Received: 19 June 2023 | Revised: 8 August 2023 | Accepted: 16 August 2023

DOI: 10.1111/andr.13515

ANDROLOGY WILEY

SHORT COMMUNICATION

Exploring rat corpus cavernosum alterations induced by finasteride treatment and withdrawal

Silvia Diviccaro | Monika Herian | Lucia Cioffi | Matteo Audano | Nico Mitro | Donatella Caruso | Silvia Giatti | Roberto Cosimo Melcangi

Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy

Correspondence
Roberto Cosimo Melcangi, Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy.
Email: roberto.melcangi@unimi.it

Abstract

Despite its efficacy for treating androgenetic alopecia, finasteride, an inhibitor of 5α-reductase (i.e., the enzyme converting testosterone, T, into dihydrotestosterone, DHT), is associated with several side effects including sexual dysfunction (e.g., erectile dysfunction). These side effects may persist after drug suspension, inducing the so-called post-finasteride syndrome (PFS). The effects of subchronic treatment with finasteride (i.e., 20 days) and its withdrawal (i.e., 1 month) in rat corpus cavernosum have been explored here. Data obtained show that the treatment was able to decrease the levels of the enzyme 5α-reductase type II in the rat corpus cavernosum with increased T and decreased DHT levels. This local change in T metabolism was linked to mechanisms associated with erectile dysfunction. Indeed, by targeted metabolomics, we reported a decrease in the nitric oxide synthase (NOS) activity, measured by the citrulline/arginine ratio and confirmed by the decrease in NO_2 levels, and a decrease in ornithine transcarbamylase (OTC) activity, measured by citrulline/ornithine ratio. Interestingly, the T levels are negatively correlated with NOS activity, while those of DHT are positively correlated with OTC activity. Finasteride treatment also induced alterations in the levels of other molecules involved in the control of penile erection, such as norepinephrine and its metabolite, epinephrine. Indeed, plasma levels of norepinephrine and epinephrine were significantly increased and decreased, respectively, suggesting an impairment of these mediators. Interestingly, these modifications were restored by suspension of the drug. Altogether, the results reported here indicate that finasteride treatment, but not its withdrawal, affects T metabolism in the rat corpus cavernosum, and this alteration was linked to mechanisms associated with erectile dysfunction. Data here reported could also suggest that the PFS sexual side effects are more related to dysfunction in a sexual central control rather than peripheral compromised condition.

KEYWORDS
5α-R type II, citrulline, NO, NOS, OTC, targeted metabolomics

1 | INTRODUCTION

Penile erection is the result of a complex neurovascular process regulated by central and local mediators such as neurotransmitters, neuropeptides, peptides, and sexual hormones that ultimately influence the corpus cavernosum.[1,2] Erectile dysfunction (ED) and overall male sexual impairment have a multifactorial etiology and may be related with psychogenic (i.e., stress, depression, anxiety), not endocrine (i.e., neurogenic, vasculogenic, and iatrogenic), or endocrine causes.[1,2] Several drugs,[3,4] including 5α-reductase inhibitors (5α-RIs),[5,6] may impair male sexual function. A flagship example of 5α-RIs is the finasteride, which was approved in 1997 for treating androgenetic alopecia (AGA) to block the conversion of testosterone (T) into dihydrotestosterone (DHT). Despite its efficacy, the drug treatment is associated with several side effects,[6–12] including those in the sexual domain.[13–17] Indeed, young men treated for AGA with finasteride showed ED, decreased libido, orgasm failure, and ejaculatory disorders.[18,19] Interestingly, these side effects may persist after the drug suspension, inducing the so-called post-finasteride syndrome (PFS).[9,20–34]

The effects of finasteride treatment were also reported in animal models. For instance, finasteride treatment in castrated rats decreased erectile response, although receiving T replacement[35,36] and in control male rats, it decreased intracavernosal pressure during electrical stimulation of cavernous nerves.[37] Treatment with finasteride or other 5α-RIs, such as dutasteride, significantly suppressed plasma DHT levels.[38,39] Additionally, drug treatment in adult male rats resulted in a loss of the weight of the corpus cavernosum,[38] reduced cross-sectional penile area,[40] and increased collagen density in the cavernosal tissue,[37] while in aged male rats resulted in morphological alterations of mitochondria and increased apoptosis in cavernous smooth muscle cells.[41] Altogether, these experimental observations suggested the corpus cavernosum as a target for finasteride-induced ED. However, the molecular mechanisms have not been explored so far in detail, and in particular, if these are also involved in the persistent ED induced by this drug.

On this basis, we have explored the effect of subchronic treatment with finasteride and its withdrawal in rat corpus cavernosum, assessing: (i) the levels of steroids, such as pregnenolone (PREG), progesterone (PROG), dihydroprogesterone (DHP), allopregnanolone (ALLO), isoallopregnanolone (ISOALLO), dehydroepiandrosterone (DHEA), T, DHT, 5α-androstane-3α, 17β-diol (3α-diol) and 17β-estradiol (17β-E) by liquid chromatography–tandem mass spectrometry (LC–MS/MS); (ii) the expression of 5α-R type II by Western blot analysis; (iii) nitrogen dioxide concentration by nitrate/nitrite colorimetric assay; (iv) nucleotide, amino acid, biogenic amine, acylcarnitine, glycolysis, and tricarboxylic cycle intermediate levels by targeted metabolomics performed with LC–MS/MS analysis.

Interestingly, finasteride also shows off-targets, such as phenylethanolamine *N*-methyltransferase (PNMT), the limiting enzyme in the formation of epinephrine from norepinephrine. Indeed, as recently demonstrated, finasteride treatment was able to inhibit this enzyme in the rat adrenal gland.[42] This is relevant since the balance of epinephrine and norepinephrine levels is crucial for achieving erection.[1,43] Therefore, the plasma levels of norepinephrine and epinephrine were assessed by LC–MS/MS after finasteride treatment and withdrawal.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult male Sprague–Dawley rats (200—225 g at arrival, Charles River Laboratories, Italy) were used. The procedures were approved by the local ethics committee and the Italian Ministry of Health (authorization 1083/2015-PR). All manipulations were performed in accordance with national (D.L. No. 26, March 4, 2014, G.U. No. 61 March 14, 2014) and international laws and policies (EEC Council Directive 2010/63, September 22, 2010: Guide for the Care and Use of Laboratory Animals, United States National Research Council, 2011). All experiments were carried out in the animal care facility of the Department of Pharmacological and Biomolecular Sciences (DiSFeB) at the Università degli Studi di Milano, Italy. The rats ($n = 24$) were acclimated to the new environment for 1 week. At sacrifice, the animals were individually placed in an induction chamber and anesthesia was induced with 2% isoflurane (ISO VET, La Zootecnica, Italy) until the righting reflex was lost. After sacrifice, the plasma and corpora cavernosa were immediately stored at −80°C until the analysis.

2.2 | Treatments

We have used the dose of 1 mg/rat/day, on the basis of our previous published observations.[44,45] In particular, this low dose of finasteride was effective in decreasing the plasma DHT levels in a persistent way.[44] Finasteride (1 mg/rat/day; Sigma–Aldrich, Italy) was dissolved in a vehicle solution of sesame oil and ethanol (5% v/v). Solutions were injected subcutaneously, at a volume of 100 μL/day for 20 days. Finasteride- and vehicle-treated rats were sacrificed at 24 h ($n = 12$) after the last injection and 1 month ($n = 12$) after drug suspension to investigate the effects of the subchronic treatment and its withdrawal, respectively.

2.3 | Liquid chromatography–tandem mass spectrometry analysis (LC–MS/MS)

2.3.1 | Reagents and chemicals

PROG-2,3,4,20,25-$^{13}C_5$ ($^{13}C_5$-PROG), 17β-E-2,3,4-$^{13}C_3$ ($^{13}C_3$-17β-E), PREG-20,21-$^{13}C_2$-16,16 D_2 ($^{13}C_2$ D_2- PREG), (±)-norepinephrine-D_6 hydrochloride (D_6-NE), PREG, PROG, DHP, ALLO, ISOALLO, DHEA, T, DHT, 3α-diol, 17β-E, norepinephrine, epinephrine, Trizma base (Tris), ethylenediaminetetraacetic acid (EDTA) disodium salt, ammonia solution 25%, ammonium acetate, 1-heptanesulfonic acid (HSA) were

purchased from Merck Life Science, Italy. Acetonitrile, acetic acid, formic acid, methanol, and water were of HPLC grade.

2.3.2 | Quantitative analysis of steroid molecules in corpus cavernosum

The levels of steroids were evaluated in corpora cavernosa (25-35 mg/sample). $^{13}C_3$-17β-E (2 ng/sample) $^{13}C_5$-PROG (0.4 ng/sample), and $^{13}C_2$ D_2-PREG (10 ng/sample) were used as internal standards and added to all samples. For quantitative analysis, corpora cavernosa tissues were homogenized using the TissueLyser, in methanol/acetic acid 1%. Corpus cavernosum was purified by organic phase extraction and the analysis was performed by liquid chromatography (LC) supplied of Surveyor LC Pump Plus and Surveyor Autosampler Plus (Thermo Fisher Scientific MA, USA) with a linear ion trap—mass spectrometer LTQ (Thermo Fisher Scientific MA, USA), operated in positive atmospheric pressure chemical ionization as previously described.[32] The chromatographic separation was achieved with a Hypersil Gold column C18 (100 × 2.1 mm, 3 μm; Thermo Fisher Scientific MA, USA) maintained at 40°C. The mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in methanol. 25 μL sample was injected at a flow rate of 0.250 mL/min. The injector needle was washed with methanol–water (1:1 v/v). Quantitative analysis was performed on the basis of freshly prepared and extracted calibration curves. LC–MS/MS data were evaluated using the software Excalibur release 2.0 SR2 (Thermo Fisher Scientific MA, USA).

2.3.3 | Quantitative analysis of norepinephrine and epinephrine in plasma

The quantitative analysis of norepinephrine and epinephrine in plasma samples was achieved as previously described.[46] Briefly, 700 μL of plasma samples were mixed with 10 μL of internal standard solution (500 ng/sample of D_6-NE) and 300 μL of buffer solution (1 mol/L Tris and 0.05 mol/L EDTA in water). Samples were purified with Sep-Pak 1cc (100 mg) Alumina B cartridges (Waters, Milano, Italy), eluted with 550 μL of elution solution (2.5% formic acid and 4% water in acetonitrile) and evaporated to 50 μL under a stream of N_2. 250 μL of 35 mmol/L HSA in aqueous 0.5% formic acid were added to samples and the solution was filtered with a 0.2 μm filter (SRC grade, regenerated cellulose membrane filter, CHMLAB Group). For the quantitative analysis 5 μL/sample were injected in API 3500 (AB Sciex, USA) mass spectrometer, equipped with an electrospray source (ESI+) and triple quadrupole analyzer, interfaced with a pump for the HPLC model EXION SL (Sciex, USA). The chromatographic separation was achieved using Luna Omega 5 μm PS C18 100 Å column (Phenomenex, USA). The mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at flow rate of 0.300 μL/min. The quantitative analysis was performed on the basis of calibration curves freshly prepared in water–acetonitrile (98:2 v/v). LC–MS/MS data were evaluated using the software Analyst software (Sciex, USA).

2.3.4 | Targeted metabolomics

Metabolomic data were obtained by LC–MS/MS. We used an API-3500 triple quadrupole mass spectrometer (AB Sciex, MA, USA) coupled with an ExionLC AC System (AB Sciex). 10 mg of tissue were smashed in a TissueLyser for 5 min at maximum speed in 250 μL of ice-cold methanol–water–acetonitrile (55:25:20 v/v). Lysates were spun at 15,000×*g* for 15 min at 4°C. Samples were then dried under N_2 flow at 40°C and resuspended in 125 μL of ice-cold methanol–water–acetonitrile (55:25:20 v/v) for subsequent analyses.

Amino acids, energy metabolites, and acyl-carnitines quantification and data processing were achieved as previously described.[47] Data processing and analysis were performed by MetaboAnalyst 5.0 web tool.

2.4 | Nitrate/nitrite colorimetric assay

10 mg of tissue was used to perform the analysis of nitric oxide (NO) metabolite detection in corpus cavernosum by Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, USA). Briefly, at the beginning nitrates (NO_3^-) have been converted to nitrites (NO_2^-), using nitrate reductase. In the next step, by the usage of Griess reagents, nitrates were transformed into a deep purple azo compound. Photometric measurement of the absorbance (540—550 nm) due to this azo chromophore accurately determined NO_2^- concentration.

2.5 | Western blot

For Western blotting, 15 mg of corpus cavernosum were homogenized using the TissueLyser in Phosphate buffer saline (PBS) without Ca^{2+} and Mg^{2+}; EDTA 0.5 M pH 8; Igepal) supplemented with a protease and phosphatase cocktail inhibitor (Cell Signaling, Italy). Then, to remove particulate matter, the tissue homogenates were centrifuged at 2000 rpm for 5 min at 4°C. The protein content was quantified using a Bradford Assay (Bio-Rad, Italy) and samples containing equal amounts of protein (20 μg) were heated to 100°C for 5 min. Samples were run on Criterion TGX 4—15% gradient gels (Bio-Rad, Italy) and transferred to nitrocellulose membranes. Stain-free technology was used to determine equal loading. Nitrocellulose membranes were blocked in 5% bovine serum albumin (BSA)–PBS–Tween for 1 h at room temperature and exposed to primary antibody (rabbit 5α-R type II; abcam 124877, 1:2000) overnight.

Then, a conjugated secondary antibody, according to the primary antibody was chosen. The protein bands were detected on membranes using the ECL method. ECL signals were acquired with a ChemiDocTM XRS+ system (Bio-Rad, Italy) and analyzed with Image LabTM software version 5.2.1. The detected protein targets were normalized with the total lane values obtained with the stain-free technology (Bio-Rad, Italy). The mean control value within a single experiment was set to 100 and all other values were expressed as a percentage.

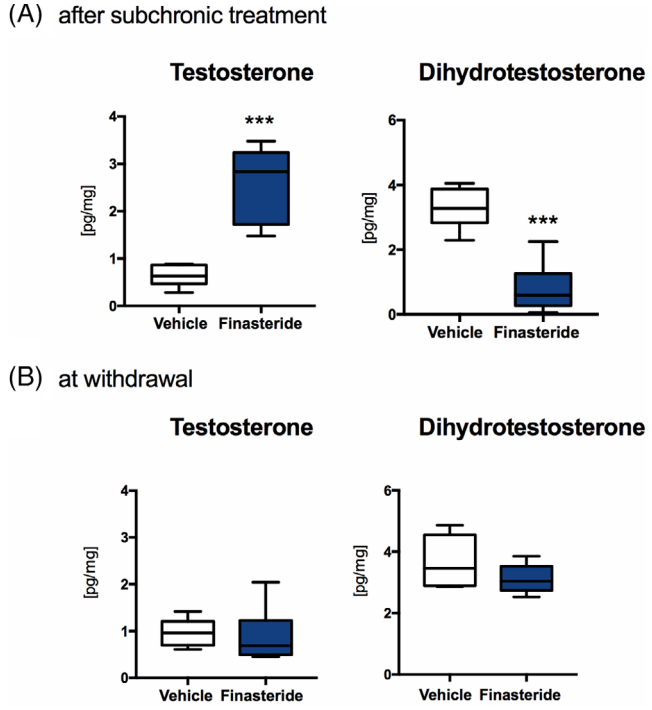

FIGURE 1 Assessment of androgen levels in the corpus cavernosum. After subchronic finasteride treatment (A) and withdrawal (B), T and DHT levels were analyzed by LC–MS/MS. Data are expressed as pg/mg ± SD with $n = 6$ for each group. Unpaired Student's *t*-test analysis: $^{***}p < 0.001$ vs. vehicle group.

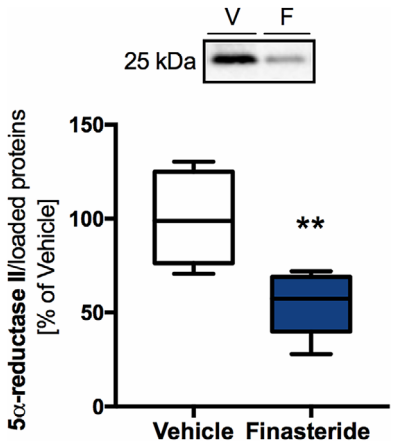

FIGURE 2 Assessment of enzyme protein expression. After subchronic finasteride treatment the relative protein expression of 5α-R type II was assessed by Western blotting. Data are expressed as % of vehicle ± SD with $n = 6$ for each group. Unpaired Student's *t*-test analysis: $^{**}p < 0.01$ vs. vehicle group.

2.6 | Statistical analysis

LC–MS/MS, Western blot data and NO_2 data were analyzed by unpaired two-tailed Student's *t*-test, after checking for the normal distribution with the Kolmogorov–Smirnov test. $p < 0.05$ was considered significant. Linear regression analysis and Pearson's correlation coefficient were computed to assess the potential relationship between two different variables. Analyses were performed using Prism, version 7.0a (GraphPad Software Inc., San Diego, CA, USA).

3 | RESULTS

3.1 | Assessment of steroid levels and 5α-R type II after finasteride treatment and its withdrawal in rat corpus cavernosum

The levels of 10 different steroids (i.e., PREG, PROG, DHP, ALLO, ISOALLO, DHEA, T, DHT, 3α-diol and 17β-E) were evaluated by LC–MS/MS in the corpus cavernosum after subchronic finasteride treatment (i.e., 24 h after the last injection, $n = 12$) and withdrawal (i.e., 1 month after drug suspension, $n = 12$). Data obtained indicate that T and DHT levels were affected by finasteride treatment (Figure 1A), while the other steroids were not significantly modified (data not shown). Indeed, a statistically significant increase in T levels was associated with a significant decrease in the levels of its metabolite, DHT (Figure 1A). The levels of T and DHT (Figure 1B), as well as the other steroids analyzed (data not shown), were restored by suspension of the drug.

Interestingly, the local alteration of T conversion induced by the finasteride treatment was supported by a significant decrease in the protein expression of 5α-R type II (Figure 2).

3.2 | Assessment of catecholamine plasma levels after finasteride treatment and its withdrawal

The levels of norepinephrine and its metabolite (i.e., epinephrine) were quantified in plasma by LC–MS/MS analysis, after subchronic finasteride treatment ($n = 12$) and withdrawal ($n = 12$). As reported in Figure 3A, subchronic finasteride treatment resulted in a significant increase in the levels of norepinephrine, associated with a decrease in those of epinephrine. In contrast, the assessment performed at finasteride withdrawal did not show significant variations in their levels (Figure 3B).

3.3 | Targeted metabolomics by LC–MS/MS coupled with nitrate/nitrite colorimetric assay after finasteride treatment and its withdrawal in corpus cavernosum

To test if and to what extent finasteride-treated rats are characterized by specific metabolic patterns, we performed targeted metabolomics to investigate nucleotide, amino acid, biogenic amine, acylcarnitine, glycolysis, and tricarboxylic acid cycle intermediate levels in the corpus cavernosum, after subchronic finasteride treatment ($n = 12$) and withdrawal ($n = 12$). As reported in the representative scheme in

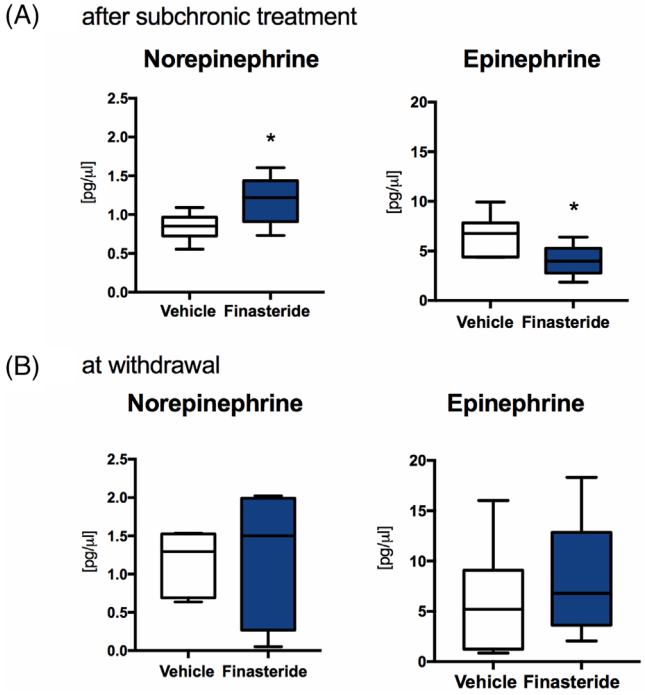

FIGURE 3 Evaluation of catecholamine levels in plasma. After subchronic finasteride treatment (A) and withdrawal (B), norepinephrine and epinephrine levels were analyzed by LC–MS/MS. Data are expressed as pg/μL ± SD with $n = 6$ for each group. Unpaired Student's t-test analysis: $^*p < 0.05$ vs. vehicle group.

Figure 4A, specific enzymatic activities were modulated by finasteride treatment. In particular, as shown in Figure 4B, the activity of ornithine transcarbamylase (OTC), catalyzing the reaction between carbamoyl phosphate and ornithine resulting in citrulline synthesis, was significantly decreased. Similarly, a decrease in the activity of the nitric oxide synthase (NOS), an enzyme that converts arginine to citrulline, was observed (Figure 4B). As a confirmation of suppressed NOS activity, the concentration of nitrogen dioxide (NO_2) was assessed in the corpus cavernosum. As shown in Figure 4C, treatment with finasteride significantly decreased the NO_2 content. No significant changes in other specific metabolic patterns after finasteride treatment and withdrawal were observed (data not shown).

4 | DISCUSSION

Data here reported indicate that the subchronic treatment with finasteride is able to directly affect the enzyme 5α-R type II in the rat corpus cavernosum and consequently the conversion of T into DHT. Indeed, we here reported a decrease in its protein expression, associated with rise in the levels of its substrate and a decrease in the levels of its first product, DHT. Therefore, our observations indicated that a subchronic treatment with this drug, in addition to what we previously reported in plasma,[44] also locally decreases the levels of DHT. As already demonstrated, androgens, particularly DHT, play a role in penile erection.[35,36,38,48,49] These effects involved changes in the NOS activity, which, as confirmed in the present study by metabolomic analysis, decreased after subchronic treatment with finasteride in the corpus cavernosum. As we reported, the decrease in the NOS activity, measured by the citrulline/arginine ratio and confirmed by the decrease in NO_2 levels, was associated with a decrease in the OTC activity, measured by citrulline/ornithine ratio. In this context, it is interesting to note that levels of T in the corpus cavernosum, which we reported to be increased after subchronic drug treatment, are negatively correlated with NOS activity ($p = 0.01$; $r = -0.69$), while tissue levels of DHT, which we demonstrated to be decreased, are positively correlated with OTC activity ($p = 0.01$; $r = +0.71$). This is particularly interesting because, even if previous observations already indicated the presence of the enzyme 5α-R type II in the corpus cavernosum,[50,51] to our knowledge this is the first observation suggesting that a local change in T metabolism is linked to mechanisms associated with ED. Indeed, to our knowledge, clinical and experimental studies evaluating ED have analyzed only steroid changes in plasma.[1] In this context, it is interesting to note that the finasteride treatment did not increase T levels in plasma,[44] suggesting that the T accumulation in corpus cavernosum could be involved in the dysfunction of the NOS activity. The finasteride subchronic treatment in male rats also confirmed alterations in the levels of other molecules involved in the control of penile erection, such as norepinephrine and its metabolite, epinephrine.[1] Indeed, we reported here that the plasma levels of norepinephrine and epinephrine were significantly increased and decreased, respectively, suggesting ED.[1,43] The inhibition of this catecholamine conversion seems to be due to a direct inhibition of the PNMT enzyme in the adrenal gland, which we have previously reported to be an off-target of the finasteride.[42] Altogether, these observations suggest that the subchronic finasteride treatment in male rats induces molecular alterations associated with ED, not only in plasma, but also directly in the corpus cavernosum. A possible limitation of this study could be the only comparison of the results obtained with finasteride treatment versus those obtained in vehicle treated animals. Thus, the lack of a baseline control group that was not exposed to finasteride. The situation seems to be very different 1 month after drug suspension, suggesting full reversibility of the effects. Indeed, in this case we did not observe alterations in the corpus cavernosum levels of T and DHT, as well as of the other steroids we have assessed (i.e., PREG, PROG, DHP, ALLO, ISOALLO, DHEA, 3α-diol, and 17β-E). In agreement, our metabolomic analysis in the corpus cavernosum and evaluation of catecholamine levels in plasma showed no changes after finasteride withdrawal. Notably, a wider distribution of catecholamine levels following drug withdrawal than during treatment was highlighted, suggesting that animals may have a variable response to finasteride.

As previously mentioned, PFS patients (i.e., at finasteride withdrawal) show ED.[1,28,43] However, the results obtained here seem to indicate that finasteride withdrawal does not alter the peripheral machinery controlling penile erection. On the other hand, it is important to highlight that observations obtained in PFS patients and in male rats after finasteride suspension show the nervous system as an important target, with alterations that could be associated with sexual dysfunction, such as depressive symptomatology and peripheral

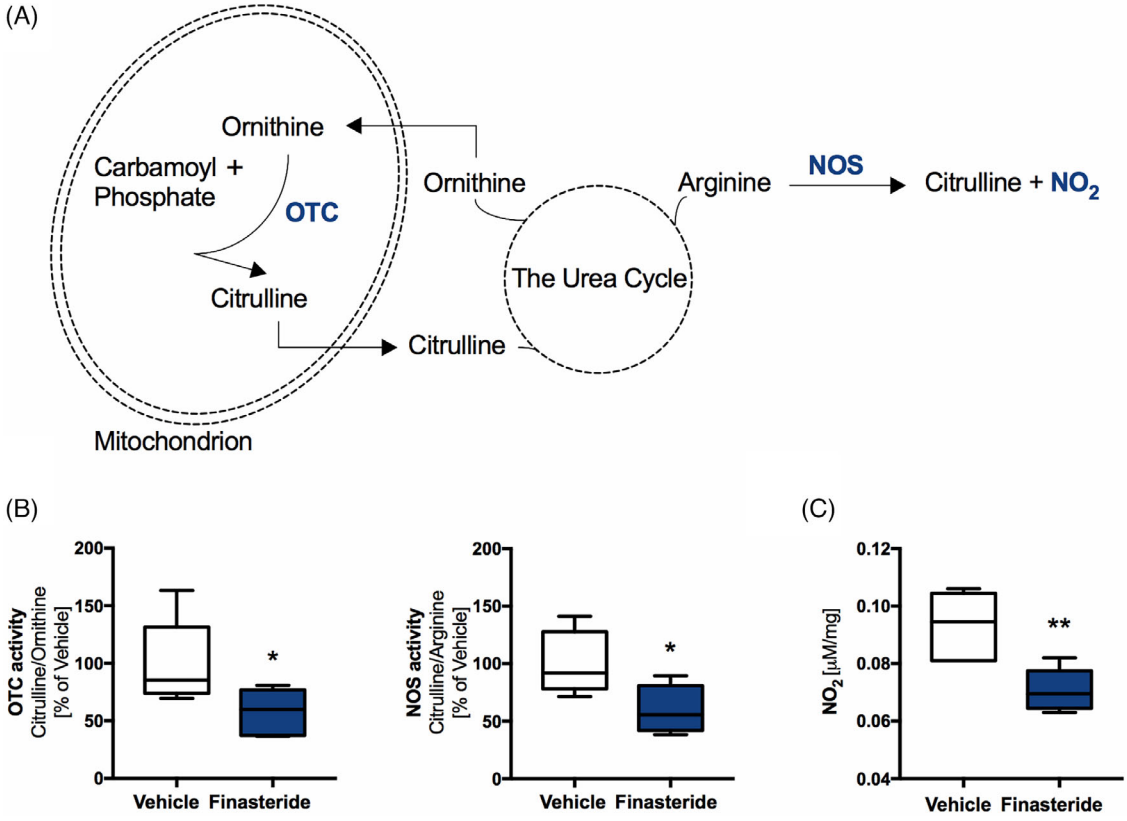

FIGURE 4 Evaluation of the metabolomic status and NO_2 production in corpus cavernosum after finasteride treatment. (A) As summarized in the representative picture, ornithine, which comes from the urea cycle goes into the mitochondria to be converted in citrulline by ornithine transcarbamylase (OTC) and carbamoyl phosphate. Citrulline can dissolve in the cytoplasm to be used in the urea cycle to produce also the substrate of nitric oxide synthase (NOS) (i.e., arginine). The last step of this pathway is the production by NOS of citrulline and nitrogen dioxide (NO_2). These pathways are altered by finasteride treatment; (B) OTC and NOS activities studied by targeted metabolomics; (C) NO_2 content analyzed by nitrate/nitrite colorimetric assay. Data are expressed as % of vehicle ± SD (B) or μM/mg ± SD (C); $n = 6$ for each group. Unpaired Student's *t*-test analysis: $^{*}p < 0.05$ vs. vehicle group; $^{**}p < 0.01$ vs. vehicle group.

neuropathy.[7,20,27,28,32,45] In addition, it is important to remember that male sexual behavior (e.g., sexual motivation and reward to sexual performance) is under the control of complex neural circuits.[1,52] Therefore, future experiments will be important to explore whether finasteride withdrawal affects these circuits and molecules involved, such as neurotransmitters and neuropeptides.[1,52]

AUTHOR CONTRIBUTIONS

Conceptualization, Silvia Diviccaro and Roberto Cosimo Melcangi; methodology, Matteo Audano, Lucia Cioffi, Silvia Diviccaro, Silvia Giatti and Monika Herian; analysis data, Donatella Caruso and Nico Mitro; writing—original draft preparation, Silvia Diviccaro, Monika Herian and Roberto Cosimo Melcangi; writing—review and editing, Silvia Diviccaro and Roberto Cosimo Melcangi; data curation, Silvia Diviccaro; supervision, Roberto Cosimo Melcangi; project administration, Roberto Cosimo Melcangi. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

We would like to thank Flavio Giavarini for his technical support for the LC–MS/MS analysis.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FUNDING INFORMATION

from MIUR Progetto Eccellenza and Post-Finasteride Foundation

DATA AVAILABILITY STATEMENT

Data will be made available on request.

ORCID

Roberto Cosimo Melcangi https://orcid.org/0000-0003-0861-8967

REFERENCES

1. Andersson KE. Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. *Pharmacol Rev*. 2011;63(4):811-859.
2. de Souza ILL, Ferreira EDS, Vasconcelos LHC, Cavalcante FA, da Silva BA. Erectile dysfunction: key role of cavernous smooth muscle cells. *Front Pharmacol*. 2022;13:895044.
3. Lambert M, Conus P, Eide P, et al. Impact of present and past antipsychotic side effects on attitude toward typical antipsychotic treatment and adherence. *Eur Psychiatry*. 2004;19(7):415-422.

4. Palha AP, Esteves M. Drugs of abuse and sexual functioning. *Adv Psychosom Med*. 2008;29:131-149.
5. Traish AM, Mulgaonkar A, Giordano N. The dark side of 5alpha-reductase inhibitors' therapy: sexual dysfunction, high Gleason grade prostate cancer and depression. *Korean J Urol*. 2014;55(6):367-379.
6. Traish AM, Melcangi RC, Bortolato M, Garcia-Segura LM, Zitzmann M. Adverse effects of 5alpha-reductase inhibitors: what do we know, don't know, and need to know? *Rev Endocr Metab Disord*. 2015;16:177-198.
7. Diviccaro S, Melcangi RC, Giatti S. Post-finasteride syndrome: an emerging clinical problem. *Neurobiol Stress*. 2020:100209.
8. Traish AM, Guay AT, Zitzmann M. 5alpha-Reductase inhibitors alter steroid metabolism and may contribute to insulin resistance, diabetes, metabolic syndrome and vascular disease: a medical hypothesis. *Horm Mol Biol Clin Investig*. 2014;20(3):73-80.
9. Giatti S, Diviccaro S, Panzica G, Melcangi RC. Post-finasteride syndrome and post-SSRI sexual dysfunction: two sides of the same coin? *Endocrine*. 2018;2(61):180-193.
10. Birch MR, Dissing S, Skakkebaek NE, Rehfeld A. Finasteride interferes with prostaglandin-induced CatSper signalling in human sperm. *Reproduction*. 2021;161(5):561-572.
11. Saengmearnuparp T, Lojanapiwat B, Chattipakorn N, Chattipakorn S. The connection of 5-alpha reductase inhibitors to the development of depression. *Biomed Pharmacother*. 2021;143:112100.
12. Rahimi-Ardabili B, Pourandarjani R, Habibollahi P, Mualeki A. Finasteride induced depression: a prospective study. *BMC Clin Pharmacol*. 2006;6:7.
13. Belknap SM, Aslam I, Kiguradze T, et al. Adverse event reporting in clinical trials of finasteride for androgenic alopecia: a meta-analysis. *JAMA Dermatol*. 2015;151(6):600-606.
14. Asanad K, Sholklapper T, Samplaski MK, Cacciamani GE. Global online interest in finasteride sexual side effects. *Int J Impot Res*. 2022.
15. Coskuner ER, Ozkan B, Culha MG. Sexual problems of men with androgenic alopecia treated with 5-alpha reductase inhibitors. *Sexual Med Rev*. 2019;7(2):277-282.
16. Shin YS, Karna KK, Choi BR, Park JK. Finasteride and erectile dysfunction in patients with benign prostatic hyperplasia or male androgenetic alopecia. *World J Mens Health*. 2019;37(2):157-165.
17. Zakhem GA, Goldberg JE, Motosko CC, Cohen BE, Ho RS. Sexual dysfunction in men taking systemic dermatologic medication: a systematic review. *J Am Acad Dermatol*. 2019;81(1):163-172.
18. Fertig RM, Gamret AC, Darwin E, Gaudi S. Sexual side effects of 5-alpha-reductase inhibitors finasteride and dutasteride: a comprehensive review. *Dermatol Online J*. 2017;23(11):13030/qt24k8q743.
19. Kaufman KD, Olsen EA, Whiting D, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. *J Am Acad Dermatol*. 1998;39(4 Pt 1):578-589.
20. Melcangi RC, Casarini L, Marino M, et al. Altered methylation pattern of the SRD5A2 gene in the cerebrospinal fluid of post-finasteride patients: a pilot study. *Endocr Connect*. 2019;8(8):1118-1125.
21. Guo M, Heran B, Flannigan R, Kezouh A, Etminan M. Persistent sexual dysfunction with finasteride 1 mg taken for hair loss. *Pharmacotherapy*. 2016;36(11):1180-1184.
22. Khera M, Than JK, Anaissie J, et al. Penile vascular abnormalities in young men with persistent side effects after finasteride use for the treatment of androgenic alopecia. *Transl Androl Urol*. 2020;9(3):1201-1209.
23. Howell S, Song W, Pastuszak A, Khera M. Differential gene expression in post-finasteride syndrome patients. *J Sex Med*. 2021;18(9):1479-1490.
24. Cauci S, Chiriaco G, Cecchin E, et al. Androgen receptor (AR) Gene (CAG)n and (GGN)n length polymorphisms and symptoms in young males with long-lasting adverse effects after finasteride use against androgenic alopecia. *Sex Med*. 2017;5(1):e61-e71.
25. Di Loreto C, La Marra F, Mazzon G, Belgrano E, Trombetta C, Cauci S. Immunohistochemical evaluation of androgen receptor and nerve structure density in human prepuce from patients with persistent sexual side effects after finasteride use for androgenetic alopecia. *PLoS One*. 2014;9(6):e100237.
26. Chiriaco G, Cauci S, Mazzon G, Trombetta C. An observational retrospective evaluation of 79 young men with long-term adverse effects after use of finasteride against androgenetic alopecia. *Andrology*. 2016;4(2):245-250.
27. Basaria S, Jasuja R, Huang G, et al. Characteristics of men who report persistent sexual symptoms after finasteride use for hair loss. *J Clin Endocrinol Metab*. 2016;101(12):4669-4680.
28. Melcangi RC, Santi D, Spezzano R, et al. Neuroactive steroid levels and psychiatric and andrological features in post-finasteride patients. *J Steroid Biochem Mol Biol*. 2017;171:229-235.
29. Irwig MS. How routine pharmacovigilance failed to identify finasteride's persistent sexual side effects. *Andrology*. 2022;10(2):207-208.
30. Melcangi RC, Caruso D, Abbiati F, et al. Neuroactive steroid levels are modified in cerebrospinal fluid and plasma of post-finasteride patients showing persistent sexual side effects and anxious/depressive symptomatology. *J Sex Med*. 2013;10(10):2598-2603.
31. Traish AM. Post-finasteride syndrome: a surmountable challenge for clinicians. *Fertil Steril*. 2020;113(1):21-50.
32. Caruso D, Abbiati F, Giatti S, et al. Patients treated for male pattern hair with finasteride show, after discontinuation of the drug, altered levels of neuroactive steroids in cerebrospinal fluid and plasma. *J Steroid Biochem Mol Biol*. 2015;146:74-79.
33. Irwig MS, Kolukula S. Persistent sexual side effects of finasteride for male pattern hair loss. *J Sex Med*. 2011;8(6):1747-1753.
34. Traish AM, Hassani J, Guay AT, Zitzmann M, Hansen ML. Adverse side effects of 5alpha-reductase inhibitors therapy: persistent diminished libido and erectile dysfunction and depression in a subset of patients. *J Sex Med*. 2011;8(3):872-884.
35. Park KH, Kim SW, Kim KD, Paick JS. Effects of androgens on the expression of nitric oxide synthase mRNAs in rat corpus cavernosum. *BJU Int*. 1999;83(3):327-333.
36. Lugg JA, Rajfer J, Gonzalez-Cadavid NF. Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. *Endocrinology*. 1995;136(4):1495-1501.
37. Kilic S, Kolukcu E, Erdemir F, Benli I, Arici A. The effects of oral 5-alpha reductase inhibitors on penile intracavernosal pressures and penile morphology in rat model. *Urol J*. 2019;16(2):205-211.
38. Zhang MG, Wu W, Zhang CM, et al. Effects of oral finasteride on erectile function in a rat model. *J Sex Med*. 2012;9(5):1328-1336.
39. Pinsky MR, Gur S, Tracey AJ, Harbin A, Hellstrom WJ. The effects of chronic 5-alpha-reductase inhibitor (dutasteride) treatment on rat erectile function. *J Sex Med*. 2011;8(11):3066-3074.
40. Da Silva MHA, Costa WS, FJ BS, De Souza DB. The corpus cavernosum after treatment with dutasteride or finasteride: a histomorphometric study in a benign prostatic hyperplasia rodent model. *Asian J Androl*. 2018;20(5):505-510.
41. Zhang MG, Wang XJ, Shen ZJ, Gao PJ. Long-term oral administration of 5alpha-reductase inhibitor attenuates erectile function by inhibiting autophagy and promoting apoptosis of smooth muscle cells in corpus cavernosum of aged rats. *Urology*. 2013;82(3):743.e749-715.
42. Giatti S, Di Domizio A, Diviccaro S, et al. Three-dimensional proteome-wide scale screening for the 5-alpha reductase inhibitor finasteride: identification of a novel off-target. *J Med Chem*. 2021;64(8):4553-4566.
43. Becker AJ, Uckert S, Stief CG, et al. Cavernous and systemic plasma levels of norepinephrine and epinephrine during different penile conditions in healthy men and patients with erectile dysfunction. *Urology*. 2002;59(2):281-286.
44. Giatti S, Foglio B, Romano S, et al. Effects of subchronic finasteride treatment and withdrawal on neuroactive steroid levels and their

receptors in the male rat brain. *Neuroendocrinology*. 2016;103(6):746-757.
45. Diviccaro S, Giatti S, Borgo F, et al. Treatment of male rats with finasteride, an inhibitor of 5alpha-reductase enzyme, induces long-lasting effects on depressive-like behavior, hippocampal neurogenesis, neuroinflammation and gut microbiota composition. *Psychoneuroendocrinology*. 2019;99:206-215.
46. Bergmann ML, Schmedes A. Highly sensitive LC–MS/MS analysis of catecholamines in plasma. *Clin Biochem*. 2020;82:51-57.
47. Audano M, Pedretti S, Ligorio S, et al. Zc3h10 regulates adipogenesis by controlling translation and F-actin/mitochondria interaction. *J Cell Biol*. 2021;220(3).
48. Waldkirch E, Uckert S, Schultheiss D, et al. Non-genomic effects of androgens on isolated human vascular and nonvascular penile erectile tissue. *BJU Int*. 2008;101(1):71-75. discussion 75.
49. Seo SI, Kim SW, Paick JS. The effects of androgen on penile reflex, erectile response to electrical stimulation and penile NOS activity in the rat. *Asian J Androl*. 1999;1(4):169-174.
50. Kim KS, Liu W, Cunha GR, et al. Expression of the androgen receptor and 5 alpha-reductase type 2 in the developing human fetal penis and urethra. *Cell Tissue Res*. 2002;307(2):145-153.
51. Ueda Y, Suzuki K, Kajimoto M, et al. Possible testosterone redundancy for 5alpha-dihydrotestosterone in the masculinization of mouse external genitalia. *Exp Anim*. 2022;71(4):451-459.
52. Argiolas A, Melis MR. Neuropeptides and central control of sexual behaviour from the past to the present: a review. *Prog Neurobiol*. 2013;108:80-107.

How to cite this article: Diviccaro S, Herian M, Cioffi L, et al. Exploring rat corpus cavernosum alterations induced by finasteride treatment and withdrawal. *Andrology*. 2023;1-8. https://doi.org/10.1111/andr.13515