Full Length Article

Bone mineral density and bone mineral content among female elite athletes

Montse Bellver[a,b,*], Luis Del Rio[c], Esther Jovell[b], Franchek Drobnic[a,d], Antoni Trilla[e]

[a] Centro de Alto Rendimiento (CAR), Av. Alcalde Barnils s/n, 08173 Sant Cugat del Valles, Barcelona, Spain
[b] Consorcio Sanitario de Terrassa, Carretera de Torrebonica s/n, 08227 Terrassa, Spain
[c] CETIR Centre Medic, c/Londres, 6, 08029 Barcelona, Spain
[d] Futbol Club Barcelona (FCB), Av. Onze de Setembre, s/n, 08970 Sant Joan Despí, Barcelona, Spain
[e] Hospital Clínic, University of Barcelona, c/Villarroel 170, 08036 Barcelona, (Spain)

ABSTRACT

Introduction: Exercise is a protective factor for the appearance of osteoporosis, but not all physical activities have the same effect on bones. Low-impact sports, like swimming, may a have a negative or no effect. The aim of the present study is to evaluate bone mineral density (BMD, g/cm^2) and bone mineral content (BMC, kg) among female high performance athletes from aquatic and non-aquatic sports and to assess possible differences with sedentary controls.
Methods: Participants are elite female athletes over 18 years, from the Spanish Olympic teams, with at least 12–15 h of weekly training on aquatic sports: swimming (n = 19), synchronized swimming (n = 24) and water polo (n = 14), and elite female athletes from non-aquatic sports: professional football players of Futbol Club Barcelona team (n = 92), Spanish national volleyball (n = 26) and field hockey teams (n = 29) as well as sedentary controls (n = 126). Bone mineral density scans was performed using dual-energy X-ray absorptiometry for whole body, segmental analysis, proximal femur and lumbar spine.
Results: Non-aquatic female athletes have total and segments BMC and BMD values higher than those of aquatic athletes, that in turn show similar BMD values compared to controls. BMD in lumbar spine and proximal femur was higher in non-aquatic sports compared to aquatic sports athletes and both were higher than controls.
Conclusion: BMD is not negatively compromised by the practice of aquatic sports at a highly competitive level. However, non-aquatic female athletes show a higher BMD. Time training out of the water improves BMD. Our findings suggest that swimming and synchronized swimming should be combined with weight-bearing, impact or strength activities, as they do not seem to be osteogenic sports.

1. Introduction

Osteoporosis affects millions of people around the world, mainly menopausal women [1]. The disease is characterized by a deterioration of bone micro architecture, low bone mass and increased risk of fractures [2]. According to the diagnostic criteria established by the World Health Organization (WHO) (T-score ≤ −2.5 SD), the number of people with osteoporosis will increase from 27.5 million in 2010 to 33.9 million in 2025 (23% increase). The number of new cases of fractures in 2010 in the European Union was 3.5 million and two thirds of these fractures occurred in women [3]. Although osteoporotic fractures occur in older ages, senile osteoporosis is a disease with paediatric origins [4,5]. Prevention of osteoporosis at early ages of life is a key factor for reducing the risk of fracture in the elderly [6–8]. The most important factors related to bone mass acquisition are endocrine and mechanical

factors, such as maintaining an active life [9], a healthy diet (with high calcium intake), good vitamin D levels [10], as well as the genetic condition, ethnic [4] and gender of the individual. Physical exercise is highly beneficial to increase bone mineral density (BMD) and bone mineral content (BMC) as a powerful protective factor to limit the emergence of osteopaenia that ends in osteoporosis development [11]. However, there is not enough evidence regarding the exercise's intensity and length required for this osteogenic stimulation to occur [12–14]. The intensity of the stimulus seems more important than its length [15]. This is the main reason that explains why not all physical activities have the same effect on bone.

Several studies assessed the osteogenic effect of physical exercise. Adolescents engaged in sports for at least 3 days per week and > 60 min per day showed lower incidence of traumatic fractures [16]. The results emphasize that non-weight bearing activities, such as swimming,

cannot provide a positive effect on bones' health. The potential effect this may have on the future development of osteoporosis is not well defined [17–19]. Even adults engaged in non-professional sport in early life have higher BMD than their inactive peers, especially women [20]. Athletes train and exercise since their childhood, with their personal goals aimed at having fun and a good performance in the short term, probably without being aware of the investment that a well-practised sport means for their future health. The stimulus of exercise in the formation of bone and the absence of it in several non-impact sports may not be as positive as accepted. We conduct a cross-sectional study to describe and evaluate the state of bone mineralization of adult high performance female elite athletes from different sports and physical activities that started at an early age. Our main goal is to evaluate the effect of high performance swimming and synchronized swimming on BMD in the whole body, lumbar spine and femoral region among female Olympic athletes over 18 years of age, compared with athletes from non-aquatic sports as well as with sedentary controls.

2. Materials and methods

This is a cross-sectional observational study.

2.1. Subjects

Female high performance ("elite") athletes practicing aquatic sports (A): Spanish Olympic swimming team (n = 19), Spanish Olympic synchronized swimming team (n = 24) and Spanish Olympic water polo team (n = 14); and non-aquatic sports (NA): first and second professional football teams of the Futbol Club Barcelona (FCB) (n = 92), Spanish volleyball team (n = 26) and Spanish field hockey team (n = 29) as well as sedentary controls (C) (n = 126). Aquatic sports are those played or practiced on or in water and non-aquatic sports are those practiced outside water.

National coaches among the best Spanish players, who compete at high-level national and international championships, selected the water polo, volleyball and field hockey players. Details of training frequency, volume, intensity and playing experience were obtained by means of a structured interview with coaches.

High competition sport means competing in World Championships (WC) and Olympic Games (OG). The Olympic swimmers were Olympic medallists (OG London 2012, OG Rio 2016) and world championship medallists (WC Barcelona 2013, WC Budapest 2017). The Spanish synchronized swimming team was silver medallist in OG London 2012 (duet free and team free routine). The Spanish water polo team won the silver medal in OG London 2012, the WC in Barcelona 2013 and grabs the 2nd place again in Budapest 2017. The Spanish volleyball team is ranks 33rd among European women's national teams. The Spanish field hockey team was 8th in the OG London 2012 and OG Rio 2016, and won the bronze medal in the 2018 WC in London. The football (soccer) players belong to the first and second Futbol Club Barcelona teams, play regularly in the Spanish Liga Iberdrola and the UEFA Champions League since 2014–15 and won the Spanish Copa de la Reina 2016 and again in 2017.

Inclusion criteria: Female athletes over 18 years of age, with > 8 years practice in their sport specialty, at least 3 years experience in high-level competition sport and at least 12–15 h of weekly training, competing at national or international championships and games. Written informed consent was obtained from all athletes prior to participation.

The sedentary controls had no formal training in any sport and did not reach the minimum physical activity recommended by WHO, i.e. they practice < 150 min/week of moderate physical activity or < 75 min/week of vigorous physical activity. They were recruited from the CETIR (Centre for Technical Studies with Radioactive Isotopes) normality registers. An epidemiological questionnaire was carried out prior to densitometry for all young women without pathology and with

normal sedentary activity typical of non–energy work or sports activity for 1–2 h week. The questionnaire gathers information about sedentary lifestyle, regular walking, sports practice and intensity degree (0: occasional or 1 h/week, 1: < 5 h/week, 2: > 5 h/week). We add comments on physical activity during life. We select sedentary or activity 0–1 individuals and we exclude those that during their youth practiced competitive sport (holders of a practising license from the Spanish Federation).

Exclusion criteria: for both athletes and non-athletes, the exclusion criteria were: 1) the diagnosis of any medical condition, 2) the use of any medication known to affect bone metabolism (corticosteroids, oral contraceptives, supplementation or other drugs), 3) any contraindication for sports practice, 4) any type of menstrual dysfunction and/or 5) abnormal (non recommended) regular food intake.

All athletes underwent regular medical examinations. To control for possible confounding variables, we administered an ad-hoc questionnaire to collect data on their physical training and sports' history, clinical history, stress fractures history, gynaecological history (menstrual cycle), average calories and calcium intake, including a detailed 5-day nutritional intake records. The athletes are controlled by the nutrition department assuring a balanced diet and the intake of all basic nutrients during the training and competitions season and were evaluated monthly.

The athletes train at the High Performance Centre (CAR), Sant Cugat del Vallés, and at the Futbol Club Barcelona facilities (Sant Joan Despí). Medical check-ups were conducted at the CAR-CST medical department and bone densitometries were performed at the FCB Medical Unit.

The Ethics and Clinical Research Committee of the Consorci Sanitari de Terrassa (CST) approved the study protocol.

2.2. Measurement of Bone Mineral Density (BMD) and body composition

BMD (g/cm^2) and BMC (kg) were assessed using dual X-ray absorptiometry (Lunar iDXATM GE Medical Systems, version 12.30). Whole body, lumbar spine and proximal femur bone mineral density were measured according to standard operating procedures.

Specific age and sex reference tables were used to calculate BMD Z-scores. Assessment by dual X-ray absorptiometry (DXA) is the reference method for measuring total BMD and diagnosing osteopaenia according to the WHO classification, specifically assessing the most susceptible sites of osteoporotic fractures, such as the lumbar spine and the head of the femur.

We assess body composition in all participants using whole body DXA. Body composition variables selected for this study are total mass (TM), fat mass (FM), percentage of fat (% FM), fat-free mass (FFM) and appendicular mass (AM). The appendicular mass corresponds to the sum of the fat-free mass of the lower and upper extremities.

Participants were measured in light clothing, barefoot and without jewellery or metal buttons. All subjects went to the toilet before test. The same technician performed all measurements. Athletes were evaluated in supine position, with their feet in slight internal rotation to have good visibility of the femoral neck.

2.3. Statistical analysis

The descriptive factors are presented as absolute and relative frequencies for the qualitative and average variables and with their mean and standard deviation for the quantitative variables. Student t has been used to compare sports in two categories of the variable (aquatic and non-aquatic). In the comparison with all sports (swimming, synchronized, water polo, soccer, field hockey, volleyball) and sedentary controls the analysis of variance has been used. In both cases the homogeneity of variances has been proven with the Levene test. In the case of non-compliance with homogeneity, the Mann-Whitney U test (for aquatic and non-aquatic) and the Kruskall-Wallis test were carried

Table 1
Elite female athletes main descriptive data (mean ± SD) grouped by aquatic, non-aquatic sports as well as for the sedentary (control) group.

	Aquatic sports (n = 57)	Non-aquatic sports (n = 147)	Sedentary controls (n = 126)
Age (years)	20.7 ± 4.3	22.4 ± 4.8[a]	21.5 ± 4.6
Height (cm)	171.6 ± 6.9[b,c]	167.7 ± 7.9[c]	163.1 ± 7.9
Weight (DEXA, kg)	61.3 ± 10.2	62.2 ± 8.5[c]	58.5 ± 11.4
BMC (kg)	2.5 ± 0.4[c]	2.7 ± 0.4[a,c]	2.3 ± 0.4
Total BMD (g/cm^2)	1.109 ± 0.1[c]	1.257 ± 0.10[a,c]	0.943 ± 0.2
FM (kg)	15.0 ± 5.0	15.5 ± 5.1	19.5 ± 8.7[a,b]
FFM (kg)	44.1 ± 5.9[c]	44.0 ± 5.5[c]	37.4 ± 4.2
AM (kg)	19.7 ± 3.3[c]	21.2 ± 3.3[a,c]	16.4 ± 1.5
% FM	24.0 ± 4.1	24.6 ± 5.6	32.3 ± 8.1[a,b]

BMC: bone mineral content, BMD: bone mineral density, FM: fat mass, FFM: fat free mass, AM: appendicular mass.

[a] Significantly greater than aquatic sports, p < 0.05.
[b] Significantly greater than non aquatic sports, p < 0.05.
[c] Significantly greater than control group, p < 0.05.

out to compare all sports. In the case of statistical significance in the ANOVA, Tukey test was performed to localize the difference for multiple comparisons. Bone density variables were further analysed using ANCOVA, with adjustments for FFM (lean mass), age and height and Bonferroni's test for multiple comparisons.

The level of statistical significance was set at 5%. The software program SPSS, version 24, was used for the statistical analysis.

3. Results

Group means for anthropometric and training data are summarised in Tables 1 and 2. Non-aquatic athletes (NA) are older than aquatic ones (A). Water polo athletes are the oldest and swimmers the youngest, without statistically significant differences with respect to the control group (C). Height and weight are higher in the water polo and volleyball groups compared to other sports. Swimmers, and especially synchronized swimmers, show the lowest BMD values.

NA athletes have greater BMC and BMD than A athletes and both groups show higher values than C. FM, is lower in athletes (A and NA) with respect to controls. FFM and AM are higher in athletes than in controls. Synchronized swimmers underwent a greater amount of water training per week than did players in other aquatic sports.

BMC is higher in all high performance athletes compared to controls, except for swimmers and synchronized swimmers who show similar values to the control group. The same happens with BMD, but in this case swimmers and synchronized swimmers have higher BMD than controls.

FM percentage is higher in the control group than in all the athletes. FFM and AM are higher in the volleyball and water polo groups, and all of them are higher than those of the C group.

Bone mineral density results are show in Fig. 1. Whole BMD values follow a decreasing order for volleyball, football, field hockey, water polo, swimming, synchronized swimming and controls.

BMD results by segments for A and NA sports as well as for C group are shown in Tables 3 and 4.

Legs, trunk, ribs, pelvis, total and Z score BMD is higher in NA than in A sports. Both groups show higher values than C group, except for legs, where A athletes and controls show similar values, mainly for swimmers and synchronized swimmers.

Water polo players show higher values than the rest of the athletes in the arms, trunk and ribs, followed by volleyball players, and have similar BMD values in legs to football players and volleyball players, while football players have higher pelvic BMD values when compared to other sports.

BMD results of the lumbar spine and proximal femur region are presented in Tables 5 and 6. BMD in the lumbar spine is higher in NA sports with respect to A and both groups, as well as all types of sports, show higher values than those of C group.

Volleyball players show superior values to the rest of the athletes in the lumbar spine. Synchronized swimmers are the athletes with the lowest BMD values. Water polo players have the highest BMD in lumbar spine among A athletes and field hockey players have the lowest BMD among NA athletes.

NA sports have a higher BMD in femur in all segments compared to A sports, and both groups show higher values in all segments compared to C group for all types of sports.

Swimmers show a lower BMD value than the rest of the athletes. NA sports have higher BMD values in the proximal area of the femur, mainly in the trochanter and diaphysis; and among them, volleyball and football have higher BMD values in the femur than field hockey. Football players had a higher BMD in the diaphysis and volleyball players in the femoral neck and trochanter. There are no statistically significant differences among A sports.

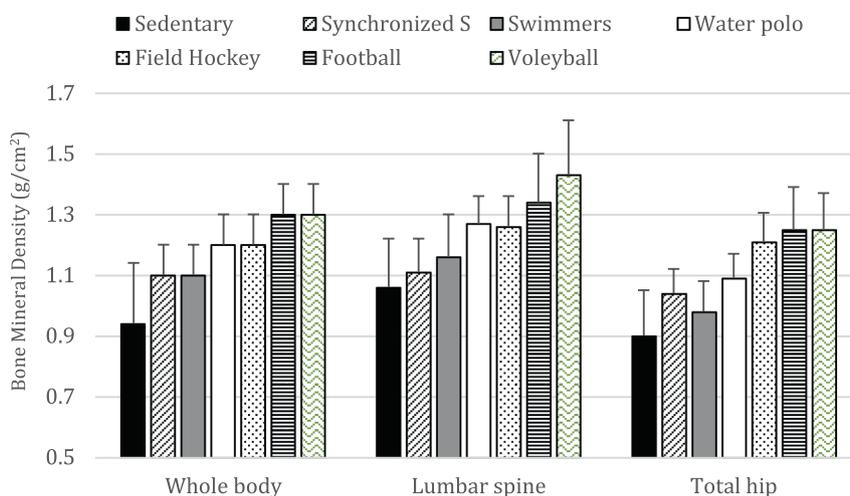After adjusting by FFM, age and height, the statistically significant

Table 2
Elite female athletes main descriptive data (mean ± SD) grouped by sport and sedentary (control) group.

	Swimmers (n = 19)	Synchronized (n = 24)	Water polo (n = 14)	Football (n = 92)	Field Hockey (n = 29)	Volleyball (n = 26)	Controls (n = 126)
Age (years)	18.4 ± 3.6	20.5 ± 3.9	24.0 ± 3.7[a,e]	22.0 ± 5.2[a]	23.8 ± 3.7[a,e]	22.5 ± 4.5[a]	21.5 ± 4.6
Height (cm)	169.4 ± 6.7[e]	170.4 ± 6.4[c,d,e]	176.5 ± 6.2[a,c,d,e]	165.9 ± 6.3[e]	164.1 ± 5.4	178.5 ± 7.2[a,b,c,d,e]	163.1 ± 7.9
Weight (Kg)	59.1 ± 6.6	57.3 ± 6.0	71.2 ± 1.4[a,b,c,d,e]	59.9 ± 6.4	61.1 ± 9.0	71.9 ± 7.8[a,b,c,d,e]	58.5 ± 11.4
BMC (Kg)	2.4 ± 0.3	2.3 ± 0.3	2.9 ± 0.4[a,b,c,d,e]	2.7 ± 0.3[a,b,e]	2.6 ± 0.3[e]	3.1 ± 0.5 [a,b,c,e]	2.3 ± 0.4
BMD (g/cm^2)	1.090 ± 0.1[e]	1.068 ± 0.1[e]	1.206 ± 0.1[a,b,e]	1.262 ± 0.1[a,b,e]	1.222 ± 0.1[a,b,e]	1.279 ± 0.1[a,b,e]	0.943 ± 0.2
FM (kg)	13.5 ± 2.9	13.8 ± 2.6	19.0 ± 7.9[a,b,c,d]	14.6 ± 3.9	15.8 ± 7.5	18.2 ± 4.7[a,b,c]	19.5 ± 8.7[a,b,c]
FFM (kg)	44.3 ± 5.1[e]	41.1 ± 4.3[e]	49.1 ± 6.2[a,b,c,d,e]	42.5 ± 4.5[e]	42.7 ± 4.3[e]	50.6 ± 5.2[a,b,c,d,e]	37.4 ± 4.2
AM (kg)	19.8 ± 2.7[e]	18.0 ± 2.1[e]	22.5 ± 3.8[a,b,c,d,e]	19.6 ± 2.1[b,e]	19.8 ± 2.4[e]	24.1 ± 3.1[a,b,c,d,e]	16.4 ± 1.5
FFM arms (kg)	4.9 ± 0.7[c,d]	4.5 ± 0.5	5.6 ± 1.1[a,b,c,d]	4.2 ± 0.6	4.1 ± 0.5	5.1 ± 0.8[b,c,d]	-
FFM legs (kg)	14.8 ± 2.1	13.5 ± 1.7	16.8 ± 2.7[a,b]	15.5 ± 1.6[b]	15.7 ± 1.9 [b]	19.0 ± 2.4[a,b,c,d]	-
Water training (hours/week)	10	36	12-15	0	0	0	-
Non water training (hours/week)	8	3	10	10-12	8-10	25	-

BMC: Bone Mineral Content, BMD: Bone Mineral Density, FM: Fat Mass, FFM: Fat Free Mass, AM: Appendicular Mass.

[a] Significantly greater than swimming, p < 0.05
[b] Significantly greater than synchronized swimming, p < 0.05
[c] Significantly greater than football, p < 0.05
[d] Significantly greater than field hockey, p < 0.05
[e] Significantly greater than control group, p < 0.05

Fig. 1. Total body, spine and total-hip bone mineral density for elite female sports groups as well as for sedentary controls.

Table 3
Mean and SD BMD (g/cm^2) values grouped by elite female athletes' aquatic and non-aquatic sports as well as for the sedentary controls.

g/cm^2	Aquatic sports (n = 57)	Non-aquatic sports (n = 147)	Sedentary controls (n = 38)
Arms	0.714 ± 0.07[b]	0.705 ± 0.07[b]	0.659 ± 0.07
Legs	1.113 ± 0.12	1.337 ± 0.11[a,b]	1.108 ± 0.12
Trunk	0.957 ± 0.11[b]	1.091 ± 0.10[a,b]	0.891 ± 0.10
Ribs	0.867 ± 0.11[b]	0.921 ± 0.09[a,b]	0.784 ± 0.09
Pelvis	1.015 ± 0.11[b]	1.219 ± 0.12[a,b]	0.924 ± 0.10
Total	1.109 ± 0.10[b]	1.257 ± 0.10[a,b]	0.943 ± 0.16
Z-score	0.586 ± 0.63	2.120 ± 0.85[a,b]	0.571 ± 0.2

[a] Significantly greater than aquatic sports, $p < 0.05$
[b] Significantly greater than control group, $p < 0.05$

differences remain for FFM and sport type. FFM explains 18.2% of the total BMD, and the type of sport explains 46.0% of the BMD (R = 0,589). FFM explains 24.5% of the total BMC and type of sport explains 33.8% (R = 0,773).

When the model is adjusted by FFM, age and height, football players show higher total BMD values than the rest of sports and sedentary controls. Statistically significant differences between swimmers, synchronized swimmers, water polo athletes and sedentary controls disappeared at total and segments BMD (Tables 7 and 8).

BMD values were still greater in the water polo group than in the rest of sports and C group at arms and ribs. However, significant differences disappeared. Football players BMD were significantly greater (p < 0.05) than BMD of swimmers, synchronized swimmers and

Table 5
Mean ± SD BMD (g/cm^2) lumbar spine and proximal femur values for elite female aquatic and non-aquatic sports athletes as well as for sedentary controls. (*) p < 0.05.

g/cm^2	Aquatic sports (n = 54)	Non-aquatic sports (n = 79)	Sedentary controls (n = 126)
"L1-L4"	1.166 ± 0.13[b]	1.364 ± 0.17[a,b]	1.057 ± 0.16
Femoral neck	1.085 ± 0.12[b]	1.243 ± 0.14[a,b]	0.903 ± 0.14
Trochanter	0.854 ± 0.99[b]	1.041 ± 0.12[a,b]	0.677 ± 0.13
Diaphysis	1.178 ± 0.12[b]	1.439 ± 0.16[a,b]	1.082 ± 0.19
Total-hip	1.034 ± 0.97[b]	1.246 ± 0.13[a,b]	0.901 ± 0.15

[a] Significantly greater than aquatic sports, $p < 0.05$
[b] Significantly greater than control group, $p < 0.05$

controls at legs and pelvis. Legs' BMD were still higher in the volleyball group than in swimmers, synchronized swimmers and controls. However, significant differences disappeared but for synchronized swimmers (Table 7).

Lumbar spine and femur in all regions BMD did not differ according to adjusted means by age, height and FFM, however significant differences disappeared between swimmers and sedentary controls.

In the comparison between A and NA and once adjusted for the covariates training time in water and training time out of water, statistically significant differences were observed in relation to BMD by training time out of water (p < 0.046). If we separate A sports in two groups: 1) swimmers plus synchronized swimmers and 2) water polo players, and compare them with NA sports adjusting for the variables training time in water and training time out of water, no statistically

Table 4
Mean and SD BMD (g/cm^2) values grouped by elite female athletes sport and sedentary (control) group.

g/cm^2	Swimmers (n = 19)	Synchronized Swimmers (n = 24)	Water polo (n = 14)	Football (n = 92)	Field Hockey (n = 29)	Volleyball (n = 26)	Controls (n = 38)
Arms	0.704 ± 0.05[c]	0.697 ± 0.05[c]	0.894 ± 0.13[c]	0.697 ± 0.05[c]	0.701 ± 0.04[c]	0.743 ± 0.14[c]	0.659 ± 0.07
Legs	1.109 ± 0.08	1.081 ± 0.10	1.346 ± 0.25[b,c]	1.346 ± 0.10[a,b,c]	1.230 ± 0.09[c]	1.345 ± 0,14[a,b,c]	1.108 ± 0.12
Trunk	0.961 ± 0.10[c]	0.926 ± 0.09	1.132 ± 0.16[c]	1.094 ± 0.10[a,b,c]	1.067 ± 0.08[c]	1.095 ± 0.12[a,b,c]	0.891 ± 0.10
Ribs	0.875 ± 0.09[c]	0.830 ± 0.08[c]	1.052 ± 0.21[b,c]	0.921 ± 0.09[b,c]	0.893 ± 0.04[c]	0.934 ± 0.12[c]	0.784 ± 0.09
Pelvis	1.019 ± 0.11[c]	0.991 ± 0.10[c]	1.149 ± 0.06[c]	1.231 ± 0.13[a,b,c]	1.185 ± 0.16[c]	1.184 ± 0.12[a,b,c]	0.924 ± 0.10
Total	1.090 ± 0.09[c]	1.068 ± 0.07[c]	1.206 ± 0.08[a,b,c]	1.262 ± 0.1[a,b,c]	1.222 ± 0.08[a,b,c]	1.279 ± 0.13[a,b,c]	0.943 ± 0.16
Z-score	0.333 ± 0.52	0.455 ± 0.52	0.833 ± 0.72	2.408 ± 0.8[a,b,d,e]	1.640 ± 0.70[a,b,d]	2.000 ± 0.94[a,b,d]	0.571 ± 0.2

[a] Significantly greater than swimming, $p < 0.05$
[b] Significantly greater than synchronized swimming, $p < 0.05$
[c] Significantly greater than the control group, $p < 0.05$
[d] Significantly greater than water polo, $p < 0.05$
[e] Significantly greater than field hockey, $p < 0.05$

Table 6
Mean ± SD BMD (g/cm²) lumbar spine and proximal femur values for elite female athletes grouped by sport.

	Swimmers (n = 18)	Synchronized swimmers (n = 22)	Water polo (n = 14)	Football (n = 46)	Field Hockey (n = 7)	Volleyball (n = 26)	Controls (n = 126)
"L1-L4"	1.161 ± 0.14[c]	1.107 ± 0.11	1.265 ± 0.09[b,c]	1.341 ± 0.16[a,b,c]	1.258 ± 0.10[c]	1.431 ± 0.18[a,b,c,d]	1.057 ± 0.16
Femoral neck	0.994 ± 0.10[c]	1.103 ± 0.09[c]	1.172 ± 0.12[a,c]	1.240 ± 0.14[a,b,c]	1.155 ± 0.11[a,c]	1.272 ± 0.14[a,b,c]	0.903 ± 0.14
Trochanter	0.811 ± 0.08[c]	0.865 ± 0.11[c]	0.889 ± 0.08[c]	1.039 ± 0.14[a,b,c,d]	1.030 ± 0.09[a,b,c]	1.048 ± 0.11[a,b,c,d]	0.677 ± 0.13
Diaphysis	1.125 ± 0.15[c]	1.164 ± 0.10[c]	1.269 ± 0.08[c]	1.453 ± 0.18[a,b,c,d]	1.397 ± 0.10[a,b,c]	1.425 ± 0.15[a,b,c,d]	1.082 ± 0.19
Total-hip	0.983 ± 0.10[c]	1.036 ± 0.08[c]	1.095 ± 0.08[c]	1.251 ± 0.14[a,b,c,d]	1.215 ± 0.095[a,b,c]	1.247 ± 0.12[a,b,c,d]	0.901 ± 0.15

[a] Significantly greater than swimming. p < 0.05
[b] Significantly greater than synchronized swimming. p < 0.05
[c] Significantly greater than the control group. p < 0.05
[d] Significantly greater than water polo. p < 0.05

significant differences were recorded. In the multiple comparison analysis, statistically significant differences were found between NA sports with respect to swimmers plus synchronized swimmers (p < 0.01) and swimmers plus synchronized swimmers with respect to water polo players (p < 0.005). No statistically significant differences were found between NA and water polo players.

4. Discussion

Physical exercise improves BMD [6,7,9,12,15]. The present study indicates that participation in NA high-level sports (football, field hockey and volleyball) is associated with higher BMD measurements than in A sports and in sedentary controls. All aquatic Spanish Olympic teams (swimmers, synchronized swimmers, water polo players) presented similar values than those of sedentary controls at total BMD and segments measurements. All athletes had higher values than sedentary controls in lumbar spine and femur measurements, which are the two assessment measures according to the WHO classification for diagnosing osteopaenia and, specifically, the most susceptible sites for osteoporotic fracture. Water polo players had the highest BMD in lumbar spine and femur in all segments among A athletes. Field hockey has the lowest BMD in lumbar spine and femur in all segments among NA athletes. In the present study, high-level athletes practice sports from childhood.

The present study shows higher BMD values among high-level female athletes who practice NA and A sports, being these values higher than those of sedentary controls. However, when we adjust by FFM, age and height, swimmers and synchronized swimmers have lower BMD than other groups of impact sports, and also analogous than those of sedentary controls, in agreement with the literature [22]. Swimmers and synchronized swimmers present higher FFM values than sedentary controls. Several authors [18,21] and a systematic review [17] show a neutral or non-effect of swimming in BMD and BMC, similar to sedentary controls and lower than impact or antigravitational sports. Thus, swimming do not affect negatively bone mass, although it may not be one of the sports to practise in order to improve this parameter, due to the hydrostatic forces against gravity (false hypogravity) and lack of

impact characteristics of this sport.

The osteogenic effect of exercise is mainly due to the impact and the mechanical load applied to the bone: the so-called mechanostat effect. The process of bone formation and remodelling is adapted to the new demand and, consequently, BMC, BMD and the architectural framework are modified [23]. According to the mechanostat theory proposed by Frost [24], the skeleton adapts to these loads, increasing its quality and strength. Several studies report an association between BMC and FFM [25–27]. It is stated that FFM and AM greatly influence BMD and BMC in adolescent women. Muscle strength applied to bone could be an important predictor of BMD [21]. In the present study, FFM and type of sport explains BMD and BMC, in both A and NA sports athletes, in agreement with the literature [21,28].

Some studies show higher arm BMD values in swimmers assumed to the combination of propulsive strokes and resistance against water [17]. It seems that muscular activity in upper extremities when swimming helps to protect the bone structure in this segment [29] with a greater FFM in the arms due to the long training swimming routines. However, this higher FFM do not correspond to a higher BMD, as it does in the present study. Elite swimmers perform a total of 16,000 to 25,000 shoulder rotations in a 1-week routine, 80% in crawl style. Other authors [29] argue that training load affects BMD negatively mainly in lower limbs among swimmers, independently of the positive effects of muscle mass on it, in agreement with our study.

In the present study, the water polo and volleyball players present the highest FFM values in arms and legs compared to the rest of the athletes and controls and have the highest BMD in arms and legs. Total FFM and AM is superior in the volleyball and water polo group with respect to the rest of the sports and controls. The volleyball and water polo groups show the greater body size with genetically determined physical characteristics that contribute to an individual's predisposition to play a particular sport (height, body shape, body composition). When the BMD is adjusted by other variables (age, height and FFM), statistical differences between volleyball and water polo players in arms and legs compared to the rest of sports and sedentary controls disappeared. Water polo players had greater BMD values that benefits overall BMD in the aquatic group than would be explained by their FFM and time

Table 7
Age, height, FFM adjusted mean ± SEM of total and segments BMD (g/cm²) for elite female sports groups as well as for sedentary controls.

g/cm²	Swimmers (n = 19)	Synchronized (n = 24)	Water polo (n = 14)	Football (n = 92)	Field Hockey (n = 29)	Volleyball (n = 26)	Controls (n = 38)
Total BMD	0.942 ± 0.04	1.089 ± 0.02	1.131 ± 0.03	1.274 ± 0.01[a,b,c,d,e]	1.232 ± 0.02[a,b,c,d]	1.216 ± 0.03[a,b,c]	1.090 ± 0.03
Arms	0.691 ± 0.03	0.677 ± 0.03	0.872 ± 0.08	0.702 ± 0.02	0.747 ± 0.05	0.689 ± 0.04	0.760 ± 0.03
Legs	1.095 ± 0.03	1.064 ± 0.03	1.257 ± 0.08	1.359 ± 0.02[a,b,c]	1.280 ± 0.05[b]	1.236 ± 0.04[b]	1.121 ± 0.03
Trunk	0.944 ± 0.03	0.909 ± 0.03	1.083 ± 0.08	1.098 ± 0.02[a,b]	1.117 ± 0.05[b]	1.017 ± 0.04	0.971 ± 0.03
Ribs	0.865 ± 0.03	0.826 ± 0.03	0.995 ± 0.07	0.921 ± 0.02[b]	0.920 ± 0.05	0.869 ± 0.03	0.829 ± 0.03
Pelvis	1.000 ± 0.03	0.982 ± 0.03	1.065 ± 0.08	1.238 ± 0.02 [a,b,c,e]	1.235 ± 0.06[a,b,c]	1.081 ± 0.04	0.983 ± 0.04

[a] Significantly greater than swimming, p < 0.05
[b] Significantly greater than synchronized swimming, p < 0.05
[c] Significantly greater than control group, p < 0.05
[d] Significantly greater than water polo, p < 0.05
[e] Significantly greater than volleyball, p < 0.05

Table 8
Age, height, FFM adjusted mean $\pm$ SEM of lumbar spine and femur BMD (g/cm^2) for elite female sports groups as well as for sedentary controls.

g/cm^2	Swimmers (n = 18)	Synchronized (n = 22)	Water polo (n = 14)	Football (n = 46)	Field Hockey (n = 7)	Volleyball (n = 26)	Controls (n = 126)
"L1-L4"	1.154 ± 0.04	1.083 ± 0.03	1.254 ± 0.4[c]	1.365 ± 0.02[a,b,c]	1.287 ± 0.06[c]	1.412 ± 0.04[a,b,c,d]	1.068 ± 0.03
Femoral neck	0.997 ± 0.03	1.105 ± 0.03[c]	1.161 ± 0.04[c]	1.245 ± 0.02[a,b,c]	1.158 ± 0.05[c]	1.260 ± 0.03 [a,b,c]	0.854 ± 0.04
Trochanter	0.808 ± 0.03[c]	0.868 ± 0.03[c]	0.873 ± 0.03[c]	1.046 ± 0.02[a,b,c,d]	1.035 ± 0.05[a,b,c]	1.026 ± 0.03 [a,b,c,d]	0.664 ± 0.03
Diaphysis	1.135 ± 0.04	1.160 ± 0.04	1.262 ± 0.05[c]	1.458 ± 0.03[a,b,c,d]	1.402 ± 0.06[a,b,c]	1.420 ± 0.04[a,b,c]	1.028 ± 0.05
Total-hip	0.985 ± 0.03	1.035 ± 0.03[c]	1.086 ± 0.04[c]	1.256 ± 0.02[a,b,c,d]	1.219 ± 0.05[a,b,c]	1.236 ± 0.03[a,b,c]	0.867 ± 0.04

[a] Significantly greater than swimming, p < 0.05
[b] Significantly greater than synchronized swimming, p < 0.05
[c] Significantly greater than control group, p < 0.05
[d] Significantly greater than water polo, p < 0.05

training out of water. FFM contains its skeletal muscle that generates force for the situations in the game where high impacts are transmitted and received. When we compare A and NA athletes and adjust for the covariates training time in water and training time out of water statistically significant differences were found between NA sports with respect to swimmers plus synchronized swimmers and those with respect to water polo players. No statistically significant differences were found between NA and water polo players. Thus, water polo players behave more like NA athletes with regard to her training and their BMD is higher than those of swimmers and synchronized swimmers.

BMD values in femur and lumbar spine are higher in NA sports. The intensity of impact (high impact loading) in sports such as volleyball and the distribution of the impact load in various directions in sports such as football (odd impact loading) increase BMD [15]. Volleyball and football players show the highest values in femur and volleyball has the highest values in lumbar spine. Football players show the highest values in legs, femur (diaphysis) and pelvis, as previously shown [30]. Football is a sport with special biomechanical characteristics: changes of direction, speed, jumps and kicks that offers additional mechanical stress in lower extremities due to the force of reaction against the ground during these actions [31] that facilitates mineral accretion, from the cortical to the trabeculae in its entire functional dimension, depending on length and intensity. Volleyball is a sport with long, fast short movements, both in defensive and offensive actions to adjust the position of the body to the position of the ball in a short period of time since the ball takes 1.2–1.4 s to go from one side of the court to the other [32], jumping, change of direction, speed and power work seems beneficial for the formation of an adequate bone from childhood and in adulthood, as happens with artistic gymnastics, volleyball or handball [33–35]. Some authors conclude that variable sports loads, like in basketball, are more effective to increasing BMD and BMC than other activities more stressful as parachuting with constant loads at specific sites [36]. Other authors [37] show that brief jumping and daily exercise increase BMD in the femoral neck, as occurs in the volleyball players included this study.

In a recent systematic review [17], children and adolescents swimmers (males and females) presented similar values of total BMD in the femur and lumbar spine than controls and lower values than other impact sports. In our study, lumbar spine and femur in all regions BDM between swimmers and controls did not differ according to adjusted means by age, height and FFM, however statistically significant differences disappeared respect to sedentary group. The authors [17] conclude that swimming practiced on a regular basis has a neutral effect on BMD in children and adolescents, and that these differences increase with age since impact sports athletes continue their practice, a situation which favours improvement in BMD. By contrast, water sports athletes continue training in a medium that does not have a positive mechanical effect on bone, and this difference is more apparent at the beginning of adulthood. The athletes included in the present study are adults training an average of 12–15 h/week at a high level of intensity and their biological age is not affected by hormonal or maturational factors, since they are adults without hormonal or menstrual disorders

(inclusion criteria).

A further study [38] carried out with university level athletes, an intermediate population between the present study and that conducted by Gomez Bruton [17], concludes that impact sports have higher BMD and BMC values than non-impact sports. Likewise, the authors observe that the only difference between controls and swimmers is that the latter present higher BMD in the arms but lower BMD values in the lumbar spine and total body than sedentary controls, unlike the findings of the present study.

Synchronized swimmers are the group that present the lowest total BMD by segments and lumbar spine, except in the femur at all sites that is slightly superior to swimmers. This discrepancy between speed swimmers and synchronized swimmers could be attributed to training differences related to the greater mechanical loading from repetitive pushing against the pool wall in speed swimming [22] and may be also the related to training work out of the water. Synchronized swimming training, which involves the execution of repeated exercises with a variety of movements of arms and legs to accelerate, decelerate, rotate, turn to pull the body out of the water and even jump, may have a positive bone effect on spine. Thus, synchronized swimmers should develop a large muscle mass in extremities that could increase bone mass, a situation that does not occur in our synchronized swimmers. Three hours per week of training out of the water seems enough for synchronized swimmers to maintain their BMD. WHO recommends at least 150 min of moderate to vigorous intensity physical activity per week to improve the health of the population. Like other studies [39], physical activity duration, frequency and load are associated with bone parameters, and length is the strongest predictor. Nevertheless, the total physical past year activity score which accounts for length, frequency and load appears to be a stronger predictor of bone parameters than any individual component of physical activity [39]. The WHO recommendations don't apply in those groups when bone health is the purpose.

The present study has several limitations. The first limitation is the relatively small sample size, determined by the small number of elite and Olympic athletes.

Secondly, the cross-sectional design of the study is a limitation because it only speculates and limits the possibility to prove causality, generating the need for follow-up measurements in the future. The design used is the main reason regarding the absence of information about time of the season training: training loads change according to the season timing and progress, and this might affect bone that is constantly adapting.

Finally, the athletes are controlled by the nutrition department in terms of their balanced diet and intake of all nutrients during the competitions and training season and monthly re-evaluated to ensure that the nutritional intake was correct. However, we do not perform statistical studies with nutritional and laboratory data variables.

The present study has also several strengths. The participants included are all high performance female athletes of different Olympic, national and international teams who train regularly at a high intensity, volume and frequency. Our participants include Olympic female

athletes from a broad sample of different sports disciplines not studied before in the literature. FFM is included in this study as it has a positive effect on bone, but few studies had presented FFM values before. The study subjects had years of practice and long weekly hours of training, emphasizing complementary training out of the water. Many swimmers, synchronized swimmers and water polo players perform complementary training out of the water in order to improve their performance, not reported in most of the studies.

5. Conclusion

In summary, mean BMD values were highest in NA athletes at all measured sites, regions and segments compared to A athletes. All sports participants, A and NA, had higher values than sedentary controls in lumbar spine and femur measures. Swimmers and synchronized swimmers had similar values to sedentary control in whole body BMD and segments. FFM and sport's type explains BMD and BMC values, in both A and NA athletes.

Time training out of the water improves BMD. Our findings suggest that swimming and synchronized swimming in female athletes should be combined with weight-bearing, impact or strength activities, as it do not seem to be osteogenic sports enough.

Acknowledgements

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