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Substantial Urinary Concentrations of Material Resembling β -Core Fragment of Chorionic Gonadotropin β -Subunit in Mid-Menstrual Cycle

Patrick Neven,^{1,4} Ray K. Iles,¹ Ian Howes,² Kiran Sharma,¹ John H. Shepherd,¹ Ray Edwards,² William P. Collins,³ and Tim Chard¹

We measured the day-to-day variations in concentrations of β -core, luteinizing hormone (LH), and α -subunit in urine during the menstrual cycle. The α -subunit concentrations showed a pattern similar to that of the LH concentrations. β -Core-like material was increased during and up to 3 to 4 days after the surge in urine LH. The urine LH concentration was associated with the presence of β -core immunoreactivity during the urine LH peak. Chromatography showed that, at the peak LH concentration and at 2 days after the LH peak, β -core immunoreactivity could be accounted for by the presence of a peptide of low molecular mass similar to the β -core molecule of hCG, but probably originating from the degradation of LH. The prolonged excretion of gonadotropin metabolites in the midcycle must be considered when β -core is being assessed as a tumor marker.

Indexing Terms: luteinizing hormone · tumor marker · variation, source of

The metabolism of circulating gonadotropins includes degradation in the kidneys and excretion in urine. The most abundant material related to human chorionic gonadotropin (hCG) in urine from pregnant women is a fragment of the β -subunit known as β -core (1-3).⁵ A similar protein of small molecular mass, which reacts in assays for luteinizing hormone (LH), is present in the urine of nonpregnant pre- and postmenopausal women (4). This cross-reactant probably results from degradation of the β -subunit of LH and is likely to have considerable sequence homology with the β -core fragment of hCG (5). Consequently, this β -LH core reacts in assays directed against the β -core of hCG.

Measurement of urinary metabolites of β -hCG, especially of the core fragment, has been advocated as a marker in some nontrophoblastic gynecological malignancies (6-11). However, the presence of β -LH core immuno-

reactivity in the urine of normal postmenopausal women must be considered when the concentration of β -core-like material is being evaluated in cancer patients (5).

There is little information on the amounts of urinary gonadotropin metabolites excreted during the normal menstrual cycle. Because gynecological malignancies may occur during the premenopausal period, the presence of such metabolites in premenopausal women would be highly relevant to an assessment of the clinical value of urinary β -core as a cancer marker. We measured β -core-like activity in early-morning urine specimens collected during the menstrual period, and present evidence that urinary gonadotropin metabolites are substantially increased during and after the midcycle LH surge.

Materials and Methods

Samples

A total of 463 early-morning urine samples were collected from 22 healthy premenopausal women (ages 19-29 years) from day 2 of a menstrual cycle up to the following menses. The procedures followed were in accordance with the ethical standards of our institution's ethical committee. Sodium azide (1 g/L) was added to all samples prior to storage at -20°C . All samples were assayed for β -core-like material by RIA. The LH peak (day 0) was measured by immunofluorometric assay (IFMA) in each of the women by using samples from day -1 to day +5, the periovulatory period. Samples from the same period from nine randomly chosen cycles were also assayed for free α -subunit (RIA) and by another LH assay (immunoradiometric assay, IRMA).

Assays

β -Core immunoreactivity was measured by RIA with a polyclonal sheep antiserum raised against purified β -core from hCG (S504) and ^{125}I -labeled purified β -core (12). Standards were calibrated against material provided by R. Wehmann and D. Blithe (NIH, Bethesda, MD). This assay shows partial cross-reaction with intact hCG (6.9%) and free β -hCG (18%), but negligible cross-reactivity with LH, thyroid-stimulating hormone (TSH), and follicle-stimulating hormone (FSH) (<0.7%). LH was measured by two methods: a time-resolved IFMA and an IRMA. The IFMA was performed in the Diagnostics Research Unit of King's College School of Medicine and Dentistry. The assay is based on the direct sandwich technique in which a polyclonal antibody is used to capture the analyte and a europium-labeled monoclonal antibody against the α -subunit is used to

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⁵ Nonstandard abbreviations: LH, luteinizing hormone; hCG, human chorionic gonadotropin; IFMA, immunofluorometric assay; IRMA, immunoradiometric assay; NIH, National Institutes of Health; TSH, thyroid-stimulating hormone; and FSH, follicle-stimulating hormone.

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monitor the reaction. There is about 60% cross-reactivity for intact hCG, 15% for β -subunit of hCG, and 50% for FSH, TSH, and the free α -subunit.

The polyclonal IRMA was provided by the North East Thames Regional Immunoassay service. For this assay the same affinity-purified polyclonal antibody as was used in the IFMA was used for the capture and detection of LH. Significant cross-reaction was found with hCG (100%), free β -hCG (20%), and the α -subunit of the glycoprotein hormones (7%); negligible cross-reactivity occurred with FSH and TSH (<0.2%).

α -Subunit was estimated by RIA with a polyclonal rabbit antibody to α -hCG provided by J. G. Pierce (UCLA School of Medicine, Los Angeles, CA). α -hCG (CR123; NIH Center for Population Research) was used as a standard and for the preparation of a labeled tracer. There was a partial cross-reaction with intact hCG (4%), LH (8%), and FSH (2%); cross-reaction with β -hCG and β -core was <1%.

Chromatography

Samples of urine (40 mL) from one woman, collected on the day of the midcycle urine LH peak and 2 days later, were concentrated by lyophilization after dialysis against deionized distilled water. Samples (0.3 mL) were loaded into a column (600 \times 9 mm) of Sephadex G-100 (Pharmacia, Uppsala, Sweden) and eluted with 0.05 mol/L phosphate-buffered saline by ascending flow at 6 mL/h at 4 $^{\circ}$ C. Fractions (0.7 mL) were collected and assayed for β -core and LH (IRMA). The column was calibrated by elution of Dextran Blue together with 125 I-labeled β -hCG and β -core.

Statistical Methods

The amounts of each analyte were not gaussian-distributed. Accordingly, the values were expressed as medians and 95% confidence intervals. The Mann-Whitney *U*-test was used to compare the relation between the LH (IFMA) concentration and β -core activity in the urine on the day of the LH peak. The Wilcoxon signed-ranks test was used to assess the concentration differences of each analyte relative to the day before the urine LH peak.

Results

In all subjects there was one major single peak of LH as measured by IFMA. All results were analyzed in relation to this day (day 0). An example of the findings of all four assays in one cycle is shown in Figure 1. The LH (IFMA) showed one distinct peak with a small rise 3 days later. α -Subunit showed a similar pattern. The presence of β -core and LH (IRMA) coincided with the LH surge but produced a major peak 3 days later.

Figure 2 shows the presence of β -core in early-morning urine samples in relation to the LH peak (IFMA) in all 22 individual cycles. β -Core concentrations were higher on days 0 to 4 than on other days ($P < 0.05$; Wilcoxon signed-ranks test). There was a difference ($P < 0.05$; Mann-Whitney *U*-test) in the LH (IFMA) concentration between the women who had measurable

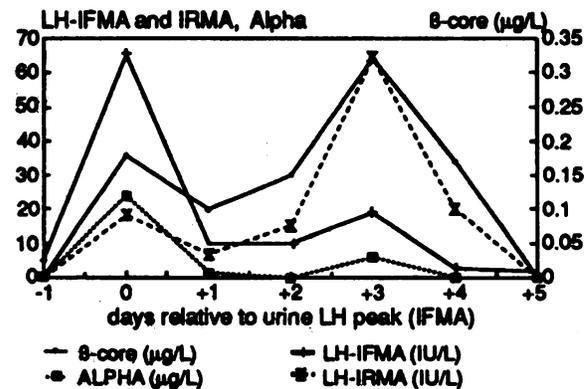


Fig. 1. Results of all four assays during one cycle

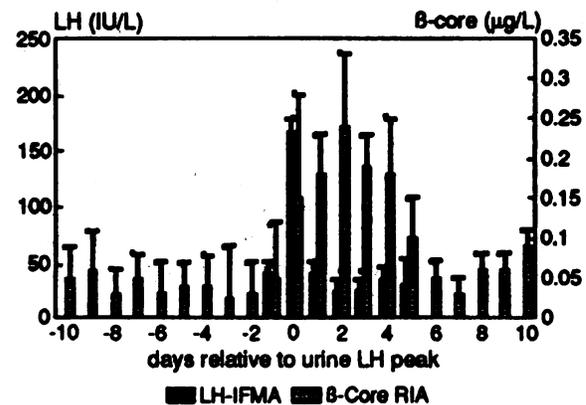


Fig. 2. β -Core immunoreactivity in relation to the LH peak

β -core concentrations ($n = 12$) and the women with $< 0.10 \mu\text{g/L}$ β -core ($n = 10$) activity at the LH peak. β -Core concentrations during the LH surge in these 12 cycles ranged from 0.17 to 0.60 $\mu\text{g/L}$ (median 0.32 $\mu\text{g/L}$). β -Core concentrations after the urine LH peak ranged from 0.34 to 0.95 $\mu\text{g/L}$ (median 0.45 $\mu\text{g/L}$). In 10 of these 12 cycles there was a separate peak of urine β -core between 1 and 5 days after the urine LH surge.

In 10 cycles there was no β -core activity at all on the day of the LH peak. Nine of these, however, had a peak of β -core activity between 1 and 5 days after the urine LH peak day. The concentrations at the peak in each case ranged from 0.15 to 0.32 $\mu\text{g/L}$ (median, 0.29 $\mu\text{g/L}$).

Figure 3 shows median concentrations for all four assays in the nine cycles. Median β -core concentrations were highest on the days after the LH peak. Median concentrations as measured by LH (IRMA) were highest on day 0 and remained high on days 1 to 5. The pattern of α -subunit concentrations closely paralleled LH (IFMA) immunoreactivity.

Chromatography of the concentrated urine (day 0) showed an early peak corresponding to intact LH and a later peak corresponding to β -core (Figure 4A). The day-2 sample (Figure 4B) showed LH and β -core activity only in the fractions containing metabolites of lower molecular mass.

Discussion

Our finding that the excretion of β -core-like material continues for several days after the urine LH peak in

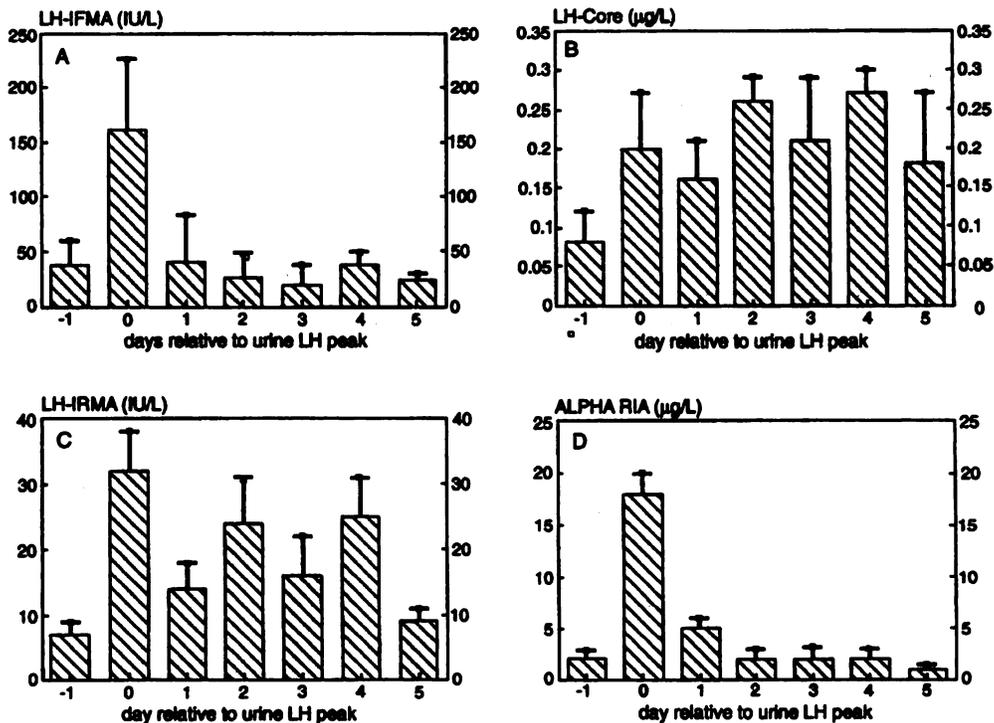


Fig. 3. Median concentrations for all four assays in the nine cycles

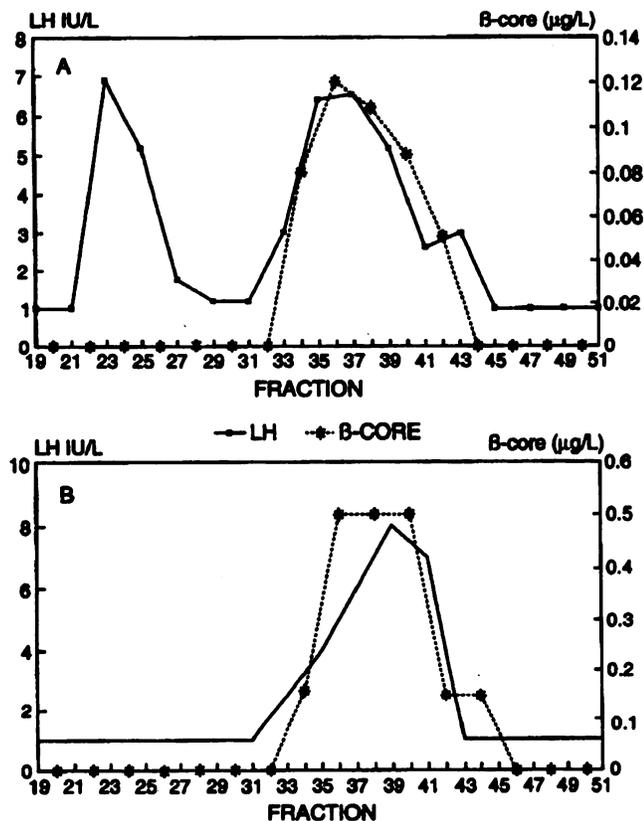


Fig. 4. Chromatography of concentrated urine

premenopausal women is important, because midcycle urine β -core concentrations were often above the 95th centile that we were using as a cutoff for the use of β -core as a tumor marker ($0.36 \mu\text{g/L}$) (manuscript in preparation). This finding weakens the value of β -core

as a tumor marker for gynecological malignancies in premenopausal women.

We regarded the IFMA as the "gold standard" for urine LH. Identification of the materials present in urine and their contribution to the results of a given assay is not straightforward. The β -core-like material measured at day 0 in 12 of the 22 women might be due to β -core derived from LH or hCG, or to the cross-reaction of intact LH or hCG in the β -core assay. The fact that women without β -core activity on the LH-peak day had lower concentrations of LH (IFMA) might support the view that cross-reactivity occurs with intact LH. However, the cross-reaction of intact LH in the β -core assay is $<0.7\%$ (12). It is also possible that some of the immunoreactivity on day 0 is related to intact hCG, because specific assays for LH and hCG in serum showed the presence of an hCG protein similar to LH (13). These authors reported that hCG is produced in a pulsatile fashion in all normal adults. In premenopausal women, they found a midcycle ovulatory peak of intact hCG coincident with the LH peak; however, the molar concentration of the hCG peak was much lower than that of the LH peak. In support of the presence of β -core material is the finding of a peak of immunoreactive material of low molecular mass in urine collected on day 0 (Figure 4A). Variation in the timing of the LH surge between individual women and the variable duration of the surge may explain why metabolites were present in some subjects but not others.

The β -core immunoreactive material present in 19 of the 22 women some days after the urine LH peak might be LH- or hCG-derived metabolites. This β -core-like material reacted in the LH IRMA, which does not recognize

the β -core fragment of pregnancy. This supports the view that it might be the β -core of LH, not hCG.

A second peak in urinary LH within 24 h of the initial surge in urine LH, originally shown by Thomas and Ferin (14), was attributed to the pulsatile nature of LH secretion (15). Other authors have also reported a secondary, smaller, increase in urinary LH (16–18). Beitins et al. (4) reported the presence of LH, FSH, free α -subunit, and an LH- β of smaller size in the urine of normal premenopausal women throughout the menstrual cycle, but did not comment on daily variation relative to the LH peak.

There are three possible explanations for the observation that urine β -core-like material is maximal after the day of peak urine LH: (a) There might be a continued and increasing secretion of a core-like material related to LH, with a peak in the luteal phase. (b) Metabolism of gonadotropin in the kidneys might lead to prolonged urinary excretion of core material from a slow-turnover pool in the renal parenchyma. The disappearance rate of both LH and hCG from serum follows a double exponential curve, with a first component reflecting clearance from blood and a slow component from a pool of organs involved in degradation (19). After administration of hCG and its β -subunit to volunteers, the appearance of core material in the urine is delayed for 12–24 h (20–22). In our subjects, β -core-like activity was maximal at days 1 to 5 after the urine LH peak. Cole et al. (23) described a deactivation mechanism for hCG, leading from hCG in the cell to β -core fragment in the urine. β -Core fragment concentrations diminished more slowly than hCG concentrations. A similar deactivation pathway might exist for LH. (c) Cross-reaction might occur with FSH, which appears in urine 1 to 4 days after the peak in urine LH (16, 24). Further, FSH metabolites could account for some of the core immunoreactivity.

The fact that urine β -core concentrations are increased for several days in the midcycle should be taken into account in considering the value of β -core measurements in premenopausal patients with cancer.

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