AGE RELATED CHANGES 
AND INTERRELATIONSHIPS BETWEEN 
PLASMA TESTOSTERONE, OESTRADIOL AND 
TESTOSTERONE-BINDING GLOBULIN 
IN NORMAL ADULT MALES 

By 
Karl M. Pirke and Peter Doerr

ABSTRACT

Testosterone (T), oestradiol-17β (Oe2) and binding capacity of the testosterone-binding globulin (TeBG) were measured in plasma of 84 adult males (22–90 years). The medians for age group I (22–61 years, n = 50) were: T 545 ng/100 ml, Oe2 1.66 ng/100 ml, TeBG capacity 1.44 μg T/100 ml; the medians for age group II (67–90 years, n = 34) were: T 459 ng/100 ml, Oe2 2.56 ng/100 ml, TeBG capacity 2.05 μg T/100 ml. The decrease of T and the increase of Oe2 and TeBG capacity with age were significant. The significant correlation ($P < 0.01$) for either age group between T and Oe2 may be ascribed to a simultaneous secretion and/or a peripheral conversion of T to Oe2. The significant correlation ($P < 0.05$) between T and TeBG for the old age group probably reflects the comparatively high percentage of total plasma T bound to TeBG. The biological meaning of the correlation between Oe2 and TeBG is discussed.

Although in adult males urinary excretion of testosterone glucuronide shows a striking decrease with age the plasma testosterone levels decrease only moderately. Vermeulen et al. (1972) recently reported plasma testosterone concentrations and binding capacities of the testosterone binding globulin for a large group of adult males (22–90 years). The elevated TeBG capacity in old age,
they found, resulted in a decrease of the unbound testosterone fraction which was in part responsible for the diminished metabolic clearance rate of testosterone. This study was undertaken to ascertain the age related change of plasma oestradiol in males and its relationship to plasma testosterone and TeBG capacity.

**MATERIALS AND METHODS**

**Subjects**

All subjects were volunteers. Group I (22–61 years) consists of college students, members of our institute and friends of them. None of them was under medical treatment. The subjects of group II (67–90 years) were inhabitants of a home for the aged. Subjects with a history of either endocrine or hepatic diseases were excluded from the study. No one took any hormonal drugs. All subjects were in generally good health. Apart from slightly elevated plasma glucose levels in 5 subjects the laboratory screening (urea, transaminases, total protein and electrophoresis) did not reveal any pathological results.

Blood was collected between 8 and 10.30 in the morning. EDTA-disodium salt was used as an anticoagulant. The plasma was separated within 15 min and frozen at −30°C.

Plasma testosterone was determined by a competitive protein binding method. The serum of an oestrogen treated woman was used as a source of the binding protein. A detailed description and the reliability of the method is given elsewhere (Pirke 1973). Plasma oestradiol was determined by a highly specific and sensitive radioimmunoassay (Doerr 1973).

**Binding capacity of testosterone binding globulin (TeBG)**

Plasma was freed from steroids according to Heyns et al. (1967). Five ml of the 1:5 diluted plasma was dialysed against 30 ml isotonic phosphate buffer pH 7.4 containing 20 mg NaN₃ and 74 mg EDTA-disodium salt per 100 ml. 500 ng cortisol, 6.75 × 10⁵ DPM 1.69 ng [1,2-³H]testosterone and 20, 30, 40, 60, 80 ng testosterone, respectively, were added to the outer medium. Details of the dialysis technique are described elsewhere (Pirke & Stamm 1972). After continuing dialysis for 21 h at room temperature 0.5 ml aliquots were removed from the diluted plasma and from the outer medium for liquid scintillation counting. The albumin concentration of each plasma sample was determined by measuring total protein content (biuret method) and the albumin fraction (cellulose acetate electrophoresis). Protein bound and free testosterone was calculated as described by Tait & Burstein (1964). The testosterone fraction bound to albumin was subtracted from the total protein bound fraction according to Mills (Tait & Burstein 1964). The Scatchard plot was used for plotting the binding data.

It could be demonstrated that under the conditions described the inner and outer volume were constant and the equilibrium was reached within 21 h. The mean recovery of the total radioactivity was 97.4 %.

**Quality control**

All assays were performed under statistical quality control. A plasma pool of normal adult males was frozen at −30°C in 0.6 ml (for testosterone assay), 5.5 ml (for oestradiol assay) and 6.0 ml (for TeBG capacity) aliquots. The plasma for the TeBG capacity
determinations was treated with charcoal (Heyns et al. 1967) before freezing to remove endogenous steroids. The between-assay precisions were as follows: testosterone at a plasma concentration of 535 ng/100 ml 9.8% (CV, n = 39), oestradiol at a plasma concentration of 2.04 ng/100 ml 9.5% (n = 22), TeBG capacity at 0.97 μg testosterone per 100 ml 8.9% (n = 21). For each kind of assay no trends were observed.

Statistical analysis
Since the plasma testosterone concentrations show neither normal nor log-normal distribution the median and the 95 percentiles were used to describe normal values. For the same reason we used Spearman's rank correlation coefficient rₚ instead of the more common product-moment correlation coefficient r.

RESULTS

Age related changes
The plasma testosterone concentration of each subject is plotted against age in Fig. 1. A steady decrease with age is apparent. The median of group I (22–61 years) was 545 ng/100 ml, the median of group II (67–90 years) 459 ng/100 ml (Table 1). The decrease of plasma testosterone with age is statistically significant as indicated by the rank correlation coefficient of the combined group (Table 2). Due to the wide scattering of the plasma testosterone the onset of this decrease cannot be precisely ascertained, but it may be around the end of the third decade.

The plasma oestradiol concentrations are given in Fig. 2. The medians of

Fig. 1.
Plasma testosterone in normal adult males as function of age.

794
Table 1.
Normal values of plasma testosterone, oestradiol-17β, ratio testosterone/oestradiol-17β, and TeBG binding capacity for 2 different age groups of normal adult males.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th></th>
<th>Group II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 22-61 (years)</td>
<td>Median</td>
<td>95 percentiles</td>
<td>Age 67-90 (years)</td>
</tr>
<tr>
<td>Testosterone (ng/100 ml)</td>
<td></td>
<td>545</td>
<td>315-965</td>
<td>459</td>
</tr>
<tr>
<td>Oestradiol-17β (ng/100 ml)</td>
<td></td>
<td>1.66</td>
<td>1.07-2.70</td>
<td>2.56</td>
</tr>
<tr>
<td>Ratio testosterone/oestradiol-17β</td>
<td></td>
<td>324</td>
<td>182-626</td>
<td>174</td>
</tr>
<tr>
<td>TeBG binding capacity (µg testosterone/100 ml)</td>
<td></td>
<td>1.44</td>
<td>0.69-2.68</td>
<td>2.05</td>
</tr>
</tbody>
</table>

group I and group II were 1.66 and 2.56 ng/100 ml, respectively. While the plasma levels did not increase up to about the fifth decade, a steady increase for the age range of group II was significant (Table 2).

Since the age related changes of testosterone and oestradiol proceed in opposite direction, the decrease of the testosterone/oestradiol ratios was most striking. Thus the median of group II was only 54% of the median of group I (Table 1).

The binding capacity of TeBG for each subject is shown in Fig. 3. A steady increase over the total age range is apparent but more pronounced for the old age group. This impression is confirmed by the correspondent rs values.

Table 2.
Correlations between age, plasma testosterone, oestradiol-17β and TeBG capacity.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Group I</th>
<th></th>
<th>Group II</th>
<th></th>
<th>Group I + II</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>rs*</td>
<td>P</td>
<td>rs</td>
<td>P</td>
<td>rs</td>
<td>P</td>
</tr>
<tr>
<td>Age-testosterone</td>
<td>-0.16</td>
<td>ns</td>
<td>0.03</td>
<td>ns</td>
<td>-0.26</td>
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</tr>
<tr>
<td>Age-oestradiol-17β</td>
<td>0.04</td>
<td>ns</td>
<td>0.39</td>
<td>&lt;0.05</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age-TeBG</td>
<td>0.36</td>
<td>&lt;0.05</td>
<td>0.37</td>
<td>&lt;0.05</td>
<td>0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone-oestradiol-17β</td>
<td>0.39</td>
<td>&lt;0.01</td>
<td>0.56</td>
<td>&lt;0.01</td>
<td>0.22</td>
<td>&lt;0.05</td>
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<tr>
<td>Testosterone-TeBG</td>
<td>0.18</td>
<td>ns</td>
<td>0.39</td>
<td>&lt;0.05</td>
<td>0.11</td>
<td>ns</td>
</tr>
<tr>
<td>Oestradiol-17β-TeBG</td>
<td>0.13</td>
<td>ns</td>
<td>0.28</td>
<td>ns</td>
<td>0.45</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Spearman's rank correlation coefficient.
Fig. 2.
Plasma oestradiol-17β in normal adult males as function of age.

Interrelations between plasma testosterone, oestradiol and TeBG capacity

Group I and group II show a significant \( (P < 0.01) \) correlation between T and Oe₂ (Table 2, Figs. 4 and 5). The \( r_s \) value for the combined group is lower
Fig. 4.
Correlation between T and Oe₂ for group I.

Fig. 5.
Correlation between T and Oe₂ for group II.
than the \( r_s \) value of either group. This finding is due to the lower testosterone/oestradiol ratio of the old age group as can be seen from Figs. 4 and 5.

A significant correlation between TeBG capacity and testosterone was found only for the old age group. This implies that the fraction of the total plasma testosterone bound to TeBG is high in this group which is the result of the high TeBG capacity at a comparatively low total plasma testosterone concentration in old age.

The correlation coefficient between TeBG capacity and oestradiol was highly significant for the combined group and just below the 5\% significance level for the old age group. A detailed analysis of the correlation between TeBG and \( \text{Oe}_2 \) for the old age group revealed that a rank correlation coefficient of 0.71 \( (P < 0.01, n = 17) \) is obtained if only the values above the median are used, whereas a \( r_s \) value of 0.28 (ns) is obtained if the TeBG values above the median are used.

**DISCUSSION**

Although it is now generally accepted that plasma testosterone decreases with age there is no agreement about the degree of this change. The discrepancies may be due to the small groups studied and to differences in physical conditions of the aged subjects. The subjects of our old age group showed an explicitly good general health. Their plasma testosterone values are in good agreement with the normal values reported by Coppage & Cooner (1965) and by Gandy & Peterson (1968) and are somewhat higher than the data given by Vermeulen et al. (1972).

Our normal values for TeBG capacity are in precise agreement with the results of Vermeulen et al. (1972) and confirm an increase of about 50\% in old age. The good agreement of these results is probably due to the fact that the TeBG capacity in either study was determined by equilibrium dialysis. TeBG capacity and other binding indices obtained by several investigators (Corvol et al. 1971; Rosenfield 1971; Rosner 1972) under non-equilibrium conditions (adsorption methods, precipitation of TeBG with ammonium sulphate, polyacrylamide gel electrophoresis) are dependent from a variety of special conditions that render a comparison between laboratories difficult and will therefore not be discussed here.

The plasma oestradiol levels of our young age group are in good agreement with the values reported by Abraham & Odell (1970) and by Jenner et al. (1972) and are somewhat lower than those given by others (Korenman et al. 1969; Chopra et al. 1972; Emmet al. 1972). The factors possibly responsible for these differences are discussed elsewhere (Doerr 1973). Apart from rather few values given by Nagai & Longcope (1971) which do not indicate but cannot exclude a possible increase of \( \text{Oe}_2 \) with age, no plasma oestradiol concentrations
in male senescence are currently available. Our data demonstrate a considerable increase of plasma oestradiol with age.

This study confirms the finding of Vermeulen et al. (1972) that TeBG capacity increases with age while the testosterone concentration in plasma falls. In addition we found a striking increase of plasma oestradiol with age and a significant correlation between TeBG and oestradiol. This correlation, however, does not prove that the increase of TeBG capacity in old age is caused by the elevated plasma oestradiol. It may only be due to the higher percentage of oestradiol bound to TeBG in old age. The hypothesis of an oestradiol dependent increase of TeBG capacity in old age is favoured only by the result that the correlation coefficient between TeBG and Oe2 for the old age group is much higher if the oestradiol values above the median are used instead of the TeBG values above the median. It is conceivable that the increase of TeBG capacity with age is due to the lower testosterone/oestradiol ratio in old age. Our study, of course, does not exclude that this increase may be due to a currently unknown cause and not at all to the hormonal changes mentioned.

The finding that the urinary oestrogen excretion remains constant up to the ninth decade while the testosterone excretion falls to about 30% (Pincus et al. 1954; Kaufmann 1968) suggests that the increase of plasma oestradiol with age may be the result of an unchanged blood production rate in connection with a decreased metabolic clearance rate. If this assumption is correct either oestradiol secretion by the testis does not change with age while the testosterone secretion falls or the peripheral conversion of testosterone to oestradiol increases with age. The correlation between TeBG and testosterone in old age is probably due to an increase of the fraction of total plasma testosterone bound to TeBG. This interpretation is in good agreement with the results of Vermeulen et al. (1972) who showed a decrease of the "apparent free plasma testosterone concentration" and a concomitant increase in TeBG capacity with age. The significant correlation between plasma T and Oe2 in either age group is not surprising if one considers that Oe2 is secreted by the testis and that about 50% of the plasma level results from the peripheral conversion of testosterone (Longcope et al. 1969).

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REFERENCES


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