

# Endogenous and Exogenous Testosterone Levels after Administration of Deuterium-Labelled Testosterone Propionate in Hypogonadotropic Hypogonadism

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A gas chromatography-mass spectrometry-selected ion monitoring technique was employed to determine simultaneously the plasma concentrations of endogenous and exogenous testosterone in three patients with hypogonadotropic hypogonadism after a single i.m. dose of testosterone propionate-19,19,19-*d*<sub>3</sub>. The plasma levels of testosterone-19,19,19-*d*<sub>3</sub> derived from testosterone propionate-19,19,19-*d*<sub>3</sub> were maintained above the normal testosterone levels (>4 ng/ml) for 48 h, while the plasma levels of endogenous testosterone changed little.

**Keywords** testosterone propionate; testosterone; hypogonadism; bioavailability; stable isotope; GC-MS-SIM

A decreased plasma testosterone level is found commonly in men with hypogonadotropic hypogonadism. The decrease in testosterone levels may be the result of primary hypothalamic-pituitary dysfunction. Although testosterone preparations are required for androgen replacement therapy in these patients,<sup>1)</sup> testicular steroidogenic responsiveness to testosterone preparations remains to be elucidated. This has been due to an inability to differentiate between endogenous testosterone and testosterone derived from administered testosterone preparations.

In an attempt to determine the influence of exogenous testosterone on endogenous testosterone levels, we have developed a stable-isotope tracer technique which can easily differentiate between endogenous and exogenous testosterone.<sup>2)</sup> In a previous study,<sup>3)</sup> the stable-isotope tracer technique was used to characterize the pharmacokinetic properties of i.m. administered testosterone propionate and the effect of testosterone propionate on the plasma levels of endogenous testosterone in normal men. It became apparent that plasma testosterone derived from testosterone propionate was transferred from the injection site in the muscle to the systemic circulation and its concentrations were maintained above the normal testosterone levels (>4 ng/ml) for 48 h, while plasma levels of endogenous testosterone changed little. In the present paper, we have studied the effects of the i.m. administered testosterone propionate on plasma testosterone levels in patients with hypogonadotropic hypogonadism using a stable-isotope tracer technique.

## Experimental

**Chemicals** Testosterone propionate-19,19,19-*d*<sub>3</sub> (testosterone propionate-19-*d*<sub>3</sub>) and testosterone-19,19,19-*d*<sub>3</sub> (testosterone-19-*d*<sub>3</sub>) were synthesized in our laboratory, as described previously.<sup>4)</sup> Their isotopic compositions were 99.0% deuterium atoms (*d*<sub>3</sub>, 97.8%; *d*<sub>2</sub>, 2.2%; *d*<sub>1</sub>, 0.0%). The testosterone propionate-19-*d*<sub>3</sub> preparation for i.m. injection was prepared by dissolving 25 mg of testosterone propionate-19-*d*<sub>3</sub> in 1 ml of

sesame oil containing 20% benzyl benzoate, followed by membrane filtration to insure sterility.

**Study Protocol** Three hypogonadal men, 33–53 years of age, were studied. Their clinical characteristics are summarized in Table I. The patients were admitted to the General Clinical Research Unit at Toho University School of Medicine, and informed consent was obtained in all cases. Subjects were given 25 mg testosterone propionate-19-*d*<sub>3</sub>, i.m., at 0800. Heparinized blood samples (5–10 ml) were taken 5 min before and 4, 8, 12, 24, 48 and 72 h after dosing. Plasma was separated by centrifugation and stored at –20 °C until analysis.

**Hormone Assays** Plasma testosterone-19-*d*<sub>3</sub> and testosterone were determined by gas chromatography-mass spectrometry-selected ion monitoring. The details of this double isotope dilution assay have been described previously.<sup>2,5)</sup> All measurements in a series were performed in a single assay. The sensitivity of the testosterone assay was 0.1 ng/ml plasma, and the inter-assay and intra-assay coefficients of variation were less than 4%.

## Results

Plasma testosterone-19-*d*<sub>3</sub> levels were measured simultaneously with endogenous testosterone levels for 72 h after the i.m. injection of 25 mg testosterone propionate-19-*d*<sub>3</sub> (Fig. 1). Plasma testosterone-19-*d*<sub>3</sub> levels increased sharply and reached maximum values (16.4 ± 0.5 ng/ml) 12 h after the injections. Thereafter, plasma testosterone-19-*d*<sub>3</sub> levels decreased very slowly and were maintained above the normal testosterone levels (>4 ng/ml)<sup>6)</sup> for 48 h. The area under the plasma concentration-time curve of testosterone-19-*d*<sub>3</sub> was 990 ± 325 ng · h/ml.

Plasma levels of endogenous testosterone just before the administration of testosterone propionate-19-*d*<sub>3</sub> were

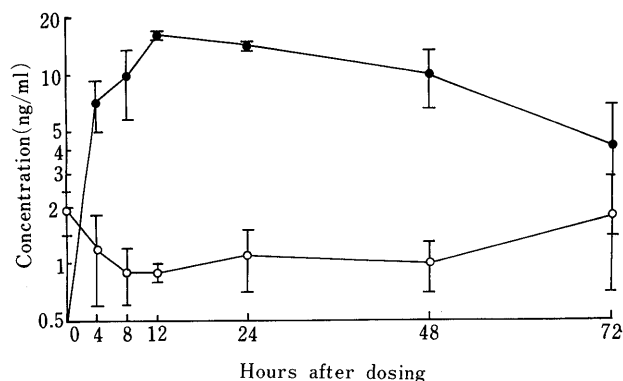


Fig. 1. Semilogarithmic Plots of Plasma Concentrations of Testosterone-19-*d*<sub>3</sub> (●—●) and Endogenous Testosterone (○—○) versus Time after a Single i.m. Dose of 25 mg of Testosterone Propionate-19-*d*<sub>3</sub> in Men with Hypogonadotropic Hypogonadism

TABLE I. Characteristics of Patients with Hypogonadotropic Hypogonadism

Patient	Age (year)	Weight (kg)	Diagnosis	Testosterone (ng/ml)	LH (mIU/ml)
A.	33	73	Fröhlich's syndrome	1.7	1.5
B.	53	76	Acromegaly	1.5	2.4
C.	49	75	Acromegaly	2.5	2.5

$1.9 \pm 0.5$  ng/ml. Endogenous plasma testosterone fell to  $0.9 \pm 0.1$  ng/ml at 12 h after the injections. The level began to rise at 48 h and reached preinjection levels at 72 h after the injections.

### Discussion

The present study was carried out to follow the plasma levels of exogenous testosterone at various times after the i.m. administration of 25 mg testosterone propionate-19- $d_3$  to three hypogonadal men and to examine the effects of testosterone propionate-19- $d_3$  on the plasma levels of endogenous testosterone.

Several investigators have measured the total plasma exogenous and endogenous testosterone levels by radioimmunoassay after the i.m. administration of testosterone esters to normal and hypogonadal men.<sup>7)</sup> However, no direct evidence is available concerning the time course of exogenous testosterone levels in plasma. Radioimmunoassay techniques cannot address this problem, since this type of assay cannot differentiate between exogenous and endogenous testosterone. With the aid of the double isotope dilution assay employed in this study, endogenous testosterone and testosterone-19- $d_3$  derived from administered testosterone propionate-19- $d_3$  could be differentiated easily. Testosterone propionate-19- $d_3$  injection resulted in plasma exogenous testosterone levels above the normal testosterone levels ( $>4$  ng/ml) for 48 h in hypogonadal men. Nieschlag *et al.*<sup>8)</sup> reported that the bioavailability of orally administered testosterone was higher in hypogonadal men than in normal men. To explain this, they suggested that the testosterone-metabolizing capacity of the liver is higher in normal men. In our present study, the maximum plasma testosterone-19- $d_3$  level, the area under the plasma concentration time curve of testosterone-19- $d_3$  and the duration of normal testosterone level were similar to those of normal men.<sup>3)</sup> These findings seemed to indicate no difference between hypogonadal men and normal men in bioavailability of testosterone after a single i.m. dose of 25 mg of testosterone propionate.

Chronic administration of testosterone propionate drastically reduces intratesticular levels of total testosterone but increases plasma levels of total testosterone.<sup>9)</sup> However, no direct evidence concerning the effects of exogenous testosterone on plasma endogenous testosterone levels is yet available. In our present study, the endogenous plasma

testosterone level just before the injection of testosterone propionate-19- $d_3$  was  $1.9 \pm 0.5$  ng/ml and fell to  $0.9 \pm 0.1$  ng/ml at 12 h after the injection. These variations represent actual changes in the plasma endogenous testosterone levels, since the present method<sup>2,5)</sup> provides accurate measurements of plasma testosterone down to 0.1 ng/ml (inter-assay coefficient of variation, 3.15%). These findings seemed to indicate that exogenous testosterone suppressed plasma levels of endogenous testosterone. However, the influence of exogenous testosterone on endogenous testosterone secretion should be interpreted cautiously, since normal men exhibit a circadian rhythm and episodic variations (about 2 ng/ml  $\cdot$  30 min) in plasma testosterone levels.<sup>2)</sup> No systematic studies of diurnal testosterone levels have been reported in men with hypogonadal hypogonadism. Thus, we cannot conclude that plasma levels of endogenous testosterone were suppressed by the single dose of testosterone propionate administered.

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