Perinatal Reference Intervals for Plasma Homocysteine and Factors Influencing Its Concentration, Claire Infante-Rivard,2,3 Georges-Etienne Rivard,2,3 Wagner V. Yostov,2,3 and Yves Théoret1,3 1Department of Epidemiology, Biostatistics and Occupational Health, Faculty of Medicine, McGill University, 1130 Pine Ave. West, Montréal, Province of Québec, H3A 1A3 Canada; 2 Research Centre, Centre Hospitalier Universitaire Mère-Enfant, and 3 Division of Hematology and Oncology, Hôpital Sainte-Justine, Université de Montréal, Montréal, Province of Québec, H3T 1C5 Canada; *author for correspondence: fax 514-398-7435, e-mail claire.infante-rivard@mcgill.ca

Moderately increased plasma total homocysteine (tHcy) concentrations have been associated with an increased risk of atherothrombotic vascular events (1). Disturbances in homocysteine metabolism have also been reported as a possible risk factor for early pregnancy loss and congenital birth defects, such as neural tube defects, as well as for maternal obstetric complications (2).

Reference intervals for healthy maternal and newborn populations are scarce; in particular, we could not find data on large samples of women at delivery. Rajmakers et al. (3) reported tHcy results for samples collected at delivery or 4 h before caesarian section on 35 women. Böhles et al. (4) and Malinow et al. (5) measured tHcy at delivery in 60 and 35 women, respectively, and Bjerke Monsen et al. (6) reported results for 169 samples collected between 96 to 108 h after birth. Available data on newborns include the results from one large study in Italy (7) and a few smaller studies (3–6), among which only one is from North America; it included 35 women and their newborns (5). Mean tHcy values were quite different among these studies.

Genetic, nutritional, and lifestyle factors are believed to influence tHcy concentrations (8, 9). Among the genetic factors, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C gene polymorphisms are potentially important. Supplementation of the diet with folate as well as smoking and caffeine consumption are among other factors that can affect tHcy concentrations. None of the studies cited above took the effect of these factors into account.

The goals of our study are (a) to provide reference values for tHcy measured within 48 h of delivery from a large unselected sample of women who gave birth to babies born at or above the 10th percentile for gestational age and sex; (b) to provide similar values for their newborns; and (c) to study the impact on maternal as well as newborn tHcy concentrations of common MTHFR genetic polymorphisms as well as nutritional factors.

We performed a hospital-based case-control study of intrauterine growth restriction in which all live-born singleton cases seen over a 2-year period (mid-1998 and mid-2000) were matched for sex, gestational age, and race to a live newborn control whose weight was at or above the 10th percentile based on gestational age and sex, as determined by Canadian population standards (10). Cases and controls were born at the same hospital and

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generally within a few days of each other. We report here on 468 mothers and their 438 babies who were the controls in our case-control study and for whom a blood sample could be obtained and tHcy determined. The study included a total of 472 mother–newborn controls. The project was approved by the ethics committee of the hospital, and informed consent was obtained from all mothers.

Venous maternal blood was obtained within 48 h of delivery (median time of collection, 25 h). Placental blood was collected from the umbilical veins of the newborns. Citrate buffer (0.5 mol/L, pH 4.3) was used as the anticoagulant (0.5 mL of citrate for 4.5 mL of blood), and samples were kept at 4 °C until centrifugation, which took place within 6 h (median, 0.58 h) for maternal samples and within 24 h for newborns (median, 6.06 h). This difference reflects the fact that the maternal specimen was obtained by the research nurse on duty, whereas the specimen from the newborn was obtained by the delivery room personnel at all hours. Under these collection and storage conditions, tHcy concentrations (in μmol/L) were stable for at least 24 h (11). After centrifugation, samples were stored at −70 °C until analyzed by HPLC as reported previously (12) with modifications. Briefly, 60 μL of 0.15 mol/L N-acetyl-L-cysteine and 30 μL of 100 g/L tri-n-butylphosphine in dimethylformamide were added to 240 μL of plasma (reducing step). After incubation at 4 °C for 30 min, the proteins were precipitated with 300 μL of 0.6 mol/L perchloric acid containing 1 mmol/L EDTA. The tubes were vortex-mixed and centrifuged for 1 min at 10 000 g.

The derivatization step was performed as follows. A 50-μL aliquot of the supernatant was transferred into another tube and mixed with 10 μL of 1.55 mol/L NaOH, 125 μL of borate buffer (125 mmol/L, pH 9.5, containing 4 mmol/L EDTA), and 50 μL of a 7-fluorobenzo-furazane-4-sulfonic acid (SBD-F) solution (freshly prepared in the same borate buffer). The mixture was incubated at 60 °C for 1 h, and then a 50-μL aliquot was mixed with 50 μL of the elution buffer. One-half of the resulting mixture (50 μL) was injected on a Kromasil C18 column (150 × 4.6 mm; 5-μm bead size; Phenomenex). SBD-F derivatives were eluted isocratically with a mobile phase containing 0.2 mol/L sodium acetate buffer (pH 4) and methanol (98:2 by volume). The HPLC analysis was performed at 1.5 mL/min. Fluorescence detection was on a Shimadzu RF551 Fluorescence Spectrometer (Shimadzu Corporation). Data were collected and analyzed with Gold software (Ver. 6.0) from Beckman-Coulter.

To determine the role of maternal smoking and eating habits (for foods rich in folic acid) as well as that of vitamin supplementation on tHcy concentrations, mothers were interviewed face-to-face after delivery. For nutritional factors, we asked about quantities (in units such as cups, cans, glasses, and so forth) consumed in a day or a week and averaged the results over a specific pregnancy trimester. In addition, MTHFR C677T and A1298G gene polymorphisms were determined for newborns and their mothers. PCR-amplification of folic acid-dependent homocysteine synthase (HcyS) was performed in 96-well plates using the following primers: 5′-CAAGAGCCGGGAAAGATG-3′ (forward) and 5′-GAGGGCTGCTCCTTCACG-3′ (reverse). The reaction mixtures were amplified in a thermal cycler (Perkin-Elmer) at 95 °C for 1 min, followed by 30 cycles at 95 °C for 30 s, 57 °C for 45 s, and 72 °C for 1 min. After the last cycle, the amplicons were kept at 70 °C for 7 min and then placed on ice. The PCR products were cleaned with a QIAquick PCR Purification Kit (Qiagen) and used for direct sequencing on an ABI Prism 377 DNA Sequencer (Perkin-Elmer).

Table 1. Postpartum and fetal umbilical vein reference intervals for plasma tHcy.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>n</th>
<th>Mean tHcy, μmol/L</th>
<th>95% CI, μmol/L</th>
<th>95% reference interval, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 25 h of delivery&lt;sup&gt;a&lt;/sup&gt;</td>
<td>468</td>
<td>5.59</td>
<td>5.41–5.76</td>
<td>1.80–9.37</td>
</tr>
<tr>
<td>26–48 h after delivery</td>
<td>234</td>
<td>5.27</td>
<td>5.03–5.52</td>
<td></td>
</tr>
<tr>
<td>Centrifugation within 1 h&lt;sup&gt;b&lt;/sup&gt;</td>
<td>234</td>
<td>5.90</td>
<td>5.66–6.15</td>
<td></td>
</tr>
<tr>
<td>Centrifugation between 1 and 6 h</td>
<td>382</td>
<td>5.57</td>
<td>5.36–5.77</td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrifugation within 6 h&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>438</td>
<td>5.06</td>
<td>4.92–5.21</td>
<td>2.02–8.09</td>
</tr>
<tr>
<td>Centrifugation between 6 and 24 h</td>
<td>216</td>
<td>4.93</td>
<td>4.72–5.14</td>
<td></td>
</tr>
<tr>
<td>Centrifugation between 6 and 24 h</td>
<td>222</td>
<td>5.19</td>
<td>4.99–5.39</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Time, in hours, between delivery and blood collection.
<sup>b</sup> Time, in hours, between blood collection and centrifugation of specimen.
<sup>c</sup> A test comparing “within 1 h” and “between 1 and 6 h” = 0.48 (P = 0.63).
<sup>d</sup> A test comparing “within 6 h” and “between 6 and 24 h” = 1.77 (P = 0.08).
were white, 23.3% were black, 2.7% were Asian, and 3.4% were Amerindian. The mean body mass index (pregnancy weight in kg divided by height in m²) was 23.1 (SD, 5.2), and the mean weight gain during pregnancy was 14.4 kg (SD, 5.6 kg). Among newborns, 55.6% were girls (more girls are affected with intrauterine growth restriction, and these controls were matched for sex), 41.8% were born before the 39th week (reflecting matching on gestational age), and mean birth weight was 3208.1 g (SD, 734.5 g; range, 795-4810 g).

The mean values and CIs for tHcy concentrations in mothers and newborns as well a reference interval for these measures are shown in Table 1. As shown in Table 1, the number of hours after delivery upwardly influenced the maternal tHcy concentration, whereas time between sample collection and centrifugation did not.

Despite the dispersion of values in the estimates because of generally small sample sizes.

In conclusion, maternal and newborn tHcy concentrations seem lower in North America than in Europe, probably because of food fortification. Nutritional and lifestyle factors seem to have a greater influence on homocysteine concentrations than do common MTHFR polymorphisms.

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References