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Patterns of Serum FSH, LH and hCG After I.M. or I.V. Administration of hMG During Pituitary Suppression

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ABSTRACT: Objective—The study was undertaken to investigate the effects of a commonly used ovarian stimulation regimen on gonadotropin levels. **Methods**—The behavior of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG) was studied after intramuscular (i.m.) and intravenous (i.v.) human menopausal gonadotropin (hMG) administration. Six female volunteers participated in the study. During pituitary suppression with a gonadotropin-releasing hormone (GnRH) agonist (Buserelin), a single dose of hMG (150 IU) was injected i.m. or i.v., in a cross-over design with an interval of 2 weeks. Blood samples were collected frequently after the injection. Serum concentrations of FSH, specific LH and hCG were determined. **Results**—After i.m. administration of hMG, a peak FSH concentration of 7.4 ± 1.3 U/L was reached after 8 (6–24) hours, with a subsequent decrease. At 0.5 hour after i.v. administration, peak FSH values of 30.5 ± 5.6 U/L were obtained, followed by a decrease to baseline levels within 48 hours. Exogenous LH and hCG were hardly detectable after i.m. administration of hMG. One-half hour after i.v. injection of hMG, a small increase in specific LH levels to 6.7 ± 2.6 U/L was shown, followed by a decline. hCG concentrations increased after i.v. hMG administration to 7.6 ± 1.6 U/L. *Int J Fertil* 40(2):86–91, 1995

KEY WORDS: FSH, LH, hCG, hMG, gonadotropins

INTRODUCTION

GONADOTROPIN PREPARATIONS have been used in clinical practice for many years, mainly for stimulation of follicular growth and induction of ovulation [1,2]. These preparations contain follicle-stimulating hormone (FSH) and luteinizing hormone (LH) activities. FSH and LH are isolated primarily from urine of postmenopausal women. Since the

amount of LH is not sufficient to reach an FSH/LH ratio of 1, human chorionic gonadotropin (hCG), purified from urine of pregnant women, may be added in small amounts to the preparation to reach the necessary LH in vivo bioactivity [3].

Although many women have been treated with these gonadotropin preparations for over 25 years, relatively little is known about their kinetic behavior after injection: how high are the peak concentrations and how fast are they reached; what is the

decrease rate after injection? Previously published studies reported on pharmacokinetics of endogenous gonadotropins [4,5] or gonadotropins purified from human pituitaries [6,7]. Hormones isolated from urine, however, might exert very different kinetic properties [8]. Two of the studies on pharmacokinetics of urinary gonadotropins reported on males [9,10], while Sharma et al [11] investigated the pharmacokinetics of a purified FSH preparation in women with suppressed pituitary function. Diczfalusy and Harlin [12] also investigated the pharmacokinetics of gonadotropins in women after a single intravenous (i.v.) or repeated intramuscular (i.m.) administration, but they did not suppress the endogenous gonadotropin activity, which complicated the estimation of the half-life of LH. The effect of administration of human menopausal gonadotropin (hMG) on serum hCG concentrations has never been reported.

In clinical practice, women undergoing ovarian stimulation treatment are routinely given daily i.m. injections of hMG, often during pituitary suppression by a gonadotropin-releasing hormone (GnRH) agonist [13]. Recently, pulsatile i.v. administration of hMG has been used for ovarian stimulation; this would mimic the physiological release of pituitary hormones [14,15].

The present study investigates for the first time the effect on serum FSH, LH and hCG levels of a single i.m. and i.v. administration of hMG in a group of women during pituitary suppression.

METHODS

Study Design

Six healthy female volunteers participated in the study. They all had regular menstrual cycles of 26–30 days, two ovaries, normal early follicular levels of FSH, LH, prolactin, testosterone and thyroxin, and had not used sex steroids in the 3 months prior to the study. The age of the women varied from 28 to 35 years; their median height was 1.68 m (1.65–1.73), and their median weight, 63 kg (55–95). The body mass index varied from 19.5 to 35.0 mg/m² (median value 21.5 kg/m²). The study was approved by the Ethical Committee of the hospital. Written informed consent was given.

On day 21 of the cycle, intranasal administration of the GnRH agonist Buserelin (Suprefact[®], Hoechst

Holland N.V., Amsterdam) was started at a dose of 300 µg three times a day. If the serum estradiol concentration on the tenth day of Buserelin administration was below 500 pmol/L, pituitary suppression was considered sufficient. If the estradiol level was above 500 pmol/L, the estradiol determination was repeated two or three days later until the required low concentration was reached. Buserelin was continued during the entire study period.

Between 8:00 and 9:00 A.M. on the day after the estradiol level was below 500 pmol/L, 150 IU hMG (Humegon[®], Organon Int. B.V., Oss) was injected intramuscularly or intravenously. The first hMG administration was i.m. in three women and i.v. in the other three women. Two different batches of Humegon were used, with similar FSH (82 IU per ampule) and LH in vivo bioactivity (66 and 61 IU per ampule, respectively). Immediately before the hMG injection, at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 96 hours, and 1 week after hMG administration, blood samples were collected. The samples were centrifuged within 15 minutes (five minutes at 2,500 g) and the sera were stored at -20°C until assayed. After a washout period of 2 weeks, a second injection of 150 IU hMG was given using the other route of administration. The same protocol for the collection of serum samples was performed. One week after the second hMG injection Buserelin administration was discontinued.

Assays

The serum estradiol concentration on the tenth day of buserelin treatment was determined by a time-resolved fluoroimmunoassay (DELFLIA[®], Wallac Oy, Turku, Finland). The limit of 500 pmol/L was chosen because of the low sensitivity of the assay in the range from 150 to 500 pmol/L. The estradiol concentration immediately before the first hMG injection was measured retrospectively by a more sensitive radioimmunoassay [16].

Serum concentrations of FSH, specific LH and hCG were measured by applying time-resolved fluoroimmunoassays (DELFLIA). These tests are solid phase, two-site assays in which two monoclonal antibodies are directed against two separate antigenic determinants on the hormone. The assays detect only intact molecules. Serum samples are first reacted with immobilized monoclonal antibodies directed against a specific antigenic site on

the β -subunit of the hormone. Europium-labeled antibodies directed against a specific antigenic site on the α - or β -subunit are then reacted with the molecule, already bound to the solid phase antibody. After dissociation of the europium ions from the labeled antibody, the amount of fluorescence is measured.

The assay for FSH had a detection limit of 0.05 U/L, with an intra-assay and inter-assay variation of less than 5%. The assay for specific LH had a cross-reactivity with hCG of less than 1.5%. The detection limit for specific LH was 0.05 U/L; the intra-assay variation was less than 6%, the inter-assay variation less than 8%. The hCG assay had a cross-reactivity for LH of less than 0.5%. The detection limit was 0.5 U/L, the intra-assay variation less than 10%, the inter-assay variation less than 11%.

Statistics

All data are presented as mean values with the standard error of the mean.

RESULTS

The mean period of Buserelin administration before reaching the estradiol limit of <500 pmol/L was 13 ± 2.9 days. The mean estradiol concentration immediately before the first hMG injection, as measured by radioimmunoassay, was 203 ± 107 pmol/L. One woman completed the first series of blood samplings after i.v. hMG administration, but did not wish to continue the study. Therefore data were obtained from six women after i.v. administration and from five women after i.m. administration. The wash-out period between the two routes of administration appeared to be sufficiently long.

FSH concentrations after i.m. and i.v. administration of a single dose of hMG are presented in Figure 1 and Table I. The mean FSH concentration at 48 hours after both i.m. and i.v. injection did not differ from the FSH concentration before injection (4.6 ± 0.9 U/L and 3.6 ± 0.6 U/L, respectively).

Specific LH concentrations in serum, measured without the cross-reactivity of hCG, after i.m. and i.v. administration of a single dose of hMG are presented in Figure 2 and Table I. In the first half-hour after i.m. administration of hMG the patterns dif-

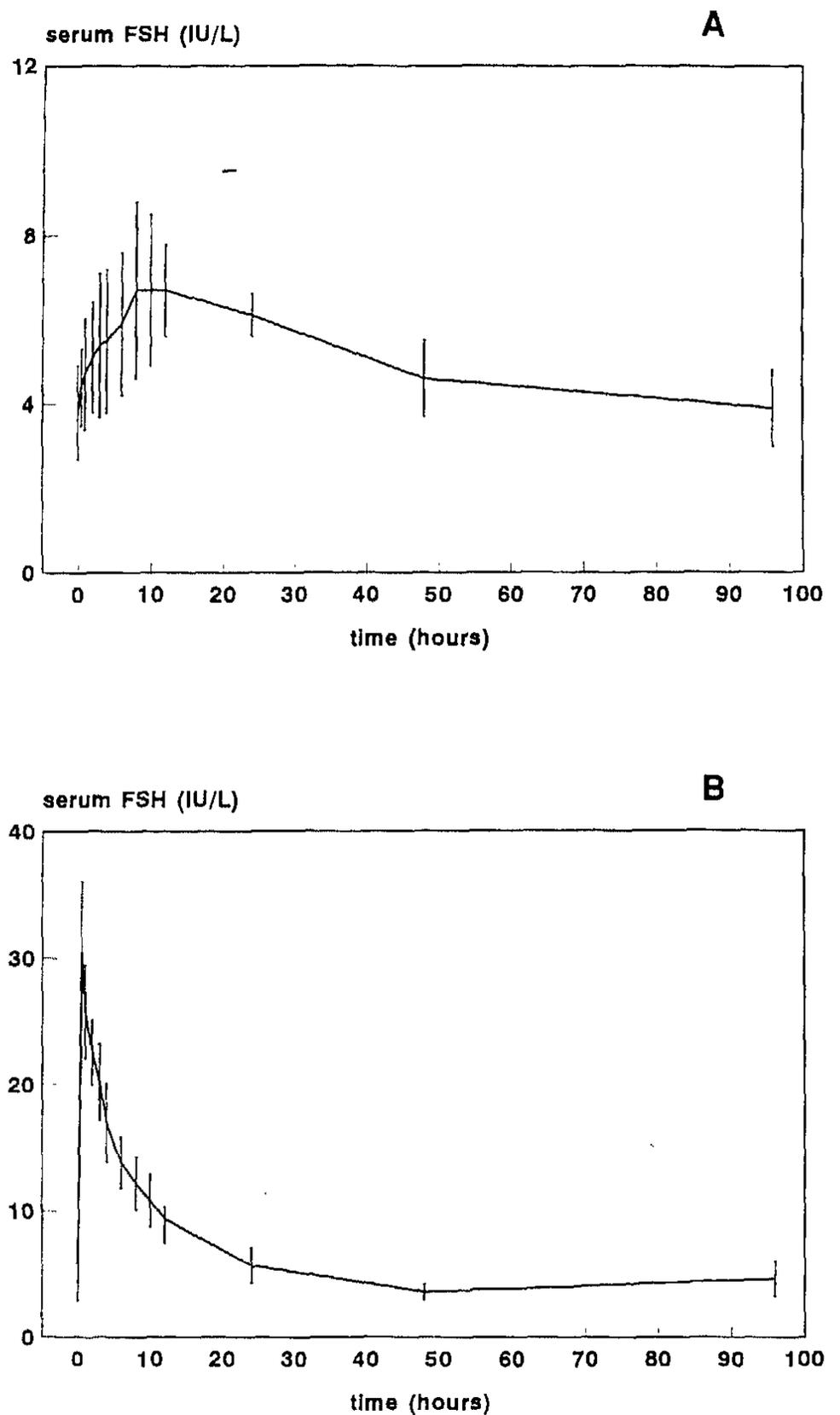


FIG. 1. Serum FSH concentrations in five women after i.m. injection (A) and in six women after i.v. injection (B) of 150 IU hMG, during GnRH agonist administration.

ferred: in two women, LH concentrations increased, whereas in three women no increase was seen. After the first half-hour a decrease of LH concentrations was present in all women, until six hours after injection, when LH concentrations returned to baseline values. Following the initial rise of LH concentrations after i.v. injection to 6.7 ± 2.6 IU/L, a decrease to 2.1 ± 1.0 U/L at four hours after injection was present. After this decrease, LH concentrations returned to baseline levels.

After i.m. administration of hMG, hCG concentrations hardly exceeded the detection limit of the

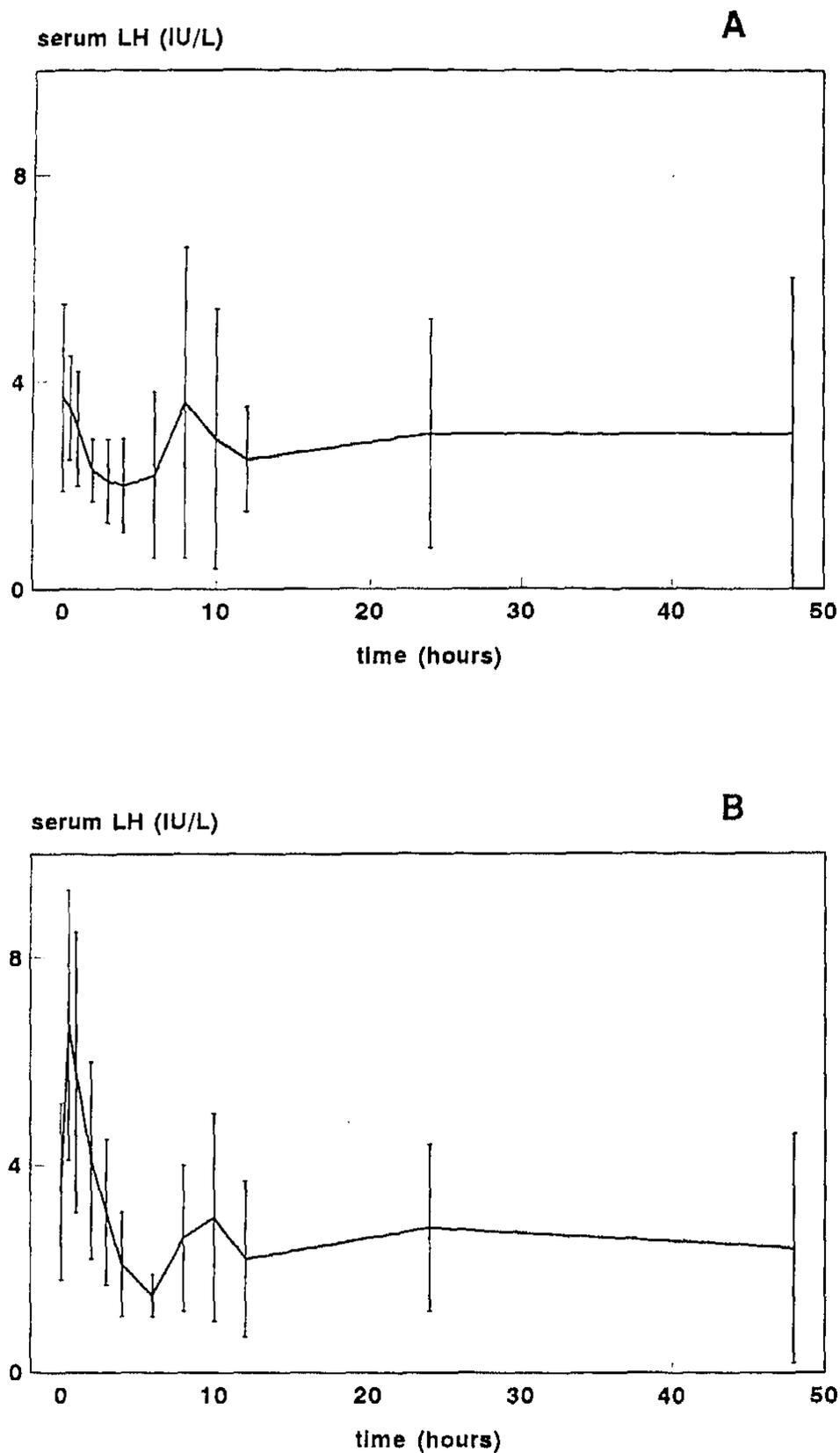


FIG. 2. Specific LH concentrations in serum in five women after i.m. injection (A) and in six women after i.v. injection (B) of 150 IU hMG, during GnRH agonist administration.

assay. Serum hCG concentrations after i.v. administration are presented in Table I.

DISCUSSION

After i.m. administration of hMG, serum FSH levels showed a smaller and more prolonged increase than after i.v. administration. The probable explanation is the formation of an intramuscular depot of hMG, with a gradual release into the systemic circulation. Since the absorption process from the

intramuscular depot would continue during the elimination of the previously absorbed amount of hMG, the decrease of FSH levels after i.m. administration was slower than after i.v. administration.

Results of previous studies in male subjects by Mizunuma et al [9] and by Jockenhövel et al [10] were in accordance with our results in females. Mizunuma et al administered a single i.m. dose of hMG and urinary FSH. They described a peak FSH value seven to eight hours after injection and half-lives of 36 and 32.6 hours, respectively. Jockenhövel et al described a peak FSH value 10 hours after the i.m. administration of urinary FSH, and a half-life of 24.6–36.2 hours. Sharma et al [11] and Diczfalusy and Harlin [12] performed studies in women. Sharma et al determined FSH after a single i.m. injection of purified urinary FSH during Buserelin administration. They observed peak FSH levels between 6 and 18 hours after injection, and a decline of FSH starting 24 hours after injection. Diczfalusy and Harlin measured FSH after a single i.v. administration of hMG without pituitary suppression. They observed a rapid decay with a half-life of 1.6 hours and a slow phase with a half-life of 11 hours. Our study and those of Jockenhövel et al and Sharma et al differ from the other studies mentioned in the application of a more sensitive assay.

We were able to differentiate between the LH and hCG components in hMG by applying two specific assays. Diczfalusy and Harlin [12] mentioned the difficulty in interpreting serum LH concentrations because of the presence of endogenous LH and the relatively low amount of administered LH. We met the same problem in our study, despite the administration of Buserelin. The measured LH activity after i.m. hMG administration was probably merely endogenous LH. The decrease of specific LH concentrations shortly after i.m. injection was previously described by Diczfalusy and Harlin [12]. Anderson et al [17] saw a decrease in serum LH levels after an i.m. injection with purified FSH, but not after an i.m. hMG injection. The most likely explanation for this decrease is a negative feedback mechanism within the hypothalamo-pituitary-ovarian axis. Exogenous FSH and LH activity may have stimulated the ovaries to produce estradiol, which in turn had a suppressive effect on the pituitary FSH and LH secretion [18]. The decrease of endogenous FSH could not be seen in the serum levels because of the presence of exogenous FSH. We consider the second LH rise in several women to be not

TABLE I

Mean basal (C_0) and maximal ($C_{t_{max}}$) concentrations of serum FSH, specific LH and hCG (with standard error) after a single intramuscular (i.m.) or intravenous (i.v.) administration of 150 IU human menopausal gonadotropin (hMG), in six healthy women during pituitary suppression with Buserelin.

		C_0	t_{max} (hours)	$C_{t_{max}}$
Serum FSH	i.m.	3.8 ± 1.1 U/L	8 (6–24)	7.4 ± 1.3 U/L
	i.v.	4.4 ± 1.5 U/L	0.5	30.5 ± 5.6 U/L
Serum LH spec	i.m.	3.7 ± 1.8 U/L	96 (0.5–168)	5.2 ± 2.7 U/L
	i.v.	3.5 ± 1.7 U/L	0.5 (0.5–96)	7.0 ± 2.6 U/L
Serum hCG	i.m.	<0.5 U/L	—	—
	i.v.	<0.5 U/L	0.5 (0.5–1)	7.6 ± 1.6 U/L

t_{max} = median interval post-injection before reaching peak value (range in parentheses).

a real rise but rather the return to baseline levels. Several other authors investigated the pharmacokinetics of LH. Yen et al [4] described a double-exponential disappearance curve of serum LH following surgical hypophysectomy in two women; Kjeld et al [7] determined a half-life of 2.3 hours after i.v. infusion of pituitary LH in males. Their study designs were not comparable with ours, and differed more from ordinary clinical practice.

In other studies, the pharmacokinetics of hCG were investigated. Damewood et al [19] studied the disappearance of hCG administered i.m. In our study, the dose of hCG in hMG was too low to detect hCG in serum after i.m. injection. Wide et al [20] administered hCG intravenously and measured serum hCG concentrations by RIA. They calculated a half-life of 6–10 hours, which is in agreement with our results.

In conclusion, we have described for the first time the different patterns of serum FSH levels over time after a single i.m. or i.v. injection of hMG during pituitary suppression. LH and hCG were hardly detectable after i.m. administration, but showed a demonstrable increase after i.v. injection.

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