Differentiation of gonadotropin and sex hormone levels in 50, 60 and 70-year old men. An attempt to indicate a normal range

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ABSTRACT Involutional changes in the androgenic activity of the male body occur gradually and exhibit high intra- and interpopulational differentiation. These differences make quantitative description of physiological ageing of the male endocrine system considerably difficult. There are also no commonly accepted normal levels for gonadotropin and sex hormone. In the present study the authors have made an attempt to determine the degree of differentiation of gonadotropin and sex hormone levels in ageing men, in three age cohorts of 50, 60 and 70-year old men, and to indicate the normal ranges for gonadotropin and sex hormones levels in the analyzed age groups. The cross-sectional survey was conducted in the years 2001-2003. The findings revealed that the knowledge about variation originating from individual differences in gonadotropins and sex hormones levels is of special importance in assessing the normal range of their levels in ageing males.

KEY WORDS males, age cohorts, involutionary changes, endocrine system

Introduction Hormonal changes play a key role in the whole body’s ageing process. Having an effect on both physiological (e.g., blood pressure changes, lipid metabolism disorders, overweight, lowered libido) and mental phenomena (e.g., central nervous system disease, male climacteric symptoms) occurring in the body, they “control” the quality of the ageing process [BRIBIESCAS 2001, 2005; KACZMAREK & SKRZYPczak 2002; ŁąCka et al. 2003; SHORES et al. 2004; ATWOOD 2005; BATES et al. 2005]. Involutional changes in the androgenic activity of the male body occur gradually and exhibit high individual differentiation. It is em-
phasized in many works that while hormonal deficiencies can be found already in some men under 50 years of age, others have the correct hormone levels even at a very old age [GRAY et al. 1991, WIKTOROWICZ-DUDEK et al. 2003]. This reflects a significant high inter-individual differentiation in the functioning of the hypothalamic – pituitary – testicular axis [BISHOP et al. 1988, BRIBIESCAS 2001]. Clinical studies indicate that men from developed countries are characterized by a wider range of differences in the level of androgens than men in underdeveloped countries that results in differences in the mean values of hormone levels [CHRISTIANSEN 1991, BRIBIESCAS 2001, CAMPBELL et al. 2003]. It has been found that testosterone level in adult men from regions inhabited by highly developed western populations is much higher (adult males in the USA: total testosterone is 589 ± 25pmol/l) as compared to men from rural regions in Africa, hunters – gatherers from South America (in the Ache tribe males, aged 17–68 years, total testosterone is 192 ± 12pmol/l) or other less developed countries [CHRISTIANSEN 1991, BRIBIESCAS 2001, CAMPBELL et al. 2003]. Nevertheless, analyses of the differences in testosterone level between populations distant in genetic and environmental terms and with different life styles (e.g., USA, Congo, Nepal and Paraguay) demonstrate a decline of differences in this hormone’s levels with age [ELLISON et al. 2002]. A problem that arises during the analysis and comparison of data presented in the literature concerning the levels of gonadotropin and sex hormones is the absence of commonly accepted reference standards for the results of these hormones determinations. The differences concern normal ranges and values of hormone levels in men that are an indication, for instance, for hormone replacement therapy (HRT) [ZGLICZYŃSKI et al. 2003].

In light of the above facts, the authors of the present study have made an attempt to determine the degree of differentiation of gonadotropin and sex hormone levels in ageing men, in three age cohorts of 50, 60 and 70-year old men, and to indicate the normal ranges for gonadotropin and sex hormones levels in the analyzed age groups.

**Materials and methods**

The group under study included cohorts of men at age 50 (49.1 to 50.9) years, 60 (59.1–60.9) years and 70 (69.1–70.9) years. The cross-sectional study was conducted in the years 2001–2003 under two interdisciplinary grants funded by Adam Mickiewicz University and the University of Medical Sciences in Poznań with the approval of the Bioethics Committee of the University of Medical Sciences in Poznań. Men who represented the urban environment (within the city of Poznań) volunteered for the study and were admitted if they were found to be free from any chronic diseases (endocrinological disorders, obesity, renal and liver failure, or cancer) which would distort the results. The numbers of men in the studied age cohorts were respectively: 50-year-olds – 69, 60-year-olds – 73 and 70-year-olds – 67 men. In order to obtain information about the subjects’ social and economic status and their life-style they were asked to complete a questionnaire including a number of detailed questions.
Each man was subjected to comprehensive medical tests to assess his health on the basis of blood pressure level, BMI, WHR, degree of adiposity and body lipids metabolism. In addition, for each subject, blood serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, estradiol and dehydroepiandrosterone sulfate (DHEA-S) were determined. Blood samples were taken from the basilic vein in the morning hours, before the first meal of the day. All serum samples were stored at -20°C until the time of determination. Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and total testosterone serum levels were determined by the immunoradiometric (IRMA) method (Polatom), while estradiol and dehydroepiandrosterone sulfate (DHEA-S) levels were determined by the immunoenzymometric EASIA method (Biosource Europe).

The database compiled and all calculations performed in the study were made using Statistica 7.0 statistical programs package (StatSoft Inc.2005 Statistica for Windows). Standard descriptive methods were used and the significance of differences was determined at the 0.05 probability level. Distributions of the analyzed hormones levels were checked for normality using the D Kolmogorov-Smirnov test. For all hormone levels, excepting the total testosterone level, variability distributions were not normal. Descriptive statistics of the studied hormones levels and andropause index in the 50, 60 and 70-year-old men are shown in Table 1. The table also shows the reference standards as indicated by manufacturers of the reagents used for determining the serum hormone levels. Testosterone levels are expressed in two units, ng/ml and nmol/l, in order to compare the results with results presented in the literature.

The ranges of variability of gonadotropic and sex hormone levels in the groups of 50, 60 and 70 year-old men were determined. In the case of total testosterone, only data for the 50 and 60-year-olds were analyzed, since for the oldest age group the level of free testosterone was determined.

Owing to the fact that the frequency distribution of the hormone levels deviated from normal, medians and centile

**Results**

The distribution of hormone levels was continuous and, for this reason, at the first stage of the analyses the character of distributions in the respective age groups was checked using D Kolmogorov-Smirnov test. For all hormone levels, excepting the total testosterone level, variability distributions were not normal. Descriptive statistics of the studied hormones and andropause index in the 50, 60 and 70-year-old men are shown in Table 1. The table also shows the reference standards as indicated by manufacturers of the reagents used for determining the serum hormone levels. Testosterone levels are expressed in two units, ng/ml and nmol/l, in order to compare the results with results presented in the literature.

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Owing to the fact that the frequency distribution of the hormone levels deviated from normal, medians and centile
distances between the 25th and 75th centile ($C_{25}$-$C_{75}$) and between the 5th and 95th centile ($C_5$-$C_{95}$) were used for the description of hormone levels in the respective age groups. The centile distances between the 25th and 75th centile ($C_{25}$-$C_{75}$) indicated changing-with-age ranges of variability and were accepted as reference values for half (50%) of the representation of men in a given age cohort. The distances between the 5th and 95th centile ($C_5$-$C_{95}$) determined the normality ranges of the hormone levels in the respective age groups. These authors found that the ranges of the centile distances $C_{25}$-$C_{75}$ and $C_5$-$C_{95}$ in successive age cohorts (50, 60 and 70) were increased for gonadotropins and decreased for sex hormones. In addition, it was found that the reference standards developed by the reagent manufacturer (determined for adult males) were radically different from the ranges we accepted either for 50% or 90% representation of men in given age cohort. In all but the DHEA-S level for the group of 50-year-olds was the calculated range was found to be consistent with the range specified by reagent manufacturer (Tab. 1, Fig. 1).

### Table 1. Descriptive statistics of gonadotropin and sex hormone levels in three cohorts of males: 50, 60, 70 years old (RS – reference standard)

<table>
<thead>
<tr>
<th>Age</th>
<th>$x$</th>
<th>$sd$</th>
<th>$Me$</th>
<th>$C_{25}$</th>
<th>$C_{75}$</th>
<th>$C_{25}$-$C_{75}$</th>
<th>$C_5$</th>
<th>$C_{95}$</th>
<th>$C_5$-$C_{95}$</th>
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<tr>
<td>LH (RS: 0.63-7.89 IU/L)</td>
<td>50</td>
<td>4.4</td>
<td>2.4</td>
<td>3.7</td>
<td>2.7</td>
<td>0.4</td>
<td>4.5</td>
<td>9.4</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.4</td>
<td>1.6</td>
<td>4.2</td>
<td>3.0</td>
<td>1.7</td>
<td>5.5</td>
<td>7.2</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>6.3</td>
<td>2.8</td>
<td>5.9</td>
<td>4.1</td>
<td>2.2</td>
<td>7.8</td>
<td>9.5</td>
<td>2.7</td>
</tr>
<tr>
<td>FSH (RS: 1.4-10.9 IU/L)</td>
<td>50</td>
<td>6.9</td>
<td>4.8</td>
<td>5.6</td>
<td>3.7</td>
<td>0.9</td>
<td>4.7</td>
<td>16.2</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>7.3</td>
<td>5.3</td>
<td>5.8</td>
<td>3.9</td>
<td>0.9</td>
<td>5.1</td>
<td>18.6</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>7.6</td>
<td>6.4</td>
<td>5.5</td>
<td>3.3</td>
<td>0.9</td>
<td>6.4</td>
<td>19.7</td>
<td>18.3</td>
</tr>
<tr>
<td>Estradiol (RS: 10-45 ng/ml)</td>
<td>50</td>
<td>24.1</td>
<td>15.9</td>
<td>21.8</td>
<td>10.2</td>
<td>36.9</td>
<td>26.7</td>
<td>55.8</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>24.7</td>
<td>15.6</td>
<td>23.4</td>
<td>10.5</td>
<td>36.6</td>
<td>26.1</td>
<td>49.6</td>
<td>45.7</td>
</tr>
<tr>
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<td>20.2</td>
<td>5.3</td>
<td>19.4</td>
<td>15.8</td>
<td>24.3</td>
<td>8.5</td>
<td>29.5</td>
<td>16.1</td>
</tr>
<tr>
<td>DHEA-S (RS: 1.8-12.5 ng/ml)</td>
<td>50</td>
<td>14.0</td>
<td>14.2</td>
<td>9.0</td>
<td>3.4</td>
<td>20.2</td>
<td>16.8</td>
<td>52.1</td>
<td>50.1</td>
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<tr>
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<td>60</td>
<td>9.6</td>
<td>12.4</td>
<td>7.1</td>
<td>2.3</td>
<td>11.0</td>
<td>8.7</td>
<td>28.3</td>
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</tr>
<tr>
<td></td>
<td>70</td>
<td>6.4</td>
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<td>8.4</td>
<td>5.2</td>
<td>14.5</td>
<td>12.8</td>
</tr>
<tr>
<td>Total Testosterone (RS: 8.2-34.6 nmol/l)</td>
<td>50</td>
<td>21.2</td>
<td>6.8</td>
<td>21.1</td>
<td>15.7</td>
<td>27.1</td>
<td>11.4</td>
<td>33.0</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20.7</td>
<td>6.9</td>
<td>21.0</td>
<td>15.2</td>
<td>25.7</td>
<td>10.5</td>
<td>31.6</td>
<td>22.6</td>
</tr>
<tr>
<td>Total Testosterone (RS: 2.4-10.1 ng/ml)</td>
<td>50</td>
<td>6.2</td>
<td>1.9</td>
<td>6.1</td>
<td>4.6</td>
<td>7.9</td>
<td>3.3</td>
<td>9.6</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6.0</td>
<td>2.0</td>
<td>6.1</td>
<td>4.4</td>
<td>7.5</td>
<td>3.1</td>
<td>9.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Andropause Index (RS: &gt;1)</td>
<td>50</td>
<td>1.7</td>
<td>0.9</td>
<td>1.5</td>
<td>1.1</td>
<td>2.2</td>
<td>1.1</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.5</td>
<td>0.7</td>
<td>1.4</td>
<td>0.9</td>
<td>1.9</td>
<td>1.0</td>
<td>2.9</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Differentiation of gonadotropin and sex hormone levels

At the next stage of the analyses, mathematical logarithmic and square root transformations of crude variables (hormone levels) were performed in order to obtain values of the studied hormone levels having normal distributions in the groups of 50, 60 and 70-year-olds. Table 2 shows the types of procedure used for transforming the skew distribution into a normal one.

Fig. 1. Comparison between reference standards and proposed normal ranges of hormone levels
Obtaining the mean values and standard deviations enabled us to calculate the coefficient of variation (CV) for each hormone in each age cohort. Analysis of the CV values showed that differentiation of hormone levels (with the exception of estradiol) and the andropause index among men in the cohorts were similar (Tab. 3).

In order to check whether there were differences in the levels of hormones between age groups, the one-way analysis of variance (ANOVA) was used. Significant differences between studied age cohorts were obtained only for the LH and DHEA-S concentrations, while other hormones levels exhibited no statistically significant differences (Tab. 4).

**Discussion**

As mentioned in the introduction, involutinal changes in the androgenic activity of the male body show high intra- and interpopulational differentiation. These differences render a quantitative description of the physiological ageing of the male endocrine system considerably difficult. The sources of interindividual differentiation in the levels of hormones are still being pursued, although according to current conclusions of studies on this subject its main causes include: diet, physical activity and environmental factors [Spratt et al. 1988, Vermeulen et al. 1996, Volek et al. 1997]. Thus, for instance, interpopulational differences in testosterone level may result from environmental factors affecting the sensitivity and activity of Leydig cells and the synthesis of sex hormone binding globulin (SHBG) [Bribiescass 1995, Ellison et al. 1998]. In a study by Ring et al. [2005] the role of genetic and environmental factors in shaping the variability of hormone levels in ageing (59–70-year old) male twins was assessed. In this study, genetic models were fitted by the method of maximum likelihood. In the case of testosterone, the role played by genetic variation in shaping total phenotypic variation was significant and was assessed at approximately 60%, whereas in the case of estradiol, this was approximately 25%.

In this study, we analyzed three groups of men at the age of 50, 60 and 70 years, assuming these age categories
Differentiation of gonadotropin and sex hormone levels

as representative for the onset and duration of the middle life crisis and the period of early old age. The creation of cohorts was deliberate and its purpose was to form groups of men of almost identical calendar age since it is well-documented that involutionsary changes in hormone levels is an age-related phenomenon. Using this method provided an increased possibility of determining the normal ranges among men of the same chronological age.

Our findings showed that frequency distributions of all except total testosterone were not normal. Therefore, in place of means and standard deviations, we used medians and lower and upper quartile values. We also calculated the 5th, 25th, 75th and 95th percentiles. This enabled us to show the range of hormone level variation at a given chronological age. We are of the opinion that the normal range of hormone levels included either 50% or 90% of the men in a given chronological age and seems to be better indicator of involutionsary changes in endocrinological system when compared with the mean values.

As noted by ZGLICZYŃSKI et al. [2003: 29] “there are also no commonly accepted norms for testosterone determination results”. In our opinion, this is a problem not only for testosterone concentrations determination, but also determination of other sex hormones or gonadotropins. Hormone replacement therapy in men is prescribed when the hormone’s blood serum level falls below a statistically specified norm which in endocrinological studies is calculated as the mean value ± 2.5 standard deviation. However, as underscored by ZGLICZYŃSKI et al. [2003], diagnosis of testosterone deficiency in elderly males with a slowly progressing decline of secondary sex traits is very difficult. This is due to the absence of sharp criteria which would separate results for healthy and nonhealthy men. One should stress that the first symptom of testosterone deficiency is an increase in gonadotropin levels. However, most often, the strict separation criteria are met neither by determinations of (total, free) testosterone nor by determinations of gonadotropins LH and FSH. In this study we also observed that despite the fact that LH and FSH levels in the group of the oldest studied men were highest, the median values remained in the normal range specified by the manufacturer while the decreasing total testosterone concentrations did not fall below the low normal limit.

VERMEULEN et al. [1999] proposed that the low normal limit should be set at the level of the mean value decreased by 2.5 standard deviation, which amounts to 11 nmol/l. They also found that 30% of men older than 70 years of age have symptoms of hypogonadism. Application of the same statistical procedure, that is the norm (mean ± 2.5SD), resulted in the age cohorts of men under study, normal ranges of respectively 4.2-38.2 nmol/l for 50-year-olds and 3.5-38.0 nmol/l for 60-year-olds. We would like to stress that results for no male in a given age category fell outside the normal range defined in this way. Assuming of the 11 nmol/l value indicated by VERMEULEN et al. [1999] as the low normal limit, it was demonstrated that in the group under study 5% of 50-year old men and 7% of 60-year old men produced results below this norm. The results obtained in the study by ZGLICZYŃSKI et al. [2003] demonstrated that
as many as 70% of men with clinically confirmed symptoms of hypogonadism had total testosterone concentrations between 3.2 ng/ml and 4 ng/ml (11.0 nmol/l –13.8 nmol/l), that is above the low normal limit as proposed by Vermeulen et al. [1999].

The andropause index proposed by Zgliczyński, based on the principle of negative feedback between luteinizing hormone LH and testosterone levels, reflects the disorders in the equilibrium of this system in each of the subjects individually. The andropause index makes it possible to segregate healthy and young men (andropause index >1) from elderly men with confirmed hypogonadism (andropause index <1), even in a situation when the total testosterone level is not outside the norm [ZGLICZYŃSKI et al. 2003]. The values of this index were calculated in the present study (Tab. 1). Differences between its values for the 50 and 60-year-olds were not statistically significant and were not lower than 1 (healthy men with no symptoms of hypogonadism).

In this study the authors also determined the degree of differentiation of hormone levels amongst men in each age cohort. CV values used for this purpose demonstrated similarity of differences in level values among men in the cohorts. Only for estradiol in the group of the 70-year olds, was the differentiation significantly smaller. The reason for this significant decrease in the differentiation of estradiol levels in the group of 70-year old men may be an increase in peripheral conversion of testosterone to estradiol in fatty tissue in elderly men, especially in smokers or men suffering from coronary heart disease [SRZEDNICKI et al. 1990; ZGLICZYŃSKI et al. 2003, after GLASS et al. 1977].

Analyses were also performed on the intergroup level, i.e., between age cohorts. We investigated whether the changes in hormone levels taking place with age were statistically significant. It is commonly known that changes typical of the ageing process of hormonal and reproductive system involves an increase in luteinizing hormone LH and follicle-stimulating hormone FSH levels with age with a simultaneous decrease in Leydig and Sertoli cells’ sensitivity to stimulation by gonadotropins. Despite the testosterone deficiency, the increased LH level usually does not exceed the upper normal limit, and results from an increased sensitivity of pituitary gonadotropin cells to the suppressing, reflexive influence of testosterone [VERMEULEN 1991, ZGLICZYŃSKI et al. 2003, BRIBIESCAS 2005]. In this study we observed only a significant increase in the LH level and a decrease in the level of DHEA-S. In addition, no significant differences were found in the value of the andropause index between men aged 50 and 60 years.

**Conclusions**

The fact that male reproductive functions decrease with age is commonly known. However, there is still a need to further improve the knowledge on the range of intra- and inter-group variability in this process. Knowledge about variation stemming from individual differences in levels of gonadotropins and sex hormones is of special importance in assessing their normal ranges in ageing males.
Acknowledgements

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Inwolucyjne zmiany w czynności androgennej ustroju męskiego przebiegają stopniowo i wykazują duże zróżnicowanie wewnątrz- oraz międzypopulacyjne. Różnice te w znacznym stopniu utrudniają i oznaczać i interpretować stężenia hormonów: luteinizującego (LH), folikulo-tropowego, estradiolu oraz siarczanu dehydroepiandrostero-

zydowych. Znajomość indywidualnych różnic w grupach mężczyzn w tym samym wieku kalendarzowym pozwala poprawnie interpretować zmiany stężenia hormonów zachodzące z wiekiem.

Streszczenie

Inwolucyjne zmiany w czynności androgennej ustroju męskiego przebiegają stopniowo i wykazują duże zróżnicowanie wewnątrz- oraz międzypopulacyjne. Różnice te w znacznym stopniu utrudniają i oznaczać i interpretować stężenia hormonów: luteinizującego (LH), folikulo-tropowego, estradiolu oraz siarczanu dehydroepiandrostero-

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