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# Plasma Levels of Epitestosterone from Prepuberty to Adult Life

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**Epitestosterone has for a long time been considered as a biologically inactive steroid. However, recently a distinct antiandrogenic activity of this naturally occurring endogenous epimer of testosterone has been demonstrated. Epitestosterone plays a role in the control of doping with testosterone, since an arbitrary ratio of testosterone to epitestosterone in urine has been accepted as a marker for testosterone abuse. For this reason, its urinary excretion has been examined intensively by several authors. On the other hand, its concentration in the blood of men was reported only randomly in a few cases. In the present study the epitestosterone level in human plasma was determined by a specific radioimmunoassay and the concentration of epitestosterone was established in age groups of males of 6 to 65 years of age. There is a clear age dependence of epitestosterone plasma concentration in males. In young boys before puberty, antiandrogenic epitestosterone prevails over testosterone, in adults a striking decline of the ratio epitestosterone:testosterone can be observed.**

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## INTRODUCTION

Androgens in males play an indispensable role in sexual differentiation, development and reproductive functions. Their concentration in body fluids has been intensively studied in the course of male puberty, in relation to reproductive functions and to the development of prostatic diseases, such as benign prostatic hyperplasia and cancer [1]. While the serum levels of androgens, their precursors and metabolites rise at puberty, in aging men a significant decrease in their levels (free testosterone, androstenedione, dehydroepiandrosterone and its sulphate, 5α-androstanediol) and an increase in sex hormone-binding globulin, LH and FSH with age was observed [2].

Epitestosterone (epiT), a naturally occurring 17α-epimer of testosterone, was for a long time considered to be devoid of any physiological function [3]. EpiT attracted attention in doping control as an internal standard in the laboratory detection of testosterone abuse in competitive sports [4–6], which is based on the nearly constant ratio of endogenous epiT to endogenous testosterone in urine.

However, recently it has been discovered that in experimental animals epiT acts as an endogenous antiandrogen [7, 8] and as an inhibitor of 5α-reductase [8, 9], testicular 17α-hydroxylase and $C_{20}$-lyase [10] and also as a modulator of pituitary production and secretion of LH [11]. For a better insight into the role of epiT in the physiology of sexual maturation, aging, and development of prostatic diseases, it seemed reasonable to determine the levels of epiT in the blood of males over their life span. Until now, in contrast to the information on epiT urinary excretion under various conditions (see [12]), and in different age groups [5, 6, 13, 14], only scarce data on the concentration of epiT in the blood of individual probands has appeared in the literature [13].

## MATERIALS AND METHODS

### *Probands*

156 healthy male subjects between the ages of 6 and 65 years old were studied. The probands were randomly selected from the population register in an industrial district as part of a regional iodine deficiency survey.

### *Sampling*

Blood was collected in the morning hours and plasma was stored in the freezer at −25°C until analyzed.

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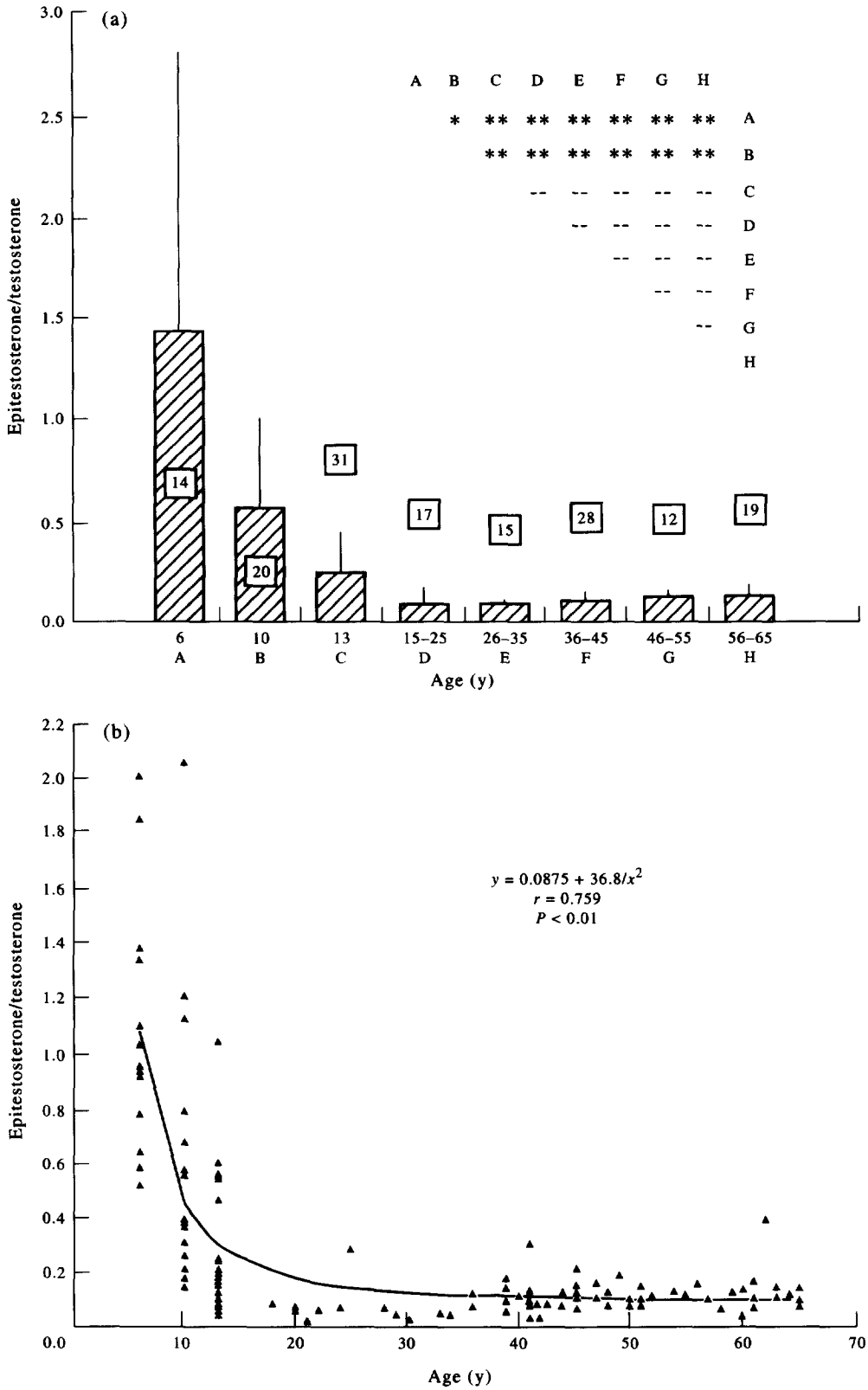


**Fig. 1. Age dependence of the ratio epiT:testosterone. (a) EpiT:testosterone ratio in different age groups and the significance of the between-group differences. Mean ± SEM, the number of cases ($n$) is given in the squares, *$P < 0.05$, **$P < 0.01$. (b) Regression curve of the age dependence of the ratio epiT:testosterone. Individual values of the epiT:testosterone ratio in plasma of the probands are given.**

*Table 1. Age dependence of plasma concentration (mean ± SEM) of testosterone, epitestosterone and dihydrotestosterone*

| Age group (yr) | No. of cases (*n*) | Testosterone (nmol/l) | Epitestosterone (nmol/l) | Dihydrotestosterone (nmol/l) |
|---|---|---|---|---|
| 6 | 14 | 0.522 ± 0.310 | 0.634 ± 0.416 | 0.604 ± 0.394 |
| 10 | 20 | 0.796 ± 0.311 | 0.470 ± 0.470 | 0.517 ± 0.250 |
| 13 | 31 | 5.51 ± 4.51 | 0.872 ± 0.772 | 1.68 ± 0.818 |
| 15–25 | 17 | 16.5 ± 3.52 | 1.57 ± 0.28 | 2.88 ± 1.72 |
| 26–35 | 15 | 18.8 ± 3.67 | 1.62 ± 0.199 | 5.91 ± 2.44 |
| 36–45 | 28 | 15.7 ± 5.49 | 1.61 ± 0.786 | 2.87 ± 1.52 |
| 46–55 | 12 | 14.4 ± 4.93 | 1.59 ± 0.251 | 3.39 ± 1.41 |
| 56–65 | 19 | 13.7 ± 6.09 | 1.54 ± 0.294 | 3.09 ± 1.87 |

*Steroid determination*

Epitestosterone was determined by a radioimmunoassay according to the original method exactly as described by Bílek *et al.* [13]. In brief, rabbit antiserum against epiT-3-(O-carboxymethyl)oxime–BSA conjugate and homologous [$^{125}$I]iodohistaminyl derivative as a tracer were used. Testosterone and dihydrotestosterone were determined by conventional RIA methods [15, 16].

*Statistical analysis*

The values are given as mean ± SEM. Prior to the evaluation of statistical significance, in order to lower the differences in variances of groups, the logarithmical transformation of the data was performed. Statistical significance of among-group differences after transformation was evaluated using the ANOVA, and between-group comparisons were made with the Scheffé test. Linearized regression analysis was performed by the weighted least-squares method.

## RESULTS

The concentrations of epiT and of the principal androgens, testosterone and dihydrotestosterone, are listed in Table 1. In childhood, the epiT concentration in plasma is comparable to that of testosterone. The epiT level increases during puberty less dramatically than that of testosterone and approximates 1/10 of testosterone concentration in adult men.

The age dependence of the ratio of epiT:testosterone in different age groups is shown in Fig. 1(a) and the regression curve in Fig. 1(b). The ratio decreases with age until maturity in a exponential-like manner and remains nearly constant in adult men until their seventies.

The dihydrotestosterone:testosterone ratio decreases with increasing epiT concentration in an exponential manner as shown in Fig. 2.

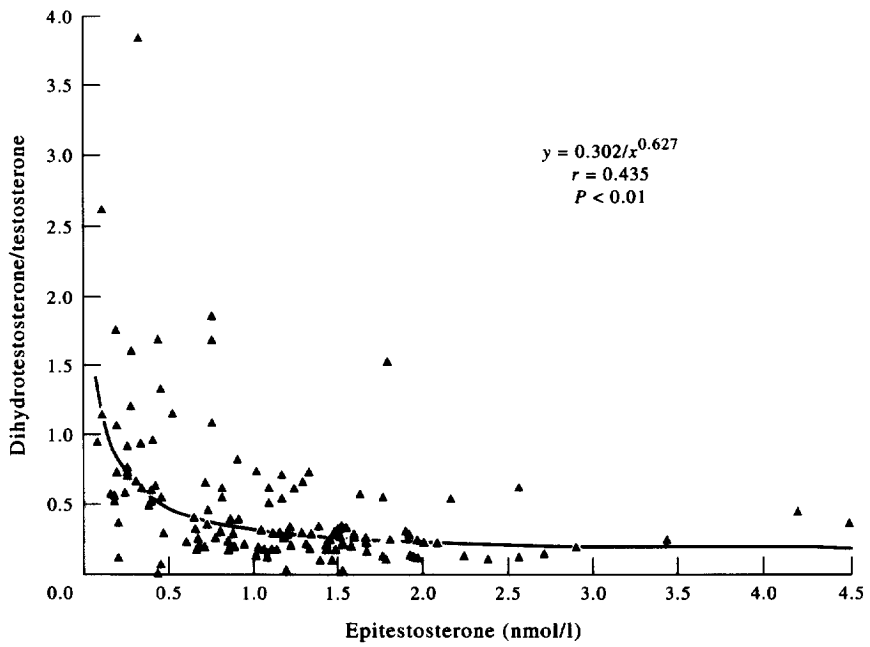


**Fig. 2. The dependence of the ratio dihydrotestosterone:testosterone on epiT concentration. Individual values are given.**

## DISCUSSION

EpiT in humans does not originate from testosterone (for review see [12]) and its excretion is independent of the latter. Its biosynthesis takes place mainly in the testes where 5-androstene-3$\beta$, 17$\alpha$-diol is ormed from pregnenolone by a one step reaction with subsequent oxidation and isomerization to epiT [17]. Though the production rate of epiT is only about 3% of that of testosterone in adults, its excretion is about 1/3 of testosterone [18].

As demonstrated here, epiT concentration in the blood increases from childhood to puberty and does not change much during adult life. There is no clear-cut decrease of epiT after the age of 50 such as reported for testosterone or dihydrotestosterone [2]. Whether this fact has any meaning for the balance of androgenic action in these important development periods of man life remains a matter of speculation.

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