Testosterone Secretion and Metabolism in Male Senescence

A. VERMEULEN, R. RUBENS, AND L. VERDONCK

University of Ghent (Belgium), Akademisch Ziekenhuis Medical Clinic, Department of Endocrinology and Metabolism

ABSTRACT. The influence of aging on plasma testosterone levels and testosterone metabolism in males was studied. It was observed that plasma testosterone levels and the apparent free plasma testosterone concentration (AFTC) remain within the same range from adolescence until the age of 50 yr, but that from the 6th decade on, the mean plasma levels decrease rather rapidly, with however a wide range of individual values. As the metabolic clearance rate (MCR) decreases in male senescence, the net result is an important decrease in the testosterone blood production rate. Within the group of subjects studied, a statistically significant correlation between the free testosterone fraction and the MCR was found. It was observed that testosterone metabolism in male senescence is characterized by a relative decrease of the formation of androstanediols, with a relative increase of 5β over 5α metabolites; moreover a statistically significant correlation between the formation of 5α-androstane-3α, 17β-diol and the free testosterone fraction was found. As the latter metabolite is largely formed in peripheral target tissues, this suggests that testosterone metabolism by target tissues becomes more important with increasing free T fraction and indirectly, that only free testosterone can be taken up by target tissues. (*J Clin Endocr* 34: 730, 1972)

ALTHOUGH on clinical grounds it is generally accepted that virility decreases in old age, data in the literature concerning Leydig cell function in senescence are rather contradictory.

Hollander and Hollander (1) reported a decrease with age of the testosterone level in human spermatic vein blood.

Coppage and Cooner (2), Kent and Acone (3) as well as Gandy and Peterson (4) reported that male plasma testosterone levels remain within the same range from adolescence until old age. However, Kent and Acone (3) observed a decrease in metabolic clearance rate in old age, accounting for a decreased testosterone production rate. Kirschner and Coffman (5) on the other hand reported significantly lower plasma testosterone levels in a small group of males (n = 6) age 55–65 yr, in comparison to males age 18–38 yr (n = 12).

In the view of these rather contradictory results we decided to reinvestigate the influence of male senescence on plasma testosterone levels, blood production rates and metabolism.

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Materials and Methods

Subjects

All male subjects studied were ambulatory and in general good health. None of the subjects studied was taking any drug known to influence testosterone metabolism.

Plasma testosterone levels

Plasma testosterone was determined as the heptafluorobutyrate by a gaschromatographic method, using an electron-capture detector as described previously (6). Plasma samples were taken in the morning between 8 and 9 AM.

Determination of the free testosterone fraction, and the binding capacity of the testosterone binding globulin. The free testosterone fraction was determined by equilibrium dialysis of plasma diluted 1:5 with phosphate buffer, and the free fraction in undiluted plasma as well as the binding capacity of the testosterone binding globulin (TeBG) were calculated as previously described (7).

The product of the free fraction and the plasma testosterone concentration, yielded the apparent free testosterone concentration (AFTC).

The metabolic clearance rate. (MCR) of testosterone was determined in the resting patient, using the continuous infusion technique as described by Horton and Tait (8). Body surface

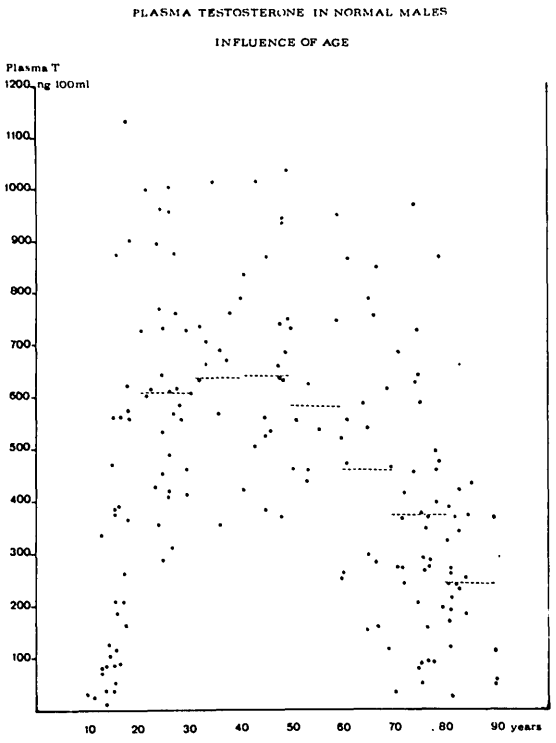

FIG. 1. Plasma testosterone levels in normal males in function of age.

was obtained from weight and length using a nomogram. The product of the plasma testosterone level, determined at the beginning of the infusion, and the MCR yielded the *blood production rate*.

Metabolism of testosterone

Metabolism of testosterone was studied after iv injection of 1,2^{3}H-testosterone SA. 55 c/mM (TRK-162-Amersham). The isotope was 99% pure as controlled by paper and thin-layer chromatography. Urines were collected for 48 hr after the administration of the tracer and stored in the deep freezer, until ready. Only the 48 hr urines containing more than 70% of the administered radioactivity were considered suitable for further analysis. A convenient fraction of the 48 hr urines was then analyzed according to the procedure described by Baulieu and Mauvais Jarvis (9,10). Radioactivity was measured in a Tricarb Scintillation counter (model 3880) with automatic quench correction (AAA-540). Urinary radioactivity was measured by adding 0.5 ml of urine to 18 ml of a scintillation fluid consisting of dioxane 1000 ml, naphthalene 100 g, PPO 5 g, dimethyl POPOP 0.2 g; radioactivity in extracts was measured using toluene 1000 ml, PPO 4 g, dimethyl POPOP 0.1 g as a scintillation liquid.

Results

Plasma testosterone levels were determined in 154 healthy adult normal males, 20–90 yr. As can be seen from Table 1, mean plasma testosterone levels remain within the same range from the age of 20 until the age of 50 yr, with a mean value of 633 ± 25 (SE) ng/100 ml; from the 6th

TABLE 1. Plasma testosterone levels, free fraction, and capacity of TeBG in males

Age yr	n		Plasma testosterone (ng/100 ml) Mean ± SE	Range	Free testosterone fraction (%) Mean ± SE	Capacity of TeBG (10^{-8}M) Mean ± SE
20–30	34	633 ± 25	616 ± 43	280–1205	2.08 ± 0.8 (n = 44)	5.2 ±0.07
30–40	22		634 ± 41	350–1010		
40–50	24		640 ± 49	255–1025		
50–60	10		582 ± 62	255–950	1.68 ± 0.09** (n = 18)	6.5 ± 0.8
60–70	12		462 ± 70*	120–870		
70–80	30		373 ± 46**	38–850	1.36 ± 0.09** (n = 22)	8.2 ± 07**
80–90	23		245 ± 26**	28–390		

* Difference with mean value in age group 20–50 yr statistically significant (* $p < 0.05$).
** $p < 0.01$.

decade on however, mean plasma testosterone values decrease progressively and from the 7th decade on, the mean values are significantly below the mean value found in the group 20–50 yr. However, individual plasma testosterone values in old age still vary over a wide range (Fig. 1): in the age group 70–80 yr, values as low as those found in normal females and as high as 850 ng/100 ml were observed, and in the 9th decade of life, six out of 23 subjects had values below 150 ng/100 ml, whereas nine had levels above 300 ng/100 ml, values which, even for young adults can be considered normal. As the free testosterone fraction decreases with age, it is not surprising that the Apparent Free Testosterone Concentration (AFTC) is similarly ($p < 0.01$) decreased in male senescence: in the age group 20–50 yr, the AFTC varies between 5.5 and 20 ng/100 ml with a mean value of 11.6 ± 0.7 ng/100 ml; it varies from 3.0–18.1 ng/100 ml in the age group 50–70 yr, and from 0.6–12.5 ng/100 ml with a mean value of 4.9 ± 1.1 ng/100 ml in patients older than 70 yr, with in the latter group, only 2 values out of 18 above the lower normal value for young adults.

The mean metabolic clearance rate in young male subjects (20–50 yr) was 640 ± 25 $l/m^2/24$ hr against 530 ± 35 $l/m^2/24$ hr in patients over 70 yr old ($n = 12$), individual values in the latter group varying between 262 and 628 $l/m^2/24$ hr. Within the group of male subjects studied, we observed a positive linear correlation between the free testosterone fraction and the MCR ($r = 0.64$, $n = 22$).

TABLE 2. Urinary metabolites of testosterone in % of administered radioactivity

	Young adults 20–50 yr (n = 6)	Old adults > 68 yr (n = 14)
Sulphate fraction		
Etiocholanolone	2.7 ± 0.3	1.6 ± 0.3
Androsterone	3.0 ± 0.4	0.6 ± 0.1
Etioch/Androst	0.7 ± 0.2	3.2 ± 0.7
Glucuronide fraction		
Etiocholanolone	12.5 ± 1.4	16.1 ± 1.3
Androsterone	11.8 ± 2.1	10.1 ± 1.5
Etioch/Androst	1.1 ± 0.2	1.85 ± 0.25
5β-androstane-3α, 17β-diol	3.7 ± 0.4	1.9 ± 0.5
5α-androstane-3α, 17β-diol	1.8 ± 0.3	0.7 ± 0.1
5β-diol/5α-diol	2.3 ± 0.3	4.0 ± 0.9

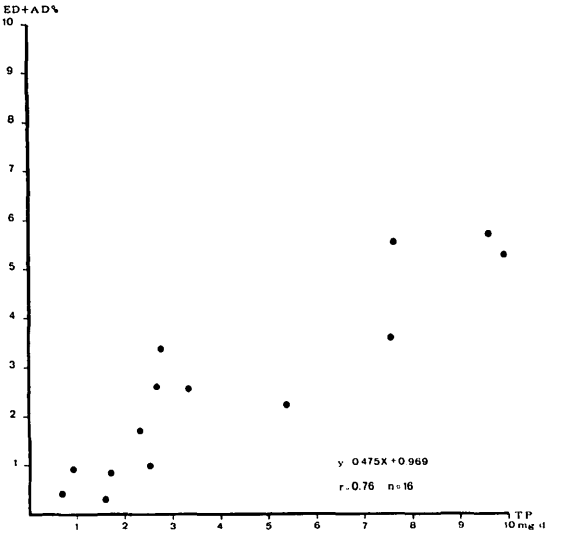

FIG. 2. Correlation between the excretion of 5α-androstane-3α, 17β-diol (A.D.) and 5β-androstane-3α, 17β-diol (E.D.) and the testosterone production rate.

The blood production rate (BPR) of testosterone, determined in ten elderly subjects, varies between 1.5 and 7.2 mg/24 hr, with a mean value of 4.0 ± 0.6 mg/24 hr against 6.6 ± 0.5 ($n = 12$) in young adults.

Testosterone metabolism was studied in 14 subjects over 68 yr old and compared to results obtained in this laboratory in a group of normal male adults ($n = 6$) ages 20–50 yr. As shown in Table 2, testosterone metabolism in male senescence is essentially characterized by a relative increase of 5β metabolites over 5α metabolites and by a relative decrease of the androstane-3α, 17β-diol metabolites; this decrease is statistically correlated with the testosterone production

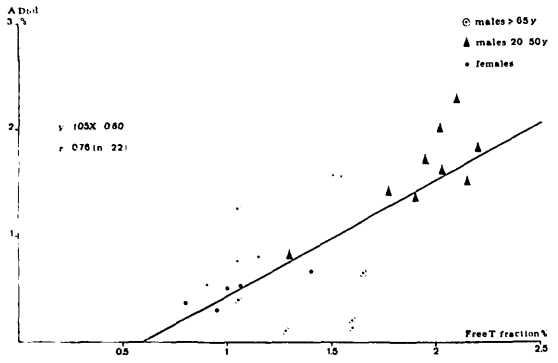

FIG. 3. Correlation between the excretion of 5α-androstane-3α, 17β-diol (A.D.) and the free testosterone fraction.

rate (Fig. 2). Moreover, we observed a significant positive correlation between the excretion of 5α-androstane-3α, 17β-diol (as percent of radioactive testosterone administered) and the free testosterone fraction (Fig. 3). No significant correlation, however, was observed between the latter and the excretion of 5β-androstane-3α, 17β-diol.

Discussion

From our results it appears that plasma T levels and the apparent free testosterone concentration remain within the same range from adolescence to the age of 50 yr, but that from the 6th decade on, the mean plasma levels decrease rapidly, however, with a wide range of individual values; even in the 8th and 9th decades, individual plasma T levels may be in the (high) normal range of younger adults. This high variation in normal T values in old age, ranging from values as low as those found in females to values that would be considered relatively high in young men, might be related to the well-known individual variation in the normal decline of male sexual vigor with age and in the rate of development of senescence.

This decrease of plasma T levels with age but with a wide range of individual values, corresponds very well with the observations of Ryan and Fairman (11) who observed in males over 50 yr old an increase in the mean plasma LH value, however, with a high variation in the individual values. The significant decrease in the mean free testosterone fraction in old age is not uniquely the consequence of the decrease in plasma T, but is also determined by the increased binding capacity (Table 1). This raises the interesting question whether this increase is the consequence of a decrease in the T levels. Before puberty and in hypogonadism the binding capacity is high, whereas after T administration binding capacity is low (12), suggesting that changes in binding capacity occur secondarily to changes in testosterone levels. As the decrease in plasma T levels in the age group 50–70 yr is only moderate, the possibility that other etiological factors, such as a shift in the estradiol/testosterone ratio in plasma, play a role in the increase TeBG capacity should be considered. The significant correlation ($r = 0.64$, $n = 22$) between the free testosterone fraction and the MCR strengthens previously reported evidence (12–14) for a more rapid metabolism of the free or non-specifically bound testosterone fraction; nevertheless, it is evident that other factors may contribute, or be responsible for the decreased MCR in old age. Previous results from this (12) and other (15) laboratories, have already shown that the difference in MCR between males and females cannot be explained exclusively by differences in testosterone binding, but is also a consequence of differences in enzyme activities. Similarly Gordon *et al.* (16), after administration of medroxyprogesterone acetate did not find any correlation between the decreased plasma binding of testosterone and the MCR, but observed an increase in the 5α reductase activity of the liver. Besides increased binding and decreased enzyme activity, an eventual decrease of specific androgen receptors in target tissues could also be responsible for the decreased MCR in senescence.

The fall in plasma testosterone levels and in MCR results in a significant ($p < 0.01$) decrease in testosterone blood production rates, from a mean value of 6.6 mg/24 hr in

young adults to 4.0 mg/24 hr in elderly males. Although Kent and Acone (3), observed a similar decrease in the testosterone blood production rate in old age, they attributed this uniquely to the lowered MCR, although in their group of elderly patients in which the BPR was determined, the mean plasma testosterone level was 0.47 μg/100 ml against 0.62 μg in the younger group. The metabolic pattern of testosterone in male senescence is essentially characterized by a relative increase of the importance of the 5β over the 5α metabolites and, perhaps more important, by a decrease of the relative importance of the 17β hydroxyl pathway, with a fall in diol formation as the testosterone production decreases. This metabolic pattern shows a strong similarity with the pattern in normal females (17,18), in male hypogonadism (8) and in estrogen-treated normal males, and it is tempting to correlate this similarity in metabolism with the similarity in free testosterone fraction (7). The latter, however, was only significantly correlated ($r = 0.76$, $n = 22$) with the percent conversion of testosterone to 5α-androstane-3α, 17β-diol. The existence of such a correlation is supported by data in the literature: 5α-androstane-3α, 17β-diol formation is low in females (17,18) in hypogonadal males (18), in testicular feminization (18,20,21), in prepubertal children (18) and after estrogen treatment of normal males, all characterized by an increased binding of testosterone; the 5α-androstane-3α, 17β-diol formation is on the other hand increased in hirsute females and in the Stein-Leventhal syndrome (10), situations in which the binding of testosterone is decreased. As there is strong suggestive evidence that at least 50% of 5α-androstane-3α, 17β-diol recovered in the urine of males arises from 5α reduction of testosterone in extrahepatic peripheral tissues (20), our results suggest that with decreasing free testosterone fraction the extrahepatic metabolism of testosterone becomes less important, possibly via a decrease of peripheral androgen receptors. Moreover, our results provide some indirect evidence that only free testosterone can be taken up by target tissues. Indeed, if, in destinction to splanchnic organs, target tissues containing specific androgen receptors could metabolize protein bound testosterone, one would expect that the relative importance of 5α-androstane-3α, 17β-diol formation would be greater at higher binding levels of testosterone, whereas the reverse is observed. Similarly, Mauvais Jarvis *et al.* (21), on the basis of results obtained in patients with the testicular feminization syndrome, concluded that peripheral tissues metabolize only the free, non-protein bound testosterone. This is also in accordance with *in vitro* experiments of Lasnitsky (personal communication) who observed that addition of pregnancy serum, with high testosterone binding capacity, to the incubation mixture, inhibited the effect of testosterone on prostatic tissue to a higher degree than male serum. Hence, it is doubtful whether the testosterone binding protein has a specific carrier function (22) rendering testosterone only available to target tissues.

In conclusion then our results show that male senescence is characterized by a decrease in plasma testosterone levels, an increase in testosterone binding and binding capacity, a decrease in MCR, a decreased T production, and a metabolic pattern of testosterone suggestive of hypogonadism, with decrease of the androstanediol formation and preponderance of the 5β over the 5α pathway.

Acknowledgments

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Personal Interviews Sponsored by the Endocrine Society's Placement Service

Through the auspices of the Society's Placement Service, personal interviews between prospective employers and employees will be possible during the Annual Meeting of The Endocrine Society and the IVth International Congress of Endocrinology in the Sheraton-Park Hotel, Washington, D.C.

Each prospective employer will be asked to indicate those periods of time when he, or his representative, will be available for interviews with prospective employees; those seeking employment will, in turn, select one of the available periods for an interview with the employer(s) of their choice. Through the Placement Service, the employers can learn about their schedule of appointments for interviews.

Prospective employers are urged to inform the Placement Service, on or before Monday, June 5, about the times that they will be available for interviews. Interviews may be scheduled at any time in the period extending from noon on Saturday, June 17, through noon on Saturday, June 24.

All participants in the interviews, whether as employers or employees, must be registered with the Placement Service; such registration can be made during the Annual Meeting. The identities of prospective employees will be divulged only to those employers with whom an interview has been requested.

Representatives of the Placement Service will be located at The Endocrine Society Placement Information Desk during the Annual Meeting.