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Preliminary Results regarding the detection of the misuse of testosterone gel
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Abstract
Since 2003 testosterone gel is admitted as therapeutic to the German market. Testosterone gel is used for the treatment of testosterone deficiency in males. To analyse the influence of a testosterone gel application on the urinary steroid profile and the carbon isotope ratios of testosterone metabolites, urine samples were collected from 2 patients, who were treated with testosterone gel for several weeks (100 mg testosterone per day). The urine samples were analysed for endogenous steroids with GC/MS and GC/C/IRMS.

The following main results were obtained: The testosterone/epitestosterone (T/E) ratios increased mainly by a decrease of the epitestosterone concentrations. After 8 days of treatment the maximum T/E ratios were 7.7 (patient 1) and 13.4 (patient 2). During the treatment the T/E ratios showed stable values with normal variations during the day. The steroid profiles of the 2 patients looked like profiles of individuals with natural increased T/E ratios based on a decreased epitestosterone excretion. The GC/C/IRMS analyses of the testosterone metabolites androsterone and etiocholanolone gave suspicious but not fully conclusive results. The results show that it is difficult or even impossible to prove the misuse of testosterone gel with the actually used methods (steroidprofiling, longitudinal studies, GC/C/IRMS).

Introduction
According to the WADA-list of prohibited substances, the use of testosterone is prohibited in sports (1). Since the year 2000 a new testosterone preparation, testosterone gel, is available on the US market and since 2003 on the European market. Testosterone gel is used for replacement therapy in men for conditions associated with low testosterone. The gel is administered by application on the skin. The daily dose is 25-100 mg testosterone and about 9-14 percent of the testosterone is absorbed through the skin (2). It has been shown that the application of testosterone gel leads to constantly increased serum-testosterone values (3). Additionally sev-
eral investigations have shown, that the application of testosterone gel leads to performance enhancing physiological effects like significant increase of the lean body mass, muscle strength and hemoglobin concentration (4). The objective of the following investigation was to find out, if an application of testosterone gel can be detected with the actual methods used in doping control.

Experimental
Two 43 years old male patients suffering from testosterone deficiency were treated 21 days with testosterone gel (Testogel®, Jenapharm, Germany, daily application of 2 sachets with 5g testosterone gel each; total testosterone amount per day = 100mg). Before and during the treatment serum testosterone and serum LH were analysed according to standard operating procedures of the Clinic and Polyclinic of Urology of the University Hospital Cologne. Additionally urine samples were collected for 24 hours before the administration of testosterone gel and 8 days after daily application of testosterone gel. The urine samples were analysed with gaschromatography/mass spectrometry for the profiles of endogenous steroids according to a modified method from Donike et al. (5, 6). Additionally the urine samples were analysed with carbon isotope mass spectrometry (GC/C/IRMS) to determine the $^{12}\text{C}/^{13}\text{C}$ carbon isotope ratios of the testosterone metabolites androsterone and etiocholanolone. The GC/C/IRMS analyses were performed according to a method from Flenker et al. (7, 8).

Results and discussion
After a daily treatment with 100 mg testosterone via a transdermal application of testosterone gel the serum testosterone concentrations of the two patients increased within 6 days from 3 to 9 ng/ml and 2 to 6 ng/ml respectively. The serum LH values decreased for patient 1 from 5.5 U/l to 1.0 U/l.

After 8 days of treatment with testosterone gel the urinary testosterone/epitestosterone (T/E)-ratios of the 2 patients increased from values of $1.3 \pm 0.15$ and $1.0 \pm 0.09$ to values of $5.3 \pm 1.6$ and $8.9 \pm 3.8$ (see fig. 1).

The increase of the T/E ratio was mainly based on a decrease of the epitestosterone concentrations and not on an increase of the testosterone concentrations (see fig. 2). The testosterone concentrations did not exceed the upper limits of the reference ranges of athletes (9). During the treatment the T/E ratios showed stable values with normal variations during the day and within several days (see fig. 1) although testosterone gel was administered every morning.
This is totally different to the oral or intramuscular application of testosterone which is followed by a strong increase of the T/E ratios and strong variations of the T/E ratios during the day (fig. 3 and 4). Based on our longtime experience in the evaluation of endocrinological and longitudinal studies the steroid profiles of the 2 patients looked like profiles of individuals with natural increased T/E ratios based on a decreased epitestosterone excretion. Based on the steroid profile data we would not give such a sample positive. An athlete, who would administer testosterone gel could keep his T/E ratios on a constant level for a long time period, so that the misuse cannot be detected by a longitudinal study or endocrinological study.

Fig.1: T/E ratios before and 8 days after the daily treatment with testosterone gel (patient 1; 100 mg testosterone per day; application at 8:00)

The GC/C/IRMS analyses of the testosterone metabolites androsterone and etiocholanolone in urine samples of patient 1 before and 8 days after the daily application of testosterone gave the following results: The $\delta^{13}$C values of etiocholanolone decreased from $-24.8\%$ to $-26.3\%$ and the $\delta^{13}$C values of androsterone decreased from $-23.1\%$ to $-25.4\%$. According to our actual criteria only a decrease of at least two $\delta^{13}$C units for an-
drosterone and three $\delta^{13}\text{C}$ units for etiocholanolone is consistent with an application of testosterone or testosterone prohormones. Therefore the GC/ClIRMS analyses delivered only suspicious but not fully conclusive results for an application of testosterone.

One reason for this weak shift of the $^{13}\text{C}$ values of the testosterone metabolites is that the testosterone in the gel has only a $\delta^{13}\text{C}$ value of $-27 \%_o$ and therefore is not as depleted as prohormones with the $\delta^{13}\text{C}$ values of about $-30 \%_o$.

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**Fig. 2:** Urinary concentrations of testosterone (rear bars) and epitestosterone (front bars) before and 8 days after the daily treatment with testosterone gel (patient 1; 100 mg testosterone per day; application at 8:00)

**Conclusion**

The results show that it is difficult or even impossible to prove the misuse of testosterone gel with the actually used methods (steroid profiling, longitudinal studies, GC/ClIRMS). To prevent the misuse of this new preparation by athletes it is necessary to develop new methods or combine several known methods for its detection.
Fig. 3: T/E ratios after repeated injection of different amounts of testosterone propionate (13).

Fig. 4: T/E ratios after repeated oral application of 40 mg testosterone undecanoate (Andriol ®) (14)
References


