

# Bioequivalence of subcutaneous injections of recombinant human follicle stimulating hormone (Puregon®) by Pen-injector and syringe

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**A randomized, single-centre, cross-over study was designed to compare the bioavailability of two pharmaceutical formulations of recombinant human follicle stimulating hormone (recFSH; Puregon®): (i) a dissolved cake injected by a normal syringe; and (ii) a ready-for-use solution injected using a device referred to as Puregon®Pen. Twenty-two healthy female volunteers underwent one of two administration sequences: Puregon®Pen/syringe or syringe/Puregon®Pen, by which they received a single subcutaneous dose of recFSH (150 IU). Endogenous gonadotrophin production had been previously suppressed using an oral contraceptive (Lyndiol®). Pharmacokinetic parameters characterizing rate [peak concentration ( $C_{max}$ ) and time of peak concentration ( $t_{max}$ )] and extent [area under the curve (AUC) and clearance (CL)] of absorption were obtained from 20 subjects. After injection with both formulations, serum FSH concentrations reached a peak of 3.4 IU/l at 13 h after injection. The elimination half-life was approximately 34 h, irrespective of formulation. A difference of approximately 18% was found between serum FSH concentrations obtained using the two formulations, which was caused by differences between the anticipated and the actual volume injected with the normal syringe. After correction for injection losses by weighing the amount injected with a normal syringe, the two formulations were found to be bioequivalent with respect to  $C_{max}$ , AUC and CL. For  $t_{max}$ , bioequivalence could not be proven due to high intra-subject variability and broad absorption peaks of FSH. Both methods were well tolerated, local reactions being generally mild and short-lived.**

**Key words:** bioequivalence/Puregon®/Puregon®Pen/recombinant human FSH/subcutaneous

## Introduction

Puregon® contains, as an active ingredient, recombinant human follicle-stimulating hormone (recFSH). The safety and efficacy of this hormonal preparation—when administered via the intramuscular (i.m.) and subcutaneous (s.c.) routes—have been proven in multicentre studies (Out *et al.*, 1997), including a

study with nearly 1000 infertility patients undergoing in-vitro fertilization (Out *et al.*, 1995).

Most urinary gonadotrophins are administered using the i.m. route. Generally, the injections are given by qualified nurses, general practitioners or other physicians, and often require frequent visits to the clinic. The high purity of recFSH (>99%) allows both i.m. and s.c. administration. RecFSH is currently supplied as a lyosphere or cake, which should be reconstituted with solvent before injection. Compared with the i.m. route, the s.c. route has the advantage that self-administration is feasible. In general, administration via the i.m. or s.c. route through a conventional syringe may give rise to local tolerance problems such as bruising and pain at the site of injection (Out *et al.*, 1997). The availability of recFSH as a ready-for-use solution supplied in an injector system for s.c. administration (Puregon®Pen) may make its administration, in particular self-administration by the patient or her partner, more convenient, as both needle size and injection volume are smaller. Additionally, the Puregon®Pen may be used for multiple administrations, and adjustment of the dose is possible.

As absorption from the injection site may be influenced by the pharmaceutical formulation, concentration of the drug and the administered volume, the present study was performed to investigate FSH pharmacokinetics after s.c. administration and to compare the bioavailability following injection with a Puregon®Pen containing ready-for-use solution with that following injection of a dissolved cake by a syringe.

## Materials and methods

### Study population

Twenty-four healthy female volunteers were initially selected. Two subjects discontinued during the pre-treatment period and consequently, 22 subjects were treated with recFSH. In order to reduce variability caused by endogenous release of FSH, all women were treated with the oral contraceptive pill Lyndiol® containing 50 µg ethinyloestradiol and 2.5 mg lynestrenol per tablet to suppress endogenous gonadotrophin production. All subjects gave written informed consent to participate in the study. The study was approved by the Ethics Committee of the Academic Hospital, Leiden and conducted in compliance with the current revision of the Declaration of Helsinki, ICH Harmonized Tripartite Guidelines, Guideline for Good Clinical Practice, and current national regulations.

The main inclusion criteria were: female volunteers using oral contraceptives for at least 3 months, which were not originally prescribed for menstrual irregularities; age between 18 and 39 years; body mass index (BMI) between 18 and 29 kg/m<sup>2</sup>. The main exclusion criteria were: history of endocrine abnormalities; abnormal (clinically relevant) blood biochemistry, haematology and/or urinalysis at screening; hypertension (diastolic blood pressure >90 mmHg and/or systolic



**Figure 1.** Puregon®Pen. The pen can be loaded with a glass cartridge containing the ready-for-use solution. The injection volume can be adjusted up to 250  $\mu$ l.

blood pressure >150 mmHg in sitting position); cardiovascular, gastrointestinal, hepatic, renal or pulmonary disease; pregnancy; positive smear result ( $\geq$ IIIa) according to the Papanicolaou classification; presence of Factor V Leiden.

#### Study design

The bioequivalence of injections of recFSH with a Pen-injector (BD 1.5 ml Classic®Pen) containing ready-for-use solution and as a dissolved cake by a syringe was investigated in a randomized, single-centre, cross-over study. After screening and inclusion in the study, subjects discontinued daily intake of contraceptive pills for a period of one week. After this pill-free period, they received daily administration of Lyndiol® (batch CP097004, NV Organon, Oss, The Netherlands) for a total period of six weeks in order to achieve full pituitary suppression. They then received recFSH in one of two formulations. One formulation was a cake in vials, each containing 75 IU in-vivo bioactivity. Two vials were reconstituted in 1 ml solvent (0.45% NaCl for injection) and immediately injected by the s.c. route using a 1-ml Luerlock (309628, Becton Dickinson, (Franklin Lakes, NJ, USA) syringe with a 25 G $\times$ 5/8 inch (0.5 $\times$ 16 mm) needle. In order to allow correction for the possible influence on the pharmacokinetics of different injection volumes with the conventional syringe, each syringe with dissolved cake was weighed just before and after injection. The correction factor was defined as the ratio of the measured weight to the weight expected if all the drug had been administered. The second formulation was a ready-for-use solution containing 833 IU/ml in-vivo bioactivity of FSH in a Puregon®Pen system, an injection device for s.c. administration that enables repeated self-injection of small volumes (Figure 1). In total, 150 IU (in a volume of 180  $\mu$ l) was administered s.c. with the Puregon Pen, using a 29 G $\times$ 1/2 inch (0.33 $\times$ 13 mm) needle.

On day 22 of Lyndiol® treatment, a single dose of one of the two pharmaceutical formulations was administered s.c. in the abdominal wall around the umbilicus. On day 36 of contraceptive use (14 days after the first injection), the procedure was repeated in a two-way, cross-over fashion with regard to the pharmaceutical formulation.

#### Assessments

Local tolerance for each injection was examined at 1 and 24 h following injection. Monitoring of the injection site included scoring of redness, itching, swelling, pain and bruising as none, mild, moderate or severe.

Blood samples for the determination of FSH were obtained at the

following times relative to injection: -5 min and 1, 2, 4, 6, 8, 10, 12, 16, 24, 30, 36, 48, 72, 96 and 120 h. For baseline assessment, an additional blood sample was obtained 1 and 2 days before each injection. Each sample was stored frozen until assessment. Immunoreactive serum FSH concentrations were determined using an automated time-resolved fluoroimmunoassay (AutoDelfia®; Wallac Oy, Turku, Finland). Inter-assay coefficients of variation were 2.3%, 3.1% and 3.1% at nominal FSH concentrations of 8.40, 12.8 and 37.0 IU/l. Accuracy ranged from 99% to 106%. Serum FSH concentrations were measured by ABL BV, Assen, The Netherlands.

#### Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated from the serum concentration versus time curves: peak serum concentration ( $C_{max}$ ), time of occurrence of peak serum concentration ( $t_{max}$ ), elimination half-life ( $t_{1/2}$ ), area under the curve from 0 to infinity ( $AUC_{0-\infty}$ ), area under the curve from 0 to the last time point at which all subjects still had measurable FSH concentrations ( $AUC_{0-tn}$ ) and apparent clearance ( $CL_{app}$ ). The peak serum concentration was also corrected for the extent of drug absorbed, by dividing  $C_{max}$  by either  $AUC_{0-\infty}$  or  $AUC_{0-tn}$ . This parameter was designated as extent-normalized (en)  $C_{max}$  (en- $C_{max, \infty}$  or en- $C_{max, tn}$ ).

The bioequivalence of the two pharmaceutical formulations was tested for the rate of absorption using  $C_{max}$ , en- $C_{max}$  and  $t_{max}$ , whereas for the extent of absorption bioequivalence was tested using  $AUC_{0-\infty}$ ,  $AUC_{0-tn}$  and  $CL_{app}$ . The dissolved cake (syringe) was taken as reference formulation and the ready-for-use solution (Puregon®Pen) as test treatment. For the reference formulation, data with and without dose correction for differences in injection volumes were used. For all parameters except  $t_{max}$ , point estimates and their 90% parametric confidence intervals derived from the ANOVA on log-transformed values were determined for the ratio 'test/reference' ( $\mu_{Pen}/\mu_{Syringe}$ ); for  $t_{max}$  the point estimate and its 90% non-parametric confidence interval were determined using the non-parametric method of Hauschke *et al.* (1992) for the true difference of 'test-reference' ( $\mu_{Pen} - \mu_{Syringe}$ ). In the ANOVA model, group (= sequence) and subjects within group (= error) were taken as the between-subject factors and treatment period, and the residual term (= error) was taken as the within-subject factor. For all parameters, except  $t_{max}$ , 0.80–1.25 was used as acceptance range. For  $t_{max}$ ,  $\pm 20\%$  of the reference mean was used as acceptance range. The formulations were defined as bioequivalent with respect to a certain parameter if the 90% confidence interval was fully contained within the acceptance range for that parameter, according to the recommendations of the International Harmonization and Consensus DIA Meeting on bioavailability testing requirements and standards (Cartwright, 1991) and current Federal Drug Agency guidelines (Chen, 1992).

FSH pharmacokinetics were calculated using non-compartmental techniques with the computer program WinNonlin V1.1 (Scientific Consulting Inc., Apex, NC, USA). Parametric bioequivalence testing and ANOVA were performed using SAS for Windows V6.10 (SAS Institute Inc., Cary, NC, USA). Non-parametric bioequivalence testing was performed using BIOEQV60 (Wijnand, 1992).

## Results

#### Study population

Twenty-four subjects were initially selected, of whom two discontinued before recFSH treatment. One subject who received recFSH treatment was excluded from evaluation because of poor compliance with Lyndiol®. Another subject was excluded from pharmacokinetic analysis because she had

**Table I.** Overall local tolerance reactions in the 22 subjects who received recFSH treatment

Method of administration	Time after administration (h)	Subjects with local tolerance reactions			
		None		Any	
		n	(%)	n	(%)
Puregon®Pen	1	20	90.9	2	9.1
	24	22	100	0	–
Syringe	1	21	95.5	1	4.5
	24	20	90.9	2	9.1

FSH baseline values above the lower limit of quantification (0.25 IU/l) before receiving recFSH on both occasions. As the elimination half-life for this subject after treatment by syringe differed greatly from the remaining study population [54.5 versus 34.2 h (range 26.1–44.0 h) in the other subjects], it was concluded that Lyndiol® treatment had not completely suppressed endogenous FSH production in this patient.

The mean age of the remaining 20 subjects was  $23.2 \pm 3.3$  (range 18–33) years; mean body weight was  $63.2 \pm 7.1$  (range 50.7–75.7) kg; mean height was  $170.2 \pm 8.6$  (range 155–187) cm; and mean BMI was  $21.8 \pm 2.2$  (range 19.0–26.6) kg/m<sup>2</sup>.

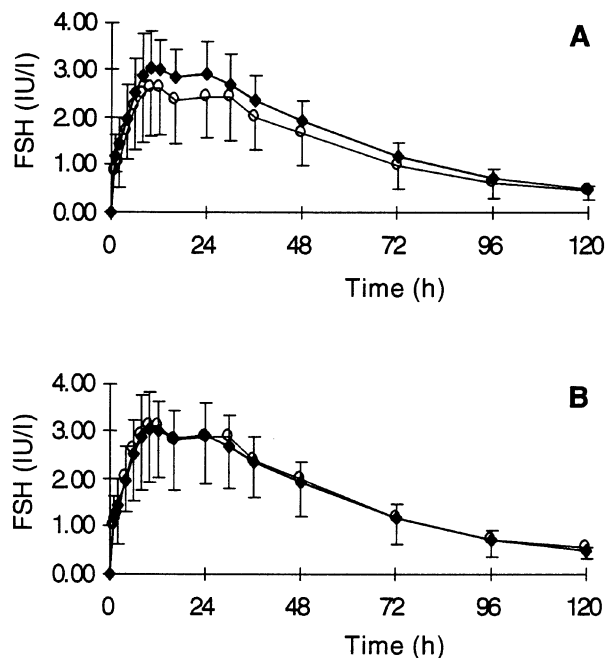
### Local tolerance

A summary of overall local tolerance reactions at 1 and 24 h after administration of recFSH is given in Table I. After injection by Puregon®Pen, redness was reported by two subjects at 1 h but no reactions were reported after 24 h. After injection by syringe, pain was experienced by two subjects, in one after 1 h and in another after 24 h. Bruising occurred in one patient, at 24 h after injection by syringe. Local reactions were all mild and generally short-lived.

### Pharmacokinetic analysis

The mean serum FSH concentrations for each pharmaceutical formulation over time are presented graphically in Figure 2. Mean serum FSH concentrations obtained after injection by syringe were not corrected for injected volume (Figure 2A). Without this correction, the serum FSH concentration versus time curves showed a similar pattern, but lower values after injection by syringe. For injection by syringe, measurements of the weights of the syringe immediately before and after recFSH injection showed that the actual amount injected was less than that anticipated (mean dose correction factor 1.18). Mean serum FSH concentrations, obtained after individual dose correction, are shown in Figure 2B. After dose correction, very similar concentration profiles were obtained after injection by syringe and Puregon®Pen. The maximum concentration of approximately 3.4 IU/l was generally reached within 13–16 h. Serum FSH concentrations remained elevated for about 18 h and then began to decline. At 120 h after injection mean serum concentrations were approximately 0.5 IU/l.

The mean values of the pharmacokinetic parameters obtained during bioequivalence testing of the two formulations are



**Figure 2.** Follicle stimulating hormone (FSH) serum concentrations versus time after injection of a dissolved cake with a normal syringe (○) or injection of a ready-for-use solution with a Puregon®Pen device (◆). (A) Syringe levels uncorrected for actual dose given. (B) Syringe levels corrected for actual dose given. Results are expressed as mean  $\pm$  SD.

**Table II.** Pharmacokinetic parameters (mean  $\pm$  SD) following recFSH treatment by two different formulations

Parameter	Puregon®Pen	Syringe	
		Uncorrected	Corrected <sup>a</sup>
$C_{\max}$ (IU/l)	$3.36 \pm 0.70$	$2.91 \pm 0.92$	$3.43 \pm 1.03$
$t_{\max}$ (h)	$12.9 \pm 6.2$	$16.2 \pm 8.0$	NA
$AUC_{0-f_{\text{fix}}}$ (IU/l•h)	$156.4 \pm 31.8$	$134.4 \pm 43.4$	$159.0 \pm 48.5$
$AUC_{0-\infty}$ (IU/l•h)	$215.1 \pm 45.8$	$185.7 \pm 58.8$	$220.3 \pm 67.0$
$en-C_{\max, f_{\text{fix}}}$ (h <sup>-1</sup> )	$0.0217 \pm 0.0038$	$0.0221 \pm 0.0042$	NA
$en-C_{\max, \infty}$ (h <sup>-1</sup> )	$0.0160 \pm 0.0038$	$0.0162 \pm 0.0040$	NA
$t_{1/2}$ (h)	$33.4 \pm 4.2$	$34.2 \pm 4.6$	NA
$CL_{\text{app}}$ (l/h/kg)	$0.0117 \pm 0.0029$	$0.0146 \pm 0.0059$	$0.0122 \pm 0.0051$

<sup>a</sup>Correction for injected dose by weighing syringe before and after administration; NA = correction not applicable;  $C_{\max}$  = peak serum concentration;  $t_{\max}$  = time of occurrence of  $C_{\max}$ ; AUC = area under the serum concentration–time curve;  $f_{\text{fix}}$  = last time point at which all subjects still have measurable FSH;  $en-C_{\max}$  = extent-normalized  $C_{\max}$  (see text);  $t_{1/2}$  = elimination half-life;  $CL_{\text{app}}$  = apparent clearance.

presented in Table II. The results of bioequivalence testing are given in Table III. Dose-dependent data in this table ( $C_{\max}$ ,  $AUC_{0-f_{\text{fix}}}$ ,  $AUC_{0-\infty}$  and  $CL_{\text{app}}$ ) were adjusted for the actual dose given with the syringe.

No sequence or period effects were found for any of the parameters tested. When pharmacokinetic parameters were used without the dose correction for injection with the normal syringe, the dose-dependent parameters ( $C_{\max}$ , AUC and  $CL_{\text{app}}$ ) were not bioequivalent. Based on point estimates after log-transformation, the observed difference for the pharmacokinetic parameters  $AUC_{0-\infty}$  and  $C_{\max}$  is 20% and 19% respectively. This corresponds to the mean dose correction factor incorporated for

**Table III.** Bioequivalence testing of recFSH administration by Puregon®Pen versus syringe

Parameter	Puregon®Pen (mean)	Syringe <sup>a</sup> (mean)	Point estimate $\mu_{\text{Pen}}/\mu_{\text{Syringe}}$	90% CI	Bioequivalence test outcome
$C_{\text{max}}$ (IU/l)	3.36	3.43	1.00	0.91–1.11	Bioequivalent
$t_{\text{max}}$ (h)	12.9	16.2	87.9 <sup>b</sup>	62.1–101.4	Not bioequivalent
$AUC_{0\text{-fix}}$ (IU/l•h)	156.4	159.0	1.02	0.93–1.11	Bioequivalent
$AUC_{0\text{-}\infty}$ (IU/l•h)	215.1	220.3	1.01	0.93–1.10	Bioequivalent
en- $C_{\text{max, fix}}$ (h <sup>-1</sup> )	0.0217	0.0221	0.99	0.95–1.03	Bioequivalent
en- $C_{\text{max, }\infty}$ (h <sup>-1</sup> )	0.0160	0.0162	0.99	0.94–1.05	Bioequivalent
$CL_{\text{app}}$ (l/h/kg)	0.0117	0.0122	0.99	0.91–1.08	Bioequivalent

For abbreviations, see Table II.

<sup>a</sup>Values for  $C_{\text{max}}$ ,  $AUC_{0\text{-fix}}$ ,  $AUC_{0\text{-}\infty}$  and  $CL_{\text{app}}$  are corrected for differences in injected dose.

<sup>b</sup>Given as % versus mean reference.

CI = confidence interval.

injections using the syringe, which was found to be 1.18. After dose correction, bioequivalence could be proven for all parameters except  $t_{\text{max}}$ .

## Discussion

This study compares two pharmaceutical formulations for the s.c. administration of the recFSH preparation, Puregon®. To distinguish between endogenous and exogenous FSH, endogenous gonadotrophin production was suppressed. For pituitary suppression, a high-dose combined oral contraceptive preparation was chosen, since it has been demonstrated that low-dose combined oral contraceptives do not ensure complete pituitary inhibition in all subjects (Dericks-Tan *et al.*, 1976). Since serum FSH concentrations in baseline samples were <0.25 IU/l, it was concluded that 3 weeks of pre-treatment with Lyndiol® is sufficient to obtain a pituitary suppression that is in almost all cases strong enough to permit accurate study of the pharmacokinetics of FSH in healthy volunteers. Several other studies using pituitary suppression with Lyndiol® have shown similar results (Out *et al.*, 1996; Duijkers *et al.*, 1997; Huisman *et al.*, 1997).

Subcutaneous injection of fluids can cause some side reactions, which may depend on a number of variables, e.g. needle size, injection volume and tonicity of the fluid. In this study, local reactions were all mild and short-lived. Injection of recFSH by both Puregon®Pen and syringe was well tolerated, and no apparent differences with respect to the local tolerance were observed. The number of local reactions found after injection with the normal syringe was low compared with a previous study in which local tolerance was assessed in 195 subjects treated with either s.c. or i.m. injection of recFSH (Out *et al.*, 1997); however, this might reflect the limited number of subjects included in the present study.

Because of the mode of administration, it was assumed that exactly 150 IU was injected by Puregon®Pen. For injection by syringe, measurements of the weight of the syringe just before and after recFSH injection showed that the actual injected amount of recFSH was 18% less than anticipated. Loss of recFSH solution can be attributed to the void volume of the syringe, and losses while filling the syringe and/or removing excess air. In clinical practice, this difference in the dose of 18% would most likely not be noticed, especially as

the Puregon® dose is individually titrated, based on ovarian response.

As there are large inter- and intra-individual variations in the response of the ovaries to exogenous gonadotrophins, it is impossible to establish a uniform dosage scheme for ovulation induction in assisted reproduction techniques. The dosage will, therefore, be adjusted individually depending on patient history and on the ovarian response as assessed by ultrasonography and monitoring of oestradiol concentration. The 18% difference in starting dose is therefore not considered to have major implications for current clinical practice.

After correction for injection losses, FSH serum profiles obtained from the two formulations were comparable (Figure 2A and B). Moreover, bioequivalence could be shown for both rate ( $C_{\text{max}}$  and en- $C_{\text{max}}$ ) and extent ( $AUC_{0\text{-}\infty}$ ,  $AUC_{0\text{-fix}}$  and  $CL_{\text{app}}$ ) of absorption-dependent parameters. For the other rate-dependent parameter,  $t_{\text{max}}$ , bioequivalence could not be proven, probably due to the high intra-subject variability which is caused mainly by the broad absorption peaks commonly found for gonadotrophin preparations (Out *et al.*, 1996; Huisman *et al.*, 1997).

The pharmacokinetic parameters derived in this study correlate very well with those found in other studies using s.c. administration of recombinant human FSH preparations (Le Cottonnec *et al.*, 1994; Mannaerts *et al.*, 1996).

In conclusion, this study suggests that injection of similar amounts of FSH using either a dissolved cake with a syringe or a ready-for-use solution with a Puregon®Pen are bioequivalent with respect to the main pharmacokinetic variables for rate and extent of absorption.

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