INTRODUCTION

In 1967 the International Olympic Committee (IOC) appointed the Medical Commission to organize and supervise fight against doping. At that time, the Commission published the first list of substances prohibited for use in sport to meet the need of anti-doping testing at the 1968 Olympic Games. The Prohibited List included stimulants, sympathomimetic amines, narcotics (narcotic analgesics), antidepressants and tranquillizers. For years the list was expanding and underwent modifications, mainly prior to successive Olympic Games. Starting from 1 January 2004, the World Anti-Doping Agency (WADA) has assumed the role of the main coordinator in fight against doping. WADA significantly modified the list of prohibited substances and methods (the Prohibited List). These modifications initiated changes, whose effects can be observed in three main areas of sport and anti-doping i.e. in athletes, doping control laboratories, and sport entourage. In Poland, the removal some substances from the List or the addition other compounds to the basic List caused an increase of usage of pseudoephedrine and caffeine by athletes and a decrease of number of positive doping cases with cannabinoids and glucocorticosteroids. The annual modification of the Prohibited List by WADA and subsequent introduction of new examples of prohibited substances strengthened the world anti-doping system. Considering the open character of the list a regular update would be expected, especially indicating prohibited or permitted status of new substances and drugs. It would be advisable to publish, on the WADA website, some additional information regarding those substances which cause the most interpretation problems.

KEY WORDS: sports, doping, prohibited substances, IOC, WADA, Prohibited List
would be to promote and coordinate fight against doping in sport. Both IOC and the governments of the interested countries agreed jointly to finance the new organization. Moreover, the governments offered such country organs as the police, customs service, financial and justice systems to be at the disposal of WADA whenever necessary [12]. WADA was officially established on 10 November 1999, but starting from 1 January 2004 it took over from the IOC Medical Commission the role of the main coordinator in fight against doping in sport.

WADA significantly modified the Prohibited List of the IOC Medical Commission, binding from the end of 2003. Since that moment the list has been regularly updated, and all changes, based mainly on scientific research, have been preceded by numerous consultations with representatives of the sport and medicine. By publishing a new version of the Prohibited List every year (Table 2) and by enlisting numerous examples of prohibited substances WADA fulfilled partly the need of publishing a complete list of prohibited substances. However, the Prohibited List still has an open character. In spite of the examples of prohibited substances or methods in particular groups, some additional substances, which are not located on the list but are characterized by “a similar chemical structure or similar biological effect(s)”, can be considered as doping. Such attitude allows for triggering of investigation procedure in case of application of new pharmacological substances by athletes, including substances specially designed for doping. The best example could be a case connected with American laboratory BALCO and its tetrahydrogestrinone (THG), the substance produced exclusively for doping purposes [17,20,24]. The immediate and proper reaction from WADA caused that THG soon appeared on the List of substances prohibited in sport.

According to the World Anti-Doping Code, a substance shall be included on the Prohibited List if it is a masking agent or meets at least two of the following three criteria:

- it has the potential to enhance or enhances sport performance,
- it represents a potential or actual health risk,
- or it is contrary to the spirit of sport [35].

However, meeting two of these three criteria does not mean that a substance is automatically placed on the Prohibited List.

### 2. Impact of the list on anti-doping laboratories

Modifications of the Prohibited List enlarged cost and work volume in anti-doping laboratories, especially at the stage of screening investigations. This was mainly caused by necessity of implementation of the following procedures [1]:

- the analysis of blood samples,
- the screening for all the synthetic glucocorticosteroids in all “in competition” samples,
- the screening for THG, gestrinone and other steroids not detectable by system of gas chromatography coupled to mass spectrometry (GC/MS),
- the screening for the new class of anti-estrogen agents,
Some aspects concerning modifications of the list of prohibited substances and methods in sport

- the reduction of threshold value from 6 to 4 in the testosterone to epitestosterone ratio (T/E),
- the confirmation of all beta-agonists and glucocorticosteroids samples, regardless of the existence of the Therapeutic Use Exemption (TUE).

One of the reasons why WADA established the procedures for obtaining and maintaining accreditation of anti-doping laboratories was to ensure rapid implementation of appropriate analytical techniques for detection of substances newly added to the Prohibited List. The procedures are included in the document called International Standard for Laboratories (ISL). As a part of the ISL, WADA has put in place the External Quality Assessment System (EQAS) that verifies the performance of the laboratories, including the ability to detect new substances introduced into the Prohibited List. The WADA-accredited laboratories are required to participate in the EQAS assessment system, which includes testing of both blind (a laboratory knows that it is an EQAS sample, but does not know the content) and double-blind (a laboratory does not know that it is an EQAS sample and does not know the content) samples [3]. For routine laboratory procedures, it became necessary to implement additionally a system of liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). This system, applied mainly for the glucocorticosteroids determination, could also be very useful for identification of some other substances such as benzoylecgonine and other stimulants, salbutamol, tetrahydrogestrinone, diuretics, and narcotics.

Other aspects of modifications of the WADA Prohibited List have been connected either with the removal of particular substances from the list (pseudoephedrine and caffeine), or with the addition of new substances to the basic list of prohibited substances (cannabinoids, glucocorticosteroids). These changes allowed for free usage of drugs which had been removed from the list and, in some cases, caused a decrease in number of positive doping cases. However, it should be taken into consideration that despite the huge number of antidoping tests performed worldwide the total number of positive samples is too limited to support any epidemiologic conclusions [2]. In 2009 the WADA accredited laboratories analyzed 277,928 samples and reported the presence of prohibited substances or evidences of the use of prohibited methods in 3,091 cases.

3. Effects of the list modifications on substances traced in athlete organisms

Testosterone (T/E ratio). Reduction of the threshold value of testosterone to epitestosterone (T/E) ratio in athlete’s urine from 6 to 4 led to a significant increase in the number of confirmed samples. For example, in Poland in the years 2004-2009, the values of the T/E ratio ranged between 4 and 6 were observed in 364 samples, which comprised 2.52% of all samples analyzed in this period (Fig. 1). Many of suspicious samples were probably an effect of variability of urinary steroid profile connected with genetic status, biological rhythm, intensity of physical activity, style of life and diet habits of the tested athletes [11].

Therefore, in many cases the final decision regarding the result of sample analyses, needed an application of isotope ratio mass spectrometry (IRMS) technique. This method allows to detect the origin of the appearance of anabolic-androgenic steroids (endogenic or exogenic) in an organism [5]. Recently, the IRMS analysis becomes a standard procedure performed for all samples with the T/E ratio greater than 4. In addition, after recent publications concerning relationship between the T/E ratio and ethnicity of the subjects [14,29], this technique may become even more commonly used in anti-doping laboratories. The human UGT2B17 gene, which protein product largely controls the process of formation of testosterone glucuronide from testosterone, varies in copy number from zero to two per individual, and also differs in mean number between populations from Africa, Europe, and East Asia [36].

\[ 
\text{FIG. 1. HISTOGRAM OF T/E RATIO VALUES IN URINE SAMPLES TESTED IN THE WARSAW ANTI-DOPING LABORATORY DURING 2004-2009.} 
\]
Lack of the UGT2B17 gene (deletion/deletion genotype) may indicate false negative results of anti-doping tests, if the T/E ratio was the only marker of doping with anabolic-androgenic steroids. In such case testosterone conversion into the glucuronide derivative would be significantly impaired, decreasing its concentration in urine. Since the associated epitestosterone glucuronide is excreted at normal levels, the T/E ratio could be low, below the threshold value of 4, even though anabolic androgenic steroids were administrated. Thus it seems that in the near future a specific T/E cut-off ratio might be applied for athletes in dependence of their UGT2B17 genotype [28]. On the other hand the IRMS technique can be used routinely to analyze samples from athletes who have both UGT2B17 alleles deletion increasing in that way the total samples work load in the anti-doping laboratory. The other solution allowing efficient detection of testosterone abuse in people is a longitudinal and retrospective evaluation of doping control samples in terms of steroid profiles which would enable the detection of abnormal alternations that trigger confirmatory analyses [18]. Application of this approach routinely could also result in limitation of number of samples, which should be analyzed by using the IRMS technique.

Caffeine. Caffeine had been restricted for many years by anti-doping rules, due to its pharmacological properties i.e. stimulation of the central nervous system, improvement of both cognitive functions and mood, which consequently increases the stimulation level of psychophysical abilities above the natural level. Until 2003 maximal caffeine concentration in urine in athlete’s urine was set at 12 μg/ml. Since 2004 caffeine consumption by athletes has not been restricted. Caffeine, as well as ethyl alcohol, nitroglycerine, opium and strychnine had already been used in the 19th century in endurance sports. It was the beginning of the doping era [9]. In European tradition, coffee and tea consumption is a common practice. However, some athletes consume caffeine before competition intentionally for its ergogenic properties as a stimulating agent. Caffeine, together with sodium bicarbonate and creatine was included by experts of the IOC Medical Commission Working Group on Sports Nutrition in the group of permitted, effective agents enhancing sport performance.

In the Department of Anti-Doping Research of the Institute of Sport (the WADA accredited laboratory), a study was performed to check whether withdrawal of caffeine from the Prohibited List would cause an increase in its usage by athletes. For this purpose, the average concentration of caffeine in urine samples collected for doping control was compared in years 2003 and 2004. The results of the study showed that the average caffeine concentration in tested samples collected in competition significantly increased from 1.24±1.59 μg/ml in 2003 to 1.56±2.04 μg/ml in 2004 (p<0.001). It seems, therefore, that stressful situation, such as competition stress, can incline athletes for usage of stimulants or psychoactive agents when they are not banned [22].

Pseudoephedrine. Another substance removed from the WADA Prohibited List in 2004 was pseudoephedrine. Pseudoephedrine is an ingredient of numerous popular medicines (e.g. Actifed, Cirrus, Gripex, Sudafed, Tabcin) which are available on the medical market over the counter (OTC drugs) and which are frequently used by athletes. Until the end of 2003 pseudoephedrine had been banned in sport with restriction that its urinary concentration should not be greater than 25 μg/ml. Then, the World Anti-Doping Agency removed pseudoephedrine from the Prohibited List. Among all positive anti-doping samples tested by IOC/WADA accredited laboratories for stimulants in the year 2003, the greatest number of cases constituted pseudoephedrine (36.6 %). Currently, starting from the 1st January 2010, this substance is again placed on the WADA Prohibited List as a specified stimulant prohibited in competition with a urinary threshold set at the level of 150 μg/ml. This decision of the WADA List Committee was based on recent research, especially on results of controlled excretion studies, indicating that application of high doses of pseudoephedrine can enhance the sport performance [34].

In any case athletes should be very cautious when using medicines containing pseudoephedrine, not only because of its known side effects, but also because of its metabolism – demethylation process leads to norpseudoephedrine (cathine), a substance prohibited in sports. According to the WADA regulations, cathine’s presence in athlete’s urine gives the positive result of anti-doping test only when its concentration exceeds 5 μg/ml. Before 2004, when pseudoephedrine had been still banned in sport, the risk of exceeding the tolerated level for cathine, by pseudoephedrine administration and its metabolism, was relatively low. Competitors educated in anti-doping regulation, generally, did not use large doses of pseudoephedrine for therapeutic purposes. For instance, between 1987 and 1998 in Poland among more than 17 thousand tests performed only 5 cases of positive samples for pseudoephedrine and none for cathine were detected [15]. Between 2004 and 2009, when pseudoephedrine

![FIG. 2. RELATIVE NUMBER OF SAMPLES CONTAINING GLUCOCORTICOSTEROIDS AMONG ALL POSITIVE SAMPLES REPORTED BY WADA ACCREDITED LABORATORIES IN THE YEARS 2004-2009 (ON THE BASIS OF LABORATORY STATISTICS PUBLISHED ON THE WADA WEBSITE: WWW.WADA-AMA.ORG).](image)
was not prohibited, it became much more “popular” in the world of sport, even though administration of high doses of it bore the risk of an unintentional violation of the anti-doping code because of cathine. In the years 2004-2007 the Department of Anti-Doping Research of the Institute of Sport reported 4 cases of positive doping results concerning presence of cathine in athletes’ urine, at the level of 5.8-11.7 μg/ml. Parallel determinations of pseudoephedrine (98.1-150.8 μg/ml) allowed to put forward a hypothesis that the prohibited substance appeared in the urine probably as a result of metabolism of the substance which was not prohibited. Additional analyses showed that the WADA threshold for cathine could be exceeded by administration of medicines containing pseudoephedrine, even when applied according to recommendations of manufacturers [23].

Glucocorticosteroids. Modifications of the Prohibited List by WADA limit also the application of some substances in sport. This concerns mainly these substances, for which effective detection methods have been developed with substantial financial support of WADA [for a review see 30 and 31] as well as those substances which have been added to the basic list (cannabinoids and glucocorticosteroids). Analyses of results of positive doping tests reported by WADA accredited laboratories in the years 2004-2009, published on the WADA website, revealed a consistent decrease in relative number of samples containing glucocorticosteroids (Figure 2). Between 2004-2009, the Department of Anti-Doping Research of the Institute of Sport in Warsaw found only four positive cases of substances in the glucocorticosteroids group (2 in 2004, and 2 in 2009). These results may suggest that adding glucocorticosteroids to the basic list and implementation of procedures connected with the Therapeutic Use Exemption (TUE) significantly reduced the abuse of glucocorticosteroids by athletes.

Cannabinoids. In 1989 marijuana was added to the list of doping substances by IOC Medical Commission in the group of substances being a subject of limitation. It was, therefore, the decision of particular sports federations whether the use of marijuana by an athlete would be penalized or not. In practice, this regulation stayed dead as international sports federations underestimated the problem and did not include any requirements to test urine samples for Cannabis derivatives (cannabinoids) in their anti-doping regulations. In consequence of such attitude a doping scandal emerged during the Winter Olympic Games in Nagano (1998) when carboxy-THC in urine sample of Canadian athlete (the gold-medal winner in the snowboarding giant slalom race) was found. Canadian competitor was disqualified but, after an appeal to the Court of Arbitration for Sport on the basis of dubious regulations, he was given back the title and the medal. This case showed that the regulations concerning presence of cannabinoids in athlete’s urine need to be more precise. Although marijuana did not change its position on the Prohibited List a clear annotation was added that “At the Olympic Games, tests will be conducted for cannabinoids. A concentration in urine of 11-nor-delta 9-tetrahydrocannabinol-9-carboxylic acid (carboxy-THC) greater than 15 nanograms per millilitre constitutes doping” [4,7,21]. In the List published by WADA and binding since 2004, cannabinoids were included in the group of substances and methods which are prohibited during competition. Now, the doping test is considered positive for cannabinoids if the concentration of carboxy-THC (the main metabolite of tetrahydrocannabinol – THC) in a urine sample is greater
than 15 ng/ml. The value of threshold for cannabinoids was set in results of studies showing that passive smoking (spending time in the company of marijuana or hashish smokers) as well as consumption of some freely available diet supplements would lead to cannabinoids presence in athlete urine [10,16,19,32].

In the years 2003-2005, the number of positive samples containing cannabinoids in Polish athletes was considerably high. As was reported by IOC/WADA accredited laboratories only in Belgium and France the relative number of positive samples with cannabinoids was greater than in Poland. The high number of cannabinoids cases in athletes might be related to social acceptance of use of such substances and to its accessibility in some countries. One of examples would be the Netherlands, where marijuana, in various forms is legally sold. Cock et al. [8] and Van Eenoo and Delbeke [33] pointed out the high social acceptance for cannabinoids in the region of Flanders where possession of small amounts of marijuana and hashish for private use does not bring any legal sanctions. It is interesting to notice, that the number of cases of Flemish athletes taking cannabinoids was relatively high, including top competitors and amateurs. In France, the number of marijuana users has been steadily growing bringing the consumption of this narcotic by young French to one of the highest level in Europe. According to the official French data, two out of three boys and every second girls had tried marijuana, and the number of teenagers between 16 and 17 years old who had smoked joints more than 10 times, tripled during the decade – from 7 to 21%. The problem became so serious that the French government launched in 2005 a special program to fight cannabinoids consumption.

In Poland, the highest number of samples containing carboxy-THC were found in 2001 (1.46%) and 2002 (1.48%). In the subsequent years, these values considerably decreased (Fig. 3).

The main reason for the decreasing tendency of cannabinoids consumption by Polish athletes was probably the decision of WADA locating marijuana and hashish among substances prohibited in competition, beginning from the January 2004. The other important factor would be educational actions undertaken by sport authorities and Polish anti-doping structures including the Department of Anti-Doping Research of the Institute of Sport in Warsaw. Physicians, coaches and athletes of the national team were informed about negative impact of narcotics use on sport performance and health as early as in 2001. For this purpose a special document entitled „New tendencies of drugs and narcotics usage in sport” was presented during seminar organized by the Polish Olympic Committee. From that event, whenever an opportunity occurred, a special attention was drawn to the problem of drug abuse in Polish sport.

### TABLE 3. DISCIPLINARY MEASURES AGAINST POLISH ATHLETES IN POSITIVE CASES WITH CANNABINOIDS.

<table>
<thead>
<tr>
<th>Year</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of AAF* (positive cases)</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Lack of sanction or reprimand only</td>
<td>62.5%</td>
<td>7.7%</td>
</tr>
<tr>
<td>Highest disqualification</td>
<td>6 months</td>
<td>1 year</td>
</tr>
</tbody>
</table>

* AAF - Adverse Analytical Findings. AAF is defined in the World Anti-Doping Code as “a report from a laboratory or other WADA-approved entity that, consistent with the International Standard for Laboratories and related Technical Documents, identifies in a sample the presence of a Prohibited Substance or its Metabolites or Markers”.

### TABLE 4. EXAMPLES OF WEBSITE INFORMATION CONCERNING LEGALITY OF SOME DRUGS IN SPORT.

<table>
<thead>
<tr>
<th>Substance or Drug</th>
<th>ASADA Australia 1</th>
<th>NADA Austria 2</th>
<th>NADA Germany 3</th>
<th>Antidoping Switzerland 4</th>
<th>Global DRO UK/CAN/USA 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follitropin /e.g. Fertavid, Gonal-F/</td>
<td>permitted</td>
<td>permitted</td>
<td>prohibited for men</td>
<td>permitted</td>
<td></td>
</tr>
<tr>
<td>Solcoseryl (protein-free dialysate of calves’ blood)</td>
<td>permitted (dental, gel, ointment)</td>
<td>prohibited (ampoules 5ml)</td>
<td>permitted (dental)</td>
<td>permitted (injections, dental, gel, ointment)</td>
<td></td>
</tr>
<tr>
<td>Actovegin (protein-free product obtained from calf blood)</td>
<td>prohibited (ampoules, infusions, dragees)</td>
<td>permitted (intravenous)</td>
<td>prohibited (injections: i.m., i.v.; local or i.a.; topical: dermal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bazedoxifen /e.g. Conbriza/ (selective estrogen receptor modulator)</td>
<td>permitted</td>
<td>permitted</td>
<td>permitted</td>
<td>permitted</td>
<td></td>
</tr>
</tbody>
</table>

Note:
- - Lack of information
2 http://www.nada.at/de/menu_2/medizin/medikamentenauffrage (accessed 15.07.2010)
In 2003, the Institute of Sport performed an extra study and published special report describing “Problems of drug abuse in sport”. This report, addressed to the Polish Sport Confederation, underlined the necessity for further educational actions aimed, especially, to young athletes. In results of the WADA regulations and the anti-narcotics campaign carried out in Poland the relative number of positive samples with marijuana detected in Polish sportsmen and sportswomen diminished attaining world - average value.

4. Other aspects of modification of the Prohibited List

Modifications of the WADA Prohibited List influence also positively on other sport organizations involved in anti-doping activity in Poland. Many sports associations and federations recognized the importance of narcotics problem for athletes and the need for strict execution of positive results in anti-doping tests. National Anti-Doping Organization in Poland (the Commission Against Doping in Sport) reported that in 2003 the anti-doping rules concerning cannabinoids were violated in 16 cases. For as many as 10 of these 16 cases, the disciplinary procedures initiated by sports associations and federations ended in no sanction or only in a reprimand. During that time the most severe punishment was a 6-month disqualification applied in two cases (by the Polish Boxing Association and by the Polish Weightlifting Federation). The situation positively changed in 2004 when 13 positive cases of cannabinoids use were reported (Table 3). Only one of them resulted in caution (the Polish Athletics Association). In all remained cases the athletes were punished by disqualification for the period from one month to one year (the Polish Boxing Association and the Polish Association of Sports Acrobatics punished their athletes most severely).

Despite of all possible efforts, the WADA Prohibited List seems still imperfect leading, sometimes, to arbitrary interpretation of anti-doping test. The case of a Polish footballer playing in the Greek League, in whom 4-methyl-2-hexaneamine was detected in 2009, can be a good illustration of this problem. The substance called 4-methyl-2-hexaneamine was not indicated on the WADA Prohibited List by WADA and subsequent introduction of new examples of prohibited substances strengthened the world anti-doping system. Considering the open character of the list a regular update would be expected, especially indicating prohibited or permitted status of new substances or drugs. Numerous national anti-doping organizations and different sports institutions publish the list of prohibited and permitted drugs in order to help the athletes, physicians and trainers in process of decision making on drug application. On the other hand, a lot of drug databases are accessible online where one can find information about the status of a drug or substance. In fact, the information coming from different sources may be contradictory, contributing to the wrong decision and possible anti-doping sanctions (Table 4).

It is worth to mention that some athletes, while inquiring about a drug, do not ask whether this substance is prohibited but they would like rather to know if the substance listed in the Prohibited List has already been detectable. However, they are not fully aware of methodological procedures requiring the store of urine samples for eight years, the time usually sufficient for development of new method of detection and its legal implementation.

Finally, it may be concluded that the annual modification of the Prohibited List by WADA and subsequent introduction of new examples of prohibited substances strengthened the world anti-doping system. Considering the open character of the list a regular update would be expected, especially indicating prohibited or permitted status of new substances or drugs. It would also be advisable to publish, on the WADA website, some additional information regarding those substances which cause the most interpretation problems.

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