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Physical activity and serum interleukin-6 in relation to bone density in young adults

Atividade física e interleucina-6 sérica em relação à densidade óssea em adultos jovens

AUTHOR'S

Francine Silva dos Santos[1,2]
Renata Moraes Bielemann[1,3]
Isabel Oliveira de Oliveira[1]
Bernardo Lessa Horta[1]
Soren Brage[4]
Denise Petrucci Gigante[1,3]

1 Universidade Federal de Pelotas, Departamento de Medicina Social, Programa de Pós-graduação em Epidemiologia, Pelotas, Rio Grande do Sul, Brasil.

2 Universidade de São Paulo, Departamento de Nutrição, Faculdade de Saúde Pública, São Paulo, Brasil.

3 Universidade Federal de Pelotas, Departamento de Nutrição, Programa de Pós-graduação em Nutrição e Alimentos, Pelotas, Rio Grande do Sul, Brasil.

4 Universidade de Cambridge, MRC Epidemiology Unit, Cambridge, United Kingdom.

CORRESPONDING

Francine Silva dos Santos
nutrifrancinesantos@gmail.com
Rua Marechal Deodoro, 1160 – 3º piso,
Pelotas, Rio Grande do Sul, Brasil.
ZIP CODE: 96020-220.

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ABSTRACT

Physical activity (PA) and inflammation influence bone density through multiple physiological mechanisms, but current evidence is not robust on the structure mediating these relationships. Therefore, the aim of this study was to investigate the associations of PA, and serum interleukin-6 (IL-6) on bone density. Cross-sectional analysis in the Pelotas (Brazil) 1982 Birth Cohort with participants aged 30-years old. PA was objectively measured by accelerometry. Bone mineral density (g/cm^2) was evaluated for the lumbar spine and femoral neck using dual-energy X-ray absorptiometry. Crude and adjusted linear regressions and mediation analyses were performed. In both sexes, the overall PA was positively associated with femoral neck bone density, but not lumbar spine. For men, the mean of femoral neck were 0.027, 0.042, and 0.032 higher in the second, third, and fourth quartiles, respectively, compared to the first quartile (reference). Among women, higher bone density values were found in the third (0.021) and fourth (0.027) quartiles of overall PA compared to the lowest quartile. Among females, moderate-to-vigorous intensity physical activity presented a positive relationship with all sites of bone density. The indirect effect through IL-6 was not significant. Physical activity was associated with gains in bone density. The findings reinforce recommendations for PA in adulthood to promote bone health.

Keywords: Accelerometry; Body composition; Epidemiology.

RESUMO

A atividade física (AF) e a inflamação influenciam a densidade óssea através de múltiplos mecanismos fisiológicos, mas a atual evidência não é robusta sobre a estrutura de mediação dessas relações. Portanto, o objetivo deste estudo foi investigar as associações de AF e interleucina-6 sérica (IL-6) na densidade óssea. Análise transversal na Coorte de Nascimentos de 1982 Pelotas (Brasil) em participantes com 30 anos de idade. AF foi medida objetivamente por acelerometria. Densidade mineral óssea (g/cm^2) foi avaliada para a coluna lombar e colo do fêmur usando absorciometria de raios-X de dupla energia. Foram realizadas regressões lineares brutas e ajustadas e análises de mediação. Em ambos os sexos, a AF total foi positivamente associada à densidade óssea do colo do fêmur, mas não à coluna lombar. Para os homens, as médias do colo do fêmur foram 0,027, 0,042 e 0,032 maiores no segundo, terceiro e quarto quartis, respectivamente, em relação ao primeiro quartil (referência). Entre as mulheres, os maiores valores de densidade óssea foram encontrados no terceiro (0,021) e quarto (0,027) quartis de AF total em comparação ao quartil mais baixo. No sexo feminino, a atividade física de intensidade moderada a vigorosa apresentou relação positiva com todos os locais de densidade óssea. O efeito indireto através da IL-6 não foi significativo. A atividade física foi associada a ganhos de densidade óssea. Os achados reforçam recomendações de AF na idade adulta para promover a saúde óssea.

Palavras-chave: *Acelerometria; Composição corporal; Epidemiologia.*

Introduction

Bone health is essential to overall health and quality of life, and bone-associated- diseases increase disabilities, mortality, and healthcare costs[1]. In this sense, low bone mass is recognized as an important risk factor for osteoporosis, therefore, it can lead to an increased risk of bone fractures[1,2]. A recent study showed that Southern Latin America is in the top four regions with the highest incidence rate of bone fractures between the 21 regions of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD)[3]. Because the bone mineral density (BMD) decreases with the age[1,2], and the life expectancy worldwide increased from 2000 to 2019, the burden of diseases related to longevity can become higher[4].

The peak bone mass is the main determinant of the amount of bone density throughout life[2]. Around late adolescence and second[5] or third[2] decade of life, this peak is attained. Several factors may modulate the bone density[1,2], and physical activity (PA) is one of the behavioural factors that can protect against losses of BMD[6,7], but the mechanisms have not been entirely elucidated[8]. Also, according to a systematic review of

prospective studies, the wide body of evidence concerning PA on BMD from population based-studies assessed the exposure using indirect instruments, such as questionnaires[6] which may be less accurate than objective measures[9], casting uncertainty on the association.

The concentrations chronically elevated of IL-6 comprise a known physiologic pathway to excessive bone resorption, leading to bone diseases, such as osteoporosis. Among the panel of pro-inflammatory cytokines, IL-6 stands out since it has a direct role in osteoclastogenesis[10]. The health-beneficial effects of PA may be mediated by the modulation of inflammatory cytokines, protecting against diseases associated with chronic inflammation status[11]. Lower concentrations of IL-6 were reported among physically active individuals[9,11]. Given there is considerable interest in exploring ways to increase BMD, and that these relationships have been less evaluated in low-and middle-income countries and among young adults which is the period of age when high bone mass is observed, this study aimed to assess the association of objectively measured PA and inflammation (evaluated by serum IL-6 concentration) on bone density at 30 years of age in the 1982 Pelotas (Brazil) birth cohort.

Methods

This study was carried out in Pelotas, a medium-sized city in southern Brazil that currently has ~330,000 inhabitants. In 1982, all maternity hospitals in the city were visited daily and the 5,914 live-borns whose families lived in the urban area of the city were examined and their mothers interviewed. These participants have been followed up many times.

From June 2012 to February 2013, when subjects were 30 years old, the entire cohort was invited to visit the Epidemiologic Research Center for clinical exams and an extensive lifestyle interview. More details on the methodology of all follow ups have been published elsewhere[12–14]. All procedures were approved by the Ethics Committee in Research of the Faculty of Medicine at the Federal University of Pelotas. The 30 years follow-up of the 1982 Pelotas Birth Cohort was approved by the Federal University of Pelotas Ethics Committee, affiliated to the *Conselho Nacional de Ética em Pesquisa* (National Research Ethics Committee – CONEP) – protocol number: of.16/12. Written informed consent was obtained from all subjects.

Areal bone mineral density (aBMD, in g/cm^2) was measured at the right femoral neck and at the lumbar spine (L1-L4) by dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE, Germany). The DXA machine was calibrated daily before each session according to manufacturer recommendation. Pregnant or suspected pregnant women, subjects weighing more than 120kg, or those with metal plates/screws inside the body or metal pieces (piercings, rings, or bracelets) that could not be removed were excluded. Lean mass (kg) was also obtained from the whole-body DXA scan. In this exam, participants wider than 60cm did not completely fit in the DXA scan area, and were submitted to half-body scans of their right side to estimate body composition. Fat mass also was assessed using DXA.

Objectively measured physical activity was assessed using accelerometers (GENEActiv ActivInsight, Kimbolton, UK). This device measures acceleration in three axes and expresses the measure in gravitational acceleration units. Participants wore the device in the non-dominant wrist all day and night, including when having a shower and performing other water activities (e.g., swimming, washing the dishes, etc). The period of use varied from four to seven days, including one weekend day. Data were recorded at a sample frequency of 85.7 Hz.

Data were processed in GENEActiv software and analyzed using the R-package GGRI (https://cran.r-project.org/web/packages/GGIR/vignettes/GGIR.html#citing-ggir). Procedures in the accelerometry analyses were made according to previous publications[15,16]. Detailed signal processing included: calibration to local gravity, verification of sensor calibration error, detection of sustained abnormally high values, non-wear detection, calculation of the vector magnitude of body acceleration and separating out the component related to movement using the Euclidian Norm minus one (ENMO: $\sqrt{x^2 + y^2 + z^2} - 1g$) with resulting negative values rounded up to zero, and imputation of invalid data segments by the average of similar time points on different days of the measurement.

Files were considered valid if data were present for every 15-minute period in a 24-hour cycle (even when scattered over multiple days) and with calibration error was lower than 0.02 *g* (following calibration). The summary measure ENMO was used as an indicator of the average magnitude of dynamic wrist acceleration over the measurement period. Results are presented in milli-*g* (1 m*g* = 0.001 *g*) for readability reasons. Time spent in moderate-to-vigorous physical activi-

ty (MVPA) per day was estimated using an intensity threshold of 100 m*g*[17,18] based on 5-second epoch data and in bouts lasting at least 5 minutes, implemented as at least 80% of data points in time-segments of 5 minutes or longer above the intensity threshold.

Non-fasting blood samples were collected at the 2012-13 follow-up. Serum interleukin-6 (IL-6) was measured for 2,988 participants by the Quantikine® HS Human IL-6 immunoassay kit (R&D Systems®, Inc.; Minneapolis, MN55413, USA) and SpectraMax 190 microplate spectrophotometer (Molecular Devices Corp, California, USA). Pregnant women did not collect blood samples. Participants with IL-6 levels ≤ 2 standard deviations (SD) [males (SD = 5.88; n = 58); and females (SD = 6.06; n = 60)] were excluded from this analysis. Intra-assay and inter-assay coefficients of variation were 1.9% and 3.4%, respectively.

Standing height was measured to the nearest 1 mm with barefooted participants using a rigid stadiometer. Current smoking and use of oral contraceptives were self-reported. Calcium intake (mg/day) and phosphorus intake (mg/day) were measured at 30 years by a semi-quantitative food frequency questionnaire. Other variables collected in different follow-ups were considered potential confounders: Skin color was evaluated by self-report in 2004-5. Birth weight measured with pediatric scales (Filizola) soon after birth; monthly family income; maternal age, schooling, and smoking during pregnancy; and breastfeeding duration were collected in the perinatal interview or during the follow-ups in childhood.

The statistical analyses were performed with Stata 14 software (StataCorp, College Station, TX, USA) following the structure of relationships among variables according to the Directed Acyclic Graph (DAG) previously designed (available at: dagitty.net/m-uUcOZ). After the interaction test, the analyses were sex-stratified. Characteristics of the individuals included in the analyses were described in absolute and relative frequencies or mean/median and standard deviation/interquartile range. Linear regressions were used in crude and adjusted analyses between exposures (overall PA and MVPA) and outcomes (femoral neck and lumbar spine aBMD), between exposures and mediator (IL-6), and between mediator and outcomes. Wald's test was used to test the statistical significance.

A mediation analysis, using IL-6 as a mediator, was carried out using the g-computation formula[19]. G-computation formula works with base confounders – variables that affect both main exposures and outcomes – and post confounders – confounders of the mediator and outcome relationship that are affected by the exposure. In this analysis, family income at birth, maternal schooling at birth, maternal age at birth, maternal smoking during pregnancy, birth weight, breastfeeding duration, skin color, oral contraceptive (females), smoking status, height, calcium intake, and phosphorus intake at 30 years were considered as base confounders. Post confounder was fat mass percentage. Direct, indirect, and total effects were estimated. Results are shown in beta coefficients and their respective 95% confidence intervals. The significance level was set at 5%.

Results

In the 2012-13 follow-up (at age ~30 years), lumbar spine and femoral neck aBMD were measured in 3,429 participants (92.7% of the 3,701 interviewed; added to the 325 known to have died the follow-up rate was 68.1%). Compared with the original cohort, the individuals included in the analysis were slightly more likely to be female, belong to the intermediate category of family income at birth, and birthweight ≥ 2,500 grams.

Characteristics of the study population are summarized in Table 1, stratified by sex. Males represented 49.2% (n = 1,687) of the study population. The means of lumbar spine and femoral neck density were [males 1.24 g/cm^2 (SD = 0.15) and 1.11 g/cm^2 (SD = 0.16); females 1.21 g/cm^2 (SD = 0.13) and 1.01 g/cm^2 (SD = 0.13)], respectively. Most participants had mothers aged 20 to 29 years, with schooling ≥ eight years, and who did not smoke during pregnancy. Low birthweight was more prevalent among females (8.5%) than males (6.0%). Approximately 30% of the study population had a breastfeeding duration ≥ of six months, and more than 70% self-reported white skin color. Females had higher calcium consumption, whereas the phosphorus intake was higher in males. Concerning main exposures, the mean overall physical activity was 38.3 m*g* (SD = 11.5) for males and 33.1 m*g* (SD = 8.7) for females. The median and interquartile range (IQR) of MVPA was 35.6 min/day (IQR = 17.6; 63.6) for males and 23.3 min/day (IQR = 11.8; 41.6) for females. On the other hand, females presented the highest IL-6 concentration.

After adjustment, we observed greater femoral neck aBMD among males and females in the three and two highest quartiles of the overall PA compared to those in the first quartile, respectively. Overall volume of ac-

Table 1 –Characteristics of participants from the 1982 Pelotas birth cohort at 30 years included in the analysis.

Subjects' characteristics	Males		Females	
	n	Mean (SD)	n	Mean (SD)
Family income at birth (tertiles)	1,687		1,742	
1st	534	31.7	546	31.3
2nd	600	35.6	611	35.1
3rd	553	32.7	585	33.6
Maternal schooling (years)	1,684		1,740	
0-4	545	32.4	565	32.5
5-8	730	43.3	735	42.2
9-11	184	10.9	189	10.9
≥12	225	13.4	251	14.4
Maternal age (years)	1,687		1,742	
<20	240	14.3	255	14.6
20-29	991	58.7	992	57.0
≥30	456	27.0	495	28.4
Maternal smoking during pregnancy	1,687		1,742	
No	1,103	65.4	1,132	65.0
Yes	584	34.6	610	35.0
Birthweight (grams)	1,687		1,741	
<2500	101	6.0	148	8.5
≥ 2500	1,586	94.0	1,593	91.5
Breastfeeding duration (months)	1,627		1,689	
<1.0	360	22.1	341	20.2
1 – 2.9	412	25.3	445	26.4
3.0 – 5.9	375	23.1	397	23.5
≥ 6.0	480	29.5	506	29.9
Skin color	1,687		1,742	
White	1,263	74.9	1,340	76.9
Non-white	424	25.1	402	23.1
Smoking status	1,670		1,725	
Never smoker	947	56.7	1,040	60.3
Former	286	17.1	314	18.2
Current smoker	437	26.2	371	21.5
Oral contraceptive	NA		1,742	
No	NA	NA	576	33.1
Yes	NA	NA	1,166	66.9
Calcium intake (mg/d)	1,672		1,732	
1st	592	291.7 (63.8)	541	287.6 (61.3)
2nd	585	489.4 (70.3)	556	497.4 (70.0)
3rd	495	882.3 (241.1)	635	908.9 (257.4)
Phosphorus intake (mg/d)	1,551		1,581	
1st	346	891.7 (173.9)	726	847.2 (190.7)
2nd	521	1392.5 (150.3)	528	1360.0 (153.7)
3rd	684	2503.3 (990.3)	327	2260.7 (754.6)
Overall PA (mg)	1,268		1,360	
1st	311	25.1 (3.8)	338	23.0 (3.4)
2nd	316	33.5 (2.0)	334	29.7 (1.5)
3rd	319	40.6 (2.3)	348	34.8 (1.7)
4th	322	53.5 (8.1)	340	45.0 (6.4)
MVPA (5-min bout: min/day)	1,265		1,357	
1st	310	8.9 (5.3)	333	5.6 (3.4)
2nd	320	26.0 (5.3)	343	17.2 (3.2)
3rd	316	46.9 (7.9)	342	32.0 (5.3)
4th	319	106.2 (44.8)	339	68.7 (28.7)
Lumbar spine (g/cm²)	1,687	1.24 (0.15)	1,742	1.21 (0.13)
Femoral neck (g/cm²)	1,687	1.11 (0.16)	1,742	1.01 (0.13)
Fat mass (%)	1,661	24.2 (8.8)	1,728	39.2 (8.6)
IL-6 (pg/mL)	1,379	1.86 (1.76)	1,486	2.03 (2.00)
Log IL-6 (pg/mL)	1,379	0.39 (0.62)	1,486	0.45 (0.66)

PA = physical activity; MVPA = moderate-to-vigorous intensity physical activity; SD = standard deviations.

tivity was not associated with lumbar spine density. Among females, MVPA was associated with aBMD at all anatomical sites. Females belonging to the highest quartile of MVPA showed greater lumbar spine density (β = 0.031; 95%CI: 0.011; 0.052). Femoral neck density was higher among females in the third (β = 0.022; 95%CI: 0.002; 0.041) and fourth (β = 0.029; 95%CI: 0.009; 0.049) quartiles of MVPA than those in the first (Table 2).

Regarding relationships between exposures and outcomes with serum IL-6, women who belong to the two highest quartiles of PA presented lower mean IL-6. The aBMD at all anatomical sites was greater between females in the third tertile of IL-6 concentration (Figure 1). Due to these results, mediation analysis was performed only for females. The mediator (IL-6) ex-

Table 2 – Crude and adjusted associations between physical activity (PA) and bone mineral density (g/cm²) at 30 years - 1982 Pelotas Birth Cohort.

	Crude		Adjusted[a]		Crude		Adjusted[a]	
	n	Lumbar spine	n	Lumbar spine	n	Femoral neck	n	Femoral neck
		P value β (95% IC)		P value β (95% IC)		P value β (95% IC)		P value β (95% IC)
Males								
Overall PA (mg)	1,268	0.518	1,147	0.095	1,268	0.002	1,147	0.006
1st		Reference		Reference		Reference		Reference
2nd		0.013 (-0.010; 0.036)		0.029 (0.005; 0.052)		0.012 (-0,012; 0,036)		0.027 (0.002; 0.052)
3rd		0.016 (-0.007; 0.039)		0.023 (-0.0005; 0.046)		0.038 (0.014; 0.062)		0.042 (0.017; 0.066)
4th		0.007 (-0.016; 0.030)		0.017 (-0.006; 0.041)		0.031 (0.007; 0.055)		0.032 (0.007; 0.057)
MVPA (5-min bout: min/day)	1,265	0.817	1,143	0.757	1,265	0.081	1,143	0.196
1st		Reference		Reference		Reference		Reference
2nd		-0.010 (-0.033; 0.013)		-0.007 (-0.030; 0.017)		0.001 (-0.023; 0.025)		0.001 (-0.024; 0.025)
3rd		-0.001 (-0.023; 0.023)		0.003 (-0.021; 0.027)		0.011 (-0.013; 0.035)		0.011 (-0.014; 0.036)
4th		-0.003 (-0.026; 0.020)		0.001 (-0.024; 0.025)		0.019 (-0.005; 0.044)		0.014 (-0.011; 0.040)
Females								
Overall PA (mg)	1,360	0.323	1,231	0.216	1,360	0.001	1,231	0.009
1st		Reference		Reference		Reference		Reference
2nd		0.003 (-0.016; 0.023)		0.005 (-0.015; 0.025)		0.017 (-0.002; 0.036)		0.019 (-0.0008; 0.038)
3rd		0.007 (-0.013; 0.026)		0.013 (-0.007; 0.033)		0.017 (-0.001; 0.036)		0.021 (0.002; 0.041)
4th		0.009 (-0.010; 0.029)		0.011 (-0.010; 0.032)		0.033 (0.014; 0.052)		0.027 (0.007; 0.047)
MVPA (5-min bout: min/day)	1,357	0.031	1,228	0.007	1,357	<0.001	1,228	0.002
1st		Reference		Reference		Reference		Reference
2nd		0.011 (-0.008; 0.031)		0.010 (-0.011; 0.030)		0.010 (-0.009; 0.029)		0.011 (-0.008; 0.031)
3rd		0.007 (-0.012; 0.027)		0.007 (-0.013; 0.027)		0.024 (0.005; 0.043)		0.022 (0.002; 0.041)
4th		0.024 (0.005; 0.043)		0.031 (0.011; 0.052)		0.032 (0.013; 0.051)		0.029 (0.009; 0.049)

[a]Adjusted for family income at birth, oral contraceptive (females), maternal schooling, maternal age, maternal smoking during pregnancy, birth weight, breastfeeding duration, skin color, smoking status, calcium intake, phosphorus intake and height (cm). PA = physical activity; MVPA = moderate-to-vigorous intensity physical activity.

plained less than 3% of the total effect of PA on aBMD (Table 3), and the indirect effect was not significant. Further analysis, indicated that fat mass, used as post confounder in the previous analysis, captured part of the effect of PA on bone mineral density in both sexes (Table 4).

Discussion

The current study in young adults from southern Brazil shows that overall PA was positively associated with femoral neck aBMD at 30 years, a relevant anatomical site to the global burden of osteoporotic fractures attributable to low bone density[1]. For females, the positive relationship between PA and aBMD at the different sites was evident considering MVPA. Furthermore, we did not observe any indirect effect through inflammation, as measured by serum IL-6.

It has previously been reported that the potential beneficial effects of PA on bone mass are largest among males when the exposure was measured using indirect methods, such as questionnaires[20], whereas a positive association also was found in women using accelerometry[21], corroborating our findings. It is possible that because most previous studies applied questionnaires that considered only leisure-time, this may not reflect habitual physical activities, such as domestic activities; such activities may be very common in certain individuals; estimates indicate that domestic PA represents more than 30% of the self-reported MVPA among women[22], and this activity would be captured by the accelerometry method. Although the relationship between PA performed in the household and health remains in de-

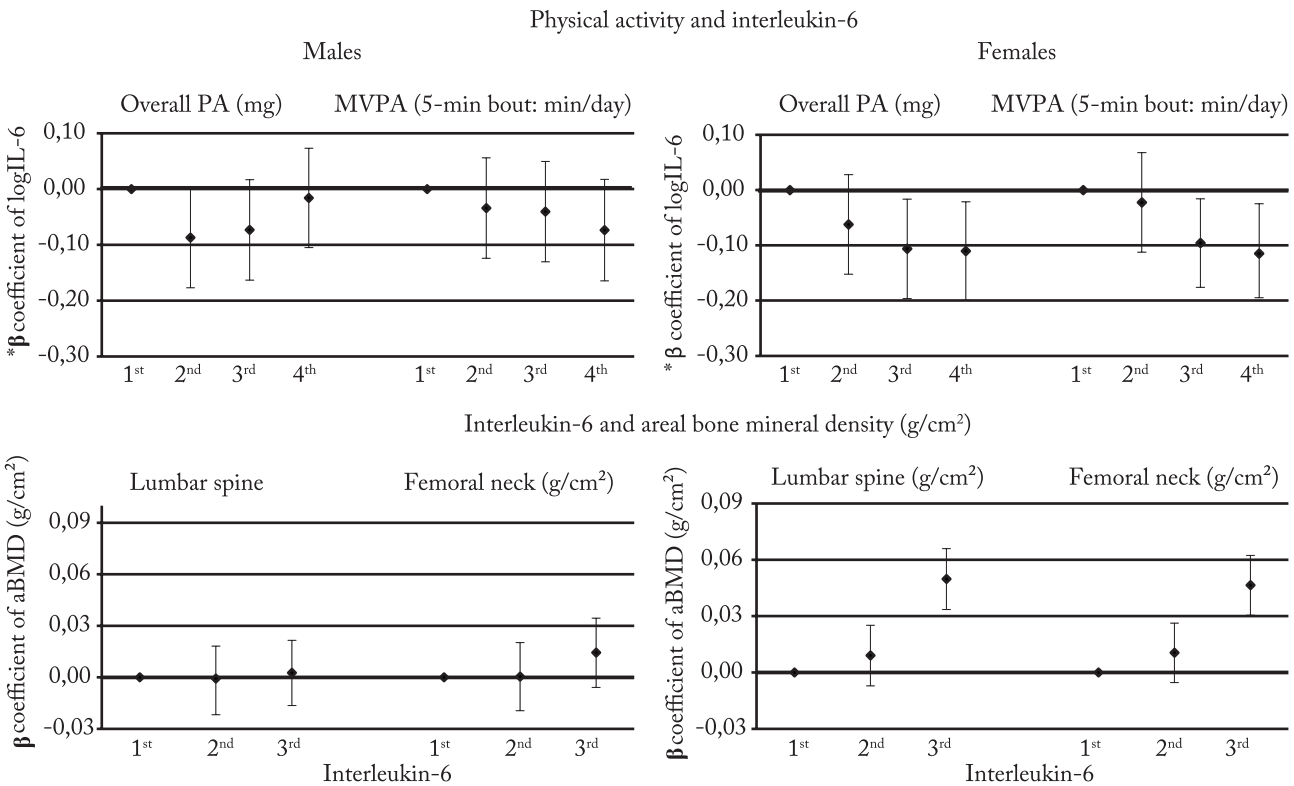

Figure 1 – Associations between physical activity and bone mineral density with interleukin-6 at 30 years - 1982 Pelotas Birth Cohort. PA = physical activity; MVPA = moderate-to-vigorous intensity physical activity.

Table 3 – Total, direct, and indirect effects of physical activity on the bone mineral density of females from the 1982 Pelotas Birth Cohort at 30 years of age.

	Total effect		Direct effect		Indirect effect		Mediated
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value	Effect (%)
Lumbar Spine							
Overall PA (mg)	0.011 (0.005; 0.018)	0.001	0.012 (0.005; 0.018)	<0.001	-0.0002 (-0.0008; 0.0004)	0.51	1.7
MVPA (5-min bout: min/day)	0.015 (0.008; 0.022)	<0.001	0.015 (0.008; 0.022)	<0.001	-0.0004 (-0.001; 0.0003)	0.24	2.6
Femoral Neck							
Overall PA (mg)	0.014 (0.008; 0.020)	<0.001	0.014 (0.008; 0.020)	<0.001	-0.00005 (-0.0005; 0.0004)	0.82	0.4
MVPA (5-min bout: min/day)	0.014 (0.008; 0.020)	<0.001	0.014 (0.008; 0.020)	<0.001	-0.0001 (-0.0006; 0.0004)	0.58	1.0

Variables included in the analysis as base confounders were family income at birth, maternal schooling at birth, maternal age at birth, maternal smoking during pregnancy, birth weight, breastfeeding duration, skin color, oral contraceptive, smoking status, height, calcium intake, and phosphorus intake. Variable included in the analysis as post-confounders was fat mass percentage. Mediator was interleukin-6. PA = physical activity; MVPA = moderate-to-vigorous intensity physical activity.

bate, it is an important domain to be considered especially in low-and middle-income countries, and may impact women's health[23].

Previous studies conducted in the Pelotas Birth Cohorts observed a beneficial effect of PA in distinct life stages on bone mass at adolescence or young adulthood[20,21]. A recent meta-analysis estimated that PA interventions may improve lumbar spine BMD (standardized effect size 0.17, 95%CI: 0.04; 0.30) among older individuals, with moderate quality of evidence according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. Concerning femoral neck density, the null value was included in the confidence interval[24].

In the current study, we also assessed the mechanisms that could elucidate the role of PA on bone density, particularly the pathways related to chronic inflammation. Longitudinal studies performed with healthy adults have found null or negative effects of inflammatory biomarkers, including IL-6, on bone

Table 4 – Total, direct, and indirect effects of physical activity on bone mineral density from the 1982 Pelotas Birth Cohort at 30 years of age.

	Total effect		Direct effect		Indirect effect	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
Males						
Lumbar Spine						
Overall PA (mg)	0.004 (-0.004; 0.012)	0.31	0.007 (-0.001; 0.015)	0.09	-0.003 (-0.005; -0.0008)	0.006
MVPA (5-min bout: min/day)	0.0007 (-0.007; 0.009)	0.86	0.003 (-0.005; 0.011)	0.44	-0.003 (-0.005; -0.0005)	0.02
Femoral Neck						
Overall PA (mg)	0.011 (0.003; 0.018)	0.01	0.014 (0.006; 0.022)	0.001	-0.003 (-0.006; -0.0007)	0.01
MVPA (5-min bout: min/day)	0.006 (-0.003; 0.014)	0.20	0.009 (0.0003; 0.018)	0.04	-0.003 (-0.006; -0.0009)	0.008
Females						
Lumbar Spine						
Overall PA (mg)	0.004 (-0.002; 0.011)	0.18	0.009 (0.002; 0.016)	0.009	-0.005 (-0.008; -0.001)	0.006
MVPA (5-min bout: min/day)	0.009 (0.002; 0.016)	0.01	0.013 (0.006; 0.020)	<0.001	-0.004 (-0.007; -0.0006)	0.02
Femoral Neck						
Overall PA (mg)	0.009 (0.003; 0.015)	0.004	0.017 (0.011; 0.024)	<0.001	-0.008 (-0.012; -0.005)	<0.001
MVPA (5-min bout: min/day)	0.009 (0.003; 0.016)	0.003	0.015 (0.008; 0.021)	<0.001	-0.005 (-0.009; -0.002)	0.003

Variables included in the analysis as base confounders were family income at birth, maternal schooling at birth, maternal age at birth, maternal smoking during pregnancy, birth weight, breastfeeding duration, skin color, oral contraceptive (females), smoking status, and height. Variables included in the analysis as post-confounders were calcium intake and phosphorus intake. The mediator was fat mass percentage. PA = physical activity; MVPA = moderate-to-vigorous intensity physical activity.

metabolism[25,26], the serum IL-6 was the inflammatory biomarker measured because it is well known by animal models that this biomarker is involved in osteoclastogenesis and thereby stimulates bone resorption[10]. As sex hormones down-regulate the IL-6 synthesis, the reduction of estrogen and testosterone after menopause or andropause may contribute to osteoporosis pathogenesis through increasing IL-6 concentration at old age[10]. Considering that our sample involved young adults, lifestyle factors such as PA (main exposure) may present a more relevant role in IL-6 concentration than sex hormones. Even after adjustment for hormonal contraceptive methods, lifestyle factors, and other possible confounders we found significant associations between IL-6 with exposure and outcome among women. As PA decreased IL-6 concentration and the IL-6 was positively associated with the aBMD, the directions of relationships may explain the negative coefficients of the indirect effect. Moreover, mediation analysis suggested that the relationship between PA and aBMD observed among women was not explained by IL-6. It is possible that others inflammatory biomarkers may be implicated as well as other factors not yet identified in the pathways of this association.

Finally, our results raised the hypothesis of a role for adiposity as a mechanism involved in the relationship of the PA, IL-6, and bone density. Adiposity modulates bone remodeling in a complex relationship, through mechanical and biochemical factors, including the secretion of cytokines[27]. The low-grade chronic inflammation described in obesity physiopathology promotes secretion of IL-6 by adipose tissue[28], and it can lead to adverse effects on the bone metabolism[27]. Nevertheless, had been shown that body mass index and fat mass are associated with bone density gain in adulthood[29], it could be explained because obesity increases the mechanical load on the skeleton[30]. We emphasized that there is not a consensus in the findings between obesity and bone density with studies conducted in different population reporting conflicting results, likely because this association is dependent on several factors, such as age, sex, levels of obesity, and regional fat deposit[27,30]. When the fat mass was analyzed as post confounder variable in the mediation analysis in the present study, the indirect effect through IL-6 was insignificant, suggesting that the association between PA and bone density could be explained by adiposity – associated to higher levels of IL-6. Additional mediation analysis showed that fat mass captured most of the association of PA with aBMD, corroborating this hypothesis. Given that PA reduces fat mass, while participants with the highest fat mass had greater BMD, the indirect effect estimate was negative.

The main limitation of this study was the cross-sectional design, hindering firm conclusions regarding causality. However, the relationships between expo-

sure, mediator, and outcome were based on previous longitudinal evidence and biological plausibility, which allowed establishing the directions of the direct acyclic graph to support the mediation analysis in the current study. As we tested the association between two exposure variables and two aBMD sites in both sexes, the observed associations might have occurred by chance and type-1 error cannot be ruled out. On the other hand, strengths of our study include exposure and outcome assessed with objective methods, minimizing the impact of measurement error. Also, the two anatomic sites (lumbar spine and femoral neck) that were investigated comprise the most studied sites in association with PA, since they are more sensitive to the mechanical stress imposed by PA, which is an important factor in osteogenesis[6]. Our findings fill an important gap in the literature on this association with objective measurements of PA in low- and middle-income countries. The DXA is considered a gold standard method to assess aBMD[1], and data from two anatomical sites (femoral neck and lumbar spine) were analyzed. Besides, we used an inflammatory biomarker to investigate possible biologic mechanisms that elucidate the relationship between PA and aBMD. The adjusted analysis considered several known confounders, reducing the probability of adjusted associations being confounded. In addition, we believe that the possibility of residual confounding is small because regression coefficients were similar or increased in the adjusted analyses compared to crude coefficients. Finally, the study includes the population-based data sets in age group with high bone mass.

In conclusion, PA was positively associated with bone density at 30 years of age. This is relevant because the findings reinforce that a low cost and modifiable health behavior can promote bone health in a period of peak bone mass, and it may impact the mineral density in later life phases. Chronic inflammation, as assessed by serum IL-6 did not explain this association; nevertheless, this study indicated other possible pathways, notably body fat, which may be explored in future researches.

Conflict of interest

The authors declare no conflict of interest.

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Author's contributions

Santos FS conceptualization, methodology, formal analysis, and writing - original draft. Bielemann RM conceptualization, methodology, supervised data collection, and writing - review and editing. Oliveira IO conceptualization, methodology, supervised data collection, and writing - review and editing. Horta BL conceptualization, coordinated and supervised data collection, writing - review and editing. Brage S conceptualization, writing - review and editing. Gigante DP supervision, conceptualization, methodology, coordinated and supervised data collection, and writing - review and editing. All authors approved the final manuscript.

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