The Type 5 Phosphodiesterase Inhibitor Tadalafil Influences Salivary Cortisol, Testosterone, and Dehydroepiandrosterone Sulphate Responses to Maximal Exercise in Healthy Men

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Context: Physical exercise-related stress activates hypothalamus-pituitary-adrenal (HPA) axis; nitric oxide is one of the mediators of the HPA axis response to stress, and phosphodiesterase type 5 inhibitors influences nitric oxide-linked biological activities.

Objective: The objective of the study was to investigate whether a single oral long half-life phosphodiesterase type 5 inhibitor (tadalafil) administration influences the HPA axis response to exercise-related stress.

Design: This was a double-blind, cross-over trial.

Participants: Participants included nine healthy male athletes.

Interventions: All subjects performed a maximal exercise test in normoxia, after which they received a single oral administration of tadalafil or placebo. Then after a 2-wk washout period, they were crossed over and repeated the exercise test. Each subject was his own control. Salivary collections, for steroid evaluations [cortisol, dehydroepiandrosterone sulphate (DHEAS), testosterone] and respective ratio calculation (DHEAS to cortisol, testosterone to cortisol, testosterone to DHEAS), were performed before each exercise (Pre-Ex), immediately after (Post-Ex), and at 30 min during recovery.

Results: As expected, mean salivary cortisol concentration increased immediately after exercise after both tadalafil and placebo ($P = 0.014$ and $P = 0.036$ *vs.* Pre-Ex, respectively); however, the cortisol increase was significantly higher after tadalafil administration ($P = 0.034$ *vs.* placebo). Furthermore, an increased salivary testosterone after exercise was observed only after tadalafil administration ($P = 0.029$ *vs.* Pre-Ex). No effects of either exercise and/or tadalafil administration on salivary DHEAS concentrations were observed. DHEAS to cortisol and testosterone to cortisol ratios significantly decreased after exercise after tadalafil administration ($P = 0.037$, and $P = 0.02$ *vs.* placebo, respectively).

Conclusion: Tadalafil administration amplified the salivary cortisol and testosterone responses to a maximal exercise-related stress in healthy trained humans. **(*J Clin Endocrinol Metab* 93: 3510–3514, 2008)**

The hypothalamus-pituitary-adrenal axis (HPA) is influenced by psychological and physical stress, depending on type, intensity, and duration of the stressor and individual characteristics (1).

One of the stress-mediators involved in HPA response to stress is nitric oxide (NO). When stress occurs, hypothalamic NO-producing neurons are activated, and the increased NO production stimulates the HPA axis (2–4).

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Abbreviations: CNS, Central nervous system; DHEAS, dehydroepiandrosterone sulphate; HPA, hypothalamus-pituitary-adrenal; NO, nitric oxide; PDE5i, phosphodiesterase type 5 inhibitors; $\dot{V}O_{2max}$, maximal oxygen uptake.

Actually, the phosphodiesterase type 5 inhibitors (PDE5i) are the most used drugs influencing NO availability and/or biological activity (5). Furthermore, anecdotal evidences reported a worldwide misuse of PDE5i in healthy athletes, as performance enhancers, and in Air Force pilots to counteract the effects of altitude.

PDE5i (*i.e.* sildenafil) has been shown to positively influence exercise capacity in both subjects affected by cardiopulmonary diseases in normoxia (6) and healthy subjects in hypoxia (7). Furthermore, whereas in healthy volunteers PDE5i (*i.e.* tadalafil) did not influence individual fitness parameters in normoxia (8), a possible action on muscles metabolism during anaerobic exercises has been hypothesized (9).

Whereas the cardiovascular effects of PDE5i might predominate, other possible actions of PDE5i, influencing exercise performance (*i.e.* metabolic, hormonal, *etc.*) should be investigated. In particular, we considered that: 1) physical stress activates HPA axis, 2) NO is one of the mediators of the HPA axis response to stress, and, 3) PDE5i influences NO-related biological activities. On these bases, the aim of this first exploratory study was to investigate whether PDE5i at pharmacological doses might influence the salivary cortisol and androgens response to exercise-related stress in normoxia in a double-blind, cross-over study design.

Subjects and Methods

We evaluated nine healthy male athletes [mean age 24 (range 23–26) yr, height 176.3 (173.3–178.2) cm, weight 71.2 (65.0–76.8) kg, body mass index 22.5 (22.0–24.8) kg/m^{-2}, maximal oxygen uptake ($\dot{V}O_{2max}$) 52.6 (46.4–62.1) $ml/min^{-1}\cdot kg^{-1}$]. Ethical committee approval and written informed consent were obtained.

Experimental protocol

All volunteers underwent a preliminary exercise test to get familiarized with the procedures and evaluate individual $\dot{V}O_{2max}$ by using a motor-driven cycle-ergometer (Excalibur Lode, Groningen, The Netherlands) and an open-circuit spirometry system (Quark b^2; COSMED, Rome, Italy) as described (10). After 1 wk, each volunteer received a single administration of one tablet of placebo or tadalafil (20 mg, Cialis; Ely Lilly, Indianapolis, IN) at 0700–0800 h and performed the first experimental exercise test at 1500–1600 h. Then after a 14-d washout period, each volunteer repeated the exercise test after receiving tadalafil or placebo, respectively.

Exercise test

After a 3-min rest period on the cycle-ergometer for baseline evaluations, the exercise started with a 1-min warm-up without any added load, and then the workload was increased 30 W every 3 min until exhaustion; the pedaling rate was set at 60 rpm. During exercise a 12-lead electrocardiogram (Mortara-Rangoni, San Giorgio di Piano, Italy) and arterial blood pressure were recorded. Fingertip capillary blood collections for lactate measurements were taken before exercise, at the third minute of each workload and during the recovery (at first, third, sixth, and 10th minute). Lactate concentration was analyzed by using an Accusport lactate analyzer (Roche Molecular Biochemicals, Mannheim, Germany).

Saliva sample collections

Saliva samples were collected by using a cotton swab and saliva-collecting tube (Salivette; Sarstedt, Germany) before exercise (Pre-Ex), immediately after (*i.e.* 3 min) the end of exercise (Post-Ex), and at 30 min of recovery (+30-Rec). Contamination with food debris was avoided by rinsing the mouth with water and delaying the first collection for 15 min after rinsing to prevent sample dilution. Blood contamination was checked (Salimetrics LLC, State College, PA). After collection, saliva-collecting tubes were centrifuged at $2000 \times g$ for 15 min (at 7 C), and then samples were stored at −20 C until they were assayed for cortisol, dehydroepiandrosterone sulfate (DHEAS), and testosterone.

Hormones analyses

Salivary cortisol and DHEAS concentration were measured by ELISA, using a DRG Instruments GmbH commercial kit (Marburg, Germany). The sensitivity for cortisol and DHEAS were 0.537 and 0.025 ng/ml, respectively (conversion factors from nanograms per milliliter to nanomoles per liter were 0.3625 for cortisol and 2.71 for DHEAS). The intraassay and interassay coefficients of variation were, respectively, 2.65 and 7.47% for cortisol and 3.7 and 5.6% for DHEAS. Salivary ranges reported for men were 1.2–14.7 ng/ml for cortisol and 0.2–2.7 ng/ml for DHEAS.

Due to possible concerns on testosterone measurement (11, 12), salivary testosterone concentration was measured by using a RIA commercial kits for serum total testosterone using a modified protocol as proposed by the producer (Orion Diagnostica Spectria, Espoo, Finland). Testosterone standard 14,400 pg/ml included in the kit was diluted with a solution of 0.1 M Tris-HCL (pH 7.5) and 0.1% BSA to obtain as testosterone standards final concentrations of 17.28, 51.84, 158.4, 480.96, and 1440 pg/ml (the conversion factor from picograms per milliliter to nanomoles per liter was 288). One hundred microliters of every standard and saliva were used for assay. The sensitivity of this method was 28.8 pg/ml. Furthermore, an in-house assay's verification (*i.e. vs.* serum) in healthy and male hypogonadal subjects was also performed. The intraassay coefficient variation was 7.5% at 460.8 pg/ml, 4.5% at 1382.4 pg/ml, 3.8% at 3283.2 pg/ml, and 5.5% at 7632 pg/ml. The interassay coefficient variation was 7.0% at 345.6 pg/ml, 5.1% at 1008 pg/ml, 4.8% at 2620.8 pg/ml, and 6710.4 pg/ml. The reference range calculated for men of aged 21–30 yr was 66.0–246.4 pg/ml.

Statistical analysis

Because most outcome measures had skewed distributions, also after log transformation, we used medians, interquartile ranges (first and third), and nonparametric tests. For each variable, the statistical analysis was based on the nonparametric Friedman's test followed, whenever significant, by Wilcoxon test for matched pairs as *post hoc* analysis. A Spearman Rho correlation was used to evaluate possible correlations between PDE5i doses in milligrams per kilogram body $weight^{-1}$, $\dot{V}O_{2max}$, and lactate concentrations and all the evaluated hormonal parameters.

Statistical analyses were performed with the SPSS statistical package (version 15.0; SPSS Inc., Chicago, IL). $P \leq 0.05$ was viewed as significant.

Results

No major adverse events were observed during the study, apart from headache in two subjects taking tadalafil. Mean tadalafil dose was 0.28 (0.26–0.31) mg/kg $body\ weight^{-1}$, and the mean exercise duration was 24 min (21–29 and 20–32 after tadalafil and placebo, respectively).

No effects of tadalafil or placebo on Pre-Ex salivary hormones and respective ratios (DHEAS to cortisol, testosterone to cortisol, testosterone to DHEAS) have been observed (Fig. 1 and Table 1); this suggests that neither modifications of resting steroids production nor their selective transfer in saliva was induced by tadalafil.

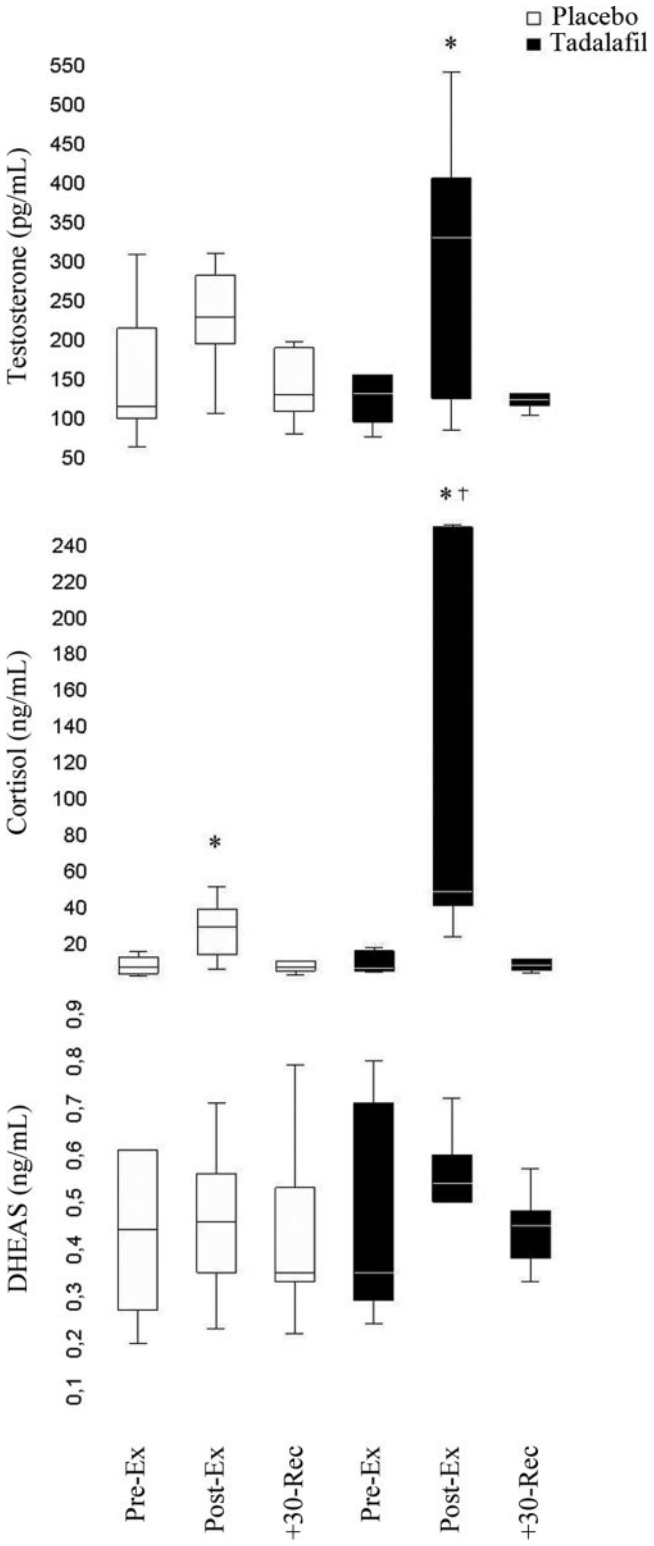

FIG. 1. Box plot of salivary steroids concentrations before (Pre-Ex), at the end (Post-Ex), and 30 min after (+30-Rec) a maximal exercise test on cycle ergometer after placebo (*open box*) and tadalafil (*dark box*) administration in healthy male athletes (n = 9). *Lower* and *upper edges of each box* represent the first and third quartile of observed data. The *line-partitioning box* corresponds to median observation and whiskers give range of data. *, $P < 0.05$ *vs.* respective Pre-Ex; †, $P < 0.05$ *vs.* placebo. (Conversion factors from nanograms per milliliter to nanomoles per liter were 0.3625 for cortisol and 2.71 for DHEAS; conversion factor from picograms per milliliter to nanomoles per liter was 288 for testosterone).

As expected, Post-Ex salivary cortisol increased after tadalafil and placebo ($P = 0.014$ and $P = 0.036$ *vs.* Pre-Ex, respectively). The Post-Ex salivary cortisol was higher after tadalafil ($P = 0.034$ *vs.* placebo) (Fig. 1). Salivary cortisol returned at Pre-Ex concentrations at 30 min of recovery in both treatments (Fig. 1).

Salivary testosterone increased immediately after exercise only after tadalafil ($P = 0.03$ *vs.* Pre-Ex) (Fig. 1).

Post-Ex salivary DHEAS to cortisol ratio decreased after tadalafil and placebo ($P = 0.003$ and $P = 0.062$ *vs.* Pre-Ex, respectively), but a lower Post-Ex DHEAS to cortisol ratio after tadalafil was observed ($P = 0.042$ *vs.* placebo) (Table 1).

After tadalafil, a decreased mean testosterone to cortisol ratio immediately after exercise was also observed ($P = 0.03$ *vs.* Pre-Ex), and this ratio was lower than respective placebo value ($P = 0.02$ *vs.* placebo) (Table 1).

No correlations between hormones and mean tadalafil dose, Pre-Ex salivary testosterone, and $\dot{V}O_{2max}$ have been observed. Furthermore, whereas the Post-Ex blood lactate after tadalafil and placebo were similar [12.3 (10.9–12.8) and 12.3 (10.9–12.8) mmol/liter, respectively; P = ns], a correlation between lactate and Post-Ex salivary cortisol and testosterone was observed after tadalafil (rho = 0.77, $P = 0.03$, and rho = 0.73, $P = 0.04$, respectively).

Discussion

The main finding of this study was that, compared with placebo, tadalafil administration has been able to amplify mean salivary cortisol and testosterone responses to maximal exercise, with concomitant further decrease of salivary DHEAS to cortisol and testosterone to cortisol ratios; and despite this, no differences in performance and lactate production has been observed between treatments.

The sites of action and the mechanisms involved in the observed effect of tadalafil on HPA axis response to physical stress are not known, and we could only speculate.

During stress events, neurons of paraventricular nuclei rapidly secrete CRH that activate HPA axis by stimulating ACTH secretion, that in turn stimulates cortisol output. Furthermore, in the central nervous system (CNS), the stress reaction is also associated with the activation of NO-producing neurons at the hypothalamic level (2) and to a stimulatory role for NO on the HPA axis (3, 4).

In the CNS tadalafil might enhance NO bioactivity during stress by inhibiting local PDE5: NO stimulates guanylate cyclase to catalyze the formation of cGMP, which intracellular levels are further increased and/or maintained by PDE5 inhibition.

A tadalafil-related increased NO biological activity in the CNS could have amplified the CRH-ACTH secretion because of NO-producing neurons are densely localized in the paraventricular nucleus and are involved in the regulation of the HPA axis. However, we also cannot confirm this hypothesis because we could not evaluate ACTH secretion.

The lack of modifications in salivary DHEAS after physical stress confirms that DHEAS secretion is not always parallel to cortisol secretion (13) but also suggests a possible selective

TABLE 1. Salivary steroid hormones ratios

	Placebo			Tadalafil		
Parameters	**Pre-Ex**	**Post-Ex**	**+30-Rec**	**Pre-Ex**	**Post-Ex**	**+30-Rec**
DHEAS to cortisol	0.07 (0.05–0.09)	0.03[a] (0.01–0.06)	0.05[b] (0.04–0.07)	0.06 (0.04–0.07)	0.009[c,d] (0.004–0.013)	0.05[e] (0.04–0.09)
Testosterone to DHEAS	424.53 (286.98–510.83)	453.18 (384.08–531.42)	414.69 (307.92–477.16)	345.60 (260.19–430.35)	363.15 (266.40–509.27)	324.38[b] (257.28–375.27)
Testosterone to cortisol	22.02 (18.17–32.46)	10.16 (6.93–19.17)	19.48 (13.90–22.37)	15.03 (9.47–23.54)	2.80[a,d] (1.81–6.21)	20.58[e] (10.24–27.25)

The parameters are evaluated before (Pre-Ex), at the end (Post-Ex), and 30 min after (+30-Rec) a maximal exercise test on cycle ergometer after placebo and tadalafil administration, in healthy male athletes (n = 9). Values are medians (interquartile range).

[a] $P < 0.05$ *vs.* respective Pre-Ex.

[b] $P < 0.05$ *vs.* respective Post-Ex.

[c] $P < 0.01$ *vs.* respective Pre-Ex.

[d] $P < 0.05$ *vs.* placebo.

[e] $P < 0.01$ *vs.* respective Post-Ex.

action of tadalafil at the adrenal level, in which PDE5 are expressed (14). A direct influence of NO on both pituitary and adrenal cortex has been reported (4), and NO has been shown to directly stimulate cortisol secretion. Our hypothesis is that tadalafil could have further stimulated cortisol secretion only by amplifying the adrenal zona fasciculata response to exercise-related ACTH increase in the bloodstream.

An increased HPA responsiveness to stress could be related to other effects of tadalafil on less known pathways, influenced by different phosphodiesterases (15), also depending on the tissues involved (16).

Furthermore, we highlight the wide variability of cortisol response to exercise after tadalafil that, at least in theory, could be related to individual responsiveness to PDE5i and exercise stress (1).

Besides heavy exercise can increase testosterone concentrations (17), in our study salivary testosterone increased after exercise only after tadalafil administration.

Whereas increased testosterone concentrations have been observed after tadalafil administration (18), no direct influences of tadalafil *per se* on the hypothalamus-pituitary-gonadal axis have been described in humans (18, 19). However, it is of interest that in animals studies, NO-producing neurons seem to influence LHRH secretion (20).

In conclusion, independently from possible effects on performance and/or on health, it is possible to argue that tadalafil influences the salivary cortisol and testosterone concentrations after physical stress.

We highlight that very few data exist, and we can only hypothesize that in our experimental conditions, tadalafil could have: 1) influenced the CNS sensitivity threshold to stress; 2) maximized, at different levels, the HPA and hypothalamus-pituitary-gonadal response to stress; and/or 3) influenced the cytochrome P450–3A-mediated steroid metabolism.

If confirmed, it would be necessary to identify whether these effects of tadalafil might be useful in specific clinical or working conditions and/or negative for health. Furthermore, at this moment we recommend a great caution in transferring our results in the areas of sport and doping.

In our opinion, this study is of importance because it represented the first investigation on the relationships between hormones, stress, and PDE5i. Further studies to verify the effects of different PDE5i treatments on hormones responses to various stressors and explore all the pathways involved are warranted.

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