Hypothalamic-Pituitary-Testicular Axis Effects and Urinary Detection Following Clomiphene Administration in Males

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Context: Clomiphene is a performance-enhancing drug commonly abused by males in sport, but the extent to which testosterone increases in healthy males following its use is unknown. In addition, evidence suggests that clomiphene, a mixture of *cis*- and *trans*-isomers zuclomiphene and enclomiphene, is detectable in urine for months following use; the isomer-specific urinary detection window has yet to be characterized in a controlled study.

Objective: To determine the effect of once-daily, 30-day clomiphene treatment on serum testosterone and gonadotropin levels in the subject population studied and the urinary clearance and detection window of clomiphene isomers following administration for antidoping purposes.

Participants and Design: Twelve healthy males aged 25 to 38 years, representing a recreational athlete population, participated in this open-label, single-arm study.

Intervention: Oral clomiphene citrate (50 mg) was self-administered once daily for 30 days. Serum and urine samples were collected at baseline and at days 7, 14, 21, 28, 30, 32, 35, 37, 44, 51, and 58; urine collections continued periodically up to day 261.

Results: Mean testosterone, LH, and FSH levels increased 146% (SEM, ±23%), 177% (±34%), and 170% (±33%), respectively, during treatment compared with baseline. Serum drug concentrations and urinary excretion were nonuniform among individuals as isomeric concentrations varied. The zuclomiphene urinary detection window ranged from 121 to >261 days.

Conclusions: Clomiphene significantly raised serum testosterone and gonadotropin levels in healthy men and thus can be abused as a performance-enhancing drug. Such abuse is detectable in urine for ≥4 months following short-term use. (***J Clin Endocrinol Metab*** **104: 906–914, 2019)**

Clomiphene (Clomid®, clomiphene citrate) belongs to a class of compounds known as selective estrogen receptor modulators, which act to alter estrogen activity via the estrogen receptor in various tissues (1). In the United States, clomiphene has a Food and Drug Administration–approved therapeutic indication to treat ovulatory dysfunction in females seeking pregnancy (2). By blocking the estrogen receptor in the hypothalamus, negative feedback of gonadotropin release is inhibited, resulting in a surge of LH and FSH, ultimately leading to follicle maturation and ovulation (3).

Because of its ability to increase gonadotropin secretion via action at the hypothalamus, clomiphene has also been a target of clinical investigation in men for the potential treatment of hypoandrogenemia. Here, the proposed mechanism of action is similar, where clomiphene inhibition of hypothalamic

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Abbreviations: CV, coefficient of variation; E, epitestosterone; LOD, limit of detection; MeOH, methanol; SG, specific gravity; T, testosterone.

estrogen receptors results in secretion of LH and FSH (4, 5). In males, these two hormones act at the testes to increase testosterone (T). Many clinical investigations of clomiphene use have resulted in significantly elevated T concentrations in the hypoandrogenemic male populations studied (6–10). For this reason, as well as the potential for T increases to enhance athletic performance, clomiphene use is prohibited by both amateur and professional sporting leagues and by the World Anti-Doping Agency (11, 12).

Despite appearing on prohibited drug lists, clomiphene is an increasingly commonly abused substance in the sporting world (13). The number of clomiphene findings worldwide has increased year over year, from 12 findings in 2012 to 57 findings in 2016 (14). These statistics do not include data from North American amateur and professional sporting organizations, where clomiphene abuse for the purpose of performance enhancement is also evident (15). In addition, the popularity and extent of clomiphene misuse can be seen with a quick Internet search of online forums within the bodybuilding, law enforcement, armed forces, fitness, recreational athlete, and antiaging communities. In these areas, clomiphene is a popular choice as a postcycle therapy (16). Following abuse of an anabolic steroid cycle (*e.g.,* bulking with trenbolone followed by cutting with stanozolol), clomiphene is used to restart natural production of T to counteract the suppression effects caused by the exogenous anabolics.

When compounded as a pharmaceutical agent, clomiphene is prepared as an approximately 40:60 isomeric mixture of *cis*- and *trans*-isomers (zuclomiphene and enclomiphene, respectively) (Fig. 1A), each with unique pharmacokinetic and pharmacodynamic properties related to clearance and activity (17, 18). Although enclomiphene is described as the active isomer promoting antiestrogenic properties, zuclomiphene has been suggested to show estrogen receptor agonist activity following administration (19–21). The half-life of each isomer also differs. In studies examining isomeric clearance, the half-life of enclomiphene was calculated around 5 hours, with the half-life of the zuclomiphene isomer calculated around 24 hours; traces of zuclomiphene in plasma were also detectable multiple weeks after dosing (3). Regardless of the clearance time window, both compounds are excreted in urine in detectable quantities, the matrix of choice for antidoping analysis. Taken together, the urinary detectability and detection window, or time from last dose until clomiphene is no longer detectable in urine samples, of each isomer is expected to vary, and these urinary detection windows have not yet been reported following clomiphene administration in a healthy male population.

In sum, this study was designed to understand the hypothalamic-pituitary-testicular axis effects and the urinary excretion pattern, including the detection window, following 30-day administration of clomiphene to healthy, recreationally active males. For the purposes of this study, *recreationally active* describes individuals training for several hours each week in high-intensity aerobic and/or anaerobic exercise.

Methods

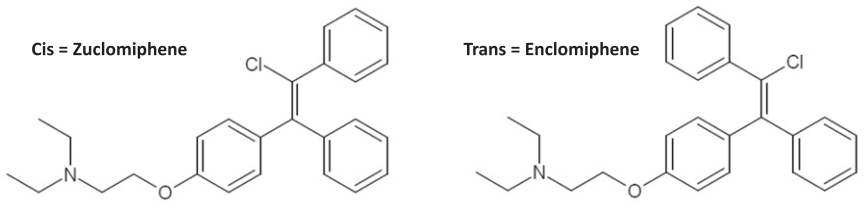
Study approval

Approval for this research was granted by the University of Utah Institutional Review Board (IRB_00097194), and the study was registered as a clinical trial with the United States National Institutes of Health (NCT03028532). Medical supervision was available for all subjects, and frequent medical checkups were performed throughout the study.

Participant selection

Twelve healthy and active male participants, aged 25 to 38 years, who were representative of a recreational athlete population participated in this study. Participants' demographics, including age, weight, and body mass index, along with measured baseline hormone levels are presented in Table 1. All participants declared no supplement or drug use that could potentially interfere with the effects or known metabolism of the study drug; this was confirmed by preliminary drug screening at the Sports Medicine Research and Testing Laboratory. Because a potentially performance-enhancing drug was

A



B

				50 mg clomiphene, once daily														
Day	BASELINE			1	7	14	21	28	30	32	34	37	44	51	58	→→→		
Blood	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
Urine	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

Figure 1. (A) Chemical structure of clomiphene. (B) Study timeline. Check marks indicate when blood and/or urine samples were collected during the study. After day 58, only urine samples were collected on various days.

self-administered in this study, participants were excluded if they were competing in sanctioned athletic events or were a member of a registered antidoping testing pool during the administration and immediate follow-up portions of the study. All participants provided written informed consent before study commencement.

Reagents

All laboratory chemicals and reagents were of analytical grade. Ethanethiol, formic acid, ammonium iodide, and components of the potassium and carbonate buffers were purchased from Sigma-Aldrich (St. Louis, MO). Methyl *tert*-butyl ether and methanol were purchased from Burdick & Jackson (Muskegon, MI), and ammonium acetate was purchased from Fluka (Honeywell International, Morris Plains, NJ). β-Glucuronidase purified from *Escherichia coli* was purchased from Roche Life Science (Indianapolis, IN). *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide was purchased from Regis Technologies, Inc (Morton Grove, IL).

Study drug

Clomiphene citrate 50-mg tablets (PAR Pharmaceutical Companies, Inc; Spring Valley, NY) were obtained from the University of Utah Investigational Drug Services pharmacy (2). Each 50-mg tablet contained ~62% enclomiphene and 38% zuclomiphene, as stated in the package insert and confirmed by independent analysis at the Sports Medicine Research and Testing Laboratory (data not shown).

Study design

A graphic of the study design is presented in Fig. 1B. Two serum and three urine samples were collected from each individual to establish baseline values for all analytes being measured. Following these baseline collections, all participants began a 30-day treatment in which 50 mg of clomiphene citrate was self-administered orally once daily. Urine and serum samples were collected once per week during the administration period, once more on the last day of administration, and periodically in the 4 weeks following the end of administration. After day 58 (28 days after administration), only urine samples were collected (weekly, biweekly, or on the basis of participant availability) until clomiphene was no longer quantifiable in the urine or until the participant no longer wished to continue providing samples. To control for diurnal variation in serum T concentration, all samples were collected at the same time of day for each individual participant (22).

Table 1. Participant Demographics and Baseline Hormone Levels

Participant Demographics	
Age, y	31.5 ± 3.6
Body weight, kg	77.9 ± 8.2
BMI, kg/m^2	24.4 ± 2.4
Baseline Hormone Levels	
Testosterone, ng/ dL	458.96 ± 159.46
LH, IU/L	4.52 ± 1.17
FSH, IU/L	4.39 ± 1.46

Data are presented as mean ± SD.

Abbreviation: BMI, body mass index.

Measurement of serum gonadotropins and SHBG

Serum T, LH, and FSH concentrations were measured simultaneously in 500 μL of sample using commercially available kits on the automated ADVIA Centaur CP Immunoassay System (Siemens Healthcare Diagnostics, Inc; Tarrytown, NY). Interassay and intra-assay coefficients of variation (CVs) for T, LH, and FSH were <10%. Commercial and established in-house controls were run with each batch to control for batch-to-batch variability.

SHBG levels were measured separately, also using commercially available kits on the ADVIA Centaur CP Immunoassay System (Siemens Healthcare Diagnostics, Inc.), in only a small cohort of the study population because of limited sample volume. In total, SHBG was measured in 46 serum samples from five subjects.

Serum quantitation of clomiphene

A standard curve for quantitation was created ranging from 0.05 to 100 ng/mL. Fifty microliters of 75 ng/mL of clomiphene-d5 (Toronto Research Chemicals; Toronto, ON, Canada) was added as an internal standard to 1.0 mL of each serum sample. Next, 20 mM of ammonium acetate (Honeywell Fluka) was added and quickly mixed to buffer the sample. Solid-phase extraction followed, using a Strata X-C 33-μm 60-mg column (Phenomenex; Torrance, CA) preconditioned with methanol [MeOH (Burdick & Jackson)] and 20 mM of ammonium acetate (pH = 4; Honeywell Fluka). Following sample addition to the columns, columns were rinsed with ammonium acetate (Honeywell Fluka) and MeOH, and sample was eluted using freshly prepared 2% NH_4OH in MeOH. Eluate was dried in a Turbovap and reconstituted in 100 μL of 95/5 formic acid/0.1% MeOH (Sigma-Aldrich, Burdick & Jackson) in preparation for reverse-phase ultra-performance liquid chromatography–tandem mass spectrometry analysis. Interassay and intra-assay CVs for quantitation of zuclomiphene and enclomiphene were <10%. A limit of detection (LOD) of 50 pg/mL was established for this study.

Urinary clomiphene quantitation

To 3 mL of urine sample, 50 μL of internal standard (clomiphene-d5; Toronto Research Chemicals) was added. Next, the sample was buffered using 0.8 M potassium phosphate buffer (pH = 7.0; Sigma-Aldrich) and incubated with β-glucuronidase (Roche) for 90 minutes at 50°C. Following incubation, 750 μL of potassium carbonate buffer (20% $K_2CO_3/KHCO_3$; pH = 9.5 to 10.0; Sigma-Aldrich) was added, and the deconjugated compounds were isolated by liquid-liquid extraction using 6.0 mL methyl-*tert* butyl ether (Burdick & Jackson). Finally, the extract was dried in a Turbovap and reconstituted in 95:5 0.1% formic acid/MeOH (Sigma-Aldrich, Burdick & Jackson) in preparation for liquid chromatography–tandem mass spectrometry analysis. An eight-point standard curve was prepared for quantitative analysis ranging from 0.05 to 100 ng/mL. Interassay and intra-assay CVs for quantitation of zuclomiphene and enclomiphene were <10%. An LOD of 50 pg/mL was established for this study. Reported values were specific gravity (SG)-normalized, using the equation

$$\text{CONCENTRATION}_{\text{SG-norm}} = \text{CONCENTRATION}_{\text{measured}} * (1.020\text{–}1)/(U_{SG} - 1)$$

where U_{SG} represents the urinary SG as determined using refractometry.

Measurement of urinary T and epitestosterone

The protocol for measurement of urinary steroids is described in detail elsewhere (23). Briefly, urine samples were buffered and β-glucuronidase (Roche) was added to cleave conjugated steroids, which were further extracted using a liquid-liquid extraction technique. Steroids were derivatized using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (Regis Technologies, Inc.) before analysis via gas chromatography–tandem mass spectrometry.

Data analysis

Because of interindividual variability in endogenous hormone levels, such as serum gonadotropins and urinary T and epitestosterone (E) concentrations, these data were normalized and reported as a percentage change from baseline within each individual. The means of each analyte in the two- and three-predrug administration collections served as baseline measurements for serum and urine samples, respectively. For the quantitation of clomiphene isomers in serum and in urine, raw values were measured and reported.

Results

Participant attrition

Three enrolled subjects ceased clomiphene treatment before the full 30-day treatment window. One patient dropped out after experiencing anxiety while taking the drug, a second dropped out because of vertigo, and a third dropped out because of "performance enhancement." During the washout phase, one subject (subject 06) dropped out at day 170 because of logistical issues. At that time, zuclomiphene was still detectable in urine at 558 pg/mL.

Serum T, LH, and FSH increased significantly throughout the duration of treatment; a small increase was also identified in SHBG

Serum T, LH, and FSH concentrations increased significantly throughout the duration of treatment. Results appear in Fig. 2; data are presented as percentage increases compared with the mean baseline values. Beginning with the first collection 7 days after the start of clomiphene treatment, all three hormone levels increased concurrently and continued to increase each week until their peaks on the last day of administration, day 30. Values peaked at increases of 146% (±23%), 177% (±34%), and 170% (±33%) compared with baseline for T, LH, and FSH, respectively. Once clomiphene treatment was stopped after 30 days, all three hormones decreased concurrently until returning to baseline levels at ~3 to 4 weeks after treatment (days 51 to 58). Of note, suppression of T, LH, and FSH following treatment did not occur. No significant differences were seen between the percentage increases in any of the variables.

Although limited to measurements taken from only n = 5 from the study population, SHBG levels showed an ~40% increase from baseline on day 7 and leveled out at around a 20% to 30% increase until drug administration stopped (data not shown).

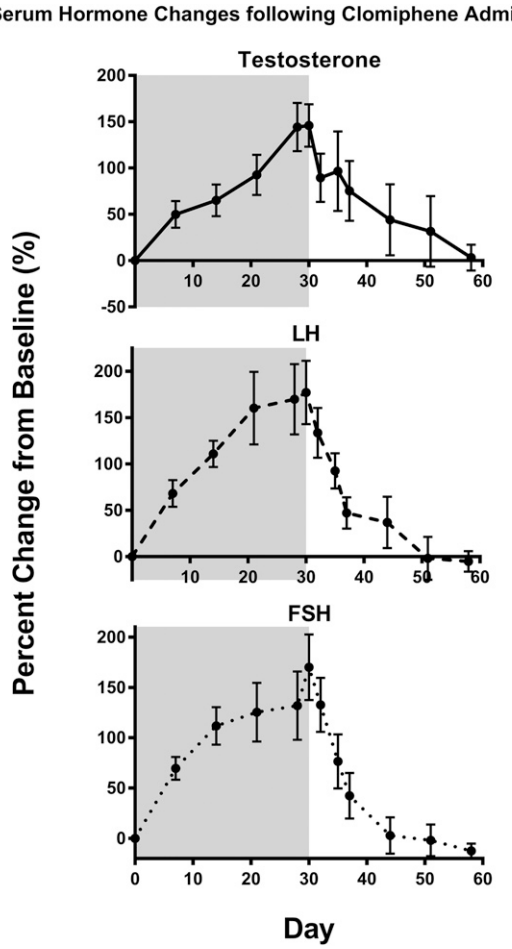


Figure 2. Serum gonadotropin changes following administration of clomiphene. Values for testosterone (top panel, solid line), LH (middle panel, dashed line), and FSH (bottom panel, dotted line) were calculated for each individual as a percentage change from individual baseline values; data on graphs are presented as the mean ± SEM of these percentage changes from baseline in the population. Area shaded in light gray represents the administration period of the drug.

A urinary increase was seen in excreted T and E, and a slight increase in T/E ratio

Throughout the study, T and E were routinely measured in urine samples to understand the effect of clomiphene administration on the longitudinal monitoring of these steroids, a common tool used in the antidoping field. Both urinary T and E concentrations increased during the administration phase, peaking at a mean 135% (±24%) increase on day 30 and a 71% (±13%) increase on day 21 for each compound compared with the average baseline, respectively (Fig. 3). Because the calculated concentrations for T increased to a greater degree than concentrations for E, an increase in the T/E ratio was also identified, peaking around the end of the administration phase at an increase of 77% (±15%) compared with baseline level (Fig. 3).

Enclomiphene cleared quickly from serum, whereas zuclomiphene was still present 4 weeks after drug administration

The quantity of each clomiphene isomer (zuclomiphene and enclomiphene) was measured in serum during the administration phase and for the 4 weeks that followed cessation. Results appear in Fig. 4. Serum concentrations of zuclomiphene rose steadily until the final day of administration, when the highest concentration was reached at 32.48 (±2.17) ng/mL, whereas the concentration of enclomiphene reached its highest concentration at day 28 at 10.47 (±8.29) ng/mL. Following discontinuation, enclomiphene concentration decreased rapidly and by day 44 was detectable in only one subject (subject 12). The last serum collection occurred on day 58, or 28 days after the end of the administration phase. At this time, zuclomiphene was present in serum at an average concentration of 15.12 (±1.38) ng/mL, whereas enclomiphene was detectable in only one subject (subject 12), at 1.31 ng/mL.

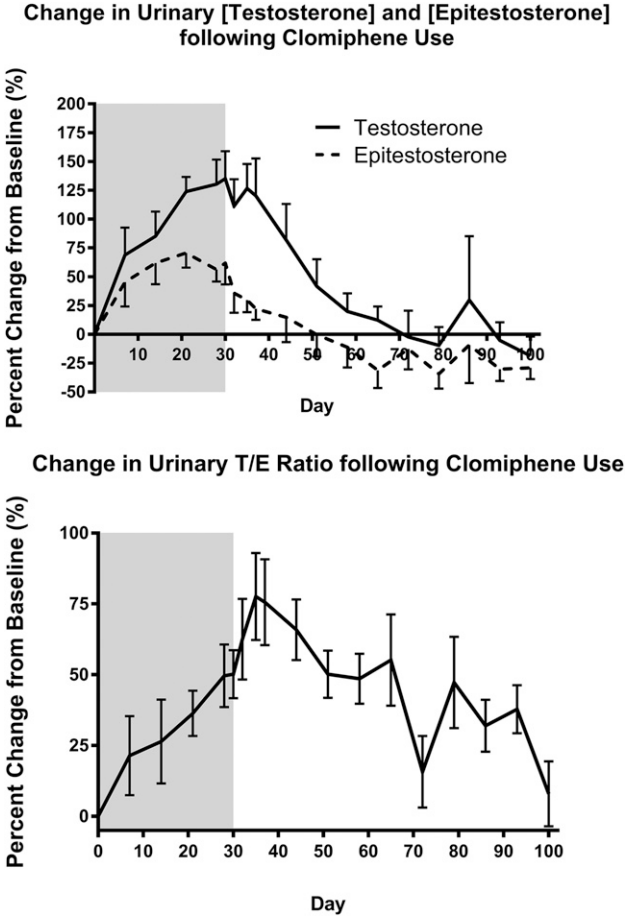


Figure 3. Urinary excretion of testosterone and epitestosterone following clomiphene use. Top panel shows testosterone and epitestosterone excretion; values are presented as percentage change compared with baseline, population mean ± SEM. Bottom panel shows testosterone/epitestosterone ratios; values are represented as percentage change compared with baseline, population mean ± SEM. Area shaded in light gray represents the administration period of the drug.

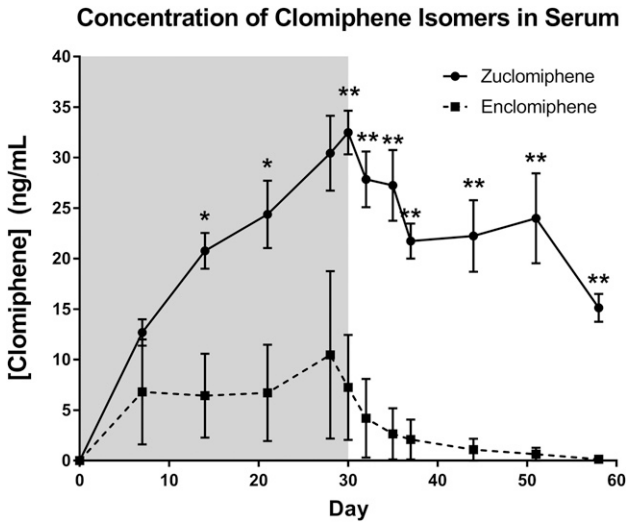


Figure 4. Concentration of clomiphene isomers measured in serum following 30-day administration. Values for zuclomiphene (solid line, circle data points) and enclomiphene (dotted line, square data points) are represented as the mean ± SEM. Area shaded in light gray represents the administration period of the drug. Significance between [zuclomiphene] and [enclomiphene] was determined using an unpaired *t* test with the Holm-Sidak method. $*P < 0.05$; $**P < 0.01$.

Significance was determined using an unpaired *t* test with the Holm-Sidak method.

Urinary clearance was nonuniform, and the detection window was variable

An LOD for the analysis of clomiphene in urine for this study was established at 50 pg/mL (using a signal/noise ratio of 3:1 for identification criteria of ion transition; data not shown). Because the enclomiphene isomer was cleared quickly from circulation, the detection window in urine was also short and thus was not fully characterized. Urinary elimination of zuclomiphene, however, was longer-lived and varied in all individuals, as shown by the urinary quantitation graphs in Fig. 5. In some subjects (subjects 01, 03, 08, and 10), the SG-normalized concentration of zuclomiphene never exceeded 10 ng/mL even during the "on-phase" of the drug. Other subjects (subjects 04, 06, 11, and 12) excreted clomiphene at relatively consistently higher values while taking the drug, with excretion concentrations dropping rapidly during the washout phase. All subjects showed consistent excretion of low concentrations of drug during the washout phase.

The urinary detection window resulting from clomiphene administration, determined by samples in which zuclomiphene was quantifiable at >50 pg/mL, also varied across all individuals, as shown in Fig. 5 and Table 2. The quickest clearance was demonstrated by subject 10, in whom clomiphene was no longer detectable at day 128 and beyond. Four subjects (subjects 01, 02, 10, and 11) fully cleared the drug by day 200, or 170 days

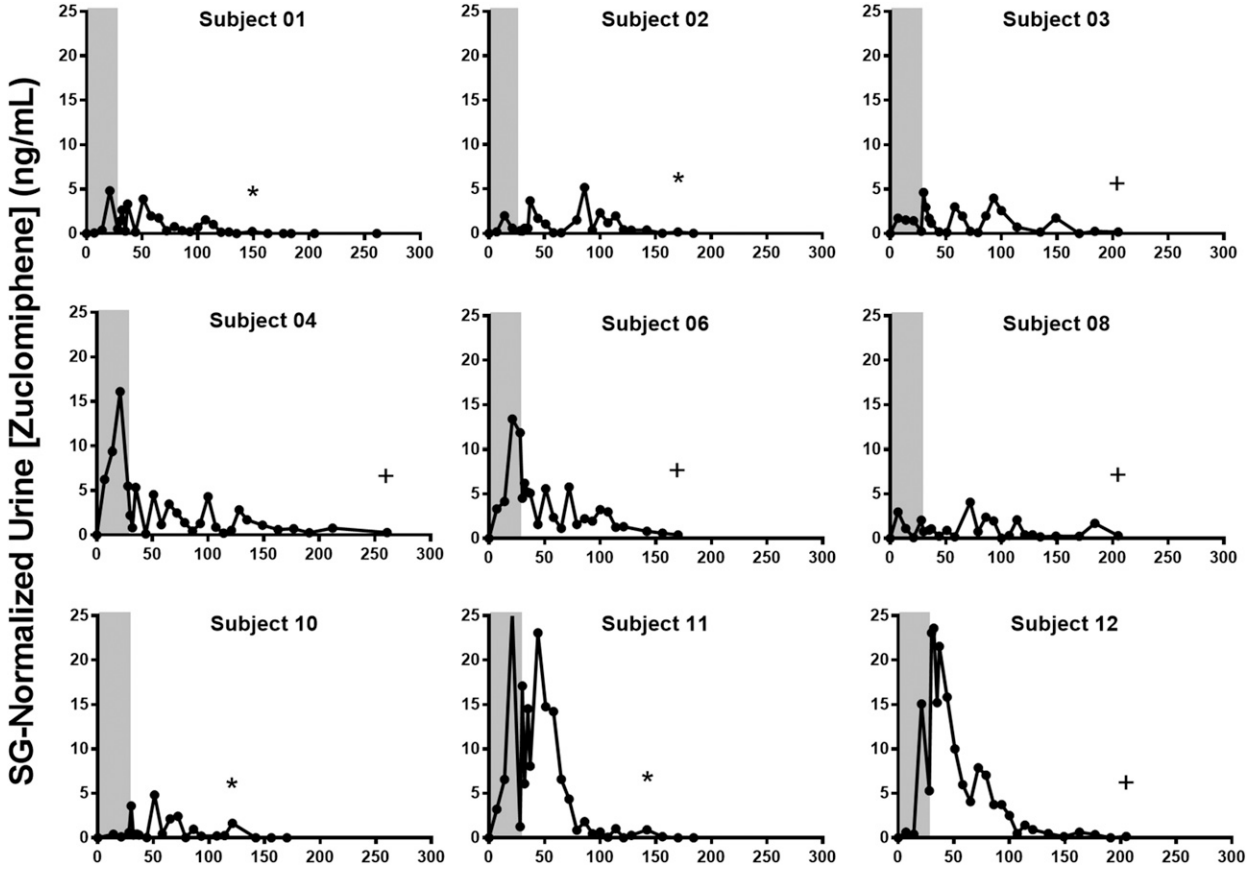


Figure 5. Urinary concentration of excreted zuclomiphene in each of the nine subjects. Zuclomiphene concentration (ng/mL) is plotted on the *y*-axis; the *x*-axis represents the timeline (day) of the study. Data are presented as the single measurement of zuclomiphene concentration (following SG normalization) in each urine sample. The area shaded in light gray represents the administration period of the drug. Asterisks (*) indicate the last urine sample in which zuclomiphene was detectable in participants who later produced a negative urine sample. Plus signs (+) indicate instances when zuclomiphene was detectable in the overall last urine sample collected by the participants.

into the washout phase. In the remaining five subjects, subjects 03, 04, 06, 08, and 12, zuclomiphene was detectable in the last urine sample collected on days 205, 261, 170, 205, and 205, respectively.

Clomiphene undetectability and redetectability in follow-up urine samples

Of the nine subjects who completed the full administration of the drug, three continued to have detectable zuclomiphene concentrations throughout the duration of the study (of note, subject 06 dropped out after day 170). In addition, four subjects had detectable zuclomiphene concentrations in urine following a negative test result (*i.e.,* zuclomiphene not detected). Urinary zuclomiphene concentration data are shown in Table 2. With subject 01, zuclomiphene was not detectable in urine on day 135, reappeared in urine on day 149 (236.9 pg/mL), and was no longer detectable in any of the follow-up collections. Subject 02 showed a similar pattern, in which drug was detectable on day 142 (384.7 pg/mL), not detectable on day 156, detectable again on day 170 (151.5 pg/mL), and not detectable in any further collections. Subject 12 produced a negative sample on day 191 and a further positive sample on day 205 (146.8 pg/mL) before ceasing collection. Subject 03 produced a negative urine sample on day 170 and followed up with positive samples on days 184 (260.3 pg/mL) and 205 (174.3 pg/mL) before ceasing collection. Subject 11 produced a negative sample on day 121 and followed up with positive samples on days 128 (288.7 pg/mL), 142 (916.1 pg/mL), and 156 (133.9 pg/mL) before producing two further negative samples on days 170 and 184. This pattern of undetectability and redetectability was not identifiable in subject 10.

Discussion

The goal of this study was to understand the hypothalamic-pituitary-testicular axis effects of clomiphene when administered to healthy, athletically active males and, importantly, to understand the urinary clearance of the drug with implications for doping detection. Clomiphene use in the sporting, bodybuilding, law enforcement, armed forces, fitness, recreational athlete, and antiaging communities was originally thought to provide a benefit as postcycle therapy, serving to more quickly restart endogenous T production

Table 2. Clomiphene Undetectability and Redetectability in Follow-Up Urine Samples

	Subject 01	Subject 02	Subject 03	Subject 04	Subject 06	Subject 08	Subject 10	Subject 11	Subject 12
Day	SG-Normalized Zuclomiphene Concentration (pg/mL)								
121	147.7	429.2		540.5	1327	414.8	1642	<LOD	934.0
128	174.8	358.2		284.1		409.1	<LOD	288.7	
135	<LOD		180.5	1727		175.4			489.9
142		384.7			802.6		<LOD	916.1	
149	236.9		1762	1112		250.0			142.0
156		<LOD			600.0		<LOD	133.9	
163	<LOD			617.6					634.5
170		151.5	<LOD		382.6	239.9	<LOD	<LOD	
177	<LOD			712.6					366.4
184	<LOD	<LOD	260.3			1699		<LOD	
191				253.7					<LOD
198									
205	<LOD		174.3			295.0			146.8
212				783.5					
261	<LOD			288.0					

SG-normalized urinary concentrations of zuclomiphene are reported in pg/mL. For this study, LOD is defined as 50 pg/mL. Empty cells indicate no urine sample was collected.

that had previously been suppressed by anabolic steroid abuse. Our results show that LH-dependent T secretion was evident even without previous suppression or previous indications of hypogonadism. Based on the proposed mechanism of action as a hypothalamic estrogen receptor antagonist, the resulting surge in LH release (and further, a T increase) following clomiphene binding was expected because the pituitary senses low estrogen levels. Previous studies have shown similar percentage increases with extended dosing up to multiple years. However, these studies were conducted mainly with males with low T levels as the subject population (8–10, 24). Of note, SHBG levels (measured in five subjects from the study population) did increase to a maximum of around 40% from baseline, showing that despite the described nearly 150% increase in total serum T concentration, this number was not entirely representative of the total increase in free T.

As described in previous work, enclomiphene is the isomer responsible for the T increases found when administered to males (19, 20). Of note, this isomer is present in lower concentrations [as also noted in a previous study (24)] and is cleared far more rapidly from circulation than zuclomiphene. In this study, by day 44, enclomiphene was detectable in the serum of only one subject. This pattern of quicker excretion of enclomiphene was also identified with urinary clearance. Thus, if an athlete were to obtain and use only the single enclomiphene isomer with the intention of enhancing athletic performance, the detectability of this compound by antidoping laboratories might be challenging in the off phase or washout phase because of the quick clearance of the drug. Closer monitoring of urinary T/E ratio increases, as shown in Fig. 4, would be beneficial in these cases.

Serum data obtained from one participant, subject 12, during and following drug administration was somewhat anomalous when compared with the data from the other eight subjects. Not only was enclomiphene detectable for a longer time in this individual, but it was also detected at significantly higher concentrations, often at least 10-fold higher, than for all other participants. The increases in T concentration detected in subject 12 were also greater than in all other subjects in the study, reaching a maximum increase of 392% from baseline on day 30. By the time blood collections finished for the study (day 58), enclomiphene was still detectable in serum at a concentration of 1.31 ng/mL, and the serum T concentration from this individual still showed a nearly twofold increase compared with baseline level. Previous studies suggested that metabolism and clearance of clomiphene may depend on the status of CYP2D6 isoforms (25–27). Because zuclomiphene concentrations measured in the serum of subject 12 were similar to those of the other eight subjects, this suggests that any underlying cause for the altered metabolism presented by this individual is specific to the enclomiphene isomer. These interindividual differences within the subject population highlight the importance of closely monitoring individual patients following therapeutic clomiphene use.

Although the serum isomeric data are important for interpretation of results, most antidoping work is conducted via urinalysis because of the extended and more

comprehensive detectability of drugs and metabolites in urine. Thus, understanding the urinary clearance of clomiphene, and especially the window of detection, was an important aspect of this study. As shown in Table 2, the soonest that zuclomiphene was no longer detectable at a concentration ≥50 pg/mL in urine in any of the subjects following administration was day 128, or 98 days following the final administration of the drug. Four of the nine subjects never produced a negative urine sample, with the longest detection window in one individual beyond day 261 (~8 months following the final dose). Many of the urine samples in the washout phase of the drug showed consistent (though nonuniform) excretion of low amounts of zuclomiphene, suggesting sequestration of the drug into bodily compartments with slow-release kinetics. The logP value of clomiphene is listed by multiple sources as >6.0 (28, 29); it is presumed that because of this high lipophilicity, clomiphene may be sequestered into adipose tissue following systemic distribution. This sequestration, in combination with enterohepatic recycling as stated in previous drug analyses (30, 31), is expected to result in the lengthy urinary detection window established in this study. Because undesirable effects of zuclomiphene may exist, it is recommended that clinical monitoring continue even in the weeks to months following cessation of treatment.

Another important finding from this work was showing the fluctuation in concentration of excreted urinary zuclomiphene, described in some instances as being undetectable (*i.e.*, below the laboratory LOD) and then detectable again in a sample collected days to weeks later. Some of this may be due in part to sample dilution. In samples with lower SG, the excreted compounds are diluted, which may result in undetectable levels of zuclomiphene in urine. However, this is not always the case. One example from this study is shown by subject 02. Zuclomiphene was undetectable in the urine on day 156, when the SG from this sample, U_{SG} = 1.0138, was in the normal range and further reappeared in the detectable range on day 170 without follow-up use. Because of the slow, nonuniform bodily clearance and likely fat sequestration of clomiphene previously suggested, it may be possible that as fat breaks down, clomiphene is released into the bloodstream and excreted in the urine, appearing in a future drug test without further administration of the drug. Similar patterns have been observed in clinically relevant monitoring substances such as cannabis (32) and in other drugs known to undergo enterohepatic circulation, such as morphine (33).

When results from this study are interpreted, it is important to include data obtained from both the serum and urine analyses. A T increase was evident following administration of clomiphene to healthy, recreationally active males. On average, this T increase returned toward baseline between 2 and 4 weeks after cessation of the drug, around the same time that enclomiphene was no longer detectable in the serum of the participants. Finally, the urinary detection window following administration of clomiphene is considerable, and this lengthened detection window is due to the slow urinary clearance of zuclomiphene.

Limitations

Although the gold-standard measurement method for T involves mass spectrometry–based techniques, T in this study was measured using validated and established immunoassay techniques. Because normal or supraphysiological T values were expected and measured in this study, immunoassay methods such as those used here are adequate for reliable measurement and are typically used by clinical laboratories for measuring T in similar ranges.

Regarding sample collection from the participants, there was no stipulation placed on fasting before collection. Although diurnal variation was addressed by collecting samples from the participants at the same time of day throughout the study, some unaccounted variation may be present because of a lack of controlling precollection conditions, such as caloric intake, stress, and activity.

The study design was open-label and single arm, meaning only a single administration pattern (and single dose) was studied, and it was not placebo controlled. Because only one dose was administered, the extrapolation of data to address longer periods of use may be difficult. In addition, because the administration was only 30 days and 50 mg per day, true peak concentrations of the analytes may not have been reached. From the antidoping perspective, however, it can be assumed that longer use would not result in a shortened detection window, still showing that its abuse in sport is detectable long after the abuser has stopped administration. Lastly, although control or placebo effects on hypothalamic-pituitary-testicular axis effects would have been beneficial, a control group would not have aided in the determination of a urinary detection window for clomiphene and thus was omitted during the study design.

Conclusions

A controlled clinical trial was designed to analyze the effects of clomiphene on the hypothalamic-pituitary-testicular axis of healthy males in a representative recreational athlete population and to understand the detectability of clomiphene from an antidoping perspective. Clomiphene ingestion at low doses increases serum total T concentration to ~150% of baseline in healthy young men, and this increase persists for weeks after drug discontinuation, clearly representing the potential for performance enhancement if misused by athletes. The use and abuse of clomiphene is detectable for weeks to months after discontinuation because of the persistent urinary excretion of the zuclomiphene isomer.

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