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Original Article

Organochlorines and Bone Mineral Density in Swedish Men from the General Population

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Abstract. Persistent organochlorines (POCs), such as polychlorinated biphenyls (PCBs) and DDT, are present at relatively high concentrations in food and show estrogenic, anti-estrogenic or anti-androgenic activity in biological test systems. Because bone mineral density (BMD) in men is influenced by sex hormones, we looked for associations between BMD and serum concentrations of POCs in 115 men (mean age 63 years, range 40–75 years) from the general Swedish population. Ten PCB congeners, five DDT isomers, hexachlorobenzene, three hexachlorocyclohexane isomers, *trans*-nonachlor and oxychlordane were analyzed by gas chromatography. Quantitative bone measurements were performed by dual-energy X-ray absorptiometry at three sites: whole body, the L2–L4 region of the lumbar spine, and the neck region of the proximal femur, as well as by quantitative ultrasound on the left os calcis (broadband ultrasound attenuation (BUA) and speed of sound (SOS)). After adjustment for confounding factors in linear regression analyses we found no strong association between serum concentrations of single POCs and the five BMD and ultrasound variables. When POCs were grouped according to hormonal activity (estrogenic, anti-estrogenic, anti-androgenic) and the study subjects were divided into organochlorine concentration quartiles, a weak association was indicated between increased serum concentrations of *p,p′*-DDE (anti-androgenic) and decreased BMD, BUA and SOS. This may suggest that *p,p′*-DDE could cause negative effects on bone density, but the findings might also be due to chance since multiple comparisons were made in the statistical analysis. Overall our results do not suggest that the studied POCs caused major effects on bone density in our study group.

Keywords: Bone mineral density; DDE; DDT; Endocrine disrupters; PCB

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Introduction

Persistent organochlorines (POCs), such as the industrial chemical polychlorinated biphenyl (PCB) and the chlorinated pesticide DDT, are ubiquitous in the environment even though the production and use of these chemicals have been banned for decades in many countries. Although the concentrations of these compounds in food have slowly decreased in some areas of the world, concentrations are still high enough to be of concern for human health [1].

PCB and chlorinated pesticides cause a variety of endocrine effects in biological test systems, including estrogenic, anti-estrogenic and anti-androgenic effects [2,3]. Experimental studies show that these effects may be caused by several mechanisms, including direct binding of the compounds to estrogen and androgen receptors and effects on activities of sex-hormone-metabolizing enzymes such as aromatase [1]. On the basis of these findings it has been hypothesized that background exposure to POCs in food may induce reproductive effects in humans [4].

Non-reproductive processes in the human body are also dependent on sex hormones, and are consequently potential targets for organochlorine toxicity. Recent studies suggest that both androgens and estrogens are involved in the regulation of bone growth and development in men, and that positive effects of androgens on bone density are at least partially dependent on aromatization of testosterone to estrogen [5–7].

Some sex-hormone-mimicking POCs might therefore affect bone mineral density (BMD) and bone quality. This hypothesis is supported by a few experimental animal studies on PCB, DDT, hexachlorobenzene (HCB) and α-hexachlorocyclohexane (α-HCH) [8–12]. The effects of POCs on bone density in humans have attracted little attention. In a mass-poisoning incident in Turkey, patients exposed to high concentrations of HCB through ingestion of HCB-contaminated bread, showed signs of low bone mass in metacarpal and phalangeal bones [13]. In Japan, indications of irregular calcification in skull bones were found in children exposed to high concentrations of PCB in utero [14]. In a similar incident in Taiwan, on the other hand, effects on skull bones were not found in exposed children and BMD measured by dual-photon absorptiometry was not affected [14,15].

Fractures are a severe and common result of osteoporosis not only in elderly women, but also in men [16]. It is important, therefore, to increase our knowledge about factors that may decrease bone density and increase the risk of development of osteoporosis in both men and women. To our knowledge no study dealing with the effects of POCs on bone density in men with background body burdens of POCs has been published. In the current study, we tested the hypothesis that serum concentrations of endocrine-disrupting PCBs and chlorinated pesticides are associated with BMD in a group of 115 men from the general Swedish population.

Subjects and Methods

Subjects

Originally, 790 men (aged 40–74 years) were randomly sampled from the population register. They lived in the county of Uppsala, in central Sweden just north of the capital Stockholm. Among these men, 286 agreed to participate in an extensive survey of food habits, to donate blood and to have their BMD measured. Serum for organochlorine analysis was sampled from the first 115 of the participating men (mean age 63 years, range 41–75 years) when they visited the hospital for blood sampling. The study was approved by the local ethics committee.

Analysis of POCs in Serum

We analyzed the following POCs in serum: 10 PCB congeners (IUPAC nos. 28, 52, 101, 105, 118, 138, 153, 156, 167 and 180), 5 DDT isomers (*p*,*p*′-DDT, *p*,*p*′-DDD, *p*,*p*′-DDE, *o*,*p*′-DDT and *o*,*p*′-DDE), hexachlorobenzene (HCB), 3 hexachlorocyclohexane isomers (α-HCH, *β*-HCH and *γ*-HCH), *trans*-nonachlor and oxychlordane. The POCs analyzed covered those present at both relatively high concentrations in food (some PCB and DDT compounds) and those present at lower concentrations (some PCB and DDT compounds, HCB, HCH, *trans*-nonachlor and oxychlordane).

Serum samples were analyzed using a method described by Atuma and Aune [17]. In short, the samples were analyzed in a gas chromatograph equipped with dual capillary columns and dual electron capture detectors (ECD, ^{63}Ni), after appropriate extraction and clean-up steps. The quality of the analysis was assured by the use of internal standards and by inclusion of quality control samples in the analytic work. The lipid content of the samples was determined gravimetrically after the extraction steps. The limit of quantification (LOQ) for the organochlorine analysis was 10 pg/g serum for the PCB congeners, HCH isomers and chlordanes, 20 pg/g for *p*,*p*′-DDD, *p*,*p*′-DDT and *o*,*p*′-DDT, 50 pg/g for HCB and 200 pg/g for *p*,*p*′-DDE. The results were expressed as nanograms per gram serum lipid, without further lipid adjustment in the statistical analysis. When concentrations were below the LOQ they were set to 50% of that limit in the statistical analysis.

The total PCB concentration in the serum (ΣPCB) was calculated as the sum of the molar concentrations of the 10 individual PCB congeners. The compounds with concentrations above the quantification limit, in at least a few individuals, were also grouped according to their possible hormonal activity. The classification was based on the hormonal activity reported most frequently in the literature [18]. We classified HCB as anti-estrogenic rather than the original classification as a substance with no known estrogenic activity [18]. Recent studies indicate that HCB is a dioxin-like substance, which binds to the dioxin receptor (Ah receptor) [19]. Substances with dioxin-like toxic actions show anti-estrogenic activity in biological test systems [2]. Molar concentrations were used when the concentrations of the substances in a group were added to each other.

We considered the following compounds to be:

1. estrogenic: CB 28, CB 52, CB 101, CB 153, *o*,*p*′-DDT, *p*,*p*′-DDT, *γ*-HCH, *β*-HCH, *trans*-nonachlor and oxychlordane;
2. anti-estrogenic: CB 105, CB 118, CB 156, CB 167 and HCB;
3. anti-androgenic: *p*,*p*′-DDE;
4. no known hormonal activity: α-HCH, CB 138, CB 180.

Quantitative Bone Measurements

Quantitative bone measurements were performed by dual-energy X-ray absorptiometry (DXA) (DPX-L, Lunar, Madison, WI) and quantitative ultrasound (QUS) (Achilles Plus, Lunar, Madison, WI). The BMD (g/cm2) of the whole body (WBBMD), the lumbar spine (LSBMD) at the L2–L4 region, and of the femoral neck (FNBMD) were measured. The measurement error in precision of DXA, as tested on a spine phantom during the study, was less than 1% (coefficient of variance, CV). We measured the propagation of the ultrasound beam by speed of sound (SOS, m/s) and the broadband ultrasound attenuation (BUA, dB/MHz) on the left os calcis. At our laboratory, the precision error was (CV) 2.5% for BUA and 0.2% for SOS.

Statistical Analysis

Simple linear regression was performed between the independent variable, 'serum organochlorine concentration', and the dependent variables, FNBMD, LSBMD, WBBMD, BUA and SOS. In multiple regressions, we considered the possible confounding variables age (continuous), body mass index (BMI, continuous), height (continuous), smoking status (never, former, current), cortisone use (never, ever), diabetes (yes, no) and long-term physical activity by tertiles, based on reports of estimated leisure physical activity on a 4-point scale in each of three periods (teenage, age 18–30 years and recent years) which were summed and condensed into tertiles. To detect nonlinear associations, the study subjects were divided into quartiles according to their concentrations of POCs in some cases. Adjusted means (least-square means) of the dependent variables were calculated for each quartile of the independent variables, on the basis of the regression estimates with all confounding factors held at their mean values. The distribution of several of the independent variables was strongly skewed, but statistical analysis of untransformed and ln-transformed organochlorine concentrations gave similar results.

Results

The characteristics and descriptive statistics of some of the study variables in the 115 men are shown in Table 1.

Among the 10 PCB congeners, CB 153 had the highest concentrations (mean 294 ng/g lipid), whereas CB 28, CB 52, CB 101 and CB 167 had the lowest concentrations (means $\leqslant$10 ng/g lipid) (Table 2). In the case of CB 52, only 37 men had concentrations above the limit of quantification (2 ng/g lipid). CB 138 and CB 28 showed the largest difference between the lowest and highest concentrations (108-fold and 39-fold).

Among DDT compounds, *p,p′*-DDE (the principal metabolite of *p,p′*-DDT) had the highest mean concentration (784 ng/g lipid) (Table 2). The average

Table 2. Serum concentrations of persistent organochlorines in 115 men from the general Swedish population

	n above LOQ[a]	Mean	SD	Range
PCBs (ng/g lipid)				
CB 28	97	5.8	9.1	<2.0–78.1
CB 52	37	4.2	3.1	<2.0–16.4
CB 101	93	4.2	3.1	<2.0–18.3
CB 105	103	6.6	4.8	<2.0–28.1
CB 118	115	41.9	23.1	4.3–143
CB 138	115	141.6	66.0	3.1–335.0
CB 153	115	294.3	120.8	22.8–627.0
CB 156	115	22.9	7.1	7.9–49.6
CB 167	111	10.0	4.7	<2.0–29.7
CB 180	115	216.1	74.2	71.4–480.0
Pesticides (ng/g lipid)				
p,p′–DDT	111	19.8	13.5	<4.0–78.6
p,p′-DDD	20	2.8	2.0	<4.0–15.4
p,p′-DDE	115	783.8	684.8	25.0–4030.0
o,p′-DDT	3	2.2	1.6	<4.0–15.7
o,p′-DDE	0			
HCB	115	84.0	136.4	23.1–1468.0
α-HCH	9	1.2	0.7	<2.0–6.1
β-HCH	115	18.1	29.0	12.4–187.0
γ-HCH	29	1.7	1.6	<2.0–12.1
trans-Nonachlor	115	30.5	15.6	7.9–89.5
Oxychlordane	115	13.7	7.0	4.3–35.7

[a]LOQ, limit of quantification. When results were below the LOQ they were set to 50% of that limit in the calculation of means and SD.

Table 1. Characteristics and descriptive statistics of some of the study variables in 115 men from the general Swedish population

	n	Mean	SD	Range
Age (years)	115	62.9	9.1	42.0–76.0
Weight (kg)	115	83.7	11.6	48–119
Length (cm)	115	176.1	6.8	162.0–195.0
BMI (kg/m^2)	115	27.0	3.0	18.1–35.2
Femoral neck BMD (g/cm^2)	115	0.98	0.15	0.63–1.50
Lumbar spine BMD (g/cm^2)	114	1.22	0.20	0.58–1.72
Total body BMD (g/cm^2)	115	1.23	0.11	0.88–1.53
Broad band attenuation (dB/MHz)	107	118	11	96–149
Speed of sound (m/s)	107	1526	35	1467–1618
Physical activity	115	Low: *n* = 29	Average: *n* = 58	High: *n* = 44
Smoking	115	Never: *n* = 14	Former: *n* = 57	Current: *n* = 44
Cortisone	115	Never: *n* = 102	Ever: *n* = 13	
Diabetes	115	Yes: *n* = 2	No: *n* = 113	

concentrations of the other pesticides and metabolites were usually lower by a factor 9 or more and the lowest concentrations were found for α-HCH, γ-HCH, *p,p′*-DDD, *o,p′*-DDT and *o,p′*-DDE (mainly below the LOQ) (Table 2). For compounds with all concentrations above the LOQ, the range of exposure was substantial (8- to 161-fold) (Table 2).

In the simple linear regression analysis, we found a few statistically significant associations between organochlorine concentrations and bone variables. Positive relationships were found between LSBMD and CB 167 concentration ($\beta = 0.0085$, $p = 0.03$), between BUA and γ-HCH concentration ($\beta = 1.38$, $p = 0.03$), and between SOS and α-HCH concentration ($\beta = 10.6$, $p = 0.03$). Moreover, a significantly negative relationship

Table 3. Regression coefficients and *p* values in multivariate regression analyses of associations between concentrations of single PCB congeners and bone variables[a]

Compound	Bone variables[b]	Coefficient ($\times 10^{-3}$)	SE ($\times 10^{-3}$)	*p*
CB 28	FNBMD	−1.0	1.5	0.50
	LSBMD	1.4	2.0	0.48
	TBBMD	−0.5	1.1	0.67
	BUA	*−292*	*120*	*0.02*
	SOS	−370	386	0.34
CB 52	FNBMD	2.3	4.6	0.61
	LSBMD	4.2	6.0	0.49
	TBBMD	0.8	3.4	0.82
	BUA	−416	402	0.30
	SOS	399	1261	0.75
CB 101	FNBMD	−0.7	4.7	0.89
	LSBMD	−0.7	6.1	0.91
	TBBMD	1.0	3.3	0.76
	BUA	25	393	0.95
	SOS	1232	1221	0.31
CB 105	FNBMD	−1.5	3.2	0.63
	LSBMD	1.0	4.2	0.82
	TBBMD	−0.5	2.3	0.83
	BUA	−125	270	0.64
	SOS	−411	843	0.63
CB 118	FNBMD	−0.4	0.7	0.51
	LSBMD	0.1	0.9	0.87
	TBBMD	−0.1	0.5	0.82
	BUA	−37	56	0.51
	SOS	−135	176	0.44
CB 138	FNBMD	−0.04	0.2	0.85
	LSBMD	−0.1	0.3	0.67
	TBBMD	−0.01	0.2	0.98
	BUA	−19	19	0.31
	SOS	35	58	0.54
CB 153	FNBMD	0.02	0.1	0.86
	LSBMD	−0.05	0.2	0.73
	TBBMD	0.05	0.08	0.54
	BUA	−8.4	9.9	0.40
	SOS	−9.4	31	0.70
CB 156	FNBMD	1.3	2.0	0.51
	LSBMD	1.7	2.6	0.51
	TBBMD	1.8	1.4	0.20
	BUA	−102	165	0.54
	SOS	69	518	0.90
CB 167	FNBMD	4.4	3.1	0.16
	LSBMD	6.6	4.1	0.11
	TBBMD	*4.4*	*2.2*	*0.05*
	BUA	181	268	0.50
	SOS	428	840	0.61
CB 180	FNBMD	0.1	0.2	0.61
	LSBMD	0.02	0.3	0.93
	TBBMD	0.2	0.1	0.21
	BUA	−13	16	0.42
	SOS	15	49	0.77

FNBMD, femoral neck bone mineral density (g/cm^2); LSBMD, lumbar spine BMD (g/cm^2); WBBMD, whole body BMD (g/cm^2); BUA, broadband ultrasound attenuation; SOS, speed of sound.
[a] Adjusted for age (continuous), BMI (continuous), height (continuous), smoking (never, former, current), cortisone use (never, ever), diabetes (yes, no) and long-term physical activity (tertiles).
[b] BMD variables: *n* = 114–115; BUA and SOS: *n* = 107.

Table 4. Regression coefficients and *p* values in multivariate regression analyses of associations between concentrations of chlorinated pesticides/metabolites and bone density variables[a]

Compound	Bone[a]	Coefficient ($\times 10^{-3}$)	SE ($\times 10^{-3}$)	*p*
p,p′-DDT	FNBMD	−0.6	1.1	0.61
	LSBMD	1.2	1.4	0.38
	TBBMD	0.01	0.8	0.99
	BUA	17	90	0.85
	SOS	113	281	0.68
p,p′-DDD	FNBMD	−3.2	7.1	0.66
	LSBMD	7.8	9.1	0.39
	TBBMD	−1.8	5.0	0.73
	BUA	−230	566	0.69
	SOS	−374	1771	0.83
p,p′-DDE	FNBMD	−0.02	0.02	0.40
	LSBMD	−0.01	0.03	0.63
	TBBMD	−0.01	0.02	0.40
	BUA	−2.3	1.8	0.21
	SOS	−4.6	5.7	0.42
HCB	FNBMD	0.05	0.1	0.59
	LSBMD	0.04	0.1	0.76
	TBBMD	0.05	0.07	0.49
	BUA	−5.7	8.2	0.49
	SOS	8.8	26	0.73
α-HCH	FNBMD	0.4	20	1.00
	LSBMD	−7.2	27	0.79
	TBBMD	−2.0	15	0.89
	BUA	2086	1628	0.20
	SOS	8443	5059	0.10
β-HCH	FNBMD	−0.6	0.5	0.28
	LSBMD	−0.5	0.7	0.45
	TBBMD	−0.5	0.4	0.18
	BUA	−66	42	0.12
	SOS	−215	130	0.10
γ-HCH	FNBMD	14	8.7	0.11
	LSBMD	19	12	0.10
	TBBMD	10	6.7	0.13
	BUA	*1432*	*718*	*0.05*
	SOS	3014	2271	0.19
Trans-nonachlor	FNBMD	−0.4	1.0	0.71
	LSBMD	−0.1	1.3	0.93
	TBBMD	−0.3	0.7	0.68
	BUA	−60	82	0.47
	SOS	−180	257	0.49
Oxychlordane	FNBMD	−1.2	2.2	0.59
	LSBMD	−0.5	2.8	0.87
	TBBMD	−1.1	1.6	0.47
	BUA	−60	82	0.47
	SOS	−557	555	0.32

Abbreviations as in Table 3.
[a] Adjusted for age (continuous), BMI (continuous), height (continuous), smoking (never, former, current), cortisone use (never, ever), diabetes (yes, no) and long-term physical activity (tertiles).
[b] BMD variables: *n* = 114–115; BUA and SOS: *n* = 107.

was found between BUA and CB 28 concentration ($\beta = -0.24$, $p = 0.03$). The multivariate analysis did not markedly change the relationship between the exposure variables and the outcome variables (still significant for CB 28, and γ-HCH) (Tables 3, 4). For a few POCs, the associations between concentration and BMD, BUA and SOS were all negative (*p,p′*-DDE, β-HCH, *trans*-nonachlor and oxychlordane) or positive (CB 167 and γ-HCH) (Tables 3, 4).

The regression analysis of associations between concentrations of POC groups and BMD, BUA and SOS did not reveal any significant associations (Table 5). The high concentration of CB 153 had a large influence on the results of the estrogenic group, and consequently we also considered the estrogenic group after exclusion of CB 153. No association was found, however, between the concentration of estrogenic compounds and quantitative bone properties after exclusion of CB 153.

When multivariately adjusted means were calculated for each concentration quartile of organochlorine groups, a few statistically significant results were found (Table 6). There were no consistent concentration-dependent changes in adjusted means, however, between the exposure quartiles, except for the only compound classified as an anti-androgen, *p,p′*-DDE. In this case a tendency for a decreased BUA (4–7%) and SOS (approx. 1%) was indicated between the lowest and the two highest concentration quartiles (not significant at the highest quartile) (Fig. 1). In the DXA measurements the adjusted mean of the femoral neck BMD decreased 5–7% between the lowest quartile and the two highest quartiles, the lumbar spine BMD decreased 6–8% and

Table 5. Regression coefficients and *p* values in multivariate regression analysis of associations between groups of chlorinated pesticides/metabolites and bone density variables[a]

Compound	Bone density	Coefficient ($\times 10^{-3}$)	SE ($\times 10^{-3}$)	*p*
SumPCB	FMBMD	2.0	18	0.91
	LSBMD	−3.1	24	0.90
	TBBMD	7.9	13	0.55
	BUA	−1614	1579	0.31
	SOS	−1138	4962	0.82
SumAll	FNBMD	−2.7	5.4	0.61
	LSBMD	−2.3	6.6	0.73
	TBBMD	−1.4	3.6	0.71
	BUA	−593	425	0.17
	SOS	−877	1339	0.51
Estrogens	FNBMD	−7.0	31	0.82
	LSBMD	−1.1	41	0.79
	TBBMD	2.7	22	0.91
	BUA	−3520	2699	0.20
	SOS	−5009	8499	0.56
Estrogens (−153)	FNBMD	−83	84	0.33
	LSBMD	−4.1	110	0.97
	TBBMD	−58	60	0.34
	BUA	−1198	6960	0.09
	SOS	−27298	21920	0.22
Anti-estrogens	FNBMD	13	26	0.63
	LSBMD	14	35	0.69
	TBBMD	14	19	0.48
	BUA	−1556	2152	0.47
	SOS	1646	6746	0.81
Anti-androgens	FNBMD	−6.5	7.6	0.40
	LSBMD	−4.3	9.0	0.63
	TBBMD	−4.1	4.9	0.40
	BUA	−723	577	0.21
	SOS	−1461	1814	0.42

Abbreviations as in Table 3.
[a] Adjusted for age (continuous), BMI (continuous), height (continuous), smoking (never, former, current), cortisone use (never, ever), diabetes (yes, no) and long-term physical activity (tertiles).
[b] BMD variables: n = 114–115; BUA and SOS: n = 107.

Table 6. Adjusted means of bone variables for each quartile of organochlorine concentration in serum[a]

Compounds	Bone variable[b]	Q1	Q2	Q3	Q4
SumPCB	FNBMD	0.988	0.979	0.972	0.976
	LSBMD	1.208	1.260	1.202	1.204
	WBBMD	1.217	1.247	1.227	1.229
	BUA	117.9	119.7	118.0	115.0
	SOS	1526	1536	1525	1522
SumAll	FNBMD	0.996	0.969	0.971	0.974
	LSBMD	1.268	1.188	1.206	1.205
	WBBMD	1.242	1.224	1.229	1.219
	BUA	120.4	117.9	116.8	115.3
	SOS	1537	1527	1520	1525
Estrogenic	FNBMD	0.979	1.002	0.952	0.975
	LSBMD	1.210	1.221	1.227	1.208
	WBBMD	1.219	1.238	1.231	1.225
	BUA	118.3	120.1	116.5	115.6
	SOS	1527	1533	1525	1523
Estrogenic (−153)	FNBMD	0.968	1.010	0.994	0.936
	LSBMD	1.194	1.221	1.240	1.211
	WBBMD	1.206	1.251	1.249	1.208
	BUA	117.9	120.0	115.4	114.7
	SOS	1528	1536	1525	1519
Anti-estrogenic	FNBMD	0.967	0.952	0.990	1.002
	LSBMD	1.193	1.162	1.232	1.279
	WBBMD	1.218	1.209	1.236	1.251
	BUA	118.4	118.4	113.7	119.3
	SOS	1532	1528	1514	1532

Abbreviations as in Table 3.
[a] Adjusted for age (continuous), BMI (continuous), height (continuous), smoking (never, former, current), cortisone use (never, ever), diabetes (yes, no) and long-term physical activity (tertiles).
[b] BMD variables: n = 114–115; BUA and SOS: n = 107.

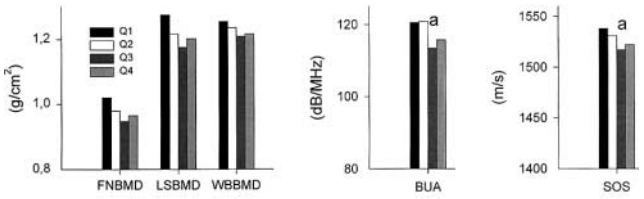

Fig. 1. Adjusted means of femoral neck BMD (FNBMD, n = 115), lumbar spine BMD (LSBMD, n = 114), whole body BMD (WBBMD, n = 115) and broad-band attenuation (BUA, n = 107) and speed of sound (SOS, n = 108) in os calcis for each exposure quartile (Q1, Q2, Q3 and Q4) of *p,p′*-DDE. The means were adjusted for age (continuous), BMI (continuous), height (continuous), smoking (never, former, current), cortisone use (never, ever), diabetes (yes, no) and long-term physical activity (tertiles). (a. Significantly different from the adjusted mean of Q1 (p<0.05).)

the whole body BMD decreased 3–4%. This decrease was, however, not statistically significant (Fig. 1).

No consistent concentration-dependent changes were found in adjusted means of BMD, BUA and SOS, when means were calculated for concentration quartiles of single substances with negative or positive associations between concentration and all five bone variables (results not shown).

Discussion

Overall, our findings do not indicate strong associations between organochlorine concentrations in serum and BMD, BUA and SOS among the study participants. The weak association between increased serum concentrations of *p,p′*-DDE and decreased BMD, BUA and SOS, may indicate that *p,p′*-DDE could cause negative effects on bone density. However, multiple tests were performed in the statistical analysis which increase the risk for type I errors. Consequently, our statistically significant findings could be due to chance.

The men in our study had no clinical signs of osteoporosis. The mean values of BMD were slightly higher than those found in a group of relatively healthy men of the same age living in southern Sweden, but the variation in BMD values was similar [20]. The slightly higher average bone density in the present study may be, at least partly, due to a slightly higher BMI among our study subjects (3–9% for different age groups).

In the statistical analyses, only a few statistically significant associations were found between single DXA or ultrasound variables and organochlorine concentrations in serum. If a stricter significance level than $p<0.05$ had been used (for instance $p<0.01$) none of the results would have been statistically significant. A true association between POCs and bone density is more likely when the associations are consistent at all sites of bone measurement. This was the case for CB 167 and γ-HCH, which showed positive associations with the five bone variables in the multiple regression analysis. Moreover, *p,p′*-DDE, β-HCH, *trans*-nonachlor and oxychlordane showed negative associations with the bone variables. In the analysis of adjusted means, however, only *p,p′*-DDE showed a weak negative association with BMD, BUA and SOS.

p,p′-DDE acts as a weak anti-androgen in biological test systems, by blockage of the androgen receptor [3]. Recent studies indicate that androgens may have positive effects on BMD by decreasing the bone resorption [7]. It is hypothetically possible, therefore, that the anti-androgenic *p,p′*-DDE causes negative effects on bone density. This hypothesis is supported by a small experimental study on male pigeons, where *p,p′*-DDE reduced the medullar bone formation during estrogen treatment [8]. *p,p′*-DDE was the single compound showing the highest serum concentrations among our men, but the decrease in adjusted means of BMD, BUA and SOS between the first and fourth quartiles of *p,p′*-DDE concentrations was not large enough to be statistically significant in our relatively small study. When the 115 study participants were divided into quartiles of serum POC concentrations, we had an 80% power to detect a 12% difference in adjusted mean of femoral BMD and lumbar spine BMD between quartiles. The corresponding percentage for whole body BMD and BUA was 8% and for SOS was 2%. The observed difference in adjusted means between the lowest and the two highest *p,p′*-DDE quartiles was not large enough, therefore, to allow a firm conclusion about the associations between body burden of *p,p′*-DDE and BMD among the participating men.

Our classification of the POCs according to assumed hormonal activity was uncertain, since the data on hormonal activity of POCs in biological test systems are scarce and data on humans are completely lacking. Moreover, some studies indicate that the hormonal activity of a single substance may differ depending on the biological test system used [3]. For instance, in ovariectomized rats, the PCB congener CB 126 caused a decreased length of the tibiae and an increased BMD, which is indicative of an estrogenic effect [12]. In sham-operated female rats, however, CB 126 appeared to exhibit anti-estrogenic activity by impairing the mineralization process of tibiae, as indicated by increases in organic content and in osteoid surface.

The potency of POCs to mimic sex hormones is low [2,3]. The body burdens of POCs in our study participants may thus be too low to cause significant biological effects. The average concentrations of PCBs found in our study participants were within the range of those reported for men from the general populations in northern Europe and North America, but several-fold higher than those found in men from non-industrialized parts of Asia and Africa [21–24]. The reverse seems to be the case for DDT and other chlorinated pesticides, i.e., average concentrations are significantly higher in men from Asia and Africa [21–24]. This is probably due to the extensive and recent use of these pesticides in the tropics. Moreover, very high body burdens of PCB and certain organochlorine pesticides are found in areas locally affected by industrial pollution and in populations with a high consumption of contaminated food (mainly fish) [22,23,25]. Studies on such highly exposed populations may reveal effects of POCs on bone density not detectable in our study population with background exposure.

In conclusion, our results indicate that the studied POCs do not exert major effects on bone density in our study group. The finding of a slightly decreased bone density among men with elevated serum concentrations of *p,p′*-DDE has to be confirmed in a larger study on the same population of men, or in studies of male populations with higher body burdens of POCs.

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Note added in proof: A study of BMD in 68 sedentary women showed a negative association between log DDE concentrations in serum and BMD in multiple regression analysis using a model including log DDE ($p = 0.018$), age ($p = 0.002$) and years of hormone replacement therapy ($p = 0.10$).

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