Disappearance of human chorionic gonadotropin and its \( \alpha \)- and \( \beta \)-subunits after term pregnancy

Juha Korhonen,* Henrik Alfthan, Pekka Ylöstalo, Johannes Veldhuis,1 and Ulf-Håkan Stenman

We have used high-specificity and precision immunofluorometric assays to measure the elimination half-times of human chorionic gonadotropin (hCG), hCG\( \alpha \), hCG\( \beta \) in serum over 21 days after delivery in six women with term pregnancies. Baseline concentrations and half-times were calculated with the use of a curve-fitting algorithm for multiexponential decay. In contrast to the two-component model, a three-component exponential function with baseline provided a fit for which predicted values could not be distinguished from the observed values by analysis of variance. Median half-times were 3.6, 18.0, and 53.0 h for hCG; 10.2, 23.4, and 194 h for hCG\( \beta \); and 0.6, 6.2, and 21.9 h for hCG\( \alpha \). The mean ratio of hCG\( \alpha \) to hCG decreased rapidly from 36.9% to 3.3% on day 3; thereafter it increased to 64.3% 21 days after delivery because of a higher baseline concentration of hCG\( \alpha \).

hCG\( \beta \) had the slowest total elimination rate, and the ratio of hCG\( \beta \) to hCG in serum increased from 0.8% before delivery to 26.7% after 21 days. If the metabolism of hCG and hCG\( \beta \) is similar in patients with trophoblastic disease, the ratio of hCG\( \beta \) to hCG must be evaluated with caution in samples taken several days after initiating therapy. We conclude that the disappearance of hCG\( \beta \) from plasma is slower than previously recognized and that the ratio of hCG\( \beta \) or hCG\( \alpha \) to intact hCG vary as a function of postpartum time. Such information may be important in clinical studies of pregnancy disorders.

Human chorionic gonadotropin (hCG)\(^2\) is a heterodimer composed of two highly glycosylated subunits, called \( \alpha \) and \( \beta \), that are noncovalently joined. The \( \alpha \)-subunits of all glycoprotein hormones of pituitary origin, including follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone, are virtually identical, but the \( \beta \)-subunits are different and thus confer biological specificity of the hormones.

In early pregnancy the concentrations of hCG in serum start to increase 7–11 days after ovulation, corresponding to 21–25 days after the last menstrual period [1,2]. After in vitro fertilization and embryo transfer, an increase of serum hCG can be observed 9 days after ovum retrieval, corresponding to 7 days after embryo transfer [3]. The increase is exponential, with a doubling time of 1.5 days during the first 6 weeks [4]. Serum hCG reaches peak concentrations of \( \sim \)100 000 IU/L (in relation to the First International Reference Preparation) at 8–10 weeks after the last menstrual period. The concentrations start to decrease after week 12 and stay fairly constant at about \( \sim \)30 000 IU/L from the 20th week until term [5].

In addition to hCG, serum and urine from pregnant women and patients with trophoblastic disease contain free \( \alpha \)-subunits (hCG\( \alpha \)) and \( \beta \)-subunits (hCG\( \beta \)). The profile of the serum concentrations of hCG\( \beta \) during pregnancy resembles that of hCG, but the concentrations are lower. During gestation, the molar ratio of hCG\( \beta \) to hCG is 1.5–4% in early pregnancy and decreases to 0.2–1% after the 10th week [3,6]. In patients with benign trophoblastic disease, the ratio is similar to that in pregnancy, whereas higher ratios are observed in trophoblastic cancer. Thus the ratio may aid in differentiating between malignant and benign trophoblastic tumors [7–10].

The concentrations of hCG\( \alpha \) increase throughout pregnancy from \(< 1\ \mu g/L (69\ \text{pmol/L}) [11]\) to 100–300 \( \mu g/L (6900–20 700\ \text{pmol/L}) \) in the third trimester [12,13]. The hCG\( \alpha \) to hCG ratio is \(< 10\%\) during the first trimester and increases to 30–60% at term [6].

When hCG is injected into humans, it reportedly has a biphasic disappearance curve with an initial fast half-time of \( \sim 5\ \text{h} \) and a slow one of 24–32 h [14–16]. Injected hCG\( \alpha \) and hCG\( \beta \) are cleared from circulation much more rapidly than hCG [16,17]. hCG\( \alpha \) has a rapid half-time of 13

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2 Nonstandard abbreviations: hCG, human chorionic gonadotropin; hCG\( \alpha \) and hCG\( \beta \), highly glycosylated subunits of hCG; AUC, area under the curve.

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min and a slow one of 76 min. The corresponding times for hCGβ are 41 and 236 min, respectively [18, 19].

Serial hCG estimations are used for detecting pregnancy-related disorders such as spontaneous abortion and ectopic pregnancy as well as following-up patients with ectopic pregnancy. In ectopic pregnancy patients selected for expectant management, decreasing concentrations usually indicate spontaneous resolution, but one-third of these still require surgery within 1–24 days [20]. Thus a decreasing hCG concentration alone is not a reliable indicator for spontaneous resolution of an ectopic pregnancy.

Because hCGα and hCGβ have been reported to have much shorter half-times than hCG, they might more rapidly reflect changes in placental function such as an impending abortion. Changes in the ratio of the subunits to intact hCG potentially could be used to evaluate trophoblastic activity in pregnancy-related disorders, e.g., a low subunit to hCG ratio might indicate the cessation of hCG production because the ratio may be expected to decrease rapidly when the production ceases such as during an abortion or after delivery.

**Materials and Methods**

**Patients**

Six women with term pregnancies were included in the study, which was approved by the local institutional review committee. One of the patients had a twin pregnancy, one had gestational diabetes (non-insulin-dependent glucose intolerance of pregnancy), and one had diabetes mellitus (insulin-dependent diabetes with nephropathy). The mean age was 37 years (range 32–48 years) and mean gestational age 38 ± 4 weeks (range 37 + 3 to 39 + 5 weeks). In singleton pregnancies the mean birth weight was 3862 g (range 3270–4360 g) and the mean placental weight 678 g (range 600–770 g). In the twin pregnancy the birth weights were 2970 g and 3325 g and placental weights 560 g and 685 g, respectively.

The first blood sample was drawn from a cannula inserted in the right cubital vein 15 min before an elective caesarean section, performed under spinal anesthesia. The infusion was continued providing the best fit of the observed concentration values. By choosing n between 1 and 3, the mathematical model for the decay curve is defined as

\[
f(t) = \sum_{i=1}^{n} C_i \cdot e^{-R_i \cdot t} + A
\]

where \( C_i \) is a coefficient (decay amplitude) and \( R_i \) is a rate constant (min\(^{-1}\)). \( t \) is the time after delivery, and \( A \) is the baseline concentration. By choosing \( n \) between 1 and 3,
Sums of up to 3 exponential terms can be used [23]. Untransformed concentration data over time are fit, such that a triphasic decay curve is defined as
\[
f(t) = C_1 \cdot e^{-R_1 t} + C_2 \cdot e^{-R_2 t} + C_3 \cdot e^{-R_3 t} + A
\] (2)
and a biphasic decay curve is defined as
\[
f(t) = C_1 \cdot e^{-R_1 t} + C_2 \cdot e^{-R_2 t} + A
\] (3)
The half-times are calculated as \( t_{1/2} = (\ln 2 / R_i) \). The integrated contribution of each exponential component to the overall disappearance curves of hCG, hCGβ, and hCGα was calculated on the basis of the area under the curve (AUC) by
\[
AUC = - \frac{C_i + A}{R_i} \cdot \left( (e^{-R_i t}) - 1 \right)
\] (4)

Significant differences between triphasic and biphasic half-time fits were evaluated by comparing the fitted variances (above) via \( F \) ratio testing with Bonferroni/Dunn correction. We estimated whether the time course over which the measurements were made affected the half-times by analyzing time intervals of 0–6 days, 0–1 day, 0–12 h, and 0–6 h. The longest half-times were not calculated when the time interval was shorter than the longest half-time component.

**Results**

The mean serum concentration of hCG before delivery was 32 275 IU/L (94 566 pmol/L) (range 1631–84 995 IU/L). The corresponding value for hCGβ was 729 pmol/L (range 48–2198 pmol/L) and for hCGα 35 862 pmol/L (range 17 432–68 145 pmol/L). The mean concentrations of hCG, hCGβ, and hCGα are shown in Figs. 1–3, and the ratios of hCGβ and hCGα to total hCG and the ratios of hCGα to hCGβ are shown in Table 1.

A three-component exponential function gave the best fit to the observed hCG decay curves (Fig. 1). In contrast to a biphasic model, this resulted in very small deviations from the observed values, and the differences between triphasic and biphasic models and between the biphasic model and the observed values were significant (Table 2). The curve for hCGβ was also optimally fit to a three-component model (Fig. 2), with significant differences from the biphasic model (Table 2). The calculated biphasic curve of hCGβ fell below the observed values during the first hour after delivery, rose above the observed values during the next 12 h, and also deviated thereafter. The curve for hCGα was also best fit to a three-component model (Fig. 3). It fit to a two-component model only when values from days 0–2 were included in the calculations. When days 3–6, 14, and 21 values were also included, the biphasic model gave a poor fit. There were no significant differences between calculated values of the triphasic half-time model and the observed values of hCGα, whereas calculated values of the biphasic model differed significantly from the observed ones and those of the triphasic model by analysis of variance (Table 2). The half-times were virtually identical when calculated for shorter time intervals.

The median half-time of the most rapid component of hCG was ~6 times longer than that of hCGα and 4 times longer than that of hCGβ (Table 3). The median half-time of the second component of hCG was 30% shorter than that for hCGβ, but for hCGα the second component was 3 times shorter. The median half-time of the third component of hCGβ was almost fourfold that of hCG, and for hCGα it was half of that for hCG (Table 3). The algorithm used was set to calculate the baseline concentrations of hCG and its subunits limiting the highest possible value to the upper reference limit of nonpregnant premenopausal women. Without this limitation two patients would have had baseline concentrations for hCG of 3.5
and 5.2 IU/L, two patients baseline concentrations for hCG of 2.8 and 2.5 pmol/L, and one patient a baseline concentration for hCG of 34.2 pmol/L.

In one patient (number 5 in Table 3) the serum concentrations of hCG, hCG_b, and hCG_a were measured before and after induction of spinal anesthesia and infusion of 1 L of saline. The infusion lowered the serum concentrations by 22%. When the postinfusion concentrations were used as initial values, the calculated half-times increased, but the median effect was small (2.2%, range 0–24%).

The mean concentrations of hCG, hCG_b, and hCG_a on day 21 after delivery were 2.6 IU/L (7.6 pmol/L) (range 0.7–4.3 IU/L), 3.2 pmol/L (range 1.2–5.1 pmol/L), and 16 pmol/L (range 9.3–25.0 pmol/L), respectively. hCG_b had the slowest total elimination rate, and decreased to a mean ± SD concentration of 1.3% ± 1.0% of the initial value after 14 days and 0.9% ± 0.8% after 21 days. The hCG concentration decreased to 0.05% ± 0.02% of the initial value at 14 days and 0.02% ± 0.01% within 21 days. hCG_a reached a concentration of 0.07% ± 0.03% within 14 days, decreasing only slightly thereafter to 0.05% ± 0.03% of the initial value on day 21 (Fig. 4).

The contributions of the various components to the disappearance curves were calculated on the basis of AUCs. For hCG, the fastest component represented 13.8% of all hCG and two slower ones were 68.8% and 17.4%, respectively. For hCG_b, the corresponding numbers were 3.1%, 78.1%, and 18.9%, and for hCG_a they were 23.9%, 59.7%, and 16.4%, respectively (Table 4). hCG contained much more of the most rapid component than hCG_b. Therefore, the total disappearance of hCG_b was clearly slower than that of hCG (Fig. 4), and after a transient decrease during the first hour after delivery, the ratio of hCG_b to hCG increased gradually from 0.8% (range 0.6–1.0%) before delivery to 15.8% (range 7.7–28.3%) after 14 days and 26.7% (range 20.6–36.9%) after 21 days (Fig. 5). hCG_a had the highest proportion of the rapid component and the lowest proportion of the slow component, explaining its most rapid total disappearance (Table 4 and Fig. 4). The mean ratio of hCG_a to hCG before delivery was 36.9% (range 15.3–78.5%). Initially the ratio decreased rapidly with a nadir of 3.3% (range 1.4–7.9%) on day 3, but thereafter it increased to 64.3% (range 42.5–83.5%) 21 days after delivery (Fig. 6). This resulted from a constant

<table>
<thead>
<tr>
<th>Table 2. Significance of the fitted variances of triphasic vs biphasic half-time curves of hCG, hCG_b, and hCG_a.</th>
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</thead>
<tbody>
<tr>
<td>Form of hCG</td>
</tr>
<tr>
<td>hCG</td>
</tr>
<tr>
<td>hCG_b</td>
</tr>
<tr>
<td>hCG_a</td>
</tr>
</tbody>
</table>

*p* Comparisons by analysis of variance are significant when the corresponding *P* is <0.0167.
background concentration of hCGα with decreasing hCG concentrations.

**Discussion**

With the use of highly sensitive and specific immunofluorometric assays, we found in this study that the disappearance of endogenous hCGβ from plasma after delivery is much slower than that observed in studies with the use of RIA and purified hCGβ infused intravenously [18, 19]. Various possible explanations exist for this. Chemical differences, mainly in the carbohydrate side chains [24–26], have been shown to exist between circulating hCGβ and that purified from pregnancy urine, which has been used for injection [18, 19]. The experimental setting in the present study is not directly comparable with that after infusion of purified hCGβ; i.e., the half-time was measured for hCGβ over a course of 3 weeks, whereas in earlier reports the time span has been only hours or a few days. However, for comparison, we also calculated half-times for shorter time frames that were comparable with those in earlier studies. This did not affect the results substantially. After pregnancy, some release of small amounts of hCGβ (and hCG) sequestered in tissues could cause an apparent increase in half-time. Another possibility is that trophoblastic cells remaining in the body continue to produce hCGβ and very little or no hCG. Trophoblasts persist in the lungs for extended periods of time after pregnancy [27], but the mass of these cells is very small in comparison with that of the placenta. Therefore, any production of hCG and hCGβ by persisting trophoblastic cells would not contribute significantly to the serum concentrations observed the first week after delivery.

![Fig. 2](https://academic.oup.com/clinchem/article-abstract/43/11/2155/5640817)

*Fig. 2. Observed disappearance of hCGβ (mean ± SD) and estimated triphasic exponential half-times of hCGβ and their predicted overall decay curve.*

![Fig. 3](https://academic.oup.com/clinchem/article-abstract/43/11/2155/5640817)

*Fig. 3. Observed disappearance of hCGα (mean ± SD) and estimated triphasic exponential half-times of hCGα and their algebraically summed curve.*
delivery, when the difference in half-times was already apparent in an increasing ratio of hCGβ to hCG (Fig. 5).

Dissociation into subunits of the hCG remaining in circulation could also affect the estimated half-time of hCGβ. This mechanism could be important if the disappearance of hCGβ were more rapid than that of hCG, but the opposite was actually true. Intact hCG is quite stable, whereas nicked hCG dissociates more rapidly. Especially in trophoblastic disease [10, 28], nicking may increase the dissociation of hCG into subunits, which may contribute to a high ratio of hCGβ to hCG. Long incubation times during the assay of hCGβ can cause dissociation of hCG, thus increasing the apparent concentration of hCGβ in the sample. However, with the incubation times used in the present assay for hCGβ, this effect is negligible [3]. Although all these mechanisms could increase the half-time of hCGβ, they probably do not explain why hCGβ in all subjects studied disappeared substantially more slowly than hCG. Therefore, other explanations need to be considered.

The most likely explanation for the longer half-time of hCGβ (vs intact hCG) is that hCGβ circulating in plasma differs from that isolated by dissociation of urinary hCG into subunits. hCG in urine is known to be less glycosylated than that in serum [24]. The carbohydrate composition, and especially the presence of terminal sialic acid, is known to affect the in vivo half-time of hCG [26]. Furthermore, the β-chain of both hCG and hCGβ in crude urinary hCG preparations has been found to be partially cleaved or nicked between residues 47 and 48 [25, 29]. In addition, under the potentially harsh chemical conditions required to dissociate the subunits, denaturation and appearance of components with shortened half-times could occur. The hCG heterodimer is unusual in that it is held together by a loop of the β-chain embracing the

<table>
<thead>
<tr>
<th>Table 3. Individual baselines and triphasic half-times (h) of hCG, hCGβ, and hCGα after delivery.</th>
<th>Half-times, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG, IU</td>
<td>Base</td>
</tr>
<tr>
<td>Patient 1</td>
<td>2.90</td>
</tr>
<tr>
<td>Patient 2</td>
<td>2.90</td>
</tr>
<tr>
<td>Patient 3</td>
<td>2.35</td>
</tr>
<tr>
<td>Patient 4</td>
<td>0.21</td>
</tr>
<tr>
<td>Patient 5</td>
<td>0.66</td>
</tr>
<tr>
<td>Patient 6</td>
<td>1.22</td>
</tr>
<tr>
<td>Mean</td>
<td>1.71</td>
</tr>
<tr>
<td>SD</td>
<td>1.17</td>
</tr>
<tr>
<td>Median</td>
<td>1.79</td>
</tr>
<tr>
<td>hCGβ, pmol/L</td>
<td>Patient 1</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1.59</td>
</tr>
<tr>
<td>Patient 3</td>
<td>1.60</td>
</tr>
<tr>
<td>Patient 4</td>
<td>0.65</td>
</tr>
<tr>
<td>Patient 5</td>
<td>0.00</td>
</tr>
<tr>
<td>Patient 6</td>
<td>0.93</td>
</tr>
<tr>
<td>Mean</td>
<td>1.06</td>
</tr>
<tr>
<td>SD</td>
<td>0.66</td>
</tr>
<tr>
<td>Median</td>
<td>1.26</td>
</tr>
<tr>
<td>hCGα, pmol/L</td>
<td>Patient 1</td>
</tr>
<tr>
<td>Patient 2</td>
<td>25.61</td>
</tr>
<tr>
<td>Patient 3</td>
<td>9.35</td>
</tr>
<tr>
<td>Patient 4</td>
<td>31.00</td>
</tr>
<tr>
<td>Patient 5</td>
<td>10.88</td>
</tr>
<tr>
<td>Patient 6</td>
<td>22.96</td>
</tr>
<tr>
<td>Mean</td>
<td>17.47</td>
</tr>
<tr>
<td>SD</td>
<td>10.43</td>
</tr>
<tr>
<td>Median</td>
<td>16.92</td>
</tr>
</tbody>
</table>

*Patient with diabetes mellitus (white F).*

**Fig. 4.** Disappearance of hCG, hCGβ, and hCGα after delivery.

The values are given as the proportion (mean + SD) of the concentration before delivery. The SD bars not visible are too small to be seen at this scale.
Disrupting the dimer in vitro might therefore change the structure of hCGβ in comparison with the circulating form, which probably never has been involved in heterodimer formation. Free hCGα in serum does not reassociate with hCGβ [31, 32].

An increased ratio of hCGβ to hCGα in most studies >10%, has been observed in trophoblastic cancer, and this has been used to differentiate between malignant and benign trophoblastic disease [7–9, 33]. In the present study, ratios >10% were observed in 5 of the 6 patients studied 14 days after delivery, and in all 4 patients studied 21 days after delivery.

### Table 4. Proportions of the various components of hCG, hCGα, and hCGβ estimated on the basis of the AUC of each component.

<table>
<thead>
<tr>
<th>Form of hCG</th>
<th>Rapid (I)</th>
<th>Medium (II)</th>
<th>Slow (III)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG</td>
<td>0.48 (13.8)</td>
<td>2.41 (68.8)</td>
<td>0.61 (17.4)</td>
<td>3.50 (100)</td>
</tr>
<tr>
<td>hCGβ</td>
<td>0.14 (3.1)</td>
<td>3.45 (78.3)</td>
<td>0.82 (18.6)</td>
<td>4.41 (100)</td>
</tr>
<tr>
<td>hCGα</td>
<td>0.13 (23.9)</td>
<td>0.32 (59.7)</td>
<td>0.09 (16.4)</td>
<td>0.54 (100)</td>
</tr>
</tbody>
</table>

*Given in parentheses is the percent of the total.

During the first day

![Graph showing changes in the ratio of hCGβ to total hCG (mean + SD) after delivery. The values are given as percentages.](image1)

During days 0-21

![Graph showing the ratio of hCGα to total hCG (mean + SD) after delivery. The values are given as percentages.](image2)

![Graph showing the ratio of hCGα to total hCG (mean + SD) after delivery. The values are given as percentages.](image3)
and hCGβ is similar in patients with trophoblastic disease, our findings suggest that the ratio of hCGβ to hCG must be evaluated with caution in samples taken several days after initiation of therapy. An increased ratio of hCGβ to hCG has actually been observed several weeks after treatment of trophoblastic disease [34, 35].

The two most rapid components of hCG had half-times similar to those observed for hCG injected into humans, i.e., 3.6 and 18 h as compared with 5 and 24–36 h, respectively [14–16]. In an earlier study three components with half-times of 15, 27, and 168 h were estimated for hCG after abortion [36]. The two latter half-times support our calculations about the third component with the half-time of several days (median 53 h for hCG). This component represented only 17% of total hCG. Therefore, it may not be detectable after injection unless a large amount of hCG is injected, or an ultrasensitive assay method is used, or very prolonged observations are carried out. However, it is possible that this component represents hCG produced by residual, gradually dying trophoblasts or that is less abundant in urinary hCG than in plasma. The half-times of the two most rapid components of disappearance of hCG, hCGβ, and hCGα were similar in all the patients studied, but there was more individual variation in the half-times of the longest component, i.e., from 38 to 64 h for hCG, 103 to 462 h for hCGβ, and 15 to 126 h for hCGα. Renal clearance accounts for 20% of the total disposal of hCG after injection of purified hCG preparations [19]. The slightly impaired kidney function in our patient with diabetes mellitus (patient 2 in Table 3) seemed to have no effect on the half-times of hCG and its subunits.

The algorithm used for calculation of half-times in the present study was based on the principles described in the EXPFIT program [23]. A two-component model has been used in most earlier studies to calculate disappearance half-times of hCG and its subunits. However, the fit of a two-component model was unsatisfactory for hCG, hCGβ, and hCGα, whereas a three-component model with baseline yielded a statistically preferred fit. In contrast to the two-component model, three exponentials with baseline provided a fit for which predicted values could not be distinguished from the observed values by analysis of variance. The baseline concentrations obtained with the algorithm were in most cases well within the range of the reference values for nonpregnant premenopausal women (Table 3) [21]. However, when the follow-up time is insufficient for analysis of the baseline, it can be restricted in the algorithm.

In conclusion, we have developed a three-component exponential model with a baseline for calculation of half-times of hCG and its subunits. Disappearance of endogenous hCGβ from plasma after delivery is slower than previously observed, and the ratios of hCGβ or hCGα to intact hCG vary as a function of postpartum time. If the metabolism of hCG and hCGβ is similar in patients with trophoblastic disease, the ratio of hCGβ to hCG must be evaluated with caution in samples taken several days after initiation of therapy. Ratios of hCGβ to hCG >10%, which are indicative for chorionic cancer, were observed in all patients 21 days after delivery. However, this needs to be evaluated in patients with trophoblastic disease. Additional studies will also reveal whether the half-times are similar in early pregnancy and whether this can be used to diagnose pregnancy-related disorders.

References