

Reprint from

RECENT ADVANCES
IN DOPING ANALYSIS
(4)

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Ketoconazole test: Blood versus urine values

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Introduction

Our laboratory was mandated by the medical commission of the UCI and by the chairman of the Austrian anti-doping Committee to perform endocrinological investigation on two different athletes with elevated T/E ratio.

Therefore, we performed a ketoconazole test in order to establish whether these high T/E results were due to a pathological / physiological state or to an intake of testosterone.

History of both competitors

The first athlete (**Case A**) is a male cyclist born in 1977. He has been controlled twice in 1995 during cycling competitions. His T/E values were as followed:

		T/E
Laboratory 1	07.07.95	9.9
UAD	10.09.95	9.3

On the basis of these results, it has been decided to perform a ketoconazole test, which started the 26 of september 1995.

The second athlete (**Case B**) is a male born in 1968. He is a mountain biker and has been controlled five time in 1995.

The samples were collected in Austria and in Switzerland, they were measured either in Laboratory 1 or in UAD at Lausanne.

		T/E
Laboratory 1	06.08.95	8.2
UAD	22.09.95	6.7
Laboratory 1	11.09.95	5.9
UAD	16.09.95	5.6
UAD	02.11.95	9.5

On the basis of these results, it has been decided to perform a ketoconazole test, which started the 7th of november 1995.

Ketoconazole test

Ketoconazole is an important oral antifungal agent with broad spectrum activity and low toxicity. One of the major secondary effect is its ability to inhibit the steroidogenesis in men, particularly the biosynthesis of testosterone.

Therefore, the following results are expected after an oral intake of ketoconazole.

If T/E ratio is naturally elevated, the T/E should decrease after Ketoconazole intake

If T/E ratio is due to Testosterone doping, the T/E should stay at the same level after Ketoconazole intake

The experiment was performed with Nizoral containing 200 mg Ketoconazole

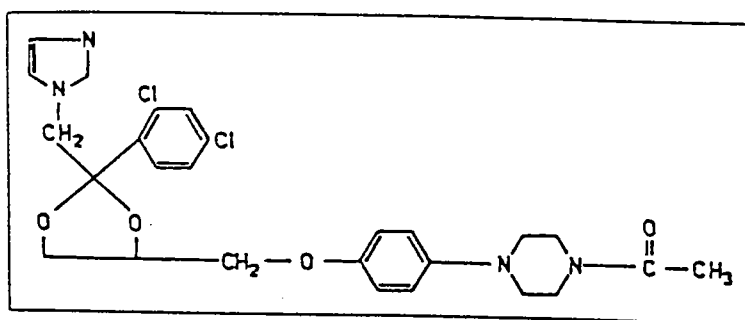


Figure 1: Structure of Ketoconazole

Protocole

The athlete is present in the laboratory for a three days period of time (2 nights included). During this period all urines emitted are collected, as well as 8 blood samples and saliva.

Flow-chart of Protocole

Day 1

08h00:	Arrival UAD
	Explanation for the test and logistic
09h00	Blood sampling 10 ml, saliva sampling 2 ml
in the morning	Housing
13h00	Blood sampling 10 ml, saliva sampling 2 ml
The all day	Urine collection
17h00	Blood sampling 10 ml, saliva sampling 2 ml

Day 2

08h15:	Physician Medical examination Explanation and consent
09h00	Blood sampling 10 ml 400 mg Ketoconazole treatment
The all day	Urine collection
13h00	Blood sampling 10 ml, saliva sampling 2 ml
17h00	Blood sampling 10 ml, saliva sampling 2 ml

Day 3

08h15:	Blood sampling 10 ml, saliva sampling 2 ml
13h00:	Blood sampling 10 ml, saliva sampling 2 ml
The all day	Urine collection
17h00	End of experiment

Methods of analysis

Urine

In order to analyse the different steroids needed for the establishment of the steroid profile, screening IV for steroid profile was performed. The urine samples and controls are derivatized, after enzymatic (E.Coli) hydrolysis and extraction with n-pentane, with MSTFA/TMSI/DTE. The analyses were performed on GC-MS 5971 fitted with an Ultra 1-HP column

The following parameters are measured:

Testosterone, Epitestosterone, Androsterone, Etiocholanolone, DHT, 5 α -androstane-3 α ,17 β -diol (α -diol) and 5 β -androstane-3 α ,17 β -diol (5 β -diol)

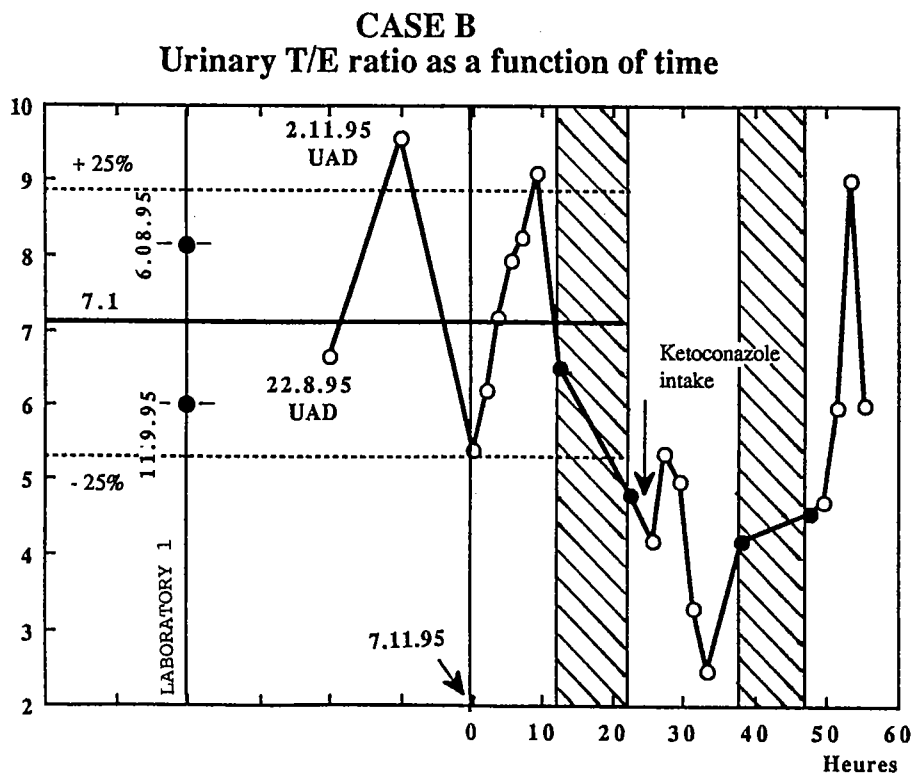
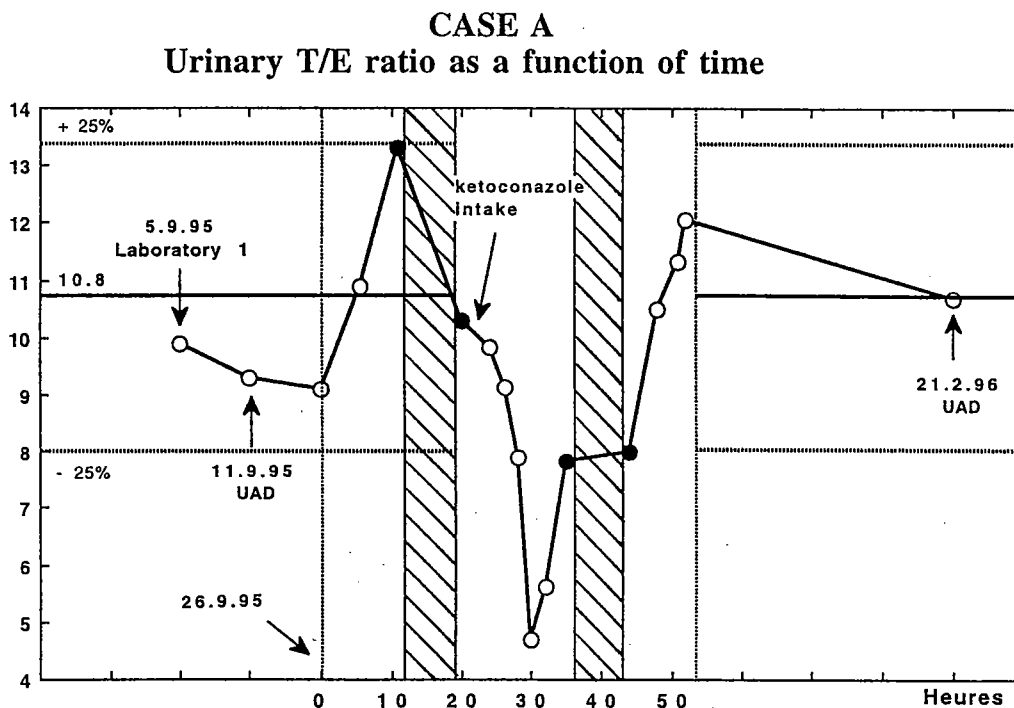
The LH, FSH and hCG hormones were measured with the Cobas Core Immunochemistry System, which is a true Random Access, fully automated instrument controlled by a multi-dialogue software.

Blood and saliva

Testosterone (blood and saliva), 17-OH-progesterone, LH and FSH (blood) were analysed by RIA from DPC

Results and comments

Figure 2 and 3: Urinary T/E ratio as a function of time. The values obtained previously in Laboratory 1 and in UAD (Lausanne) are plotted before T0. The black shadows are for the night periods.



Despite the rather unstability of the T/E ratio for the case B, the "natural" (before Ketoconazole intake) T/E values are almost all included in plus minus 25 % from the mean values, which is considered as a normal variation. After ketoconazole intake, a diminution of the T/E value is observed. In the morning of the third day, the T/E ratio are recovering the natural values.

These primary results are showing expected responses of the Ketoconazole test.

The urinary testosterone excretion (Figure 4) for both athletes are quite similar. A decrease of the testosterone excretion after the intake of ketoconazole can be observed, although it is not so spectacular. Reporting the testosterone concentration on the creatinine concentration (Figure 5) shows different results. For the case A, the diminution is now more pronounced, whereas for the case B, the suppression of the testosterone production is not so well marked.

Epitestosterone or its report on creatinine (Figure 6 and 7) are slightly affected by the intake of ketoconazole.

The urinary excretion of creatinine (Figure 8) varies a lot during the experiment. It even seems that it might be affected by the intake of ketoconazole. This must be taken with caution as the athlete has to produce regular urine miction during the day and therefore is obliged to drink a lot which dilute his urine and then affects the creatinine concentration.

To avoid this problem of dilution, other ratio than T/E are taken into consideration as A/T ratio (Figure 9). The absolute values of the A/T ratio are really different in both cases (case A, the maximum A/T value is 28.8; case B is 110). When ratio's values are represented in percent from the maximum, the curves are fully superimposable and an increase, after ketoconazole intake, is well marked with about 60 % of elevation, which is quite spectacular.

In blood as well as in saliva the testosterone concentrations (Figure 10 and 11) are subject to variation during the observation day with minimum values of about 10 nmol/l for blood and around 250 pmol/l in saliva. During the second day, the variation profile is the same, but after the treatment, the diminution is really much more pronounced with minimum values at 4 for blood and < 20 for case A and almost 80 for case B in saliva. The next morning all values are comparable with those of the two previous mornings.

The LH values as well as in urine than in blood are difficult to interprate (Figure 12 and 13)

As for urine, in blood, ratios give meaningfull values as it can be seen in figure 14 representing the ratio Testosterone to 17-OH-Progesterone in % of the maximum value. As for the urinary A/T ratio, the results of the two athletes are matching well together.

Discussion

On the basis of the following parameters (table 1), we conclude for these two cases that these athletes have a naturally elevated T/E ratio and that it was not due to any drug intake or other kind of manipulation.

Table 1: Parameters involved for the decision

	CASE A	CASE B
<i>Urinary</i>	T/E	T/E
	A/T	A/T
	T/crea	?
	T	T
<i>Blood</i>	T/17-OH-P	T/17-OH-P
	T	T
	not measured	Delta 4/ 17-OH-P
<i>Saliva</i>	T	T

7 parameters were taken into account to decide whether or not the ketoconazole test was a success.

For urine, the concentration of the different compounds is dependant from the dilution and, because of the creatinine results, the best parameters are the ratios T/E and A/T.

In blood the question of dilution is not present, then ratios and testosterone concentration are useful results. So including saliva, the number of significative parameters are the same in urine than in blood and saliva.

Therefore and also for different parameters, as for example time consuming (RIA), costs and the fact that it is more difficult to cheat with blood than with urine, it could be decided to work only with blood and saliva. Anyway it is important to measure these different parameters to give additional informations from those in urine.

Conclusion

As well as for the urinary testosterone (figure 4) than for the blood testosterone (figure 10), it needs to be confirmed that the diminution of concentration is really due to the effect of the intake of ketoconazole and not due to a circadien cycle. Therefore it will be necessary to perform a double blind study with placebo and testosterone administration.

In conclusion, and even if there are still some unsolved questions, this test is from a great help to take a decision on elevated T/E ratio. After some more experiments, a number of criteria and their variations should be specified to validate the test. Then the decision on the result will be taken from clearly defined parameters and not from an empiric evaluation.

References

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Figure 4

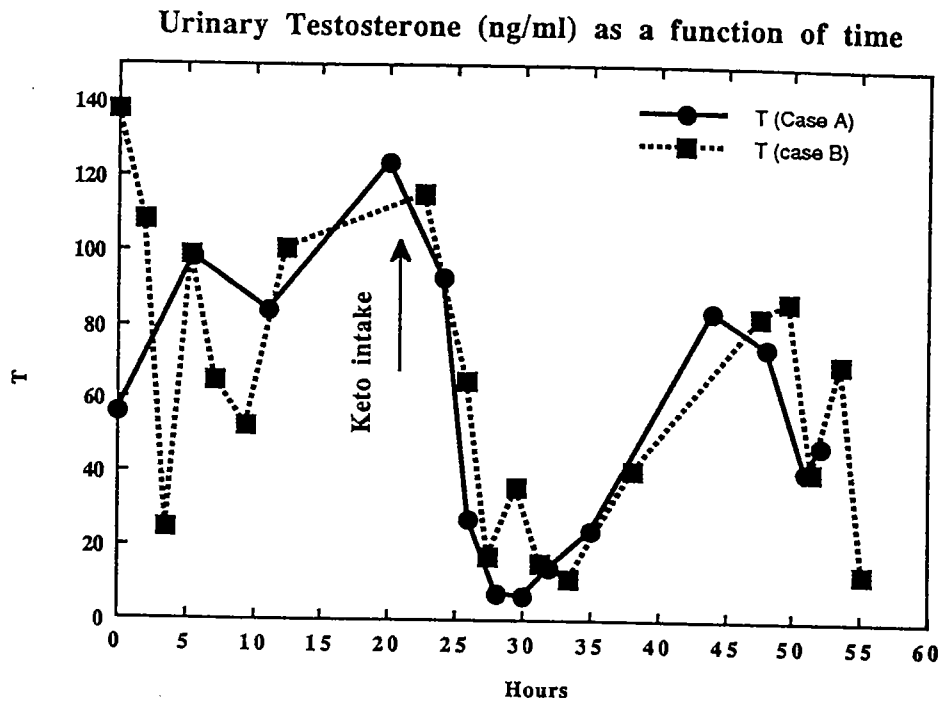


Figure 5

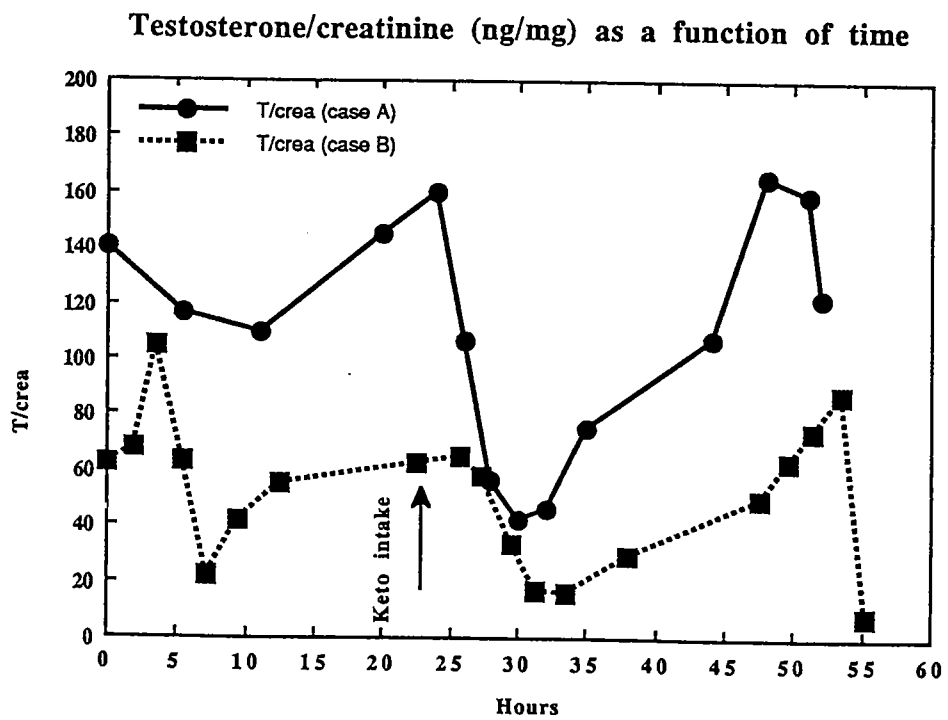


Figure 6

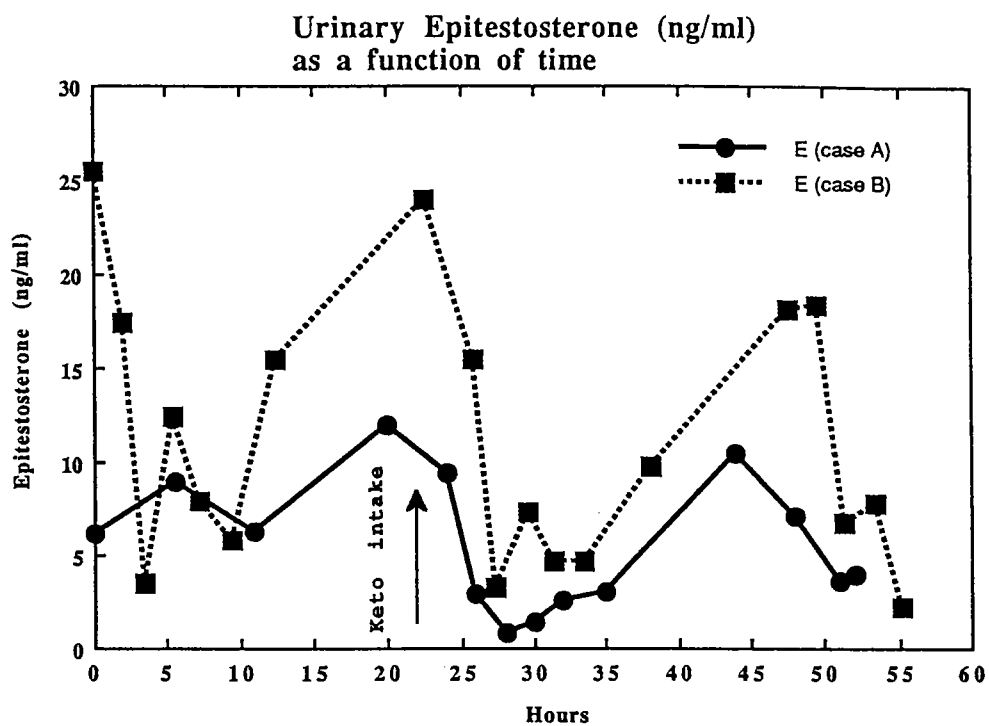


Figure 7

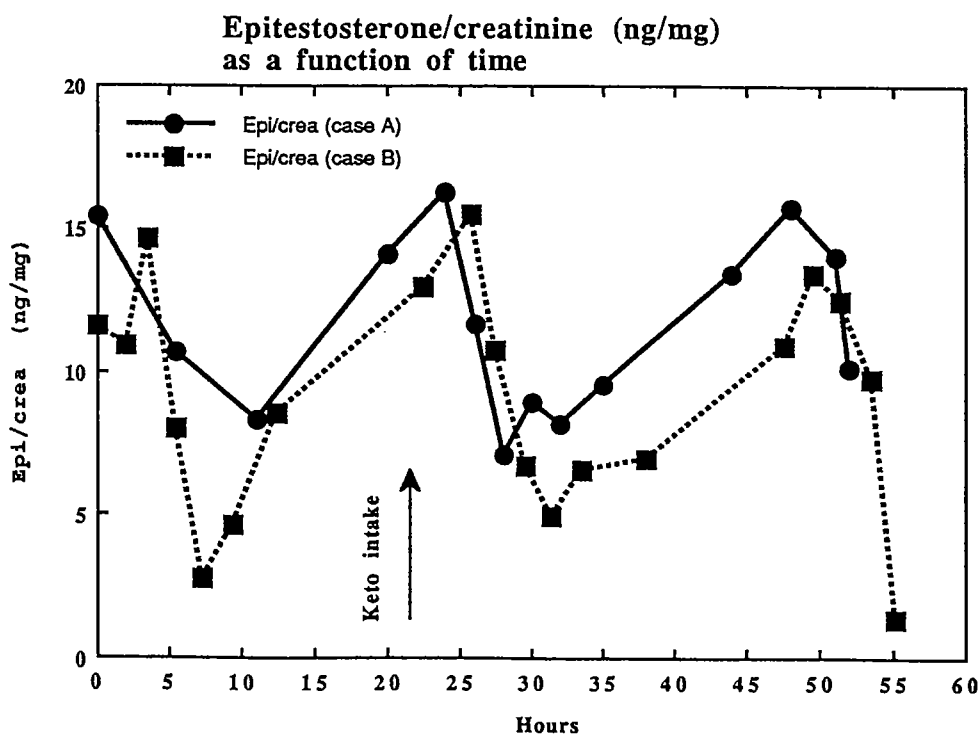


Figure 8

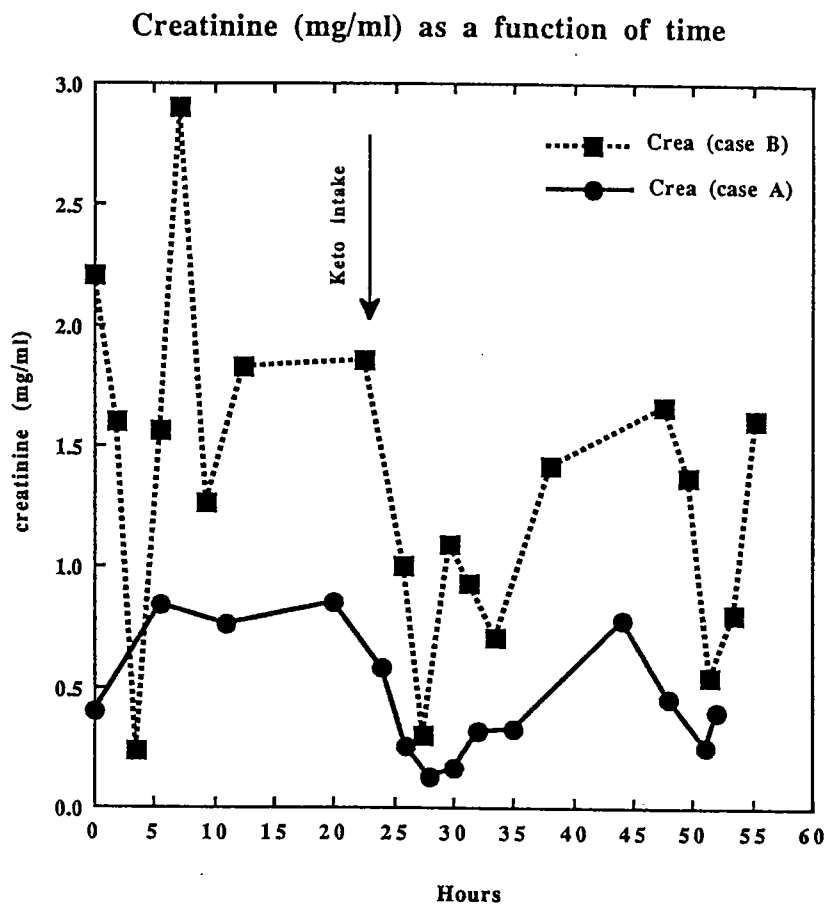


Figure 9

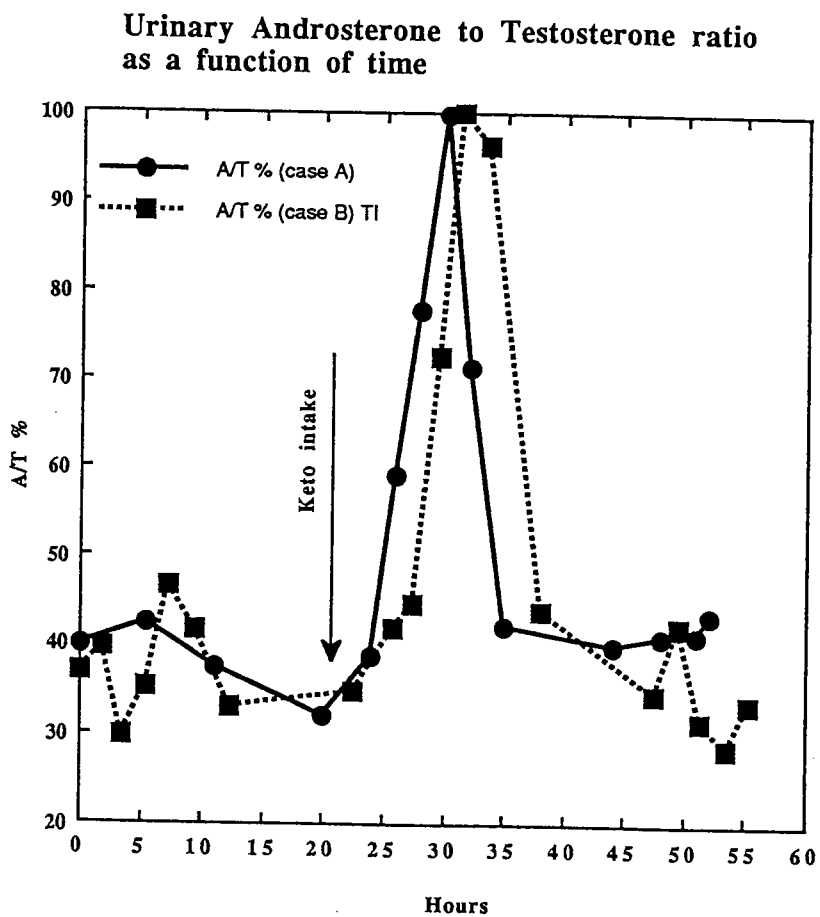


Figure 10

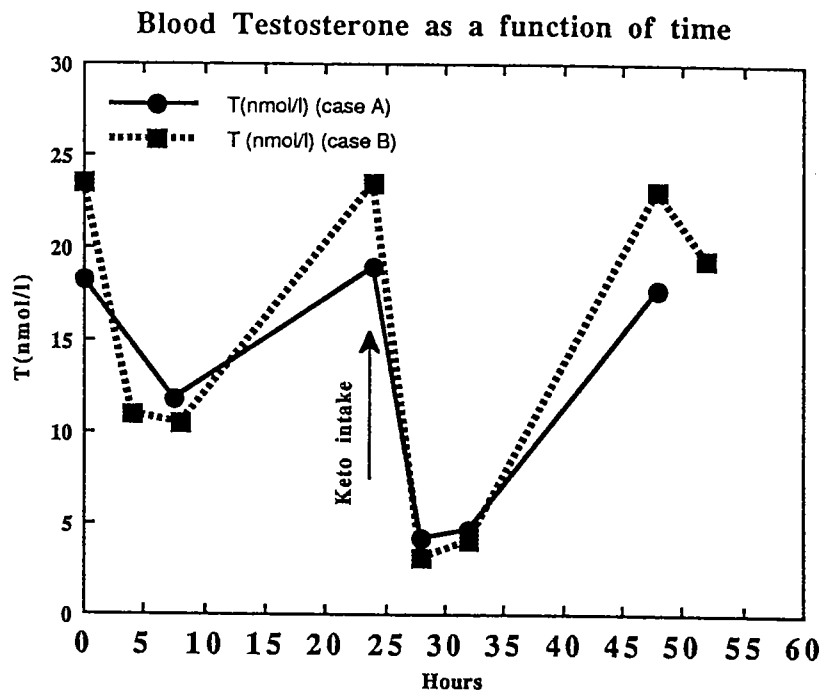


Figure 11

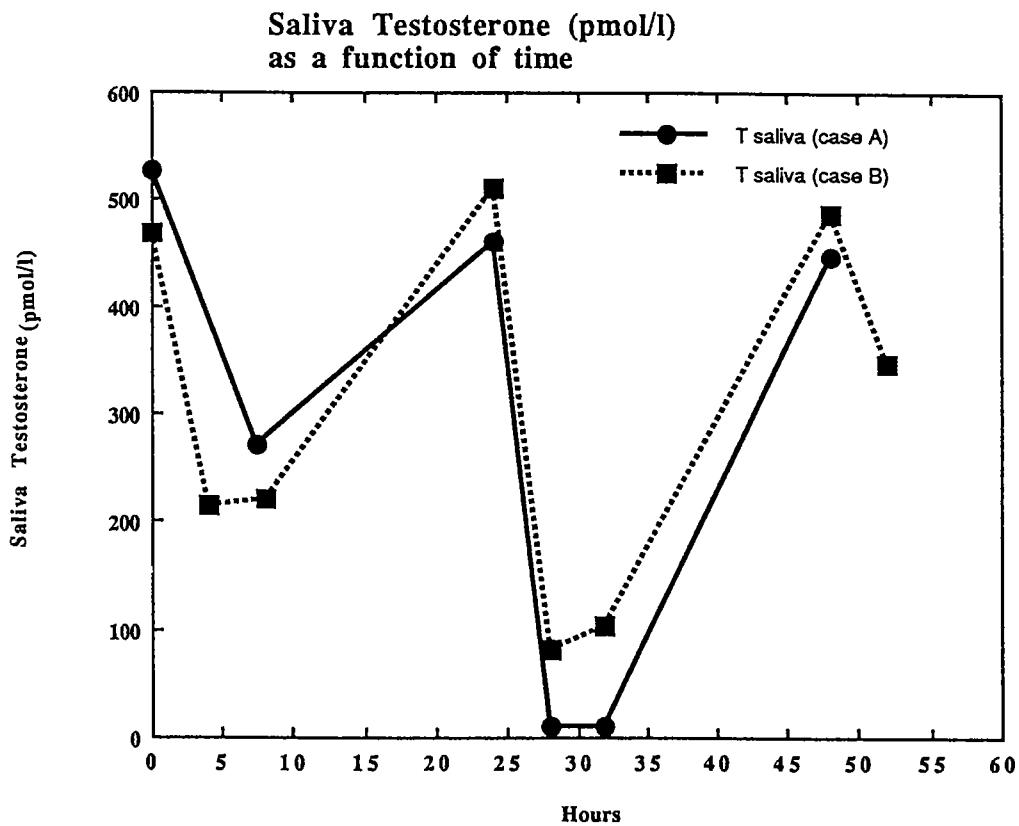


Figure 12

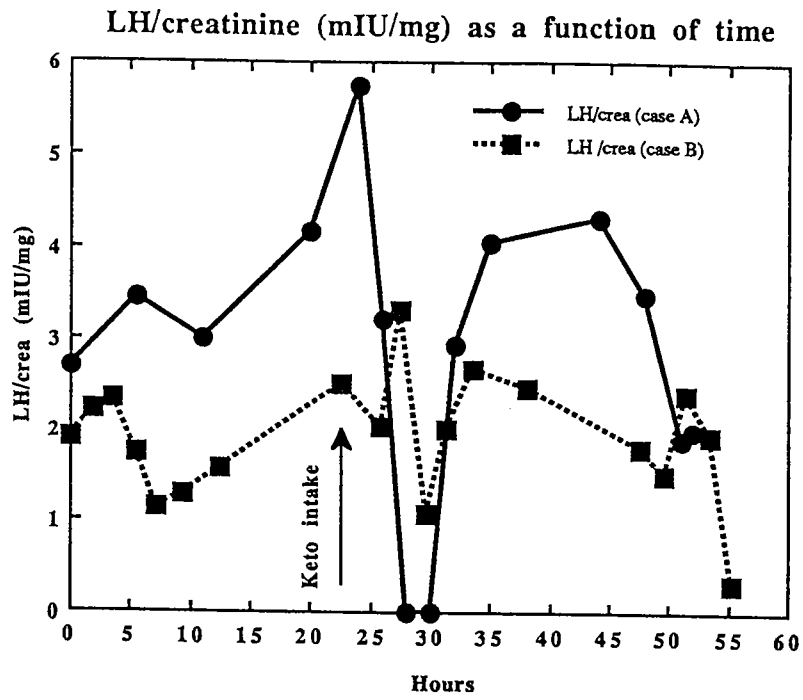


Figure 13

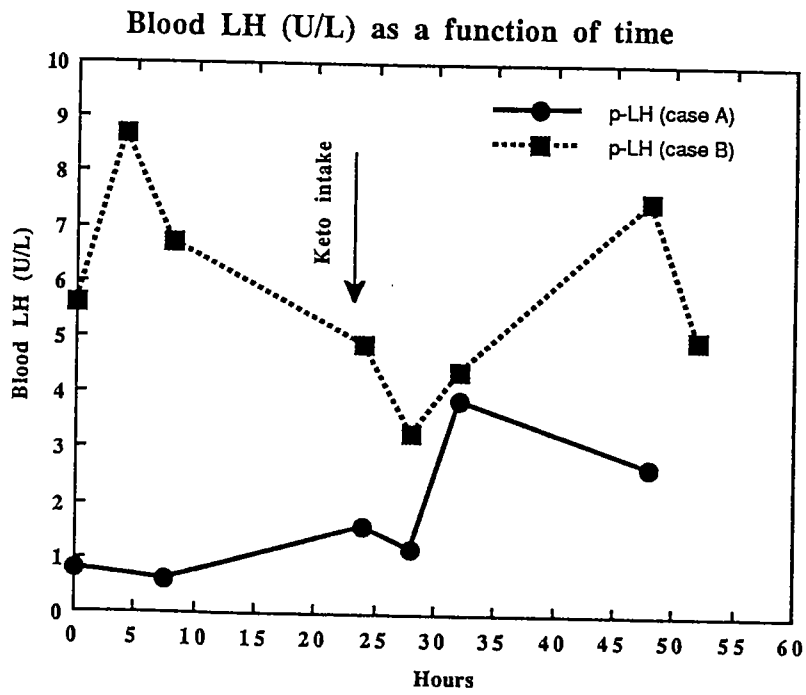


Figure 14

Blood Testosterone/17 OH-Progesterone in %
as a function of time

