Short communication

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The problem of anti-doping control of luteinizing hormone in boxing

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Luteinizing hormone (LH) is physiologically produced by the anterior pituitary gland. Male athletes may use pharmaceutical LH for doping since it increases the production of testosterone by testes. This hormone is thus on the World Anti-Doping Agency (WADA) list of substances prohibited for males. Anti-doping laboratories perform the assay of this hormone in urine and report abnormally elevated results. We observed a highly significant prevalence of abnormal results in samples taken after a boxing match. Comparison of the descriptive statistics for 426 LH values observed in boxing and other sports showed significant differences. An experimental study comparing urinary LH levels in 17 boxers before and after a match demonstrated a clear increase after the match. The same observation was made for urinary follicle stimulating hormone (FSH) in all of the eight boxers tested for this other pituitary gonadotropin. These observations have consequences for anti-doping controls, as the reference range for urinary LH levels must take into account the specificities of boxers. They also suggest consequences for the health of boxers. Although to our knowledge such observations have never been described, other pituitary disorders have been reported. Our results deserve further investigation from a medical point of view. Copyright © 2013 John Wiley & Sons, Ltd.

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Introduction

The World Anti-Doping Agency (WADA)[1] has put luteinizing hormone (LH) on its list of prohibited substances for male athletes. Indeed, this gonadotropin, which is one of the numerous hormones produced by the anterior pituitary gland, stimulates the conversion of cholesterol to testosterone in the Leydig cells of testes.[2] Pharmaceutical LH is obtained either by extraction from the urine of postmenopausal women or by genetic engineering to produce recombinant LH.

No method is currently able to differentiate between endogenous and pharmaceutical LH. Anti-doping control is based on the assay of this gonadotropin in urine in order to detect abnormally elevated levels. This approach requires a reliable threshold value for the limit of physiological levels. Since there is no consensus value, each anti-doping laboratory has to estimate a reference interval (normal range) that depends on the assay method. In the course of conducting this analysis, our laboratory observed disturbing results related to boxing.

Experiment and discussion

At the time of these observations, an upper limit of 40 IU/L had been calculated for the reference interval of the urinary LH level. This had been estimated from 407 log transformed data obtained from urine samples analyzed for anti-doping control either in or out of competition. These samples had been collected from male athletes from various sports and were free of the aromatase inhibitors, aminogluthetimide and formestane; the selective estrogen receptor modulator, tamoxifen; and the anti-oestrogen drugs, clomiphene, and cyclofenil.

After centrifugation of the urine samples for 10 min at 1500 g, the LH levels were determined using microparticle enzyme immunoassay (MEIA) on an Axsym apparatus (Abbott). Since this assay is intended for determination of LH in serum or plasma, its application for urine had been fully validated in our laboratory before being used for anti-doping control (intermediate precision $< 10\%$, limit of quantification: 0.4 IU/L, linearity and accuracy tested in the 2–60 IU/L range). The data were not corrected for the specific gravity of urine, in line with the WADA rules in force. The upper limit of the reference interval was much higher than that corresponding to the 95% reference interval classically used in clinical biology. It was much more conservative since it corresponded to a risk of 1/50 000 of a physiological level being reported as abnormally elevated.

Over two years, 13 600 LH results were obtained in our laboratory. Only ten results were more than 40 IU/L. Strikingly, eight of them corresponded to urine samples taken after a boxing match from seven athletes, one of them having been controlled twice on two different days. The total number of LH controls performed in boxers during this period was 225, with the result being that the percentage of abnormal LH values was 3.5% (8/225) in boxing, whereas it was only 0.015% (2/13,375) in other sports.

As shown in Table 1, normal levels of urinary testosterone (determination by gas chromatography-mass spectrometry (GC-MS)) were observed in the eight samples with high LH levels, in comparison with the data described by Van Renterghem *et al.* from a population of 2027 male athletes.[3] This finding was neither in favour of doping, where high levels of testosterone would have

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Table 1. LH and testosterone urinary concentrations in the eight samples from boxers reported with abnormally elevated LH. The numbers in brackets have been corrected for the specific gravity of urine. Samples 7 and 8 came from the same boxer after two different matches.

Sample	1	2	3	4	5	6	7	8
LH (IU/L)	62 (75.8)	44.9 (82.3)	60.1 (60.1)	51.6 (43.9)	46.1 (46.1)	49 (51.3)	40 (36.7)	65.3 (53.2)
Testosterone (ng/mL)	27 (33)	7 (12.8)	36 (36)	55 (46.8)	29 (29)	25 (26.2)	25 (22.9)	20 (16.3)
Testosterone (nM/L)	93.7 (114.6)	24.3 (44.4)	125 (125)	191 (162.5)	100.7 (100.7)	86.8 (91)	86.8 (79.5)	69.4 (56.6)

been expected, nor in favour of a pituitary response to a pathological low level of testosterone.

In order to further compare the urinary LH levels in boxing and the other disciplines, a histogram showing the distribution of 426 data collected over four years from boxers was compared with that of a same number of data randomly collected from athletes in other sports. Figure 1 shows that these distributions were significantly different, with means of 7.52 (s = 9.46) and 3.11 (s = 3.02) and medians of 4.2 and 2.4 IU/L for boxing and the other sports, respectively. It was clear that the previously noted prevalence of abnormally elevated LH values exceeding the limit of 40 IU/L in boxing was only the tip of the iceberg and that the boxing population was different from a population of athletes from a range of other sports.

These results were not related to the red blood cells and/or haemoglobin that are frequently present in the urine of boxers. Intact red cells and/or membranes are in fact removed by centrifugation of the urine samples before LH determination. The absence of interference from haemoglobin indicated by the manufacturer for assay of serum LH had been verified in urine by enriching ten urine samples at 2 and 5 g/L of haemoglobin. No significant differences were observed when we compared the results from the samples without haemoglobin and those with the two enrichments (paired t-test).

Other non-specific matrix interferences were investigated. The urine samples from boxers presenting high LH levels were diluted (1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 7/10, 8/10, 9/10), with both the dilution buffer of the assay and urine from non-boxers with normal LH levels. In both cases, the results were perfectly linear. The recoveries were from 94% to 117%.

Enrichments of the samples from boxers by standard addition (at 4 different concentrations) or recombinant LH (Luveris at five different concentrations) gave rise to recoveries never exceeding 109.4%, indicating the absence of positive interferences.

Since the physician attending the matches had observed that most of the abnormally elevated LH results reported by the laboratory belonged to boxers who had performed a very hard match, the influence of a boxing match on the LH level was investigated. This study was approved by the institutional medical board of the national French boxing federation.

Two urine samples were taken from 17 male boxers. The first one was taken in the evening of the day before a match and the second one during the first hour after the end of the match (the boxers did not agree to give urine just before the match).

All the urine samples were submitted to LH determination and eight of them were assayed for the FSH level using the same method as for LH (MEIA, Axsym from Abbott). For the purpose of this study, the results were then corrected for the specific gravity of the urine samples.

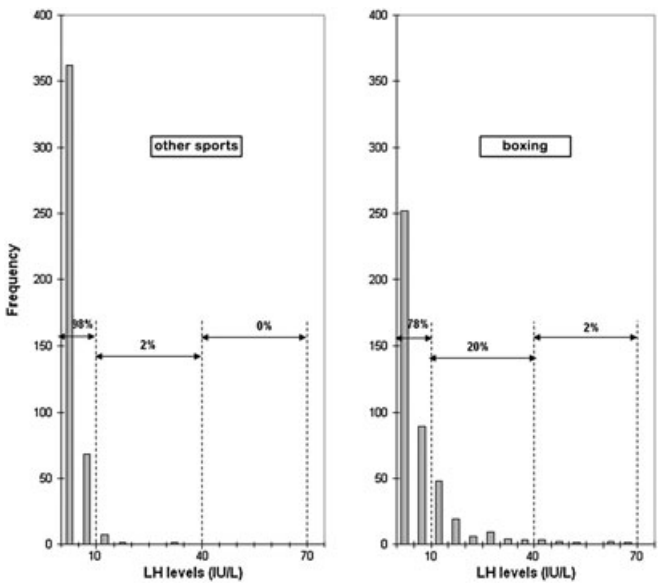

Figure 1. Distribution of urinary LH concentrations in boxing and other sports. For the two histograms, the distribution was established from 426 results obtained from urine samples analysed during four years and were not corrected for specific gravity.

Table 2. LH and FSH urinary concentrations in boxers after a match (a) and in the evening of the day before (b). All the concentrations have been corrected for the specific gravity of urine.

Subject	LH (IU/L)			FSH (IU/L)		
	Before (b)	After (a)	a/b	Before (b)	After (a)	a/b
1	2.57	3.03	1.18			
2	0.63	1.40	2.23			
3	1.44	2.54	1.76			
4	4.14	8.14	1.97			
5	1.95	4.53	2.32			
6	1.76	17	9.66			
7	0.91	3.80	4.19			
8	3.73	2.75	0.74			
9	0.37	2.85	7.76			
10	1.84	3.16	1.72	4.21	5.71	1.36
11	3.87	7.50	1.94	9.73	16.57	1.70
12	1.08	5.15	4.77	4.28	9.34	2.18
13	2.94	9.80	3.33	5.63	11.64	2.07
14	0.72	8.73	12.19	5.59	10.18	1.82
15	0.76	2.60	3.42	3.53	5.02	1.42
16	1.90	7.23	3.81	8.22	12.12	1.48
17	3.17	5.06	1.59	15.51	24.42	1.57
median	1.84	4.53		5.61	10.91	
range	3.77	15.6		11.98	19.4	

As shown in Table 2, in 16 of the 17 boxers tested for LH and in all 8 of the boxers tested for FSH, there was a clear increase in these gonadotropins after the match in comparison with the day before. Of the 17 urine samples collected after the match, none gave rise to a LH level exceeding 40 IU/L and this was not surprising since our previous study of 426 values collected from boxers over four years had shown that only 2% of the urine samples gave rise to LH levels more than 40 IU/L. The essential observation was the significant increase (paired Student's t-test, $p < 0.001$) proving that the urinary LH level was indeed affected by the match.

Conclusion

These observations have two consequences. The first is related to anti-doping control. If the threshold value for reporting an abnormally elevated concentration of urinary LH is determined from sports other than boxing, there is a clear risk of misinterpreted results in boxing. This must be taken into account in the future technical document from WADA, and a recommendation might be made to perform only out-of-competition controls in boxing.

The second is related to health in boxing. Brain damage has been extensively described in this sport.[4] Pituitary dysfunction has been reported and, in particular, it has been shown that chronic head trauma may lead to growth hormone (GH) and/or adrenocorticotropic hormone (ACTH) deficiencies.[5–7] To our knowledge, the observation of an increased urinary level of LH and FSH after a match has never been reported and deserves further investigation. Many urine samples are collected for anti-doping control after intensive physical exercise and many of them show high protein levels due to effort proteinuria of both glomerular and tubular origin. In addition to boxing, many other sports induce proteinuria, which seems to indicate that the prevalence of high urinary LH levels in boxing is not related to increased excretion of this hormone in urine. However, it will be important to investigate LH in blood. In addition, the other hormones produced by the anterior pituitary gland should be explored: GH, ACTH, prolactin, TSH), as well as those secreted by the posterior pituitary gland: oxytocin and vasopressin. It would also be interesting to assess the implication of hypothalamic releasing factors with regard to our observations.

Whatever the origin of the high urinary LH levels after a boxing match, from an anti-doping control perspective, they need to be taken into account in order to reduce the risk of reporting false positive results.

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