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Self-Declared and Measured Prevalence of Glucocorticoid Use in Polish Athletes

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Abstract: Glucocorticoids (GCs) are included in the list of prohibited substances and methods in sport published annually by the World Anti-Doping Agency (WADA). In its 2022 update, the WADA list prohibits all injectable routes of administration of GCs for use during in-competition periods. Previously, GCs were prohibited in-competition when administered by oral, intravenous, intramuscular, or rectal routes, but local injections (in addition to topical applications) were allowed. This study first investigated the prevalence of GC use by athletes in Poland, declared in 2130 doping control forms, and the related 2130 urine samples analysed at the Polish Anti-Doping Laboratory. Second, the validity of the analytical methodology to detect GCs was evaluated with the updated WADA requirement for substance-specific minimum reporting levels and considering the proposed washout periods. Despite the new regulation in place, the use of 30 different GC preparations were declared in a total of 162 occurrences (8% of the tests) with therapeutic purposes. Laboratory analyses resulted in the presence of GCs in 16 occurrences with only two samples with a concentration triggering an adverse analytical finding. Our study allowed us to confirm that the applied methodology for the determination of GCs in urine samples (ultra-high-performance liquid chromatography–tandem mass spectrometry) remains fully valid after the latter regulation change while the challenge to assess the timing and administration route for GCs persists.

Keywords: glucocorticoids; doping control; prevalence; urine samples; LC-MS/MS

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1. Introduction

Glucocorticoids (GCs) are commonly used to treat a range of conditions as a very effective medication, administered for their anti-inflammatory and immune-suppressive effects. They are among the most commonly used drugs in athletes, especially for the treatment of musculoskeletal injuries and diseases, asthma, and allergies [1,2]. As steroid hormones secreted by the adrenal glands and whose synthesis originates from cortisol, the "steroid" appellation refers to their chemical structure with none of the common effects of androgenic anabolic steroids.

GCs are included in the list of prohibited substances and methods in sport (S9 Class) published and updated annually by the World Anti-Doping Agency (WADA). Interestingly, GCs represented 6% of all adverse analytical findings (AAF) (n = 124 occurrences) in 2021 for the drugs on WADA's prohibited list [3]. Of these adverse findings, 89% were due to the presence of only five substances, namely, prednisolone (n = 28 occurrences, 23%), prednisone (n = 25, 20%), triamcinolone acetonide (n = 20, 16%), dexamethasone (n = 18,

15%), and betamethasone (n = 18, 15%). Beyond a therapeutical use, GCs may enhance physical performance [4], and GCs are prohibited during the in-competition period only. This period is defined as the period commencing just before midnight (at 11:59 p.m.) on the day before a competition in which the athlete is scheduled to participate until the end of the competition and the sample-collection process. Before 1 January 2022, GCs were prohibited only when administered by oral, intravenous, intra-muscular, or rectal routes. In the latest 2022 update of the WADA list, all injectable routes of administration of GCs are prohibited for GC use during the in-competition period, including local intra-articular injections [5]. The prohibition of all injectable routes of administration of GCs during the in-competition period had already been included in the 2021 draft of the WADA Prohibited List while it was implemented on 1 January 2022 to allow sufficient time for information and education on the rule changes. This one-year period was intended to allow, inter alia, laboratories to update their procedures to account for the updated and new substance-specific reporting values [6]. The authorized out-of-competition administration of GCs may, however, ultimately produce an adverse analytical finding for a sample collected in competition because of a lack of sufficient washout period after the treatment. For the sake of clarity and to accompany the latter evolution of the rule, WADA published a document delineating the various washout periods for specific GCs and administration routes [7]. Interestingly, most of the GCs have a short washout period of 3–5 days when administered orally, while triamcinolone acetonide requires between 10 days (oral administration) and 60 days (intramuscular) of washout. This should be put in the context of the increasing ability of the anti-doping laboratories to detect GCs with more sensitive instruments and methods.

Various analytical techniques and their combination can be used for the detection and determination of GCs in biological samples, e.g., radioimmunoassay [8], gas chromatography–mass spectrometry [8–10], gas chromatography–combustion–isotope-ratio mass spectrometry [11], high-performance liquid chromatography–photodiode array [12], high-performance liquid chromatography–high-resolution tandem mass spectrometry [13], ultra-high-performance liquid chromatography–ion mobility–high-resolution mass spectrometry [14], liquid chromatography–mass spectrometry [15], liquid chromatography–tandem mass spectrometry [16–20], or liquid chromatography–ion mobility mass spectrometry [21]. From the point of view of the practical application of various analytical techniques in anti-doping laboratories, the annual banned substance review published over the years by Thevis et al. in Drug Testing and Analysis are very helpful [22,23]. This also applies to glucocorticoids.

The obligation to determine GCs in all urine samples from doping control collected in competition, introduced by WADA in 2003, forced anti-doping laboratories to use liquid chromatography–tandem mass spectrometry (LC-MS/MS) in routine praxis. This approach is very sensitive and selective, and thus it is possible to monitor very low concentrations of the prohibited compounds in question, down to 1–2 ng/mL [24]. Furthermore, a specific reporting limit for urinary concentrations of GC parent compounds and their metabolites was defined at 30 ng/mL. It was the same value as the minimum required performance limit (MRPL) for GCs at which all WADA accredited laboratories must be able to operate [25]. It is worth noting that, by the end of 2021, GCs were not to be reported at levels below the MRPL of 30 ng/mL [26]. As of 1 January 2022, a new WADA technical document entered into force. It adds the new concept of minimum reporting levels (MRL), defining a cut-off level below which laboratories should not report an AAF for certain classes of or for some specific non-threshold substances. The MRL currently in force to declare an adverse analytical finding is substance-dependent for GCs with levels between 15 ng/mL to 300 ng/mL, with most GCs needing to be reported for concentrations beyond 30 ng/mL [27].

The aim of the study was first to evaluate the prevalence of GC use by athletes in Poland, self-declared in doping control forms, with a particular emphasis on the amended regulations, which, from 2022, prohibit all injectable routes of administration of GCs during the in-competition period. Taking into consideration the commonly prevailing opinion

about the popularity of local injections with GCs, in order to enable athletes to participate in competitions, we hypothesized that the tightened rules would result in an increased number of laboratory adverse analytical findings. Second, this study investigated whether the UPLC-MS/MS method used so far required any modification in the determination of the presence of GCs and the discrimination between prohibited and permitted use in an anti-doping context.

2. Materials and Methods

Doping control forms (DCFs) from anti-doping tests carried out by the Polish Anti-Doping Agency (POLADA) in the years 2020 and 2021 were assessed to investigate the list of all medications with GCs the tested athletes reported on the DCFs for the 7 days prior to controls. Out of 2130 DCFs, 1321 (62%) were from out-of-competition tests, and 809 (38%) from in-competition tests. Statistical analyses were performed using the non-parametric Chi-squared test with significance set for $p < 0.05$.

The declarations of the athletes were first compared with the appropriate results of laboratory tests carried out by the Polish Anti-Doping Laboratory (PLAd), which is accredited by WADA. Second, despite the fact that the use of GCs is prohibited in the in-competition period only, all urine samples from the latter anti-doping tests were tested for the presence of these compounds, including samples collected in out-of-competition period, as GCs were part of the WADA Monitoring Program.

2.1. Chemicals and Reagents

All solvents and inorganic chemicals were of analytical grade. The GCs of certified standard were purchased from TRC (Toronto, ON, Canada), LGC Standards (GB), Dr. Ehrenstorfer GmbH, and Steroids (Johnstown, PA, USA), respectively; 17-epitestosterone-d3, testosterone-d3 glucuronide, and 19-D3-testosterone as internal standards were purchased from NMIA (Lindfield, Australia).

LC/MS-grade of acetonitrile was purchased from Fisher Chemical (Hampton, NH, USA). β-glucuronidase (*Escherichia coli*) was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Formic acid (98–100%), disodium hydrogen phosphate, and sodium dihydrogen phosphate, 99% purity, were obtained from Honeywell. Potassium carbonate, potassium bicarbonate, and 1-chlorobutane-HPLC purity were purchased from POCH S.A. (Gliwice, Poland), respectively. Methyl tert-butyl ether (MTBE) was from JT.Baker (Deventer, Holland).

The Millipore DirectQ UV3 system (Darmstadt, Germany) was used as the source of water (R > 18 MWcm). Stock solutions of standard substances were prepared at the concentration of 1 mg/mL in methanol and stored at −20 °C.

2.2. Sample Preparation

Samples were prepared according to the method involving enzymatic hydrolysis and single extraction described previously by Grucza et al. (2018) [28]. First, 3 mL of urine was spiked with internal standards. After an addition of 1 mL of 0.8M phosphate buffer (pH 6.5) and β-glucuronidase (from *E. coli*), the samples were incubated at 50 °C for 1 h. Afterwards, samples were cooled down to room temperature. Next, after an addition of 1 mL of K_2CO_3/$KHCO_3$, the extraction with 6 mL of methyl tert-butyl ether was performed (20 min). Samples were then centrifuged (5 min/16495 RCF), and the ether phase was recovered and evaporated under a nitrogen flow at 55 °C. The dry residue was reconstituted in 100 µL of acetonitrile/H_2O mixture (*v*/*v*, 1:1).

2.3. Hydrolysis Control

Hydrolysis control was described previously by Kwiatkowska et al. (2018) [29].

2.4. Liquid Chromatography

Chromatographic separation was achieved on a Waters Acquity UPLC system equipped with a BEH C18 (100 mm × 2.1 mm, 1.7 µm; Waters, Milford, MA, USA). Table 1 shows the solvent gradient used for separation. Samples were stored at 4 °C in an autosampler. Injection volume was 15 µL.

Table 1. Mobile phase gradient of liquid chromatography method.

Time [min]	Flow Rate [ml/min] *	Mobile Phase [%]	
		A	B
Initial	0.3	5	95
2	0.3	35	65
8	0.3	50	50
9	0.3	100	0
10	0.3	100	0
11	0.3	5	95

* Flow rate: 0.3 ml/min at 45 °C; A—0.1% formic acid in acetonitrile; B—0.1% formic acid in water.

2.5. Mass Spectrometry

Multiple reaction monitoring (MRM) of the studied substances (Table 2) was traced with a Micromass Quattro Premier XE mass spectrometer equipped with an ESI source. All analytes were investigated in the ESI+ mode. Based on fragmentation patterns, ions with the highest intensity were used for the identification of investigated analytes. All data were acquired and processed using MassLynxTM software version 4.1 SCN805 (Waters, Milford, MA, USA).

Table 2. Multiple reaction monitoring (MRM) transition, collision energy, and retention times for tested glucocorticoids.

Substance	Precursor and Product Ion (*m*/*z*)/Ion Ratio (%)	Collision Energy (eV)	Retention Time (min)
Beclometasone	409.00→391.00 (100)	10	4.16
Betamethasone	393.00→373.00 (100)	10	3.92
Budesonide	431.00→323.00 (100)	15	6.02/6.17
Budesonide metabolite (6β-hydroxy-budesonide)	447.24→120.87 (100)	30	3.66/3.72
Ciclesonide	541.10→147.10 (100)	15	10.26
Ciclesonide metabolite (desisobutyrylciclesonide)	471.58→453.30 (100)	12	8.98
Clobetasol	411.17→391.29 (100)	10	6.41
Dexamethasone	393.00→373.00 (100)	10	3.97
Fludrocortisone	381.20→239.20 (100)	25	3.37
Fludrocortisone (acetate)	423.00→239.00 (100)	25	4.80
Fluticasone (propionate)	501.20→293.20 (100)	20	9.33
Fluticasone (furoate)	539.17→313.15 (100)	10	9.36
Hydrocortisone	363.00→121.00 (100)	20	3.37
Loteprednol (etabonate)	467.18→265.16 (100)	20	9.07
Mometasone	427.10→408.96 (100)	10	7.09
Prednisone	359.19→341.14 (100)	15	3.30
Triamcinolone	395.00→375.00 (100)	10	2.83

The desolvation gas flow was set at 800 L/h at a temperature of 400 °C and the source temperature was 120 °C. The capillary voltage applied was 3.0 kV. The cone and collision gas flows were set at 50 L/h and 0.20 mL/min, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were defined as 0.2 ng/mL and 0.5 ng/mL respectively.

The validation process was performed in accordance with the WADA technical document TD2021IDCR [30], in an analogous way to that described previously by Wicka et al. (2023) [31] and Kwiatkowska et al. (2023) [32].

3. Results

The surveyed athletes declared the use of GCs in the week preceding doping control in 7.6% (n = 162) of the cases. There were 30 different medicines containing 13 GCs. The most frequently used preparations were Alvesco® (34.5%), Symbicort® Turbuhaler (15.0%), and Dymista® (14.0%). It was found that 21% of athletes using GCs take two preparations from this group at the same time, and 1.2% use three medicines at the same time. The most frequently used substances are cyclesonide (34.0%) and fluticasone (22.5%) (Figure 1). We did not observe a different prevalence of GC use between in- and out-of-competition periods (p = 0.662) while injections were only reported during out-of-competition controls.

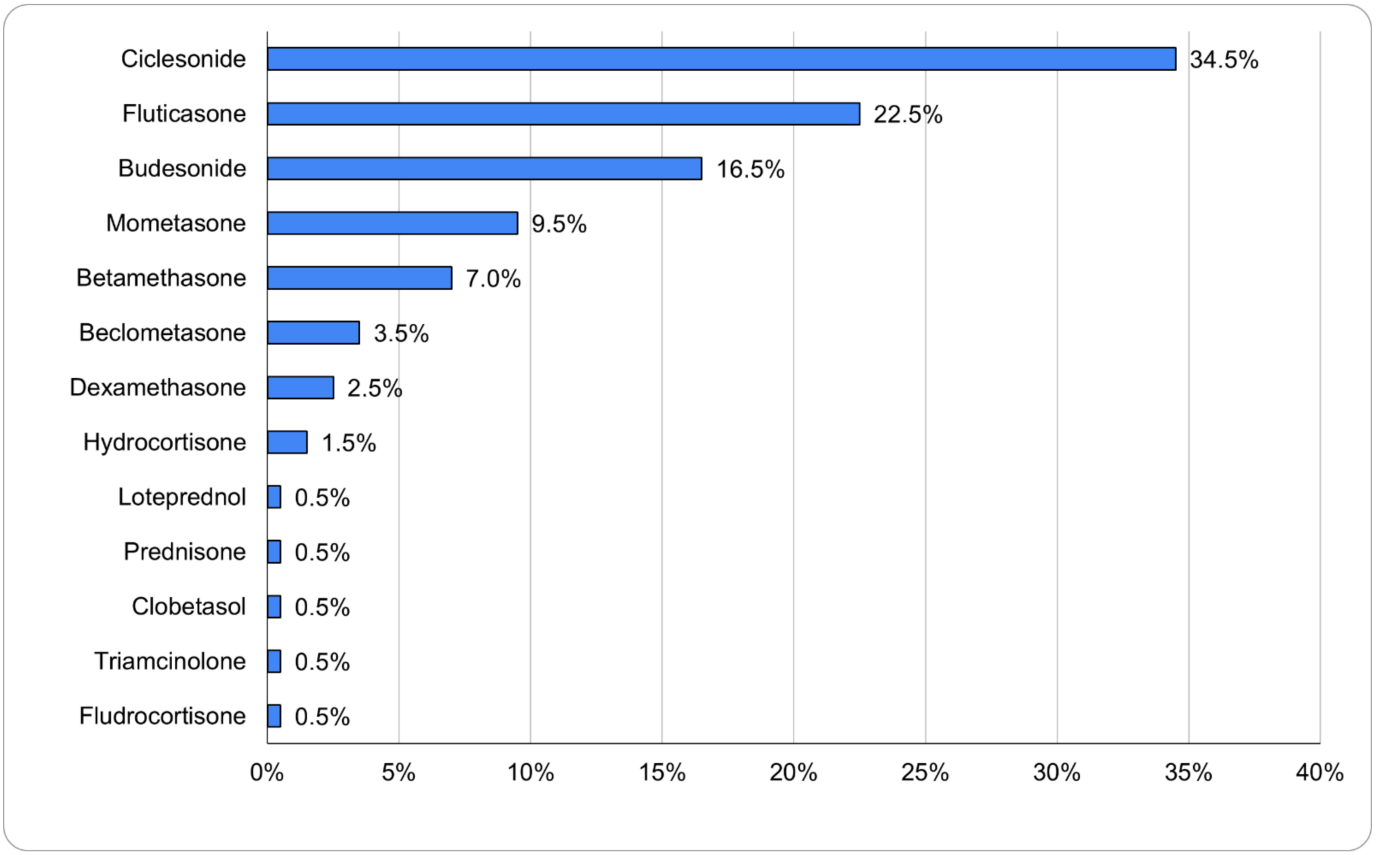

Figure 1. Glucocorticoids declared by athletes in doping control forms.

Most medications with GCs used by athletes are inhaled drugs (57%) and nasal sprays (26%). Injections (7%), ointments (5%), capsules/tablets (3%), eye drops (1%), and medicated shampoos (1%) were also reported.

The presence of GCs was found in 16 urine samples with estimated concentrations measured down to 1.27 ng/mL as detailed in Table 3, with two samples where the positivity criteria were met according to the WADA regulation with levels above a minimum reporting level of 30 ng/mL [26,27,33]. A substance from the GC class but differing from the athlete's declaration was detected in three occurrences.

Table 3. Cases in which the use of declared glucocorticoids was confirmed by laboratory tests.

No.	Athletes				DCFs		Laboratory Results		
	Sex	Age	Sport	Period	Declared Substance(s)	Drug Form	Substance	Estimated Concentration (ng/mL)	Remarks
1.	M	16	weightlifting	OOC	budesonide, fluticasone	nasal spray	budesonide	1.27	negative
2.	F	17	weightlifting	IC	fluticasone	inhalation aerosol	fluticasone metabolite	2.29	negative
3.	F	18	skating	IC	budesonide	capsules / tablets	budesonide, budesonide metabolite	15.00 **56.74**	**AAF**
4.	M	18	skiing	IC	budesonide	inhalation aerosol	budesonide, budesonide metabolite	5.02 15.44	negative
5.	M	19	biathlon	IC	ciclesonide	inhalation aerosol	ciclesonide metabolite	2.00	negative
6.	F	19	skating	IC	budesonide	capsules / tablets	budesonide, budesonide metabolite	12.97 **62.02**	**AAF**
7.	M	19	weightlifting	OOC	betamethasone	injection	betamethasone	1.29	negative
8.	M	20	athletics	IC	budesonide	inhalation aerosol	budesonide	2.29	negative
9.	M	21	biathlon	IC	ciclesonide, fluticasone	inhalation aerosol	ciclesonide metabolite	3.14	negative
10.	M	22	canoe/kayak	OOC	betamethasone	injection	betamethasone	10.37	negative
11.	F	24	cycling	IC	fluticasone	nasal spray	fluticasone metabolite	7.31	negative
12.	M	25	canoe/kayak	OOC	ciclesonide	inhalation aerosol	ciclesonide metabolite	1.41	negative
13.	F	25	skating	OOC	betamethasone	injection	betamethasone	2.35	negative
14.	F	27	athletics	OOC	dexamethasone	capsules / tablets	methylprednisolone	7.18	negative
15.	F	31	para-sport	OOC	ciclesonide	inhalation aerosol	dexamethasone	4.23	negative
16.	F	32	athletics	OOC	dexamethasone	injection	methylprednisolone	2.26	negative

AAF—Adverse Analytical Finding; DCFs—Doping control forms; F—female; M—male; IC—in competition; OOC—out of competition; budesonide metabolite—6β-hydroxy-budesonide; ciclesonide metabolite—desisobutyrylciclesonide; fluticasone metabolite—fluticasone propionate-17β-carboxylic acid.

4. Discussion

The primary result of our study was that 8% of the surveyed athletes declared the use of GCs in the week preceding doping control, with 30 medications containing 13 different GCs. From the laboratory analyses, GCs were detected in 0.8% of the urine samples. Interestingly, the LOD and LOQ values for the determination of GCs in urine samples by UPLC/MS/MS are much lower than the specific minimal laboratory reporting limits used to decide whether an athlete has violated the anti-doping rules or not. Of all the samples in which the presence of GCs was found, only in two cases were concentrations of GCs or their metabolites the basis for a positive result of the analysis (AAF—adverse analytical finding). However, in both cases, the athletes had an appropriate therapeutic use exemption (TUE). A TUE ensures that athletes can be treated for medical conditions, even if the treatment involves using a prohibited substance or method, while avoiding the risk of being sanctioned. The frequency of use of GCs was not different during in-competition and out-of-competition periods. More importantly, injections were only reported by athletes during out-of-competition controls. From a subject perspective, the reported use of these drugs was not related to pharmacological support for performance enhancement, but solely for therapeutic purposes. Based on the data available in the Summary of Product Characteristics of the individual medicinal products, the basic indications for the use

of drugs declared by athletes are bronchial asthma (57% of cases), rhinitis (26%), pain blockade (7%), inflammatory skin diseases (5.5%), Achilles tendinitis (1.5%), Crohn's disease (1.5%), eye infections (1%), and Addison's disease (0.5%).

As already mentioned, since 2022, the use of a GC by any injectable, oral, or rectal route requires a therapeutic use exemption. However, after the administration of GCs, the urinary minimum reporting levels (MRL) which would result in an AAF can be reached at different periods of time after administration (ranging from days to weeks), depending on the glucocorticoid administered and the dose. To reduce the risk of an AAF, athletes should follow the minimum washout periods, expressed from the time of administration to the start of the in-competition period (Table 4). These washout periods are based on the use of these medications according to the maximum manufacturer's licensed doses and allow us to check the elimination time of the glucocorticoid below the reporting level. WADA notes that, according to the International Standard for Therapeutic Use Exemptions, an athlete may apply retroactively for a TUE if the athlete used out of competition, for therapeutic reasons, a prohibited substance that is only prohibited in competition. Athletes are strongly advised to have a medical file prepared and ready to demonstrate their satisfaction of the TUE conditions set out, in case an application for a retroactive TUE is necessary following sample collection [34].

Table 4. Minimum washout periods of glucocorticoids [34].

Route	Glucocorticoid	Washout Period
Oral (include e.g., oromucosal, buccal, gingival and sublingual)	All glucocorticoids;	3 days
	Except: triamcinolone acetonide	30 days
Intramuscular	Betamethasone; dexamethasone; methylprednisolone	5 days
	Prednisolone; prednisone	10 days
	Triamcinolone acetonide	60 days
Local injections (including periarticular, intraarticular, peritendinous and intratendinous)	All glucocorticoids;	3 days
	Except: triamcinolone acetonide; prednisolone; prednisone	10 days

Since substances with different half-lives are prescribed to treat athletes, washout periods for such substances shall be carefully accounted for, even though some administration routes are allowed out of competition. Guidelines for the International Standard for Therapeutic Use Exemptions are paramount for athletes and their medical staff to cope with the current WADA regulations and prevent adverse analytical findings for GCs [35]. Interestingly, the concept of MRL introduced with the 2022 regulation [27] allows specifically for GCs with an increased sensitivity (i.e., defining true positives) and a better specificity (i.e., confirming true negatives). In the case of GCs, most substances in the S9 class, e.g., beclomethasone, ciclesonide, flumethasone, flunisolide, fluocortolone, fluorometholone, methylprednisolone, mometasone, triamcinolone, as well as desacetyldeflazacort (metabolite of deflazacort), and 6β-hydroxy-budesonide (metabolite of budesonide), have an MRL at 30 ng/mL (comparable to the required reporting level before 2022). However, for triamcinolone acetonide, the MRL is as low as 15 ng/mL, 45 ng/mL for 6β-hydroxy-budesonide (metabolite of budesonide), 60 ng/mL for betamethasone and dexamethasone, 100 ng/mL for prednisolone, and up to 300 ng/mL for prednisone. Such discrepancies were explained with studies outlining the need to establish compound-specific reporting levels given the diversity of administration routes and doses, as well as pharmacokinetic and pharmacodynamic properties between the different GCs [27]. As Ventura et al. (2020) rightly pointed out, new washout periods enable clinicians to use glucocorticoids safely and to avoid the risk of athletes testing positive for a doping test. In turn, the new substance-specific laboratory reporting values allows us to better distinguish between prohibited and permitted

use in sport [36]. Using these criteria in our study, we noted no false positives that would suggest that an athlete legally treated with GCs would actually be sanctioned for doping.

This is very important because there has been a history of reporting positive laboratory results as a result of the use of authorised forms and/or doses of drugs containing GCs. Kaliszewski et al. (2016) presented the results of five studies of controlled budesonide administration carried out on professional athletes [25]. The samples were analyzed by using a quantitative LC-MS/MS method for 16α-hydroxyprednisolone, the most abundant budesonide metabolite in urine. Their results indicated that the use of budesonide by inhalation within 12 h before and during competition could lead to a positive result from anti-doping testing if the WADA rules effective till 1 September 2014 were applied. The only way to prove that budesonide had been taken by a non-prohibited route was a controlled excretion study. This vindicated the decision of WADA, which allowed the participation of athletes in controlled excretion studies in order to prove that they did not violate anti-doping rules. To avoid similar cases, for the detection of budesonide administration via systemic routes, WADA has decided that laboratories shall target the detection of the 6β-hydroxy-budesonide metabolite. Our study confirmed that this metabolite is a good marker when budesonide is used orally, as declared by the athletes in the DCFs. In two cases, the concentration of 6β-hydroxy-budesonide met the criteria for the test result to be considered positive. However, in both cases, the athletes had a relevant TUE.

The above is evidence that the presence of a prohibited substance or its markers in an athlete's biological sample collected during doping control may not be linked with intentional pharmacological support, but may constitute an anti-doping rule violation (ADRV). The other reasons for the unintended use of a prohibited substance, rather than the imperfect analytical methods or criteria for the evaluation of laboratory results, can be the consumption of food products containing prohibited substances, e.g., dietary supplements containing or adulterated with doping agents [37,38], or products containing hemp (cannabis) extract or poppy seeds with morphine as a natural ingredient [39,40]. It may also happen that meat consumed by an athlete is obtained from illegal slaughter or from animals treated with anabolic agents, which may result in the detection of prohibited substances in the athlete [38]. Most athletes use supplements, despite the fact that experts have been convincing athletes for years that dietary supplements can only play a small role in an athlete's sports nutrition plan, and improper supplementation may even weaken training effects [41]. The huge popularity of products from this group seems to not be even influenced by the fact that approximately 6 to 9% of adverse analytical findings worldwide are the result of consuming supplements containing prohibited substances, in most cases not declared on the label of products [42]. An interesting fact is that the Polish pharmacist Alfons Bukowski, widely regarded as a pioneer in anti-doping research, also worked on examining food and detecting the adulteration of foodstuffs. That was in the 19th century, but it resembles the present-day problem of contamination of dietary supplements with doping agents [43]. Athletes may as well be exposed passively to smoke, e.g., marijuana or hashish (containing tetrahydrocannabinol—THC), crack (cocaine), and ice or crystal meth (methamphetamine), possibly resulting in positive anti-doping test results [44]. However, WADA decided in 2013 to increase the threshold level allowed for carboxy-THC to 150 ng/mL and this allowed numerous ADRVs to be avoided.

The use of substances not present on the prohibited list for medical purposes may also result in prohibited compounds in the body after the metabolization of the allowed compounds into forbidden agents, e.g., oxethazine to phentermine and mephentermine [45], codeine or ethylmorphine to morphine, or lomerizine to trimetazidine [46]. A case was also described of the detection of the diuretic hydrochlorothiazide in a doping control urine sample as a result of a non-steroidal anti-inflammatory drug tablet with ibuprofen contamination [47]. Some positive anti-doping tests could be accounted for by the use of permitted generic prescription drugs contaminated with diuretics. The contamination levels found in the medications are reported and were below the United States Food and Drug Administration limits for manufacturers that are based primarily on safety

considerations [48]. In recent years, the number of positive cases has also been increasing due to sexual intercourses, during which prohibited substances taken by partners enter the athlete's body, which may be at least partially due to urine contamination by semen [49].

Nevertheless, it is up to the athlete and his or her support (medical) team to check whether the adequate treatment is still authorised. Fully aware of the athletes' rights to treat any acute or chronic condition, WADA defends the concept of TUE for certain prohibited substances or methods insofar as the athlete would suffer significant health damage if the adequate treatment was not administered. The WADA Code explicitly states that a TUE can only be granted if it is highly unlikely that the therapeutic use of the prohibited substance or method will result in an improvement in performance beyond that which would be achieved by returning the athlete to a state of normal health following treatment of the acute or chronic condition. In addition, there must be no authorised therapeutic alternative to the prohibited substance or prohibited method. The athlete must therefore be able to count on the proven support of his or her treating physician to assess either the possible risk of wanting to participate in his or her sport in the presence of a pathology, or the effective possibility of a TUE. In a recent study, 58% (of 775) of athletes feared the side effects of using prohibited products, even though 18% of them were interested in using them, to the detriment of their health [50]. The latter is, finally, particularly interesting in the current analysis of GCs use in athletes, and remarkably topical when questioning the spirit of sport even beyond the medical ethics and deontology.

Overall, our results confirm that the method used in the Polish Anti-Doping Laboratory to date for glucocorticoid determination in urine samples collected for doping controls, using ultra-high-performance liquid chromatography–tandem mass spectrometry, does not give false-positive results and does not need to be modified with the updated anti-doping regulations which prohibit all injectable routes of administration of GCs during the in-competition period.

5. Conclusions

In conclusion, the previously used methodology for the determination of GC in urine samples (ultra-high-performance liquid chromatography–tandem mass spectrometry) remains fully valid after the recent regulatory change which prohibits all injectable routes of administration of GCs for use in the in-competition periods. Polish athletes declared a wide use of various forms of glucocorticoid medicinal preparations with therapeutic purposes, applied to the body by different routes, containing substances with different half-lives. Newly established minimum reporting levels for GCs that are substance-specific in addition to recommended washout periods do support the justified therapeutical use of GCs in a sporting context. The regulation set in place by the World Anti-Doping Agency combined with the analytical expertise in anti-doping laboratories may definitely help to distinguish between the prohibited and permitted use of GCs in sport; in addition, the early submission of the applicable therapeutic use exemption, and the possibility for a retroactive therapeutic use exemption may prevent adverse analytical findings for GCs. Even though some administration routes for GCs are allowed out of competition for elite athletes, substance-specific washout periods shall however be carefully accounted for to avoid sanctions by anti-doping authorities. It is equally important for athletes to remember the exact names of the preparations they are taking, because it happens that, in the doping control forms, they sometimes declare medicines containing different substances than those detected in laboratory tests.

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