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Systematic Review

Exogenous Versus Endogenous Nandrolone in Doping Investigations: A Systematic Literature Review

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Abstract: Nandrolone, or 19-nortestosterone, is an anabolic steroid derived from testosterone, known for its androgenic and anabolic effects. Often used illicitly by athletes to boost performance, its use is banned by the World Anti-Doping Agency (WADA) in and out of competition. Nandrolone's main metabolites, 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE), are typically detected in urine. This systematic review, registered with PROSPERO and following PRISMA guidelines, examines nandrolone's metabolism, factors affecting its natural production, and the analytical methods used in doping tests. Searches on PubMed, Scopus, and Web of Science yielded 517 studies, of which 57 were selected for analysis after excluding duplicates and unrelated articles. Descriptive statistics were applied to assess data on metabolic pathways, endogenous production influences, and detection techniques. Based on this review, it clearly emerges that the only technique that can distinguish endogenous production from an exogenous intake is gas chromatography/combustion/isotope ratio mass spectrometry (GC-C-IRMS). In addition, factors influencing endogenous production are considered and explored. Overall, this review provides useful information regarding nandrolone and its main metabolites.

Keywords: nandrolone; 19-norandrosterone; 19-noretiocholanolone; metabolic factors; doping

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1. Introduction

Nandrolone, or 19-nortestosterone, is an anabolic steroid originating from testosterone, known for its androgenic and muscle-building effects. It was the first anabolic steroid to be synthesized in 1953. Since 1959, it has been used in clinical practice to promote tissue growth and it is applicable in adjunctive therapy for osteoporosis, anemias, burn injuries, and muscle hypotrophy [1]. Soon, its use became an international drug abuse problem, as athletes discovered its evident anabolic properties, especially in sports requiring greater muscular strength, since it is known to increase muscle mass and strength [2]. Due to these performance-enhancing effects, the World Anti-Doping Agency (WADA) prohibits the use of nandrolone in and out of competition and has included it in its Prohibited List of substances [3]. Despite the evolution of analytical techniques that allow for an increasingly precise determination of urinary levels of nandrolone and its main metabolites, issues relating to the endogenous production of this steroid have yet to be comprehensively evaluated.

Nandrolone can be administered orally or by intramuscular injection. It is metabolized by extensive oxidative biotransformation to form glucuronide and sulfate conjugates. The main metabolites detectable in urine are 19-norandrosterone (19-NA), 19-norethiocholanolone (19-NE), and 19-norepiandrosterone [4]. The 19-NA is usually the most predominant urinary metabolite, and, for this reason, the detection of nandrolone is usually centered on the identification of this primary metabolite. One problem related to the identification of

nandrolone in anti-doping controls is that this anabolic steroid is produced endogenously by the body, which means traces can be found in the urine of healthy subjects who have not metabolized exogenous nandrolone. It is well documented that during pregnancy, there is an increase in 19-norandrosterone levels in urine [5]. In addition, some contraceptive pills contain norsteroids, such as norethisterone, and can lead to urinary excretion of 19-NA [6]. Another source of contamination is the consumption of meat containing anabolic steroids due to the treatment or the natural production of animals such as boars and stallions [7]. Positive tests can also result from the ingestion of dietary supplements contaminated with steroids and prohormones not included in the list of ingredients [8]. For this reason, the World Anti-Doping Agency has issued a technical document [9] to standardize the analysis of 19-norsteroids associated with nandrolone. For instance, gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) is generally not required when the concentration of 19-NA exceeds 15 ng/mL, except in cases of pregnancy, where the laboratory must report an adverse analytical finding. GC/C/IRMS analysis is required for pregnant individuals if the 19-NA concentration is over 15 ng/mL. Additionally, GC/C/IRMS may be conducted when 19-NA levels range from 2.5 to 15 ng/mL to determine if nandrolone is of endogenous or exogenous origin.

The aim of this systematic review is to focus on the metabolism of nandrolone, the different factors that affect endogenous production leading to the urinary excretion of this steroid or its metabolites, and the different techniques that can be applied to detect nandrolone and discriminate between the exogenous and endogenous forms. For this purpose, all articles in the scientific literature regarding the above-mentioned issues were evaluated. The ultimate goal of this work is to produce an updated paper containing the main predisposing factors for an increased presence of nandrolone and its metabolites in urine that may lead to positives in athletes. In addition, we identify the main second-level analytical tools that can discriminate between endogenous production and exogenous intake of nandrolone.

2. Materials and Methods

This systematic review adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines [10], with the study protocol registered on PROSPERO (CRD42024512477).

A comprehensive literature search of PubMed, Scopus, and Web of Science was conducted to identify studies published in English through May 2024.

This review aimed to analyze existing research on nandrolone metabolism and factors that may affect its production, potentially leading to positive doping results. Additionally, it examined analytical methods that help differentiate between endogenous and exogenous sources of nandrolone.

The generic free-text search terms were as follows: (“Nadrolone” [All Fields] OR “19-norandrosterone” [All Fields] OR “19-noretiocholanolone” [All Fields]) AND (“endogenous” [All Fields]) AND (“human” [All Fields]) AND (“urine” [All Fields]) AND (“doping”) [All Fields]) AND (“athletes”) [All Fields]).

Two researchers independently conducted searches in PubMed, Scopus, and Web of Science, while three other researchers evaluated whether the chosen articles met the inclusion criteria. The following information was recorded from the selected studies: authors, publication year, title, analytical techniques, production and metabolism mechanisms, factors influencing endogenous production, and cut-off values. The search results were further refined based on language (English), as well as relevance in titles, abstracts, methods, and keywords. Only studies meeting the following specified inclusion criteria were ultimately selected for analysis:

- Analysis performed on human urine;
- Clarifications on the metabolism of nandrolone;
- Detection of nandrolone and its metabolites;
- Factors influencing the production of nandrolone;

- Analytical techniques used to identify and quantify nandrolone and its metabolites.

Non-inclusion and exclusion criteria were the following:

- Analysis performed on human tissues and fluids;
- Analysis performed on animals.

This study followed PRISMA guidelines, as illustrated in Figure 1, and descriptive statistics were applied to analyze the data. The selected articles were examined to explore factors influencing endogenous nandrolone production and to assess analytical techniques that differentiate between endogenous and exogenous nandrolone.

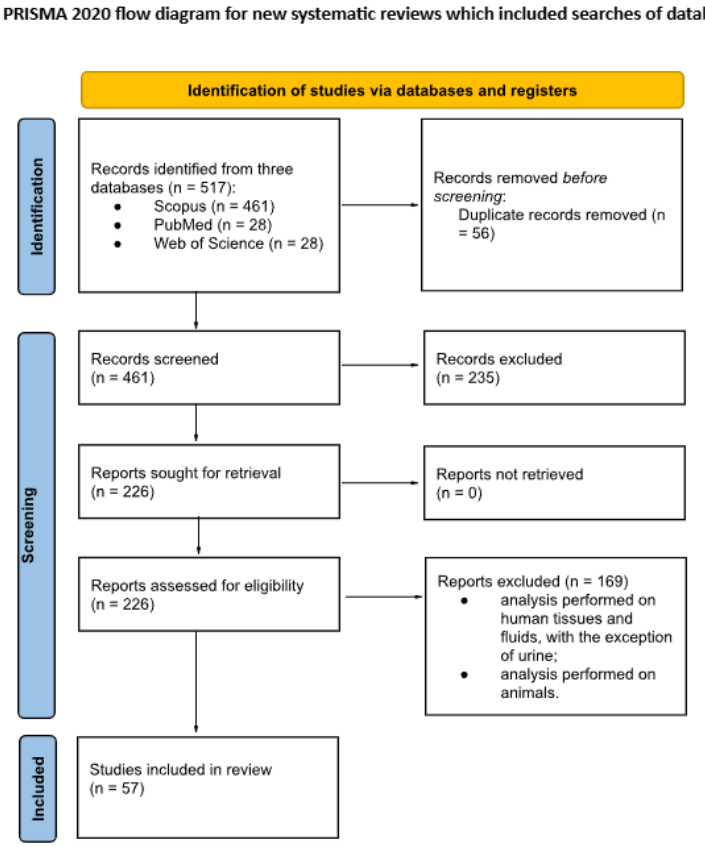

Figure 1. Descriptive diagram of the paper selection process.

Before beginning the data extraction process, the items were subjected to an assessment of their quality. Specifically, the Johanna Briggs Institute's (JBI) evaluation criteria were used. Each question on the checklist was assigned a score of 0 when the answer was "no", 1 when it was "yes", and "NA" when the criterion was not applicable to the work. Eighteen articles were not submitted for quality assessment because they were method validation papers. The summary table of the quality study is given in the Supplementary Materials in Table S1. We decided to include articles that reached a score of at least 4.

Data collection involved both study selection and data extraction. As noted, three researchers independently screened titles and abstracts for inclusion criteria. In cases of discrepancy in the evaluation of papers, the researchers involved conducted a joint examination of the disputed papers. These were reviewed together until a unanimous consensus was reached. Two researchers initially extracted the data, which was then reviewed by two others and subsequently validated by an additional pair of investigators.

3. Results

A total of 517 publications initially met the search criteria. After removing 56 duplicates, 235 additional studies were excluded based on primary inclusion criteria, resulting

in 226 full-text articles. Studies specifically examining nandrolone's presence in human urine were selected, leading to the exclusion of 169 more articles. Thus, 57 full-text articles ultimately met all inclusion criteria for this review. Figure 1 provides a summary of the article selection process.

Of the selected studies, authors, year of publication, analytical technique used, found concentrations of metabolites, mechanisms of production and metabolism, and factors influencing endogenous production of nandrolone are summarized in Tables 1–3.

Table 1. Table showing the analytical techniques used in the works reviewed and the concentrations of 19-NA and 19-NE obtained with them.

Authors	Analytical Techniques	Concentrations Found
Le Bizec B. et al. (1999) [11]	GC-MS	On a total of 32 urine, 11 had a concentration of 19-NA between the limit of detection (LOD) and 0.3 ng/mL, 7 had concentrations between 0.2 ng/mL and 0.6 ng/mL, and, in 14, it was not detected. 19-NE was not observed above the detection limit (LOD) of the method (0.02 ng/mL).
Dehennin L. et al. (1999) [12]	GC-MS	Not clearly reported
Le Bizec B. et al. (2000) [13]	LC-MS/MS	Some traces of 19-NA and 19-NE are present in low concentrations in the reference urine (H0), which correspond to the endogenous 19-NA and 19-NE (<0.01 ppb) levels. One hundred and five minutes after meal intake, levels of the two metabolites slightly increased to reach 0.02 and 0.01 ng/mL for 19-NA and 19-NE, respectively. The concentration progressed drastically three hours and fifty minutes after boar ingestion, to attain 3.2 and 0.8 ppb, respectively. Twenty-four hours after meal consumption, the levels returned to the endogenous values, i.e., around 0.1 ppb.For the three individuals, the maximal values reached for 19-NA were 7.5, 3.7, and 3.1 mg/L. For 19-NE, the highest concentrations were 1.0, 0.5, and 1.2 mg/L.
Catlin D. H. et al. (2000) [14]	HRMS	All urine samples from participants treated with androstenedione contained 19-norandrosterone, while no samples from the no-androstenedione group *did*. Urinary concentrations were averaged from day 1 vs. day 7 measurements; mean 19-NA concentrations in the 100 mg/d and 300 mg/d groups were 3.8 ng 7 mL and 10.2 ng/mL.
Van Eenoo P. et al. (2001) [15]	GC-MS/MS	19-NA was detected in 7 samples at concentrations ranging between 0.2 and 0.5 ng/mL. Traces of 19-NE could only be detected in a sample.
Galán Martín A. M. et al. (2001) [16]	GC-MS/MS	Of 54 samples, 9 were positive for 19-NA: 22 ng/mL, 14 ng/mL, 8 ng/mL, 4 ng/mL, 6 ng/mL, and 5 ng/mL, while the remaining 3 had concentrations between LOD and LOQ. 7 of these samples were also positive for 19-NE: 6 ng/mL and 6.5 ng/mL, while the other 5 had concentrations between LOD and LOQ.
Reznik Y. et al. (2001) [17]	GC-MS	19-NA was detected in all 10 baseline urine samples at concentrations ranging between 1 ng/mL and 14 ng/mL. 19-NE was detected in 4 samples at a concentration range of 2 ng/mL and 7 ng/mL. After metabolic stress, no significative variations were found.
Robinson N. et al. (2001) [18]	GC-MS/MS	Out of 358 samples taken after effort, 336 had a 19-NA concentration lower than 0.2 ng/mL and 22 higher than 0.2 ng/mL. Out of 137 samples retrieved before effort, none had 19-NA. Of the same 137 samples retrieved after effort, 129 had a 19-NA concentration lower than 0.2 ng/mL and, in only 8, it was higher than 0.2 ng/mL.
Schmitt N. et al. (2002) [19]	GC-MS	Baseline urinary 19-NA concentrations varied widely across individuals, from undetectable levels to 0.250 ng/mL. Exhaustive exercise *did* not significantly increase endogenous nandrolone secretion.
Le Bizec B. et al. (2002) [20]	A quadrupole GC-MS	Before administration, the concentration of 19-NA phase II metabolites was lower than 0.1 ng/mL. After nandrolone administration, the urinary concentrations of 19-Na were between 2 and 10 ng/mL.

Table 1. *Cont.*

Authors	Analytical Techniques	Concentrations Found
Gambelunghe C. et al. (2002) [21]	GC-MS and GC-MS/MS	Out of the 18 samples retrieved from professional footballers, only 5 had a 19-NA concentration higher than 0.2 ng/mL, while in the group of sedentary, no trace of 19-NA was found. 19-NE was not found in any sample.
Mareck-Engelke U. et al. (2002) [5]	GC-MS	In the first trimester, all pregnant women had 19-NA concentrations between 1 and 2 ng/mL.
Desroches M. C. et al. (2002) [22]	IAC followed by GC/C/IRMS or GC/MS	Method validation
Le Bizec B. et al. (2002) [23]	GC-HRMS	Out of 40 samples, 27 had a 19-NA concentration lower than 0.1 ng/mL, while the remaining 13 samples had the following concentrantions: 1.79 ng/mL, 1.50 ng/mL, 1.43 ng/mL, 1.41 ng/mL, 0.99 ng/mL, 0.86 ng/mL, 0.83 ng/mL, 0.43 ng/mL, 0.42 ng/mL, 0.37 ng/mL, 0.27 ng/mL, 0.24 ng/mL, and 0.23 ng/mL. The same 13 samples had 19-NE concentrations higher than 0.1 ng/mL. Their concentrations were as folows: 0.85 ng/mL, 0.82 ng/mL, 0.76 ng/mL, 1.42 ng/mL, 0.26 ng/mL, 0.40 ng/mL, 0.43 ng/mL, 0.73 ng/mL, 0.18 ng/mL, 0.12 ng/mL, 0.15 ng/mL, 0.16 ng/mL, and 0.08 ng/mL.
de Geus B. et al. (2004) [24]	GC-MS/MS	19-NA and 19-NE concentrations were lower than the detection limit in all samples but one. This sample had a pre-exercise 19-NA concentration of 0.13 ng/mL. After exercise, neither 19-NA nor 19-NE could be detected.
Baume N. et al. (2004) [25]	GC-MS	Not clearly reported
Bagchus W. M. et al. (2005) [26]	LC-MS	In most cases, 19-NA and 19-NE concentrations were below the limit of quantification. However, in 5 of 37 subjects, 19-NE concentrations were between 0.6 and 0.9 ng/mL. In one subject, 19-NA had a concentration of 0.6 ng/mL.
Grosse J. et al. (2005) [27]	HRMS	Not clearly reported
Baume N. et al. (2005) [28]	GC-MS	Not clearly reported
Tseng Y. L. et al. (2005) [29]	GC-MS	Not clearly reported
Hemmersbach P. et al. (2006) [30]	GC-MS	Out of 345 samples, the 19-NA maximum concentrations varied between 0.31 and 0.60 ng/mL. The absolute highest concentration was 0.83 ng/mL.
Baume N. et al. (2006) [31]	GC-MS	Not clearly reported
Hebestreit M. et al. (2006) [32]	GC-C-IRMS	Method validation
Avois L. et al. (2007) [33]	GC-MS	Regarding 19-NA, out of the 28 samples of volunteer 1, 4 had concentrations between LOD and 2 ng/mL, and the other 24 had concentrations between 2 ng/mL and 415.3 ng/mL. For volunteer 2, 6 samples had concentrations between LOD and 2 ng/mL, while the concentrations of the remaining 22 samples were between 2 ng/mL and 252.1 ng/mL. For volunteer 3, 10 samples had concentrations between LOD and 2 ng/mL, while the remaining 18 had concentrations between 2 ng/mL and 458.1 ng/mL.Regarding 19-NE, in volunteer 1, 17 samples had concentrations between LOD and 2 ng/mL, while the remaining 11 had concentrations between 2 ng/mL and 11.3 ng/mL. Volunteer 2 had 20 samples that ranged between LOD and 2 ng/mL, while the other 8 concentrations were between 2 ng/mL and 4.8 ng/mL. Volunteer 3 had 20 samples that ranged between LOD and 2 ng/mL, while the remaining 8 ranged from 2 ng/mL to 66.8 ng/mL.
Cheng W. et al. (2007) [34]	IDGC-HRMS	Method validation

Table 1. *Cont.*

Authors	Analytical Techniques	Concentrations Found
Goyal R. N. et al. (2007) [35]	Voltammetric analysis with fullerene-C60-modified GCE	Method validation
Strahm E. et al. (2007) [36]	LC-MS/MS	Method validation
Strahm E. et al. (2008) [37]	LC-MS/MS	Method validation
Torrado S. et al. (2008) [38]	GC-MS	Method validation
Guay C. et al. (2009) [39]	GC-MS, GC-HRMS, GC-C-IRMS	In the group of volunteers who took a "dietary supplement", the highest levels of 19-NA were 240 ng/mL after 2 days and 14 ng/mL after 105 h. In the group of positive athletes, the 19-NA concentrations ranged from 3.7 ng/mL and 36,500 ng/mL. In the group of pregnant women, the highest concentration of 19-NA found was 15 ng/mL. In the group of those who consumed 300 gr of pig, the registered levels of 19-NA ranged between 20 and 130 ng/mL.
Walker C. J. et al. (2009) [40]	GC-MS	1202 samples of urine from women not using anabolic steroids but including those using oral contraceptives: most have a 19-N concentration of less than 1 ng/mL (19-NA/d4NA ratio < 1); 42 samples (19-NA/d4NA ratio > 1) were confirmed by GC-MS/MS analysis. Of these samples, 14 matched the wada identification criteria (max. 4.1 ng/mL, min. 0.5 ng/mL). 9 samples were from women using contraceptives and 5 from women not using contraceptives.
Walker C. J. et al. (2009) [41]	GC-MS	19-NA concentrations ranged from 51 ng/mL to 63 ng/mL
Goyal R. N. et al. (2009) [42]	Fullerene voltammetric analysis (OSWV)	Method validation
Graham M. et al. (2009) [43]	GC-MS	19-NA and 19-NE reached, respectively, a concentration of 450 ng/mL and 70 ng/mL.
Strahm E. et al. (2009) [44]	LC-MS/MS and GC/MS	Not clearly reported
Saito K. et al. (2010) [45]	On-line in-tube SPME/LC-MS	Method validation
Enea C. et al. (2010) [46]	GC-MS	Not clearly reported
Gårevik N. et al. (2011) [47]	GC-MS	Not clearly reported
Van Eenoo P. et al. (2011) [48]	GC-MS with triple quadrupole	Method validation
de la Torre X. et al. (2011) [49]	GC-C-IRMS	Method validation
Palermo A. et al. (2016) [50]	GC-MS LC-MS/MS	Not clearly reported
Piper T. et al. (2016) [51]	GC-MS/MS and GC-C-IRMS	Not clearly reported
Palonek E. et al. (2016) [52]	GC-C-IRMS	In most cases, 19-NA and 19-NE were not detectable. 120 days after the administration of nandrolone decanoate, out of 11 samples, 4 had a 19-NA concentration lower than 2 ng/mL. The remaining 7 had concentrations between 2 ng/mL and 22 ng/mL. After 270 days, 6 samples had a concentration lower than 2 ng/mL, while the other 5 had concentrations between 2 ng/mL and 9.42 ng/mL.

Table 1. *Cont.*

Authors	Analytical Techniques	Concentrations Found
Podolskiy I. I. (2018) [53]	GC-C-IRMS	Not clearly reported
Mullen J. et al. (2018) [54]	GC-MS/MS	Not clearly reported
Hülsemann F. et al. (2018) [55]	GC-MS/MS	Before consumption of the test meal, no 19-NA was detected in urine samples. The concentrations after one hour were 1 ng/mL. Highest 19-NA concentrations were reached after 4 h with 4 ng/mL and 8 ng/mL.
Brailsford A. D. et al. (2018) [56]	GC-C-IRMS	Method validation
de Oliveira F. et al. (2019) [57]	GC-C-IRMS	Method validation
Protti M. et al. (2020) [58]	LC-MS/MS	Not clearly reported
Hülsemann F. et al. (2020) [59]	GC-MS/MS and GC-C-IRMS	19-NA was not detected in the urine samples before the ingestion of the boar meat. After consumption, the two highest concentrations were 1.9 ng/mL and 2.1 ng/mL. In the other three samples, the concentrations were lower than 1 ng/mL.
Honesova L. et al. (2021) [60]	GC-C-IRMS + 2D/3D-HPLC purification	Method validation
Iannella L. et al. (2021) [61]	GC-C-IRMS	Method validation
Wen C. et al. (2021) [62]	GC-C-IRMS + 2D-HPLC purification	Method validation
Iannella L. et al. (2021) [63]	GC-C-IRMS	Not clearly reported
Shkembi X. et al. (2022) [64]	Aptamers for in situ analysis, colorimetric testing, use of gold nanoparticles	Method validation
Nair V. S. et al. (2023) [65]	GC-MS/MS and GC-C-IRMS	After the consumption of boar, in two cases, the 19-NA concentrations were 2.8 ng/mL and 3.8 ng/mL. After the consumption of a supplement, the 19-NA concentration reached 3500 ng/mL.
Chen C. et al. (2023) [66]	Electrochemical sensors	Method validation

GC-MS: gas chromatography–mass spectrometry; LC-MS/MS: liquid chromatography with tandem mass spectrometry; GC-MS/MS: gas chromatography with tandem mass spectrometry; IAC: immunoaffinity chromatography; GC-HRMS: gas chromatography/high-resolution mass spectrometry; GC-C-IRMS: gas chromatography/combustion/isotope ratio mass spectrometry; IDGC-HRMS: isotope dilution gas chromatography–high-resolution mass spectrometry; GCE: fullerene-modified glassy carbon electrode; SPME/LC-MS: solid-phase microextraction coupled to liquid chromatography–mass spectrometry.

Interest in nandrolone, its metabolism, and factors influencing its endogenous production has always remained high. This review focused on a 24-year period (from 1999 to 2023), and Figure 2 shows the distribution of the publication years of the articles included in this systematic review.

Table 2. Table showing the main information on metabolism found in the articles reviewed.

Authors	Mechanisms of Production and Metabolism
Reznik Y. et al. (2001) [17]	The data suggested that the aromatization process contributes to nandrolone production and its metabolism, therefore reinforcing a long-held hypothesis that norandrogen synthesis is linked to the androgen-to-estrogen conversion.
Le Bizec B. et al. (2002) [20]	When nandrolone is administered, 19-norandrosterone is primarily excreted in a form conjugated to glucuronic acid, with almost no sulfate conjugates detectable in the samples. The levels of endogenous metabolites are minimal in comparison to the concentration of metabolites specifically associated with nandrolone administration. When produced endogenously, 19-norandrosterone is excreted in urine as a conjugate with both glucuronic acid and sulfuric acid. For endogenously produced 19-noretiocholanolone, it is most often conjugated with glucuronic acid (approximately 84%), though a considerable proportion is also excreted as a sulfuric acid conjugate. These findings suggest that the presence of 19-NA and 19-NE can be attributed to endogenous production if sulfate conjugates are detected; however, their detection as glucuronide conjugates points specifically to nandrolone administration.
Hemmersbach P. et al. (2006) [30]	During the process of aromatization, which involves the conversion of testosterone or estrogens, nandrolone and/or norandrostenedione may be produced as secondary byproducts. This means that these compounds can form indirectly as a result of biochemical changes occurring when testosterone or estrogens are aromatized.
Baume N. et al. (2006) [31]	Two precursors (4-norandrostenediol and 5-norandrostenediol) form 4-norandrostenedione, which can convert into 19-nortestosterone. It has two main metabolites: 19-notethiocholanolone and 19-norandrosterone.
Strahm E. et al. (2008) [37]	The 19-NA/19-NE glucuronide ratio was stable in the first 24 h after administration of 100 mg of 19-nor-4-androstenedione.
Torrado S. et al. (2008) [38]	The main metabolites found were NA and NE glucuronides and sulfates, and ENA sulfate. The relative amounts of NA and NE glucuronides and sulfates varied over time, but after the entire collection period, NA sulfate was the main metabolite.
Gårevik N. et al. (2011) [47]	19-NA remains in the urine for quite a long time, in some cases for a period of one year after the last intake.
Palonek E. et al. (2016) [52]	19-NA concentration was higher than that of 19-NE (about fourfold).
Chen C. et al. (2023) [66]	Sulfoconjugate metabolites can be detected for a longer time than their respective glucoconjugates.

Table 3. Table showing the main factors affecting the endogenous production of nandrolone.

Authors	Factors Influencing Endogenous Production
Le Bizec B. et al. (2000) [13]	Wild boar meat
Catlin D. H. et al. (2000) [14]	Androstenedione as an over-the-counter supplement
Van Eenoo P. et al. (2001) [15]	Women who experience elevated levels of estradiol production, such as during the middle of their menstrual cycle, following intense physical activity, or throughout the various stages of pregnancy
Galán Martín A. M. et al. (2001) [16]	In the group of post-menopausal women analyzed, the results indicated that one individual exhibited notably high levels of metabolites, while three others displayed the presence of two specific metabolites. This detection was further validated using gas chromatography–tandem mass spectrometry (GC-MS/MS), which confirmed the findings. In all four samples taken from post-menopausal women, the concentration of 19-norandrosterone (19-NA) consistently exceeded that of 19-noretiocholanolone (19-NE). Among the male athletes studied, only 4 out of the 36 urine samples analyzed showed significant levels of both 19-NA and 19-NE metabolites, with 19-NA appearing as the predominant metabolite in these samples. In the samples collected from female athletes, 19-NA was detected in just one of the four samples, whereas 19-NE was not detectable in any of these samples.

Table 3. *Cont.*

Authors	Factors Influencing Endogenous Production
Schmitt N. et al. (2002) [19]	Exhaustive exercise did not significantly increase endogenous nandrolone secretion in trained male athletes.
Mareck-Engelke U. et al. (2002) [5]	Pregnancy
de Geus B. et al. (2004) [24]	Submaximal standardized exercise does not influence endogenous nandrolone production.
Baume N. et al. (2004) [25]	C13-labeled nandrolone
Bagchus W. M. et al. (2005) [26]	Nandrolone decanoate
Grosse J. et al. (2005) [27]	In situ demethylation of endogenous steroids in stored urine samples
Tseng Y. L. et al. (2005) [29]	Over-the-counter food supplements
Hemmersbach P. et al. (2006) [30]	NA was excreted in female volunteers not treated with nandrolone, norandrostenedione, or norandrostenediol. Additionally, the NA urinary excretion pattern during the menstrual cycle followed the essential hormonal changes, especially just before and during ovulation.
Baume N. et al. (2006) [31]	Unlabeled supplements containing creatinine
Avois L. et al. (2007) [33]	Ophthalmic solution containing nandrolone
Guay C. et al. (2009) [39]	Offal of uncastrated pigs
Walker C. J. et al. (2009) [41]	Norethisterone
Graham M. et al. (2009) [43]	Keratyl eye drops
Strahm E. et al. (2009) [44]	19-nor-4-androstenedione administration
Enea C. et al. (2010) [46]	Nandrolone excretion did not increase after exercise in women with a normal flow cycle. No significant difference was found in nandrolone concentration between eumenorrheic women and those taking oral contraceptives, nor was an increase in nandrolone metabolites seen following exercise.
Palermo A. et al. (2016) [50]	Antifungal drugs, benzodiazepines, and nonsteroidal anti-inflammatory drugs not on the WADA's Prohibited List
Mullen J. et al. (2018) [54]	Pregnancy
Hülsemann F. et al. (2018) [55]	Wild boar entrails
Hülsemann F. et al. (2020) [59]	Wild boar meat
Nair V. S. et al. (2023) [65]	Wild boar meat

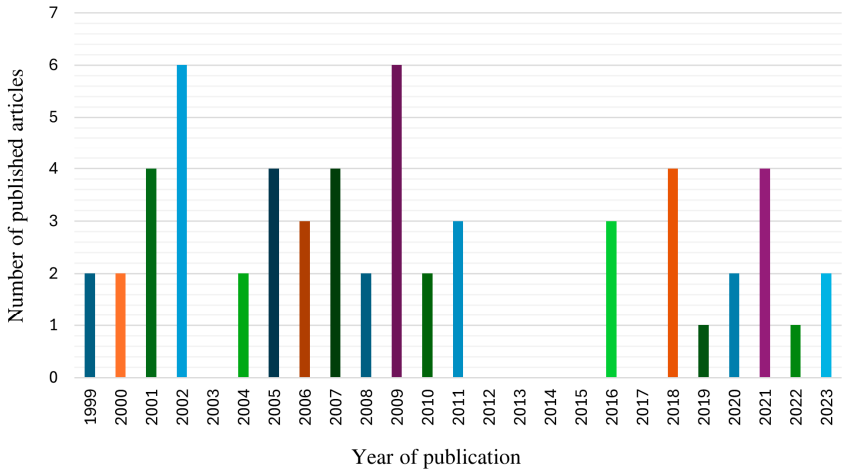

Figure 2. Distribution of publication years.

Out of a total of 57 articles, only 24 were concerned with factors influencing endogenous nandrolone production. The factors investigated in these studies are shown in Figure 3. These are as follows: wild boar meat or pork offal (5); supplements (3); mixed (post-menopausal women and male athletes) (1); physical exercise (1); exogenous nandrolone (2); stored urine samples (1); ophthalmic solutions (2); women, pregnancy, and contraceptive pills (6); antifungal, BDZs (benzodiazepines), and NSAIDs (nonsteroidal anti-inflammatory drugs) not featured on the WADA's Prohibited List (1); and foodborne doping, including foods, drinks, and supplements (2).

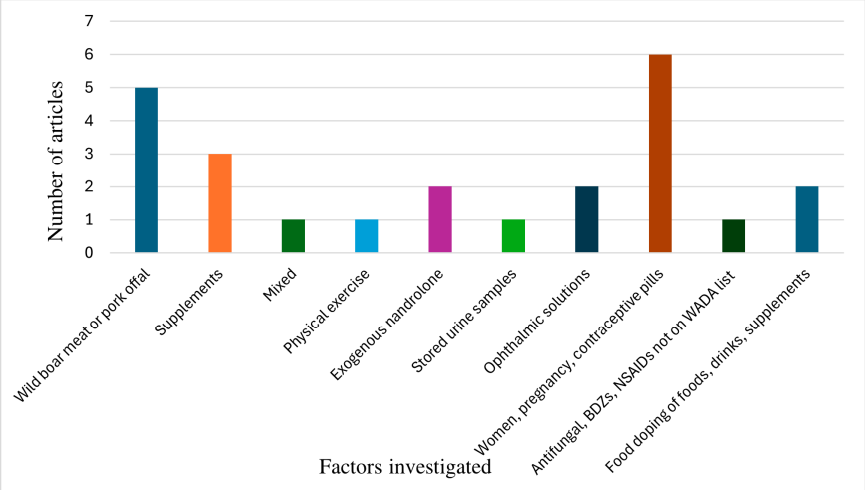

Figure 3. Factors influencing endogenous production.

4. Discussion

In this systematic review, all 57 scientific articles examined used urine as the primary biological matrix for detecting nandrolone and its metabolites. The majority of these studies concentrated solely on analytical methods designed to identify and measure molecules of interest. Eight of the studies also considered dietary variations as a potential factor that could influence urinary concentrations of nandrolone, investigating how different diets might affect these levels [13,14,29,31,39,55,59,65]. Additionally, six articles specifically addressed the endogenous production of nandrolone in women, examining physiological factors like pregnancy and the menstrual cycle that might alter metabolite concentrations [5,15,16,30,41,46,54]. Some other studies focused on measuring nandrolone and its metabolites in urine following the administration of drugs containing 19-nortestosterone or other pharmaceutical masking agents, aiming to understand how these substances impact metabolite levels. Finally, a significant portion of the selected publications reviewed and expanded upon previous research regarding nandrolone's metabolic pathway, with an emphasis on the precise quantification of its metabolites throughout the metabolic process.

The metabolic pathway of nandrolone is well documented, culminating in its elimination through urine. While a small portion of nandrolone may be excreted unchanged, it is more commonly converted into its primary metabolites, 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE), in forms conjugated either to glucuronic acid or sulfate. However, the detected concentrations of these metabolites alone are insufficient to definitively distinguish between nandrolone naturally produced by the body and nandrolone that may result from the intake of synthetic steroids. To make this determination with high accuracy, advanced analysis using GC-C-IRMS is required [32,40]. An important finding in this area of research is the increased excretion of conjugated 19-NA sulfate in urine over time, suggesting that time-related factors may influence urinary levels of this metabolite. This observation could be particularly relevant for improving retrospective analyses in doping control by considering time-based variations in metabolite excretion [38]. Additionally, research conducted on professional athletes has explored changes in the urinary

ratio of 19-NA glucuronide to 19-NA sulfate. The findings showed that when athletes were administered exogenous nandrolone, 100% of the detected 19-NA was present in the glucuronidated form. By contrast, under normal physiological conditions, at least 30% of 19-NA was excreted as a sulfate conjugate [23]. This differentiation could offer a useful indicator in anti-doping efforts to help distinguish between endogenous production and the intake of synthetic nandrolone.

In exploring the mechanisms behind nandrolone production and metabolism, it is worth noting that several biological and lifestyle factors can significantly impact the body's natural production of nandrolone and its metabolites. This review considered various parameters, including biological sex, pregnancy status, and levels of physical activity. For instance, it appears that pregnancy can elevate the production of 19-nortestosterone metabolites to such an extent that it suggests the need for a higher cut-off threshold for female athletes [54]. Furthermore, studies also analyzed endogenous nandrolone production following intense physical exertion or engagement in professional sports [23]. However, results from these studies displayed considerable variability between individuals, preventing researchers from establishing a clear correlation between physical activity and the increase or decrease in urinary concentrations of 19-NA and 19-NE. Additional factors were investigated for their potential effects on 19-nortestosterone metabolism, including dietary habits, the use of steroid-based medications, and drugs acting as masking agents for the main metabolites 19-NA and 19-NE. An increased presence of nandrolone metabolites in urine can create serious challenges for doping control measures, where the accepted limit is set at 2 ng/mL. For example, consuming wild boar meat has been shown to produce detectable levels of 19-NA and 19-NE in urine, which could result in disqualification for an athlete under doping regulations [65]. Similarly, certain dietary supplements containing steroids—sometimes available as over-the-counter products—can raise urinary levels of 19-NA and 19-NE [14,29]. Medications prescribed to manage menstrual cycle disorders, including those containing norethisterone, have also been found to potentially increase levels of nandrolone metabolites in urine [41]. Additionally, certain types of eye medications (ophthalmic drugs) can produce similar effects [33,43]. The use of these products may unintentionally lead to elevated concentrations of nandrolone metabolites in an athlete's urine, thereby heightening the likelihood of a positive result in doping tests. This unintended increase in metabolite levels could pose a serious risk for athletes, as it may result in them being flagged for substances that they did not intentionally consume as performance enhancers.

What has been discussed so far is reflected in the concentrations found in the samples analyzed by the various studies. In fact, only trace amounts of 19-NA were found in most cases. However, significant increases were found following the consumption of wild boar meat. Regarding exercise, it was found that 19-NA levels generally remained low, suggesting that while exercise might influence some metabolic pathways, it does not significantly elevate nandrolone secretion. In conclusion, these results collectively underscore the complexity of analyzing nandrolone and its metabolites in urine samples.

Gas chromatography and liquid chromatography, when combined with mass spectrometry, have firmly established themselves as the preferred analytical methods for detecting nandrolone and its metabolites in urine. Specifically, techniques such as GC/MS or GC/MS/MS are frequently employed for the precise identification of these substances. Although LC/MS or LC/MS/MS methods are also utilized, they are less commonly used as stand-alone techniques for this purpose. The most significant advancement in this field is the application of GC-C-IRMS, which stands out as the only technique capable of accurately differentiating between the endogenous (naturally produced) and exogenous (externally introduced) forms of nandrolone [32]. This differentiation is based on detecting abnormal carbon-13 (13C) isotope values of 19-NA in urine. Values outside the typical endogenous steroid range, approximately between −17% and −25%, signal the intake of synthetic nandrolone [49,55]. Therefore, GC-C-IRMS offers a high degree of reliability for anti-doping testing by identifying synthetic sources of nandrolone with precision [57].

Moreover, the steps taken in sample pretreatment are crucial for ensuring the accuracy of GC-C-IRMS results, as they must prevent any potential contamination. Effective purification techniques include solid-phase extraction (SPE), liquid/liquid extraction, and immunoaffinity chromatography (IAC), often used after enzymatic hydrolysis to prepare the sample. Additionally, high-performance liquid chromatography (HPLC) separation and fractionation serve as vital pre-analytical purification steps, ensuring that the sample is sufficiently purified for reliable analysis. Together, these techniques strengthen the detection capabilities in anti-doping control by enabling clear differentiation between naturally occurring and synthetic nandrolone sources.

Study Limitations

We decided to exclude scientific works that considered biological matrices other than urine for the identification of nandrolone and its metabolites. We selected three different parameters on which to base the systematic research: the metabolic pathway of nandrolone, considering its metabolites in glucuronidated or sulfated form; the various factors that alter endogenous physiological levels; and, finally, the analytical techniques aimed at identifying this steroid. None of the scientific articles included in our review addressed these parameters simultaneously; instead, studies dealt with only one or at most two of them. It is important to highlight that the only analytical technique capable of distinguishing between exclusively endogenous quantities and exogenous intakes of nandrolone is GC-C-IRMS. In fact, in papers that addressed anti-doping controls, this technique was regularly reported. The research reported in other types of publications was not conducted in the context of competition in professional sports and, therefore, the technique was not considered essential. However, its use would have been appropriate given that different quantities of nandrolone and metabolites following drug intake were investigated.

5. Conclusions

This systematic review underscores the critical importance of accurately distinguishing between the endogenous and exogenous sources of nandrolone and its metabolites in the context of anti-doping tests. A range of factors influence the levels of 19-NA and 19-NE found in urine samples. These factors include dietary habits, physical activity, hormonal fluctuations, and the potential use of anabolic steroids or dietary supplements. Given that these metabolites can be detected in both naturally occurring levels and those resulting from supplementation, it is imperative that doping control authorities carefully take these variables into account when assessing urine samples for signs of anabolic steroid use. Moreover, the significant variability in individual responses and the temporary increases in metabolite levels following dietary consumption further complicate the interpretation of test results. This variability emphasizes the necessity for employing advanced analytical techniques, such as GC-C-IRMS, which are capable of accurately distinguishing between metabolites produced endogenously and those introduced exogenously through supplementation. It is crucial for organizations responsible for doping controls, particularly those affiliated with the WADA, to take these complexities into serious consideration. They should adopt increasingly sensitive and specific analytical methods to ensure accurate detection and interpretation of nandrolone levels. Additionally, athletes themselves need to be cognizant of the various factors that could potentially influence their urinary levels of nandrolone and its metabolites. By understanding these variables, athletes can better navigate compliance with regulatory standards and avoid unintentional violations related to anabolic steroid use.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app142210641/s1, Table S1: Quality assessment.

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