regulatory proteins between MAs and SEDs, there were several interesting correlations between these proteins and other variables. For example, VO_2max and fetuin-A were positively correlated in our male, but not female, subjects (Fig. 2). Fetuin-A is a systemically acting inhibitor of VC that has been estimated to account for 50% of the calcium precipitation inhibitory capacity of serum [26]. Its circulating levels are reduced in patients with renal failure [27], a condition associated with greatly elevated VC [1], and are inversely associated with atherosclerosis and CVD mortality [28]. Fetuin-A is a reverse, acute phase protein, meaning that inflammation itself reduces its circulating levels [29]. On the other hand, chronic exercise has potent anti-inflammatory effects [30] and so should increase fetuin-A levels. Indeed, the positive correlation between fetuin-A and VO_2max in men in this study supports this theory and indicates a potential mechanism by which exercise may correlate negatively with CAC.

There were several limitations to our study, the most prominent being the limited sample size and cross-sectional design. Because of the small sample size, the study was extremely underpowered (eg, β = .25 for CAC, our primary outcome). Furthermore, the cross-sectional design prevents us from making conclusive determinations about cause and effect between our outcomes.

4.1. Summary

To our knowledge, this is the first study to investigate the relationship between habitual endurance activity, aerobic fitness, CAC, and BMD. In men, but not women, we found a modest inverse relation between VO_2max and the number of calcified coronary artery lesions, and a trend for a correlation between VO_2max and logCAC score. Furthermore, fetuin-A, a potent circulating inhibitor of VC, was positively correlated with VO_2max in men, indicating a potential mechanism by which exercise may inhibit VC. The relationship between physical activity, CAC, BMD, and circulating levels of mineral regulatory proteins is complex. Additional studies with larger sample sizes will be needed to determine if the protective effects of endurance exercise on CAC and BMD are sex specific or are mediated through changes in mineral regulatory proteins or other common mechanisms.

Acknowledgment

This research was supported by the University of Illinois Urban-Champaign Campus Research Board.

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