Sex, Gestational Age, and Weight Dependency of Adult Hemoglobin Concentration in Normal Newborns

By F. Galacteros, M. Guilloud-Bataille, and J. Feingold

We have measured by cation exchange high pressure liquid chromatography adult hemoglobin (HbA) concentration at birth in 6,123 unselected single newborn individuals. Probably because of the high precision of the analytical method used, we could demonstrate a relationship between HbA concentration and, respectively, gestational age and birth weight. We also demonstrated a significant difference in the Hb switching process between male and female newborns. Reference percentile distribution curves are given that could be used to define more precisely those children having slow or fast Hb switching.

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The adult hemoglobin (HbA) fetal hemoglobin (HbF) switching process during the perinatal period had been the subject of many in vivo and in vitro studies. This topic has been deeply investigated, and up to date reviews are regularly published.

Numerous reports have been focused on the relative rates of HbF and HbA synthesis during the first 6 months of life. However, available data on physiologic HbA concentration range in normal newborns are contained in a small number of reports. These studies did not give satisfactory reference data that would allow clear identification of the small subgroup of newborns having HbA concentration out of the ±2 SD limits of normal range. These methodologic inaccuracies came mainly from imprecise laboratory methods, uncertainties in regard to gestational age, which in many case was not taken into account, and insufficiently population-studied newborn groups.

Many pathologic circumstances have been shown to modify Hb phenotype (mostly by slowing the switching process) in this period of life. Among them are maternal anoxia, placental insufficiency, prematurity, some chromosomal abnormalities, hyperinsulinemia secondary to maternal diabetes mellitus, and the sudden infant death syndrome, although with some controversy. The β-thalassemia trait is a genetic condition at the heterozygous state that could be used to define more precisely those children having slow or fast Hb switching.

Laboratory investigations included thin-layer isoelectric focusing, citrate agar electrophoresis when an abnormality was detected by isoelectric focusing or cation exchange HPLC. This latter method was applied according to referenced methods. The equipment used was a VARIAN 5000 liquid chromatograph. The column was a Brownlee 3CM cartridge (Acquapore CX-300, 0.4 mm × 30 mm; Brownlee Laboratories, Inc, Santa Clara, CA). The gradient used was similar to that referenced with a slight modification permitting Hb Bart’s detection and quantitation. We have not included in this study results obtained when an abnormal Hb fraction was detected either by isoelectric focusing or by HPLC. The only excluded infants either carried an abnormal Hb (4%) or had more than 0.5% Hb Bart’s (5%). This policy was applied because we had no information about the possible influence of these traits on adult Hb A synthesis, gestational age, or birth weight. Ethnic distribution in the studied cohort was 60% white Caucasians, 15% from North Africa, 13% from French West Indies, 9% from different regions of Sub-Saharan Africa, and 3% from Asia. According to reported data on β-thalassemia gene frequencies in these ethnic subgroups, the overall incidence of this trait must be lower than 1%.

All HbA values were expressed as the mean ± standard deviation. Comparisons of HbA means between males and females were made using the Student’s t-test. A P value below .05 was considered significant. In gestational age (in weeks) groups and in birth weight groups, the percentiles were computed. The correlations between either gestational age or birth weight and HbA concentration were also computed.

RESULTS

Hb studies were performed on 6,123 blood samples from unselected single newborns without detectable Hb abnormality. The studied group was composed of 3,168 males and 2,955 females; the sex ratio was 51.74%. Their respective mean gestational ages were 39.07 ± 1.67 and 39.06 ± 1.70 weeks of amenorrhea. Corresponding mean birth weights were 3,306.2 ± 521.4 g and 3,166.6 ± 505.1 g. These data were very close to corresponding, published reference...
values obtained for newborns in France. The classical relationship between sex, birth weight, and gestational age, observed in French newborns, was found again in the studied group of newborns.

HbA concentrations were obtained by cation exchange HPLC. The relationship between HbA concentration and gestational age was studied in newborns having over 41 weeks of gestational age were insufficiently numerous to permit accurate statistical calculations. Figure 1A (males) and B (females) illustrates the relationship between those two parameters, as well as the percentile distribution curves. The correlation coefficients were, respectively, 0.39 for males ($P < .0001$) and 0.36 for females ($P < .0001$). However, the HbA concentration cannot be used to define gestational age, because the part of the variance explained by the HbA concentration is small. The corresponding percentile data for HbA concentration are given in Table 1. A significant difference between males and females was evidenced. Mean HbA concentrations were, respectively, 18.0% ± 6.7% in males and 19.2% ± 7.2% in females. The sexual difference in HbA concentration was significantly present from 37 to 41 weeks of amenorrhea.

By the same time, the relationship between HbA concentration and birth weight according to sex was studied. Results are given in a similar way in Fig 2A and B and Table 2. The correlation coefficients were, respectively, 0.30 for males ($P < .0001$) and 0.27 for females ($P < .0001$). A clear relationship was observed between birth weight and HbA concentration, and at birth weight ranging from 2,000 to 4,500 g, the HbA concentration differed significantly between the sexual groups. Although the male newborns were heavier, they had synthesized less HbA than females.

### Table 1. HbA Concentrations in Male and Female Newborns According to Gestational Age

<table>
<thead>
<tr>
<th>Gestational Age (wk)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤33</td>
<td>311.2 ± 10.0</td>
<td>3314.1 ± 12.8</td>
</tr>
<tr>
<td>34-35</td>
<td>5411.7 ± 6.1</td>
<td>5911.5 ± 4.3</td>
</tr>
<tr>
<td>36</td>
<td>9912.5 ± 4.1</td>
<td>8713.4 ± 4.1</td>
</tr>
<tr>
<td>37</td>
<td>25614.1 ± 6.0</td>
<td>23215.2 ± 5.1</td>
</tr>
<tr>
<td>38</td>
<td>51715.6 ± 5.4</td>
<td>45416.8 ± 5.0</td>
</tr>
<tr>
<td>39</td>
<td>80717.8 ± 5.9</td>
<td>77618.9 ± 6.8</td>
</tr>
<tr>
<td>40</td>
<td>78219.7 ± 6.4</td>
<td>74921.0 ± 6.8</td>
</tr>
<tr>
<td>41</td>
<td>50921.5 ± 6.5</td>
<td>46622.6 ± 7.1</td>
</tr>
<tr>
<td>≥42</td>
<td>7022.5 ± 8.1</td>
<td>6022.9 ± 7.9</td>
</tr>
<tr>
<td>Total</td>
<td>3,12518.0 ± 6.7</td>
<td>2,91619.2 ± 7.2</td>
</tr>
</tbody>
</table>

Abbreviations: NB, numbers; NS, not significant.

### Table 2. HbA Concentration in Male and Female Newborns According to Birth Weight

<table>
<thead>
<tr>
<th>Birth Weight (g)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1,500</td>
<td>97.1 ± 1.3</td>
<td>1413.2 ± 11.3</td>
</tr>
<tr>
<td>1,501 to 2,000</td>
<td>3612.2 ± 6.2</td>
<td>4413.1 ± 10.4</td>
</tr>
<tr>
<td>2,001 to 2,500</td>
<td>13912.6 ± 5.2</td>
<td>20614.9 ± 6.5</td>
</tr>
<tr>
<td>2,501 to 3,000</td>
<td>63515.8 ± 6.4</td>
<td>77617.8 ± 6.2</td>
</tr>
<tr>
<td>3,001 to 3,500</td>
<td>1,28918.2 ± 6.5</td>
<td>1,20419.8 ± 6.8</td>
</tr>
<tr>
<td>3,501 to 4,000</td>
<td>80320.0 ± 6.6</td>
<td>59020.9 ± 7.2</td>
</tr>
<tr>
<td>4,001 to 4,500</td>
<td>20018.7 ± 6.3</td>
<td>9422.8 ± 8.6</td>
</tr>
<tr>
<td>≥4,500</td>
<td>4521.7 ± 7.0</td>
<td>2020.9 ± 7.2</td>
</tr>
</tbody>
</table>

Abbreviations: NB, numbers; NS, not significant.

![Graph A](image1.png)

![Graph B](image2.png)

Fig 1. Relationship and percentile distribution curves of HbA concentration values according to gestational age (in week of amenorrhea) in male (A) and female (B) newborns.

![Graph A](image3.png)

![Graph B](image4.png)

Fig 2. Relationship and percentile distribution curves of HbA concentration values according to birth weight (in grams) in male (A) and female (B) newborns.
having identical mean gestational age. The partial correlations between HbA concentration and gestational age, birth weight being the fixed variable, are 0.29 in males and 0.27 in females \((P < .0001)\). On the contrary, the partial correlations between HbA concentration and birth weight, gestational age being the fixed variable, are 0.12 in males and 0.10 in females \((P < .0001)\). The comparison of these values indicates that the important correlation concerns gestational age and HbA concentration.

**DISCUSSION**

This work was initiated during the course of an experimental program for hemoglobinopathy detection at birth. Because it had been reported that HbA concentration is significantly lower when measured in \(\beta\)-thalassemia carrier newborns, our aim was to define the fifth percentile threshold of HbA concentration allowing to recognize the subgroup of newborns at risk for having \(\beta\)-thalassemia or hereditary persistence of HbF synthesis traits.

In fact, it became evident that it was necessary to take into account gestational age, birth weight, and sex simultaneously to provide correct interpretation of HbA concentration at birth.

Although electrophoretical analysis of newborn Hb is the method of choice in most of the clinical laboratories, HPLC is a reliable and reproducible alternative procedure that can be used as a mass screening assay. It also allows for the precise quantitation of Hb fractions, which was probably necessary to obtain the present results. Differences were more significant when HbA concentration was matched to birth weight and sex probably because newborn weight measure is more precise than gestational age determination.

Results were not demonstrative for extreme gestational age and birth weight groups probably because they were not sufficiently populated, but it could also result from pathologic or therapeutic interferences (therapeutic transfusion, maternal to fetal transfusion, maternal diabetes). In fact, in the most premature infants, transfusion therapy was often used very soon after birth. This therapy produced a paradoxical elevation of the 95th percentile value in these subgroups of newborns (Figs 1A and B and 2B).

The results reported here could be used as reference values to define slow and fast Hb switching in newborn individuals. These results will be useful in properly characterizing or further studying factors that may slow the Hb switching process like maternal diabetes, maternal chronic hypoxemia, or intrauterine growth disturbances. They may also help to define the subgroup of babies with an accelerated Hb switch that has been reported to occur, for instance, in some chromosomal abnormalities and \(\gamma\)-thalassemia traits.

Hb studies have been proposed to precisely determine fetal maturity and correct estimation of gestational age.\(^{10,27,28}\) Previously described methods were difficult to use routinely. The present report shows that HbA concentration reference values could be useful in that way, all the more so because it could appear as a routine secondary result of newborn screening for hemoglobinopathies by HPLC procedure.

We were able to find only one previous report that mentioned a sexual difference in Hb switching in the neonatal period.\(^{22}\) The authors comparing HbF concentration between 47 male and 74 female newborns with no Hb abnormality found significantly less HbF (67.6%) in females than in males (72.6%). The present results suggest that a sex-associated factor affects Hb switching. This observation could be brought together with some recent reports. One report described X-linked dominant control of F cells in normal adult life\(^{29}\) and the second located the major human erythroid-specific DNA-binding protein (GF) on the X chromosome.\(^{30}\) It is tempting to speculate on a relationship between these sets of results after authors have suggested that GF, is implicated in human Hb switching.\(^{31,32}\)

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**REFERENCES**


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