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Pharmacokinetic Evaluation of Three Different Intramuscular Doses of Nandrolone Decanoate: Analysis of Serum and Urine Samples in Healthy Men

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The pharmacokinetics of nandrolone in serum and urine were investigated in healthy young men after a single im injection of 50 mg (n = 20), 100 mg (n = 17), or 150 mg (n = 17) nandrolone decanoate. Blood samples were collected before treatment and for up to 32 d after dosing. In addition, in the 50- and 150-mg groups, 24-h urine samples were collected before treatment and on d 1, 7, and 33 after treatment; in the 150-mg group, additional samples were collected after 3 and 6 months. Serum concentrations and the area under the curve of nandrolone increased proportionally with the dose administered. The peak serum concentration ranged from 2.14 ng/ml in the 50-mg group to 4.26 ng/ml in the 100-mg group and 5.16 ng/ml in the 150-mg group. The peak serum concentration was reached after 30 h (50 and 100 mg) and 72 h (150 mg), whereas the terminal half-life was 7–12 d. In urine, pretreatment concentrations of 19-norandrosterone (19-NA) and/or 19-noretiocholanolone (19-NE) were detected in five of 37 subjects (14%). In the 50-mg group, 19-NA and/or 19-NE could be detected at least until 33 d after injection in 16 of 17 subjects (94%). In the 150-mg group, who were presumed to have not previously used nandrolone, nandrolone metabolites could be detected for up to 6 months in eight of 12 subjects (67%) for 19-NE and in 10 of 12 subjects (83%) for 19-NA. (*J Clin Endocrinol Metab* 90: 2624–2630, 2005)

NANDROLONE DECANOATE, AN ester for im injection of the anabolic-androgenic steroid nandrolone, is indicated as supportive therapy in pathological conditions characterized by a negative nitrogen balance and also for the treatment of osteoporosis and anemia. Studies have demonstrated that treatment with nandrolone decanoate increases lean body mass and body weight in wasting or cachexia associated with HIV infection (1–5), chronic obstructive pulmonary disease (6, 7), renal failure (8), and long-term use of glucocorticosteroids (9). Therefore, a clinical development program was started to develop nandrolone decanoate as a supportive therapy to increase lean body mass and body weight in patients with wasting or cachexia related to such conditions.

Nandrolone decanoate has long attracted extensive attention from the scientific community, regulatory authorities, and media. It has been demonstrated that when used in trained athletes in the right regimen and dose, nandrolone decanoate injections increase muscle mass (10) as well as muscle strength in one study (11), whereas in another study no increase in muscle strength was observed (12). As a consequence, nandrolone decanoate has been implicated in relation to doping (especially in sports, where muscle mass and strength are deciding factors) and was banned by the International Olympic Committee Medical Commission in 1974. Recently, several elite athletes have tested positive for 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE), two nandrolone metabolites that are used as marker metabolites of use of nandrolone doping. However, the urine levels of these metabolites were often only slightly above the threshold level of 2 ng/ml, and there has been discussion and controversy about whether such borderline levels are valid proof of nandrolone abuse for performance enhancement (13, 14). These positive doping tests have been attributed to endogenous production of nandrolone, consumption of certain meat products, or use of contaminated food supplements, because in most cases the urinary levels of 19-NA and/or 19-NE only just exceeded the maximally allowed concentration (15). In particular, it has been shown that up to 15% of nutritional supplements may indeed contain detectable amounts of nandrolone (or nandrolone precursors and metabolites) (16). Oral intake of such contaminated nutritional supplements has been shown to result in a positive doping test for up to 6 d after intake (17).

Although several pharmacokinetic studies have been performed with nandrolone decanoate over the years (18–20), a formal dose-proportionality study has been lacking. Data are also lacking on the relationship between the im nandrolone decanoate dose and the urinary excretion of 19-NA and 19-NE. In view of this, it would not only be of interest to have a more detailed overview of the pharmacokinetic profile of nandrolone in serum, but also to explore the urinary excretion of nandrolone metabolites.

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Abbreviations: AE, Adverse event; amu, atomic mass unit; AUC, area under the curve; BMI, body mass index; C_{max}, peak serum concentration; ECG, electrocardiogram; LC-MS, liquid chromatography-mass spectrometry; 19-NA, 19-norandrosterone; 19-NE, 19-noretiocholanolone; SAE, serious adverse event; t_{max}, time of peak serum levels.

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The objective of this study was to assess the pharmacokinetic profile of nandrolone after a single im injection of three different doses of nandrolone decanoate. In addition, urinary excretion of the nandrolone metabolites 19-NA and 19-NE was assessed.

Subjects and Methods

This randomized, single-blind, group-comparative, parallel-design study was undertaken in young healthy male volunteers at a single center from August to December 2001. The study was approved by the institutional review board and was conducted in accordance with the principles contained in the Declaration of Helsinki, the International Conference on Harmonization Guideline for Good Clinical Practice, and regulatory requirements. Written informed consent was obtained from all subjects before study-related procedures were performed.

Subjects and medication

A total of 54 subjects were selected from the volunteer database of FOCUS Clinical Drug Development. These subjects are involved in studies on a regular basis and were aware that they would be checked for drugs of abuse before inclusion. Subjects were randomized and treated with a single im dose in the gluteal muscle of nandrolone decanoate in 1 ml arachis oil (Deca-Durabolin, Organon International, Inc., Roseland, NJ). All subjects were Caucasian, and there were no differences in mean age or body mass index (BMI) among the three groups. Subjects in group 1 (n = 20) received 50 mg nandrolone decanoate, subjects in group 2 (n = 17) received 100 mg nandrolone decanoate, and subjects in group 3 (n = 17) received 150 mg nandrolone decanoate. To obtain a power of at least 80% at $\alpha = 0.05$, a group size of at least 17 subjects/dose group was required to detect a difference in the dose-normalized area under the curve (AUC) of 20% between the dose groups, because the coefficient of variation was approximately 22%. It was decided to keep the injection volume constant and increase the dose of nandrolone decanoate by increasing the concentration. As a consequence, the effects of dose and concentration cannot be separated statistically.

All subjects provided informed consent, were between 18 and 40 yr of age, had a BMI between 18 and 28 kg/m^2, did not smoke excessively, were in good physical and mental health, and refrained from use of caffeine and alcohol from 48 h before until 24 h after dosing. Subjects were excluded from the study if they had a history of sensitivity or idiosyncrasy to nandrolone decanoate or to chemically related compounds or excipients; a relevant history or presence of any clinical disorder; clinically significant abnormal laboratory, electrocardiogram (ECG), or physical findings at screening; a history or presence of substance abuse (each volunteer was subjected to urinary drug screening); use of any medication from 14 d before dosing until the first follow-up visit; use of any medication influencing P450 enzymes from 4 wk before dosing until the first follow-up; use of androgen replacement or any other anabolic agents within the past 3 months; use of im injection of nandrolone decanoate within the past 6 months; inability to understand the nature and extent of the trial and its procedures; participation in another investigative drug trial or donation of blood with the past 3 months or during the trial; or febrile illness within 3 d before dosing or if they were endurance sportsmen.

Study design

At the screening visit, subjects had their medical history taken, a physical examination (including vital signs and ECG), an alcohol breath test, as well as hematological, biochemical, and urinary investigations (including urine drug screen). Subsequently, subjects were randomized.

On the day before the dosing day, the subjects had blood sampled for hematology and biochemistry, had to provide a urine sample for urinalysis and drug screening, had an alcohol breath test, and had a medical review. An ECG was performed, and blood pressure and pulse rate were measured. On d 33, another blood sample was drawn for hematology and biochemistry, and a physical examination (including vital signs and ECG) was performed. Before dosing; 2, 4, 6, 8, 10, 12, 18, and 24 h after injection on d 1; and subsequently in the morning of d 3, 4, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, and 33, blood samples (at least 5 ml) were taken for determination of serum nandrolone levels. The serum samples were stored at −20 C, thawed 1 h before analysis, and extracted by liquid extraction with hexane-ethylacetate (50:50) and on-line solid phase extraction on HySphere C_8 cartridges using a Prospekt 2 system (Spark Holland, Emmen, The Netherlands). Separation was performed by reverse phase HPLC using a 4-μm Phenomenex Synergy MAX-RP 80A column (150 × 2.0 mm; conditioned at 30 C) (Phenomex, Torrance, CA) and a mobile phase gradient elution with 5.0 mM ammonium formate buffer, pH 5.0, and methanol in a linear gradient from 65–95% methanol. [$^{13}C_2$]Nandrolone was used as an internal standard. Quantitation was performed with tandem mass spectrometry using positive turboionspray ionization. Nandrolone and internal standard were monitored using 275.2 and 277.2 amu as precursor ions and 109.0 and 111.0 atomic mass unit (amu) as product ions, respectively.

From 24 h before until the time of dosing and also on d 1, 7, and 33 after treatment, the subjects in the 50- and 150-mg groups had to collect their urine. After the follow-up examination on d 33, the subjects in the 150-mg group were asked to return for two additional visits at 3 and 6 months after dosing. Before each of these visits, 24-h urine samples were collected for investigation of levels of 19-NA and 19-NE. Urine samples were frozen and stored at −20 C. Thawed urine samples were treated with β-glucuronidase and extracted by solid phase extraction on GV-65 polymer columns, and the extracts were introduced to the liquid chromatography-mass spectrometry (LC-MS)/MS system after extraction and separation as described above with slight modifications (*i.e.* 10.0 mM ammonium formate buffer and a 65–100% gradient). Quantitation was performed with tandem mass spectrometry using positive Turboionspray ionization. 19-NA, 19-NE, and internal standards (d6–19-NA and d6–19-NE) were monitored using 277.2 and 283.3 amu as precursor ions and 241.2 and 247.2 amu as product ions.

Analysis of nandrolone, 19-NA, and 19-NE was performed by Xendo Laboratories (Groningen, The Netherlands) using validated LC-MS assays under supervision of the Department of Drug Metabolism and Kinetics of Organon. All laboratory observations (hematology, blood chemistry, and urinalysis) were performed at FOCUS Clinical Drug Development (Neuss, Germany). All assays were carried out in full compliance with Good Laboratory Practice regulations.

Pharmacokinetic parameters

From the nandrolone serum concentrations, the following noncompartmental pharmacokinetic parameters were calculated for each subject: the peak concentration (C_{max}) and the time of its occurrence (t_{max}) were taken from the measured serum concentration data.

The terminal half-life ($t_{1/2}$) was calculated as $-\log_e 2/\beta$, where β is the slope of the terminal log-linear phase of the concentration *vs.* time curve, determined by linear regression.

The AUC from zero to infinity was calculated as: $AUC_{0-\infty} = AUC_{0-t_{last}} + AUC_{t_{last}-\infty}$. $AUC_{0-t_{last}}$ was calculated by means of the linear trapezoidal rule, where t_{last} represents the last time point with a measurable concentration within a subject. $AUC_{t_{last}-\infty} = C_{t_{last}}/-\beta$, where $C_{t_{last}}$ is the fitted concentration at time t_{last} using the regression line from which β was calculated.

The serum clearance (CL) after single-dose injection equals f × dose/$AUC_{0-\infty}$, where f is the fractional absolute bioavailability of the im preparation. Because f cannot be calculated from the present trial, CL/f = (274.4/428.6) × dose/$AUC_{0-\infty}$ was calculated and denoted the apparent clearance (CL_{app}). This formula includes a correction for the difference in molecular mass between nandrolone decanoate (428.6) and nandrolone (274.4).

The individual nandrolone serum concentration data were also analyzed with a nonlinear regression fit using the restricted flip-flop model described by Wijnand *et al.* (19). This is a one-compartment model with first-order absorption and elimination according to the equation: Ct = Z × [e(−Kdesc × t) − e(−Kasc × t)], in which Ct is the nandrolone concentration in serum at time t, Z is the scaling factor, Kdesc is the rate constant in descending phase (equal to the absorption rate constant), Kasc is the rate constant in ascending phase (equal to the elimination rate constant), and t is time. Individual concentration *vs.* time points were weighted with the reciprocate of the nandrolone concentration. Analysis was performed using SAS procedure NLIN (SAS Institute, Inc., Cary, NC). From the model parameters of each best fit, the derived parameters

TABLE 1. Demographic details (mean and range) per treatment group

Treatment group	Age (yr)	Height (cm)	Body weight (kg)	BMI (kg/m^2)
50 mg (n = 20)	29.8 (21–41)	183 (170–203)	80.9 (69–104)	24.1 (20.9–27.4)
100 mg (n = 17)	29.6 (20–39)	180 (166–195)	78.8 (64–102)	24.3 (19.4–27.8)
150 mg (n = 17)	30.2 (21–40)	180 (172–194)	76.4 (61–89)	23.6 (19.9–27.7)
Overall (n = 54)	29.8 (20–41)	181 (166–203)	78.8 (61–104)	24.0 (19.4–27.8)

$t_{1/2,descending}$ and $t_{1/2,ascending}$ were calculated according to the equations: $t_{1/2,descending} = \log_e 2 / Kdesc$ and $t_{1/2,ascending} = \log_e 2/Kasc$.

Safety assessments

Adverse events (AEs) and serious adverse events (SAEs) were recorded according to standard methods. Local tolerability at the administration site was assessed directly and at 2 and 24 h after dosing as an additional safety measure.

Statistical analyses

The dose independence of the pharmacokinetic parameters $t_{1/2}$, Cl_{app}, $t_{½,ascending}$, and $t_{½,descending}$ was analyzed statistically by one-way ANOVA with factor dose after $\log_e$-transformation of the parameters. The dose proportionality of C_{max} and $AUC_{0-\infty}$ was tested using the same approach on the dose-normalized parameters. The dose independence of t_{max} was tested using the nonparametric Kruskal-Wallis test. Effects were considered statistically significant at $P < 0.05$ (two-sided tail probability).

Results

A total of 54 subjects received a dose of nandrolone decanoate. Subjects had a mean age of 29.8 yr (range, 20–41 yr) and a mean BMI of 24.0 kg/m^2 (range, 19.4–27.8 kg/m^2; Table 1). Three subjects dropped out (all in the 50-mg group) and were replaced (one due to poor compliance on d 5, one due to personal reasons on d 17, and one with severe back pain who took disallowed medication on d 18). All 54 subjects were included in the safety analysis, whereas 53 subjects were included in the pharmacokinetic analysis: the subject that dropped out on d 5 was eliminated from the pharmacokinetic analyses due to insufficient pharmacokinetic data.

Pharmacokinetics of nandrolone in serum

The pharmacokinetic analysis was performed in 53 evaluable subjects. The AUC for nandrolone increased dose-proportionally. The t_{max} of nandrolone in healthy male volunteers after a single im dose of nandrolone decanoate occurred between 30 and 72 h after injection, reaching a C_{max} of 2.14 ng/ml in the 50-mg group, 4.26 ng/ml in the 100-mg group, and 5.16 ng/ml in the 150-mg group (Table 2). Subsequently, nandrolone levels decreased, but were still measurable 32 d after dosing in approximately half the subjects in the 50-mg group and in all subjects in the 100- and 150-mg groups (Fig. 1). The $t_{1/2}$ was calculated and ranged from 7.1 d (50 mg) to 11.7 d (100 mg) and 11.8 d (150 mg), respectively. The $t_{1/2}$ in the 50-mg group was significantly shorter than those in the other two dose groups ($P < 0.05$; Table 2). In contrast, the half-lives of the ascending and descending phases of the curves, as determined by model fitting, were not significantly different between dose groups ($P > 0.05$; Table 2).

Excretion of 19-NA and 19-NE in urine

The main pharmacokinetic parameters of nandrolone metabolites 19-NA and 19-NE in urine are presented in Tables 3 and 4. From the tables it appears that before injection, urine levels of 19-NA and 19-NE were in most cases below the limit of quantification. However, in five of 37 subjects (14%), 19-NE was detectable in urine (range, 0.6–0.9 ng/ml), whereas in one subject (3%), 19-NA was also detected (0.6 ng/ml). Initially, 19-NA levels in urine were 3- to 4-fold higher than 19-NE concentrations, whereas later 19-NE levels became relatively more important.

In the 50-mg group, urinary metabolites could be detected on d 33 (final assessment) in 16 of 17 subjects (94%). The urinary excretion rate of 19-NA was highest on d 1 and 7 after injection, with urinary excretion of 1–2%/d of the administered dose. The urinary excretion of 19-NE was also highest on d 1 and 7, with 0.4–0.7% of the administered dose/d. After 33 d, the urinary excretion rate was low (<0.05%/d). In the 150-mg group, urinary metabolites could be detected

TABLE 2. Summary of pharmacokinetic parameters for nandrolone in serum

Treatment group	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng/ml · h)	Cl_{app} (l/h)	$t_{1/2}$[a] (d)	$t_{1/2}$ desc.[b] (d)	$t_{1/2}$ asc.[c] (h)
50 mg nandrolone decanoate (n = 19)							
Mean	30	2.14	400	80.0	7.1	4.8	4.7
CV (%)		64.3	21.3	21.3	98.5	53.1	58.4
Range	4–98	0.59–7.21	254–567	56.4–126	1.4–37.9	1.6–13.2	1.1–11.8
100 mg nandrolone decanoate (n = 17)							
Mean	30	4.26	862	74.3	11.7	5.1	6.0
CV (%)		27.3	19.5	19.5	47.0	22.5	44.1
Range	8–96	2.86–6.93	607–1269	50.4–105	6.8–35.5	3.4–8.4	2.8–16.0
150 mg nandrolone decanoate (n = 17)							
Mean	72	5.16	1261	76.2	11.8	5.8	5.8
CV (%)		27.4	21.9	21.9	29.3	25.0	45.2
Range	8–216	2.74–7.29	801–2008	47.8–120	6.7–18.3	3.7–8.6	2.6–12.0

The geometric mean and coefficient of variation (CV) are presented.
[a] Terminal half-life.
[b] Half-life in descending phase (absorption half-life) determined using model fitting.
[c] Half-life in ascending phase (elimination half-life) determined using model fitting.

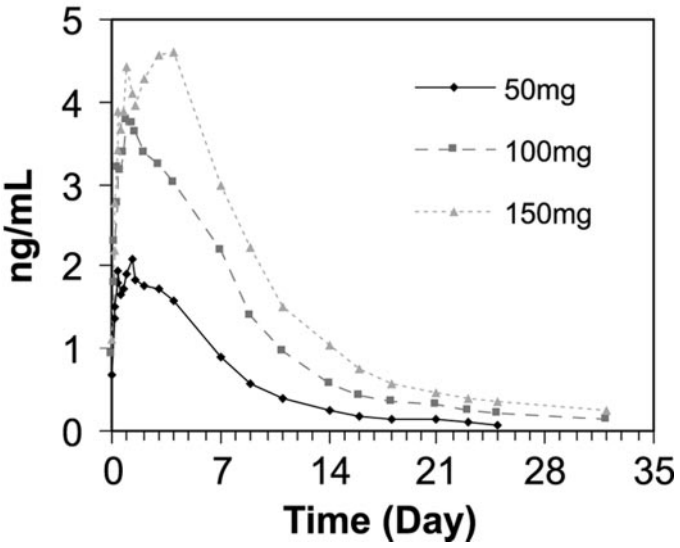

FIG. 1. Mean serum concentration profiles for nandrolone after single im injection of 50, 100, or 150 mg nandrolone decanoate in healthy men.

after 6 months (final assessment) in eight of 12 subject (67%) for 19-NE and 10 of 12 subjects (83%) for 19-NA. Moreover, 6 months after injection, urinary levels of 19-NA (four of 12 subjects) and 19-NE (one of 12 subjects) were occasionally still sufficiently high (>2 ng/ml) to test positive for doping (Figs. 2 and 3).

In general, renal clearance of 19-NA and 19-NE was higher during the first week after injection compared with 33 d after injection. The urinary $t_{1/2}$ was approximately 29 d for 19-NA and 34 d for 19-NE (Table 5).

Safety parameters

A total of 36 subjects (66.7%) reported AEs in this study, and none of the subjects reported an SAE. The most frequently reported AE was headache, which was reported by 18.5% of the subjects (20%, 24%, and 12% after 50, 100, and 150 mg/ml, respectively), followed by rhinitis (14.8%), back pain (14.8%), and rash (11.1%). Most of these events were of mild intensity. In all cases, the relation to study drug was judged unlikely or none. There were 13 drug-related AEs reported in 10 subjects: testicular pain (one subject), injection site pain (one subject), injection site reaction (one subject), back pain (one subject), fatigue (five subjects), hot flushes (one subject), and leg pain (one subject). The intensity of all drug-related AEs was assessed as mild.

Local tolerance was good. No itching, swelling, or bruising was observed after injection. Two subjects showed mild redness directly after dosing. Pain at the injection site was reported in 13 subjects 2 h after injection. Mild pain at the injection site was still present in one subject in each dose group 24 h after injection.

Discussion

In this study it has been shown for the first time that there is a rapid and dose-proportional increase in nandrolone serum levels across a dose range after a single im injection of 50–150 mg nandrolone decanoate in healthy young men. Peak serum levels of nandrolone were reached 2–3 d after injection, and the maximum serum level ranged from 2.14 ng/ml in the 50-mg group to 5.16 ng/ml in the 150-mg group. Moreover, because the AUC of nandrolone increases linearly with the amount administered, a predictable serum level and therefore a predictable clinical response in relation to the dose administered is possible. It was also demonstrated for the first time that the nandrolone metabolites 19-NA and 19-NE are detectable in urine samples for at least 6 months after a single im injection of 150 mg nandrolone decanoate, which is still above the threshold of 2 ng/ml in one third of subjects. Moreover, this study shows that a significant proportion of men presumably not exposed to nandrolone decanoate injections may present with detectable amounts of 19-NA and/or 19-NE in urine, although the limit for a positive doping test (2 ng/ml) was never reached.

Nandrolone displays so-called flip-flop pharmacokinetics. This means that the ascending phase of the curve represents the disposition of nandrolone, and the descending part of the curve represents the rate-limiting process of release of nandrolone decanoate from the muscle into the general circulation (19). Therefore, the half-life in the descending phase of the curve is an estimate of the absorption half-life rather than

TABLE 3. Urinary levels of nandrolone metabolites after single im injection of nandrolone decanoate

Treatment group	n	19-NA (ng/ml)			19-NE (ng/ml)		
		Geometric mean	CV	Range	Geometric mean	CV	Range
50 mg nandrolone decanoate							
−1 d	20			BLQ			BLQ–0.8
1 d	20	322	98.8	45.6–899	94.8	91.9	21.1–523
7 d	19	312	75.0	38.0–691	86.6	55.5	21.4–204
33 d	17	9.2	176.8	BLQ–40.7	3.9	111.6	BLQ–19.7
150 mg nandrolone decanoate							
−1 d	17			BLQ–0.6			BLQ–0.9
1 d	17	742	71.4	216–1887	232	67.5	95.8–590
7 d	17	1244	65.5	496–4572	322	67.7	109–1026
33 d	17	67.6	107.3	26.7–380	19.7	82.4	7.6–100
90 d	15	5.1	150.1	BLQ–39.7	2.5	158.2	BLQ–16.6
180 d	12	1.2	100.6	BLQ–4.1	0.7	80.7	BLQ–2.1

CV, Geometric coefficient of variation (percentage); BLQ, below limit of quantification (0.6 ng/ml). For calculation of the mean, BLQ values were replaced by 0.3 ng/ml (½ × LOQ). If more than one third of the samples were BLQ, no mean value was calculated.

TABLE 4. Urinary excretion rate of nandrolone metabolites after single im injection of nandrolone decanoate

Treatment group	n	19-NA				19-NE			
		Geometric mean		CV (%)	Range (μg/d)	Geometric mean		CV (%)	Range (μg/d)
		μg/day	%/day			μg/d	%/d		
50 mg nandrolone decanoate									
−1 d	20				BLQ				BLQ–1.32
1 d	20	696	2.16	87.7	189–2668	205	0.635	84.8	66.6–957
7 d	18	522	1.62	85.3	49.0–1272	140	0.435	52.2	27.6–268
33 d	15	14.5	0.0450	189.5	BLQ–72.4	5.38	0.0167	126.0	BLQ–35.1
150 mg nandrolone decanoate									
−1 d	17				BLQ–1.19				BLQ–3.67
1 d	17	1359	1.40	65.1	376–2903	424	0.438	55.7	134–987
7 d	15	1677	1.73	58.2	727–4440	446	0.461	55.7	190–1210
33 d	17	80.3	0.083	90.2	20.3–437	23.4	0.0242	88.6	7.00–115
90 d	14	8.58	0.00887	91.1	1.64–38.9	3.93	0.00406	128.7	BLQ–16.3
180 d	12	1.73	0.00179	92.0	0.408–5.08	0.993	0.00103	82.9	BLQ–2.66

CV, Geometric coefficient of variation; BLQ, below limit of quantification (0.6 ng/ml). For calculation of the mean, BLQ values were replaced by 0.3 ng/ml (½ × LOQ). If more than one third of the samples were BLQ, no mean value was calculated.

the elimination half-life. The fact that the terminal half-life for the 50-mg dose was significantly shorter than those for the higher doses may be a result of the fact that nandrolone concentrations do not decrease in a monoexponential way, but, rather, with an increasing half-life over time. In the 50-mg group, the last nandrolone concentrations of the time curve were often below the limit of quantification. As a result, these time points were not taken into account for calculation of the terminal half-life. Consequently, in the low-dose group, the terminal half-life was, on the average, calculated over an earlier time interval than in the higher dose groups. The $t_{1/2,descending}$, which is based on a fit of the entire concentration *vs.* time curve, is probably a better estimate of the absorption half-life of nandrolone. The fact that no significant difference between dose groups was observed for $t_{1/2,descending}$ and $t_{1/2,ascending}$ indicates that the underlying processes of absorption and elimination are dose independent in the tested dose range.

The pharmacokinetics of nandrolone decanoate in men have been studied in three previous trials. In one study, the pharmacokinetics of nandrolone decanoate after single im injection were studied in male volunteers. After a dose of 200 mg in men, a t_{max} of 9 h and a C_{max} of 3.7 ng/ml were found, whereas the $t_{1/2}$ was 5.9 d (19). In another study, after a single im injection of 50 mg nandrolone decanoate in six healthy men, serum nandrolone levels increased rapidly to a peak of 1.3 ng/ml at 24 h after injection, whereas serum nandrolone levels remained elevated for 15–20 d. The $t_{1/2}$ of nandrolone in serum was approximately 8 d (18). In a third study, 23 healthy men were randomized into four groups receiving a single dose of 100 mg nandrolone esters: nandrolone phenylpropionate in 4 ml arachis oil injected into the gluteal muscle (group 1), nandrolone decanoate in 4 ml arachis oil injected into the gluteal muscle (group 2), nandrolone decanoate in 1 ml arachis oil injected into the gluteal muscle (group 3), or nandrolone decanoate in 1 ml arachis oil injected into the deltoid muscle (group 4). Absolute bioavailability was higher after single-dose injection of 100 mg nandrolone decanoate in 1 ml arachis oil into the gluteal muscle (73%) than in the other three groups (53–56%). In this former

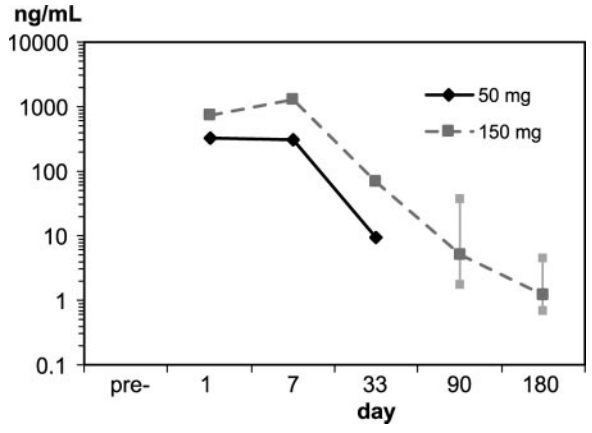

FIG. 2. Mean urine concentration profiles for 19-NA after single im injection of 50 or 150 mg nandrolone decanoate in healthy men (semilogarithmic scale). On d 90 and 180, in addition to the mean, the lowest (1.84 on d 90 and 0.72 on d 180) and the highest (39.7 on d 90 and 4.06 on d 180) measurable levels (nanograms per milliliter) are depicted.

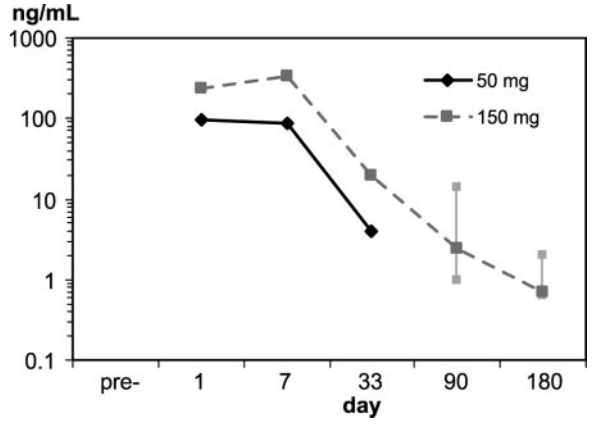

FIG. 3. Mean urine concentration profiles for 19-NE after a single im injection of 50 or 150 mg nandrolone decanoate in healthy men (semilogarithmic scale). On d 90 and 180, in addition to the mean, the lowest (1.01 on d 90 and 0.67 on d 180) and the highest (16.6 on d 90 and 2.07 on d 180) measurable levels (in nanograms per milliliter) are depicted.

TABLE 5. Renal clearance of 19-NA and 19-NE

Treatment group	19-NA (l/h)				19-NE (l/h)			
	d 1	d 7	d 33	$t_{1/2}$ (d)	d 1	d 7	d 33	$t_{1/2}$ (d)
50 mg nandrolone decanoate								
n	20	18	8[a]		20	18	8[a]	
Mean	21.1	22.7	11.0		6.22	6.08	3.96	
CV	47.0	66.8	91.2		44.8	47.4	98.5	
150 mg nandrolone decanoate								
n	17	15	17	10	17	15	17	7
Mean	18.8	23.3	15.7	28.7	5.88	6.20	4.57	34.3
CV	48.0	56.8	71.3	21.3	43.2	58.0	71.6	24.1

CV, Geometric coefficient of variation (percentage).
[a] n = 8 because the serum concentrations of nandrolone in all other subjects were less than the LOQ.

group, the C_{max} of nandrolone was 4.4 ng/ml, the t_{max} was 1.6 d, and the $t_{1/2}$ was 7.7 d (20).

Studies have shown that nandrolone decanoate and its precursor 19-norandrostenedione are metabolized in humans to 19-NA and 19-NE, the two major metabolites that can be detected in urine (21). After a single im injection of 150 mg nandrolone decanoate, urinary 19-NA and 19-NE were detectable for up to 6 months after administration using LC-MS. The urinary excretion of nandrolone metabolites after im injection of nandrolone esters has been examined in three studies, all with only a single subject included. In an early study, it was reported that 19-NA was detectable using gas chromatography-MS in urine for up to 6 wk in a man after a single injection of 25 mg nandrolone decanoate, reaching a peak level of approximately 400 ng/ml 5 d after injection (22). In another study, the urinary excretion of 19-NA was studied using gas chromatography-MS after a single injection of 50 mg nandrolone decanoate. In this subject, a peak level of 19-NA in urine of 570 ng/ml was measured 5 d after injection, and 19-NA was detectable for at least 50 d (23). In the third study, a single injection of 50 mg of a similar nandrolone ester (nandrolone undecanoate) resulted in detectable 19-NA and 19-NE levels in urine for 8 months (24). The results of these three studies fit the more detailed analysis presented in this manuscript. The clinical significance of these findings may be considerable. In view of the long-term elevated levels of 19-NA and/or 19-NE in urine after im injection of nandrolone decanoate, as demonstrated in this study, it is probable that an elite athlete abusing nandrolone decanoate injections will test positive in a doping test; the probability depends mainly on the frequency of testing. In contrast, it may be questioned whether elite athletes (undergoing regular doping tests with negative findings) who suddenly test positive with slightly elevated urinary levels of 19-NA and/or 19-NE have been abusing nandrolone decanoate injections for enhancement of sports performance (14). The issue of allegedly false positive doping tests for nandrolone abuse was first raised in the early 1990s in an investigation where elevated 19-NA levels were associated with consumption of contaminated meat from cattle that had been illegally treated with nandrolone esters to increase meat yield (25). Since then, several alternative explanations for elevated 19-NA and/or 19-NE levels in urine have been proposed, such as contaminated nutritional supplements (16, 17, 21, 26), consumption of boar meat (27, 28), endogenous production of nandrolone (13, 29–31), and exercise-induced concentration of urine (13, 32) (although the latter explanation has been questioned) (33). The World Anti-Doping Agency is supporting a number of analytical and clinical studies with the objective to gain more scientific data about the ability to dissociate between true positive doping tests related to abuse of anabolic-androgenic steroid, on the one hand, and false positive cases, on the other hand. The results obtained in our study also add to the above-mentioned objective. One potential additional method of distinguishing between endogenous and exogenous urinary metabolites may be the ratio of 19-NA/19-NE in urine. In our study before treatment, this ratio was typically less than 1, whereas after injection, the excretion pattern changed, and more 19-NA was excreted relative to 19-NE, changing the ratio to levels greater than 1.

It was concluded that after a single im dose of nandrolone decanoate, serum levels of nandrolone increase in a linear fashion across a dose range of 50–150 mg (the dose range that is also used to treat HIV wasting). In addition, in the 50-mg group, urinary metabolites 19-NA and/or 19-NE were detectable in 16 of 17 subjects for at least 33 d after injection. In the 150-mg group, in subjects presumed to have not previously used nandrolone, nandrolone metabolites were detectable for up to 6 months after injection in a significant proportion of subjects.

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