The Common Genetic Variant of Luteinizing Hormone Has a Longer Serum Half-Life than the Wild Type in Heterozygous Women

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Context: The common genetic variant of human LH has two mutations and an extra N-linked oligosaccharide chain, a modification expected to affect the half-life in the circulation.

Objectives: Our objectives were to determine the half-lives of variant and wild-type forms of LH during GnRH receptor blockade in heterozygous women and to determine the time-related changes in isoform composition.

Design and Participants: Serum samples were obtained from three healthy women heterozygous for variant LH before and up to 20 h after administration of the NAL-GLU GnRH antagonist.

Main Outcome Measures: The half-lives were estimated by monoexponential decay. The number of sialic acid and sulfonated N-acetylgalactosamine residues per wild-type and variant LH molecule and the distribution of molecules with zero, one, two, or three sulfonated residues were measured.

Results: The variant LH had a half-life that was approximately 40% longer than the corresponding forms of wild-type LH (148 vs. 108 min; P < 0.001). Variant LH had more sialic acid residues per molecule than wild type (3.6 vs. 2.4; P < 0.05), whereas the number of sulfonated residues was similar (1.0 vs. 0.98). The decline in the variant LH during GnRH receptor blockade was associated with a decrease in sulfonated and an increase in sialic acid residues similar to that for in wild-type LH. Isoforms of either variant or wild-type LH with two to three sulfonate groups per molecule had the shortest half-life.

Conclusion: Variant LH remains longer in circulation than wild type during GnRH receptor blockade in heterozygous women, in accord with its higher content of sialic acid. (J Clin Endocrinol Metab 95: 383–389, 2010)

A common genetic variant form of LH was first detected in Finland (1) and subsequently in Japan (2, 3). Nucleotide sequencing showed that variant LH had two mutations (Trp^8Arg and Ile^15Thr) on the \(\beta\)-subunit (2–4). A consequence of one of the mutations is an extra glycosylation site (Asn^13-Ala-Thr) compared with the wild-type LH.

Individuals with the variant LH can be detected both by an immunoassay technique and by hybridization assay (5). The genetic variant of LH has a worldwide distribution, and the carrier frequencies in different populations vary from 0–54% (5, 6). Clinical implications of the variant LH have been reported to include a higher frequency of reproductive disorders in women including unexplained infertility, ovulatory disorders, and premature ovarian failure (7, 8) and a slower progression through puberty in boys (9).

Human wild-type LH is secreted from the pituitary and circulates in blood as spectra of different isoforms. These isoforms vary with respect to the number of terminal sialic acid and sulfonated N-acetylgalactosamine (SO_3-GalNAc)
residues on the oligosaccharides (10). The composition of isoforms in serum varies during the menstrual cycle, changes after menopause, and is different in women with polycystic ovarian syndrome and in healthy men compared with healthy women (10).

Modifications of the oligosaccharides on the wild-type LH molecule determine its half-life in the circulation. Sulfonation decreases and sialylation increases the half-life as estimated during GnRH receptor blockade in women (11). The oligosaccharide chain at the additional glycosylation site on variant LH is therefore expected to modify the half-life in the circulation. A shorter half-life for variant LH or recombinant human variant LH compared with wild-type LH was reported in three studies in which half-life was estimated in the rat circulation (12–14). However, the half-life of variant LH has not previously been characterized in the human circulation.

In the current study, we have characterized endogenous variant and wild-type LH and examined their half-life in three women heterozygous for the variant LH by determining their decays in peripheral blood during GnRH receptor blockade (11, 15). In this model, the variant and wild-type LH are thus synthesized and secreted within an individual in whom potentially confounding factors such as the endocrine environment, body weight, and age are controlled. The average number of sialic acid and sulfonated GalNAc residues per variant and wild-type LH molecule in each blood sample and the concentrations of isoforms with zero to three sulfonated GalNAc residues were measured with a method based on neuraminidase treatment and electrophoresis (10). These studies also allowed us to determine whether there are time-related changes in the numbers of sialic acid and sulfonated GalNAc residues per variant LH molecule when LH declines after GnRH receptor blockade, similar to those reported for wild-type LH (11).

Subjects and Methods

Subjects and experimental design of the GnRH receptor blockade study

Three healthy women who were heterozygous for variant LH participated; one woman was in the early follicular phase (EFP), one at the gonadotropin midcycle surge (MCS), and one woman was postmenopausal (PM) and off any estrogen replacement for at least 6 months. The principle underlying the use of the GnRH receptor blockade model to assess the pharmacodynamics of endogenous LH and FSH has been described previously (15). The subjects were admitted to the General Clinical Research Center of the Massachusetts General Hospital. For each study, blood was sampled every 10 min for 4 h before and 8 h after sc administration of 150 μg/kg of the NAL-GLU GnRH antagonist followed by hourly samples for an additional 12 h. Samples were pooled for the following time periods: 4–2 h before (basal level) and 2–4 h (3-h level), 6–8 h (7-h level), and 16–20 h (18 h level) after antagonist administration. The study was approved by the Institutional Review Board of the Massachusetts General Hospital, and all subjects provided written informed consent.

Assay of variant and wild-type LH

The LH concentrations in serum samples and fractions eluted after agarose suspension electrophoresis were measured using two different sandwich fluorimunnoassays (5, 6). The total (variant plus wild-type) LH concentration was measured using LHspec (Delfia, PerkinElmer-Wallac Oy, Turku, Finland), as previously described (16). This method recognizes equally variant and wild-type LH (6). Gonadotropin values were expressed in international units per liter using the International Standards for pituitary LH (80/552) as reference standards. The detection limit in serum was 0.02 IU/liter, and the interassay coefficient of variation was less than 3%, estimated at concentrations between 0.6 and 60 IU/liter.

The other assay using monoclonal antibody-I3 recognizes only wild-type LH (17). The ratio between wild-type and total LH concentration was calculated. Sera of individuals homozygous for wild-type LH were assayed simultaneously. All ratios were adjusted to obtain a geometric mean ratio of one for wild-type LH to eliminate variations due to differences in the concentrations of variant LH and free LH β-subunits in the LH standard preparations. The concentrations of variant and wild-type LH were calculated using the adjusted ratio value.

The ratios between the serum concentrations of variant and wild-type LH were estimated in a reference group of 141 women heterozygous for variant LH, with a median age of 50 yr and range of 15–91 yr. The heterozygous individuals were classified using the immunoassay ratio method (5, 6). The reference group of heterozygous women comprised 34 (15.7%) of 216 healthy female medical students at Uppsala University and 107 (15.4%) of 696 female ambulatory and hospitalized patients in Uppsala Community. The reference group was obtained from discard specimens from clinical serum TSH assays, all with a normal TSH level.

Neuraminidase treatment and electrophoresis

Neuraminidase treatment was performed as previously described (10) with the exception that the mixture was incubated for 24 h at 4 C instead of 37 C because the recovery of wild-type LH activity, estimated using the antibody-I3, was 96.0 ± 2.2% (n = 23) at 4 C compared with 60% at 37 C. The desialylation of LH was equally effective at the two temperatures. All serum samples were analyzed before and after neuraminidase treatment with an electrophoretic technique using a 0.10% agarose suspension in veronal buffer (pH 8.7) (10, 18). The net charge was estimated as electrophoretic mobility in albumin mobility units (AMU); one AMU = 1 cm2 × s−1 × V−1 × 106. The mobility of wild-type LH without sulfonate and sialic acid groups was −49 mAMU, and the increase in mobility per charged group on LH was 81 mAMU as determined by analyses of the electrophoretic LH peak patterns of serum samples from 48 individuals homozygous for wild-type LH. The corresponding mobility for variant LH was −53 mAMU with an increase per charged group of 66 mAMU. These figures were determined from analyses of LH peak patterns of sera from 20 individuals homozygous for variant LH.

The amount and the pattern of wild-type LH eluted after electrophoresis were determined using the antibody-I3. The amount and pattern of variant LH were obtained by subtracting...
the wild-type LH values from those obtained with the assay of total LH. The average number of sialic acid and sulfonated GalNAc per LH molecule and the distribution of molecules with zero to three sulfonated GalNAc were estimated, as previously described (10).

The majority (about 60%) of variant LH isoforms had four or five negatively charged groups and the variation was from one to eight. The corresponding variation for wild-type LH isoforms was from one to six, and the majority (about 80%) had three or four negatively charged groups per molecule.

Data analyses

The half-lives in the circulation of endogenous variant and wild-type LH and different isoforms of both variant and wild-type were estimated after GnRH receptor blockade using single-phase exponential decay as previously described (11). Values before and at 3 and 7 h after GnRH antagonist administration were used for calculation of the half-life. A nadir value of 7.5% of baseline, estimated from analyzing the decays of sulfonated LH, was used in the calculations.

Data were analyzed using two-tailed Student’s t test with paired observations where applicable. Serum levels of variant and wild-type LH, ratios between number of sialic acid and sulfonated residues per molecule, and half-life values were log transformed before statistical analysis. A P value < 0.05 was considered to be significantly different. If not otherwise indicated, data are presented as mean ± SEM.

Results

Serum concentrations of variant and wild-type LH: basal levels and reference group

The basal levels of total, variant, and wild-type LH of the three women are shown in Fig. 1 (upper panels). The ratios of the basal serum concentrations of variant to wild-type LH levels in the individual subjects were 1.64 (EFP), 1.14 (MCS), and 1.16 (PM).

The serum concentration of the variant LH was higher than that of wild-type LH in 84% of the reference group of 141 heterozygous women. The geometric mean (95% confidence limits of mean) of the concentration ratio of variant to wild-type LH of these heterozygous women was 1.21 (1.17–1.26), which was significantly (P < 0.001) different from 1.

The ratios of the basal serum concentrations of variant to wild-type LH for the three women were within the 95% confidence limits for single values (0.81–1.81) of the women in the reference group.

Serum levels of variant and wild-type LH during GnRH antagonist treatment

The changes in serum concentration of the total, the variant, and the wild-type LH after administration of the GnRH antagonist are shown in Fig. 1, expressed in international units per liter (upper panels) and as a percentage of the basal level (lower panels). Sera of three women heterozygous for variant LH are represented as follows: PM by squares, MCS by triangles, and EFP by circles. Variant LH is represented by open symbols, wild-type LH by filled symbols and variant plus wild-type LH by half-filled symbols.

FIG. 1. Changes in serum concentration of LH (left panels) and of variant and wild-type LH (right panels) after GnRH antagonist administration, expressed in international units per liter (upper panels) and as a percentage of the basal level (lower panels). Sera of three women heterozygous for variant LH are represented as follows: PM by squares, MCS by triangles, and EFP by circles. Variant LH is represented by open symbols, wild-type LH by filled symbols and variant plus wild-type LH by half-filled symbols.

The concentration of LH decreased up to 7 h after GnRH antagonist administration but was not significantly different between 7 and 18 h (P > 0.05).

The variant to wild-type LH ratios increased after GnRH antagonist injection to a maximum at 7 h of 2.10 for EFP and 1.49 for MCS and at 18 h of 1.82 for PM, consistent with a more rapid disappearance of wild-type LH after GnRH receptor blockade.

Electrophoretic mobility

Both variant and wild-type LH were more anionic, i.e. more negatively charged, in serum samples taken after administration of the GnRH antagonist, consistent with more rapid disappearance of more basic isoforms. The change of the mean mobility was 10.9 ± 3.1 mAMU (P < 0.05) at 3 h and 14.8 ± 3.4 mAMU (P < 0.05) at 7 h.

Number of sialic acid and sulfonated GalNAc residues per LH molecule at baseline

The average number of sialic acid and sulfonated GalNAc residues per variant and wild-type LH molecule
in serum was estimated for each individual before and after GnRH antagonist (Fig. 2). Before administration of the GnRH antagonist, the mean number of sialic acid residues per LH molecule was greater for variant compared with wild-type (3.62 ± 0.40 and 2.40 ± 0.20, respectively; \( P < 0.05 \)), whereas the mean number of sulfonated residues was not different for variant and wild-type LH (1.00 ± 0.24 and 0.98 ± 0.12, respectively).

The total number of negatively charged residues (sialic acid plus sulfonated residues) on the oligosaccharides was 35.9 ± 2.6% higher on the variant than on the corresponding wild-type LH (4.62 vs. 3.38).

**Number of sialic acid and sulfonated GalNAc per LH molecule after GnRH antagonist**

The number of sialic acid residues per LH molecule in the circulation was greater after GnRH receptor blockade with a maximum at 7 h (Fig. 2). The mean increase for variant LH, 0.44 ± 0.11, was similar (\( P > 0.05 \)) to that for wild-type LH, 0.30 ± 0.04.

During GnRH receptor blockade the number of sulfonated residues per LH molecule in the circulation decreased and at 7 h this was significant (\( P < 0.05 \)) and similar for wild-type and variant LH, minus 0.18 ± 0.03 and minus 0.15 ± 0.03, respectively (Fig. 2).

The increase in number of sialic acid residues per LH molecule was larger than the decrease in sulfonated residues at all time points after GnRH antagonist, leading to an increase in negatively charged LH molecules in serum, as also reflected in the electrophoretic mobility.

The mean ratio of sialic acid to sulfonated residues per variant LH molecule increased during the first 7 h after GnRH antagonist administration from a mean value of 4.16 ± 1.29 to 5.64 ± 1.53 (\( P < 0.05 \)). The corresponding ratio for wild-type LH also increased significantly, from 2.55 ± 0.41 to 3.43 ± 0.57 (\( P < 0.05 \)).

**Serum levels of LH isoforms**

The time course of the disappearance after GnRH antagonist of isoforms with different numbers of sulfonated GalNAc residues per molecule are presented in Fig. 3. Values of isoforms with two and three sulfonated residues per molecule were pooled. The serum levels are expressed in percentage of baseline.

The variant and the wild-type LH isoforms with two to three sulfonated residues disappeared faster than those with zero or one. Wild-type LH isoforms disappeared faster than the corresponding variant LH isoforms, consistent with the greater number of sialic acid residues on variant LH.

**Half-life of wild-type and variant LH and of different LH isoforms as estimated during GnRH receptor blockade**

The estimated half-life of wild-type and variant LH and of their isoforms with different numbers of SO₃GalNAc residues per molecule are given in Table 1 for each individual. The ratio between the concentrations of wild-type LH and total LH in the serum samples was determined six times for the EFP and 10 times for the PM sera. These determinations were made on serum and diluted serum and on activity eluted after electrophoresis. The half-life was calculated for each determination and presented as geometric means in Table 1. The half-life of variant LH was approximately 40% longer (\( P < 0.001 \)) than that of corresponding forms of wild-type LH.

The isoforms with two to three sulfonated residues had the shortest half-life of both variant and wild-type LH. The mean half-life of these LH isoforms was 70 ± 6.5% of the nonsulfonated isoforms (\( P < 0.01; n = 6 \)). Isoforms with one sulfonated residue had a half-life that was 98% of the nonsulfonated.
Discussion

In this study, we have examined the half-life in peripheral blood of variant and wild-type LH during GnRH receptor blockade in women heterozygous for variant LH. Our results indicate that the variant form of LH has a half-life in the circulation that is approximately 40% longer than that of wild-type LH. A likely explanation for the longer half-life is the higher content of sialic acid residues per molecule on the variant form compared with the corresponding wild-type LH.

Using an identical model, we have shown recently that the numbers of terminal sialic acid and sulfonated GalNAc residues on the oligosaccharide chains of the LH molecule regulate its disappearance rate in the human circulation (11). In the current study, the endogenous variant and wild-type LH forms were analyzed in serum of individuals heterozygous for the variant form of LH. This experimental model makes it possible to compare the two forms of LH within an individual, thus controlling for potential differences such as steroid milieu, body weight, renal and hepatic function, and age.

The results of the present study stand in contrast to previous studies in which the human variant LH was found to have a shorter half-life than wild-type LH when tested in a rat model (12–14). In two of the previous studies, recombinant preparations of variant and wild-type LH were used (13, 14). The half-life of both the variant (5–9 min) and wild-type (12–22 min) LH in the rat was significantly shorter than previous estimates of half-life of endogenous LH in the human (11, 15). The short half-lives may be explained by differences in glycosylation of the recombinant preparations compared with the LH forms in the human blood and/or species differences with respect to the clearance of LH from peripheral circulation. It is not known whether the clearance from the circulation of variant compared with wild-type LH differs in the rat and in the human, making it important to compare their clear-

![FIG. 3. Mean ± SEM of serum levels after GnRH antagonist administration of isoforms of variant (var) and wild-type (wt) LH with zero (left panel), one (middle panel), and two to three (right panel) sulfonated GalNAc residues per molecule, expressed as a percentage of the basal level. Mean values of three women heterozygous for variant LH are shown. Variant LH is represented by open symbols and wild-type LH by filled symbols.](image)

| TABLE 1. Half-lives in minutes, estimated after GnRH receptor blockade in three heterozygous women, of wild-type LH and variant LH and isoforms with zero, one, and two to three SO₃-GalNAc per molecule |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Serum level     | Half-life (min) |                 | Serum level     | Half-life (min) |                 |                 |                 |
|                 | (IU/liter)      | Geometric       | 95% confidence  | (IU/liter)      | Geometric       | 95% confidence  |                 |                 |
|                 |                 | mean            | limits          |                 | mean            | limits          |                 |                 |
| **Wild-type LH**|                 |                 |                 |                 |                 |                 |                 |                 |
| EFP (n = 6)     | 1.18            | 94.2            | 89.1–99.6       | 1.94            | 116⁵           | 112–120         |
| MCS (n = 2)     | 8.31            | 88.8            | 79.5–99.3       | 9.49            | 112            | 101–124         |
| PM (n = 10)     | 12.0            | 163             | 151–175         | 13.8            | 257⁵           | 239–276         |
| Isoforms       |                 |                 |                 |                 |                 |                 |                 |                 |
| EFP             |                 |                 |                 |                 |                 |                 |                 |                 |
| 0               | 0.30            | 109             |                 | 0.31            | 108            |                 |
| 1               | 0.33            | 99.4            |                 | 0.75            | 131            |                 |
| 2–3             | 0.55            | 82.5            |                 | 0.88            | 101            |                 |
| MCS             |                 |                 |                 |                 |                 |                 |                 |                 |
| 0               | 2.95            | 110             |                 | 4.39            | 123            |                 |
| 1               | 3.88            | 88.3            |                 | 2.39            | 137            |                 |
| 2–3             | 1.48            | 60.3            |                 | 2.71            | 89.8           |                 |
| PM              |                 |                 |                 |                 |                 |                 |                 |                 |
| 0               | 3.15            | 202             |                 | 7.76            | 283            |                 |
| 1               | 7.20            | 161             |                 | 2.80            | 282            |                 |
| 2–3             | 1.59            | 103             |                 | 3.29            | 188            |                 |
| All 12 estimates| 108             | 87.2–133        |                 | 148⁵           | 114–192        |                 |

⁵ Number of half-life estimations using several different determinations of the ratio between wild-type LH and total LH in serum.

⁵ P < 0.01 vs. wild type, paired observations.

⁵ P < 0.001 vs. wild type, paired observation.
ances in a human model. In the third study, blood samples were collected 20 min after GnRH administration in men and young women homozygous for variant LH or wild-type LH (12) and injected iv into the rat circulation. The estimated mean half-life was shorter for variant compared with wild-type LH for both female (44 vs. 53 min) and male (26 vs. 48 min) samples. This study is complicated by unknown factors associated with the injection of human sera into rats and by known influences of GnRH on LH isoforms in blood. GnRH stimulation has been shown to induce disparate responses of variant and wild-type LH in heterozygous individuals (19). Some of the newly released LH isoforms circulating in blood after GnRH administration are less anionic (acidic) and, thus, are expected to have a different glycosylation pattern leading to a shorter half-life (20).

In our study, the comparisons between variant and wild-type LH were made on a pool of serum samples taken every 10 min during a 2-h period, and the half-lives of the variant and wild-type LH were analyzed simultaneously in the same individual exposed to the same endocrine environment. The higher serum levels of variant LH compared with wild-type LH in the three women and in 84% of the 141 heterozygous women in the reference group are in accord with a longer half-life of the variant form, provided that the secretion rate is similar for the two forms of LH.

The estimated half-life for wild-type LH of the three women are close to those previously reported for regularly cycling and postmenopausal women using the same model of GnRH receptor blockade (11, 15). During GnRH receptor blockade, the increase in sialic acid and decrease in sulfonated residues of variant and wild-type LH are similar to those reported for the wild-type LH (11). LH isoforms with two to three sulfonated residues disappeared faster from the circulation than those with zero or one of both the variant and the wild-type, also in agreement with our previous study on wild-type LH (11). LH molecules, both variant and wild-type, with two or more oligosaccharides terminating with SO3−-GalNAc are most likely removed from the circulation by a human hepatic receptor similar to that found in rodents (21, 22).

The variant LH has an extra glycosylation site at position Asn13 leading to four branched oligosaccharide chains on variant LH compared with three on wild-type LH. Theoretically, this increases the number of possible positions for terminal sialic acid or sulfonated GalNAc residues on variant LH by 33%. Consistent with this prediction, the observed total number of negatively charged residues (sialic acid plus sulfonated residues) on the oligosaccharides was 35.9 ± 2.6% higher on the variant than on the corresponding wild-type LH in the current study.

The biochemical events leading to terminal sialylation or sulfonation of LH have been reviewed by Baenziger (23). The sulfonation pathway leading to terminal SO3−-4GalNAc is first regulated by a peptide-specific β1-4GalNAc-transferase adding GalNAc to the subterminal GlcNAc residue on the biantennary glycan chains. This enzymatic activity occurs in competition with a β1-4galactosyltransferase adding galactose to the same subterminal GlcNAc in a sialylation pathway leading to terminal sialic acid. A Pro-Leu-Arg tripeptide motif on the β-subunit of LH and a cluster of cationic amino acids (Pro-Leu-Arg-Ser-Lys-Lys) within an α-helix on the α-subunit are recognized by the peptide-specific β1-4GalNAc-transferase leading to a high rate of GalNAc transfer on the LH molecule (24, 25).

In the present study, the number of sulfonated residues was similar on variant and wild-type LH. The larger number of negatively charged residues on variant LH was exclusively due to a greater number of sialic acid residues. This suggests that the three oligosaccharides with identical positions on variant and wild-type LH are sulfonated to the same degree on the two forms of LH, whereas the fourth oligosaccharide at position Asn13 is mainly sialylated. This could occur if the peptide-specific β1-4GalNAc-transferase adding GalNAc to the fourth oligosaccharide chain at the position Asn13 cannot simultaneously reach and recognize the peptide motifs on the α- or β-subunit of LH.

There is hitherto no good explanation for the higher frequency of reproductive disorders in women with the variant form of LH (7, 8). We propose that the longer half-life observed for variant LH could be a contributing factor to some of these disorders. Pulsatile exposure to LH at the granulosa cells is essential for the control of ovulation and also for the luteinization of these cells (26). The longer half-life of variant LH will result in a decrease of the pulsatile amplitudes of LH at the granulosa cells, which could disturb the precise control of these ovarian functions.

In conclusion, the common genetic variant of human LH having two mutations and an extra glycosylation consensus site is cleared less rapidly from peripheral circulation during GnRH receptor blockade than wild-type LH in heterozygous women. The longer half-life of variant LH, consistent with the finding of a larger number of sialic acid residues per molecule, is suggested to be a contributing factor to the higher frequency of reproductive disorders in women with variant LH.

Acknowledgments

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