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Free and bioavailable fractions of sex steroids may influence bones in young men, depending on age and oestradiol level

Wolne i biodostępne frakcje steroidów płciowych mogą wpływać na kości u młodych mężczyzn w zależności od wieku i stężenia estradiolu

Dorota Szyska-Skrobot[1, 2*], Katarzyna Marchlewska[1*], Renata Walczak-Jędrzejowska[1], Elżbieta Oszukowska[1], Eliza Filipiak[1], Piotr Kula[3], Ryszard Mężyk[2], Aldona Kowalska[2], Ryszard Jaszewski[3], Jolanta Słowikowska-Hilczer[1], Krzysztof Kula[1]

**These authors contributed equally to this work*
[1]*Department of Andrology and Reproductive Endocrinology, Medical University of Łódź, Poland*
[2]*Division of Endocrinology, Świętokrzyskie Centrum of Oncology in Kielce, Poland*
[3]*Department of Cardiosurgery, Medical University of Łódź, Poland*

Abstract

Introduction: Longitudinal bone growth ceases by the end of puberty, and it is thought to be a result, in both sexes, of increased pubertal oestrogen serum concentrations. Since peak bone mass is achieved by the third decade of life or later, the aim of this study was to relate sex steroid hormones and sex hormone binding globulin (SHBG) levels to bone quality in men during their third and fourth decades of life.
Material and methods: Eighty men, healthy volunteers aged between 18 and 39 years, were subjected to an interviewer-administered questionnaire, body mass index (BMI) measurement, blood sample and calcaneal quantitative ultrasound (QUS) (Hologic-SAHARA). Blood was assessed for testosterone (T), oestradiol (E2), dehydroepiandrosterone sulfate (DHEAS), SHBG, luteinising hormone (LH) and follicle stimulating hormone (FSH). Free and bioavailable T and E2 levels were calculated knowing SHBG and albumin levels.
Results: While T, E2, DHEAS, LH and FSH levels were not related, free and bioavailable fractions of T and E2 were positively associated with QUS readings. SHBG level was associated negatively. After dichotomisation for age, the associations remained significant only for younger subjects (18–30 years, n = 47). After adjustment for other co-variants, only SHBG in younger subjects retained its negative association with QUS. Older subjects (31–39 years, n = 33) revealed higher BMI and lower serum concentrations of total (–17 %), free (–18.5%) and bioavailable (–22.5%) levels of E2 than younger subjects.
Conclusion: Free and bioavailable fractions of sex steroids may influence bones in young men, depending on age and E2 level. **(Endokrynol Pol 2014; 65 (5): 357–364)**

Key words: *testosterone; oestradiol; SHBG; bone; ultrasound; men*

Streszczenie

Wstęp: Wzrost kości na długość ustaje wraz z końcem dojrzewania płciowego i wykazano, że u obu płci jest to wynik wzrostu stężenia estrogenów we krwi. Skoro przyjęto, że szczytowa masa kostna jest osiągana dopiero w trzeciej dekadzie życia lub po trzydziestce, badano związki pomiędzy stężeniami steroidów płciowych i białka wiążącego steroidy płciowe (SHBG) a jakością kości u mężczyzn w trzeciej i czwartej dekadzie życia.
Materiał i metody: Osiemdziesięciu mężczyzn, zdrowych ochotników w wieku 18–39 lat wypełniło kwestionariusz z wywiadem, zmierzono u nich wskaźnik masy ciała (BMI) i wykonano ilościową analizę ultrasonograficzną kości piętowej (QUS) (Hologic-SAHARA). We krwi oznaczono stężenia testosteronu (T), estradiolu (E2), siarczanu dehydroepiandrosteronu (DHEAS), SHBG, hormonu luteinizującego (LH) i hormonu folikulotropowego (FSH). Znając stężenia SHBG i albumin, wyliczano stężenia wolnego i biodostępnego T i E2.
Wyniki: Podczas gdy stężenia T, E2, DHEAS, LH i FSH nie wykazywały powiązań, stężenia wolnych i biodostępnych frakcji T i E2 były dodatnio związane z parametrami QUS. Stężenie SHBG wykazywało związek ujemny. Relacje te zależały od wieku. Mianowicie, po podziale na dwie grupy wiekowe, relacje pozostały znamienne tylko wśród młodszych mężczyzn (18–30 lat, n = 47). Analiza wieloczynnikowa wykazała, że tylko stężenie SHBG u młodszych mężczyzn zachowało znamiennie ujemny związek ze stanem kości. Starsi (31–39 lat, n = 33) wykazali wyższy BMI, a niższe stężenie całkowitego (–17%), wolnego (–18,5%) i biodostępnego (–22,55%) E2 w porównaniu z młodszymi badanymi.
Wnioski: Wolne i biodostępne frakcje steroidów płciowych mogą wpływać na kości u młodych mężczyzn, w zależności od wieku i stężenia E2. **(Endokrynol Pol 2014; 65 (5): 357–364)**

Słowa kluczowe: *testosteron; estradiol; SHBG; kości; ultrasonografia*

Prof. Krzysztof Kula M.D., Ph.D., Department of Andrology and Reproductive Endocrinology, Medical University of Łódź, Sterlinga St. 5, 91–425 Łódź, Poland, tel.: +48 42 633 07 05, e-mail: krzysztof.kula@umed.lodz.pl

Introduction

Testosterone (T) and oestradiol (E2) are the two most important sex steroids in men and women respectively. In the circulation they are bound or unbound to serum proteins. Their serum protein-bound fractions are biologically inactive. The bioactive fractions of circulating hormones are either free (non-bound to sex hormone binding protein, SHBG, and albumin) or bioavailable (non-bound to SHBG) [1]. Studies on individuals with androgen insensitivity support the role of T in maintaining trabecular bone [2], and androgen receptors have been reported in human osteoblasts [3]. However, in a large cohort of middle-aged and elderly men, total and free E2, but not T, blood levels were found to positively correlate with bone health as estimated by quantitative ultrasonography (QUS) at the calcaneus [4].

Longitudinal bone growth ceases by the end of puberty and it is thought to be a result, in both males and females, of the actions of increased pubertal oestrogen concentrations to induce epiphyseal maturation and closure [5–8]. However, bone development progresses beyond puberty and it is believed that peak bone mass, a significant predictor of osteoporosis, is achieved by the early- to mid-20 s or after 30 years of age [9–12]. In the present study, we investigated the association between serum concentrations of sex steroids, SHBG, luteinising hormone (LH) and follicle stimulating hormone (FSH), the pituitary hormones that control gonadal function, and the bone status of men in the age-span of 18–39 years.

Material and methods

Subjects

The study was performed in the outpatient clinic of the University Hospital at Medical University of Łódź, Poland. The study protocol was approved by the Ethical Committee of the Medical University in Łódź (RNN/145/05/KE) and informed consent was obtained from all participants. A group of 300 men was randomly recruited from a population-based sample of the Łódź city population register for participation in this study. The criterion was age between 18 and 39 years. Subjects were invited by letter to attend a screening visit at the Department of Andrology and Reproductive Endocrinology between March 2006 and June 2007. Eighty men agreed to participate, with an overall response rate for participation of 26.7%. The subjects were asked to complete a postal questionnaire and attend an interviewer-assisted questionnaire, which included questions about physical activity and lifestyle. Other data from these individuals including blood biochemistry data has previously been described [13].

Study questionnaires and clinical data

The postal questionnaire included questions concerning current smoking and alcohol consumption in the previous year (response set = every day/5–6 days per week/3–4 days per week/1–2 days per week/less than once a week/not at all). The main study questionnaire included general health, physical activity, assessed as frequency and duration of walking. Frequency per week was categorised as 1) none, 2) seldom, 3) from time to time or 4) frequent. Duration of walking per day was categorised as 1) less than 1 hour, 2) about 1 hour but less than 2 h, 3) 2–4 hours, or 4) more than 4 hours. Height and weight were measured in a standardised fashion. Body weight was measured to the nearest 0.1kg using an electronic scale (SECA, model no. 8801321009, SECA UK Ltd) and height to the nearest 1mm using a stadiometer (Leicester Height Measure, SECA UK Ltd). Body mass index was calculated (BMI = weight/height^2).

Hormone measurements

After a single fasting morning (before 10am), a venous blood sample was obtained from all subjects. The serum was separated immediately after phlebotomy and stored at –80°C until single run assays. Determinations of T, E2, DHEAS, SHBG were performed using chemiluminescence immunoassay (Immulite 1000, DPC, USA). Detection limits were 0.5 nmol/L for T, 55 pmol/L for E2, 0.081 μmol/L for DHEAS, and 0.2 nmol/L for SHBG. The coefficients of variation within runs were 13% for T, 15% for E2, 13% for DHEAS and 6.9% for SHBG. FSH and LH concentrations were determined by an immunometric technique (Vitros System, Ortho-Clinical Diagnostics, USA) with a sensitivity of 0.5 mIU/mL for both, and with an intra-assay precision of 2.2% (FSH) and 2.6% (LH). The free and bio-available T and E2 levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and association constants of Vermeulen et al. [1] and Van Pottelbergh et al. [14].

QUS of the heel

QUS of the left heel was performed with the Sahara Clinical Sonometer (Hologic, Bedford, MA, USA) using a standardised protocol. Outputs included the rate of loss of ultrasonic intensity with frequency (broadband ultrasound attenuation [BUA] measured in dB/MHz using a Fourier transformation of the recorded signal) and the velocity of ultrasound transmission through bone (speed of sound [SOS] measured in m/s from the sound propagation time between the transducers). In addition, the quantitative ultrasound index (QUI), a measure of stiffness, was derived from the BUA and SOS measures: QUI = 0.41 (SOS) + 0.41 (BUA) – 571.

Table I. *Subject characteristics*
Tabela I. *Charakterystyka grupy*

Variable	N = 80 Mean (SD)
Age at interview (years)	28,2 (5,9)
Height [cm]	179.8 (4,6)
Weight [kg]	81.7 (13.4)
Body mass index [kg/m^2]	25.2 (4.6)
Broadband ultrasound attenuation (BUA) (dB/MHz)	88.63 (18.4)
Speed of sound (m/s) (SOS)	1561.2 (38.5)
Quantitative ultrasound index (QUI)	104.9 (22.3)
	%
Currently smoke (yes *vs.* no)	33.7
Alcohol consumption ≥ 1day/week	82.5
Physical activity (walking) (yes *vs.* no)	58.7

Table II. *Hormone descriptives*
Tabela II. *Stężenia hormonów*

Variable	N = 80 Mean (SD)	Reference range
Testosterone [nmol/L]	18.3 (5.2)	14.4–22.5
Free testosterone [pmol/L]	424.0 (129.7)	200–500
Bio-available testosterone [nmol/L]	10.8 (3.3)	
Oestradiol [pmol/L]	113.5 (41.1)	88–142
Free oestradiol [pmol/L]	2.4 (0.9)	1.7–2.6
Bio-available oestradiol [pmol/L]	63.8 (24.9)	44–73
SHBG [nmol/L]	25.7 (10.6)	16–35
DHEAS [mmol/L]	7.2 (2.7)	2.2–13.9
LH [IU/L]	4.5 (1.8)	0.8–7.6
FSH [IU/L]	4.1 (2.2)	1.5–9.7

The short-term precision of the method was based on duplicate measurements performed in 20 randomly-selected cohort members in the reference centre of the Department of Andrology and Endocrinology, Catholic University of Leuven (Prof. Dirk Van der Schueren). The *in vivo* coefficients of variation were 2.8% and 0.3% for BUA and SOS, respectively, and 2.3% for QUI.

Statistical analysis

The data is expressed as mean ± standard deviation (SD) for parametric, or median (25–75% interval and range) for nonparametric, distributions. The distribution of data was assessed according to the Shapiro-Wilk test. One-way ANOVA was applied to assess the statistical significance for normally distributed data, while the Kruskal-Wallis or Mann-Whitney test was used for data which was not normally distributed. Differences were considered significant at $p < 0.05$. The associations between each of the ultrasound parameters and the different hormone levels were expressed as β coefficients and 95% confidence intervals (CI). Analysis was performed using Statistica version 8.0.

Results

Eighty men aged from 18–39 years (mean age 28.2 years) were included in the analysis. All reported good general health and revealed a normal development of primary and secondary sexual characteristics. Of the subjects, 33.7% reported that they currently smoke, 82.5% that they consume alcohol more than one day per week and 58.7% reported physical activity. Subject characteristics are presented in Table I. Mean values for the hormones are shown in Table II. The level of T found in the entire group ranged between 8.2 and 27.7 nmol/L; the same for E2 was 45.5 and 208.8 pmol/L.

Table III shows that while T, E2, DHEAS, LH or FSH levels were not related, the levels of free and bioavailable T, and E2 were significantly positively associated with QUS parameters, whereas SHBG level was associated negatively.

When the subjects were divided by age, the associations remained significant for sex steroids in younger subjects (18–30 years, n = 47), but not in the older subgroup (31–39 years, n = 33) (Table IV). In the multivariate model including free and bio- available T, free and bio available E2, and SHBG as continuous variables, after adjustment for age, BMI, physical activity, current smoking and alcohol consumption, only SHBG remained significantly associated with all QUS parameters among younger subjects ($\beta = -1.0024$, $p = 0.037$).

Older subjects (31–39 years) revealed significantly higher BMI and lower levels of total (–17%), free (–18.5%) and bio-available (–22.5%) E2 than the younger group. The total T, free T, SHBG, LH or FSH, BMI and QUS parameters were not found to be different between subgroups (Table V). There was no difference with respect to smoking habits, alcohol consumption or physical activity, and none of these factors influenced QUS (data not shown).

To further characterise the influence of age on hormone levels, the subjects were stratified for 2-3 year age ranges as follows: 18-20 (n = 8), 21–23 (n = 16), 24–26 (n = 11), 27–29 (n = 9), 30–32 (n = 17), 33–35 (n = 8), and 36–39 (n = 11). Significant changes concerned serum levels of BMI, free E2, bio-available E2 and SHBG. Figure 1 demonstrates a stepwise increase of BMI between

Table III. *Association between hormones or SHBG and QUS parameters in 80 men aged 18–39 years*

Tabela III. *Relacje pomiędzy stężeniami hormonów lub SHBG a parametrami QUS u 80 mężczyzn w wieku 18–39 lat*

	BUA (dB/Mhz) β-coefficient (95% CI)	**SOS (m/s) β-coefficient (95% CI)**	**QUI β-coefficient (95% CI)**
Testosterone [nmol/L]	0.059 (–0.738, 0.857)	0.624 (–1.035, 2.284))	0.256 (–0.710, 1.223)
Free testosterone [pmol/L]	0.029 (–0.016, 0.061)	0,080 (0.015, 0.144)*	0.042 (0.005, 0.080)*
Bioavailable testosterone [nmol/L]	1.061 (–0.166, 2.289)	3.016 (0.494, 5.538)*	1.561 (0.084, 3.038)*
Oestradiol [pmol/L]	0.040 (–0.059, 0.141)	0.124 (–0.08, 0.333)	0.055 (–0.067, 0.177)
Free oestradiol [pmol/L]	5.026 (0.760, 9.292)*	12.151 (3.353, 20.950)*	6.305 (1.140, 11.470)*
Bioavailable oestradiol [pmo/L]	0.169 (0.007, 0.331)*	0,423 (0.089, 0.757) *	0.214 (0.018, 0.411)*
SHBG [nmol/L]	–0.470 (–8.451, –0.096)*	–0,950 (–1.734,–0.166)*	–0.550 (–1.00,–0.095)*
DHEAS [mg/dL]	0.353 (–1.132, 1.840)	1.051 (–2.047, 4.150)	0.327 (–1.478, 2.132)
LH [IU/L]	–0.604 (–2.871, 1.662)	–0,445 (–5.185, 4.295)	–0,617 (–3.370, 2.135)
FSH (IU/L)	–0.963 (–2.844, 0.916)	–2.380 (–6.295, 1.535)	–1.287 (–3.562, 0.998)

*p<0.05

Table IV. *Association between sex hormones or SHBG and QUS parameters in younger (18–30 years) and older (31–39 years) men*

Tabela IV. *Relacje pomiędzy stężeniami hormonów lub SHBG a parametrami QUS u młodszych (16–30 lat) i starszych (31–39 lat) badanych*

	BUA (dB/Mhz) β-coefficient (95% CI)	**SOS (m/s) β-coefficient (95% CI)**	**QUI β-coefficient (95% CI)**
Testosterone [nmol/L]			
Younger	0.398 (–1.816, 1.617)	1.989 (–0.646, 4.624)	0.911 (–0.612, 2.435)
Older	–0.183 (–1.303, 0.936)	–0.360 (–2.360, 1.720)	–0.216 (–1.143, 1.088)
Free testosterone [pmol/L]			
Younger	0.050 (0.009, 0.091)*	0,145 (0.058, 0.232)*	0.075 (0.024, 0.126)*
Older	0.004 (–0.046, 0.054)	0,001 (–0.095, 0.091)	0.001 (0.055, 0.057)
Bioavailable testosterone [nmol/L]			
Younger	1.602 (–0.009, 3.195)*	4.875 (1.490, 8.259)*	2.483 (0.505, 4.461)*
Older	0.233 (–1.812, 0.817)	0.101 (–3.704, 3.907)	0.142 (–2.157, 2.443)
Oestradiol [pmol/L]			
Younger	0.027 (–0.103, 0.158)	0.150 (–0.136, 0.438)	0.056 (–0.109, 0.222)
Older	0.045 (–0.137, 0.229)	–0.002 (–0.345, 0.339)	0.018 (–0.188, 0.225)
Free oestradiol [pmol/L]			
Younger	5.614 (0.302, 11.199)*	15.991 (3.991, 27.992)*	7.813 (0.786, 14.840)*
Older	3.940 (–3.967, 11.847)	3.393 (–11.494, 18.282)	3.015 (–5.948, 11.979)
Bioavailable oestradiol [pmo/L]			
Younger	0.172 (–0.004, 0.386)	0.531 (0.070, 0.991)*	0.248 (–0.020, 0.517)
Older	0.160 (–0.145, 0.465)	0,138 (–0.436, 0.713)	0.122 (–0.223, 0.468)
SHBG [nmol/L]			
Younger	–0.890 (–1.487, –0.294)*	–1.824 (–3.164, –0.483)*	–1.049 (–1.817, –0.281)*
Older	–0.206 (–0.728, 0.315)	–0.325 (–1.298, 0.647)	–0.215 (–0.802, 0.371)

*p < 0.05

Table V. *Means (SD) of hormone levels, SHBG and QUS parameters in younger (18–30 years, n = 47) and older (31–39 years, n = 33) age groups. Comparisons with ANOVA or Mann-Whitney test*

Tabela V. *Średnie (SD) stężenia hormonów, SHBG i parametrów QUS u młodszych (18–30 lat, n = 47) i starszych (31–39 lat, n = 33) badanych. Porównania z zastosowaniem testu ANOVA lub testu Manna-Whitneya*

Variable	18–30 yrs	31–39 yrs	P value
Total T [nmol/L]	18.1 (4.5)	18.5 (6.1)	0.922
Free T [pmol/L]	429.0 (125.2)	415.0 (137.3)	0.463
Bio T [nmol/L]	11.1 (3.3)	10.4 (3.4)	0.218
Total E2 [pmol/L]	122.2 (41.8)	101.3 (37.4)	0.031
Free E2 [pmol/L]	2.7 (0.9)	2.2 (0.9)	0.008
Bio E2 [pmol/L]	70.2 (25.3)	54.4 (22.2)	0.006
SHBG [nmol/L]	24.1 (8.4)	28.0 (13.1)	0.256
DHEAS [μmol/L]	7.8 (2.9)	6.6 (2.6)	0.053
LH [IU/L]	4.6 (1.8)	4.5 (1.9)	0.660
FSH [IU/L]	4.1 (2.3)	4.1 (2.1)	0.860
BMI [kg/m²]	24.6 (3.9)	26.1 (3.2)	0.034
SOS [m/s]	1565.6 (40.5)	1554.6 (34.9)	0.215
BUA [dB/MHz]	89.8 (18.3)	86.9 (18.8)	0.256
QUI	106.9 (23.2)	102.0 (21.1)	0.358

Bio — bioavailable

18 and 26 years of age, before the BMI becomes stable in later ages (A). Free (B) and bio-available E2 (C) levels declined beginning at the 30–32 year range and reaching their lowest points in the 36–39 age range. In turn, SHBG level increased in the 26–29 age range and in the 36–39 age range (Fig. 2).

Discussion

The immunoassay (IA) methods for gonadal sex hormone determination used herein have their limitations as to accuracy and precision, and switching to more specific mass spectrometry (MS) — based methods has been advocated. However, it has been recently shown that serum T levels show a high correlation over a broad concentration range when measured by IA and MS, indicating that IA is acceptable for T determination [15]. Although the IA/MS correlation was weaker in measuring E2 at lower concentrations, the correlation between IA and MS was high (R = 0.74, P < 0.001) at E2 concentrations higher than 40.8 pmol/L, and the IA sensitivity was shown to be 88.4% and specificity 88.6% at E2 concentrations above 120 pmol/L [15]. The levels of total E2 reported here ranged between 45.5 and 208.8 pmol/L, indicating the reliability of IA determinations. When comparing the clinical applicability of E2 data

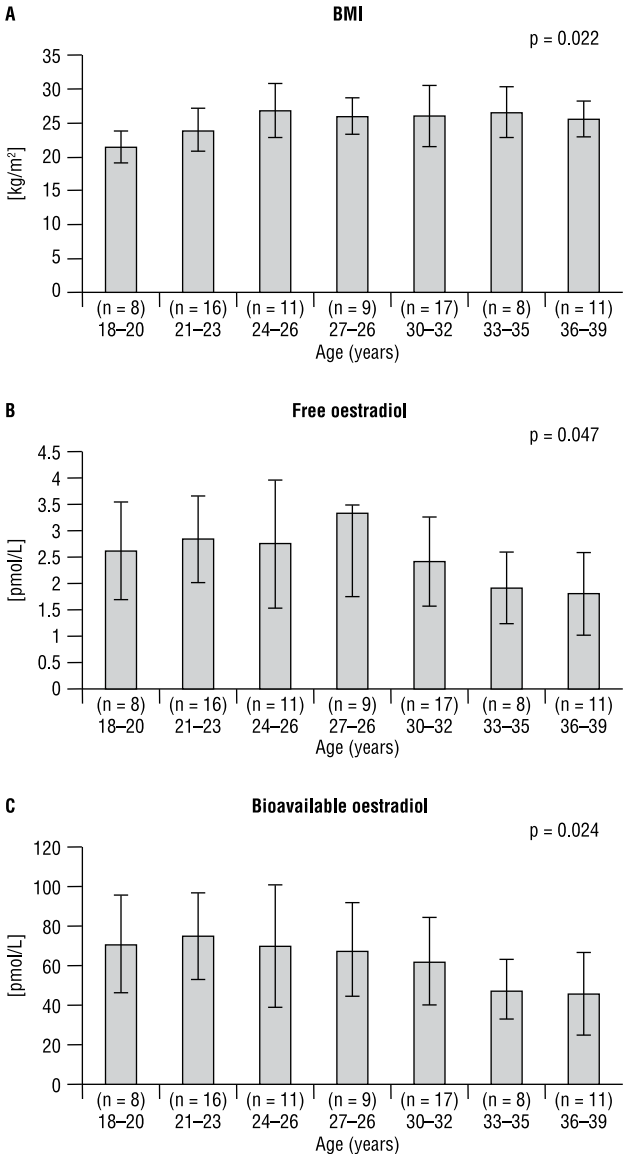

Figure 1. *Changes in body mass index (BMI) (**A**), serum level of free oestradiol (**B**) and bioavailable oestradiol (**C**) (mean ± SD) with dependence of age. P — one-way ANOVA test. Number of participants per age interval in parenthesis*

Rycina 1. *Zmiany wskaźnika masy ciała (BMI) (**A**), stężenia wolnego estradiolu w surowicy (**B**) i stężenia estradiolu biodostępnego (**C**) (średnia ± SD) w zależności od wieku. P — reprezentuje test ANOVA. W nawiasach podano liczbę badanych w poszczególnych przedziałach wiekowych*

in studies that included bone mass density, Kholsa et al. [16] and Ohlsson et al. [17] have concluded that although the MS data provides more accurate measurements, the use of IA for E2 determination in bone analysis is still valid.

We have shown that the levels of free and bioavailable fractions of both T and E2, but not other examined hormones, were positively associated with QUS parameters. Age influenced this issue because after dichotomisation for age, the associations re-

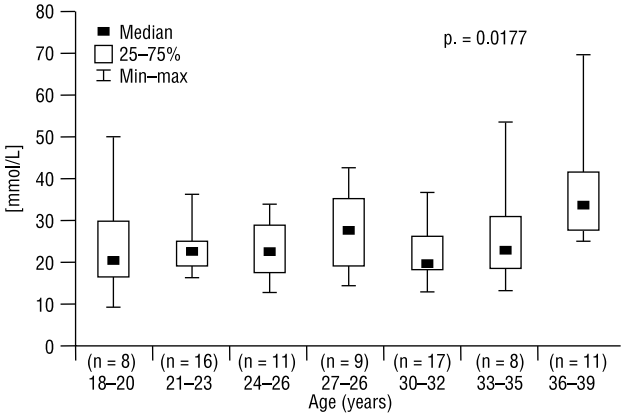

Figure 2. *Changes in serum level of sex hormone binding globulin (SHBG) with dependence of age (median, 25th and 75th percentile and minimum and maximum). P – Kruskal-Wallis test. Number of participants per age interval in parenthesis*

Rycina 2. *Zmiany stężenia białka wiążącego steroidy płciowe (SHBG) w surowicy w zależności od wieku (mediana, wartości 25. i 75. percentyli oraz wartości najniższej i janwyższej). P — reprezentuje test Kruskal'a-Wallis'a. W nawiasach podano liczbę badanych w poszczególnych przedziałach wiekowych*

mained significant only in individuals younger than 30 years. Differences in the number of cases recruited as either younger or older than 30 years (n = 47 in 18–30 years, and n = 33 in 31–39 years) might influence the findings, but it is more probable that during the third decade of life, which reportedly surrounds peak bone mass attainment [9–12], bones present stronger sensitivity to sex steroids than after the 30s and hence express significant associations with circulating biologically-active T and E2.

In contrast to other studies, we were not able to demonstrate a decline in total [18], free [13] or bioavailable [19] T levels in young men with dependence on an age. This discrepancy may arise from the different numbers of men examined (more numerous in our study) and differently selected age-spans [13, 18]. A new finding in our study is a decline in serum level of total E2 in the fourth decade of life. A decreased level of total E2 was followed by lowered blood concentrations of free and bioavailable fractions of E2. A decrease in aromatase activity, an enzyme converting T into E2, might be responsible. Scarce clinical material available in the literature has indicated that although aromatase activity in men does not correlate with age, the highest levels of aromatase in the blood are observed in men aged 18–20 years [20]. An age-dependent decrease in total and bioavailable E2 levels between young (22–39 years), middle-aged (40–59 years) and elderly (60–90 years) men has been described previously and attributed to the increased serum SHBG levels [21]. Our data suggests that these changes may initiate earlier, as soon as the 30th year of life. A decrease of total and free E2 levels in ageing men was described recently in the age span 55–90 years [22].

All fractions of E2 may play a crucial role in the maintenance of bone health in men, more important than T. In middle-aged and elderly men, total and free E2, but not T, blood levels positively correlated with bone health [4]. Aromatase deficiency (a rare autosomal recessive disorder) in young men, despite normal or elevated T levels, results in osteopenia and osteoporosis [5–8]. Our findings may suggest that age or age-related change in blood level of E2 influences the association between sex steroid levels and bone status in young men. E2 may facilitate the effect of T in bones. It cannot be excluded that a specific E2 threshold may exist in young men to facilitate the promotion of bone health by sex steroids. It has been suggested than age-related decreases in bioavailable E2 levels to less than 40 pmol/L may be the major cause of bone loss in elderly men [21].

The disappearance of the associations between sex hormones and bone quality in men older than 30 years observed herein may be transient or relative. It has been demonstrated that in men older than 40 years, T and E2 may act in concert to improve bone status. Dual-energy x-ray absorbtiometry (DEXA) of the radius revealed that while bioavailable E2 was responsible for the maintenance of cortical and trabecular bone mass density (BMD), bioavailable T affected bone health through associations with muscle mass and bone area in men [23].

The fact that SHBG binds, but also inactivates, sex steroids is a determinant of the bioavailability for tissue action. In accordance with most [24–27] but not all earlier observations [28, 29], circulating SHBG was found to negatively correlate with bone parameters. After adjustment for other co-variants, SHBG retained its negative association with bone status only in the younger subgroup. This suggests that SHBG has a special importance in the regulation of bones in men under 30. The increase of SHBG level between 18 and 29 years of age and in the oldest age range (36–39 years) may be in accordance with previous reports [30, 31]. Although experimental studies have shown that SHBG limits access of T to some target tissues such as brain, salivary gland, lymph node and prostate, some clinical data on BMD investigation renders against free hormone hypothesis. Namely, in some studies, higher levels of SHBG have been shown to be associated with higher BMD in men. Accordingly, SHBG may not only be an inhibitor, but also a facilitator (or both) of sex steroid action [32].

LH and FSH levels did not relate to QUS parameters, suggesting that SHBG and albumins, but not supratesticular stimulating hormones, play a primary role in regulating the availability of sex steroid in bones. No association between adrenal sex steroid DHEAS and QUS parameters was present, but it has been shown that DHEAS is active mostly in men of less than 20 years of age. Indirect evidence exists that an association between DHEAS and bone accrual would be better demonstrated in young women rather than men. Namely, the strength of the negative correlation between DHEAS level and bone turnover is higher in young females than in males [18].

The limitation of this study is the low number of cases investigated. The overall response rate for participation was 26.7%. This might be because young men were invited. In the European Male Ageing Study, exploring the relationship between gonadal steroid status and bone health in the older population, the response rate was 45% [4, 33]. Any factors influencing participation, however, are unlikely to have influenced the association between hormones and QUS parameters, which are based on an internal comparison of those who participated.

The main strength of our study is that it is based on the use of standardised methods to assess bone health and that hormone measurements were performed in one run to minimise assay variability. Our results are based on assessment of the calcaneus, a trabecular bone which may be more sensitive to sex hormone levels than cortical bone. The results may therefore be difficult to extrapolate to other skeletal sites. Unlike DEXA, there are no published methods for cross calibration between QUS scanners, but the results reported are the data obtained at one centre. In ageing men, QUS measurements predict the risk of any non-spine fracture almost as well as hip DEXA measurements [9].

In summary, free and bioavailable fraction levels of T and E2 in the circulation are associated positively, and SHBG level negatively, with the bone status of men until the end of their third decade of life and until the blood level of E2 begins to decline. The influence of gonadal steroids on bones in young adult men may depend on age and blood level of E2.

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