

## REGULAR RESEARCH ARTICLE

# Does Methylphenidate Reduce Testosterone Levels in Humans? A Prospective Study in Children with Attention-Deficit/Hyperactivity Disorder

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## Abstract

**Background:** Animal studies and case reports have suggested that methylphenidate exerts adverse effects on gonadal hormones. This study aimed to determine whether methylphenidate alters testosterone levels in children with attention-deficit/hyperactivity disorder through comparison of those with or without methylphenidate treatment.

**Methods:** This 4-week, nonrandomized, prospective study conducted in Taiwan included 203 attention-deficit/hyperactivity disorder patients with a mean age of 8.7 years (boys: 75.8%). After the initial recruitment, 137 received daily methylphenidate treatment (medicated group) and 66 were assessed through naturalistic observation (nonmedicated group). The saliva samples of attention-deficit/hyperactivity disorder patients were used to quantify testosterone levels at baseline and the endpoint by using the chemiluminescence immunoassay. At the 4th week, 86 patients in the medicated group and 46 patients in the nonmedicated group were eligible for statistical analyses.

**Results:** During the study period, salivary testosterone levels did not significantly change in the medicated group ( $P = .389$ ) or in the nonmedicated group ( $P = .488$ ). After correction for the potential confounding effects of age and sex, salivary testosterone levels still remained unchanged in the medicated and nonmedicated groups during the 4-week follow-up. In the medicated group, changes in salivary testosterone levels over 4 weeks were not significantly correlated with the methylphenidate daily dose (mean daily dose: 18.1 mg).

**Conclusions:** Findings suggest that short-term treatment with methylphenidate at usual doses does not significantly alter salivary testosterone levels in attention-deficit/hyperactivity disorder patients. Future studies should clarify whether long-term methylphenidate treatment disrupts testosterone production as well as the function of the reproductive system.

**Keywords:** ADHD, endocrinology, stimulant, reproductive system, testosterone

## Significance Statement

Animal studies and case reports have suggested that methylphenidate (MPH) exerts adverse effects on the reproductive system or gonadal hormones. However, it is unclear whether MPH treatment reduces testosterone levels in children with attention-deficit/hyperactivity disorder (ADHD). In this prospective study, we provide the first evidence that short-term treatment with MPH at usual doses does not significantly alter salivary testosterone levels in ADHD patients, after correction for age and sex. In addition, MPH exerted no dose-dependent effect on testosterone levels. We suggest that caution should be exercised when indiscriminately applying the results of animal studies to clinical practice.

## Introduction

Methylphenidate (MPH) is a widely used stimulant for treating attention-deficit/hyperactivity disorder (ADHD) (Burcu et al., 2016). MPH increases dopamine and norepinephrine neurotransmissions through reuptake inhibition (Wilens, 2008). The neurobiological mechanism clinically manifests as improvements in the core symptoms of ADHD (Thapar and Cooper, 2015). Although the therapeutic effectiveness of MPH for children with ADHD has been well established, its safety and appropriateness have been debated for decades (Vaughan et al., 2012; Storebo et al., 2015). MPH treatment may lead to several adverse effects, including decreased appetite, sleep disturbance, headache, anxiety, irritability, and rarely occurring cardiovascular adverse events (Cortese et al., 2013; Martinez-Raga et al., 2013). Moreover, researchers have noticed that MPH treatment potentially affects the endocrine system of patients (Maayan et al., 2003; Lee et al., 2008; Wang et al., 2011, 2014). More recently, controversies have emerged regarding whether MPH interferes with the gonadal function of children, particularly testosterone levels.

Several animal studies have investigated the potential influence of MPH on the reproductive system, although their findings are contradictory. In rats that underwent adrenalectomy-testectomy, plasma testosterone levels decreased after MPH administration (Kaneyuki et al., 1979). Cansu et al. (2011) reported that MPH exerts dose-dependent negative effects on rat spermatogenesis. Several studies have consistently found that consecutive MPH exposure influences testis growth and negatively affects testosterone metabolism in adolescent rats (Adriani et al., 2006; Fazelipour et al., 2012; Montagnini et al., 2014). Moreover, chronic MPH exposure seems to impair appendicular bone quality in adolescent rats, and the possible mechanism underlying skeletal impairment is testosterone suppression (Komatsu et al., 2012). Mattison et al. (2011) indicated that MPH administration to juvenile male rhesus monkeys reduces serum testosterone levels and impairs pubertal testicular development. Avital et al. (2011) reported that environment enrichment in adolescent rats is a moderating factor between MPH administration and corticosterone and testosterone levels in their adulthood. By contrast, some studies have indicated that perinatal or postnatal MPH exposure does not significantly affect serum testosterone levels in rats (Ferguson and Boctor, 2010; Panos et al., 2014) and can even increase spermatogonia and testosterone levels (Fazelipour et al., 2014).

Relatively few studies have investigated the relationship between MPH and testosterone in humans. A clinical study revealed that youth who took MPH did not exhibit the normal diurnal variation in salivary testosterone levels throughout the day (Hibel et al., 2007). Schmid et al. (2015) reported that MPH increased adult participants' sexual arousal for explicit sexual stimuli, and the appraisal of sexual stimuli was not correlated with plasma testosterone levels. Seibert et al. (2014) found that acute administration of a single dose of MPH (60 mg) to adults did not affect their plasma testosterone levels. However,

2 case reports have indicated that MPH may reduce testosterone levels and delay sexual maturation (Ramasamy et al., 2014; Akaltun, 2016). A longitudinal study revealed that prolonged treatment (more than 3 years) with stimulants may delay the development of puberty (Poulton et al., 2013). Nonetheless, whether MPH alters testosterone levels in humans is under debate.

Testosterone is the main sex hormone produced by the testicles and the adrenal glands and is crucial for the proper development of male sexual characteristics (Albin and Norjavaara, 2013). If MPH does disrupt endogenous testosterone and exerts adverse effects on the reproductive system of children, this would be a serious public health issue that should be addressed. To date, no prospective study has examined whether MPH treatment reduces the testosterone levels in children with ADHD in the naturalistic clinical setting. To fill this research gap, this study examined the changes in testosterone levels in children with ADHD receiving 4-week MPH treatment through comparison with their counterparts without MPH treatment. In addition, this study investigated the potential dose-dependent effect of MPH on changes in testosterone levels.

## Methods

The study protocol was approved by the Institutional Review Board of Chang Gung Hospital in Taiwan (IRB no. 101-4835A3). All procedures performed in this study involving participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration. This clinical study has been registered at ClinicalTrials.gov (trial registration: NCT02392169, <https://register.clinicaltrials.gov/>).

## Study Participants

We recruited eligible outpatients with ADHD receiving treatment at the Department of Child Psychiatry at Kaohsiung Chang Gung Memorial Hospital in Taiwan from August 2013 to December 2015. The inclusion criteria were (1) clinical diagnosis of ADHD by a senior child psychiatrist based on the criteria of the DSM-IV-TR after structured interviews based on the Chinese version of the Schedule for Affective Disorders and Schizophrenia for School-Age Children, epidemiologic version (K-SADS-E) (Kaufman et al., 1997); (2) age between 6 and 16 years; (3) Han Chinese ethnicity; (4) new diagnosis of ADHD in a drug-naïve patient or a patient who had an existing diagnosis but had not used ADHD medication in the previous 6 months or longer; and (5) no history of comorbid autistic spectrum disorders, intellectual disability, major depressive disorder, bipolar disorder, psychosis, epilepsy, or brain injury. In total, 203 ADHD patients (mean age,  $8.7 \pm 2.0$  years) met the inclusion criteria and were included in this study.

## Laboratory Testing of Testosterone Levels

To measure testosterone levels, the saliva samples of participants were collected at 8:00 AM in the outpatient department by using the passive drool method. Participants were instructed to avoid excessive levels of physical activity in the preceding 24 hours and to fast overnight before saliva collection. Saliva samples were collected in collecting tubes, immediately placed on ice, and stored at  $-80^{\circ}\text{C}$  until further analysis. The chemiluminescence immunoassay (IB57403; IBL-America) was used to quantify salivary testosterone levels. The sensitivity of detection of this method is 1.0 pg/mL. The intra- and interassay CVs are 2.9% to 7.0% at 21 to 557 pg/mL and 1.5% to 3.2% at 38 to 541 pg/mL, respectively.

## Clinical Measurements

The K-SADS-E is a semistructured diagnostic interview that is designed to assess current and past episodes of psychopathology in children and adolescents according to DSM-III-R and DSM-IV criteria (Kaufman et al., 1997). The K-SADS-E is administered by interviewing the parent(s) and the child and finally achieving summary ratings that include all sources of information. The validity and reliability of the Chinese version of the K-SADS-E have been established in Taiwan (Gau and Soong, 1999).

The Chinese version of the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) is an individually administered and norm-referenced instrument designed to measure the intelligence of children aged from 6 to 16 years (Yang et al., 2013). The WISC-IV contains 10 core and 5 supplemental subtests. The core subtests are used to form 4 factor indices, and the Full Scale Intelligence Quotient is also formed from the 10 core subtests. The factor indices and Full Scale Intelligence Quotient each has a population mean of 100 and a standard deviation of 15 (Baron, 2005).

The Swanson, Nolan, and Pelham Version IV Scale (SNAP-IV) is a 26-item questionnaire used to evaluate ADHD symptoms and severity; the SNAP-IV is completed by parents or teachers (Bussing et al., 2008). The 26 items comprise 18 for ADHD symptoms (9 for inattentive and 9 for hyperactive/impulsive) and 8 for oppositional defiant disorder symptoms, as defined in DSM-IV. Each item is scored on a 0 to 3 Likert scale. The Chinese versions of the SNAP-IV parent form (Gau et al., 2008) and the SNAP-IV teacher form (Gau et al., 2009) have satisfactory levels of reliability and concurrent validity.

The Conners' Continuous Performance Test (CPT) is a 14-minute computerized test that primarily assesses attention and impulse control (Conners, 1985). Among the multiple dependent measures used, omissions, commissions, and detectability are the most commonly used indices. The T-score of the CPT is commonly used in research analyses, and a higher T-score indicates poorer performance. The Confidence Index (percentile) integrates all CPT data obtained from test administration to provide a chance out of 100 that a significant attention problem exists (Conners, 2004).

## Study Procedures

In this 4-week, nonrandomized, observational, prospective study, each participant was initially interviewed using the K-SADS-E diagnostic tool by a senior psychiatrist and was then administered the WISC-IV by an experienced child psychologist. At visit 1 (V1; pretreatment), saliva samples were collected from ADHD patients at 8:00 AM in the outpatient department. Subsequently, the patients' parents or caregivers and their teachers completed

the SNAP-IV parent form and the SNAP-IV teacher form, respectively. Individual patients were administered the CPT by an experienced child psychologist in a room at approximately 9:00 AM to reduce variability in testing conditions.

After completion of testing, the patients and their parents or caregivers were counseled regarding information on ADHD, adequate parenting skills, and possible treatment options. If the parents or caregivers opted for drug therapy (medicated group), the patients were prescribed oral immediate-release MPH once, twice, or thrice per day, with each dose ranging between 0.3 and 1.0 mg/kg, determined based on the clinical symptoms, as well as the patient's age, height, and body weight. The dosage was adjusted every 1 or 2 weeks to properly improve ADHD symptoms. Those who required less frequent dosing interval were switched to once daily use of osmotic controlled-release formulations of MPH. Patients could not take a drug holiday, and concomitant medications were not permitted in the protocol. The MPH dosage may have been modified for some patients during the 4-week follow-up. Drug compliance was confirmed at each visit based on reports of the patients' parents or caregivers and presentation of the remaining MPH medication. If the parents or caregivers opted to not receive drug therapy (nonmedicated group), patient care was left to the discretion of the psychiatrists' usual practice in the outpatient department. Reeducation and supportive psychotherapy were allowed, but no psychotropic drug was permitted during the follow-up period.

At visit 2 (V2), which occurred 4 weeks after V1, the procedures performed at V1 were repeated. The saliva samples of ADHD patients were collected at 8:00 AM. Subsequently, the patients' caregivers and teachers completed the SNAP-IV parent form and the SNAP-IV teacher form, respectively. The patients were administered the CPT at approximately 9:00 AM.

Figure 1 summarizes the study procedure and patient allocation. At the initial recruitment, of the 203 patients, 137 (67.5%) received MPH treatment (medicated group) and 66 (32.5%) did not (nonmedicated group). The exclusion criteria were (1) lost to follow-up or were ineligible for providing saliva samples at V2 ( $n = 47$ ); (2) the amount of saliva sample was insufficient, or testosterone levels were undetermined ( $n = 11$ ); (3) salivary testosterone levels were outliers ( $n = 3$ ); (4) drug compliance was  $<80\%$  of total prescription ( $n = 3$ ); and (5) premature discontinuation of MPH treatment because of ineffectiveness or intolerable side effects ( $n = 7$ ). Finally, 86 (62.8%) patients in the medicated group and 46 (69.7%) patients in the nonmedicated group were included in statistical analyses.

## Statistical Analyses

Data were analyzed using SPSS, version 16.0 (SPSS Inc). Variables are expressed as the mean  $\pm$  SD or frequency. Categorical variables were compared between medicated group and nonmedicated group by using the chi-square test or the Fisher's exact test, depending on the sample size. Continuous variables were compared between the groups by using an independent  $t$  test.

Repeated-measure ANOVA was applied to analyze the differences in changes in testosterone levels between medicated and nonmedicated groups over 4 weeks. The within-group effect (time effect) and the between-group effect were analyzed. The hypothesis that a differential change exists in testosterone levels between medicated group and nonmedicated group over 4 weeks would be supported by a group  $\times$  time interaction on dependent measures. We subsequently controlled for the potential confounders of age, sex, and ADHD subtypes to verify the results obtained from aforementioned repeated-measure ANOVA analyses.

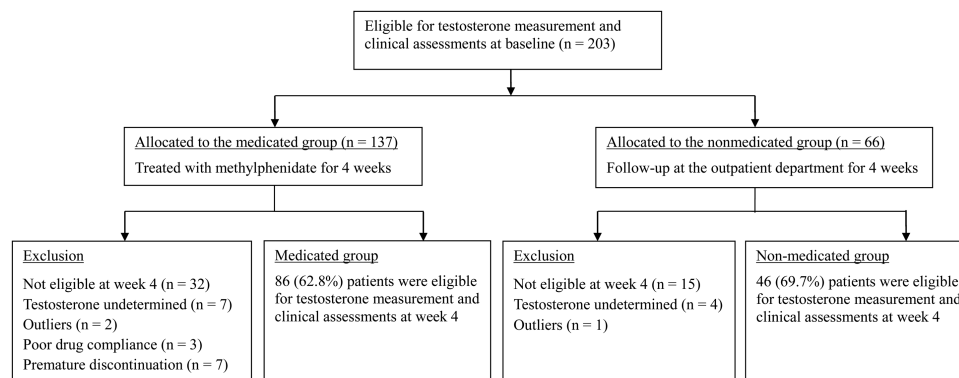


Figure 1. Flowchart of the study procedure and patient allocation.

The paired *t* test was used to examine whether salivary testosterone levels changed in each group during the 4-week observation. To exclude the confounding effects of age and sex, sensitivity analyses were performed to test the robustness of the results. First, we stratified patients into a younger group and an older group based on the median age of 8.3 years; subsequently, we used the paired *t* test to examine the changes in testosterone levels between the 2 age groups. Second, the paired *t* test was applied to investigate the changes in testosterone levels between boys and girls. Finally, we used the propensity score matching technique to create a matching medicated cohort with a ratio of 1:1 for the nonmedicated group. The propensity scores were determined using multiple logistic regression with age, sex, and ADHD subtypes as the confounding covariates. Subsequently, we used the paired *t* test to examine the changes in testosterone levels between the 2 matched groups.

Moreover, the paired *t* test was used to examine whether the behavioral symptoms (scores of SNAP-IV) and neuropsychological performance (indices of CPT) changed in each group during the 4-week observation. Pearson correlation was performed to analyze the relationships among changes in testosterone levels, MPH daily dose, changes in behavioral symptoms, and CPT performance over the 4-week follow-up. Two-tailed *P* values of  $< .05$  were considered statistically significant.

## Results

### Patient Characteristics

Of the 86 patients in the medicated group (mean age:  $8.9 \pm 2.0$  years), 69 (80.2%) were boys and 17 (19.8%) were girls. Of the 46 patients in the nonmedicated group (mean age:  $8.0 \pm 1.6$  years), 31 (67.4%) were boys and 15 (32.6%) were girls. Compared with the nonmedicated group (Table 1), patients in the medicated group were older ( $t = 2.66$ ,  $P = .010$ ) and had higher oppositional scores on the parent-rated SNAP-IV ( $t = 2.89$ ,  $P = .005$ ).

### Trends of Salivary Testosterone Levels

Figure 2 summarizes the changes in salivary testosterone levels between the medicated group and nonmedicated group over 4 weeks. Repeated-measure ANOVA revealed a significant group effect in testosterone levels (medicated group  $>$  nonmedicated group,  $F = 4.097$ ,  $P = .045$ ). However, no significant time effect ( $F = 1.143$ ,  $P = .287$ ) or group  $\times$  time interaction was observed in testosterone levels ( $F = 0.001$ ,  $P = .971$ ). After controlling for age, sex, and ADHD subtypes, repeated-measure ANOVA revealed

that receiving MPH treatment or not was not associated with testosterone levels ( $F = 1.372$ ,  $P = .244$ ). Moreover, no significant time effect ( $F = 1.89$ ,  $P = .655$ ) or group  $\times$  time interaction was observed in testosterone levels ( $F = 0.174$ ,  $P = .677$ ). However, age was significantly associated with salivary testosterone levels ( $F = 12.506$ ,  $P = .001$ ).

The paired *t* test revealed that salivary testosterone levels did not change in the medicated group ( $48.0 \pm 65.6$  pg/mL at V1;  $42.9 \pm 58.4$  pg/mL at V2;  $t = 0.866$ ,  $P = .389$ ) or in the nonmedicated group ( $30.2 \pm 48.1$  pg/mL at V1;  $24.9 \pm 28.0$  pg/mL at V2;  $t = 0.699$ ,  $P = .488$ ) during the study period (Figure 2A). Figure 2B and C, respectively, depict the trends of testosterone levels in patients stratified by age and sex. Irrespective of age or sex, salivary testosterone levels did not significantly change in the medicated and nonmedicated groups during the 4-week follow-up. The propensity score matching cohorts (supplementary Table 1) also showed that salivary testosterone levels did not significantly change in the medicated group ( $34.9 \pm 46.2$  pg/mL at V1;  $32.9 \pm 46.3$  pg/mL at V2;  $t = 0.224$ ,  $P = .824$ ) or in the nonmedicated group ( $30.2 \pm 48.1$  pg/mL at V1;  $24.9 \pm 28.0$  pg/mL at V2;  $t = 0.699$ ,  $P = .488$ ) during the study period.

In the medicated group, the average daily dose of MPH administered for treating ADHD was  $18.1 \pm 10.5$  mg, and the ratio of the MPH dose to body weight was  $0.57 \pm 0.30$  mg/kg. Changes in salivary testosterone levels over 4 weeks were not significantly correlated with the MPH daily dose ( $r = 0.005$ ,  $P = .964$ ) or the ratio of the MPH dose to body weight ( $r = 0.036$ ,  $P = .742$ ) (Figure 3).

### Trends of Clinical Measurements

In the medicated group (Table 2), 4-week MPH treatment significantly improved the inattentive, hyperactive/impulsive, and oppositional defiant disorder symptoms rated by patients' caregivers and their teachers. In the nonmedicated group, all behavioral symptoms, except for caregivers' rated inattentive symptoms, also significantly improved in 4 weeks. Regarding the CPT assessment, the commission errors were significantly decreased in the medicated group ( $t = 2.728$ ,  $P = .008$ ), whereas none of the CPT indices significantly improved in the nonmedicated group. In all patients, changes in salivary testosterone levels were not significantly correlated with changes in any behavioral symptom or index of the CPT ( $P > 0.05$ ).

## Discussion

The main finding of this study is that short-term treatment with MPH at usual doses did not significantly alter salivary

**Table 1.** Characteristics of ADHD Patients Treated with Methylphenidate (Medicated) and Those Who Were Nonmedicated at Baseline

|  | Medicated<br>(n = 86) | Nonmedicated<br>(n = 46) | Statistical values | P value |
|--|-----------------------|--------------------------|--------------------|---------|
| Age (month)                                    | 106.5 ± 23.6          | 96.0 ± 19.3              | t = 2.660          | .010*   |
| Sex, n (%)                                     |                       |                          | $\chi^2 = 2.691$   | .101    |
| Male   | 69 (80.2)             | 31 (67.4)                |                    |         |
| Female   | 17 (19.8)             | 15 (32.6)                |                    |         |
| ADHD subtypes, n (%)                           |                       |                          | $\chi^2 = 3.821$   | .051    |
| Inattentive type                               | 23 (26.7)             | 20 (43.5)                |                    |         |
| Hyperactive/impulsive or combined type         | 63 (73.3)             | 26 (56.5)                |                    |         |
| Comorbidities, n (%)                           |                       |                          |                    |         |
| Oppositional defiant disorder/conduct disorder | 17 (19.8)             | 4 (8.7)                  | $\chi^2 = 2.746$   | .134    |
| Tic disorders                                  | 8 (9.3)               | 5 (10.9)                 | $\chi^2 = 0.083$   | .773    |
| Anxiety disorders                              | 3 (3.5)               | 1 (2.2)                  | $\chi^2 = 0.176$   | .675    |
| Height (cm)                                    | 132.6 ± 11.9          | 129.4 ± 11.4             | t = 1.517          | .132    |
| Weight (kg)                                    | 32.2 ± 9.5            | 30.0 ± 10.6              | t = 1.204          | .231    |
| FSIQ of the WISC                               | 94.6 ± 12.8           | 97.8 ± 13.4              | t = -1.351         | .179    |
| Behavioral symptoms                            |                       |                          |                    |         |
| SNAP-IV parent form (I)                        | 15.8 ± 5.2            | 14.2 ± 4.7               | t = 1.639          | .104    |
| SNAP-IV parent form (H)                        | 13.7 ± 5.6            | 12.3 ± 5.6               | t = 1.351          | .179    |
| SNAP-IV parent form (O)                        | 11.9 ± 5.1            | 9.0 ± 5.8                | t = 2.889          | .005**  |
| SNAP-IV teacher form (I)                       | 13.5 ± 5.9            | 13.5 ± 6.0               | t = 0.000          | 1.000   |
| SNAP-IV teacher form (H)                       | 10.5 ± 7.2            | 10.4 ± 7.2               | t = 0.068          | .946    |
| SNAP-IV teacher form (O)                       | 7.1 ± 5.9             | 6.6 ± 6.4                | t = 0.375          | .709    |
| Indices of CPT                                 |                       |                          |                    |         |
| Confidence index                               | 58.9 ± 23.1           | 53.9 ± 23.8              | t = 1.112          | .268    |
| Omission                                       | 61.6 ± 30.2           | 58.3 ± 28.4              | t = 0.586          | .559    |
| Commission                                     | 51.0 ± 10.0           | 49.8 ± 10.9              | t = 0.620          | .536    |
| Detectability                                  | 51.2 ± 9.9            | 50.9 ± 10.1              | t = 0.181          | .857    |

Abbreviations: CPT, Conners' Continuous Performance Test; FSIQ, Full Scale Intelligence Quotient; H, hyperactivity/impulsivity scores; I, inattention scores; O, oppositional scores; SNAP-IV, the Swanson, Nolan, and Pelham–Version IV Scale for ADHD; WISC-IV, the Wechsler Intelligence Scale for Children–Fourth Edition.

\*P < .05, \*\*P < .01.

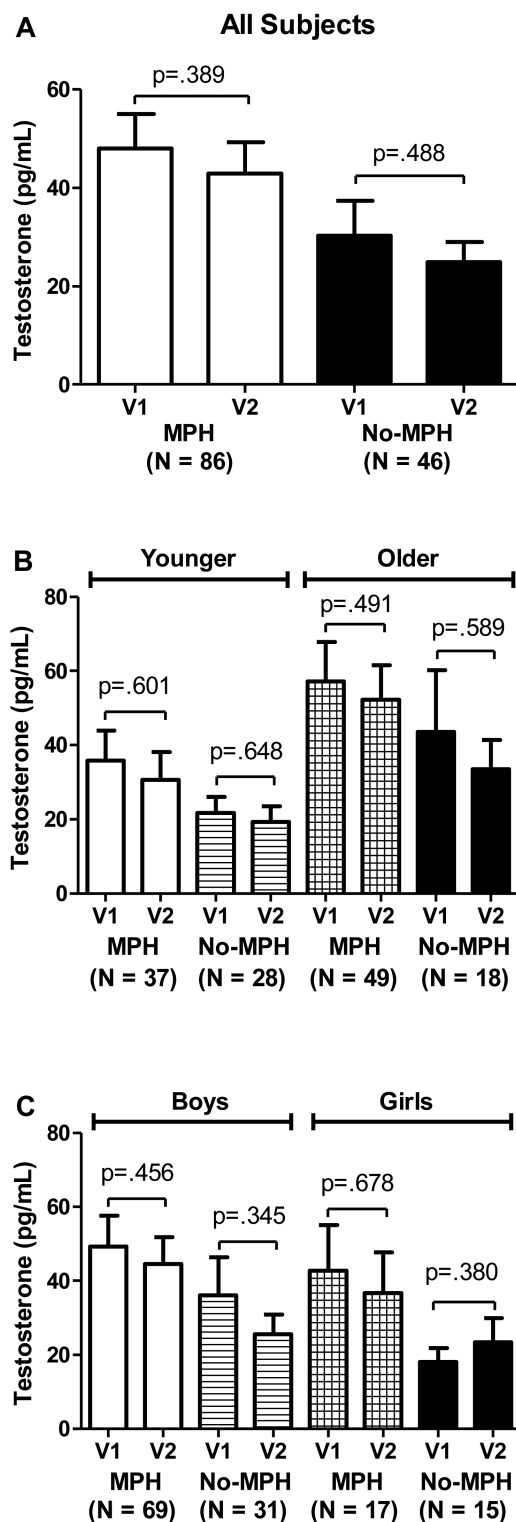
Data are expressed as mean ± SD or n (%).

testosterone levels in drug-naïve ADHD patients in the clinical setting after correction for the potential confounding effects of age and sex. Moreover, MPH exerted no dose-dependent effect on changes in salivary testosterone levels. In addition, changes in testosterone levels were not correlated with patients' behavioral symptoms or neuropsychological function.

Although repeated-measure ANOVA initially showed that the medicated group had a higher testosterone level than the nonmedicated group ( $P = .045$ ), this difference disappeared after controlling for the confounding effects of age, sex, and ADHD subtypes. We suggest that age is the main factor correlated with salivary testosterone levels ( $r = 0.288$ ,  $P = .001$ ). Notably, the patients' allocation in this study was through clinical judgment and self-selection in a naturalistic setting, but not random assignment. The distributions of age, sex, and clinical manifestations in the medicated and nonmedicated groups were not balanced. Therefore, we used stratification and propensity score-matching strategies to reduce the potential confounding effects of age and sex. Our data show that 4-week MPH administration did not significantly alter salivary testosterone levels in patients. Consistent with our results, animal studies have shown that short-term perinatal or postnatal MPH exposure did not significantly affect the serum testosterone levels of Sprague–Dawley rats (Ferguson and Boctor, 2010; Panos et al., 2014). Another study indicated that acute administration of MPH (60 mg) did not influence the plasma testosterone levels of healthy adult subjects (Seibert et al., 2014). These results generally demonstrate that

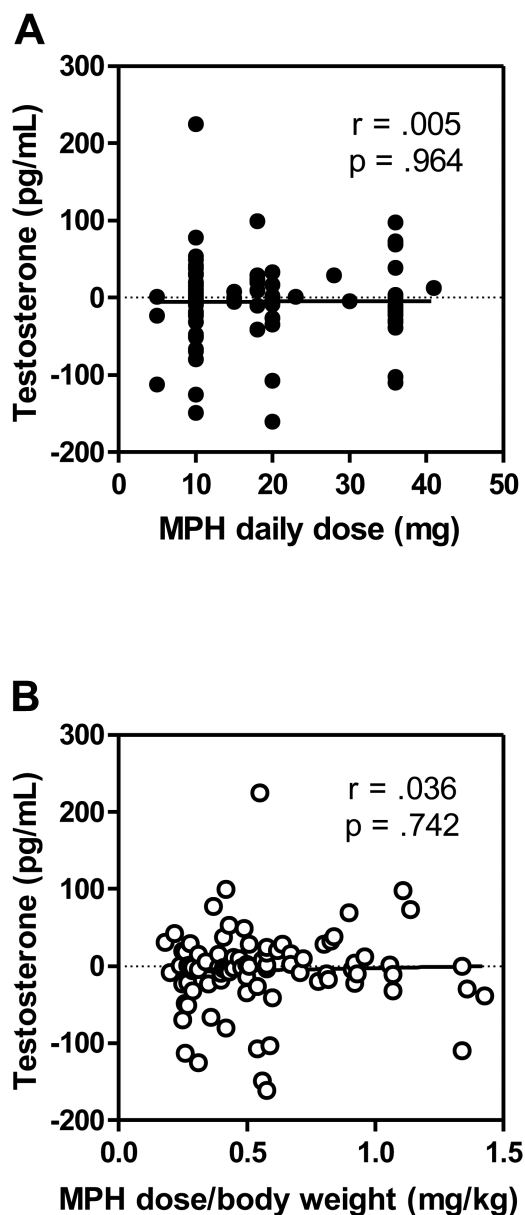
MPH at effective therapeutic doses does not alter testosterone levels.

By contrast, several studies have reported the reduction of serum testosterone levels in rats (Kaneyuki et al., 1979; Adriani et al., 2006; Fazelipour et al., 2012; Montagnini et al., 2014) and rhesus monkeys after MPH exposure (Mattison et al., 2011). The most plausible explanation for this phenomenon is that MPH suppresses testicular secretion of testosterone. Suppression by MPH can be explained by various mechanisms. First, MPH suppresses the testicular secretion of testosterone by increasing p53 expression and apoptosis of germ cells (Cansu et al., 2011). Second, MPH influences the function of the hypothalamus by interfering with the pulsatile release of gonadotropin-releasing hormone in the hypothalamus (Chatterjee-Chakrabarty et al., 2005). Third, MPH facilitates hepatic enzyme induction and increases testosterone degradation (Adriani et al., 2006). However, some discrepancies exist in the dosing strategy and the duration of treatment between animal studies and the current study in the naturalistic clinical setting. For example, the effective dose of MPH ranges from 0.3 to 1.0 mg/kg in humans (Feldman and Reiff, 2014), and the average MPH dose administered to our study population was 0.57 mg/kg. The equivalent dose of MPH in rats to a clinically relevant dose in humans is 1.7 to 5.5 mg/kg (Solanto, 2000). However, doses higher than this range (10–30 mg/kg) were administered for rats in some studies (Fazelipour et al., 2012, 2014; Komatsu et al., 2012), whereas a median to high dose (5–10 mg/kg) was used in another study (Cansu et al., 2011). In juvenile rhesus monkeys with



**Figure 2.** Trends of salivary testosterone levels in the medicated group (MPH) and the nonmedicated group (No-MPH). (A) All patients. (B) Stratification by age: The younger group included patients aged  $\leq 8.3$  years; the older group included patients aged  $> 8.3$  years. (C) Stratification by sex. Note: V1 (visit 1) represents the baseline in the pretreatment status, and V2 (visit 2) occurred 4 weeks after V1. No significant differences were observed in any pairwise comparison using paired t test.

the observed pubertal delay, MPH was administered with 2.5 to 12.5 mg/kg twice per day since approximately 2 y old, for a



**Figure 3.** Relationship between changes in salivary testosterone levels and dose of methylphenidate (MPH). (A) The relationship between changes in salivary testosterone levels over 4 weeks and the daily dose of MPH ( $n = 86$ ). (B) The relationship between changes in salivary testosterone levels and the ratio of the daily MPH dose to body weight (mg/kg) in ADHD patients.

total duration of 40 months (Mattison et al., 2011). The dosage administered for rhesus monkeys is somewhat lower than the human equivalent doses clinically used in pediatric patients (Yang et al., 2014). Nonetheless, deficits in testicular volume and testosterone secretion noticed in monkeys were only transient (disappeared over the 40-month observation period), suggesting that the impact of MPH on pubertal development is not permanent. Altogether, the MPH doses, the age at MPH treatment, and the duration of treatment are considerably heterogeneous across the aforementioned studies. Species-specific neurologic effects are also likely to be involved in the observed discrepancies. In sum, we suggest that caution should be exercised when indiscriminately applying the results of animal studies to clinical practice.

**Table 2.** Changes in Clinical Measures of ADHD Patients Treated with Methylphenidate (Medicated) and Those Who Were Nonmedicated over the 4-Week Follow-Up

|                            | Medicated   |             |       |          | Nonmedicated |             |        |         |
|----------------------------|-------------|-------------|-------|----------|--------------|-------------|--------|---------|
|                            | Baseline    | Week 4      | t     | P value  | Baseline     | Week 4      | t      | P-value |
| <b>Behavioral symptoms</b> |             |             |       |          |              |             |        |         |
| SNAP-IV parent form (I)    | 15.7 ± 5.3  | 11.7 ± 5.4  | 6.744 | <.001*** | 14.2 ± 4.7   | 12.8 ± 4.5  | 1.839  | .073    |
| SNAP-IV parent form (H)    | 13.6 ± 5.6  | 9.8 ± 5.5   | 5.367 | <.001*** | 12.3 ± 5.6   | 10.1 ± 5.7  | 2.937  | .005**  |
| SNAP-IV parent form (O)    | 11.7 ± 5.1  | 9.1 ± 5.5   | 4.158 | <.001*** | 9.0 ± 5.8    | 7.4 ± 5.4   | 2.600  | .013*   |
| SNAP-IV teacher form (I)   | 13.7 ± 6.0  | 10.7 ± 5.8  | 3.820 | <.001*** | 13.3 ± 6.1   | 11.4 ± 5.6  | 2.329  | .025*   |
| SNAP-IV teacher form (H)   | 10.4 ± 7.0  | 8.1 ± 6.4   | 2.755 | .007**   | 10.9 ± 7.3   | 9.2 ± 6.2   | 2.125  | .040*   |
| SNAP-IV teacher form (O)   | 7.1 ± 5.8   | 5.0 ± 4.7   | 3.166 | .002**   | 7.0 ± 6.6    | 5.5 ± 5.5   | 2.154  | .037*   |
| <b>Indices of CPT</b>      |             |             |       |          |              |             |        |         |
| Confidence index           | 58.7 ± 23.1 | 56.9 ± 23.5 | 0.590 | .557     | 53.7 ± 24.0  | 55.5 ± 24.6 | -0.560 | .578    |
| Omission                   | 61.8 ± 30.8 | 56.9 ± 21.8 | 1.297 | .199     | 58.5 ± 28.7  | 56.9 ± 29.3 | 0.452  | .654    |
| Commission                 | 50.9 ± 10.1 | 47.4 ± 13.2 | 2.728 | .008**   | 49.4 ± 10.8  | 47.8 ± 10.6 | 1.466  | .151    |
| Detectability              | 51.1 ± 10.0 | 48.8 ± 12.8 | 1.594 | .115     | 50.6 ± 10.1  | 50.0 ± 9.4  | 0.373  | .711    |

Abbreviations: CPT, Conners' Continuous Performance Test; H, hyperactivity/impulsivity scores; I, inattention scores; O, oppositional scores; SNAP-IV, the Swanson, Nolan, and Pelham-Version IV Scale for ADHD. \*P < .05, \*\*P < .01, \*\*\*P < .001.

Hibel et al. (2007) reported that MPH treatment resulted in a flattened diurnal variation in the salivary testosterone levels of children aged 6 to 13 years throughout the day. The diurnal changes in salivary testosterone levels were not identified in the current study; therefore, we could not validate or controvert the finding of Hibel et al. (2007). Instead, in this study, we determined the salivary testosterone levels at the same time point (8:00 AM) to minimize the variation in diurnal testosterone levels. Moreover, 2 case reports have suggested that MPH adversely affects gonadal hormones (Ramasamy et al., 2014; Akaltun, 2016). The first case report involved a 20-year-old man in the United States who received MPH treatment since the age of 3 years (Ramasamy et al., 2014). The second case report involved a 14-year-old boy in Turkey who underwent surgery for his undescended testis at the age of 6 years. His blood testosterone level decreased after 2 weeks of MPH treatment (Akaltun, 2016). The participants recruited to the current study were aged from 6 to 16 years and were physically healthy. Therefore, the characteristics of our study population are different from those of the 2 patients in the aforementioned case reports. Furthermore, in evidence-based medicine, case reports are generally regarded as a type of anecdotal evidence, because they lack control groups and exhibit limited statistical validity (Evidence-Based Medicine Working Group, 1992). The findings for our study population cohort provide the first evidence that short-term MPH treatment does not significantly change salivary testosterone levels in ADHD patients in the clinical setting. However, in the current study, the MPH doses (18.1 ± 10.5 mg) were low, and the duration of treatment was short (4 weeks). Additional studies should investigate whether long-term treatment with medium or high doses of MPH influences the testosterone levels or the reproductive system of children.

In the medicated group, 4-week MPH treatment significantly improved all behavioral symptoms. Most symptoms also improved in the nonmedicated group. Because of the open-label, nonrandomized study design, behavioral symptoms rated subjectively by parents or teachers can be easily inflated by the placebo effect or reporting bias. By contrast, neuropsychological performance (the CPT) is less likely to be influenced by the placebo effect or reporting bias. We found that the commission errors of the CPT were significantly decreased in the medicated group, but not in the nonmedicated group. This finding implies that MPH treatment is beneficial for ameliorating impulsivity

levels of ADHD patients. In all patients, changes in salivary testosterone levels were not significantly correlated with changes in any behavioral symptom or index of the CPT. Some studies have suggested that testosterone causes physiological alteration but does not necessarily cause ADHD-like manifestations (Dorn et al., 2009; Rice, 2015; van der Meij et al., 2016). The finding of our study supports this point of view.

This study has several limitations. First, patients in the current study were observed for only 4 weeks. Additional prospective studies with a longer follow-up period are warranted to identify whether long-term MPH treatment alters testosterone levels. Second, patients' allocation to the medicated or nonmedicated group was not through random assignment. The main appeal of the randomized controlled design is the reduction of potential selection bias. Therefore, although stratification and propensity score-matching strategies were carried out, selection bias might not be totally eliminated from this study. Third, sexual maturation (i.e., Tanner Stage) and morphology of the sexual organ (i.e., testis size) were not assessed in this study. Therefore, we lacked this information to assist in dealing with potential bias or to understand the effect of MPH administration on sexual maturation. Fourth, this study did not include a placebo control group or a healthy control group for comparison. We could not determine whether testosterone had been influenced by a placebo effect; meanwhile, we could not compare the testosterone levels in ADHD patients with those in children without ADHD. Fifth, saliva samples might not be the most ideal specimen for determining testosterone levels. Compared with blood samples (i.e., plasma or serum), the testosterone level in saliva is lower and more variable (Albrecht and Styne, 2007). However, collecting saliva is a noninvasive approach and is more acceptable for children and their parents. Finally, testosterone was quantified using an enzyme immunoassays method in this study. Relative to enzyme immunoassays, a newer technique (i.e., liquid chromatography tandem mass spectrometry) has higher accuracy for determining salivary testosterone levels (Welker et al., 2016). Hence, the possibility of overestimation of testosterone levels cannot be excluded in this study.

## Conclusion

Despite study limitations, this study provided the first evidence of the trend of testosterone levels in children with ADHD

receiving MPH treatment in the clinical setting. The findings of the current study revealed that short-term treatment with MPH at usual doses does not significantly alter salivary testosterone levels in ADHD patients. The MPH doses administered for treating ADHD are not associated with changes in testosterone levels. In summary, the findings of previous animal studies might not be excessively generalized to humans. However, future studies should clarify whether long-term MPH treatment disrupts testosterone production as well as the function of the reproductive system.

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## Statement of Interest

None.

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