Bioavailability of testosterone enanthate dependent on genetic variation in the phosphodiesterase 7B but not on the uridine 5′-diphospho-glucuronosyltransferase (*UGT2B17*) gene

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Objective **To study the disposition of serum testosterone and seven of its metabolites before and after 2 days of an intramuscular dose (500 mg) of testosterone enanthate in relation to the phosphodiesterase (PDE7B) and the uridine 5′-diphospho-glucuronosyltransferase (UGT2B17) genotypes.**

Methods **Patients were genotyped for UGT2B17 deletion polymorphism and single nucleotide polymorphisms in the *PDE7B* gene. The involvement of PDE7B in hydrolysis of enanthate was assessed in human liver homogenates.**

Results **Genetic variation in the *PDE7B* gene was found to be associated with the serum level of testosterone. Individuals homozygous for *PDE7B* rs7774640 G allele had a smaller increase (2.5-fold) in the serum testosterone levels compared with carriers of the A allele (3.9-fold, $P=0.0006$). In addition, genetic variation in the *PDE7B* gene significantly influences the testosterone/epitestosterone ratio, a biomarker of testosterone doping. Our in-vitro incubation studies confirmed that PDE7B serves as a catalyst of the hydrolysis of testosterone enanthate. The UGT2B17 deletion polymorphism did not show any significant association with serum testosterone levels or the other androgen metabolites investigated.**

Conclusion **We have shown that PDE7B is involved in the hydrolysis of testosterone enanthate and that genetic variation in the *PDE7B* gene is a determinant of the systemic levels of testosterone after administration of testosterone enanthate. It is reasonable to believe that the genetic variation in testosterone bioavailability may be correlated to varying effects of this androgen, whether it is used for replacement therapy or abused in doping. Thus our results may be important to consider in doping test programmes and in therapeutics with androgens and other esterified drugs. *Pharmacogenetics and Genomics* 21:325–332 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: phosphodiesterase, testosterone, testosterone glucuronide and epitestosterone glucuronide ratio, uridine 5′-diphospho-glucuronosyltransferase

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Introduction

Testosterone is responsible for normal growth and development of the male reproductive system and for maintenance of secondary sex characteristics. It has been in clinical use for approximately 60 years, and testosterone replacement therapy in men with hypogonadism is a widespread and growing practice. There are a number of pharmaceutical formulations approved for this treatment including buccal tablets, oral capsules, intramuscular depot preparations and gels (reviewed in [1]). To date, injectable testosterone esters, that is, testosterone enanthate and testosterone decanoate, are the most common formulations used. These drugs have a prolonged depot action due to slow release of the lipophilic steroid ester from the injection site. The ester is hydrolyzed to active testosterone, but it is not known which esterases are involved.

As administration of exogenous androgens may enhance muscle mass and strength, these drugs are popular among young people and in the society and in sports [2]. This study programme for testosterone doping is based on determination of the urinary ratio of testosterone glucuronide and epitestosterone glucuronide, often referred to as the T/E ratio [3,4]. International rules for doping in sports stipulate that a T/E ratio above 4.0 gives reason to suspect testosterone doping and such samples must be subjected to further analyses for confirmation.

The major enzyme responsible for testosterone glucuronidation is uridine 5′-diphospho-glucuronosyltransferase (UGT2B17) [5,6]. A gene deletion polymorphism of

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UGT2B17 [7] was shown by us to be concordant with the rate of urinary testosterone excretion [5]. We were subsequently able to show that testosterone doping test results are highly dependent on the *UGT2B17* deletion genotype as investigated in healthy volunteers [8,9].

Individuals homozygous for the *UGT2B17* deletion polymorphism (*del/del*) have a markedly compromised testosterone glucuronide excretion capacity, both physiologically [5] and after testosterone administration [8]. Therefore, it is conceivable to assume that testosterone concentration in the circulation would be higher in patients devoid of the enzyme (*del/del*) compared with those with the UGT2B17 enzyme (*del/ins*, *ins/ins*), after testosterone administration.

Here, we have investigated the serum levels of testosterone and seven of its metabolites in 51 patients before and after 2 days of a single intramuscular dose of testosterone enanthate. Interestingly, a large interindividual variation and a bimodal distribution of the serum androgen levels were observed on day 2 indicating a genetic influence on the bioavailability. Affymetrix (Mercury Park, UK) analysis identified the phosphodiesterase (*PDE7B*) gene as potentially causative. Genotyping of selected single nucleotide polymorphisms (SNPs) in the *PDE7B* gene disclosed the PDE7B as a determinant of testosterone serum levels. Our data are further supported by studies of ester cleavage of testosterone enanthate in human liver homogenates.

Materials and methods

Study participants included 51 healthy male volunteers aged between 18 and 50 years (mean: 30.6 ± 7.0 years). All participants underwent a medical examination including laboratory tests before enrolment to exclude the possibility of any infections, hepatic or renal disease. Medical records were taken and participants were excluded if they had taken drugs that interfere with the synthesis, metabolism or excretion of steroids. Three participants reported to have taken antidepressants (paroxetin, escitalopram and sertralin) throughout the study. One participant took antibiotics (metronidazole and phenoxymethylpenicillin) on days 11–15. Three participants took one or two doses of nonsteroidal anti-inflammatory drugs (diclofenac and ibuprofen) and one patient took one dose of acetylsalicylic acid during the course of the study. Further inclusion criteria included negative screening tests for illegal drugs, anabolic androgenic steroids, HIV, and hepatitis B or C virus. For inclusion it was also required that the participant was not a member of any organization belonging to the Swedish Sports Confederation, or had had a malignancy within the past 5 years, or an allergy to the study substance. All participants gave informed consent consistent with the approval of the Ethics Review Board in Stockholm.

The participants were given 500 mg of testosterone enanthate as a single intramuscular dose (Testoviron-Depot, kindly provided by Schering Nordiska AB, Solna Sweden) equivalent to 360 mg testosterone. Before administration (day 0) and on day 2, serum samples were collected for analyses. All samples were collected between 07 : 00 h and 11 : 00 h. Moreover, morning urine samples were collected on days 0, 1–9, 11, 13 and 15. Adverse drug reactions were monitored from the time of screening until day 15 after administration of testosterone. The study was conducted according to the Helsinki declaration and the ICH Harmonised Tripartite Guideline for Good Clinical Practice.

Assessment of steroids in serum

Serum concentrations of testosterone, dihydrotestosterone, androsterone, 5α-androstane-3β,17β-diol and the glucuronidated androgen metabolites 5α-androstane-3α,17β-diol-17-glucuronide (3α-Adiol-17G), 5α-androstane-3α, 17β-diol-3-glucuronide (3α-Adiol-3G), androsterone-glucuronide (ADT-G), and etiocholanolone glucuronide (Etio-G) were analysed as described earlier [10,11].

Genome-wide analysis

Ten DNA samples obtained from individuals with high ($N = 5$; > 20 ng/ml) and low serum ($N = 5$; < 10 ng/ml) testosterone levels on day 2 were analysed using Affymetrix. DNA was prepared using Qiamp DNA Blood Mini Kit (Hilden, Germany). The extracted DNA samples were analysed by Bioinformatics and Expression Analysis Core Facility, Karolinska Institutet, where they were processed and hybridized to Affymetrix Human Mapping SNP6.0.

Sequence analysis

One hundred nanogram of genomic DNA obtained from each participant ($N = 10$) were used in PCR reactions together with elongation DNA polymerase according to the manufacturer's manual (Invitrogen, Carlsbad, California, USA). The PCR products were purified using PCR purification kit (Macherey-Nagel, Düren, Germany) and the promoter region, and exons 1–5 were sequenced in both directions using the following oligonucleotides: exon 1, promoter region, forward 5′-cagtcagttggtctgggca-3′, reverse 5′-agaaaactgagcttgccgct-3′; exon 2, forward 5′-tta tggtaatgtcaggtcac-3′, reverse 5′-accacaaaagaacctatcag-3′; exon 3, forward 5′-attgaggatttacaggggaa-3′, reverse 5′-aagttcagagggaaatgagg-3′; exon 4, forward 5′-gtacaacatact caggggag-3′, reverse 5′-cttgcttgtccaaggtagtc-3′; exon 5, forward 5′-attcatttgcactagatggt-3′, reverse 5′-aaaggaatag cagtgtttaa-3.

Genotyping of phosphodiesterase and uridine 5′-diphospho-glucuronosyltransferase polymorphisms

Two SNPs, rs7774640 and rs4896187, in the intron 1 region of *PDE7B* were investigated by 5′nuclease activity method using Taqman SNP Genotyping Assays

C__1843374_10 and C__1843403_10 (Applied Biosystems, Foster City, California, USA). The PCR reaction was carried out in 25 µl volume including 25 ng genomic DNA, 2 × Taqman universal master mix, and run on an ABI 7500 Fast (Applied Biosystems). The PCR profile consisted of an initial denaturation step of 95°C for 10 min followed by 40 cycles of denaturation for 15 s and annealing/elongation at 60°C for 1 min. The UGT2B17 deletion polymorphism was assayed by real-time PCR analysis as described earlier [8].

Urinary testosterone/epitestosterone ratio

The urinary levels of T/E were determined by gas chromatography-mass spectrometry after hydrolysis of the conjugates with β-glucuronidase [12]. The morning T/E ratio was monitored for 15 days after the testosterone administration [8].

In-vitro studies in liver homogenates

Fourteen human liver homogenate samples were obtained from Caucasian donors to our human liver bank (approved by the Ethics Review Board in Stockholm/at Karolinska Institutet). The liver samples were homogenized in 50 mmol/l potassium phosphate buffer (pH 7.4) and stored at −80°C until use. The protein concentration was determined according to Lowry *et al.* [13]. Testosterone enanthate was dissolved in ethanol to a final concentration of 5 mmol/l and diluted in 50 mmol/l Tris-HCl buffer (pH 7.4). The incubation was performed at 37°C using 10 µmol/l testosterone enanthate and 20 µl liver homogenate. At 1, 2, 4 and 8 min, the reaction mixture was stopped by immediate freezing. Testosterone esterase activity was determined by monitoring the testosterone formation by high-performance liquid chromatography as described by Narayanan *et al.* [14] with slight modifications. The analyses were carried out by high-performance liquid chromatography (231 XL Sampling injector, Gilson, P580 Pump, Dionex, Middleton, Wisconsin, USA) using a Zorbax SB-CN column (150 × 4.6 mm inner diameter, 5 µm; Agilent Technologies, Palo Alto, California, USA). The mobile phase consisted of acetonitrile and ammonium phosphate buffer (50 mmol/l, pH 4.5; 30 : 70) and the flow rate was 1 ml/min. Testosterone was detected by ultraviolet at 250 nmol/l (Lambda 1010, Bischoff, Leonberg, Germany). The inhibition studies were carried out by adding increasing concentrations (10–1000 µmol/l) of BRL50481. BRL50481 was dissolved in dimethyl sulfoxide and the final dimethyl sulfoxide concentration was 0.5% in all assays. The inhibition of hydrolyse activity was expressed as the percentage of control activity.

Data analysis

The statistical analyses were carried out using GraphPad Prism Software version 4.03 (GraphPad, San Diego, California, USA). The serum levels of steroid metabolites before and after testosterone administration were compared by paired Student *t*-test. The increase and the differences in serum steroid levels between *PDE7B* genotypes and UGT2B17 deletion polymorphism were compared by unpaired Student's *t*-test and analysis of variance. Spearman's rank-correlation coefficient was used in the evaluation of association between the body weight and testosterone serum levels.

Results

Serum steroid profile of exogenous testosterone

The serum levels of testosterone and the metabolites such as dihydrotestosterone, 5α-androstane-3β,17β-diol, ADT, Etio-G, 3α-Adiol-17G, 3α-Adiol-3G and ADT-G on days 0 and 2 are shown in Table 1. On day 2, a significant increase in the serum concentrations of all androgens was observed. For most of the metabolites analysed an approximately 100% increase was observed, whereas for testosterone and 3α-Adiol-17G a 200% increase was observed. The concentrations of all metabolites were positively-associated with testosterone level both on days 0 and 2.

There was a large interindividual variation in serum testosterone level 2 days after testosterone administration. The level was associated with body mass on day 2 ($R^2 = 0.16$, $P = 0.003$). When the increase (nanogram/millilitre) was corrected for body weight, the distribution of natural logarithms of serum testosterone level showed a multimodal (bi-modal or tri-modal) pattern (Fig. 1), suggesting a genetic contribution to the interindividual variation in serum testosterone concentration.

Genetic analysis

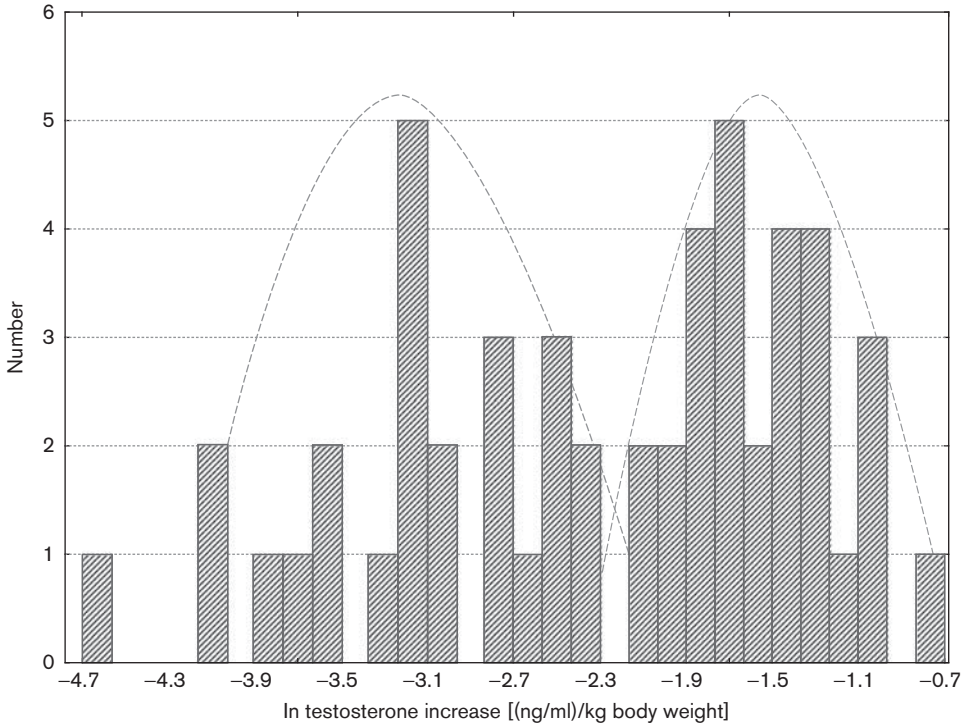
The results from the Affymetrix analysis of five individuals each with low or high-rise in testosterone concentration yielded approximately 300 hits (Supplementary Table 1, Supplemental digital content 1 *http://links.lww.com/FPC/A244*). Of particular interest, 12 of the SNPs were localized in the *PDE7B* gene. Two of them

Table 1 Serum levels of testosterone and its metabolites before (day 0) and after 2 days (day 2) of the administration of 500 mg testosterone enanthate

	Day 0	Day 2	Increase (%)
Testosterone (ng/ml)	5.07 (4.66–5.49)	15.00 (12.29–17.72)	196***
DHT (pg/ml)	400.7 (351.3–450.0)	752.6 (667.3–837.9)	88***
3β-Adiol (pg/ml)	64.81 (50.2–79.4)	150.7 (125.4–176.0)	132***
ADT (pg/ml)	418.1 (358.2–478.0)	764.2 (659.0–869.5)	83***
3α-Adiol-3G (ng/ml)	2.19 (1.6–2.8)	4.78 (3.75–5.8)	118***
ADT-G (ng/ml)	59.92 (50.18–69.6)	120.7 (97.1–144.3)	101***
3α-Adiol-17G (ng/ml)	4.4 (3.5–5.3)	14.86 (12.2–17.5)	237***
Etio-G (ng/ml)	24.5 (20.7–28.3)	50.8 (40.6–61.0)	107***

Results are mean with 95% confidence interval in parentheses.
3β-Adiol, 5α-androstane-3β,17β-diol; 3α-Adiol-3G, 5α-androstane-3α,17β-diol-3-glucuronide; 3α-Adiol-17G, 5α-androstane-3α,17β-diol-17-glucuronide; ADT, androsterone; ADT-G, androsterone-glucuronide; DHT, dihydrotestosterone; Etio-G, etiocholanolone glucuronide.
***$P < 0.0001$.

Fig. 1



Distribution of the increase in testosterone when corrected for body weight.

were randomly chosen (rs7774640 and rs4896187) for further analysis, and Taqman reactions (Applied Biosystems) were used to genotype all participants ($N = 51$). The *PDE7B* genotypes were in Hardy–Weinberg equilibrium. The two *PDE7B* polymorphisms investigated showed the same genotype distribution in all the individuals indicating that they are in linkage disequilibrium. Haplotype analysis indicated that all the intron 1 SNPs identified from the Affymetrix analysis were in linkage disequilibrium with each other (data not shown). Therefore, only the analysis of the rs7774640, an A>G substitution, is presented here.

Individuals homozygous for the *PDE7B* G allele displayed significantly lower total testosterone concentrations on day 2 (13.5 ng/ml ± standard deviation 8.0) than individuals with one or two A alleles (19.3 ng/ml ± standard deviation 6.7) ($R^2 = 0.15$, $P = 0.0053$) (Fig. 2a). Individuals homozygous for the G allele showed a significantly smaller increase (2.5-fold increase) in serum testosterone levels compared with carriers of the A allele (3.9-fold increase) ($R^2 = 0.22$, $P = 0.0006$). As expected, the baseline testosterone levels were not dependent on the *PDE7B* genotype.

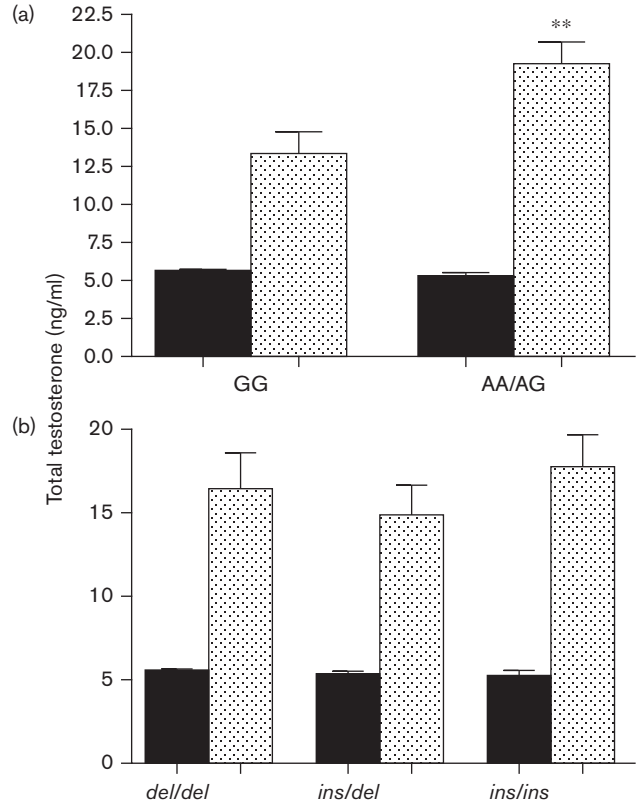
To investigate whether the intron *PDE7B* SNP was linked with any other polymorphism in the near coding/regulatory region of the gene, the exons 1–5 and the core promoter were sequenced. However, no polymorphism was identified in the 20 individual alleles sequenced.

There was no association between the *UGT2B17* deletion genotype and total testosterone serum level on day 0 or day 2 (Fig. 2b). Subsequently, the increase in total testosterone serum levels 2 days after the testosterone dose did neither differ between the genotype panels nor was there any association between the serum concentrations of the glucuronidated metabolites, that is, Etio-G, 3α-Adiol-17G, 3α-Adiol-3G on day 0 or day 2 and the *UGT2B17* deletion polymorphism (data not shown).

Inhibition studies

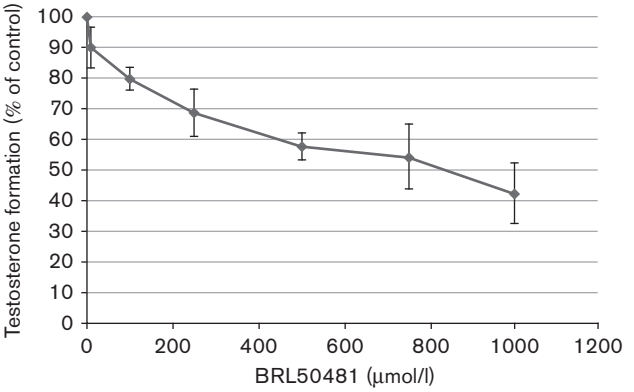
The esterase activity was determined in human liver homogenates by monitoring the release of testosterone from its enanthate salt. A peak in testosterone level was observed after 2 min (data not shown). To assess if PDE7B exhibits testosterone enanthate hydrolysis activity, inhibition studies using BRL 50481 (Sigma–Aldrich, St. Louis, Missouri, USA), a specific PDE7B/7A inhibitor, were carried out. BRL50481 was found to inhibit the testosterone formation in human liver homogenates in a concentration-dependent manner indicating that PDE7B is involved in the hydrolysis of the testosterone enanthate ester (Fig. 3).

Fig. 2



(a) Serum levels of testosterone in relation to the phosphodiesterase intron 1 G>A polymorphism before (day 0; filled bars) and after 2 days (day 2; dotted bars) of testosterone administration. The total level of serum testosterone was significantly higher in A-carriers ($N=27$) compared with individuals homozygous for the G allele ($N=24$) on day 2. (b) Serum levels of testosterone and uridine 5′-diphospho-glucuronosyltransferase deletion polymorphism before (day 0; filled bars) and after 2 days (day 2; dotted bars) of testosterone administration. The UGT2B17 deletion polymorphism was not associated with total serum testosterone levels on day 0 and day 2.

Fig. 3



Inhibition studies in human liver homogenates. Testosterone enanthate was incubated with human liver homogenates for 2 min and the formation of testosterone was monitored by high-performance liquid chromatography. When increasing concentration of phosphodiesterase specific inhibitor BRL50481 was added, a decrease in testosterone enanthate hydrolyse activity was observed.

Phosphodiesterase genotype and testosterone/epitestosterone ratio

The T/E ratio, an important biomarker in international doping control programme, was monitored in the urine after testosterone administration. The T/E ratio was associated with the *PDE7B* polymorphism, that is, individuals homozygous for the *PDE7B* G allele had lower T/E ratio after testosterone administration. This may have clinical relevance in *UGT2B17 del/del* individuals who rarely reach the T/E cut-off value of 4 (Fig. 4a). After the first 5 days of testosterone administration, *del/del* participants homozygous for the *PDE7B* G variant had lower T/E ratio compared with carriers of *the PDE7B* A allele (significant on days 1 and 2, $P=0.02$ and 0.04, respectively). The opposite was found on days 13 and 15, A-carriers had lower T/E ratio compared with GG ($P=0.09$ and 0.03, respectively). Also, among individuals expressing UGT2B17 enzyme the T/E ratio was significantly lower in individuals homozygous for *PDE7B* G allele compared with individuals homozygous for AG/AA on day 2 ($P=0.03$), day 4 ($P=0.03$) and day 5 ($P=0.009$) (Fig. 4c).

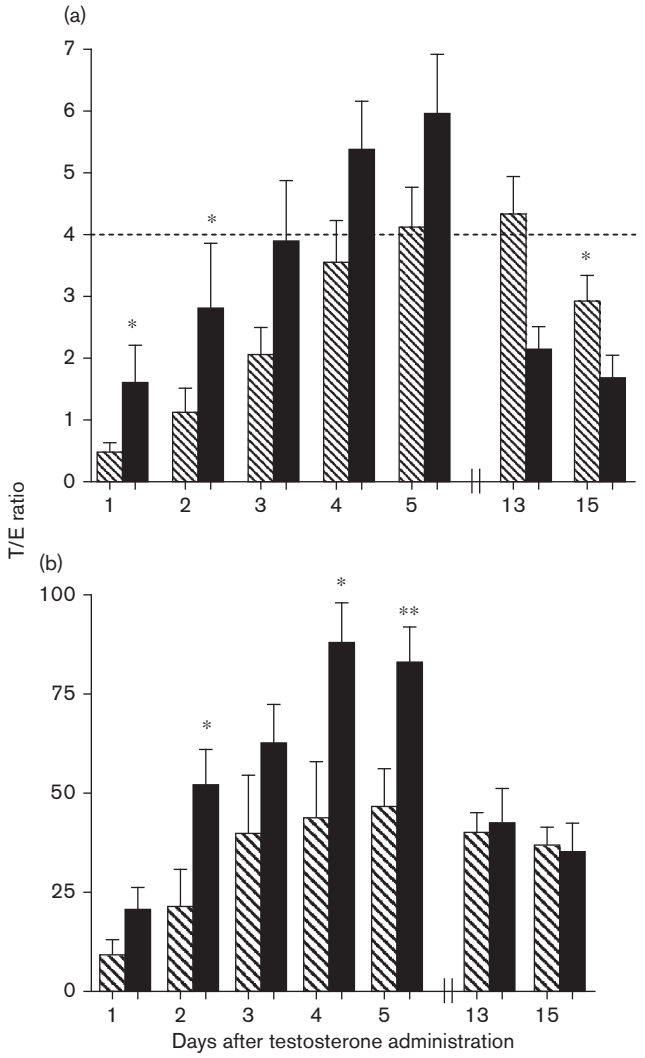
Discussion

The large interindividual variation in the increase of testosterone serum levels 2 days after a single dose observed by us may be ascribed to genetic variation in an esterase enzyme, as we suggested earlier [15]. Our hypothesis was supported by the Affymetrix hit at the *PDE7B* gene, which was subject to further investigation. Interestingly, the serum level of testosterone was associated with genetic variation in the *PDE7B* gene. Our findings were corroborated in experiments of human liver samples in which we observed an inhibition of the hydrolysis of testosterone enanthate by the PDE7B/7A specific inhibitor, BRL50485. To our knowledge this is the first time an enzyme involved in testosterone enanthate hydrolysis has been studied and identified. It is likely that other enzymes are also involved in the hydrolysis as there was a residual activity after inhibition of the specific PDE7B/7A inhibitor.

A multiple regression analysis showed that the body weight and *PDE7B* genotype together explained 36% of the variation observed in serum testosterone level 2 days after testosterone administration. Other factors are known to influence the release of depot drugs. Thus, it has been shown that the body fat may serve as a depot for injected steroids, resulting in a retarded release [16]. Moreover, exercise will increase the blood flow through the tissue near the site of injection and hence increase the release rate.

As we did not find any linkage between the investigated intronic SNPs of the *PDE7B* gene and the near coding sequence of the gene it is not likely that these intron

Fig. 4



Mean (±standard error of the mean) urinary testosterone glucuronide and epitestosterone glucuronide (T/E) levels in (a) individuals homozygous for the uridine 5′-diphospho-glucuronosyltransferase deletion (*del/del*; *N*=16). (b) Individuals expressing UGT2B17 (*ins/del and ins/ins*; *N*=36). Individuals homozygous for the phosphodiesterase G allele (striped bars) have lower T/E ratio compared with carriers of the A-allele (filled bars) for 5 days after testosterone administration. On days 13 and 15 the GG individuals display a higher T/E ratio in *del/del* individuals.

SNPs are in close linkage disequilibrium with a coding SNP in the *PDE7B* gene. Moreover, a HapMap analysis also confirmed that the 12 intronic SNPs are in linkage disequilibrium and that they are not linked with a SNP in the coding or regulatory region of the *PDE7B* gene. In fact, there are over 4000 intronic SNPs in the *PDE7B* gene identified in the National Centre for Biotechnology Information SNP database to date, but no one has been associated with an altered phenotype. It is possible that the intronic SNPs modulate the abundance and/or stability of *PDE7B* specific mRNA and hence the protein expression.

PDE7B is a cyclic AMP-specific PDE7B mediating degradation of intracellular cyclic AMP by hydrolysis and is highly expressed in the brain, heart and liver [17–19]. Zhang *et al.* [20] found that the peripheral blood mononuclear PDE7B was selectively overexpressed in chronic lymphocytic leukaemia, highlighting PDE7B as a potential therapeutic target in this disease. Our novel finding that PDE7B also exhibits activity towards exogenous substrates may have clinical pharmacological importance. Interestingly, *PDE7B* was recently identified as a candidate gene for treatment response to risperidone [21].

In addition to our findings of a large variation in testosterone levels in healthy volunteers after testosterone administration, Di Luigi *et al.* [22] observed inter-individual variation in testosterone levels in hypogonadal volunteers after testosterone treatment 1 week after intramuscular administration of testosterone enanthate. Other investigations have also described large differences in serum concentration of testosterone among patients after androgen replacement therapy in hypogonadal men [23,24]. It is of great interest to identify the genetic reasons for the variation in bioavailability of testosterone after androgen replacement therapy. Any measures to compensate for the variation in bioavailability of testosterone and presumably also other esterified drugs such as neuroleptic depot preparations, will improve the outcome of treatment and diminish the risk of side-effects. The effect of repeated testosterone administrations as in chronic treatment or abuse of anabolic androgenic steroids remains to be studied. In addition to polymorphisms in the androgen receptor known to modulate the androgenic effects [25], we believe that genetic variation in the *PDE7B* gene also modulates the response to testosterone therapy or abuse.

Significant implications of genetic variation for interpretation of testosterone doping test results, were earlier founded by us for the *UGT2B17* gene [8]. Individuals devoid of the *UGT2B17* gene rarely reach the international accepted cut-off limit after testosterone injection in contrast to individuals expressing UGT2B17, who highly exceed the cut-off value. Here, we show the influence of yet another gene pathway on the T/E ratio. We show that the *PDE7B* genotype is associated with the excretion profile of testosterone and consequently the T/E ratio in *UGT2B17 del/del* individuals. Our result indicates that the *PDE7B* rs7774640 A allele is associated with faster excretion rate of testosterone compared with individuals homozygous for the G allele, which appear to excrete the testosterone slower and for a longer period of time. Thus it is conceivable that in *UGT2B17 del/del* participants, *PDE7B* G allele may yield an ‘advantage genotype’ further decreasing the risk of being identified as a doped subject. These findings need to be considered in the international doping test programs.

It is not known how the exogenous testosterone is metabolized in individuals homozygous for *UGT2B17* deletion polymorphism (*del/del*). As *del/del* individuals have poor ability to excrete testosterone in the urine, it may be reasonable to think that they may have higher serum levels after administration. However, as shown here there was no association between the *UGT2B17* genotype and serum levels of testosterone or the other androgen metabolites investigated. In contrast, a recent publication has described a correlation between the *UGT2B17* polymorphism and the 5α-androstane-3α,17β-diol-glucuronides (= 3αdiol-3G + 17G) [26]. We did not find such a relation in our study including 51 participants. It may be possible that in a larger sample population such a correlation would be detected.

Today, the urinary level of etiocholanolone sulphate is the only testosterone metabolite known to be significantly increased after testosterone administration in *del/del* individuals compared with individuals expressing the *UGT2B17* gene (unpublished results). This increase is however not large enough to account for the difference between individuals with or without a functional UGT2B17 enzyme. The overall fate of testosterone in individuals devoid of the UGT2B17 enzyme is yet to be identified.

Of note, the serum level of the 3α-Adiol-17G metabolite, which is known to be mainly glucuronidated by UGT2B17 [27–29], was not associated with the *UGT2B17*gene deletion polymorphism, suggesting a shift to glucuronidation of this steroid substrate toward UGT2B15. In addition to UGT2B15, there are however other UGT enzymes known to exhibit 17-OH activity *in vitro*, namely UGTs 2B15, 2A2, 2A1, 1A10, 1A8 and 1A4 [30]. It is possible that the high load of testosterone induces the expression and/or activity of some of these UGTs in the *del/del* individuals and thereby compensates for the deficient capacity in *del/del* individuals to glucuronidate at position 17.

Other testosterone preparations than testosterone enanthate are available, that is, undecanoate, cypionate and propionate. In addition, many other anabolic androgenic steroids are provided as esters, for example, nandrolone decanoate, drostanolone propionate, trenbolone enanthate, etc. It is not known which enzymes catalyze the hydrolysis activity on these esters, but it is possible that PDE7B, or other PDE family members, may be involved. Importantly, a large number of other drugs are esterified with enanthate, for example, antipsychotic drugs (haloperidol, perfenazine, zuklopentixol, etc) and antiasthmatic drugs (flutikason, beklametason). Dose requirement and injection interval of these drugs differ widely between patients, and it is possible that genetic variation in the PDE pathway may contribute to this interindividual variation. This has great interest in clinical medicine, in particular in therapeutics with neuroleptic and androgenic drugs, and many drugs to be introduced in the market in the future.

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References

1 Nieschlag E. Testosterone treatment comes of age: new options for hypogonadal men. *Clin Endocrinol* 2006; **65**:275–281.
2 Sjoqvist F, Garle M, Rane A. Use of doping agents, particularly anabolic steroids, in sports and society. *Lancet* 2008; **371**:1872–1882.
3 Donike M, Brwald KR, Klostermann K, Schänzer W, Zimmerman J. Detection of exogenous testosterone. Nachweis von exogenem testosteron Sport: Leistung und Gesundheit, Kongressbd. Dtsch Sportarztekongress. Köln: Deutcher Ärtzte-Verlag; 1983. pp. 293–298.
4 Donike M, Ueki M, Kuroda Y, Geyer H, Nolteernsting E, Rauth S, *et al.* Detection of dihydrotestosterone (DHT) doping: alterations in the steroid profile and reference ranges for DHT and its 5 alpha-metabolites. *J Sports Med Phys Fitness* 1995; **35**:235–250.
5 Jakobsson J, Ekstrom L, Inotsume N, Garle M, Lorentzon M, Ohlsson C, *et al.* Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. *J Clin Endocrinol Metab* 2006; **91**:687–693.
6 Sten T, Bichlmaier I, Kuuranne T, Leinonen A, Yli-Kauhaluoma J, Finel M. UDP-glucuronosyltransferases (UGTs) 2B7 and UGT2B17 display converse specificity in testosterone and epitestosterone glucuronidation, whereas UGT2A1 conjugates both androgens similarly. *Drug Metab Dispos* 2009; **37**:417–423.
7 Wilson W III, Pardo-Manuel de Villena F, Lyn-Cook BD, Chatterjee PK, Bell TA, Detwiler DA, *et al.* Characterization of a common deletion polymorphism of the UGT2B17 gene linked to UGT2B15. *Genomics* 2004; **84**:707–714.
8 Schulze JJ, Lundmark J, Garle M, Skilving I, Ekstrom L, Rane A. Doping test results dependent on genotype of uridine diphospho-glucuronosyl transferase 2B17, the major enzyme for testosterone glucuronidation. *J Clin Endocrinol Metab* 2008; **93**:2500–2506.
9 Schulze JJ, Lundmark J, Garle M, Ekstrom L, Sottas PE, Rane A. Substantial advantage of a combined Bayesian and genotyping approach in testosterone doping tests. *Steroids* 2009; **74**:365–368.
10 Labrie F, Belanger A, Belanger P, Berube R, Martel C, Cusan L, *et al.* Metabolism of DHEA in postmenopausal women following percutaneous administration. *J Steroid Biochem Mol Biol* 2007; **103**:178–188.
11 Hsing AW, Stanczyk FZ, Belanger A, Schroeder P, Chang L, Falk RT, *et al.* Reproducibility of serum sex steroid assays in men by RIA and mass spectrometry. *Cancer Epidemiol Biomarkers Prev* 2007; **16**:1004–1008.
12 Chung BC, Choo HY, Kim TW, Eom KD, Kwon OS, Suh J, *et al.* Analysis of anabolic steroids using GC/MS with selected ion monitoring. *J Anal Toxicol* 1990; **14**:91–95.
13 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**:265–275.
14 Narayanan R, LeDuc B, Williams DA. Determination of the kinetics of rat UDP-glucuronosyltransferases (UGTs) in liver and intestine using HPLC. *J Pharm Biomed Anal* 2000; **22**:527–540.
15 Schulze JJ, Rane A, Ekstrom L. Genetic variation in androgen disposition: implications in clinical medicine including testosterone abuse. *Expert Opin Drug Metab Toxicol* 2009; **5**:731–744.
16 Back DJ, Breckenridge AM, Chapman CR, Crawford FE, Olsen KA, Orme ML, *et al.* Studies on the enzymatic cleavage of norethisterone oenanthate. *Contraception* 1981; **23**:125–132.
17 Hetman JM, Soderling SH, Glavas NA, Beavo JA. Cloning and characterization of PDE7B: a cAMP-specific phosphodiesterase. *Proc Natl Acad Sci U S A* 2000; **97**:472–476.

18 Sasaki T, Kotera J, Yuasa K, Omori K. Identification of human PDE7B: a cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun* 2000; **271**:575–583.

19 Gardner C, Robas N, Cawkill D, Fidock M. Cloning and characterization of the human and mouse PDE7B, a novel cAMP-specific cyclic nucleotide phosphodiesterase. *Biochem Biophys Res Commun* 2000; **272**:186–192.

20 Zhang L, Murray F, Zahno A, Kanter JR, Chou D, Suda R, *et al.* Cyclic nucleotide phosphodiesterase profiling reveals increased expression of phosphodiesterase 7B in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2008; **105**:19532–19537.

21 Ikeda M, Tomita Y, Mouri A, Koga M, Okochi T, Yoshimura R, *et al.* Identification of novel candidate genes for treatment response to risperidone and susceptibility for schizophrenia: integrated analysis among pharmacogenomics, mouse expression, and genetic case-control association approaches. *Biol Psychiatry* 2010; **67**:263–269.

22 Di Luigi L, Sgro P, Romanelli F, Mazzarino M, Donati F, Bragano MC, *et al.* Urinary and serum hormones profiles after testosterone enanthate administration in male hypogonadism: concerns on the detection of doping with testosterone in treated hypogonadal athletes. *J Endocrinol Invest* 2009; **32**:445–453.

23 Kelleher S, Conway AJ, Handelsman DJ. Blood testosterone threshold for androgen deficiency symptoms. *J Clin Endocrinol Metab* 2004; **89**:3813–3817.

24 Zitzmann M, Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. *J Clin Endocrinol Metab* 2007; **92**:3844–3853.

25 Zitzmann M. Mechanisms of disease: pharmacogenetics of testosterone therapy in hypogonadal men. *Nat Clin Pract Urol* 2007; **4**:161–166.

26 Hu DG, Gardner-Stephen D, Severi G, Gregory PA, Treloar J, Giles GG, *et al.* A novel polymorphism in a forkhead box A1 (FOXA1) binding site of the human UDP glucuronosyltransferase 2B17 gene modulates promoter activity and is associated with altered levels of circulating androstane-3alpha,17beta-diol glucuronide. *Mol Pharmacol* 2010; **78**:714–722.

27 Belanger A, Pelletier G, Labrie F, Barbier O, Chouinard S. Inactivation of androgens by UDP-glucuronosyltransferase enzymes in humans. *Trends Endocrinol Metab* 2003; **14**:473–479.

28 Chouinard S, Pelletier G, Belanger A, Barbier O. Isoform-specific regulation of uridine diphosphate-glucuronosyltransferase 2B enzymes in the human prostate: differential consequences for androgen and bioactive lipid inactivation. *Endocrinology* 2006; **147**:5431–5442.

29 Sten T, Kurkela M, Kuuranne T, Leinonen A, Finel M. UDP-glucuronosyltransferases in conjugation of 5alpha- and 5beta-androstane steroids. *Drug Metab Dispos* 2009; **37**:2221–2227.

30 Kuuranne T, Kurkela M, Thevis M, Schanzer W, Finel M, Kostiainen R. Glucuronidation of anabolic androgenic steroids by recombinant human UDP-glucuronosyltransferases. *Drug Metab Dispos* 2003; **31**:1117–1124.