Research on the Application of Isotope Ratio Mass Spectrometry Technology in Traceability Detection of Stimulants

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Abstract: Endogenous substances, which are produced by the body itself, have become a focus of concern due to their potential abuse as stimulants in sports. The chemical structure and physicochemical properties of externally synthesized endogenous substances are similar to those produced internally, making them difficult to detect. In response to this challenge, it is crucial to develop effective methods for detecting both endogenous and exogenous substances. One promising approach leverages the differences in stable carbon isotope abundance between internal and external sources, leading to changes in the Compound-Specific Isotope Ratio (CIR) after ingestion. The GC-C-IRMS method has emerged as a commonly used and highly accurate technique for tracing the origin of substances. Recognizing its significance in the fight against doping, the World Anti-Doping Agency (WADA) has standardized this method and established relevant benchmarks. This review article examines the advancements made over the past decade in detecting S1 anabolic steroids, S4 hormones and metabolic modulators, and S9 glucocorticoids. By offering a comprehensive overview of progress in the detection of endogenous substances using isotope ratio mass spectrometry, the aim is to provide valuable insights for enhancing existing anti-doping measures. This research serves as a critical reference point for ongoing efforts to combat the misuse of endogenous substances in the realm of sports.

Keywords: Endogenous Substance; Detection Method; Isotope Ratio Mass Spectrometry; Tracing the Origin of Doping

1. Introduction

Stable isotopes refer to nuclei with the same atomic number but different mass numbers and are non-radioactive, such as ^{13}C and ^{12}C, ^{18}O and ^{16}O. In nature, substances constantly exchange matter with the external environment, and their stable isotope characteristics undergo fractionation influenced by factors such as climate, environment, and metabolic pathways, thereby forming unique stable isotope fingerprints. These fingerprints serve as natural labels for substances, closely related to the environment in which the substances exist, and remain unaffected by changes in chemical additives. Consequently, stable isotope techniques offer unparalleled advantages in traceability, authenticity assessment, and characterization of conversion reactions compared to other methods. They have increasingly demonstrated wide applicability and vast potential across various research fields in the natural sciences.

1.1 Isotopic Abundance

Typically, the composition of isotopes in a substance is expressed in terms of absolute abundance and relative abundance. Absolute abundance is determined by overcoming all measurement errors within a reasonable uncertainty range, which can be achieved through the application of mass spectrometry coupled with calibration coefficients. Relative abundance, on the other hand, refers to the relative content of each isotope of the same element, such as ^{12}C at 98.892% and ^{13}C at 1.108%. In nature, monoisotopic elements are scarce, with only 20 known, such as ^{19}F at 100%.

The elemental isotopic abundance variations in plants, particularly between C3 and C4 plants, constitute the earliest subjects of observation and study. A classic example is found in steroid hormones, where significant

differences in the $^{13}C/^{12}C$ values exist between exogenous and endogenous steroids. Initially, some pharmaceutical companies utilized plant sterols as raw materials for semi-synthetic steroid formulations due to their low cost and ready availability [1]. Most plants in nature utilize carbon fixed in the form of CO_2 as raw material to synthesize the nutrients and metabolic precursors they need. However, due to the different forms of photosynthesis in C3 and C4 plants, there are local deficiencies or enrichments of carbon elements. C3 plants absorb CO_2 through the Calvin cycle, exhibiting the ability to enrich ^{12}C. Typical C3 plants include wheat and soybeans, with $\delta^{13}C$ ranging from -23.0 to -31.3‰. C4 plants absorb CO_2 via the Hatch-Slack photosynthetic cycle, resulting in higher $^{13}C/^{12}C$ values compared to C3 plants. Typical C4 plants include maize, sugarcane, and sorghum, with $\delta^{13}C$ ranging from -8.0 to -20.0‰. CAM plants combine the Calvin cycle with the Hatch-Slack photosynthetic cycle, generally exhibiting higher $^{13}C/^{12}C$ values than C3 plants. A typical CAM plant is pineapple, with strong CAM showing $\delta^{13}C$ values of -8 to -20‰, while values greater than -20‰ indicate the possibility of some degree of CAM activity in C3 plants [2-3].

1.2 Principle of IRMS Tracing Analysis

Isotope ratio mass spectrometry (IRMS), recognized as a novel and specialized technique, finds wide application. The local enrichment and depletion of elements are influenced by both kinetic and thermodynamic factors, leading to variations in isotopic ratios. For instance, isotopic ratios like $^{13}C/^{12}C$, $^{18}O/^{16}O$, $D/^{1}H$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$ differ due to these influences. IRMS leverages the differences in isotopic abundances of constituent elements in various substances to differentiate materials with identical chemical compositions or their sources [4].

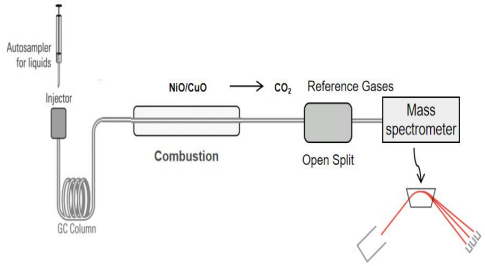

Figure 1. Isotope Mass Spectrometry Operational Principles

The working principle is shown in Figure 1: the substrate is analyzed and separated by gas chromatography (GC), and then introduced into a combustion furnace (C) through a three-way valve. Inside the furnace, under the action of catalysts and oxygen suppliers, it undergoes complete oxidation to form CO_2, N_2, and H_2O. During the combustion process, nitrogen-containing compounds will produce a small amount of nitrogen oxides, which will subsequently be reduced to N_2. Finally, the samples enter the IRMS for isotopic ratio analysis. The results are represented by δ, indicating the isotopic difference between the sample and the international standard substance, using carbon isotopes as an example (1).

$$\delta^{13}C(‰)=(R_{SPL}-R_{STD})/R_{STD}\times 1000 \quad (1)$$

This article focuses on the anti-doping control in sports, where IRMS is used to distinguish between endogenous and exogenous substances and their metabolites in urine. The WADA mandates a reanalysis using IRMS to confirm positive test results for false endogenous steroids before disclosing them in the list of prohibited substances.

For each individual undergoing testing, it is necessary to select appropriate Endogenous Reference Compound (ERC) for comparison. The parameter $\Delta^{13}C$ represents the difference between the $\delta^{13}C$ values of the detected substance and ERCS. The $\delta^{13}C$ value of ERCS remains unaffected by exogenous compounds. With the use of exogenous substances, the difference in $\delta^{13}C$ values between the two increases, resulting in a higher $\Delta^{13}C$, while its absence indicates a lower $\Delta^{13}C$. According to relevant regulations of WADA, $\Delta^{13}C > 3‰$ serves as clear evidence of abuse of endogenous steroids. Currently, substances used as ERCs mainly include PD, PT, 16-en, 11-OH-A, and corticosteroids (11-oxo-Etio).

1.3 Applications in Common Fields

1.3.1 Applications in forensic science

In the past decade, the combination of IRMS technology with progressively improved chromatographic techniques has greatly assisted various fields of forensic science. Identification of human remains. Isotope analysis technology is highly valuable for forensic anthropological case studies, analysis of postmortem changes, and examination of antemortem injuries, while also aiding in the

search for missing persons and more [5]. For example, water participates in the global ecological cycle, with its constituent elements being H and O, and stable isotopes including ^{1}H, ^{2}H, ^{16}O, ^{17}O, and ^{18}O[6]. During measurement, the isotopic composition of water exhibits systematic spatial variations. This can be used to determine the residency history and origin of remains, among other things. Illegal wildlife trade forensics: IRMS can successfully trace the geographic origins and movements of animals between different landscapes [7]. Environmental forensics (illegal pollution in protected areas, arson) [8], illegal drug detection (cannabis and other drugs) [9], flammable liquid analysis, and more.

1.3.2 Applications in food science

As a "natural fingerprint" of the environment in which animals and plants grow, GC-C-IRMS technology is highly favored for traceability and adulteration analysis of animal and plant products, with promising development prospects. GC-C-IRMS technology can determine the stable isotope ratios of C and N in different matrices of food, known as compound-specific isotope analysis (CSIA), where the combustion furnace only burns specific components, analyzing single compounds within the components. Compared to EA-IRMS, this method is more precise. Once exceeding the range of the established isotope database, food adulteration can be suspected. Research by Da Silva et al. in 2016 indicated that IRMS can be used to detect the amount of prohibited by-product protein (ABP) ingested by animals, thereby reducing the transmission of viruses (such as prions) in biopharmaceuticals and products. In the same year, Camin et al. pointed out that IRMS technology has been successfully applied in the field of animal products such as meat, dairy products, and fish.

Several examples can be provided to illustrate the application in Food Science. For instance, when identifying the regional origins of South African lambs and the fat ratio of loin muscles, stable isotope ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ are measured, allowing for an understanding of the dietary characteristics in different regions. Similarly, this method can be utilized to authenticate the true origins of certain fruits (e.g., strawberries, mangoes) [10], facilitating better classification and marketing.

1.3.3 Applications in archaeology

Although archaeology and forensic science are two distinct fields, they sometimes face remarkably similar challenges. Therefore, there is a close connection between IRMS and both disciplines. For instance, in cases such as tracing individual origins/residences based on remains, both criminal investigations and archaeology utilize IRMS extensively. As early as 2013, Nehlich et al. first provided sulfur isotope data of modern and archaeological cod collagen (8th to 18th centuries), aiming to establish baseline values for human dietary studies and to identify potential variables and spatial variability in fish habitat and fish trade [11]. In 2016, Göhring further investigated fish bones and found that the GMM clustering method was more suitable for complex multidimensional stable isotope datasets established by IRMS. This typical application can reveal habitat preferences and identify non-local individuals. In addition to collagen analysis, another major application is organic residue analysis, where artifacts (mostly from the Stone Age) are typically extracted for TAG to conduct IRMS analysis, thus studying ancient diets and food origins. This can help extend to data analysis for tracking ancient trade routes, which is highly meaningful [12-13].

2. Applications of IRMS in the Field of Endogenous Doping Detection

Steroid hormones, also known as corticosteroids, are a type of hormone secreted by the human body, divided into sex hormones (estrogens, androgens) and corticosteroids (glucocorticoids, mineralocorticoids). Research has shown that steroid hormones play roles in maintaining life, regulating immunity, and regulating reproductive function. They also hold significant positions in the neuroendocrine axis, treatment of skin diseases, and birth control [14-17]. In competitive sports, anabolic-androgenic steroids (AAS) are often used to promote muscle growth, accelerate physical performance and fatigue recovery, thereby improving athletic performance [18]. However, cessation of use often leads to neurodegenerative diseases, withdrawal depression and addiction, as well as many adverse reactions [19].

Steroid hormones can be classified as endogenous and exogenous. Exogenous

steroid hormones are not synthesized within the human body, the urine sample is deemed positive, indicating doping violations by the athlete. Based on current technological means, the determination of endogenous steroids primarily relies on the threshold. When surpassing the threshold, further confirmation of their origin is required through GC/C/IRMS. Common AAS include testosterone (T), dihydrotestosterone (DHT), and epitestosterone (ET), among others.

The $\delta^{13}C$ data obtained from IRMS are primarily based on diet. However, the metabolism of steroids in the human body is also influenced by factors such as gender, age, and geographical environment. Therefore, the Athlete Biological Passport (ABP) established internationally incorporates a steroid module for long-term longitudinal monitoring.

2.1 Methods for Detecting Anabolic-Androgenic Steroids

The entire testing process currently comprises several stages, including sample pretreatment, substance isolation, and IRMS analysis. For samples with complex matrices such as urine, additional HPLC purification is required. In the case of blood samples, Putz et al. proposed the use of immunoadsorption chromatography (IAC) in 2019 to replace existing purification methods, significantly reducing pretreatment time. In doping detection, GC is commonly combined with EI for steroid analysis, but EI can disrupt the analytes. In 2017, the Cha team developed GC-ESI-HRMS for AAS analysis in human urine, improving ionization efficiency.

The use of LC-ESI-HRMS is also quite common, for example, in the analysis of glucuronides, sulfates, and so on. In 2020, G€oschl et al. introduced a novel method for detection of stanozolol in urine, with a minimum detection limit of 75 pg/mL [20].

Since these esters are entirely exogenous, their detection in the blood constitutes evidence of doping [21]. Piper et al. have demonstrated that compared to common target compounds like 5α-diol, 5β-diol, or testosterone itself, EpiA-S can extend the detection window for exogenous steroids by 2-3 times.

As early as 2013, this issue was addressed by LC-MS analysis [22]. In 2020, Van Renterghem and colleagues proposed a method which involves sample preparation using liquid-liquid extraction (LLE), HPLC separation, and MSTFA derivatization, enabling the detection of testosterone and nandrolone esters.

In 2021, Kintz and colleagues conducted a retrospective steroid testing on human nail clippings. When samples such as hair are not available, nail clippings can serve as substitutes. Nail sampling is more convenient, less prone to melanin interference, and offers a longer detection window, with finger clippings providing a window of 3-8 months and toenail clippings 4-12 months [23].

2.2 Methods for Detecting Glucocorticoids

Glucocorticoids (GCs) are the most important regulatory hormones in the body's stress response, regulating development, growth, metabolism, and immune function. Widely used in clinical settings, they are highly effective anti-inflammatory and immunosuppressive agents [24], effective for virtually any type of allergic or inflammatory disease. GCs are the preferred drugs for treating asthma, rheumatoid arthritis, allergic rhinitis, and pain caused by chronic musculoskeletal injuries. Some athletes apply for Therapeutic Use Exemptions (TUE) to use GCs for therapeutic purposes. Common clinical GC drugs include prednisolone, Betamethasone, hydrocortisone, and dexamethasone.

Research indicates that GCs can delay central nervous system fatigue; their anti-inflammatory and analgesic effects can suppress muscle pain during exercise, raising the fatigue threshold; their metabolic effects can accelerate the breakdown of fats and glycogen induced by catecholamines and growth hormone, thereby allowing muscles to utilize energy more efficiently. Consequently, WADA has set the urine detection threshold for GCs at 30 ng/mL. Long-term use may lead to various side effects, particularly affecting bone tissue (such as osteoporosis), metabolism (such as insulin resistance), and the cardiovascular system (such as hypertension and atherosclerosis).

Current GCs detection methods primarily utilize SPE and LC-MS/MS, with sample materials including hair, saliva, serum, and urine [25], constituting a major focus of various analytical studies. In 2017, Shah quantified four types of GCs in camel hair using LC-MS/MS. Subsequently, in comparison with

commercial ELISA kits, it was found that results from ELISA kits tended to overestimate, with LC-MS/MS proving to be the more reliable method. In 2020, Protti employed dried urine spots (DUS), quantifying three endogenous (cortisol, cortisone, and corticosterone) and three exogenous corticosteroids. Sample stability at room temperature was satisfactory, with less than 15% analyte loss after three months. A study in 2021 demonstrated the feasibility of screening GCs using online SPE and LC-HRMS, utilizing an internally developed low-cost monolithic column for automated and high-throughput detection [26]. When quantitative analysis fails to distinguish between endogenous and exogenous sources, IRMS detection methods will be employed for determination.

3. Methods for Detecting Metabolic Modulators

3.1 Methods for Detecting Formestane

Formestane is a derivative of androstenedione and belongs to type I aromatase inhibitors. It is commonly used to treat estrogen-dependent breast cancer, with minimal toxic side effects and good tolerance. Aromatase is a composite enzyme in the cytochrome P450 enzyme system, which is a membrane-bound protein located in the endoplasmic reticulum. It can catalyze the removal of carbon-19 from androstenedione and testosterone and cause aromatic conversion of the A ring, forming estrone and estradiol respectively. It is a key enzyme in estrogen biosynthesis. Aromatase inhibitors specifically lead to aromatase inactivation, blocking aromatization reactions, inhibiting estrogen production, and reducing estrogen levels in the blood.

Before menopause, estrogen in women mainly originates from the ovaries. After menopause, estrogen in the body is primarily derived from androgens secreted by the adrenal glands, which undergo aromatization by aromatase in sites such as adipose tissue, liver, and kidneys. The main estrogen produced is estrone. Formestane, structurally similar to androstenedione, can bind to the active site of aromatase, competitively inhibiting its activity. This leads to a reduction in estrogen levels and a relative increase in testosterone levels in the body [27].

Utilizing this characteristic of formestane, many athletes often use it as a performance-enhancing drug. Because formestane lacks the androgenic properties and primarily works by inhibiting the conversion of endogenous androgens, its side effects are relatively minor. Combining it with testosterone or androstenedione can further reduce aromatization-related side effects, such as male breast development. It was classified as a prohibited substance by WADA in 2004.

In 2013, Polet proposed that due to WADA setting the detection threshold for formestane at 150ng/mL, and considering that formestane can be produced in small amounts by the human body, trace amounts of formestane can be detected in almost every urine sample, posing challenges for IRMS confirmation. Therefore, he suggested a lower limit of 25 ng/mL, where formestane detected within this range would be considered endogenous and not require further confirmation. In 2014, a study confirmed that among the extensive metabolites of formestane, 4OH-EA had the longest detection window. Therefore, it is recommended to monitor 4OH-EA in analyses as a biomarker to determine subsequent IRMS analysis.

In recent years, intramuscular injection of formestane has led to peak blood drug concentrations within 1-2 days, accompanied by various adverse reactions such as headache, rash, and drowsiness. The oral route exhibits low bioavailability (approximately 4%), with 20-45% excreted in the form of glucuronic acid after first-pass metabolism, leaving only 50% after 4 hours. Additionally, the substance is primarily available in the market in the form of topical gel. Therefore, transdermal application appears to be the most likely route of administration for athletes.

In 2014, WADA redefined the detection thresholds for fulvestrant. Concentrations of fulvestrant detected below 50 ng/mL are classified as negative, while concentrations exceeding 150 ng/mL are deemed positive. For concentrations falling between these two thresholds, GC-C-IRMS analysis is required, with a $\Delta\delta$ value greater than 4 units indicating a positive result. Currently, there is limited research on fulvestrant, and new detection methods have yet to be fully established.

3.2 Methods for Detecting AICAR

5-Aminoimidazole4-carboxamide ribonucleoside (AICAR), commonly known as "exercise pill," is an activator of AMP-activated protein kinase (AMPK), which can penetrate cell membranes. AMPK is a key protein in metabolic regulation [28]. When energy supply is insufficient, the ratio of AMP/ATP increases, leading to AMPK activation and inhibition of synthetic metabolism. AICAR activates AMPK without affecting ATP, ADP, and AMP levels, while promoting glucose uptake in skeletal muscles independently of insulin, a process not blocked by PI3K inhibitors [29]. Consequently, AICAR significantly enhances athletes' endurance, leading to its inclusion in the WADA list of prohibited substances in 2009.

Since AICAR is an intermediate product in the purine synthesis pathway, its endogenous and exogenous sources need to be considered in doping tests. Currently, there is limited international research on this topic, with studies mainly following the GC-C-IRMS method prescribed by WADA to confirm its endogenous and exogenous origin.

So far, only a few studies have conducted quantitative analysis of urinary AICAR. In 2013, Thomas analyzed blood samples from 459 athletes using LC-MS/MS to measure AICAR-ribotide in RBC concentrates. The normal physiological level of this substance is 10-500 ng/mL, and it was proposed that AICAR concentrations exceeding 20 μg/mL are considered exogenous [30]. Subsequently, Piper and colleagues, after studying 499 urine samples, proposed a lower threshold of 3500 ng/mL, with an extended detection time of up to 40 hours [31]. In 2017, Bussion optimized the GC-C-IRMS method. Due to the molecular structure of AICAR, traditional derivatization methods cannot be used, with silylation being the preferred method to improve the stability of derivatives. This adjustment resulted in a repeatable derivatization pattern with 3-TMS as the main derivative. Additionally, SPE washing steps, microfiltration, and internal sample preparation for steroid components were used instead of concentration steps.

In 2019, Sobolevsky's experiment found that compared to conventional C18 chromatographic columns, the retention chromatographic column Cortecs generated less matrix effect. The average concentration of AICAR was correlated with gender, exercise, and competition status, with higher concentrations in male athletes than female athletes, and higher in samples taken during competition than those taken outside of competition. The threshold was set at 2500 ng/mL, and could even be set at 2000 ng/mL, with concentrations exceeding this value requiring IRMS analysis. In the same year, Dmitrieva's team conducted quantitative analysis of AICAR using UHPLC-MS/MS. The LOD was 5 ng/mL, and within the concentration range of 50 to 5000 ng/mL, a good linear relationship was observed [32].

4. Other Confounding Factors

The steroid hormone module (SP) enables longitudinal monitoring of steroid hormone levels for each individual, hence it is imperative to ensure the integrity of each data point within this module. Various confounding factors (CFs) may affect the SP, leading to misinterpretation of GC-C-IRMS results; for instance, ethanol intake can significantly elevate the T/E ratio within a few hours.

This review mainly focuses on masking agents. 5α-reductase inhibitors can reduce the generation of DHT by inhibiting the activity of 5α-reductase, treating benign prostatic hyperplasia and male pattern baldness, and are effective means for treating androgen-dependent diseases. Common drugs include finasteride and dutasteride. Studies have found that the use of finasteride, especially in long-term users, can decrease the δ13C values of both 19-NA and 19-NE when simultaneously ingesting them. This is equivalent to using multiple stimulants concurrently, producing a masking effect during urine testing [33]. Therefore, developing detection methods for 19-NA and 19-NE to enhance sensitivity is crucial.

Athletes often abuse diuretics to increase urine volume, expel large amounts of fluid, rapidly reduce weight. Currently, the detection of diuretics is relatively mature compared to 5α-reductase inhibitors. Steroid analogs and benzodiazepines have been studied and proven to act as inhibitors of AKR1C3, affecting the measurement of testosterone concentration.

5. Conclusion

The characteristics of doping drug use include low doses and local administration to avoid

peak concentration excretion. The diversity of detection matrices leads to differences in the extraction efficiency of target substances in different matrices. In order to improve detection capabilities, research and improvements are being made based on pre-treatment schemes. Controlling the abuse of doping drugs poses a significant challenge in analysis. Currently, the detection of doping drugs involves the integration of multiple disciplines, and detection instruments are gradually moving towards automation. In the future, research will continue to develop new, accurate, and rapidly evolving detection technologies.

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References

[1] Araghiniknam M, Chung S, Nelson-White T, et al. Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans. Life Sciences, 1996, 59(11): PL147–PL157.
[2] Winter K, Holtum J A M, Smith J A C. Crassulacean acid metabolism: a continuous or discrete trait? New Phytologist, 2015, 208(1): 73–78.
[3] Winter K. Ecophysiology of constitutive and facultative CAM photosynthesis. Cushman. Journal of Experimental Botany, 2019, 70(22): 6495–6508.
[4] Muccio Z, Jackson G P. Isotope ratio mass spectrometry. The Analyst, 2009, 134(2): 213–222.
[5] Bartelink E J, Chesson L A. Recent applications of isotope analysis to for ensic anthropology. Forensic Sciences Research, 2019, 4(1): 29–44.
[6] Gat J R. Oxygen and hydrogen isotopes in the hydrologic cycle. Annual Review of Earth and Planetary Sciences, 1996, 24(1): 225–262.
[7] Seminoff J A, Benson S R, Arthur K E, et al. Stable Isotope Tracking of Endangered Sea Turtles: Validation with Satellite Telemetry and $\delta^{15}N$ Analysis of Amino Acids. R. Reina. PLoS ONE, 2012, 7(5): e37403.
[8] Shin W-J, Lee S-W, Heo S-Y, et al. Stable Isotopic Fingerprinting for Identification of the Methyl Tert-Butyl Ether (MTBE) Manufacturer. Environmental Forensics, 2013, 14(1): 36–41.
[9] Kim H, Lee D-H, Go A, et al. Differentiation of endogenous and exogenous γ-Hydroxybutyrate in rat and human urine by GC/C/IRMS. International Journal of Legal Medicine, 2019, 133(6): 1785–1794.
[10] Strojnik L, Potočnik D, Jagodic Hudobivnik M, et al. Geographical identification of strawberries based on stable isotope ratio and multi-elemental analysis coupled with multivariate statistical analysis: A Slovenian case study. Food Chemistry, 2022, 381: 132204.
[11] Nehlich O, Barrett J H, Richards M P. Spatial variability in sulphur isotope values of archaeological and modern cod (Gadus morhua): Sulphur isotopes of cod. Rapid Communications in Mass Spectrometry, 2013, 27(20): 2255–2262.
[12] Colonese A C, Farrell T, Lucquin A, et al. Archaeological bone lipids as palaeodietary markers: Lipids as dietary markers. Rapid Communications in Mass Spectrometry, 2015, 29(7): 611–618.
[13] Oras E, Vahur S, Isaksson S, et al. MALDI-FT-ICR-MS for archaeological lipid residue analysis. Journal of Mass Spectrometry, 2017, 52(10): 689–700.
[14] Benagiano M, Bianchi P, D'Elios M M, et al. Autoimmune diseases: Role of steroid hormones. Best Practice & Research Clinical Obstetrics & Gynaecology, 2019, 60: 24–34.
[15] Zubeldia-Brenner L, Roselli C E, Recabarren S E, et al. Developmental and Functional Effects of Steroid Hormones on the Neuroendocrine Axis and Spinal Cord. Journal of Neuroendocrinology, 2016, 28(7): jne.12401.
[16] Mehta A, Nadkarni N, Patil S, et al. Topical corticosteroids in dermatology. Indian Journal of Dermatology, Venereology, and Leprology, 2016, 82(4): 371.
[17] Geyer J, Bakhaus K, Bernhardt R, et al. The role of sulfated steroid hormones in reproductive processes. The Journal of Steroid Biochemistry and Molecular Biology, 2017, 172: 207–221.
[18] Mędraś M, Brona A, Jóźków P. The Central Effects of Androgenic-anabolic

Steroid Use. Journal of Addiction Medicine, 2018, 12(3): 184–192.
[19] Albano G D, Amico F, Cocimano G, et al. Adverse Effects of Anabolic-Androgenic Steroids: A Literature Review. Healthcare, 2021, 9(1): 97.
[20] Smit D L, de Hon O, Venhuis B J, et al. Baseline characteristics of the HAARLEM study: 100 male amateur athletes using anabolic androgenic steroids.:9.
[21] World Anti-Doping Agency (WADA). TD2021APMU. 2020.
[22] Forsdahl G, Vatne H K, Geisendorfer T, et al. Screening of testosterone esters in human plasma: Screening of testosterone esters. Drug Testing and Analysis, 2013, 5(11–12): 826–833.
[23] Kintz P, Gheddar L, Raul J. Testing for anabolic steroids in human nail clippings. Journal of Forensic Sciences, 2021, 66(4): 1577–1582.
[24] Yang M, Chen J, Wei W. Dimerization of glucocorticoid receptors and its role in inflammation and immune responses. Pharmacological Research, 2021, 166: 105334.
[25] Mezzullo M, Fanelli F, Fazzini A, et al. Validation of an LC–MS/MS salivary assay for glucocorticoid status assessment: Evaluation of the diurnal fluctuation of cortisol and cortisone and of their association within and between serum and saliva. The Journal of Steroid Biochemistry and Molecular Biology, 2016, 163: 103–112.
[26] Yan Y, Ai L, Zhang H, et al. Development an automated and high-throughput analytical platform for screening 39 glucocorticoids in animal-derived food for doping control. Microchemical Journal, 2021, 165: 106142.
[27] Onizuka Y, Nagai K, Ideno Y, et al. Association between FSH, E1, and E2levels in urine and serum in premenopausal and postmenopausal women. Clinical Biochemistry, 2019, 73: 105–108.
[28] Rodríguez C, Muñoz M, Contreras C, et al. AMPK, metabolism, and vascular function. The FEBS Journal, 2021, 288(12): 3746–3771.
[29] Chen M-M, Li Y, Deng S-L, et al. Mitochondrial Function and Reactive Oxygen/Nitrogen Species in Skeletal Muscle. Frontiers in Cell and Developmental Biology, 2022, 10: 826981.
[30] Thomas A, Vogel M, Piper T, et al. Quantification of AICAR-ribotide concentrations in red blood cells by means of LC-MS/MS. Analytical and Bioanalytical Chemistry, 2013, 405(30): 9703–9709.
[31] Piper T, Thomas A, Baume N, et al. Determination of $^{13}C/^{12}C$ ratios of endogenous urinary 5-amino-imidazole-4-carboxamide 1β-D-ribofuranoside (AICAR): Determination of $^{13}C/^{12}C$ ratios of endogenous urinary AICAR. Rapid Communications in Mass Spectrometry, 2014, 28(11): 1194–1202.
[32] Dmitrieva E V, Temerdashev A Z, Azaryan A A, et al. Application of Solid-Phase Extraction for the Quantification of Urinary AICAR by Ultra-High Performance Liquid Chromatography–Tandem Mass-Spectrometry. Journal of Analytical Chemistry, 2019, 74(9): 861–864.
[33] Iannella L, Colamonici C, Curcio D, et al. 5α-reductase inhibitors: Evaluation of their potential confounding effect on GC-C-IRMS doping analysis. Drug Testing and Analysis, 2021, 13(11–12): 1852–1861.

