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Phthalates Content in Urine Samples of Swiss Athletes during Routine Doping Controls in 2012

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Abstract

Blood manipulation is often used in endurance sports since the beginning of modern competition. After the misuse of erythropoietin was detectable around 2002 by isoelectric focusing; athletes turned to autologous blood transfusions to boost their performance. Autologous blood transfusion is not yet directly detectable, but can be showed indirectly by analyzing athlete's biological passport values. Another approach to indicate autologous blood transfusion is the analysis of metabolites of di-(2-ethylhexyl)phthalate (DEHP) in urine. DEHP is an authorized plasticizer and is widely used in blood pouches for storage of blood for transfusions.
For two years, 1'153 urine samples from 827 athletes in 54 sports have been analysed for metabolites of DEHP. However there was no evidence for potential blood transfusion in the blood profile of athletes with a highly variable phthalate values.

Introduction

Autologous blood transfusion is one of the most challenging doping problems in endurance sports. Direct detection of this practice is still not possible in doping analysis. An encouraging approach to indicate autologous blood transfusion is the analysis of metabolites of di-(2-ethylhexyl)phthalate (DEHP) in urine. DEHP is an authorized plasticizer in several medical devices, including blood pouches for storage of blood for transfusions. There are reports on the analysis of DEHP metabolites [1-3] or population concentrations of DEHP metabolites [4]. During routine urine doping controls in Switzerland, we analysed urine samples for metabolites of DEHP for their potential usefulness and informative value when measuring phthalates in routine urine controls

Experimental

We analysed 1'153 urine samples from 827 athletes in 54 sports between January 2011 and January 2013 for metabolites of DEHP according to standard analytical methods [1]. That means:
To an aliquot of 200 µL of urine 25 µL of β-glucuronidase was added and icubated for 10 minutes at ambient temperatures. Then 105 µL of the hydrolisate was transferrd to a new tube and 100 µL internal standards $^{13}C_4$-MEHP, $^{13}C_4$-5-oxo-MEHP and $^{13}C_4$-5-OH-MEHP as well as 795 µL of ultrapure water were added. An an liquot of 10 µL was injected into the LC-MS instrument. For quantification, the peak area ratios of the quantifier ion transitions of the analytes and the respective internal standards (ISTDs; MEHP to $^{13}C_4$-MEHP, 5-oxo-MEHP to $^{13}C_4$-5-oxo-MEHP and 5-OH-MEHP to $^{13}C_4$-5-OH-MEHP) were used. For quantification purposes, the most suitable ion transitions were m/z 277/134 for MEHP, m/z 291/143 for 5-oxo-MEHP and m/z 293/121 for 5-OH-MEHP.

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Results and Discussion

The resulting values of the three main metabolites MEHP, 5-OH-MEHP and 5-oxo-MEHP were normalized for specific gravity (Table 1 above). In addition, the measured samples were categorized according to different aspects like sex, type of sport, in- and out-of-competition as well as season (Table 1 below).

	original values (ng/ml)			normalized values (ng/ml)		
N = 1153	MEHP	5-OH-MEHP	5-oxo-MEHP	MEHP	5-OH-MEHP	5-oxo-MEHP
arithmetic mean	**5.12**	**21.13**	**12.06**	**6.15**	**23.86**	**13.71**
std error of mean	0.28	1.26	0.66	0.27	1.28	0.67
std deviation (σ)	9.51	42.81	22.43	9.16	43.61	22.69
skewness	12.25	11.89	11.60	9.06	11.78	11.86
10th percentile	0.30	2.80	2.00	0.58	5.50	3.72
25th percentile	1.60	6.50	3.80	2.25	9.65	6.00
50the percentile (median)	**3.40**	**12.70**	**7.80**	**4.35**	**16.00**	**9.78**
75th percentile	6.00	23.30	13.50	7.20	25.14	14.67
90th percentile	9.90	39.00	22.36	11.86	41.96	23.67
share of values > mean+σ	4.7%	4.5%	4.9%	6.2%	3.7%	3.9%
highest value	201.1	907.7	482.1	160.88	907.7	482.10

	MEHP (ng/ml)	@50HMEHP (ng/ml)	@5oxoMEHP (ng/ml)	n	%
Sex				**827**	**100**
female	5.96 (5.12-6.79)	21.90 (18.80-25.01)	14.17 (12.23-16.11)	196	23.7
male	6.27 (5.45-7.08)	24.51 (20.87-28.15)	13.55 (11.72-15.38)	631	76.3
Type of sport				**827**	**100**
endurance	5.71 (5.04-6.38)	22.94 (19.96-25.92)	13.31 (11.62-15.00)	240	29.0
Technical / Power / Team	6.17 (5.44-6.91)	24.32 (20.27-28.38)	13.81 (11.77-15.86)	558	67.5
Paralympics	10.61 (-0.51-21.74)**	23.47 (16.88-30.04)	14.73 (10.54-18.93)	29	3.5
Test Setting				**1153**	**100**
in competition	6.14 (5.09-7.18)	27.87 (21.64-34.09)	15.24 (12.17-18.30)	377	32.7
out of competition	6.16 (5.55-6.76)	21.92 (19.71-24.13)	12.98 (11.72-14.24)	775	67.3
Season				**1153**	**100**
winter	5.29 (4.92-5.66)	21.62 (19.60-23.64)	13.24 (12.16-14.31)	457	39.6
spring	7.28 (6.07-8.48)	26.11 (20.99-31.23)	14.37 (11.59-17.15)	457	39.6
summer	6.37 (4.27-8.47)	27.55 (14.61-40.49)	16.40 (9.50-23.30)	33	2.9
fall	5.50 (4.60-6.41)	23.23 (16.37-30.07)	12.89 (9.93-15.94)	206	17.9

Mean values with 95% confidence interval in brackets

** Level of significance p < .05

Table 1: Overview of the measured doping control urine samples

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It is noticeable that the level of phthalates found in urine of Swiss athletes are distinctively lower than the levels found in Spanish athletes or in the Spanish control group [2]. However, it is not clear if these differences are due to different analytical methods or a different lifestyle (e.g. diet).
The distribution of the percentiles of the original MEHP-values in Switzerland and Spain [2] is shown. The distribution of the data is skewed to the right, demonstrating that lower metabolites concentrations are more frequent than the higher ones. The share of values that lay more than a standard deviation above the arithmetic mean are also significant and lay between 3.7 and 6.2. (Table 2 and 3).

	Switzerland		Spain	
	samples (n=1153	athletes (n=827)	sportsmen (n=464)	control group (n=30)
10th percentile	0.30	0.5	2.5	5.1
25th percentile	1.6	1.8	3.5	8.1
50the percentile (median)	3.4	3.5	5.5	16.0
75th percentile	6.0	6.0	9.4	22.7
90th percentile	9.9	9.6	15.3	26.4

Table 2: Distribution of percentiles of the original MEHP-values in Switzerland and Spain

	MEHP	@50HMEHP	@5oxoMEHP
arithmetic mean of all athletes (n=827)	6.19	23.89	13.70
one sample (n=658)			
arithmetic mean	6.24	24.04	13.75
two samples (n=110)			
arithmetic mean	5.84	22.49	12.69
range	5.78	20.22	10.26
three and more samples (n=59)			
arithmetic mean	6.43	24.87	15.02
range	10.52	48.04	26.93

Table 3: Distribution of the normalized DEHP values of athletes that had provided one or more samples (n=827)

For 16 athletes (2 female and 14 male) 5-12 doping control samples were obtained and analyzed during the given time period. Thereof two athletes (male, both cycling) had larger variations than average of MEHP. To show an eventual correlation between an Athlete Biological Profile and the phthalate values, the phthalate values of one of these athletes were plotted against hemoglobin values and %reticulocytes values but there was no indication of a possible misuse of autologous blood transfusion (Figure 1).

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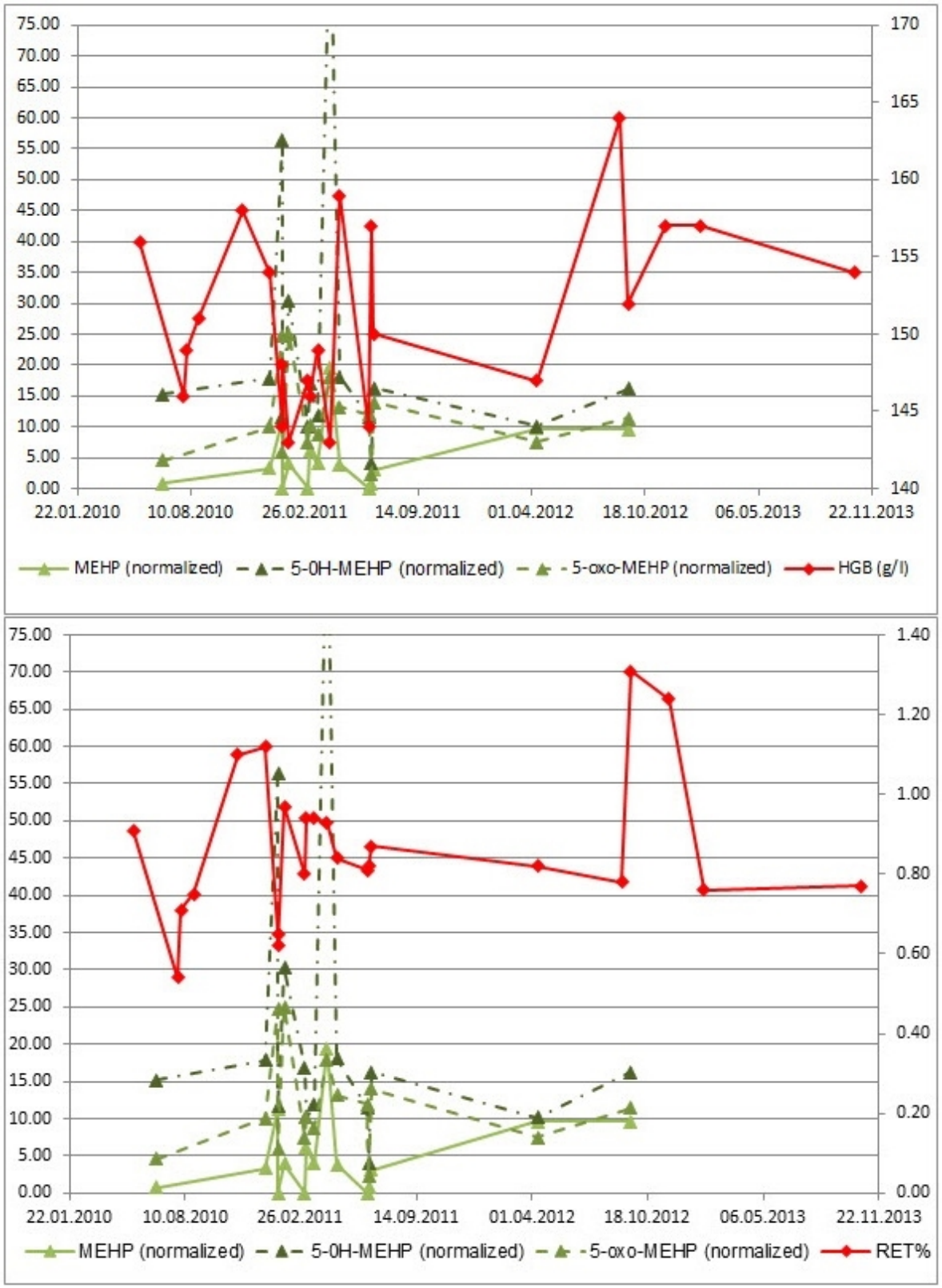

Figure 1: Endurance athlete with 15 urine samples taken for phthalate measures and 23 blood samples for blood profiles. Phthalate values plotted with hemoglobin (above) and %reticulocytes (below)

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Conclusions

Our results provide further insight for the potential usefulness of phthalate metabolites measurements in urine doping controls. It is to mention that the phthalate values measured in the Swiss athletic population was overall lower than in a comparable Spanish population. The reason for this difference, however, remains unknown. There were no differences between endurance sports on one hand and team / technique and power type of sports on the other hand. However, we found significantly higher values of MEHP for Paralympic sports, which may be due to higher exposure of these athletes to plastic material (e.g. catheters). These findings have to be accounted for when testing such sports. We did not find any evidence for potential blood transfusion in samples of athletes with a highly variable blood profile.
Our data suggest that there is no need to measure phthalates in routine doping controls. However, phthalates measurements may provide additional information on athletes within a blood passport program.

References

[1] Solymos E., Guddat S., Geyer H. et. al. (2011) Rapid determination of urinary di(2-ethylhexyl) phtalate metabolites based on liquid chromatography/tandem mass spectrometry as a marker for blood transfusion in sports drug testing. Anal. Bioanal. Chem. **401**, 517-528.
[2] Monfort N., Ventura R., Latorre A. et. al. Urinary DEHP metabolites in athletes as screening measure for illicit blood doping: A comparison study with patients receiving blood transfusion. Transfusion 2010;50:145-149.
[3] Monfort N., Ventura R., Platen P. et. al. Plasticizers excreted in urine: indication of autologous blood transfusion in sports. Transfusion 2012;52:647-57.
[4] Monfort N., Ventura R., Valvi D. et. al. (2013) Phtalates in urine as markers of blood transfusion in sports: population concentrations of five DEHP metabolites and reference limits. In: Schänzer W, Thevis M, Geyer H, Mareck U. (eds) Recent Advances in Doping Analysis (21), Köln, pp 177-181.

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