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The reference values of sex hormones and SHBG serum levels in subjects over 65 years old — The PolSenior Study

Wartości referencyjne stężenia hormonów płciowych oraz SHBG w surowicy u osób powyżej 65. roku życia — badanie PolSenior

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Abstract

Introduction: Over the last decade, average life expectancy has continuously increased. There has been no data on normal sex hormone (SH) levels in a Polish elderly population. In this study, we assessed SH in the PolSenior cohort to determine normal reference ranges in relation to gender, age, and cardiovascular disease risk factors (CVDRFs).

Material and methods: The study was performed with 4,352 participants (2,168 men and 2,088 women), aged from 55 to over 90 years, stratified in five-year age groups. Pre-elderly subjects (55–59 years of age) served as the reference group. We assessed total testosterone (TT), estradiol (TE_2) and DHEA-S (by RIA) SHBG and FSH (by IRMA) and calculated free androgen and free estrogen indices (FAI and FEI). Percentage body fat (%BF) was measured by bioelectric impedance analysis.

The CVDRFs assessment included blood pressure and biochemical (blood glucose, high-density lipoproteins, triglycerides) and anthropometric (waist circumference) components of the metabolic syndrome.

Results: TT was low in 19.9%, normal in 78.2%, and high in 1.8% of men. TE_2 was low in 94.6% of women. Age and CVDRFs significantly influenced values of SHBG, FSH, TT, FAI, FEI, and DHEA-S in men, while in women values of FSH, TT and TE_2 did not change. BMI and %BF affected SH regardless of the age groups and CVDRFs.

Conclusions: Our findings suggest that the reference ranges stratified by the five-year age bands seem more accurate than those given for the overall population over 60 years of age. The clinical relevance of these reference ranges increases when they are considered in relation to CVDRFs, BMI and %BF. **(Endokrynol Pol 2013; 64 (2): 82–93)**

Key words: *testosterone, estradiol, indices of free sex hormones, SHBG, DHEA-S, elderly people, reference values*

Streszczenie

Wstęp: W ostatniej dekadzie wzrasta przewidywana długość życia społeczeństw. W Polsce brak danych o stężeniach hormonów płciowych (SH) u starzejących się osób. Celem naszej pracy było przedstawienie wartości referencyjnych SH w grupie osób uczestniczących w badaniu PolSenior w zależności od płci, wieku i czynników ryzyka chorób układu sercowo-naczyniowego (CVDRFs).

Materiał i metody: Badania wykonano u 4352 osób (2168 mężczyzn i 2088 kobiet) w wieku od 55. do powyżej 90. roku życia, podzielonych na 5-letnie przedziały wieku. Osoby na przedpolu starości (wiek 55–59 lat) stanowiły grupę referencyjną. Oznaczano testosteron całkowity (TT), estradiol całkowity (TE_2) i DHEAS metodami RIA, FSH i SHBG metodą IRMA wyliczono wskaźniki wolnych androgenów (FAI) i estrogenów (FEI). Odsetek tłuszczu ciała (%BF) oznaczano metodą bioimpedancji. CVDRFs analizowano w odniesieniu do wartości ciśnienia tętniczego oraz biochemicznych (stężenie glukozy, HDL i triglicerydów) i antropometrycznych (obwód talii) składowych zespołu metabolicznego.

Wyniki: Prawie 20% mężczyzn miało stężenia TT poniżej normy, 78,2% w zakresie norm a 1,8% powyżej górnego zakresu normy. Stężenia TE_2 poniżej normy dla kobiet po menopauzie miało 94,6% kobiet. Wiek i liczba CVDRFs istotnie zmieniały stężenia SH za wyjątkiem TE_2 u mężczyzn i FSH i TT u kobiet. BMI i %BF istotnie wpływało na większość oznaczanych hormonów niezależnie od wieku.

Wnioski: Przedstawiono wartości referencyjne dla stężeń SH u mężczyzn i kobiet od 65. do powyżej 90. roku życia. Nasze badania sugerują, że wartości referencyjne przedstawione w 5. letnich przedziałach wieku wydają się bardziej precyzyjne niż dla całej populacji powyżej 60. roku życia. Ich przydatność kliniczna zwiększa się, jeśli uwzględni się liczbę CVDRFs oraz BMI i %BF. **(Endokrynol Pol 2013; 64 (2): 82–93)**

Słowa kluczowe: *testosteron, estradiol, wskaźniki wolnych hormonów, SHBG, DHEA-S, ludzie w podeszłym wieku, wartości referencyjne*

Introduction

Over the last decade, average life expectancy has continuously increased. In Poland, compared to the middle of the last century, men live longer by approximately 16 years and women by almost 19 years, including individuals over 60 years old. In 2010, the predicted life expectancy at age 75 was 9.47 years for men and

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11.85 years for women [1]. Therefore, there have been increasing numbers of multidisciplinary studies aiming to assess the age-dependent health risk factors that affect life expectancy in the elderly. The PolSenior Study is the first cross-sectional, national, and multicentre health survey designed to evaluate the medical, psychological, and socio-economic aspects of ageing in Poland [2].

The ageing process, which is still poorly understood, seems to be associated, among a variety of mechanisms, with a decline in the activity of the hypothalamic-pituitary-gonadal axis in postmenopausal women, and, to a lesser extent, in men over 65 years old. Many symptoms observed in elderly people such as the decrease in physical activity associated with decline in muscle mass and the weakening of muscle contraction, mood changes, impaired cognitive processes, impaired sexual performance, increased rate of obesity and bone mass deterioration that lead to disability, may be associated, at least partially, with impaired gonad function. The main sex hormones, including testosterone in men and estrogen in women, are transported in the blood to target cells by carrier proteins, mainly by sex hormone-binding globulin (SHBG) and albumin. The sex hormones' biological activity is associated with albumin-bound and albumin-free fractions that decide on their bioavailability. In men over 60 years of age, the main sources of estrogens are the liver, skin, and adipose tissue, where biosynthesis of estrogen is based on aromatisation of their adrenal precursors [3]. In women, estrogens are synthesised also in ovarian theca cells [4].

The few studies performed on subjects aged 60–65 years have demonstrated that, in men, SHBG levels increase and free testosterone (FT) decreases with age. The reports on total testosterone (TT) in men over 65 have yielded conflicting results. Some studies have shown a progressive decrease in TT levels [5, 6], while others have not found significant age-dependent changes in TT [7, 8]. Similarly, there have been only a few studies that have provided inconsistent results regarding total estradiol (TE_2) levels and free estradiol index (FEI) in ageing men [5].

In women over 60 years of age, as in men, studies have shown increases in SHBG levels with age [9, 10]. In women over 70 years of age, some studies have demonstrated decreases in TT, FT, and dehydroepiandrosterone sulfate (DHEA-S) levels [11]. On the other hand, van Geel et al. [12] found that, in women aged 55–84 years, SHBG, TT, FAI, and TE_2 did not change, while FEI significantly decreased with age.

SHBG and sex hormone levels in the elderly are influenced by many factors. Aside from chronic kidney and liver diseases, obesity, thyroid diseases, hypopituitarism, dyslipidaemia, insulin resistance and type 2 diabetes, metabolic cardiovascular risk factors (CVDRFs), heart failure, and coronary heart disease may influence serum concentrations [13–15]. Increasing evidence suggests that the normal reference values for sex hormones in elderly subjects should be determined because they could help both in the assessment of hypogonadism rates [16] and in monitoring the safety and efficacy of hormone replacement therapy in elderly patients.

In this study, we assessed sex hormone levels in the PolSenior cohort, stratified by five-year age groups, to determine reference ranges in relation to gender, age, and cardiovascular disease risk factors (CVDRFs).

Material and methods

Study population

This study was performed with 5,695 subjects (2,899 men and 2,796 women) participating in the PolSenior Study, who were randomly selected from 16 administrative centres of Poland by the three-stage, proportional, and stratified-by-age group selection process, as described elsewhere [2]. In brief, stratified random sampling was used with the aim of recruiting elderly men and women in six five-year age groups: 65–69, 70–74, 75–79, 80–84, 85–89, and 90 years and over. Additionally, from the same cohort, pre-elderly subjects aged 55–59 years served as the reference group. Subjects were invited by letter to attend a session for an interviewer-administered questionnaire; assessment of height, weight, and waist and hip circumference, and blood sampling. The structured questionnaire (available at http://polsenior.iimcb.gov.pl/ankiety/) included details about medical history and socio-economic status of the subjects. Blood sampling, blood pressure and measurements of height, weight, waist circumference, and hip circumference were performed according to the standard protocol, as described elsewhere [17]. We included only those responders who were able to complete study procedures, and excluded immobilised patients.

The study complied fully with all applicable institutional and governmental regulations concerning the ethical use of human volunteers and with the terms of the Helsinki Declaration. The institutional review board approved the study protocol, and all the recruited subjects gave their written informed consent.

Anthropometric measurements

Height, waist circumference (WC) and hip circumference were measured to the nearest 0.5 cm. Percentage body fat (%BF) was measured by bioelectric impedance (Tanita BC-536, Tanita Corporation, Japan). %BF was calculated from the measurements of resistance made at 50 kHz using the formula provided by the manufacturer.

Assays

We collected blood samples from 2,168 men and 2,088 women. Biochemical assessments included SHBG, FSH, TT, TE_2, DHEA-S, lipid profiles, fasting glucose and insulin were measured in all subjects in one batch of serum or plasma that had been thawed for the first time. Blood samples were divided in aliquots and frozen at –20°C until further analyses. Sex hormones, DHEA-S and insulin were assessed by radioimmunological methods using Wallac 1470 Wizard gamma counter. SHBG and FSH were assessed by IRMA using commercially available assays (Immunotech, Prague). TT, TE_2, DHEA-S, and insulin were assessed using the Siemens assays (Los Angeles, USA). The free androgen index (FAI) and FEI were calculated from SHBG, TT, and TE_2 using the following formulas: FAI = TT(nmol/L) × 100/SHBG(nmol/L); FEI = 100 × E_2 (pmol/L)/SHBG (nmol/L) [18, 19]. Blood glucose and lipid profiles, including total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG) were assessed by automatic methods (Modular PPE, Roche Diagnostics). Insulin resistance was calculated from fasting glucose and insulin using the following formula: HOMA-IR (Homeostasis Model Assessment– Insulin Resistance) = glucose (mmol/L) × insulin (mIU/L)/22.5 [20].

The CVDRFs assessment included waist circumference, blood pressure, blood glucose, HDL, and TG, according to current guidelines and diagnostic criteria for metabolic syndrome [21].

Statistical analysis

The distribution of continuous variables was tested for normality by the Kolmogorov-Smirnov test. Because the majority of the studied variables showed markedly skewed distribution, logarithmic transformations of these measurements were performed before calculations, and the results were presented as median (range), median (25–75 quartiles) or mean (95% CI). Differences among the groups were evaluated by Mann-Whitney U test or Wilcoxon signed rank test as appropriate. Intra-gender differences (between age bands, separately for men and women) were evaluated using ANOVA/MANOVA followed by Tukey's post-hoc test. Linear Spearman's rank correlation coefficients and multiple regression analyses were used to determine relationships between studied variables. We used the 5% significance levels for all tests. All calculations were performed with the Statistica 9.0 software package (StatSoft, Tulsa, OK, USA).

Results

Baseline characteristics are shown in Table I. As expected, men had higher TE_2, FEI and DHEA-S levels than women. They had also more favourable lipid profile. SHBG concentrations were comparable in both sexes. Medical history revealed that overall prevalence of known diabetes was 14.5% (308 subjects) in men and 18% (396 subjects) in women. However, in the studied cohort, as many as 33.8% of individuals (1,202 men and women) had increased fasting glucose concentration, of whom nearly two thirds had glucose levels above 7.0 mmol/L (126 mg/dL). TT was low in 19.9%, normal in 78.2% and high in 1.8% of men compared to the normal_reference range for men aged 60 years. Moreover, almost all men (99.6%) had TE_2 levels below the normal reference range. On the other hand, only 3.6% of females had TE_2 levels above reference values for postmenopausal women. Compared to the normal reference ranges, TT was lowered in 46.5%, normal in 50.1%, and increased in 3.4% of women. Sex hormones, SHBG, and DHEA-S significantly correlated with the majority of anthropometric and biochemical CVDRFs; the results of these analyses have been published elsewhere [22].

The results of multiple regression analyses that assessed the associations of sex hormones and DHEA-S with selected anthropometric (Model 1) and biochemical (Model 2) parameters as independent variables are shown in Table II. Sex hormones (except for FSH in women) and DHEA-S were strongly associated with age.

Changes in SHBG, TT, FAI, FEI, FSH, and DHEA-S levels within age groups analysed by one-way ANOVA are shown in Table III and Figure 1. In men, compared to the pre-elderly and across some of the elderly age groups, mean values of FSH, TT, FAI, FEI, and DHEA-S decreased and SHBG increased. In women, as in men, SHBG increased and DHEA-S and free sex hormone indices decreased, while FSH and TT did not change with ageing.

Among all the factors that influenced sex hormone levels, the majority were CVDRFs. Overall, more than three CVDRFs were present in as many as 41.4% of men and 58.6% of women. Although the majority of CVDRFs — adjusted for gender, age, and BMI — were strong single predictors of sex hormone levels in multiple regression analysis, further analyses using MANOVA revealed that, in women, only age (stratified by age groups) and number of CVDRFs (< 3 or ≥ 3) were significant determinants of sex hormone levels (Table IV, Figure 2). In contrast, in men, the number of CVDRFs and age were associated with sex hormone levels only in a MANOVA model that also included %BF ($p = 0.04$). In women, %BF (< 30% or ≥ 30%) was a third factor that significantly modified sex hormone levels.

Median values of SHBG, TT, FAI, TE_2, FEI and DHEA-S in subjects with more and less than three

Table I. *Baseline characteristics. Values are expressed as median and Q25–Q75*
Tabela I. *Charakterystyka badanych. Wartości przedstawiono jako mediana i Q25–Q75*

	Men (n = 1,649)	Women (n = 1,547)	Z test
Age (years)	76.0 [69.0–85.0]	74.0 [67.0–83.0]	ns
Height [cm]	170.0 [164.0–173.6]	156.0 [152.0–161.0]	$p < 0.000$
Body mass [kg]	77.7 [69.0–87.2]	69.5 [60.4–80.0]	$p < 0.000$
Waist circumference [cm]	100.0 [93.0-109.0]	96.0 [87 0–105.0]	$p < 0.000$
Hip circumference [cm]	104.0 [100.0–110.0]	108.0 [101.0–116.0]	$p < 0.02$
BMI [kg/m^2]	27.2 [24.6–30.1]	28.3 [25.1–32.4]	$p < 0.000$
%BF	27.7 [22.6–32.9]	37.4 [31.8–41.9]	$p < 0.000$
SHBG [nmol/L]	51.7 [38.6–70.2]	58.2 [41.8–80.8]	ns
FSH [IU/L]	10.1 [6.4–19.5]	59.7 [44.0–75.0]	$p < 0.000$
TT [nmol/L]	15.3 [11.3–19.4]	0.8 [0.4–1.1]	$p < 0.000$
FAI	29.8 [21.7–39.6]	1.3 [0.7–2.2]	$p < 0.000$
TE_2 [pmol/L]	48.4 [36.0–63.4]	23.8 [18.3–31.2]	$p < 0.000$
FEI	91.6 [59.3–141.2]	41.3 [26.1-66.2]	$p < 0.000$
DHEA-S [mg/dL]	766.0 [463.0–1,169.0]	543.0 [335.0–886.0]	$p < 0.001$
Total cholesterol (TC) [mmol/L]	5.1 [4.3–5.8]	5.5 [4.7–6.3]	$p < 0.004$
LDL [mmol/L]	2.9 [2.3–3,7]	3.2 [2.5–4.0]	ns
HDL [mmol/L]	1.2 [0.9–1,5]	1.4 [1.1–1.6]	$p < 0.000$
Triglycerides (TG) [mmol/L]	1.2 [0.9–1.6]	1.4 [1.1–1.82]	$p < 0.000$
Glucose [mmol/L]	5.3 [4.8–6.1]	5.2 [4.8–5.9]	ns
Insulin [mIU/L]	5.1 [2.8–9.4]	6.3 [3.6–10.6]	ns
HOMA IR	1.2 [0.6–2.4]	1.5 [0.8–2.7]	ns
Diabetes (%)	14.5	18.0	
Hypertension (%)	57.0	41.3	

Z — Wald-Wolfowitz test

CVDRFs are given in Table V. In both genders, all studied parameters (except for estradiol in men) significantly differed between subjects with ≥ 3 and < 3 CVDRFs. Sex hormones and SHBG concentrations significantly changed with age in men; however, changes in SHBG and FEI were more pronounced in individuals with more than three CVDRFs. FSH and TT in women did not change with age, and CVDRFs and individual significant changes in other parameters were not observed in all age groups. Overall, we found significant differences in sex hormone levels (except FSH and TT) between women with less and more than three CVDRFs, but these differences were less pronounced than in men (Fig. 2).

In univariate analysis, the associations of BMI and %BF with SHBG, FSH, FEI, and DHEA-S, although statistically significant, explained only a small percentage of the variation in sex hormones, except for SHBG level. In women, as in men, BMI markedly influenced the variation in SHBG. However, the impact of BMI on variations in other sex hormones (including FAI) was relatively low (Table VI).

Discussion

In this study, we determined, for the first time, reference ranges for SHBG, TT, TE_2, FAI, FEI, FSH, and DHEA-S in Polish men and women over 65 years of age, stratified by five-year age groups and including subjects over 90 years of age. In the literature, there have been only a few reports on the reference values of sex hormones in people over 65 years of age [5, 11, 23–25]. In our study, sex hormone levels showed high individual variations, which has also been reported in earlier studies [5, 23]. These variations may result, at least partially, from the relatively less strict inclusion criteria applied in our study. However, they may also result from well-known environmental and metabolic factors as well as less-known genetic factors, which account for approximately 30-60% of the variation in

Table II. *Most relevant associations between SHBG, DHEA-S, FSH, TT, TE_2, FAI, FEI, age and anthropometric (β1) and metabolic (β2) measurements*

Tabela II. *Najważniejsze zależności między SHBG, DHEA-S, TT, FSH, TT, TE_2, FAI, wiekiem i pomiarami antropometrycznymi (β1) oraz metabolicznymi (β2)*

	Men							Women						
	SHBG	FSH	TT	FAI	E_2	FEI	DHEA-S	SHBG	FSH	TT	FAI	E_2	FEI	DHEA-S
	[nmol/L]	[IU/L]	[nmol/L]		[pmol/L]		[mg/dL]	[nmol/L]	[IU/L]	[nmol/L]		[pmol/L]		[mg/dL]
	β1	β1	β1	β1	β1	β1	β1		β1	β1	β1	β1	β1	β1
Age (years)	0.25[c]	0.37[c]	−0.23[c]	0.36[c]	−0.12[c]	−0.27[c]	−0.49[c]		0.23[c]	ns	−0.10[c]	−0.07[b]	−0.21[c]	−0.27[b]
BF%	−0.08[b]	ns	−0.08[c]	ns	ns	ns	ns		−0.06[a]	ns	0.08[a]	ns	ns	ns
WC [cm]	−0.13[b]	−0.08[b]	−0.10[b]	ns	ns	ns	ns		−0.17[c]	−0.14[c]	ns	ns	0.09[a]	ns
BMI [kg/m^2]	−0.18[c]	ns	ns	0.08[a]	ns	0.17[c]	ns		−0.24[c]	−0.22[c]	0.21[c]	0.26[c]	0.33[c]	ns
	β2	β2	β2	β2	β2	β2	β2		β2	β2	β2	β2	β2	β2
TC [mmol/L]	ns	0.14[b]	ns	ns	0.06[a]	ns	0.24[c]		0.21[c]	ns	ns	ns	−0.18[b]	ns
LDL [mmol/L]	ns	ns	ns	ns	ns	ns	ns		−0.15[b]	ns	ns	ns	0.16[b]	ns
HDL [mmol/L]	0.07[b]	ns	ns	ns	ns	−0.07[a]	0.08[b]		ns	0.18[c]	ns	ns	ns	ns
TG [mmol/L]	−0.22[c]	−0.07[a]	−0.14[c]	ns	ns	0.15[c]	0.10[c]		−0.25[c]	ns	0.09[b]	ns	0.17[c]	ns
HOMA–IR	−0.07[c]	ns	ns	ns	ns	ns	ns		−0.25[c]	−0.12[c]	0.22[c]	0.17[c]	0.28[c]	0.12[c]

[a]$p < 0.05$; [b]$p < 01$; [c]$p < 0.001$; Model 1 (β1) includes age, BF%, waist circumference, and BMI; Model 2 (β2) includes TC, LDL-C, HDL-C, TG, and HOMA IR; TC — total cholesterol

SHBG [26] — a globulin that determines both binding and bioavailability of sex hormones. We have demonstrated that, compared to the pre-elderly and across the elderly age groups, mean values of TT, DHEA-S, FAI, and FEI decreased, SHBG increased, and TE_2 decreased only in men over 85 years of age. In women, as in men, SHBG increased, free sex hormones indices and DHEA-S decreased, but TT, FSH and TE_2 (except for women 90 years of age) did not change with age. Overall, these findings are consistent with the results of earlier studies [5, 7], although, unlike our study, they could not find any associations between age and TT in men. We also confirmed previous reports that have found increases in SHBG levels with age in women [9, 10]. On the other hand, in line with some studies [11, 12], but in contrast to other studies [24, 23], we could not demonstrate the impact of ageing on TT levels in women. As in men, we observed age-dependent decreases in DHEA-S, FAI, and FEI in women. These discrepancies may result from differences in methods of hormone assessment and their free fraction calculation, ethnicity, age of studied populations, and sample size.

The cross-sectional nature of our study does not allow exploration of a cause-effect relationship; thus, our results do not explain age-dependent increases in SHBG levels in the ageing population. However, our results seem to confirm that some of these changes might be associated with changes in insulin sensitivity and BMI, which progressively decrease with age (data not shown). Free sex hormones indices seem to reflect changes in SHBG levels. Moreover, the progressive FSH increase and TT decrease observed in our study may suggest that the age-related decline of gonadal function in elderly men is rather a slow process, while in women, the pituitary-gonadal axis seems to remain relatively stable throughout the entire postmenopausal period up to 85 years of age.

Our results suggest that both in men and women, CVDRFs may influence sex hormone levels. Men with more than three CVDRFs had decreased values of SHBG and TT, higher FAI and FEI, but similar TE_2 compared to those with fewer CVDRFs. These findings are consistent with the majority of previous studies [8, 9, 27] and Maggio et al. [28], who found lowered TE_2 concentration in older men in the InCHIANTI Study. In our study, women with three or more CVDRFs, like men, had lowered SHBG levels but higher TT, TE_2, and FAI, which has also been reported by previous studies [9, 29] and confirmed in a recent meta-analysis of 52 observational studies [27]. In addition to these observations, we also found in this population higher FEI and DHEA-S concentrations.

Based on our results and the results of previously cited reports, we present, for the first time in

Table IIIA. *Mean and 95% confidence intervals (antilogarithms) values of SHBG, FSH, TT, FAI, TE_2, FEI and DHEA-S levels in five-year age bands in men*

Tabela IIIA. *Średnie i 95% przedziały ufności (antylogarytmy) stężenia SHBG, FSH, TT, FAI, TE_2, FEI i DHEA-S w 5-letnich przedziałach wiekowych u mężczyzn*

Age group (years)	n	SHBG [nmol/L]	FSH [UI/L]	TT [nmol/L]	FAI	E2 [pmol/L]	FEI	DHEA-S [ng/mL]
55–59	233	41.6	6.9	16.1	38.6	50.8	122.0	1,397.8
		39.3–44.1	6.3–1.1	14.6–17.1	35.0–42.6	47.8–53.9	112.5–132.4	1,288.2–1,516.8
65–69	273	45.3	8.5	15.7	34.6	49.0	108.0	973.1***
		42.0–47.9	7.7–9.3	14.3–17.2	31.5–38.1	446.2–51.9	99.8–116.8	899.1–1,053.2
70–74	317	50.3***	10.1	14.2	28.2**	46.6	92.6***	802.8***
		47.7–53.0	9.2–11.1	13.0–15.5	25.8–30.9	44.1–49.2	85.9–99.8	744.4–865.8
75–79	312	53.2***	12.1***	15.0	28.2**	49.3	92.6***	737.9***
		50.6–56.0	11.0–13.2	13.8–16.4	25.8–30.8	46.7–52.0	86.1–99.5	685.9–793.8
80–84	286	57.0***	13.1***	12.5***	22.0***	45.3	79.4***	590.1***
		53.9–60.3	11.9–14.5	11.4–13.8	19.9–24.2	42.7–48.1	73.3–86.1	544.1–640.0
85–89	315	61.7***	17.1***	10.8***	17.6***	44.4**	72.0***	510.6***
		65.0–65.0	15.6–18.8	9.9–11.9	16.0–19.2	42.0–47.0	66.8–77.6	473.5–550.6
> 90	219	68.5***	22.8***	9.5***	13.8***	41.4**	60.5	408.1
		64.5–72.6	20.6–25.3	8.5–10.5	12.5–15.3	38.9–44.1	55.6–65.8	374.8–444.3
p (ANOVA)		p < 0.000	p < 0.000	p < 0.000	p < 0.000	p < 0.000	p < 0.000	p < 0.000

Differences in serum SHBG, FSH, TT, FAI, TE_2, FEI, and DHEA-S levels between age groups were calculated using Tukey's post hoc test ; **p > 0.00 *v.* group of 55–59 year; ***p > 0.000 *v.* group of 55–59 year

Table IIIB. *Mean and 95% confidence intervals (antilogarithms) values of SHBG, FSH, TT, FAI, TE2, FEI, and DHEA-S in five-year age bands in women*

Tabela IIIB. *Średnie i 95% przedziały ufności (antylogarytmy) stężenia SHBG, FSH, TT, FAI, TE2, FEI i DHEA-S w 5-letnich przedziałach wiekowych u kobiet*

Age group (years)	n	SHBG [nmol/L]	FSH [IU/L]	TT [nmol/L]	FAI	E2 [pmol/L]	FEI	DHEA-S [ng/mL]
55–59	288	49.7	56.3	0.72	1.45	26.5	53.2	779.6
		47.1–52.4	52.8–60.1	0.66–0.79	1.31–1.61	25.1–27.9	49.1–57.6	719.4–844.8
65–69	285	51.9	55.5	0.71	1.37	24.6	47.4	647.2***
		49.0–55.0	51.7–59.5	0.65–0.78	1.23–1.53	23.2–26.0	43.4–51.6	593.4–706.0
70–74	319	55.8	56.3	0.75	1.34	23.8	42.7	534.0***
		52.7–59.1	52.5–60.3	0.68–0.82	1.21–1.50	22.5–25.2	39.2–46.6	489.9–582.2
75–79	262	59.3***	49.4	0.72	1.21	24.4	41.2***	451.3***
		55.8–63.1	45.8–53.2	0.65–0.80	1.08–1.36	23.0–26.0	37.6–45.1	411.4–494.9
80–84	223	65.6***	53.7	0.70	1.06*	23.1	35.2***	395.9***
		61.6–70.0	49.7–58.0	0.63–0.77	0.94–1.19	21.7–24.6	32.0–38.7	359.7–435.8
85–89	237	71.8***	53.9	0.71	0.99***	23.3	32.4***	444.0***
		67.6–76.3	50.0–58.0	0.64–0.78	0.88–1.11	21.9–24.8	29.6–35.5	405.3–486.4
> 90	201	80.0***	59.4	0.74	0.93***	22.2*	27.8***	378.4***
		75.0–85.3	55.0–64.2	0.67–0.83	0.82–1.05	20.8–23.7	25.2–30.5	343.7–416.6
p (ANOVA)		p = 0.000	p = 0.0799	p = 0.8903	p = 0.002	p = 0.000	p = 0.000	p = 0.000

Differences in serum SHBG, FSH, TT, FAI, TE_2, FEI, and DHEA-S levels between age groups were calculated using Tukey's post hoc test ; *p < 0.05 *v.* group of 55–59 yr; ***p < 0.000 *v.* group of 55–59 yr

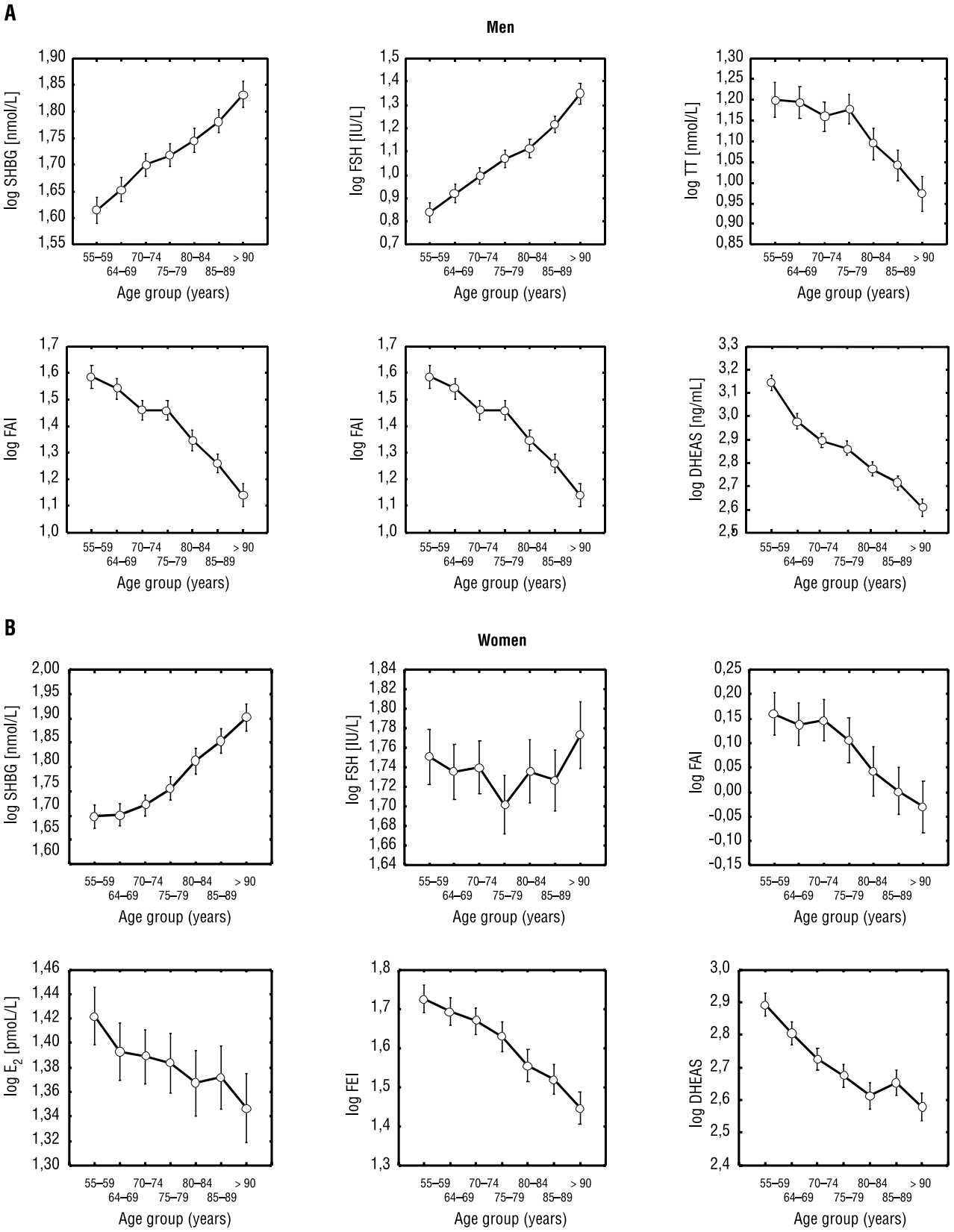

Figure 1A. *Distribution of values of SHBG, FSH, TT, FAI, FEI and DHEA-S concentrations (mean and 95% CI; logarithms) by age groups in men. TE_2 decreased significantly only in subjects aged > 85 (not shown);* **B**. *Distribution of values of SHBG, FSH, FAI, TE_2, FEI and DHEAS concentrations (mean and 95% CI; logarithms) by age groups in women. FSH and TT (not shown) did not change. TE_2 decreased significantly only in subjects aged > 90*

Rycina 1A. *Rozkład stężeń SHBG, FSH, TT, FAI, FEI i DHEA-s (średnia i 95% CI; logarytmy) w grupach wieku u mężczyzn. Stężenia TE_2 zmniejszały się tylko u osób > 85. roku życia (nie przedstawione);* **B.** *Rozkład stężeń SHBG, FSH, FAI, TE_2 , FEI i DHEA-S (średnia i 95% CI; logarytmy) w grupach wieku u kobiet. Stężenia FSH i TT (nie przedstawione) nie zmieniały się. Stężenia TE_2 zmniejszały się istotnie dopiero > 90. roku życia*

Table IVA. *Mean and 95% confidence intervals (antilogarithms) values of SHBG, FSH, TT, FAI, TE_2, FEI and DHEA-S in five-year age bands in men with CVDRF < 3 and CVDRF ≥ 3. Differences between age groups were calculated using Tukey's post hoc test. MANOVA; the Wilks test = 0.97; p = 0.04*

Tabela IVA. *Średnie i 95% przedziały ufności (antylogarytmy) stężenia SHBG, FSH, TT, FAI, TE_2, FEI i DHEA-S w 5-letnich przedziałach wiekowych u mężczyzn z CVDRF < 3 i CVDRF ≥ 3. Różnice między przedziałami wieku oceniano testem post hoc Tukey'a. MANOVA; test Wilksa = 0.97; p = 0.04*

	Age group (years)	n	SHBG [nmol/L]	FSH [IU/L]	TT [mmol/L]	FAI	TE2 [pmol/L]	FEI	DHEAS [ng/mL]
CVDRF < 3									
	55–59	122	52.7	7.3	19.7	37.4	52.9	100.3	1,401.8
			48.4–57.5	6.2–8.6	16.9–23.0	32.0–43.8	48.1–58.3	88.3–114.0	1,231.2–1,596.1
	65–69	113	53.6	8.6	16.7	31.1	46.6	87.0	969.6
			48.6–59.1	7.2–10.3	14.0–19.9	26.0–37.2	41.8–52.0	75.2–100.5	836.5–1,123.7
	70–74	144	63.2	9.6	17.6	27.9	44.7	70.8	767.6
			58.0–68.8	8.3–11.3	15.2–20.5	23.9–32.6	40.7–49.2	62.4–80.3	675.1–872.8
	75–79	146	59.8	11.0	18.2	30.3	55.8	93.2	774.2
			54.1–66.2	9.2–13.3	15.2–21.7	25.3–36.4	49.9–62.3	80.4–108.0	666.3–899.6
	80–84	134	62.2	12.6	12.9	20.7	44.0	70.7	582.6
			57.4–67.4	10.9–14.7	11.1–14.8	17.8–23.9	40.2–48.1	62.7–79.6	516.1–657.6
	85–89	173	68.2	15.7	13.1	19.1	44.7	65.4	500.4
			63.5–79.6	13.8–17.9	11.5–14.8	16.8–21.8	41.2–48.4	58.8–72.8	449.1–557.6
	> 90	122	70.9	23.0	10.2	14.3	45.0	63.5	392.5
			65.3–76.9	19.8–26.7	10.2–11.7	12.4–16.6	41.1–49.3	56.3–71.6	347.4–443.5
CVDR ≥ 3									
	55–59	92	31.1	5.8	12.2	39.2	56.4	181.8	1,229.7
			25.8–37.5	4.1–8.2	8.8–17.0	27.9–55.1	45.9–69.5	138.0–239.5	928.8–1,628.0
	65–69	126	48.4	8.0	16.3	33.7	55.6	114.8	1,286.0
			39.4–59.5	5.5–11.6	11.3–23.5	23.2–49.1	44.2–69.9	84.7–155.7	943.2–1,753.5
	70–74	145	47.0	9.2	14.4	30.6	48.6	103.5	671.4
			41.4–53.4	7.3–11.6	11.5–18.0	24.3–38.6	42.2–56.1	85.7–124.9	554.5–813.0
	75–79	130	49.1	11.6	15.6	31.7	50.9	103.7	666.0
			41.4–58.2	8.5–15.8	11.5–21.0	23.3–43.2	42.2–61.5	80.7–133.2	516.0–859.4
	80–84	111	54.6	12.8	12.6	23.1	50.7	92.8	447.2
			46.0–64.8	9.4–17.5	9.3–17.1	17.0–31.5	41.9–61.3	72.1–119.3	346.2–577.8
	85–89	97	50.2	14.5	8.6	17.1	45.5	90.7	497.3
			42.3–59.6	10.6–19.8	6.4–11.7	12.6–23.4	37.6–55.1	70.4–116.7	384.5–643.1
	> 90	43	61.9	13.2	5.1	8.3	33.2	53.7	453.0
			48.5–79.0	8.5–20.7	3.3–7.9	8.3–12.9	25.4–43.6	37.5–76.8	314.4–652.7

the literature, reference ranges for SHBG, TT, TE_2, DHEA-S, FSH, FAI, and FEI in elderly Polish men and women, stratified by five-year age groups and number of CVDRFs. Our results suggest that the reference ranges for sex hormones are not only gender- and age-specific, but also should be evaluated in relation to the number of CVDRFs. In particular, this might facilitate decisions regarding the application of hormone replacement therapy in elderly cases with hypogonadism.

The strength of this study is that we analysed a large, representative, randomly selected sample of elderly Polish people recruited from different administrative centres of Poland using the three-stage, proportional and stratified-by-age group selection process. However, some potential limitations should be considered in interpreting our results. First, the cross-sectional nature of the study is problematic for differentiating cause and effect from simple association. Second, we did not use strict exclusion criteria,

Table IVB. *Mean and 95% confidence intervals (antilogarithms) values of SHBG, FSH, TT, FAI, TE$_2$, FEI, and DHEA-S in five–year age groups in women with CVDRF < 3 and CVDRF ≥ 3. Differences between age groups were calculated using Tukey's post hoc test. MANOVA; the Wilks test = 0.97; p = 0.004*

Tabela IVB. Średnie i 95% przedziały ufności (antylogarytmy) stężenia SHBG, FSH, TT, FAI, TE2, FEI i DHEA-S w 5–letnich przedziałach wiekowych u kobiet z CVDRF < 3 i CVDRF ≥ 3. Różnice między przedziałami wieku oceniani testem post hoc Tukey'a. MANOVA: test Wilksa = 0.97; p = 0,004

Age group) [years]	n	log SHBG [nmol/L]	log FSH [IU/L]	log TT [nmol/L]	log FAI [nmol/L]	log E2 [pmol/L]	log FEI	log DHEAS [ng/mL]
CVDRFs < 3								
55–69	148	56.1	58.7	0.67	1.19	25.9	46.1	770.7
		52.1–60.5	53.6–64.2	0.6–0.8	1.03–1.36	24.0–27.9	41.2–51.5	689.0–862.1
65–69	90	56.7	58.7	0.76	1.34	24.1	42.5	686.1
		51.4–62.4	52.3–65.9	0.6–0.9	1.12–1.60	21.9–26.5	36.8–49.0	594.3–792.1
70–74	85	63.3	59.0	0.77	1.22	22.5	35.6	539.7
		57.4–69.8	52.4–66.5	0.7–0.9	1.02–1.47	20.4–24.8	30.7–41.2	465.5–625.7
75–79	77	65.5	47.0	0.70	1.08	24.9	38.1	404.2
		59.1–72.6	41.5–53.3	0.6–0.8	0.89–1.31	22.5–27.6	32.6–44.4	346.0–472.1
80–84	77	68.1	51.3	0.64	0.93	22.6	33.2	353.0
		61.5–75.5	45.3–58.2	0.5–0.8	0.77–1.13	20.4–25.1	28.4–38.7	302.3–412.4
85–89	90	74.0	56.1	0.68	0.91	23.3	31.4	410.4
		67.3–81.4	50.0–63.0	0.6–0.8	0.76–1.09	21.1–25.6	27.3–36.2	355.5–473.8
> 90	97	83.7	63.0	0.70	0.83	19.9	23.8	336.3
		76.4–91.8	56.3–70.4	0.6–0.8	0.70–0.99	18.2–21.8	20.7–27.3	292.9–386.3
CVDRFs ≥ 3								
55–59	140	44.0	54.1	0.78	1.78	27.1	61.5	788.6
		40.8–47.5	49.3–59.4	0.7–0.9	1.54–2.05	25.1–29.2	54.8–68.9	702.8–884.0
65–69	195	47.5	52.5	0.67	1.40	25.1	52.8	610.5
		44.5–50.7	48.5–56.8	0.6–0.7	1.24–1.59	23.5–26.8	47.9–58.2	553.8–673.1
70–74	234	49.1	53.6	0.72	1.47	25.2	51.4	528.5
		46.3–52.1	49.9–57.6	0.7–0.8	1.32–1.65	23.8–26.8	47.0–56.1	483.4–577.7
75–79	185	53.7	51.8	0.73	1.37	23.9	44.5	503.8
		50.3–57.4	47.8–56.2	0.7–0.8	1.21–1.55	22.4–25.5	40.3–49.1	455.7–556.9
80–84	146	63.2	56.2	0.76	1.20	23.7	37.4	444.0
		58.7–68.2	51.3–61.5	0.7–0.9	1.04–1.38	22.0–25.5	33.5–41.8	296.7–497.1
85–89	147	69.7	51.7	0.74	1.07	23.3	33.5	480.3
		64.6–75.1	47.2–56.6	0.7–0.8	0.93–1.23	21.7–25.1	30.4–37.5	429.2–537.4
> 90	104	76.4	56.1	0.79	1.04	24.8	32.4	425.7
		70.0–83.5	50.4–62.5	0.7–0.9	0.88–1.23	22.7–27.1	28.4–37.0	372.4–486.5

which might have potentially influenced the variability of hormonal assessments. Like Orwoll et al. [5] and Yeap et al. [7], we did not exclude cases with benign prostatic hyperplasia (2.5% of men in the PolSenior cohort), prostate cancer (0.85%) that might be treated with antiandrogens [30], or hypothyroidism (10.3% of women and 5.6% of men, including 60% with asymptomatic disease) [31]. We also did not exclude patients with diabetes because in the multiple regression analysis, history of diabetes, along with BMI and age bands, did not significantly influence sex hormone levels (data not shown). Third, in this study we evaluated TT with the RIA method. It has been suggested that liquid chromatography-mass spectrometry has the highest sensitivity and selectivity in the determination of total and free testosterone. However, recent studies have not shown significant advantages for either method [32].

In conclusion, in this study, we have presented normal reference values for sex hormones in Polish elderly

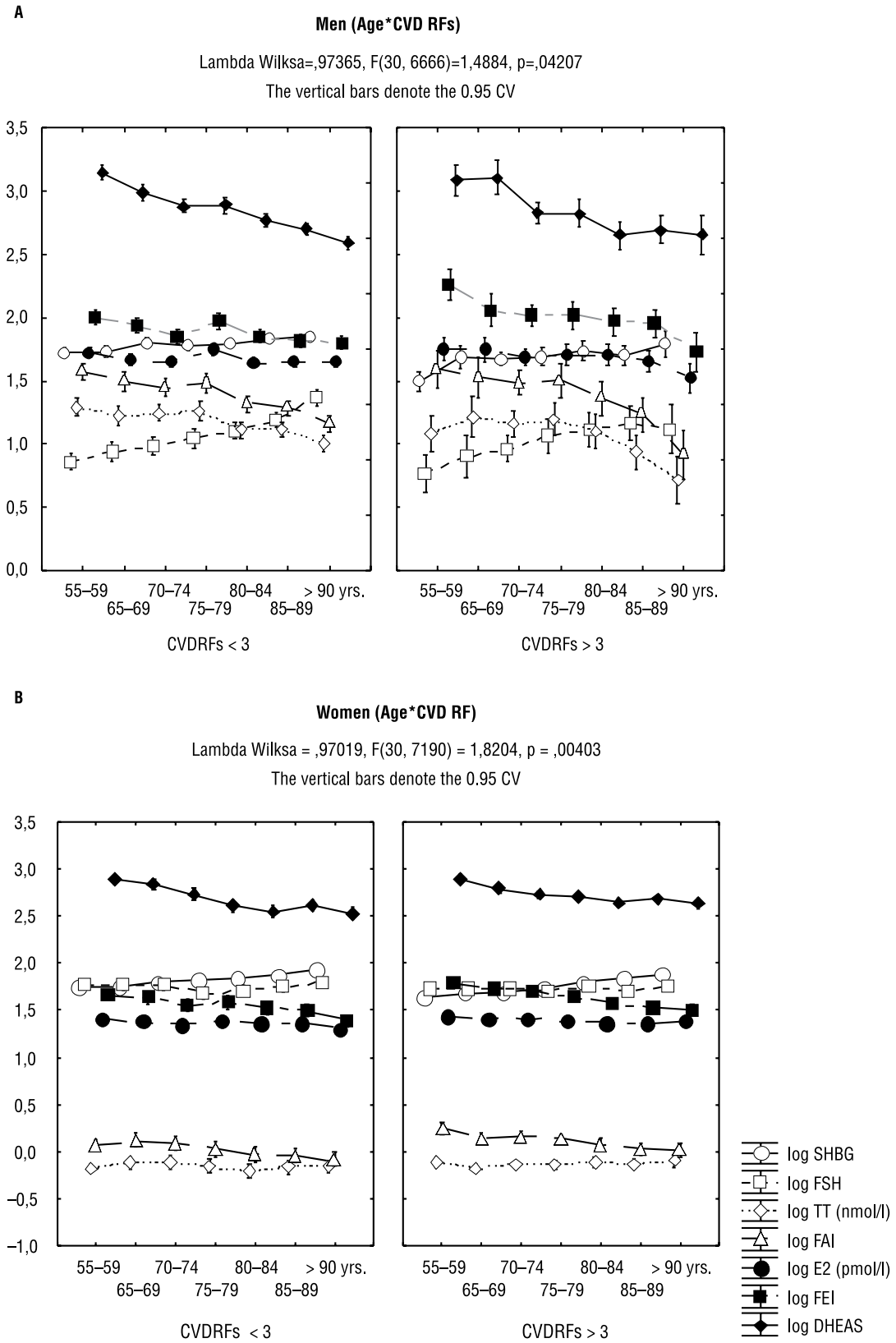

Figure 2A *(men),* **B** *(women) — Distribution of sex hormones SHBG and DHEA-S concentrations (mean and 95% CI; logarithms) by age groups in persons with CVDRFs < 3 and CVDRFs ≥ 3*

Rycina 2A *(mężczyźni),* **B** *(kobiety) — Rozkład stężeń hormonów płciowych, SHBG i DHEA-S w grupach wieku (średnia i 95% CI; logarytmy) u osób mających < 3 CHDRFs oraz ≥ 3 CHDRFs*

Table V. Median values (range) of SHBG, TT, FAI, TE2, FEI, FSH, and DHEA-S concentrations in men and women with CVDRF < 3 and CVDRF ≥ 3

Tabela V. Mediany (rozstęp) stężeń SHBG, TT, FAI, TE2 ,FEI, FSH i DHEA-S u mężczyzn i kobiet z liczbą czynników ryzyka sercowo-naczyniowego poniżej 3 i powyżej 3 (CVDRF < 3 i CVDRF ≥ 3)

	Men		p	Women		p
	CVDRFs < 3	**CVDRFs ≥ 3**		**CVDRFs < 3**	**CVDRFs ≥ 3**	
SHBG [nmol/L]	60.1 (321.7)	45.4 (215.9)	p = 0.000	65.5 (226.2)	54.9 (228.8)	p = 0.000
FSH [IU/L]	10.7 (166.6)	9.7 (106.9)	p = 0.002	63.2 (210.7)	57.6 (275.7)	p = 0.000
TT [nmol/L]	16.2 (62.9)	14.0 (36.9)	p = 0.000	0.7 (27.2)	0.8 (65.8)	p = 0.04
FAI	28.3 (124.9)	31.4 (119.3)	p = 0.000	1.1 (38.8)	1.3 (89.4)	p = 0.000
E_2 [pmol/L]	47.7 (231.2)	48.8 (261.0)	ns	22.7 (367.0)	24.2 (472.3)	p = 0.002
FEI	79.1 (595.1)	105.9 (715.6)	p = 0.000	34.2 (398.2)	43.9 (624.2)	p = 0.000
DHEA–S [mg/dL]	723.0 (6,473.0)	772.0 (4,942.0)	p = 0.007	503.0 (4,100.0)	554.0 (4,786.0)	p = 0.02

Table VI. *Associations between BMI, %BF, SHBG, TT, FAI, TE2, FEI, FSH, and DHEA-S in men and between BMI, SHBG, TT, FAI, TE2, FEI, FSH, and DHEAS in women*

Tabela VI. *Zależności między BMI, BF%, SHBG, TT, FAI, TE2, FEI, FSH i DHEA-S u mężczyzn oraz BMI, SHBG, TT, FAI, TE2, FEI, FSH i DHEA-S u kobiet*

			log BMI		
Men	β	**SE**	**p**	**R2**	**The regression equation**
SHBG [nmol/L]	–1.00	0.06	0.000	0.12	log SHBG = 3.15–1.00 * log BMI
FSH [IU/mL]	–0.44	–0.44	0.000	0.01	log FSH = 1.65–0.44 * log BMI
TT [nmol/L]	–0.51	0.11	0.000	0.01	log TT = 1.84–0.51* log BMI
FAI	0.51	0.11	0.000	0.01	log FAI = 0.67 + 0.51* log BMI
TE_2 [pmol/L]	0.09	0.07	ns		
FEI	1.11	0.09	0.000	0.07	log FEI = 0.365 + 1.10* log BMI
DHEAS [ng/mL]	0.39	0.10	0.000	0.01	log DHEAS = 2.28 + 0.39 * log BMI
			log %BF		
Men	β	**SE**	**p**	**R2**	**The regression equation**
SHBG [nmol/L]	–0.40	0.04	0.000	0.07	log SHBG = 2.29–0.40 * log %BF
FSH	–0.17	0.06	0.008	0.00	log FSH = 1.30–0.17 * log %BF
TT [nmol/L]	–0.31	0.06	0.000	0.01	log TT = 1.57–0.31* log %BF
FAI	0.09	0.06	ns		
TE_2 [pmol/L]	–0.03	0.04	ns		
FEI	0.38	0.05	0.000	0.03	log FEI = 1.40 + 0.38 * log %BF
DHEAS [ng/mL]	0.14	0.06	0.011	0.00	log DHEAS = 2.66 + 0.14 * %BF
			log BMI		
Women	β	**SE**	**p**	**R2**	**The regression equation**
SHBG [nmol/L]	–0.97	0.06	0.000	0.13	log SHBG = 3.19–0.97 * log BMI
FSH [IU/L]	–0.58	0.07	0.000	0.04	log FSH = 2.57–0.58 * log BMI
TT [nmol/L]	0.28	0.09	0.002	0.00	log TT = –0.51 + 0.28 * log BMI
FAI	1.25	0.10	0.000	0.07	log FAI = –1.701 + 1.2295 * log BMI
TE_2 [pmol/L]	0.50	0.06	0.000	0.04	log E2 = 0.61 + 0.50 * log BMI
FEI	1.48	0.08	0.000	0.14	log FEI = –0.57 + 1.48 * log BMI
DHEAS [ng/mL]	0.27	0.09	0.004	0.00	log DHEAS = 2.37 + 0.27 * log BMI

men and women. Our data suggests that the reference ranges stratified by five-year age bands seem more accurate than those given for the overall population over 60 years of age. The clinical relevance of these reference ranges increases when they are considered in relation to CVDRFs, BMI, and %BF.

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