

## CLINICAL STUDY

# GH responsiveness varies during the menstrual cycle

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## Abstract

**Objective:** The GH–IGF-1 axis is affected by oestrogen. Both endogenous and exogenous oestrogen facilitates the central drive of pulsatile GH secretion. However, the effect on IGF-1 levels is more subtle, and a reduction in GH sensitivity has been proposed. The IGF generation test has confirmed reduced GH sensitivity with high doses of exogenous oestrogen. It is not known, however, whether fluctuant levels of endogenous oestrogen modify GH sensitivity. To investigate this further, women were challenged with the IGF-1 generation test at different stages of the menstrual cycle.

**Methods:** Nine women (age 38(6) years (mean (s.d.)) with regular menstrual cycles were recruited. An IGF-1 generation test, s.c. injection of 7 mg GH, was performed in the early-follicular (EF), periovulatory (PO) and midluteal (ML) phases. IGF-1, insulin-like growth factor binding protein (IGFBP)-3 and acid-labile subunit (ALS) levels were measured at baseline and 24 h after GH administration.

**Results:** Oestradiol levels were lower in the EF than PO or ML phases (32.6(7.8) vs 69.6(16.2) vs 66.6(23.6) pg/ml respectively (repeated measures ANOVA,  $P < 0.001$ )). Baseline IGF-1 was lower, but increment IGF-1 (peak minus baseline) was higher in the EF than PO or ML phases (baseline: 291.8(56.6) vs 335.0(55.2) vs 346.6(78.2) ng/ml ( $P = 0.008$ ); increment IGF-1: 234.6(59.2) vs 194.7(37.8) vs 185.2(37.3) ng/ml ( $P = 0.008$ )).

**Conclusions:** Increased endogenous oestrogen levels are associated with only a modestly elevated baseline IGF-1 from midcycle onward despite a previously reported twofold increase in GH secretion. In parallel with this apparent GH insensitivity, increased endogenous oestrogen levels are associated with reduction in GH sensitivity evidenced by reduction in increment IGF-1. This may have clinical implications for women with isolated GH deficiency with regular menstrual cycles on a fixed dose of GH. This possibility requires further study.

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## Introduction

There are clear gender differences in the growth hormone (GH)–insulin-like growth factor (IGF)-1 axis. Women have higher mean 24-h GH levels than men as a result of greater GH secretory burst mass and higher degree of disordered GH release (1–5). Despite this excess of GH secretion, serum IGF-1 levels are not significantly different between the genders. Similarly, in GH-deficient (GHD) adults, despite the use of the same biochemical threshold for defining severe GHD by provocative testing, untreated GHD females have significantly lower serum IGF-1 levels (6), and require significantly higher doses of GH replacement to maintain equivalent serum IGF-1 values, than males of similar GH status (7). These observations in normal and GHD individuals imply relative GH resistance in women.

Oestrogen affects GH action at the level of the GH receptor expression and function (8). More recently, oestrogen has been shown to suppress GH-induced

Janus kinase 2 (JAK2) phosphorylation, leading to reduction in transcriptional activity of signal transducer and activator of transcription signaling (STAT), and stimulate expression of suppressor of cytokine signalling-2 (SOCS-2), which in turn inhibits JAK2 action (8).

Thus, oestrogen provides one explanation for the relative GH resistance observed in women. Clinical studies, however, have shown that exogenous oestrogen increases GH levels, but IGF-1 levels have been reported to be reduced, unchanged or increased. Consistent with the concept of oestrogen-modulated GH resistance, peripheral responsiveness to GH determined by an IGF-1 response to a single bolus of GH has been shown to be reduced in postmenopausal women on exogenous oestrogen compared with the unreplaced menopausal state (9).

Reduction in GH sensitivity due to exogenous oestrogen is clinically relevant, as the use of, in particular, oral oestrogen in a GH-replaced GHD individual results

in a higher dose requirement of GH to maintain IGF-1 levels in the normal range. It is, however, unknown whether endogenous oestrogen has a similar impact on the GH-IGF-1 axis, and whether monitoring of IGF-1 in a GH-replaced individual should take into consideration the phase of the menstrual cycle.

To investigate this possibility further, normal women were challenged with the IGF-1 generation test at different stages of the menstrual cycle.

## Subjects and methods

Nine healthy subjects (age 38.6 (6.5) years (mean (s.d.)); body-mass index (BMI) 25.2 (3.7) kg/m<sup>2</sup>) were recruited from hospital staff. Ethical approval was obtained from the local ethical committee, and all subjects gave informed, written consent. All subjects had regular menstrual cycles. No subject had either a condition (such as diabetes, liver disease or pituitary disease) or was taking any medication (such as oestrogen replacement or opioids) known to affect the GH-IGF-1 axis. All screening biochemistry (e.g. liver function tests, random glucose, haemoglobin (Hb)A1c and thyroid function tests) was normal.

### IGF-1 generation test

An IGF-1 generation test was performed at the following three time points during the menstrual cycle in each subject: the early follicular phase (EF) (days 2-5 after the initiation of menstruation), the periovulatory phase (PO) (days 13-15) and the midluteal phase (ML) (days 20-22). The IGF-1 generation tests were performed at each phase in random order in different menstrual cycles.

The IGF-1 generation test consisted of the administration of a single s.c. injection of 7 mg recombinant GH (Genotropin 1 mg = 3 IU; Pfizer, New York, USA). It is also the largest dose of GH to have been used in previous studies without side effects (10, 11).

Blood samples were taken before the injection of GH and 24 h after; serum oestradiol and progesterone levels were estimated on samples taken before the injection, whereas serum IGF-1, IGF-binding protein (IGFBP)-3 and acid-labile subunit (ALS) levels were measured on samples at both time points. This timing for blood tests was chosen because IGF-1 levels peak 18-24 h after s.c. injection of GH (11).

There is evidence of significant correlation in IGF-1 and IGFBP-3 responses in individuals undergoing sequential IGF-1 generation tests (12).

## Assays

**Serum oestradiol** Serum oestradiol was measured by RIA (Diagnostic Systems Laboratories, Webster, TX, USA) with a sensitivity of 2.2 pg/ml. The intra-assay

coefficients of variation for mean oestradiol concentrations of 5.3, 24.9, 40.4 and 92.6 pg/ml were 8.9%, 6.5%, 7.6% and 6.9% respectively. The interassay coefficients of variation for mean oestradiol concentrations of 5.3, 28.0, 42.3 and 108.7 pg/ml were 7.5%, 9.7%, 8.0% and 12.2% respectively.

**Serum progesterone** Serum progesterone was measured by RIA (Diagnostic Systems Laboratories) with a sensitivity of 0.12 ng/ml. The intra-assay coefficients of variation for mean progesterone concentrations of 0.50, 5.93 and 15.61 ng/ml were 8.0%, 6.9% and 4.8% respectively. The interassay coefficients of variation for mean progesterone concentrations of 0.16, 5.18 and 18.48 ng/ml were 13.1%, 12.9% and 9.2% respectively.

**Serum IGF-I** Serum IGF-I was measured by an immunoradiometric assay (Diagnostic Systems Laboratories), utilising an acid-alcohol extraction with a sensitivity of 0.8 ng/ml. The intra-assay coefficients of variation for mean IGF-I concentrations of 9.3, 55 and 263 ng/ml were 3.4%, 3.0% and 1.5% respectively. The interassay coefficients of variation for mean IGF-I concentrations of 10.4, 53 and 256 ng/ml were 8.2%, 1.5% and 3.7% respectively.

**Serum IGFBP-3** Serum IGFBP-3 was measured by an immunoradiometric assay (Diagnostic Systems Laboratories) with a sensitivity of 0.5 µg/l. The intra-assay coefficients of variation for mean IGFBP-3 concentrations of 1.0, 2.2 and 9.8 mg/l were 6.1%, 4.1% and 4.4% respectively. The interassay coefficients of variation for mean IGFBP-3 concentrations of 0.9, 3.5 and 11.0 mg/l were 9.0%, 4.6% and 3.8% respectively.

**Serum ALS** Serum ALS was measured by an enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories) with a sensitivity of 0.7 µg/l. The intra-assay coefficients of variation for mean ALS concentrations of 1.65, 7.72 and 29.17 mg/l were 6.1%, 7.5% and 3.8% respectively. The interassay coefficients of variation for mean ALS concentrations of 2.22, 7.90 and 30.13 mg/l were 8.6%, 2.8% and 8.9% respectively.

### Molar IGF-1/IGFBP3 ratio

The molar ratio of IGF-1/IGFBP-3, as an estimate of free IGF-1, was calculated by multiplying the IGF-1/IGFBP-3 ratio (ng/ml) by 3.7.

## Analysis and statistics

Data are presented as mean (s.d.). Increment IGF-1, IGFBP-3, ALS and molar ratio IGF-1/IGFBP-3 was calculated by subtracting baseline from peak levels. Similarly, percentage increase in IGF-1, IGFBP-3, ALS

or molar ratio IGF-1/IGFBP-3 was calculated by the following formula: increment divided by baseline multiplied by 100. One-way repeated-measures ANOVA was used to compare different phases of the menstrual cycle. Repeated measures regression analysis was used to determine whether the changes seen in baseline and increment IGF-1 were associated with the covariates, oestradiol and progesterone. Statistical significance was assumed for  $P < 0.05$ .

## Results (Table 1)

### Serum oestradiol and progesterone levels

Oestradiol levels were significantly lower in the EF phase than the PO or ML phase, and progesterone levels were significantly higher in the ML than EF and PO phases.

### Serum total IGF-1 levels

Baseline IGF-1 was significantly lower but increment IGF-1 was significantly higher in the EF than PO or ML phase (Fig. 1A and B). Peak IGF-1 was unchanged throughout the menstrual cycle. Repeated-measure regression analysis found that baseline IGF-1, increment IGF-1 and percentage increase in IGF-1, but not peak IGF-1, were predicted by levels of oestradiol (Rsqr. 0.20,  $P < 0.05$ ; Rsqr. 0.42,  $P = 0.05$ ; Rsqr. 0.29,  $P < 0.05$  respectively), but not by progesterone levels.

### Molar ratio IGF-1/IGFBP-3 levels (estimate of free IGF-1)

Molar ratio of IGF-1/IGFBP-3 demonstrated a similar trend although it reached statistical significance only in percentage increase of molar ratio IGF-1/IGFBP-3

and on individual comparisons with EF in baseline measurements.

### Serum IGFBP-3 and ALS levels

Peak IGFBP-3 was unchanged through the menstrual cycle. However, baseline IGFBP-3 was significantly higher, increment IGFBP-3 and percentage increase in IGFBP-3 significantly lower in the ML than the EF phase. Baseline, increment and peak ALS was unaffected through the menstrual cycle.

## Discussion

The GH-IGF-1 axis alters through the menstrual cycle. We believe that this is the first study to investigate how varying endogenous oestrogen levels might affect peripheral responsiveness to GH. We have demonstrated that, in association with increased endogenous oestrogen levels, from midcycle onward there is a reduction in peripheral responsiveness to GH.

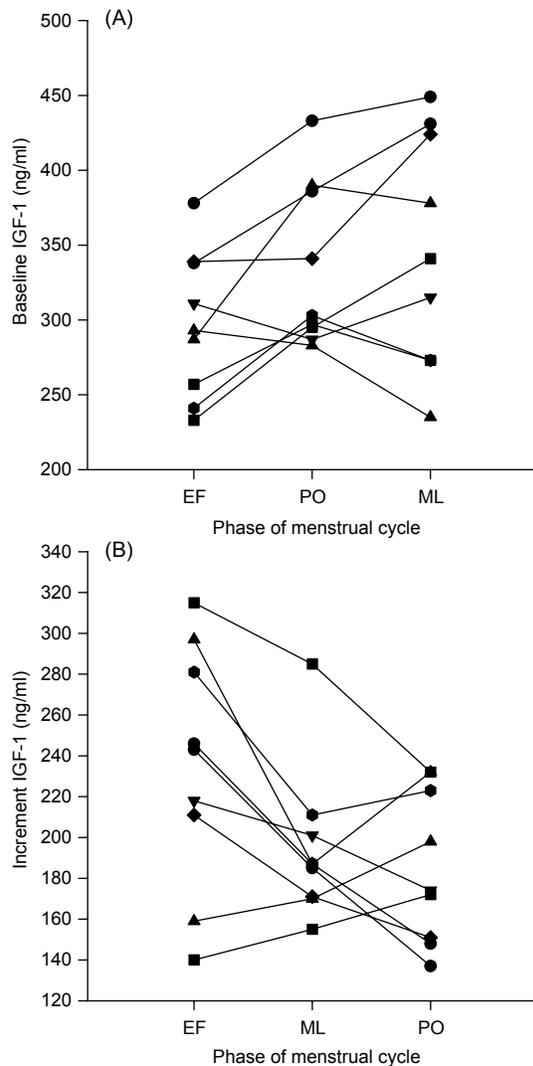
Ovesen *et al.* (13) have previously demonstrated elevated GH levels in 10 women during the periovulatory phase of the menstrual cycle, mediated by increased GH production and burst frequency, compared with the early follicular phase. They interpreted their findings of elevated GH levels, in association with a concomitant increase in serum IGF-1 in the periovulatory phase, as suggestive of central stimulation of the GH-IGF-1 axis alone, mediated by endogenous oestrogen levels. This effect may be modulated by either direct action of oestrogen on the hypothalamic pituitary axis or reduced IGF-1-dependent negative feedback, or both. However, the elevation in baseline IGF-1 from midcycle onward is modest (only 15% increase in this study above early

**Table 1** Oestradiol, progesterone, IGF-1, IGFBP-3, ALS and molar ratio of IGF-1/IGFBP-3 levels.

		EF	PO	ML	ANOVA
Oestradiol (pg/ml)	Baseline	32.6 (8)	66.6 (16)*	66.6 (24)*	$P < 0.0005$
	Progesterone (ng/ml)	1.12 (0.3)	2.20 (1.2)	15.81 (5.7)*	$P < 0.0001$
IGF-1 (mg/l)	Baseline	297.4 (48)	335.0 (55)*	346.6 (78)*	$P < 0.05$
	Increment	234.4 (59)	194.7 (37)*	185.2 (37)*	$P < 0.01$
	Peak	531.9 (70)	529.7 (60)	531.8 (67)	ns
	% increase	82.9 (28)	59.8 (17)*	57.1 (19)*	$P < 0.01$
	IGFBP-3 (mg/l)	Baseline	3.99 (0.3)	4.10 (0.4)	4.18 (0.3)*
ALS (mg/l)	Increment	0.39 (0.2)	0.27 (0.2)	0.18 (0.2)*	$P < 0.05$
	Peak	4.39 (0.3)	4.38 (0.3)	4.36 (0.3)	ns
	% Increase	10.0 (5)	7.0 (5)	4.5 (5)*	$P < 0.05$
	Baseline	24.6 (4)	26.8 (3)	25.6 (3)	ns
	Increment	7.86 (4)	6.61 (5)	7.73 (4)	ns
IGF-1/IGFBP-3 (moles)	Peak	32.5 (4)	33.4 (3)	33.3 (5)	ns
	% Increase	34.1 (19)	26.7 (22)	30.2 (13)	ns
	Baseline	0.271 (0.05)	0.303 (0.3)*	0.308 (0.7)*	$P < 0.06$
	Increment	0.174 (0.05)	0.147 (0.03)	0.146 (0.04)	$P < 0.08$
	Peak	0.445 (0.06)	0.450 (0.04)	0.454 (0.05)	ns
% Increase	66.3 (22)	49.2 (12)*	50.5 (18)*	$P < 0.05$	

Early-follicular phase (EF); periovulatory phase (PO); midluteal phase (ML).

\* Compared with EF phase  $P < 0.05$ .



**Figure 1** Serum levels of baseline (A) and increment (B) IGF-1 during IGF-1 generation test at different phases of the menstrual cycle. Early-follicular phase (EF); periovulatory phase (PO); midluteal phase (ML). Each linked symbol represents one study participant.

follicular levels) in comparison with the reported twofold increase in GH secretion; therefore, their findings left open the possibility that increased endogenous oestrogen levels result in not only increased central stimulation of the GH-IGF-1 axis but also reduced GH sensitivity (13). We therefore further explored the GH-IGF-1 axis during the menstrual cycle by tackling the potential effects of endogenous oestrogen on GH sensitivity in terms of IGF-1 status and reserve. Our results are in concert with the suggestion that GH insensitivity is associated with increased endogenous oestrogen levels, as there is a decrease in peripheral responsiveness to GH evidenced by a reduction in increment and percentage increase in IGF-1 from midcycle onward. Interestingly, however, there is no alteration in peak IGF-1

levels throughout the menstrual cycle, suggesting a fixed potential reserve capacity of IGF-1 generation.

If we use the molar ratio of IGF-1/IGFBP-3 as an estimate of free biologically active IGF-1, a similar trend to total IGF-1 is observed in baseline, increment and percentage increase in molar ratio IGF-1/IGFBP-3. The percentage increase in molar ratio of IGF-1/IGFBP-3 is 10% less than for total IGF-1.

Parallels can be drawn from a similar study looking at the modulating influence of exogenous oestrogen on IGF-1 generation, which proved to be both dose and route dependent. Baseline IGF-1 was unchanged after transdermal oestrogen but reduced after oral oestrogen, suggesting a reduction in peripheral responsiveness to GH. High doses of exogenous oestrogen via either the transdermal or oral route resulted in a reduction in peak IGF-1, whereas with low-dose transdermal oestrogen, chosen to mimic oestrogen levels in the follicular phase of the menstrual cycle, peak IGF-1 was unaffected (9), suggesting that at low levels of oestrogen the capacity to generate IGF-1 is undiminished.

The effect of changing progesterone levels on the GH-IGF-1 axis is unclear. Several studies (14–16) have examined the effect of exogenous progestogens on IGF-1 levels. The most recent study found that, when used in combination with oral or transdermal oestrogen, the more androgenic progestogens resulted in either no reduction of IGF-1 levels with oral oestrogen or an actual increase in IGF-1 levels with transdermal oestrogen (16). This suggests that oral progesterone, at least in its most androgenic form, may increase GH sensitivity. In this current study, it was not possible to determine whether higher levels of progesterone in the ML phase had an effect on IGF-1 levels due to the effect of oestrogen.

In conclusion, the changing sex-steroid milieu of the menstrual cycle alters peripheral sensitivity to GH. The clinical implication for women with isolated GHD with regular menstrual cycles on a fixed dose of GH is unknown, but in these women one might expect a reduced IGF-1 level from midcycle onward due to reduced peripheral responsiveness secondary to increased endogenous oestrogen levels; this could result in GH-dose adjustments that are influenced by the timing of the IGF-1 estimation during the menstrual cycle. It is a possibility that requires further study.

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