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RECENT ADVANCES IN DOPING ANALYSIS (4)

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T/E ratio variations through puberty in male adolescents .

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Abstract

In the last few years, because the mean age of top level athletes is decreasing in some sports, our laboratory was faced to the evaluation of the T/E ratios and more generally to the steroid profile interpretation of adolescents in sport. In order to set up a database and to collect reference values for different stages of the pubertal development, about 100 young male athletes (practising track and field and gymnastics) were selected. A classical morphological examination of each adolescent was performed allowing then to distribute them into 5 classes of development .
The T/E ratio and other endogenous steroids were measured in a night urine and the mean values of these compounds for each class was related to their morphological development.
Testosterone and Epitestosterone concentrations are increasing in urine with the biological age of the adolescents. Whereas, T/E ratios are not significantly different from one class to the other with a slight tendency to decrease and to stabilize with the age.
A part of that population was re-examined 6 monthes later. The evolution through the development stages has been then evaluated and confimed what was observed in the former transversal experiment .

Introduction

According to the National Comity for the Elite Sport in Switzerland (Personal Communication, 1993), 25% of the elite athletes who can be controlled for doping analysis are young people of 19 years old or less. In this population the subjects are at different stage of development which could lead to important modifications in the steroid profile. In our laboratory, we observed that low concentration of Testosterone and Epitestosterone which led to some problem in the quantification of the T/E ratio could be attributed to adolescents.
A few works were done on the adolescents and their excretion of Testosterone and Epitestosterone [1-5]: Raynaud (1993) [2] concluded that there was a sharper increase of Testosterone than that of Epitestosterone, but this was not confirmed by Dehennin (1994) [4] who observed a progressive increase of the glururonides of Testosterone and Epitestosterone, but no variation in the T/E ratio during the pubertal development [4-5].

In this study the evolution of the different development stages was followed to see if there is a probability that the T/E ratio exceed the critical value of 6.

Aim of the study

The aim of this study was to analyse the steroid profile of sportive male adolescents in order to follow the evolution and the variability in their hormonal state and so to collect references values. Some of the adolescents were examined twice in an interval of 6 months to observe the variations in their development.

Material and methods

Young Volunteers

100 young male adolescents (between 10 and 17 years old) with regular sport activity were chosen in different regional clubs (gymnastics, track and fields) with the consent of the parents and of the ethical commission. All subjects were examined in order to establish the state of development: age, height, weight, testicular volume, Tanner.

40 young volunteers in track and field were examined 6 month later for the semi-longitudinal study.

Urinaralysis

The night urine was collected to be analyse. Excretion volume were measured and a 20 ml aliquot was frozen at -80°C until analysis.
Creatinine was determined by a colormetric test with a Cobas Mira (Hoffmann-La-Roche, Basel, Switzerland).
The luteinising hormone (LH) and the follicle stimulating hormone (FSH) were measured by an enzyme immunoassay test with a Cobas Core (Hoffmann- La-Roche, Basel, Switzerland).

Steroids

Several parameters were analysed in the urine: Testosterone, Epitestosterone, Androsterone, Etiocholanolone, 5α–androstan-3α,17β-diol, 5β-androstan-3α,17β-diol, Androstanolone in order to establish the ratios T/E, A/T, DHT/E, DHT/Etio *1000, α/β diols.

Sample preparation for steroid profiles

The method of preparation and analysis of the endogenous steroids in urine was similar to what is performed by the Cologne group [6-7] with some modifications [8].
For sample preparation, the purification of the sample prior to hydrolysis was performed by using 6 ml bakerbond spe (C-18) columns (J.T.Baker, Pilippsburg, NJ, USA) instead of the XAD-2 columns.
The derivatization mixture was MSTFA/TMSI/DTE (1000:5:5) instead of MSTFA/NH4I/ethanethiol and the injection was done on GC-MSD.

GC/MS parameters

GC/MS: HP 5890/HP 5971 (Hewlett Packard)
Column: HP Ultra 1 (OV-1) 17 m, 0.2 mm i.d., 0.33 µm film thickness
carrier gas: 1 ml/min helium at 180°C, splitless
Temperature program: 170°C, 1 min, 1.8°C per min, 229°C, 30 °C per min 310, 5 min.

Results and discussion

Clinical data

Group	*Subject*	*Age*	*Height*	*Weight*	*Vol of testes*	*Tanner*
	n	in years ±SD	in cm ± SD	in kg ± SD	in ml ± SD	
1	**34**	**10.2 ±1.2**	**139.5 ± 7.0**	**31.8 ± 4.3**	**2.4 ± 0.8**	**I**
2	**22**	**12.4 ±1.1**	**149.6 ± 6.7**	**38.1 ± 5.4**	**4.7 ± 1.9**	**II**
3	**12**	**13.7 ± 1.4**	**158.0 ± 5.3**	**48.3 ± 6.2**	**9.7 ± 3.0**	**III**
4	**20**	**14.9 ±1.2**	**168.5 ± 6.6**	**57.3 ± 4.8**	**14.4 ± 3.9**	**IV**
5	**12**	**16.4 ± 1.2**	**179.1 ± 4.7**	**67.7 ± 6.4**	**19.4 ± 3.1**	**V**

Table 1: The distribution of the 100 adolescents in 5 stages according their physical parameters and Tanner.

The Table 1 gives the distribution of the 100 adolescents in five different groups according to the Tanner stages and the means of the morphological parameters measured for the five stages. The Figure 1 shows where our groups are situated on the growth curve, well distinctive from each other.
In the Figures 2 and 3 are represented the increase of height, weight and the volume of testes through puberty.

Results of the steroid profile

The statistical distribution of the T/E ratios of all 100 subjects showed that the distribution is not different than that of sportive adults (Figure 4): the mean (1.8 ± 1.3) is slightly lower than the T/E ratio of the samples analysed in our laboratory (1.7 ± 1.4: data of 1995). But the majority of the subjects has not a T/E ratio higher than 2.6. Some subjects show a T/E ratio around 6, due to a lower Epitestosterone at the stages I and II.

When we represent the T/E ratio according to the Tanner (through puberty), we observe a slight decrease of the T/E ratio between stage II and III (Figure 5). But in fact the variation of the T/E ratio from a class to another is not significant as Dehennin had showed it already [4-5].
In the stages I and II, T/E ratio exhibit a great variation, and in these groups, the most elevated ratios were found: it is certainly due to lower concentrations of Testosterone and especially of Epitestosterone (Figure 6).

The concentrations of Testosterone and Epitestosterone increase progressively during the development of the adolescents until to stabilize at the end of puberty (Figure 6). The most important increase can be observed between the stage III and IV. Great inter-stages variations of the concentrations of Testosterone and Epitestosterone begin at the pubertal stage III, but the T/E ratios in these stages do not vary a lot.
Moreover an increase in the concentrations of others metabolites such as Androsterone and Etiocholanolone is observed through the pubertal development (Figure 6).
Interestingly the concentration of the luteinizing hormone follows the curve of growth (Figure 1) with a highest value at stage III which correponds to the peak of growth (Figure 7).

Semi-longitudinal studies

In the semi-longitudinal studies 40 adolescents were re-examined 6 months later: this experience showed that some adolescents moved to a category to another with the most important shift from the stage III to the stage IV (Figure 8).

Mean T/E ratios (Figure 9) from the Tanner groups shows similar tendancy that what was observed in the transversal study (see Figure 5). The ratio decreases with time (mostly in the lower classes) as well as it does from the younger to the older groups, confirming that the stable T/E ratio value is not reached before the latest stages of development.
Testosterone and Epitestosterone concentrations (Figure 10) both increase with time, reflecting the same pattern that during the pubertal development (see Figure 6). The highest increase is obtained by individuals from the class 3, similarly to the evolution from classes to classes.

Conclusions

- The T/E ratio is slighly higher in the first stages of puberty and the T/E ratio become similar to adults in the lattest stage. This tendancy can be observed in individuals.
- The concentration of Testosterone and others steroids in urine area increasing during the development of male adolescents to stabilize at the end of puberty.
- The LH concentration is higher in the third group corresponding to the peak of growth.
- Similar experiments should certainly be done in female athletes and also as a function of volume of training.

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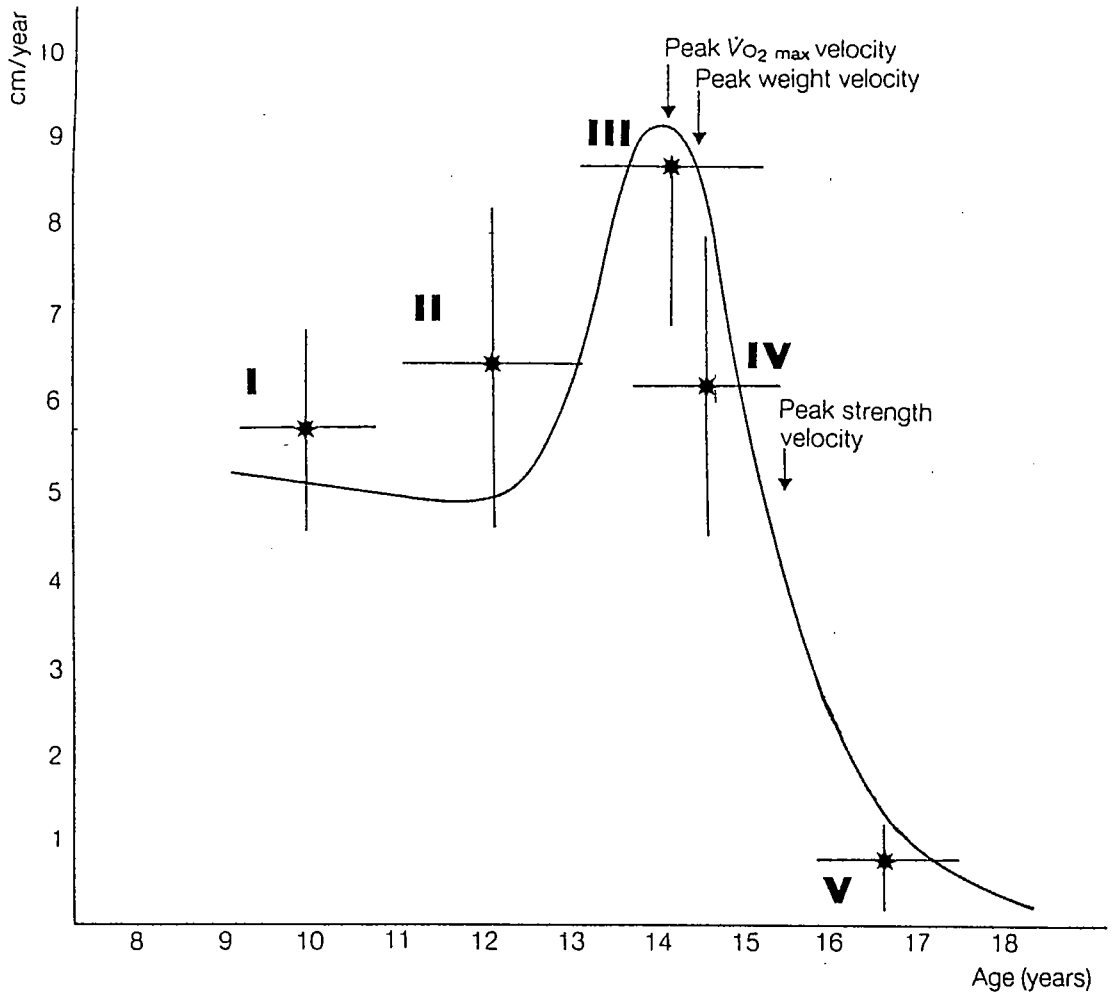


Figure 1: The location of the five groups on the growth curve.

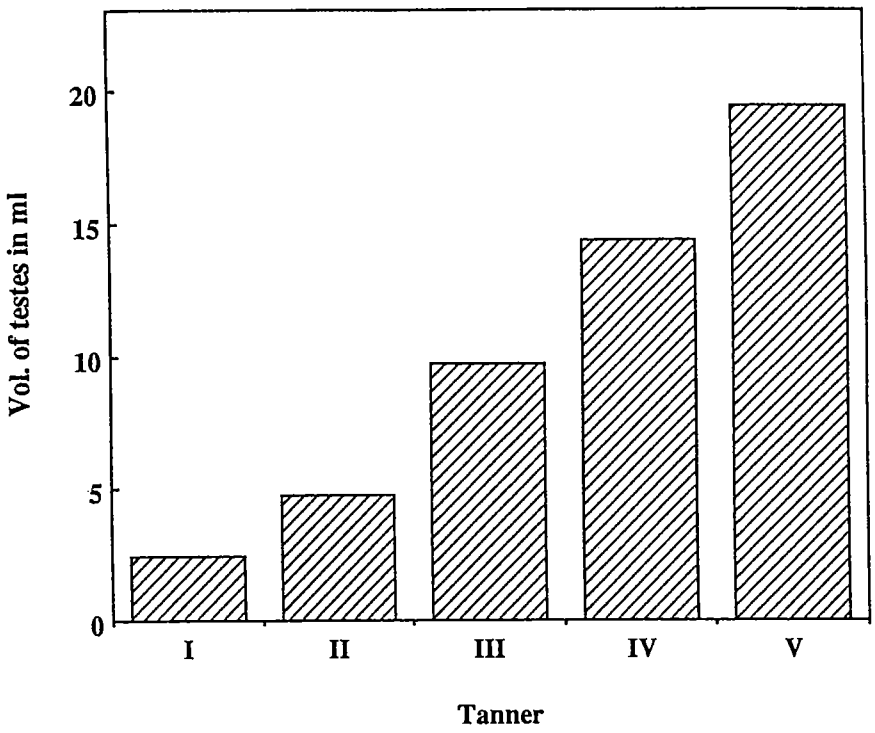


Figure 2: Volume of testes through puberty.

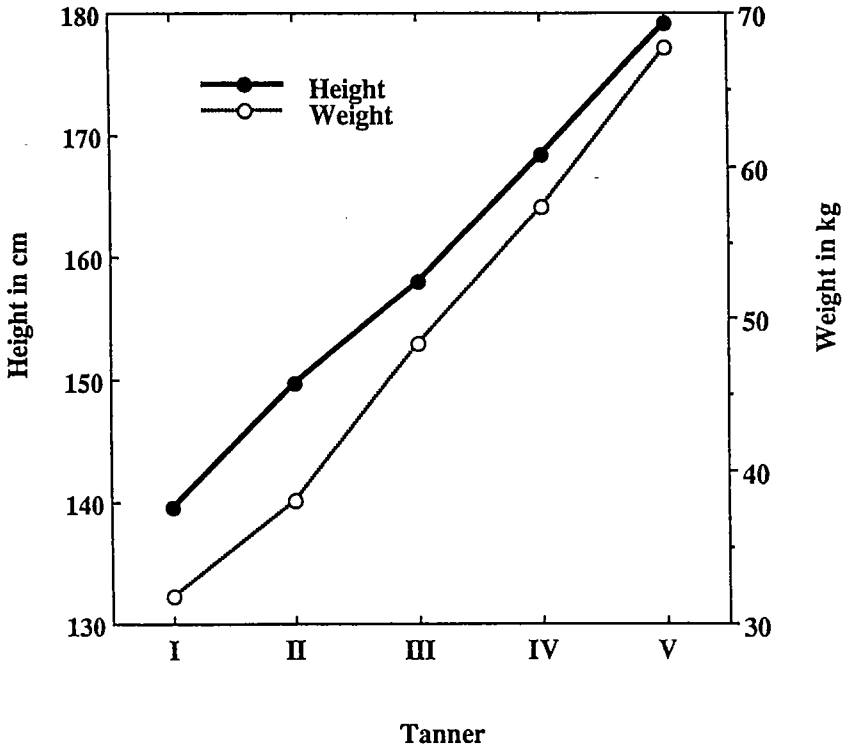


Figure 3: Height and weight through puberty.

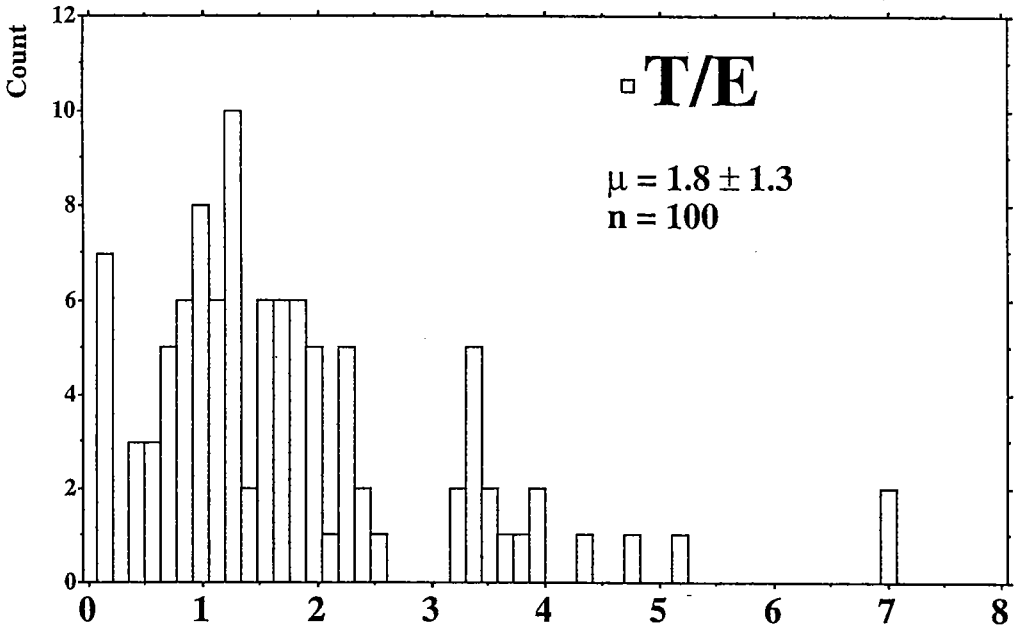


Figure 4: Statistical distribution of the T/E ratio of 100 adolescents.

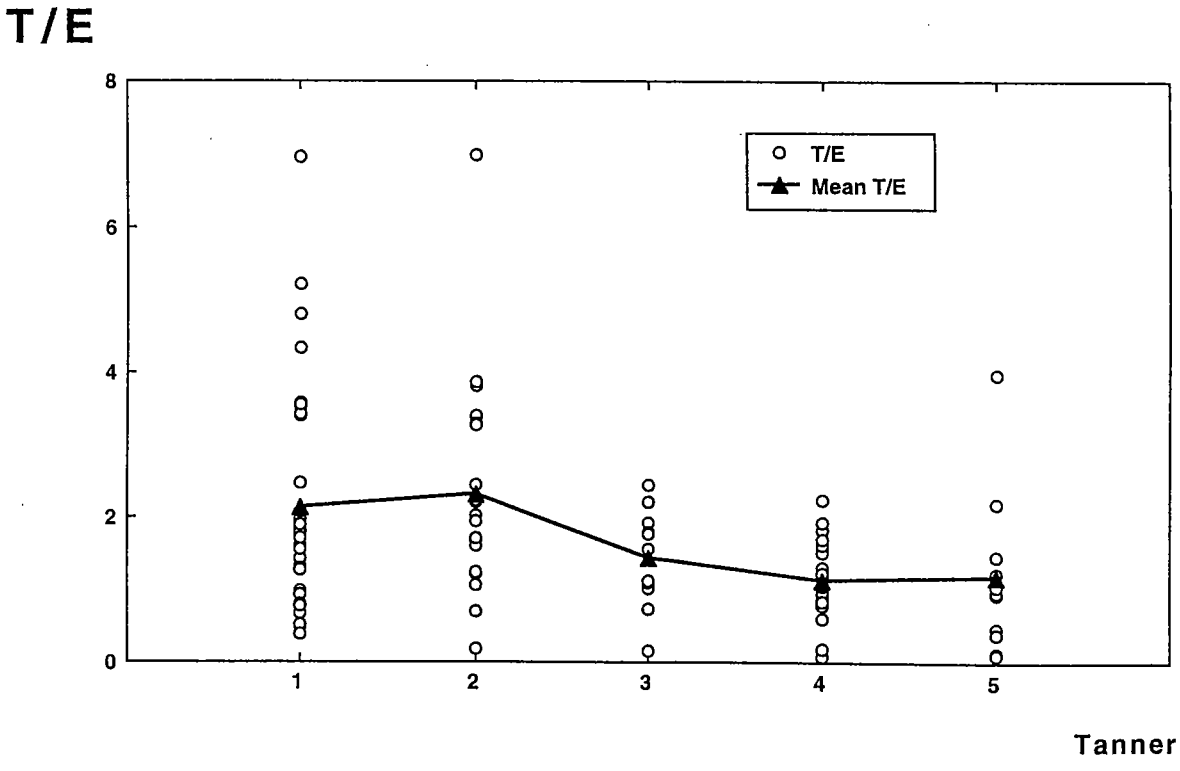


Figure 5: The T/E ratio through puberty.

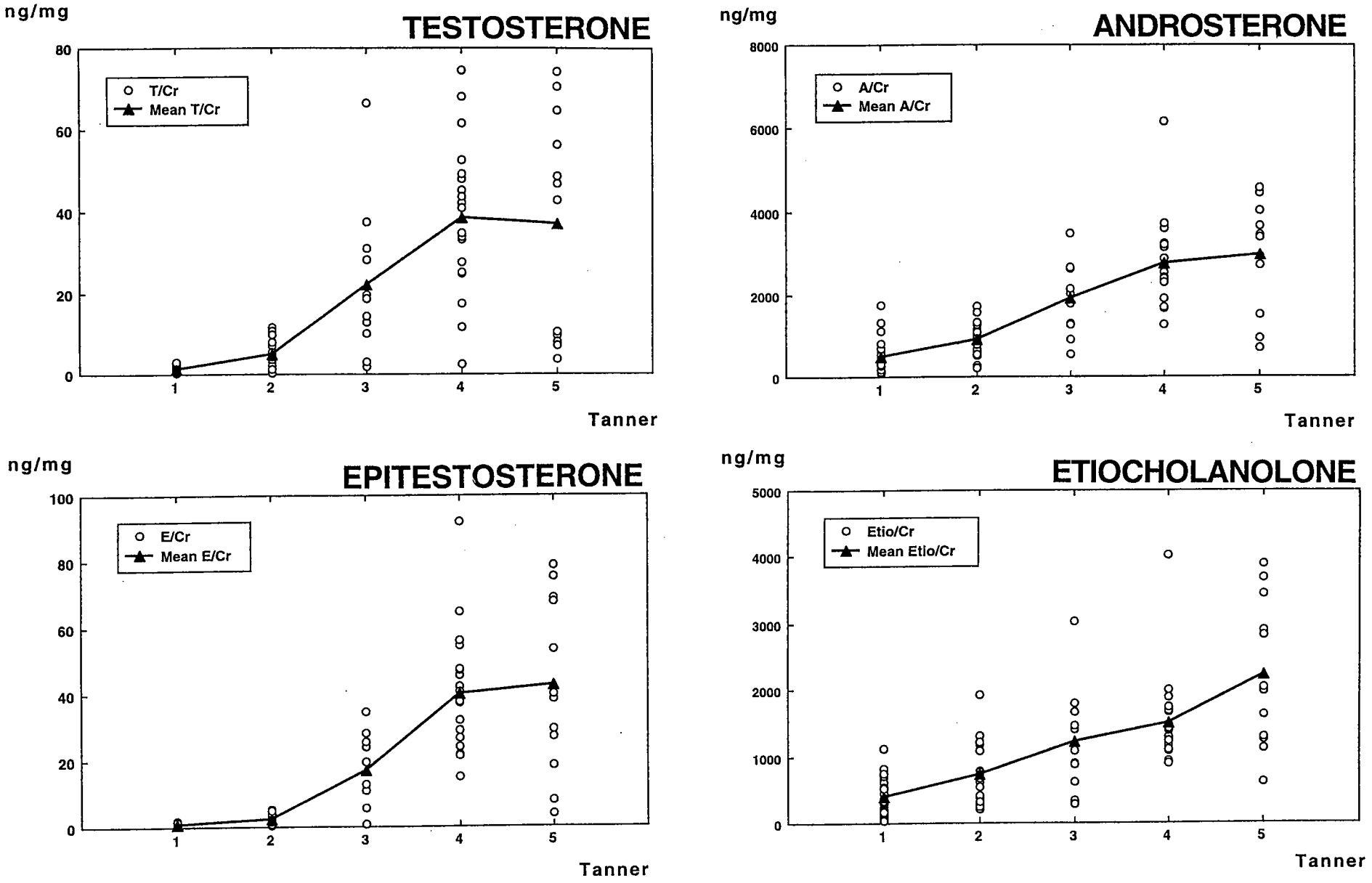


Figure 6: The concentrations of Testosterone, Epitestosterone, Androsterone and Etiocholanolone through puberty.

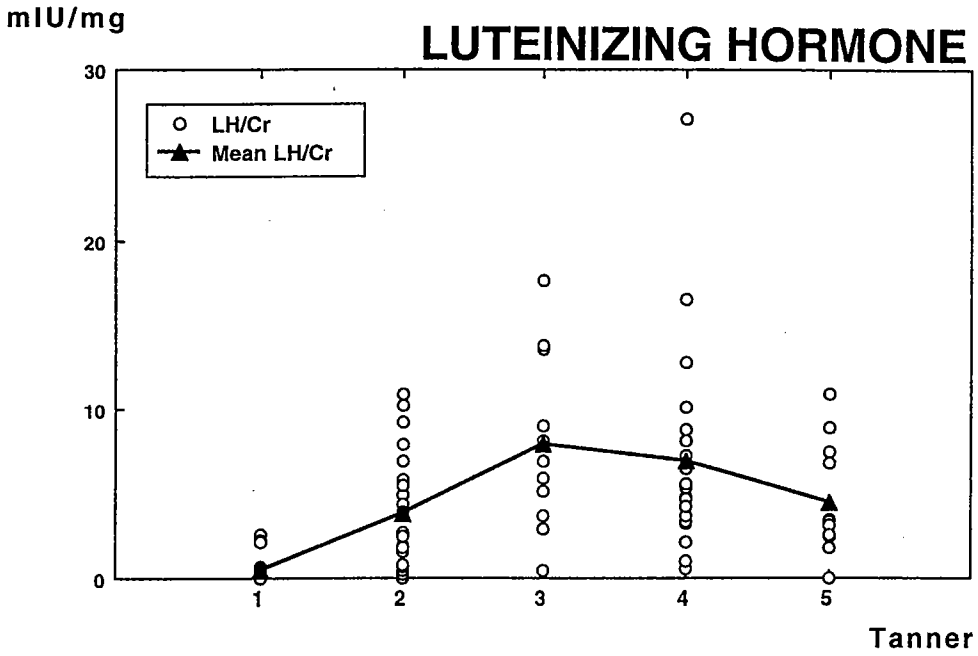


Figure 7: The concentration of the luteinizing hormone (LH) through puberty.

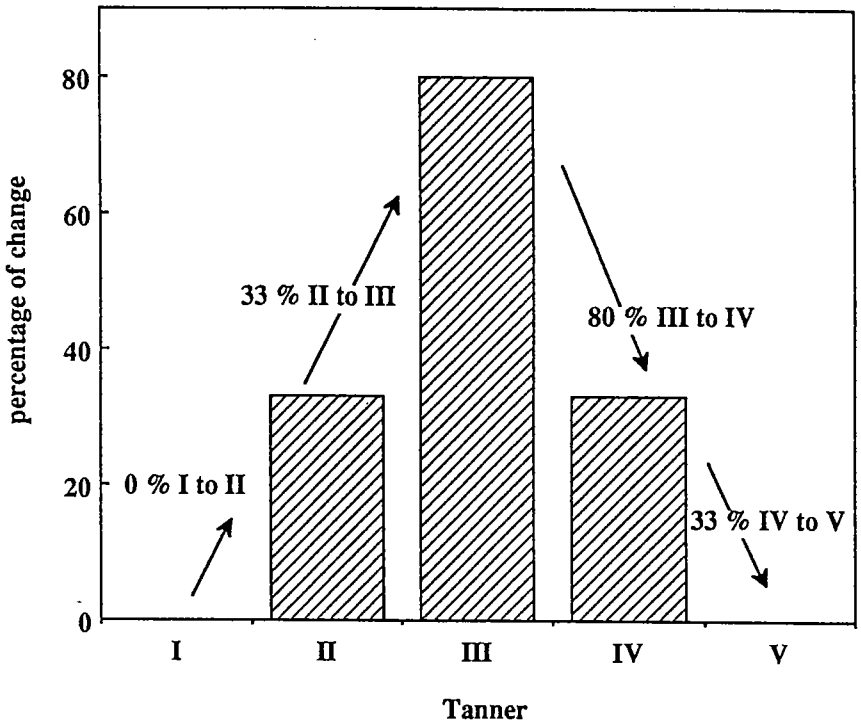


Figure 8: Percentage of change during the 6 month period.

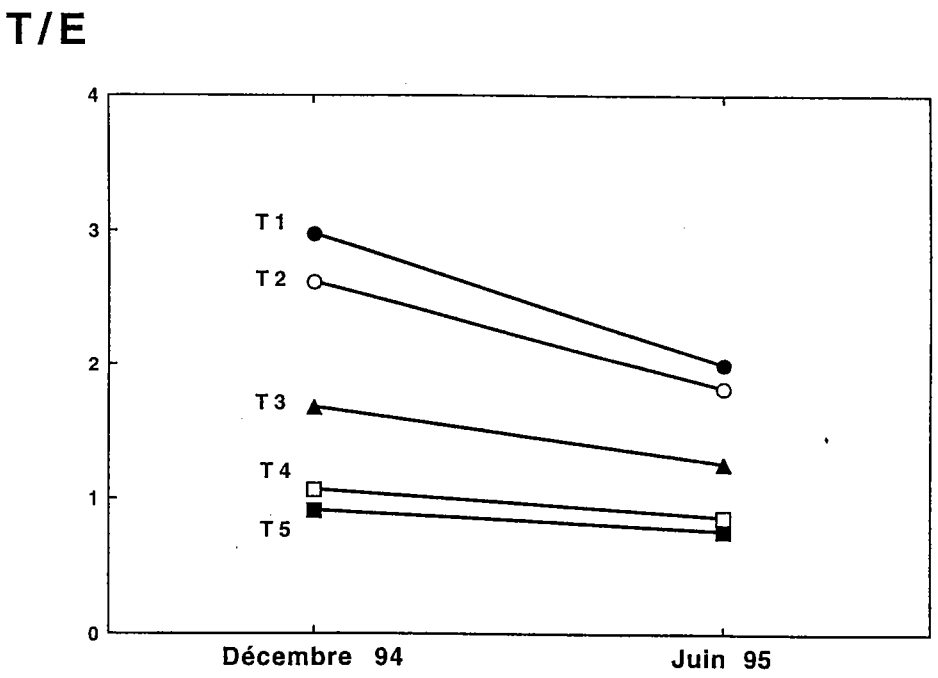
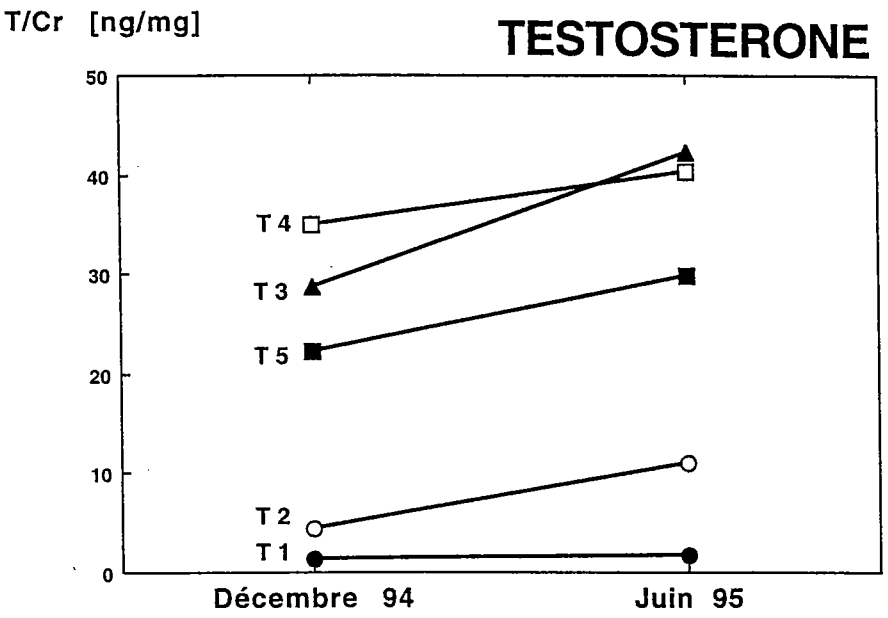


Figure 9: Variation of the T/E ratio of the different pubertal stages (T) after 6 month.



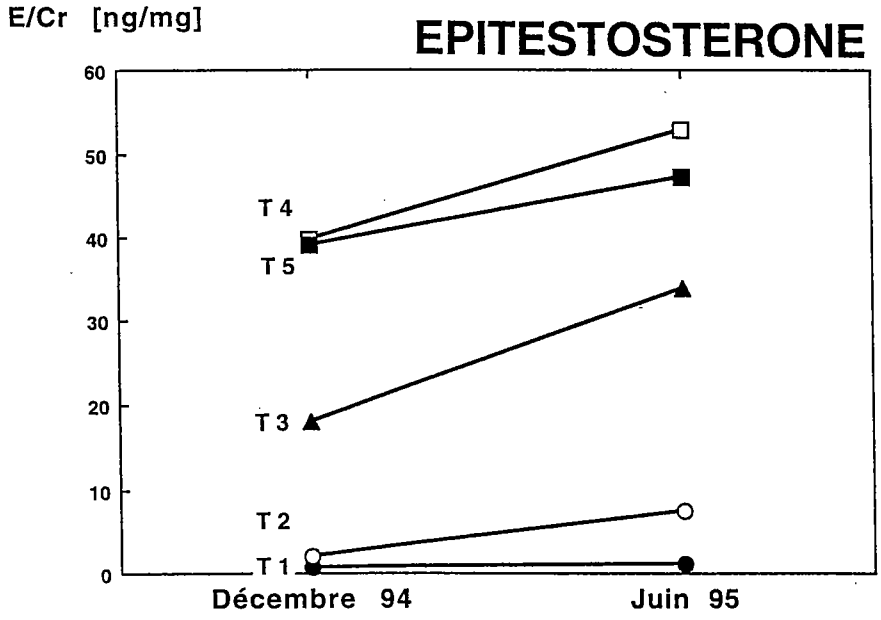


Figure 10: Variation of the concentration of Testosterone and Epitestosterone of the different pubertal stages (T) after 6 month.