Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (6)

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Sport und Buch Strauß, Köln, 1999

C. SCHWEIZER, C. CARDIS, M. CAUDERAY, L. RIVIER, M. SAUGY:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (6). Sport und Buch Strauß, Köln, (1999) 205-221

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Profile variations through puberty in young adolescent girls.

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Abstract

Steroid profiles were examined in young sportive girls in order to set up references values for different stages of the pubertal development. About 184 young girls exercising gymnastics (8-16 hours/week) were selected and put through a morphological examination in order to distribute then into 4 classes of development. Morning urine samples were collected and analysed for steroid profile by GC-MS.

There was no difference in growth and weight between the gymnasts and the control group composed of 69 girls: the development was normal for the sportive girls. For the steroid profile the same observations could be made.

For all the girls, the T/E ratio, significantly higher in the first stage, decreased with the development and tended to stabilize with the age. It appears clearly that Epitestosterone increase is responsible for that T/E ratio decrease through the different groups. This phenomena is more significant than what was observed previously with the boys [1].

A part of the population was re-examined 6 months later. The evolution through the development stages has been then evaluated and confirmed what was observed in the former transversal experiment.

Introduction

Two years ago, a study was already presented with male sportive adolescents following their steroid profile during the pubertal development, because it was observed elevated T/E ratio due to low concentrations of Testosterone and Epitestosterone in some young athletes [1]. The conclusions of the study were, that the T/E ratio was slighly higher in the first stages of

puberty to become similar to the T/E of adults in the lattest stage, but it never exceeded the critical value of 6. The concentration of Testosterone and others steroids in urine were showing a significant increase during the development of male adolescents.
The second part of the study about adolescents and steroid profiles was to do the same experiments with sportive girls, to follow the evolution of the steroid profiles in the different development stages, and to detect a predilected stage in which a risk to have elevated T/E ratios might be present.

Aim of the study

The aim of this study was to set up references values in young sportive girls (the litterature is very poor about steroid profile and women), to see the evolution and the variability of the T/E ratio, of the concentrations of steroids according the different stages of the pubertal development. The sportive girls were compared with a control group. A number of them were also examined a second time in an interval of 6-7 months to measure the variations in their development.

Material and methods

Young Volunteers

About 256 young girls between 6 and 17 years old were examined.
- Sportive girls group: 187 gymnasts practisizing 8 to 16 hours gymnastic a week. They were choosen in different clubs in the french part of Switzerland.
- Control group: 69 normal girls with normal sport activities (but no gymnastics in club).
All the subjects were examined by a doctor in order to establish the state of development. Different parameters were measured: weight, height, height when sitting, wingspan, Tanner (secondary sexual characters: breast and pubic hair). For all the volunteers the consent of the parents and of the ethical commission was obtained.

117 young girls were reexamined 6-7 months later for the semi-longitudinal study.

Urinanalysis

The night urine was collected to be analysed. Excretion volume were measured and a 20ml aliquot was frozen at -20°C until analysis.
Creatinine was determined by a colormetric test with a Cobas Mira (Hoffmann-La-Roche, Basel, Switzerland).
The Luteinising Hormone (LH), the Follicle Stimulating Hormone (FSH) and Estradiol were measured by an enzyme immunoassay test with a Cobas Core (Hoffmann- La-Roche, Basel, Switzerland).

Steroids

Several endogenous steroids were analysed in the urine: Testosterone, Epitestosterone, Androsterone, Etiocholanolone, 5α–androstan-3α,17β-diol, 5β-androstan-3α,17β-diol, Androstanolone (DHT), Androstenolone (DHEA) 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 [2-3] with some modifications [4].
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 extraction was made with n-pentane.
The derivatization mixture was MSTFA/TMSI/DTE (1000:5:5) instead of MSTFA/NH4I/ethanethiol and the injection was done on GC-MSD.
The internal standard was composed of a mixture of deuterated standards (furnished by the Cologne group).

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 for 1 min, then to 230°C at 1.8°C/min, then to 310°C (5 min) at 30°C/min.

Results and discussion

Clinical data

Table 1: The distribution of the girls in 4 stages according their physical parameters and Tanner.

Tanner	*Group* n	*Subject*	*Age* in years ± SD	*Height* in cm ± SD	*Weight* in kg ± SD	*BMI* * in kg/m^2
I	Gymnasts	124	8.9 ± 1.3	128.8 ± 8.8	26.2 ± 4.1	15.7 ± 1.3
	Controls	32	9.4 ± 1.5	134.5 ± 7.6	29.8 ± 4.1	16.4 ± 1.1
II	Gymnasts	32	12.0 ± 1.0	145.2 ± 5.7	35.6 ± 4.3	16.8 ± 1.4
	Controls	15	11.0 ± 1.4	145.7 ± 7.4	35.7 ± 4.7	16.7 ± 1.2
III	Gymnasts	18	12.6 ± 1.2	152.1 ± 6.1	41.0 ± 4.2	17.7 ± 1.4
	Controls	14	12.6 ± 1.4	156.6 ± 8.4	44.5 ± 8.4	18.0 ± 2.3
IV	Gymnasts	12	15.1 ± 1.2	160.4 ± 5.7	52.8 ± 4.8	20.5 ± 1.1
	Controls	8	14.5 ± 1.5	161.8 ± 5.3	53.3 ± 4.6	20.4 ± 1.6

* Body Mass Index

The Table 1 gives the distribution of the the gymnasts group and the control group in four different groups according to the Tanner stages and the means of the morphological parameters for the stages. There was no morphological difference between the gymnasts and the control groups. The gymnasts were slighly smaller and thinner than the controls, but at stage IV they caught up the control group (Figure 1). The development was the same for the gymnasts than for the other girls: there was a normal increase of weight and height. In this case moderate sport (< 15h) didn't affect the growth of the sportive girls [5-7].

Results of the steroid profile

When looking at the representation of the T/E ratio according to the Tanner through puberty, an elevated T/E ratio can be seen at the pubertal stage I, which tended to decrease significantly and to finally stabilize between stages III and IV (Figure 2). In the stage I a large variability of the T/E ratio could be seen: in this Tanner group the most elevated ratios were found with values approaching the limit of 6. The absolute variability tended to decrease with the pubertal development.

This variability and these high T/E ratios could be explained by the concentrations of Epitestosterone which are very low at stage I (Figure 3).

The concentrations of Testosterone and Epitestosterone increased progressively during the development until to stabilize at the end of puberty, with an important increase during the passage from the pubertal stages II to III (Figure 3). But the urinary excretion of Testosterone and Epitestosterone weren't exactly similar: the excretion of Epitestosterone seemed to be more important (a steeper slope) during this passage.

The same observations could be made as for the sportive group as for the control group.

This pattern was nearly similar for the adolescent boys, although a parallelism of the excretion between the concentrations of Testosterone and Epitestosterone could be seen (Figure 4). These results were also noticed by Dehennin [8]. Also the stability of the T/E ratio was reached later, only at stage V (the girls reached it at stage III).

Moreover an increase in the concentrations of others metabolites such as Androsterone and Etiocholanolone was shown through the pubertal development, with a higher increase of Androsterone in the two group of girls (Figure 5). No lowered Androsterone in the older trained girls was noticed compared to the controls group, as it was related by Juricskay [9].

The Luteinizing Hormone, as for the boys or for the girls, followed the curve of growth with a hightest values at stage III which correponds to the peak of growth (Figure 6).

For the two groups, there were no difference in the T/E ratios, in the concentrations of steroids, of Luteinizing Hormone between the gymnasts and the control group in each step of the pubertal development (Figure 7). There was only a clear difference between the stage I and the stages III, IV with a decrease of the T/E ratio for the two groups.

When compared, the statistical distributions of the T/E ratios of all the adolescent subjects, gymnasts and controls, (mean: 2.0 ± 1.1) and of female athletes analysed in our laboratory in 1997 (mean 1.5 ± 1.1) showed a T/E ratio slighltly higher for the adolescents: this could be explained that the stage I contained a lot of prebupertal girls with elevated T/E ratios (Figure 8).

Semi-longitudinal studies

117 girls were reexamined 6-7 months later in order to observe the variations and the change in their pubertal development. Two important shifts were established from stage II to II and III to IV (Figure 9).

Considering only the lapse of time during which the girls changed stages , the percentage of variation of the concentrations of Testosterone and Epitestosterone could be calculated (Figure 10). During the move from stage I to stage II, there was a parallelism in the increase of Testosterone an d Epitestosterone, which was related by a very small decrease of T/E ratio (Figure 10). On the contrary during the move from stage II to stage III, there was a much more increase of Epitestosterone than of Testosterone, so the T/E ratio decreased strongly in this pubertal period. During the move from stage III to stage IV, the T/E ratio and the concentrations of Testosterone and Epitestosterone tended to a stabilization (no change in the T/E ratio). So this confirmed the hypothesis of the transversal study, during the passage from stage II to stage III a larger urinary excretion of Epitestosterone could be noticed, which produced a significant decrease of the T/E ratio.

Conclusions

- There were no significant differences between the gymnasts and the control group as well as in physical parameters as in the steroid profile. Moderate level of sport (< 15h) had no influence in the pubertal development.
- The T/E ratio was significantly higher in the first stage (Tanner 1) and decreased with the development and tended to stabilize at the end of puberty (stage IV).
- There was no parallelism in the excretion of Testosterone and Epitestosterone: the excretion of Epitestosterone was more important between the stages II to III (no similitude with the boys).

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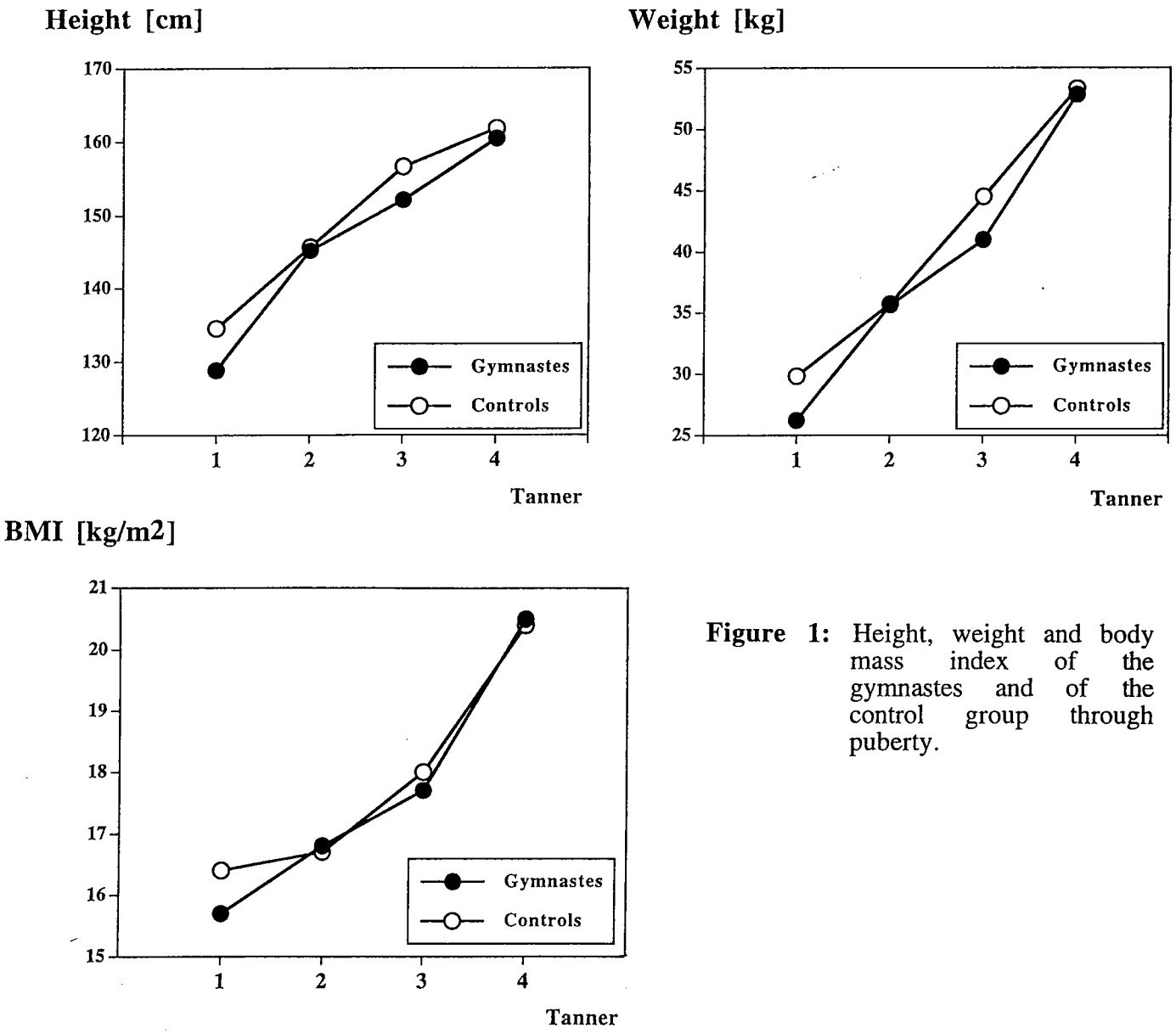


Figure 1: Height, weight and body mass index of the gymnastes and of the control group through puberty.

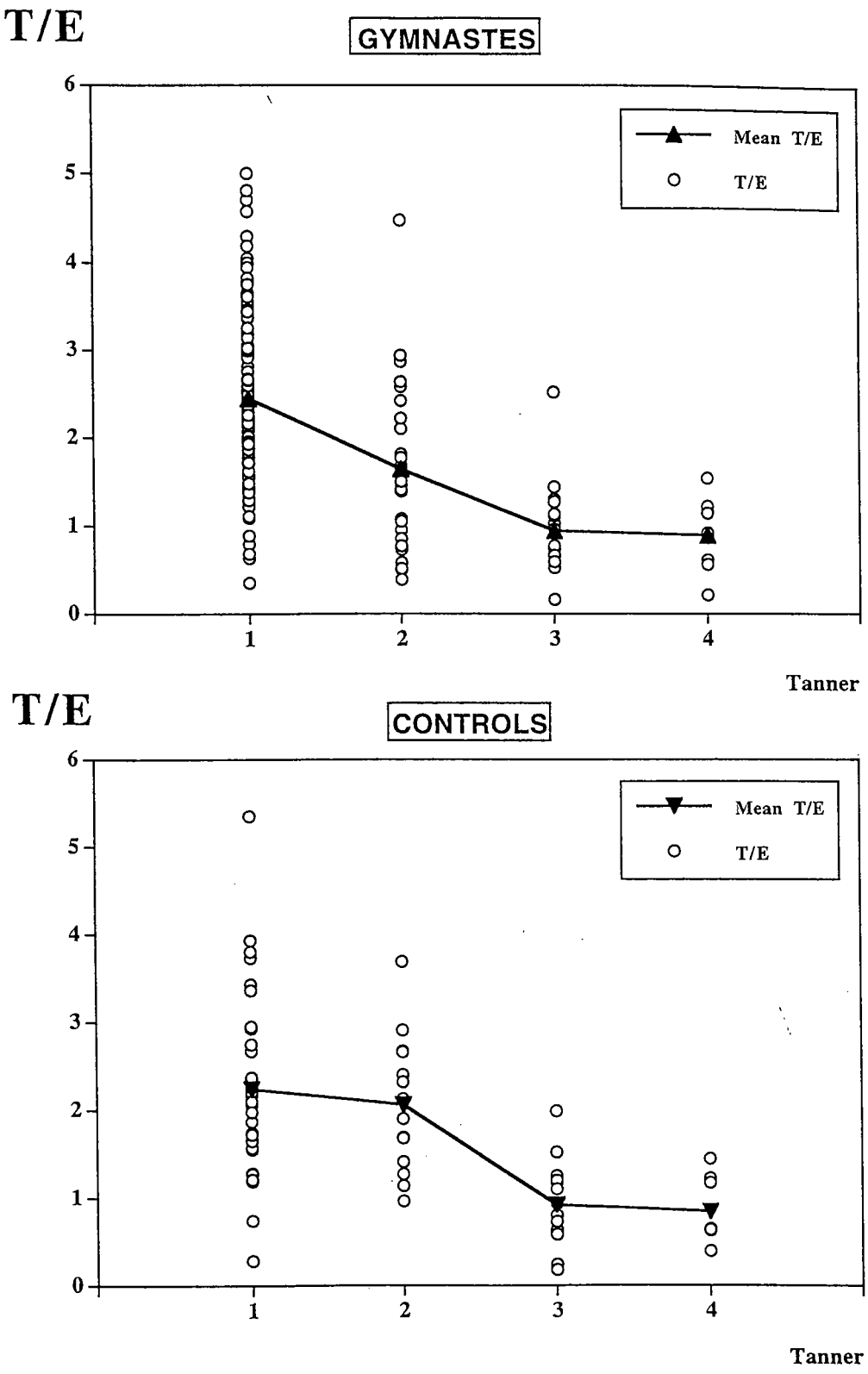


Figure 2: The T/E ratio of the gymnastes and of the control group through puberty. Black line: The mean ot the T/E ratio for each pubertal stages.

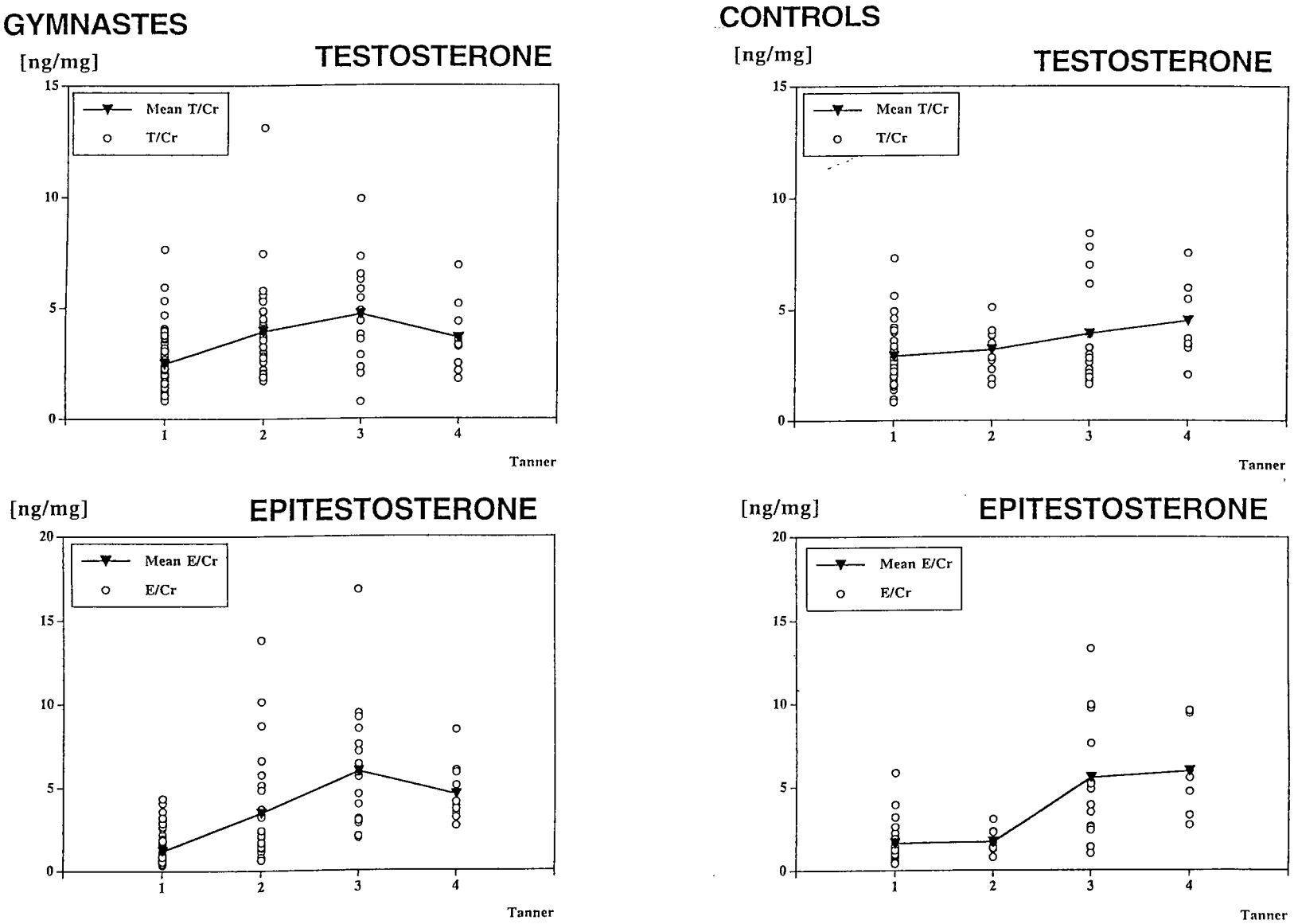


Figure 3: Concentrations of Testosterone and Epitestosterone through puberty. Left: Gymnastes; Right: Control group. Black line: The mean ot Testosterone and Epitestosterone concentrations for each pubertal stages.

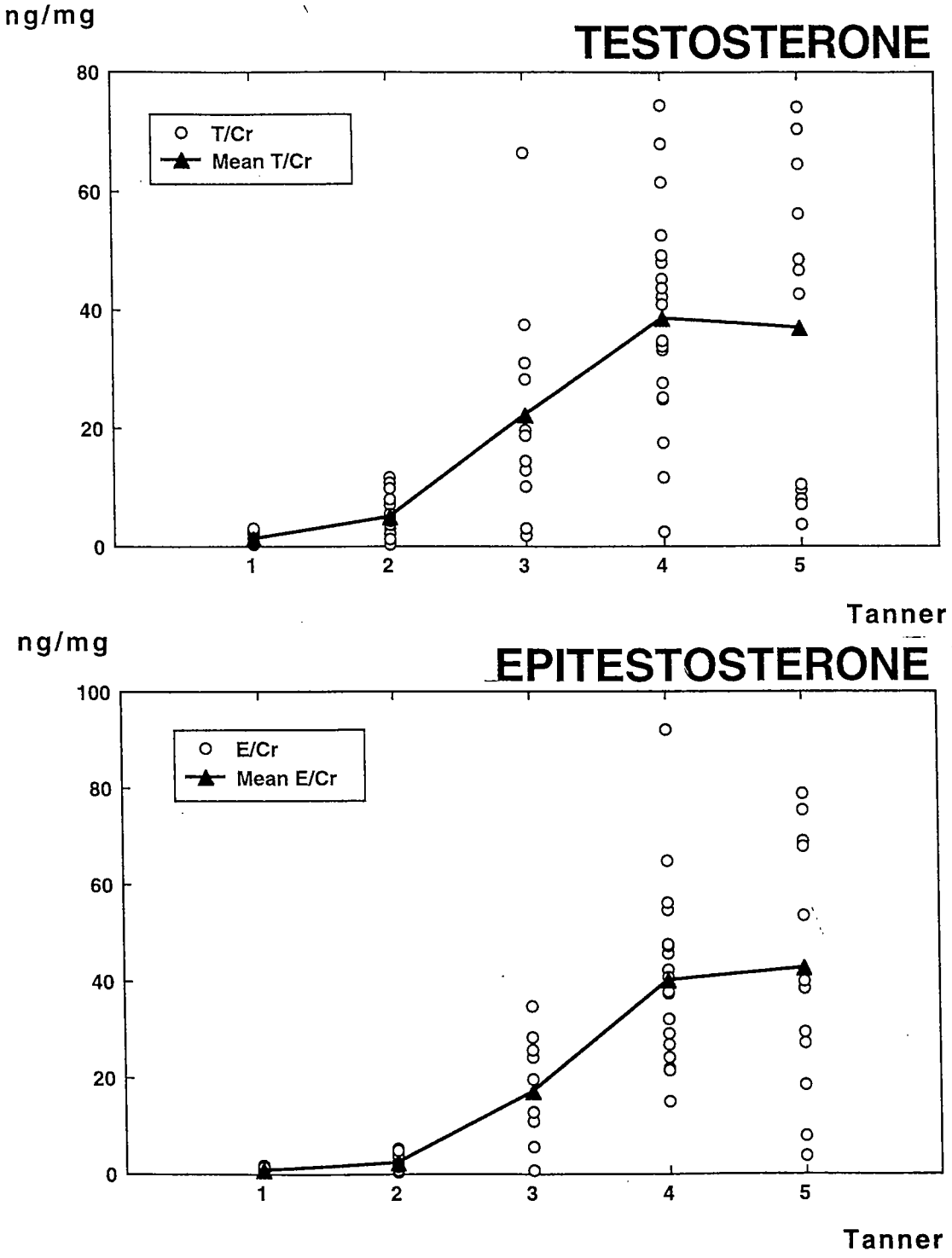


Figure 4: Concentrations of Testosterone and Epitestosterone of the adolescents boys through puberty.
Black line: The mean ot the T/E ratio for each pubertal stages.

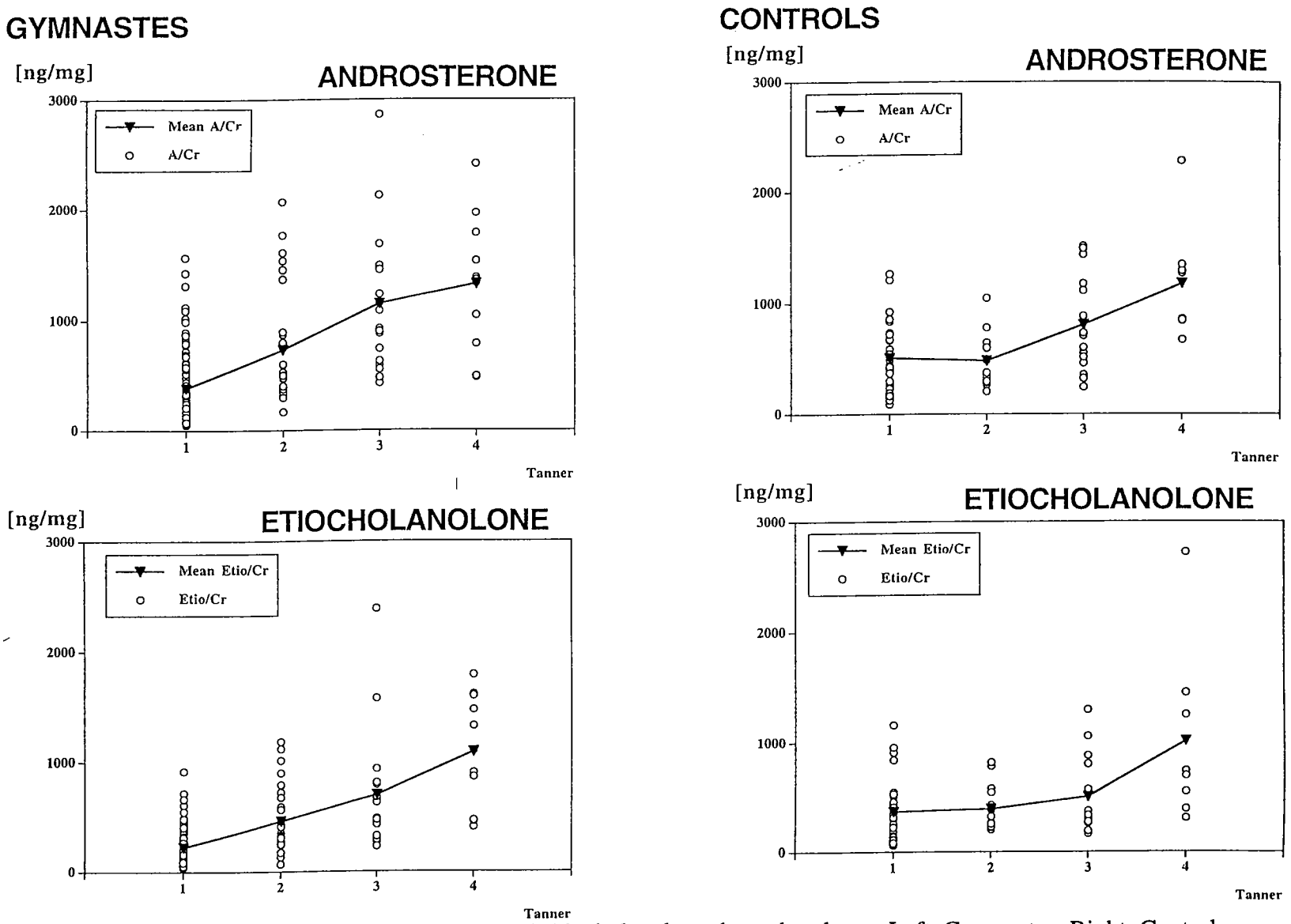


Figure 5: Concentrations of Androsterone and Etiocholanolone through puberty. Left. Gymnastes; Right: Control group. Black line: The mean of Androsterone and Etiocholanolone concentrations for each pubertal stages.

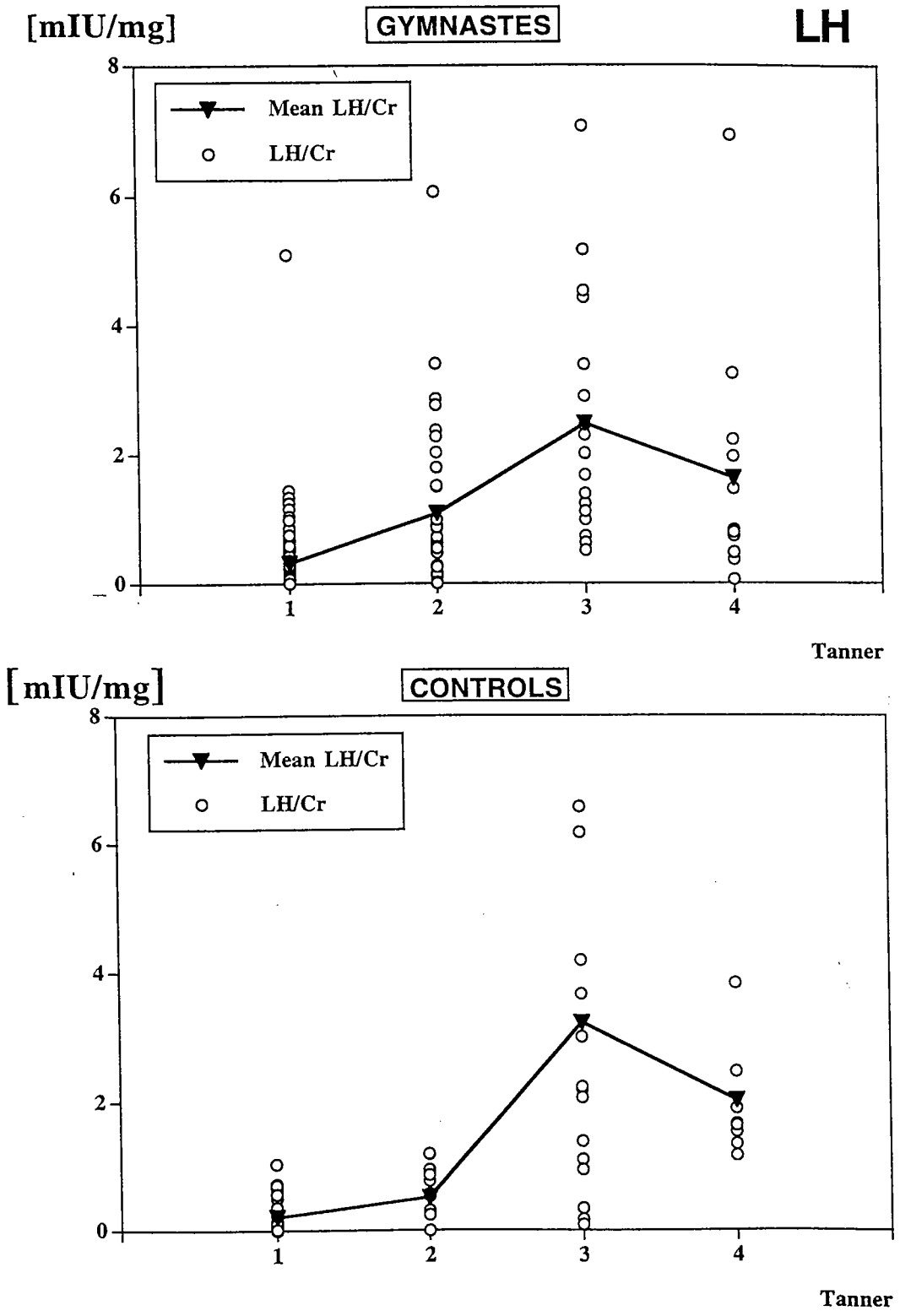


Figure 6: Concentration of the Luteinizing Hormone (LH) through puberty. Up: Gymnastes; Below: Control group.

T/E

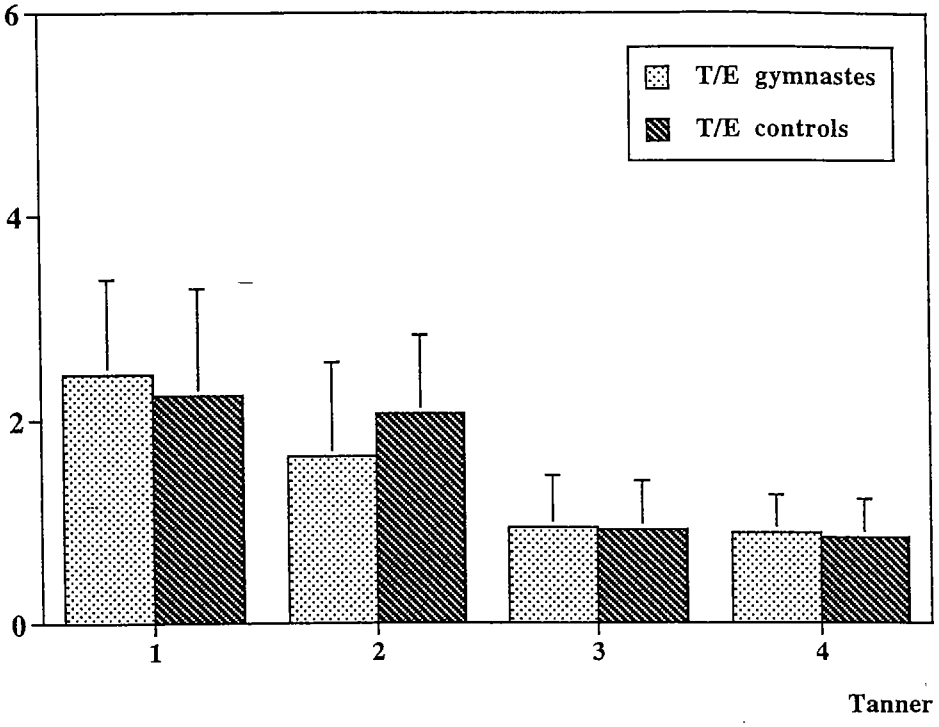


Figure 7: Comparison between the gymnastes and the control group of the T/E ratio's mean for each pubertal stage.

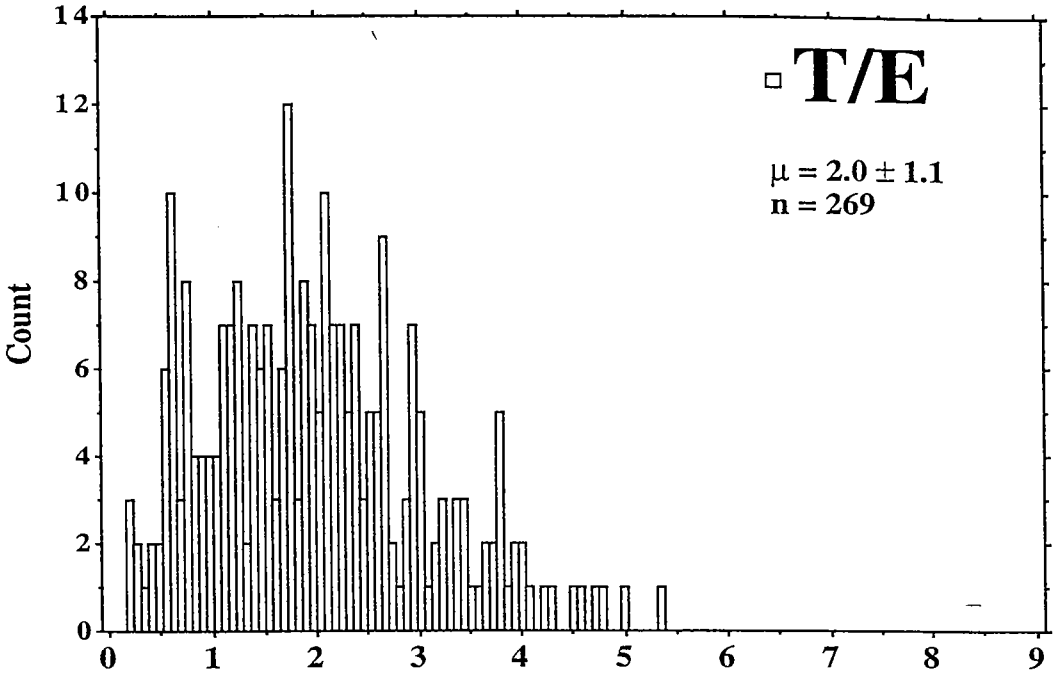


Figure 8 a: Statistical distribution of the T/E ratio of Adolescent girls.

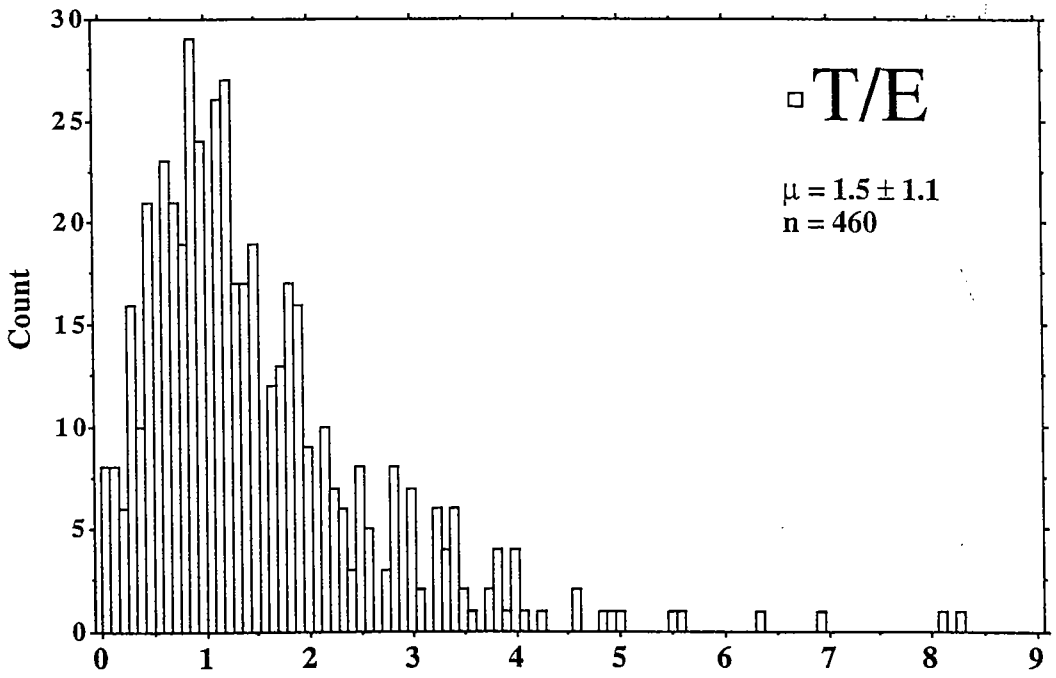


Figure 8 b: Statistical distribution of the T/E ratio of female Athletes in 1997.

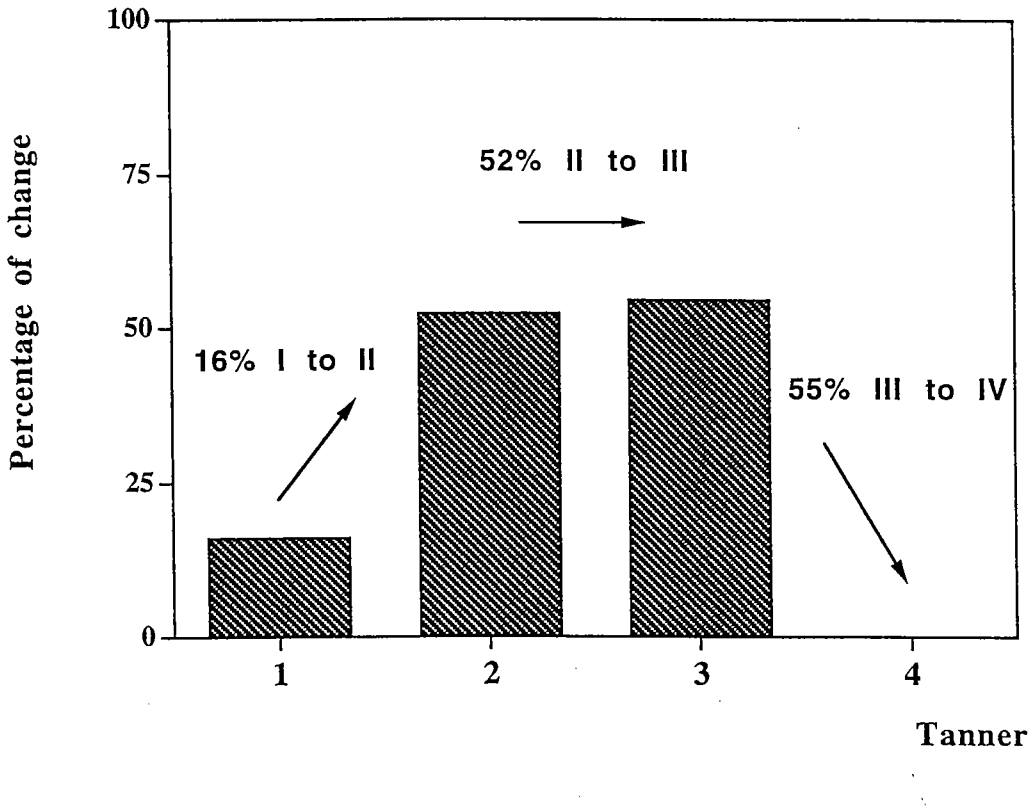
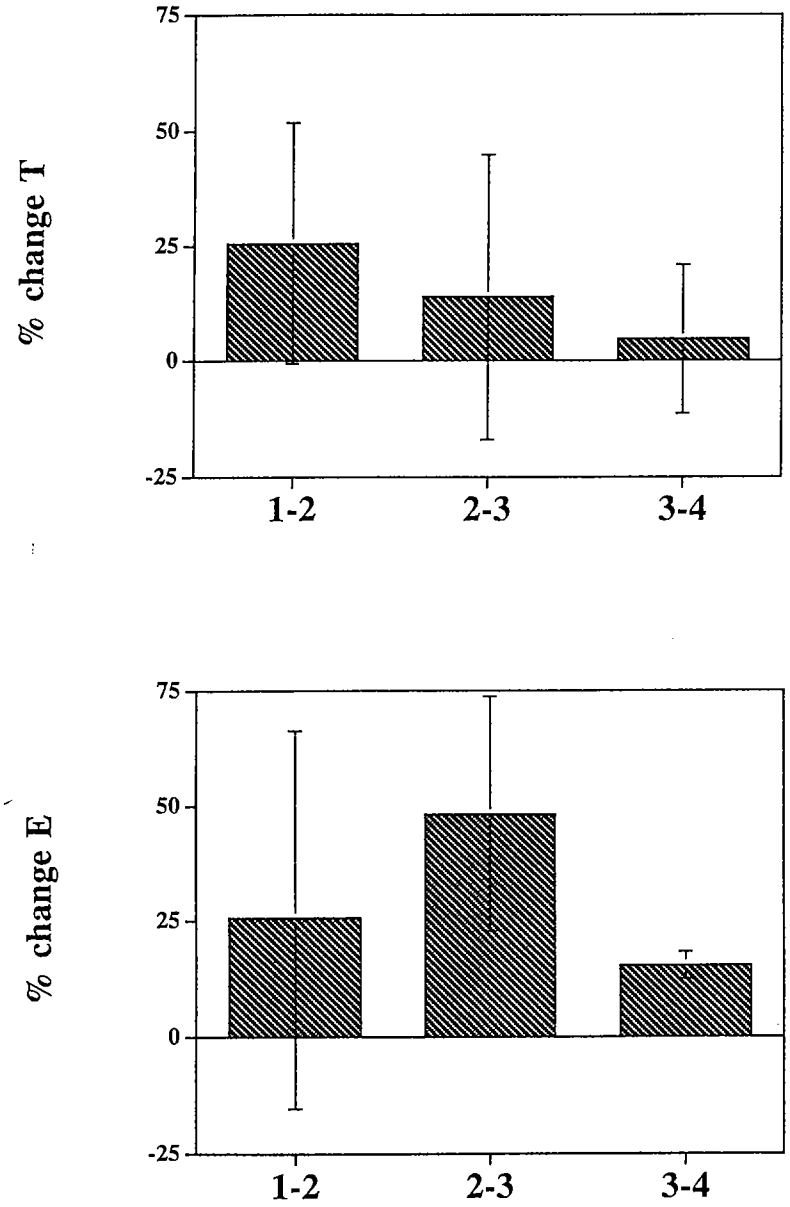


Figure 9: Percentage of stage's change during the 6-7 months period.



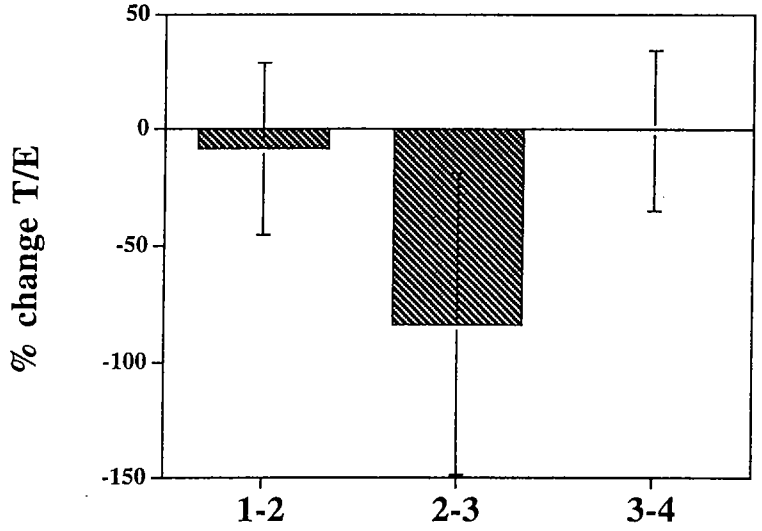


Figure 10: Variation of the concentrations of Testosterone, Epitestosterone and T/E ratio during the 6-7 months period.