Age-Dependent Change of Serum 5α-Dihydrotestosterone and its Relation to Testosterone in Man

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Synopsis

Serum 5α-dihydrotestosterone (DHT) and testosterone (T) were measured in 78 normal men aged between 12 and 89 years, separating DHT and T by celite micro-column and using reliable radioimmunoassay.

In four age groups of young (20-39 years), middle aged (40-59 years) and old (60-79 years and 80-89 years) men, the mean±SEM for serum DHT were 98±12, 91±9, 82±7 and 54±15 (ng/100 ml), respectively. The corresponding values for T were 696±27, 698±18, 624±20 and 457±21 (ng/100 ml). DHT and T showed significant correlation in each age group: r=0.625, p<0.05 (young men), r=0.727, p<0.02 (middle aged men), r=0.673, p<0.05 and r=0.734, p<0.02 (old men), respectively. There was a significant decline in DHT and T levels of 80- to 89-year-age group compared those of 30- to 39-year-age group (p<0.05 and p<0.005 respectively). But there was no significant changes in DHT and T levels between other age groups. After 3 daily im injections of 4,000 IU human chorionic gonadotropin (hCG) the levels of serum DHT of the young group (20-32 years) and old group (72-78 years) increased 2.6 times and 1.9 times more, respectively. T increased 2.7 times and 1.4 times more, respectively.

Various reports on the influences of aging on plasma T levels have been made Coppage and Cooner (1965), Kent and Acone (1966), Frick and Kinol (1969) and Stearns et al. (1974) reported that male plasma T levels remained within the same range from adolescence till old age. However, Kirschner et al. (1968) and Vermeulen et al. (1972) reported lower plasma T levels in old males.

DHT, the main metabolite of T in male target organ, is the most potent endogenous androgen. In human males most of DHT in the plasma is derived from peripheral conversion of T. The change of serum DHT with age and its relation to serum T remain uncertain.

This paper reports the change of serum DHT with age and during the stimulation with hCG and the relation between serum DHT and T assayed simultaneously from the same serum.

Materials and Methods

Materials

All reagents and solvents were of analytical grade. [1-2-3H]T (45 Ci/mmol) and [1-2-3H] DHT (44 Ci/mmol) were purified by thin layer chromatography of silica gel, Merck. These labeled steroids were obtained from New England Nuclear Corporation. Celite (No. 545) was purchased from Jones-Manville. This celite was activated by washing in nitric acid and heating for 12 hours at 550°C. HCG was obtained from Teikoku Hormone MFG, Co., LTD. The antiserum against T-3-oxime-BSA was gift of Abraham.

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Radioimmunoassay of DHT and T

Radioimmunoassay which simultaneously measured both T and DHT in the same serum was done by the modified method of Coyotupa et al. (1972) as follows. We reported this modified method elsewhere (1973). To 0.5 to 1.0 ml of serum was added 2,000 cpm $^3$H-T and $^3$H-DHT. Extraction was carried out with 10 vol. of ether by vortex mixer for 1 min. The ether was evaporated to dryness under nitrogen. The dried residue was then transferred to the celite microcolumn (5×0.5 cm) by rinsing once with 2 ml of isooctane. Elution was carried out stepwise with the solvent system of isooctane and 10% and 20% ethylacetate in isooctane to separate T from DHT completely. DHT and T fractions were dissolved in 2 ml of methanol.

Estimation of the recovery was made from 0.5 ml. Two aliquots of 0.5 ml were dried under nitrogen and 0.3 ml of antiserum diluted 1:10,000 in 0.1 M phosphate buffer (pH 6.8) containing 0.1% gelatin and 0.9% sodium azide and $^3$H-T (20,000 cpm) or $^3$H-DHT (20,000 cpm) were added and mixed. Standard T and DHT of 10-500 pg were run together with unknown serum. After overnight incubation at 4°C separation of free from bound steroid was accomplished by the dextran-coated charcoal procedure. After centrifugation for 15 min at 4°C the supernatants were transferred to the counting vial with scintillation fluid and counted in Aloka liquid scintillation counter. The within-assay variance of T and DHT evaluated on human pooled serum gave coefficients of variation of 9.3±5.0% and 11.8±10.9%, respectively. The between-assay variance was 12.8±8.9% and 11.1±8.9%, respectively.

Following ether extraction and celite column, the recovery of $^3$H-T and $^3$H-DHT ranged 57±10% and 63±8%, respectively. The reagent blanks of T and DHT were below 5 pg per test tube. When increasing known amounts of both T and DHT were added to human serum, T and DHT curves were found to be within the coefficient of variation as determined by precision experiments. DHT cross-reacted 67% of T with the antiserum generated T-3-oxime-BSA.

Paired t test was employed to assess the statistical significance of the data.

Subjects

Seventy-eight healthy male volunteers aged 12 to 89 served as subjects. Each was free from any endocrine, hepatic and renal disease. In 8 healthy males divided into 2 groups (young group, 20-32 years, n=4 and old group, 72-78 years, n=4), hCG test was done. This was performed in the morning after overnight fast. hCG of 4,000 units were administered intramuscularly daily for 3 days. Blood samples for the determination of T and DHT were drawn before the first injection and on 1st, 2nd and 3rd days after injection. Blood samples were centrifuged as soon as possible. Then serum was frozen at -20°C.

Results

Serum DHT and T levels in normal males in various age group

The serum concentration of DHT and T was plotted against age in Fig. 1. Serum T and DHT levels were low before puberty and then increased and remained within the range of 300 to 1,100 ng/100 ml (647±19 ng/100 ml, M±SEM) and 20 to 150 ng/100 ml (82±5 ng/100 ml, M±SEM) respectively from 20 years to 79 (n=68). The DHT and T for each age group were listed in Table 1, in which all males were grouped into four age periods; young (20-39), middle aged (40-59) and old (60-79 and 80-89). This demonstrated that the mean serum DHT and T levels did not change until the 7th decade and became statistically significantly lower in the old age group (80-89) than in any other young, middle and old (60-79) group (p<0.01 and p<0.01, respectively). The mean of DHT: T ratio of the same individual serum was 0.12±0.02 and the significant correlation between T and DHT was almost the same in young, middle aged and old group (r=0.625, p<0.05; r=0.727, p<0.02; r=0.673, p<0.05 and r=0.734, p<0.02, respectively).

Response of serum T and DHT to hCG

The mean T levels in young and old groups increased from 481±71 ng/100 ml (M±SEM) to 1303±186 ng/100 ml and 523±54 ng/100 ml to 765±128 ng/100 ml respectively after intramuscular injection of hCG of 4,000 units daily for 3 days. The mean relative increase of T in the young group (270%) was statistically higher (p<0.001) than that in the old group (140%). It is interesting that the response of DHT was observed to the same extent as T.
The mean relative increase of DHT in the young group (260%) was statistically higher (p<0.05) than that in the old group (190%) (Table 2).

![Fig. 1. Serum T and DHT levels in normal males](image)

Table 1. Age-dependent change of T and DHT

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>T (ng/100 ml)</th>
<th>DHT (ng/100 ml)</th>
<th>r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-39 years</td>
<td>(17)</td>
<td>696±27</td>
<td>98±12</td>
<td>0.625 (p&lt;0.05)</td>
</tr>
<tr>
<td>40-59 years</td>
<td>(19)</td>
<td>698±18</td>
<td>91±9</td>
<td>0.727 (p&lt;0.02)</td>
</tr>
<tr>
<td>60-79 years</td>
<td>(18)</td>
<td>624±20</td>
<td>82±7</td>
<td>0.673 (p&lt;0.05)</td>
</tr>
<tr>
<td>80-89 years</td>
<td>(7)</td>
<td>457±21</td>
<td>54±15</td>
<td>0.734 (p&lt;0.02)</td>
</tr>
</tbody>
</table>

The value represents Mean±SEM.

* represents the correlation between T and DHT in the same age group.

Table 2. Response of serum T and DHT to hCG (4,000 IU) for 3 days

<table>
<thead>
<tr>
<th>Days</th>
<th>T (ng/100 ml)</th>
<th>DHT (ng/100 ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>young (2)**</td>
<td>old (2)</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>481±71*</td>
<td>523±54</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>835±90</td>
<td>616±70</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1055±80</td>
<td>722±79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III</td>
<td>1303±186</td>
<td>765±128</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>young (2)</th>
<th>old (2)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>67±9</td>
<td>74±5</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>110±16</td>
<td>107±9</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>114±24</td>
<td>120±12</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>III</td>
<td>178±27</td>
<td>141±19</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Mean±SEM, ** numbers of cases, *** compared with the value between young and old males.
Discussion

A specific and reliable radioimmunoassay for DHT and T has been developed using antiserum against T-3-oxime-BSA. Normal values obtained with this method are in good agreement with the data in the literature (Ito and Horton, 1971; Gupta et al., 1972; Pirke et al., 1975; Lewis et al., 1976). We reported that serum T levels remained within the same range from adolescence to the age of the 7th decade, then from the 8th decade its levels rapidly decreased. This corresponds very well the observation of Coppage and Cooner (1975), Kent and Acone (1966), Frick and Kinol (1969) and Stearns et al. (1974). In contrast to T the change of serum DHT with age has not been established. We have demonstrated the statistically significant decline in the level of DHT from the 8th decade, although its levels remain within the same range from adolescence to the age group of 60–79 years. The decline in the concentration of serum DHT in old males is in accordance with the findings of Lewis et al. (1976). Meanwhile Pirke et al. (1975) demonstrated that DHT did not decrease in old age, although T fell by 20.6% in old age. DHT has been reported to be involved in pathological conditions of the prostate and serum DHT was elevated in the elderly men with prostatic hypertrophy (Horton et al., 1975). This suggests that we must be careful to evaluate high serum DHT levels in old males.

We found a good correlation between serum DHT and T in both young and old males ($r = +0.72, p<0.01$) (Fig. 2). The mean of DHT: T ratio was $0.12\pm0.02$ from adolescence to the 8th decade. Pirke et al. (1975) demonstrated the much better correlation between DHT and T in old age than in young age. We demonstrated a significant correlation between T and DHT in all age groups. Pirke et al. explained that the better correlation in old age was due to the higher binding to TeBG, which increased in old age. Hosaka (1976) also demonstrated the significant correlation between DHT and T in normal males. Ito et al. (1971) clarified...
the peripheral conversion of T at about 70% of plasma DHT, remaining 30% converted by androstenedione, which was mainly secreted by the adrenal cortex, in males. Direct secretion by the testis plays only a minor role (Plazzagli et al., 1974). Thus it was not surprising that plasma DHT and T showed a significant correlation. Moreover, the close correlation between DHT and T following hCG stimulation in both young and old males was observed. The DHT increase was to the same extent as T after hCG stimulation. This is in agreement with the observation of Mauer et al. (1973), Pirke et al. (1975) and Hosaka (1976). These results suggest that DHT is converted from T at a rapid rate depending on the serum T concentration. The mean relative increase of T and DHT in the young group was statistically higher than that in the old group.

In conclusion, it was shown that the decreased testicular function in old males found its origin in the testis itself by the hCG stimulation. The serum T and DHT levels remained with the same range from adolescence to the 7th decade, then from the 8th decade decreased. The statistically significant correlation between serum T and DHT in both young and old males in the resting level and following hCG tests was found. Serum DHT levels were about one-tenth of T concentration.

References