## ORIGINAL ARTICLE

# Risk of hepatitis B infections in Olympic wrestling

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**Objective:** First, to investigate the prevalence of the hepatitis B virus (HBV) and occult HBV infection (OC-HBV) in Turkish Olympic wrestlers. Second, to examine the relationship between HBV DNA values in sweat and blood.

Methods: A total of 70 male Olympic wrestlers were recruited as the study sample.

**Results:** As a result of the standard monoclonal antibody based hepatitis B surface antigen (HBsAg) detection, none of the Olympic wrestlers carried HBsAg in this study. On the other hand, according to real time PCR for serum HBV DNA detection in this study, 9 (13%) of the wrestlers had OC-HBV infection. Eight (11%) of the participants had HBV DNA in their sweat. In addition, there was a significant relationship between HBV DNA values in the blood and sweat of the wrestlers (r=0.52, p<0.01).

**Conclusions:** In addition to bleeding wounds and mucous membranes, sweating may be another way of transmitting HBV infections in contact sports. An HBV test should be done and each wrestler should be vaccinated at the start of his career.

Publicity about HIV infection in athletes has focused attention on the potential for transmission of bloodborne pathogens during sports and athletic competitions. Chronic hepatitis B virus (HBV) is also classified as a bloodborne pathogen and one of the 10 leading causes of death.<sup>1</sup> The HBV is more likely than HIV to be transmitted because it is present in higher concentrations in the blood and more stable in the environment. Some organisations, such as the International Federation of Associated Wrestling Styles<sup>2</sup> and the International Boxing Federation,3 have ordained that an AIDS detection test should be compulsory for participants in their sports, but mandatory testing of athletes for HBV is not even recommended. Furthermore, the International Federation of Sports Medicine<sup>4</sup> and World Health Organisation also<sup>5</sup> do not recommend immunisation against HBV for athletes. On the other hand, some influential groups, such as the National Collegiate Athletic Association6 has recommended hepatitis B immunisation for all student athletes since 1994, and Sports Medicine Australia,7 have strongly recommended that "all participants involved in contact/collision sports and playing under adult rules be vaccinated against hepatitis B".

An outbreak of hepatitis B in members of a high school sumo wrestling club in Japan in 1980, caused an increase in the amount of research into HBV infections in athletic settings. In this outbreak, the putative source for infection was an asymptomatic wrestler who tested positive for both hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg).8 However, this outbreak was not the first epidemic appearance of HBV in an athletic population. According to Karjalainen and Friman a much larger epidemic occurred among Swedish orienteers (track-finders) in the early 1960s.9 In that epidemic, more than 600 infected competitors had clinical symptoms, and at least as many subclinical cases occurred.<sup>10</sup> Another reported outbreak of HBV occurred among members of Okayama University's American football team in Japan.<sup>11</sup> In this study, the rate of occurrence of HBV infection in the American football team was 20%, which was significantly higher than the 1.8% recorded for a comparison group of non-footballing students (p<0.01). There is no clear evidence on the magnitude and the exact risk of transmission of HBV in sports.

Other than bleeding wounds and mucous membranes,<sup>12</sup> sweating might be another way of transmitting HBV infections, especially in contact sports like wrestling. To maintain thermal balance, sweating is vital. If the amount of sweat produced by Olympic wrestlers is taken into account, the importance of testing the hypothesis can be appreciated. That would increase the risk of HBV transmission on and off of the wrestling mat. However, the levels of HBV DNA in sweat have not been investigated in either an athletic or clinical setting.

Traditional HBV infection testing was based on the result of serological tests for HBV antigens, and antibodies were produced for them. Advances in molecular testing challenge conventional understanding of HBV infection. A PCR assay seems to be the most sensitive.13 PCR methods led to the identification of an increasing number of people carrying HBV DNA as the only marker of active infection. Detection of HBV DNA without HBsAg is the definition of occult HBV (OC-HBV) infection. The real place of OC-HBV infection in an athletic setting and the biological spectrum of HBV infection are not well known. HBV DNA was found in low levels in liver tissue and in circulating blood in unclassified chronic hepatitis and in patients with a high risk of hepatocellular carcinoma.14 Therefore, the main purpose of our study was to investigate the prevalence of HBV and OC-HBV in Olympic wrestlers. The second was to examine the relationship between HBV DNA levels in sweat and blood.

#### **METHODS**

#### Sources of data

A total of 70 men, aged 18–30 years, were recruited as the study sample. The potential pool of participants comprised wrestlers who competed at the Turkish National Championship. All competitive weight classes (except for heavyweights) were represented in this sample. The wrestlers of this study had at least four years' wrestling experience. The participants

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBU, Celal Bayar University; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; OC-HBV, occult HBV infection

	No.	Mean	SD	
3MI	70	23.75	3.94	
Body fat (%)	70	11.16	5.14	
FW (kg)	70	61.76	10.93	

participated voluntarily, after having been informed about the aims of the study. Before testing, all participants signed an informed consent form and completed a medical questionnaire. The study had been reviewed and approved by Celal Bayar University (CBU) ethics committee.

#### Procedures

All testing was conducted as follows: upon arrival in the CBU Performance Laboratory of the School of Physical Education and Sport, gross body weight and standing height were measured. Standing height was measured to the nearest 0.5 cm at mid-inspiration using a stadiometer, with the participants barefoot and standing erect with arms and hands at their sides. The researcher recorded total body weight to the nearest 0.1 lb (0.045 kg). In addition, the percentage of body fat was determined by an bioelectrical impedance device (Tanita 300 MA, Tokyo, Japan).

Sweat (200 µl) was loaded into composite plastic tubes straight after wrestlers were exhausted during the main part of the training. During sweat collection, the researchers collected dropping sweat into plastic tubes rather than scratching drops out of the skin. Then, blood samples (10 ml) were drawn for liver biochemistry (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), anti-hepatitis C virus (HCV), HBV serology (HBsAg, anti-HBsAg, and antibody to hepatitis B core antigen (anti-HBcAg)), and HBV DNA testing. HBV DNA testing was also carried out for sweat samples. Testing was performed at the laboratory of Microbiology, CBU Faculty of Medicine. Anti-HCV testing was performed by Microparticle Enzyme Immunoassay (MEIA; Abbott Laboratories Inc, USA). Testing for HBsAg was performed by monoclonal enzyme immunoassay, according to the manufacturer's instructions (Ausyzme Monoclonal Diagnostic Kits; Abbot Laboratories, North Chicago, Illinois, USA). The sensitivity of the assay was 0.3-0.7 ng/ml. Testing for anti-HBcAg was carried out by the Core IMx system and anti-HBsAg testing by the AusAb IMx system.

#### Isolation of total DNA in serum samples and sweat

DNA was extracted from 200  $\mu$ l of serum and sweat by the Nucleospin Virus kit (Biogene, Kimbolton, UK), according to the manufacturer's instructions.

#### Real time PCR for HBV DNA detection

Ten microlitres of the extracted DNA was detected with the sequence detector system (ABI Prism 7700; Applied

Table 2      Liver biochemistry test results in Olympic wrestler						
Test (normal range)		Mean (SD)	Range			
ALT (0-30 AST (10-3	U/l) 2 U/l)	25.30 (5.49) 28.01 (4.79)	4.00-37.00 19.00-39.00			

Biosystems, Foster City, California, USA) in 50  $\mu$ l of a PCR mixture containing TaqMan PCR core reagents with Ampli-Taq Gold and AmpErase uracil-N'-glycosylase (Applied Biosystems), 45 pmol of each primer, and 15 pmol of the probe.

All isolations and amplification reactions were performed in duplicate. Amplification and detection were performed with an ABI Prism 7700 Sequence Detection System (PE Biosystems). After incubation for 2 minutes at 50°C, which enables uracil N'-glycosylase (present in the 2× Universal MasterMix) to inactivate possible contaminating amplicons, incubation for 10 minutes at 95°C allowed AmpliTag Gold polymerase to activate and inactivate the uracil N'-glycosylase. The PCR cycling programme consisted of 45 two-step cycles of 15 seconds at 95°C and 60 seconds at 60°C. During the PCR amplification, the amplified products were measured continuously by determination of the fluorescence emission. After real time data acquisition, the cycle threshold value was calculated by determining the point at which the fluorescence exceeded the arbitrary threshold limit. This limit was manually set to cross the fluorescent signal of the standard in the exponential phase. The five standards cover a range of four logs to enable generation of a standard curve by the ABI Prism 7700 Sequence Detection System over approximately 1×103 to 1×107 copies/ ml. Both standards and negative controls were included in every experiment.

#### Statistical analysis

Statistical analyses of the study were carried out with the SPSS 10 package program, working under Windows XP. Distributions of the variables were examined for potential outliers. Univariate outliers were defined as scores falling more than 3SDs from the mean score of their cell and were discontinuous from the scores of their closest neighbours. No univariate outliers were found in the standardised residuals plot. Mean, standard deviation, frequencies and case summaries of each variable were analysed. Pearson correlation analysis was used to define the relationship between HBV DNA samples of the blood and sweat. Statistical significance was defined as p<0.05.

#### RESULTS

The mean (SD) height and gross body weight of the study sample were 170 (6.84) cm and 70.09 (14.67) kg, respectively. Table 1 presents the participants' body composition for body mass index (BMI), body fat and fat free weight.

According to the results of the medical questionnaire completed by the wrestlers of the study, 26 (37.1%) of the participants had bleeding wounds or exudative skin injury during training or matches; 33 (47.2%) of the wrestlers had an episode of bleeding—that is, crash, accidents during off the field activities; 14 (20%) of the wrestlers underwent an operation during their sporting life; and 49 (70%) of the participants had had a dental visit at least once in the previous year.

Table 2 shows the results of liver biochemistry tests from the study group. Raised AST was found in 13.2% of the study group. Also, 12% of wrestlers were in the upper limit of the normalised ratio numbers. Moreover, 11.8% of the study participants had raised ALT; 14.7% of the study group had a borderline value according to the normal test range.

The HBV serology results indicated that no wrestler had a positive HBsAg result. On the other hand, nine of the study participants were HBsAg negative, blood HBV DNA positive. Therefore, nine (12.9%) of the 70 the Olympic wrestlers had OC-HBV infection. Table 3 indicates liver biochemistry, anti-HCV, anti-HBcAg, anti-HBsAg and HBsAg values of the wrestlers whose blood and sweat HBV DNA were positive.

Table 3 Liver biochemistry, anti-HCV, anti-HBcAg, anti-HBsAg, HBsAg values of the wrestlers whose blood and sweat HBV DNA were positive

Wrestler No	AST (IU/I)	alt (IU/I)	HBsAg	Anti-HBsAg	Anti-HBcAg	Anti-HCV	Sweat HBV DNA (copies/ml)	Blood HBV DNA (copies/ml)
1	37	30	Negative	Negative	Negative	Negative	390	580
2	37	30	Negative	Negative	Positive	Negative	7500	7900
3	30	31	Negative	Negative	Negative	Negative	620	88
4	31	30	Negative	Negative	Negative	Negative	2.4	680
5	26	20	Negative	Negative	Negative	Negative	530	210
6	20	23	Negative	Negative	Negative	Negative	1500	Negative
7	28	26	Negative	Negative	Negative	Negative	450	530
8	32	23	Negative	Negative	Negative	Negative	150	880
9	27	20	Negative	Negative	Negative	Negative	Negative	550
10	22	18	Negative	Negative	Negative	Negative	500	230
11	19	28	Negative	Negative	Positive	Negative	Negative	1800
12	30	24	Negative	Negative	Negative	Negative	2400	110
13	22	20	Negative	Negative	Negative	Negative	Negative	550
14	32	30	Negative	Negative	Negative	Negative	2400	110
15	26	32	Negative	Positive	Negative	Negative	Negative	9700
16	38	36	Negative	Negative	Negative	Negative	820	Negative

HBV DNA in sweat was detected in eight (11.4%) of the 70 anti-HBcAg and anti-HBsAg Olympic wrestlers.

Table 4 presents descriptive statistics of the serum HBV DNA and sweat HBV DNA values.

The correlation between levels of HBV DNA of blood and sweat samples of Olympic wrestlers was significant (r = 0.52, p < 0.01).

#### DISCUSSION

The purpose of this study was to investigate the prevalence of HBV in Olympic wrestlers. Even though, the HBsAg seropositivity in a sedentary population of Turkey was shown to be  $12\%^{15}$  using the standard monoclonal antibody based HBsAg detection, none of the Turkish Olympic wrestlers in this study had HBV.

On the other hand, HBV infections exist in at least three distinct clinical states of viral persistence.<sup>16</sup> They have been defined based on the serological findings as: chronic hepatitis B, the silent or "healthy" carrier, and occult hepatitis (OC-HBV). Chronic hepatitis B is defined clinically as the repeated detection of HBsAg for six or more months after acute infection.<sup>17</sup> Chronic HBV is associated with high levels of HBV DNA, high risk of transmission liver inflammation, raised liver enzymes and the highest risk of cirrhosis.<sup>17</sup> <sup>18</sup> The healthy carrier state refers to people in whom HBsAg remains detectable in serum samples, but who have repeatedly normal liver enzymes and negative tests for the HBeAg.<sup>19 20</sup> The final viral persistence group, OC-HBV, has been identified by sensitive PCR assays that may detect low levels of HBV DNA in the serum samples of people who are HBsAg negative.<sup>16 21</sup>

In this study, according to HBV-DNA in the serum of the Olympic wrestlers, 13% of the wrestlers have OC-HBV. This proportion was 11% in the sweat HBV-DNA of the participants. A single multination investigation<sup>22</sup> recorded the prevalence of OC-HBV in liver tissue as 11% in Italy, 6–9% in Hong Kong, and

0% in the UK. However, those values represent the prevalence of OC-HBV in a sedentary population and are difficult to compare with the prevalence for the wrestlers of this study. Sedentary and athletic populations differ significantly in factors such as sanitary conditions of training area, incidence of bleeding wounds or exudative skin injury during training and competitions, which may change the prevalence of OC-HBV. Also, it was not possible to compare the prevalence of OC-HBV of Olympic wrestlers in this study either with other athletic populations or with wrestlers of other nations, because no study giving the OC-HBV prevalence in athletic settings has been published.

The pathogenic role of OC-HBV is unclear in the metabolism of wrestlers because OC-HBV infection is more commonly found in patients with chronic HCV infection, HIV infection, cryptogenic advanced liver fibrosis, cirrhosis or hepatocellular carcinoma than in those with minimal liver injury or healthy populations.<sup>23 24</sup> In replying to the medical questionnaire, none of the wrestlers in this study indicated any kind of former liver disease or dysfunction.

Several mechanisms conducive to OC-HBV have been suggested, such as an altered immune response, infection of peripheral blood mononuclear cells by HBV integration in the chromosomes of the host or co-infection with other hepatotropic viruses and mutations in HBV genes.<sup>17 25 26</sup> The real place of OC-HBV infection in an athletic setting and the biological spectrum of HBV infection is not well known. One of several mechanisms conducive to OC-HBV is an altered immune response. Therefore, the suppressed immune function of elite wrestlers, owing to the high intensity and long duration of their training sessions, might be the reason for the high prevalence of OC-HBV in this study. Training and competition sessions usually last for several hours, and it is well established that prolonged exercise may induce a temporary immunosuppression, termed the "open window", with a presumed increased susceptibility for infection.27 28 Studies have consistently found

Table 4      Mean, SD, minimum and maximum values of serum and sweat HBV DNA						
	No.	Minimum	Maximum	Mean	SD	
Sweat HBV DNA (copies/ml)	70	0.00	7500.00	297.34	974.95	
Sweat HBV DNA (copies/ml) Blood HBV DNA (copies/ml)	70	0.00	9700.00	377.51	1487.57	

#### What is already known on this topic

- The HBV is more likely than HIV to be transmitted because it is present in higher concentrations in the blood and more stable in the environment.
- Most sports organisations have decided that an AIDS detection test should be compulsory for participants in their sports, but mandatory testing of athletes for HBV is not even recommended.

#### What this study adds

- With increasing technology and use of real time PCR in athletic settings, evidence is emerging that the incidence of OC-HBV in Olympic wrestling is higher than expected and that transmission of HBV may also occur through sweat
- The advice of the sports organisations about HBV testing should be changed, making it obligatory for all participants involved in contact sports and playing under adult rules to be vaccinated against hepatitis B.

lower total leucocyte counts, lymphocyte counts and immunoglobulins in well trained athletes at rest and during exercise.<sup>29 30</sup> Some sports like wrestling, which has prolonged body contact accompanied by bleeding injuries and sweating during intense exercise, have a greater risk of HBV and OC-HBV infection than any other contact sport or sedentary populations.

Another explanation for the high prevalence of OC-HBV infection might be non-documented anabolic steroid use by the wrestlers. During the past decade the non-therapeutic use of anabolic steroids by athletes has increased. That may induce hepatocellular carcinoma, peliosis hepatitis, and general liver dysfunction.<sup>31 32</sup> The wrestlers were not asked about anabolic steroid use during data collection in this study.

Unfortunately, there are no published guidelines indicating which athletes should be screened for OC-HBV. An important concern of these findings is whether OC-HBV infection can be transmitted to others. Previous data indicated that infection can occur in susceptible chimpanzees, infants and transfusion or organ recipients after exposure to HBsAg negative, HBV DNA positive blood.33

Many serum enzymes have been proposed as indicators of hepatocellular injury. Of these, AST and ALT activities have proved most useful. In this study, increased AST and ALT activity was detected in the Olympic wrestlers. It is unclear if this rise in enzyme activity is due to hepatocellular injury or due to damaged striated muscles. Harrington claimed that this rise in enzymatic activity was most probably muscular in origin and not from the liver.34

Various experts have produced guidelines on the management of players with bleeding wounds. However, the results of this study suggest that sweating may be another way of transmitting HBV infection. The correlation between sweat and blood HBV DNA was statistically significant in this study. Also, the incidence of HBV in sweat DNA (11.4%) was close to its incidence in blood (12.9%). As far as we know, no previous published study has examined HBV DNA in sweat and the incidence of transmission. If the origin of the HBV in the wrestlers who had OC-HBV were identified by secant analysis that would strengthen the hypothesis that HBV could be transmitted by sweat in contact or collision sports.

#### CONCLUSION

According to the results of this study an HBV test should be done and wrestlers should be vaccinated at the start of their wrestling career. Clinicians and staff of athletic programmes should aggressively promote HBV immunisation. The advice of sports organisations should be changed, making HBV immunisation obligatory for contact sports. Further studies are necessary to answer a variety of questions such as: (a) Should screening for HBV and OC-HBV infections be mandatory in different types of contact sports? (b) What is the pathogenic role of OC-HBV in the metabolism of wrestlers?

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# EDITORIAL BOARD MEMBER

### Lindy Castell

back injury put paid to Lindy's plans for a farming career. Instead she became a technician in the University of Oxford's Human Physiology group, for Drs Dan Cunningham and Brian Lloyd. From 1985 to 1987 she studied for an Open University undergraduate science degree part-time. In 1986 she was invited to work at a US university.

In 1990, Lindy joined Professor Eric Newsholme's biochemistry group at Oxford to work on amino acids in clinical situations. Eric encouraged her to do a higher degree at the university; she was a very mature student—a grandmother at matriculation! Lindy's clinical research interests were put on hold when she was asked to investigate the role of glutamine in exercise. Happily, she discovered that endurance athletes often made themselves as ill as some patients, albeit transiently! Exercise-induced immunodepression proved to be an endless source of fascination.

When Eric Newsholme took early retirement in 1996, Lindy decided to keep the Cellular Nutrition Research Group (CNRG) going. She received MA status at Oxford in 2000, and is a member of Green College. In 2001 she became an Honorary Research Associate at the Nuffield Department of Anaesthetics, University of Oxford, where she is also head of the CNRG. She lectures on the Oxford Sports MSc course, and is visiting lecturer at three other universities. For the past 10 years, she has supervised PhD, MSc and medical student research projects, including immunodepression, fatigue and amino acids in exercise, and in stroke and intensive care patients. Her interest in these projects continues.



COMMENTARY

I believe that this study makes an important contribution to the

field of sports medicine. Results show that athletes risk

hepatitis B infection in contact sports, especially wrestling.

Therefore, athletes in contact sports should be vaccinated at the

beginning of their careers, and other precautions should be

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taken very seriously.

Figure 1 Lindy Castell