Changes in serum concentrations of growth hormone, insulin, insulin-like growth factor and insulin-like growth factor-binding proteins 1 and 3 and urinary growth hormone excretion during the menstrual cycle

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Few studies exist on the physiological changes in the concentrations of growth hormone (GH), insulin-like growth factors (IGF) and IGF-binding proteins (IGFBP) within the menstrual cycle, and some controversy remains. We therefore decided to study the impact of endogenous sex steroids on the GH-IGF–IGFBP axis during the ovulatory menstrual cycle in 10 healthy women (aged 18–40 years). Blood sampling and urinary collection was performed every morning at 0800 h for 32 consecutive days. Every second day the subjects were fasted overnight before blood sampling. Follicle stimulating hormone, luteinizing hormone (LH), oestriadiol, progesterone, IGF-I, IGFBP-3, sex hormone-binding globulin, dihydroepiandrosterone sulphate and GH were determined in all samples, whereas insulin and IGFBP-1 were determined in fasted samples only. Serum IGF-I concentrations showed some fluctuation during the menstrual cycle, with significantly higher values in the luteal phase compared to the proliferative phase (P < 0.001). Mean individual variation in IGF-I concentrations throughout the menstrual cycle was 13.2% (SD 4.3; range 0.1–18.3%). There were no cyclic changes in IGFBP-3 serum concentrations and no differences in IGFBP-3 concentrations between the luteal and the proliferative phases. Mean individual variation in IGFBP-3 concentrations throughout the menstrual cycle was 8.8% (SD 2.7; range 3.2–14.1). IGFBP-1 concentrations were inversely associated with insulin concentrations, and showed a significant pre-ovulatory increase that returned to baseline at the day of the LH surge. Fasting insulin concentrations showed some fluctuations throughout the menstrual cycle without any distinct cyclic pattern. No cyclic changes in urinary GH excretion during menstrual cycle were detected. We conclude that, although IGF-I concentrations are dependent on the phase of the menstrual cycle, the variation in IGF-I concentrations throughout the menstrual cycle is relatively small. Therefore, the menstrual cycle does not need to be considered when evaluating IGF-I or IGFBP-3 serum values in women suspected to have GH deficiency.

Key words: growth hormone/IGF-binding proteins/insulin/insulin-like growth factors/menstrual cycle

Introduction

The pulsatile secretion of growth hormone (GH) stimulates the production of insulin-like growth factor (IGF)-I. Circulating IGF-I is primarily derived from the liver and is bound to specific IGF-binding proteins (IGFBP) in plasma. The majority of IGF-I is bound to the GH-dependent IGFBP-3, whereas a minor fraction is bound to the insulin-dependent IGFBP-1. Serum IGF-I and IGFBP-3 concentrations as well as urinary GH excretion correlate significantly with the 24-h integrated GH secretion in children and adolescents (Blum et al., 1993). IGF-I stimulates cellular growth and differentiation and mediates the majority of mitogenic effects of GH, although direct metabolic actions of GH also exist. The bioavailability of IGF-I is dependent on alterations of circulating IGFBP as well as on specific proteolysis of these IGFBP, which decreases their affinity for the IGF.

In children, the physiological increase of gonadal steroids in puberty stimulates GH secretion (Kerrigan and Rogol, 1992). Subsequently, IGF-I and IGFBP-3 serum concentrations as well as urinary GH excretion are high in normal (Juul et al., 1994a, 1995a; Main et al., 1994) and precocious (Juul et al., 1995b) puberty.

In adults, less is known about the impact of endogenous sex steroids on serum IGF-I and IGFBP-3 concentrations. IGF-I and IGFBP-3 decrease with increasing age in both sexes, with no apparent difference according to gender (Juul et al., 1994b; Landin-Wilhelmsen et al., 1994). Postmenopausal women have lower IGF-I concentrations than premenopausal women, probably as a result of a diminished oestrogen concentration. This hypothesis has been strengthened by the observation of Ho et al. (1987), who found that endogenous oestrogen concentrations accounted for most of the age-related variation in circulating IGF-I in an adult population. IGF-I and IGFBP-3 determinations are useful in the primary diagnostic evaluation of short children suspected of GH deficiency (Blum et al., 1990), whereas the diagnostic value in adults suspected of GH deficiency is more controversial (Hoffman et al., 1994; Juul et al., 1997).

In order to optimize the diagnostic value, it may be important to consider factors other than GH secretion for describing the
variation in circulating IGF-I and IGFBP-3 concentrations. While an increasing number of reports on the GH–IGF–IGFBP system in relation to reproductive function have emerged (for review, see Homburg and Ostergaard, 1995), few studies exist on the physiological changes of the circulating GH–IGF axis within the menstrual cycle. Therefore, further information on the potential impact of the stage of the menstrual cycle on circulating IGF-I concentrations is required.

Methods and materials

Subjects
A total of 12 healthy women (aged 18–40 years) agreed to participate in the study. Two of the subjects were, however, subsequently excluded due to irregular bleeding (n = 1) and a lack of increase in progesterone during the luteal phase (n = 1), leaving data on 10 women for further analyses. All were menstruating regularly [mean (SD) length of cycle: 29.9 (5.1) days, mean (SD) length of menstrual period: 5.6 (0.8) days]. The mean (SD) standing height was 167.6 (4.8) cm, mean weight was 67.1 (13.4) kg and mean body mass index was 23.9 (4.5) kg/m² (range 18.1–32.8). None had a history of endocrine or metabolic disease. None was taking any medication or oral contraceptives at the time of the study.

Ethics
The study was approved by the local ethical committee (approval no. 01–331/93) and the subjects gave their informed written consent.

Study
Blood sampling and urinary collection was performed every morning at 0800 h for 32 consecutive days. Every second day the subjects were fasted overnight before the blood sampling. Follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, progesterone, IGF-I, IGFBP-3, sex hormone-binding globulin (SHBG), dihydroepiandrosterone sulphate (DHEA-S) and GH were determined in all samples, whereas insulin and IGFBP-1 were determined in fasted samples only. For each individual, the 32 consecutive days were transformed to days in the individual cycle in relation to the midcycle LH surge and menstrual periods. Hereby, intra-individual cyclic variations could be studied. Furthermore, mean values of menstrual, follicular, and luteal phases were defined as means of days −15 to −10, −10 to ovulation, and from ovulation to +15 respectively. No urine was collected during the menstrual period.

Analyses
IGF-I was determined in all subjects with a radioimmunoassay as originally described by Bang et al. (1991) with some modifications (Juul et al., 1994a). Serum was extracted by acid/ethanol and cryoprecipitated (AEC) prior to analysis in order to remove interfering binding proteins. It is well known that the low molecular weight IGFBP are not completely removed by AEC, but addition of exogenous IGFBP-1 to -2 revealed no significant interference with the tracer, which was a truncated monoiodinated IGF-I [Tyr31-des(1–31)IGF-I; Juul et al., 1994a]. Inter- and intra-assay variation was 8.7 and 3.9% (at B/B₀ of 0.4) respectively.

IGFBP-1 was determined by a commercially available sandwich enzyme immunoassay (Mediagnost GmbH, Tübingen, Germany) using a monoclonal anti-IGFBP-1 antibody.

IGFBP-3 was determined in all subjects by radioimmunoassay as described by Blum et al. (1990). IGFBP-3 was measured on unprocessed serum using a polyclonal rabbit antiserum and a purified human IGFBP-3 fragment as standard and radioligand. Reagents for the analysis were obtained from Mediagnost GmbH (Tübingen, Germany). Values are expressed as ng/l. Inter- and intra-assay variation was 7.3 and 3.5% respectively.

GH was determined in serum by a specific monoclonal enzyme-linked immunosorbent assay (ELISA) (Novo Nordisk, Denmark). Inter- and intra-assay variation was 10.0% (at 1.4 µg/l) and 7.7% (at 1.68 µg/l) respectively. The detection limit was 0.02 µg/l (Andersson et al., 1995). Values were calibrated against the WHO International Standard (IRP 80/505) and expressed as mIU/l (conversion factor: µg/l × 3.6 = mIU/l) for purposes of standardization.

Serum insulin concentrations were measured by means of radioimmunoassay as described by Kühn (1975).

The other analyses were also performed. The data on the oestradiol, progesterone, SHBG, DHEA-S, FSH and LH analyses were summarized in Table I.

Statistics
To assess whether or not the menstrual cycle had any effect on the variable of interest, we fitted a random effect model where the variable of interest, we fitted a random effect model where

Statistics

Results
All women were considered to have an ovulatory cycle, based on their luteal progesterone concentrations.

IGF-I concentrations showed some day-to-day fluctuations during the menstrual cycle (Figure 1), with significantly higher concentrations in the luteal phase compared to the proliferative (follicular) phase (see Table II and Figure 2). The mean (SD) individual variation in IGF-I concentrations throughout the menstrual cycle was 13.2 (4.3)% (range 10.1–18.3). The individual IGF-I and IGFBP-3 concentrations were not correlated with oestradiol or progesterone concentrations (data
Menstrual cycle and the GH–IGF–IGFBP system

Figure 1. Mean concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol (E2) and progesterone (left panels), insulin-like growth factor-I (IGF-I), IGF-binding protein (IGFBP)-3, IGFBP-1 and insulin (middle panels) as well as single spot serum growth hormone (GH), urinary GH excretion, dihydroepiandrosterone sulphate (DHEA-S) and sex hormone-binding globulin (SHBG) (right panels) during the menstrual cycle in 10 healthy women (solid lines). Dotted lines represent the 95% confidence intervals.

not shown). There were no cyclic changes in IGFBP-3 serum concentrations (Figure 1) and no differences in IGFBP-3 concentrations between the follicular and luteal phases (Table II). The mean (SD) individual variation in IGFBP-3 concentrations throughout the whole menstrual cycle was 8.8 (2.7)% (range 3.2–14.1).

Fasting IGFBP-1 concentrations were inversely associated with insulin concentrations when pooling all data from the 10 women, with pronounced clustering of IGFBP-1/insulin values according to each individual (not shown). IGFBP-1 concentrations showed large variation during the menstrual cycle (coefficient of variation 61.6%), with no differences between the follicular and luteal phases. There was a pre-ovulatory increase in IGFBP-1 concentration that returned to baseline on the day of the LH surge. IGFBP-1 concentrations were negatively associated with SHBG concentrations (data not shown). Fasting insulin concentrations fluctuated significantly throughout the menstrual cycle (coefficient of variation 26.8%), without any significant cyclic pattern. There was a significant negative association between insulin and SHBG concentrations when the data from all 10 women were combined. No cyclic changes in urinary GH excretion during the menstrual cycle were
Table II. Mean (SD) values of the various parameters in the different phases of normal menstrual cycle defined as follows: menstrual phase (days –15 to –10), follicular phase (days –10 to 0) and luteal phase (days 0 to 15). The mean coefficient of variation (CV) throughout the entire cycle is also given.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Menstrual phase</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>Mean CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I (ng/ml)</td>
<td>202 (78)</td>
<td>209 (78)</td>
<td>227 (80)</td>
<td>13.2 (4.3)</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>3.4 (1.9)</td>
<td>3.0 (1.9)</td>
<td>3.2 (1.9)</td>
<td>61.6 (36.2)</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>3774 (780)</td>
<td>3825 (811)</td>
<td>3742 (682)</td>
<td>8.8 (2.7)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>76 (31)</td>
<td>96 (38)</td>
<td>99 (60)</td>
<td>26.8 (7.1)</td>
</tr>
<tr>
<td>Serum GH (ng/ml)</td>
<td>1.1 (0.7)</td>
<td>1.4 (1.3)</td>
<td>1.8 (2.2)</td>
<td>106.4 (33.4)</td>
</tr>
<tr>
<td>Urinary GH (pg/h)</td>
<td>ND</td>
<td>308 (156)</td>
<td>293 (112)</td>
<td>48.9 (23.5)</td>
</tr>
</tbody>
</table>

IGF = insulin-like growth factor; IGFBP = IGF-binding protein; GH = growth hormone; ND = not determined.

Figure 2. Mean insulin-like growth factor-I (IGF-I) concentrations in the menstrual phase, follicular phase and luteal phase in 10 healthy women. n.s. = non-significant difference.

Discussion

This study showed that IGF-I serum concentrations were significantly higher in the luteal phase as compared with the follicular phase during the normal menstrual cycle. In contrast, IGFBP-3 did not change. Furthermore, urinary GH excretion exhibited no cyclic variation.

The results concerning IGF-I are in accordance with those of one study demonstrating higher IGF-I concentrations in the luteal than in the follicular phase in five healthy women (Caufrizz et al., 1986), but are at variance with other studies in which unchanged IGF-I concentrations throughout the menstrual cycle were demonstrated (Evans et al., 1984; Guidoux et al., 1986; Wang et al., 1995; Thierry van Dessel et al., 1996). Our findings of unchanged serum IGFBP-3 concentrations are in accordance with the only two previous studies on IGFBP-3 concentrations during the menstrual cycle (Wang et al., 1995; Thierry van Dessel et al., 1996). Taken together, these findings suggest that IGFBP-3 and IGF-I are differentially regulated. This differential regulation could simply reflect that they are synthesized in two different cell types of the liver: IGF-I in the hepatocyte, and IGFBP-3 in the Kupffer cells (Arany et al., 1994). Despite the fact that most of the circulating IGF-I is believed to be of hepatic origin, it has been suggested that locally produced IGF-I contributes to the circulating pool and accounts for some of the observed cyclic variation. However, a recent study demonstrated no differences between the concentrations of IGF and their binding proteins in peripheral vein as compared to concentrations from venous effluents of ovary and adrenal gland (Martikainen et al., 1997). These findings would argue against an ovarian contribution to the circulating pool of IGF, but do not exclude other peripheral sources (e.g. endometrial).

Endogenous oestrogen plays an important role in determining the sexually dimorphic pattern of GH secretion. However, contrasting effects of transdermal and oral oestrogen replacement therapy on GH secretion and IGF-I concentrations have been shown in postmenopausal women (Dawson-Hughes et al., 1986; Stege et al., 1987; Bellantoni et al., 1991; Weissberger et al., 1991; Slowinska-Srzednicka et al., 1992; Kelly et al., 1993; Massa et al., 1993; Goodman-Gruen and Baret-Connor, 1996). The inhibiting effect of oral oestrogen on IGF-I concentrations is probably mediated by an inhibition of hepatic IGF-I production during the first pass through the hepatic portal system, whereas transdermal oestrogens deliver oestradiol directly into the circulation. We found no cyclic changes in urinary GH concentrations throughout the menstrual cycle despite varying concentrations of endogenous oestradiol in plasma. To our knowledge, urinary GH excretion has not previously been described during the menstrual cycle, but our finding is in accordance with previous reports of no cyclic changes in spontaneous 24-h GH secretion in 23 healthy women (Zadik et al., 1985). In addition, most studies demonstrate no differences in single spot serum GH values between the follicular and the luteal phase of the menstrual cycle (Spellacy et al., 1969; Genazzani et al., 1975; Toth et al., 1987), whereas Frantz and Rabkin (1965) found a significant rise in ambulatory (but not basal) GH concentrations in the early luteal phase shortly after ovulation in nine healthy women, which is in agreement with the present findings. It has been suggested that endogenous GH might relate to ovarian responsiveness to FSH (Stone and Marrs, 1991; Wilson et al., 1991).
Some controversy exists concerning the variation of stimulated GH secretion throughout the menstrual cycle. In some studies, higher peak GH concentrations in the luteal phase compared to the follicular phase have been described following stimulation with pyridostigmine (O’Keane and Dinan, 1992) or dexamethasone (Thakore and Dinan, 1995), whereas other studies were unable to detect any differences in the maximal GH response among the different menstrual phases following administration of hpGRF-40 (Evans et al., 1984) or gonadotrophin-releasing hormone (Genazzani et al., 1975; Gelato et al., 1984; Benito et al., 1991). In summary, it appears that differences in stimulated GH during different phases in the menstrual cycle depend on the type of stimulus.

We found no differences in fasting insulin concentrations between the follicular and the luteal phases. The demonstrated pre-ovulatory increase in fasting IGFBP-1 that significantly decreased on the days of the LH surge may suggest a regulatory role of circulating IGFBP-1 in the process of follicular maturation. Our present finding is in accordance with that of Wang et al. (1995), who also found a pre-ovulatory IGFBP-1 peak. In addition, they demonstrated the lack of a pre-ovulatory IGFBP-1 peak in women with inadequate luteal function based on mid-luteal progesterone concentrations below 10 ng/ml. These findings were in contrast to those of others, who did not find cyclical changes in IGFBP-1 in healthy menstruating women (Suikkari et al., 1987; Thierry van Dessel et al., 1996). However, none of these studies was based on fasting serum samples, although IGFBP-1 is regulated by food intake and insulin concentrations (Suikkari et al., 1988; Mogul et al., 1996). IGFBP-1 is thought to modify the free fraction of IGF-I, thereby affecting biological activity of IGF-I. IGFBP-1 and SHBG are both regulated by insulin in similar fashion in many physiological and pathological situations (Holly et al., 1989, 1991; Weaver et al., 1990). However, the observed pre-ovulatory increase in IGFBP-1 was not accompanied by changes in insulin or SHBG concentrations, suggesting that IGFBP-1 may be involved in insulin-independent follicular maturation and possibly in luteal function in women.

We conclude that, although IGF-1 concentrations are significantly dependent on the phase of the menstrual cycle, the individual variation in IGF-1 concentrations throughout the menstrual cycle is relatively small. Therefore, the menstrual cycle does not need to be considered when evaluating IGF-1 or IGFBP-3 serum values in women suspected of GH deficiency.

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