Biochemical Markers in the Assessment of Protein–Calorie Malnutrition in Premature Neonates

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We studied 135 premature newborns of 26 to 36 weeks gestation, divided into three groups: the control group, 66 premature infants with uncomplicated course; 51 premature neonates with appropriate birth weight for gestational age (AGA), who suffered from clinical problems that delayed oral feeding; and 18 premature infants with small birth weight for gestational age (SGA). When neonates of the same postnatal age were compared, prealbumin concentrations were the lowest in the SGA group at the third and fourth postnatal week. Although the AGA group had the most infants with serious illnesses and the lowest protein–calorie intakes, their prealbumin concentrations did not differ significantly from those of the control group. But when the infants of each group were subdivided on the basis of intakes and weight gain regardless of postnatal age, those with greater intakes showed significantly higher prealbumin values; however, in all groups, the infants with higher intakes were also significantly older. Total proteins and albumin showed similar changes in all groups. Prealbumin concentrations showed great interindividual variability in infants of the same postnatal age. We conclude that prealbumin, albumin, and serum total proteins are not sufficiently sensitive biochemical markers to assess alterations of the nutritional status of premature infants.

Additional Keyphrases: prematurity · total proteins, albumin, prealbumin in serum · anthropometric measurements · nutritional status

Modern neonatal care enables the survival of an increased number of premature or small-for-gestational-age infants. One of the main objectives in caring for these newborn infants is to provide appropriate nutrition to assure normal development of the central nervous system and adequate growth (1).

Although anthropometric methods (weight gain, head circumference, crown-to-heel length) are used to evaluate the nutritional status of the newborns, most are rendered inaccurate by specific problems (fluid retention, imprecision of the measurements, etc.) (2). Sensitive techniques dealing specifically with the metabolic aspects of the nutritional condition of the newborn infants are still lacking or have been inadequately studied.

Albumin and prealbumin (transthyretin) in serum, and nitrogen balance, are the most common nutritional biochemical markers used in adults—prealbumin only recently having been shown to be a sensitive marker of the nutritional status in adults and children (3–11). Corresponding information on normal changes in biochemical markers of protein–calorie intake during the neonatal period, and their correlation to adequate nutrition and growth, are almost nonexistent.

Prealbumin is an obvious candidate for indicating the nutritional status of newborns. Synthesized in the liver, this tryptophan-rich tetramer of 54 980 Da has a short half-life (1.9 days). It is a thyroxin-binding protein and circulates in plasma bound to retinol-binding protein, forming a complex for the transport of retinol to the target tissues (12–14). Recognition of these functions led to its newer appellation of transthyretin (15). Recent studies evaluating the use of prealbumin to assess protein–calorie status in small groups of premature infants have given contradictory results (16, 17).

We have evaluated the use of transthyretin as a biochemical marker of the protein–calorie nutritional status of premature infants and report here our results in comparison with those for total proteins and albumin in serum and with anthropometric measurements.

Subjects and Methods

Selection of Subjects

From a group of 143 infants evaluated in the Neonatal Intensive Care Unit of Saint-François-d'Assise Hospital, we studied 135 premature infants from birth to 12 weeks of hospitalization; the other eight infants, who were suffering from bacterial infection, were omitted from the study because infection per se decreases the concentrations of prealbumin in serum (18). Gestational age ranged from 26 to 36 weeks, as determined from the date of the last menstrual period and substantiated by clinical examination following the criteria of Ballard et al. (19). The subjects were classified into three groups: 66 "control" premature infants, 51 ill infants with appropriate birth weight for gestational age (AGA), and 18 infants with small birth weight for gestational age (SGA).

Birth weight classification followed the anthropometric standards for newborn Caucasian infants proposed by Usher and McLean (20). To be classified in the control group, the infants had to meet the following criteria: appropriate birth weight for gestational age, absence of evidence of congenital anomalies, absence of illness, and enteral feeding within 10 days postpartum. The AGA group was composed of premature infants with major illnesses (respiratory distress syndrome, severe anemia and bradycardia, cardiac malformations with cardiac failure), delayed oral feeding, and (or) receiving parenteral nutrition. The SGA group comprised premature infants with intra-uterine growth retardation, including those with major illnesses and those without evident disease. The characteristics of each group are summarized in Table 1. The study was approved by the Ethics Committee of the Hospital.
Nursery Care and Feeding Regimen

All infants were unclothed and kept in controlled isolets at thermoneutral temperature; all received dextrose and electrolyte solution intravenously during the first postnatal days. Nasogastric tube feeding was started as soon as possible after birth. Thereafter, oral feeding was commenced, with the mother’s milk when possible. Otherwise, infant formula with a high protein (18 g/L; whey casein ratio: 60/40 by wt) and calorie (680 kcal/L) content (Special Care; Ross Laboratories, Division of Abbott Laboratories, Montréal, Canada) was used. Nasogastric and bottle feedings were started at a later age in the SGA and AGA groups; 35% of the infants of the AGA group and 33% of those of the SGA group received parenteral nutrition (Vamin-N, Intra-Lipids, Pharmacia Canada, Dorval; Dextrose 5%, Travenol Canada, St-Laurent, Montréal; electrolytes and trace elements, IMS International, Mississauga; and vitamins, USV Canada, Mississauga, Canada).

When the mothers’ milk was used for oral feeding, calorie and protein intakes were calculated as the following values: calories = 710 kcal/L, proteins = 11 g/L (21). Calorie (kcal/kg) and protein intakes (g/kg) were calculated each day, and average daily intakes for each week were compared with the biochemical values and the anthropometric measurements.

Anthropometric Measurements

All infants were weighed daily, and the rate of weight increment was calculated from the mean daily weight gain for each week. Crown-to-heel length and head circumference were measured consistently every week and the results were expressed in centimeters gained per week. Triceps skinfold thickness was measured weekly by a single observer (M.C.) and the results were expressed in millimeters gained per week.

Biochemical Assessments

Blood samples were collected from all infants from birth (within 2 h) throughout their hospitalization. Samples for prealbumin measurements were obtained only when infants were having blood drawn for other tests during the last three days of each week. Prealbumin was not measured in serum specimens from infants who had had blood transfusions within the last four weeks. Serum was frozen at -70 °C until analyzed. Prealbumin concentrations in 2-μL serum samples were measured by radial immunodiffusion with in-house reagents from Le Centre de Recherche de l’Hôtel-Dieu de Québec. The prealbumin standard solution and control sera were from Behring Diagnostic (standard human serum, lot no. 041011, and control plasma for immunodiffusion plates, lot no. 047009; Hoechst Canada Inc., Montréal, Québec, H4R 1R6). Total imprecision of the method (CV) was 12% at a low prealbumin concentration (24.4 mg/L) and 7.9% at a high concentration (155 mg/L) (22). Total serum proteins were determined by refractometry, and albumin concentrations were measured with an Hitachi 705 (Boehringer Mannheim of Canada, Dorval, Québec, H4R 1V8) and the bromocresol-green method (Abbott, Montréal, Québec, H4P 1A5).

Statistical Methods

We compared the three groups of premature infants in terms of their protein–calorie intakes, anthropometric variations, and changes in biochemical analytes (prealbumin, albumin, and total proteins) to determine a potential marker of malnutrition. One-way variance analysis was used to assess the three groups simultaneously, and the Bonferroni t-test was applied to determine the source of statistically significant difference (23). A p-value of <0.05 was to indicate a significant difference. We determined the Pearson product–moment correlation coefficient (r) to evaluate the degree of correlation between biochemical markers, anthropometric measurements, and protein–calorie intakes. The Kolmogorov–Smirnov test for normality was used to test the distribution of the results of biochemical and anthropometric measurements. Differences between birth parameters of the various groups were assessed by Student’s t-test.

Results

The gestational age of group 1 (control group: uncomplicated prematurity) was significantly greater (p <0.01) than that of group 2 (sick AGA) but not significantly different from that of group 3 (SGA). The anthropometric measurements of group 1 at birth were also significantly different from those of groups 2 and 3 (Table 1).

During the first postnatal week, the mean daily weight loss was significantly more important (p <0.01) in the AGA group (23 g per day) than in the two other groups (Figure 1). Subsequently, the mean daily weight gain was greater in group 1 than in the two other groups, who showed similar mean daily increase in weight. The difference was statistically significant (p <0.01), however, only for the third week.
During group SGA groups than postpartum. During the same period, head circumference and body length increased more rapidly in the SGA group than in the two other groups (results not shown).

Protein–calorie intakes increased progressively in all groups (Figure 2). Although the mean daily intake of the SGA group was less than that of group 1, the difference was not statistically significant, whereas the intake of the AGA group was significantly (p < 0.01) less than that of group 1 during the first four weeks for calorie intake (Figure 2) and during the first two weeks for protein intake.

The concentrations of total proteins and albumin in serum showed similar changes in all three groups, decreasing by 10 to 15% by the fifth week (results not shown). Prealbumin increased with time in the control group to peak at 132 mg/L by the fourth week, which was 43% greater than the prealbumin concentration at birth (Figure 3). In the sick AGA group, prealbumin was slightly higher at birth than in the control group and remained relatively constant during the study period. The results were not significantly different from those of group 1, even though the AGA infants had lower protein and calorie intakes during the first two postnatal weeks. SGA infants had prealbumin values lower than those of the control group throughout the study, the differences being statistically significant at the third and fourth weeks (p < 0.001). Protein intake of the SGA group was only slightly lower than that of the control group through the investigation, calorie intake was 10 to 15% less than that of the control group (not statistically significant).

Reference intervals were established for total proteins, albumin, and prealbumin, which showed a normal distribution by the normality test of Kolmogorov–Smirnov. There being no great variation in total proteins and albumin concentrations during the study period, we used all the values of the control group. The reference intervals were 37.9 to 61.1 g/L (mean ± 2SD) for total proteins and 23.8 to 37.6 g/L for albumin. For prealbumin the reference interval was calculated by combining results at birth and one week later; the results, 33 to 152 mg/L, showed a great interindividual variability.

Linear regression equations and Pearson product–moment correlation coefficients were calculated to determine the relation between the changes in biochemical markers and the anthropometric measurements for the AGA and SGA groups combined over the first 12 weeks postpartum; no correlation was found (results not shown). Similarly, we obtained no correlation between the variations in total serum proteins and albumin and the calorie or protein intake. The correlations of prealbumin concentration with the calorie intake (y = 0.27x + 70.48; r = 0.28; p < 0.001) and protein intake (y = 8.83x + 76.98; r = 0.28; p < 0.001) were weak and without apparent clinical usefulness.

To determine whether the biochemical markers could differentiate between high and low calorie intake, protein intake, and (or) weight gain, we further subdivided each study group into classes: calorie intake of less than or more
than 120 kcal/kg per day, protein intake of less than or more than 2 g/kg per day, and weight gain of less than or more than 20 g/day. This comparison included all infants in the first 12 postnatal weeks. The concentrations of total proteins and albumin showed no statistical differences among these subgroups. Prealbumin concentrations apparently differed significantly according to the calorie and protein intakes in all three groups (Table 2). This difference may however be attributable, in part at least, to age (Table 3) because the normal group, which was well nourished, also showed significant differences in relation to intakes and weight gain (Table 2).

Discussion

Our results demonstrate that biochemical analytes such as total proteins, albumin, and prealbumin are not sensitive indicators of nutritional intake in premature infants of the same postnatal age. Changes in concentration of these markers did not correlate with anthropometric measures. Concentrations of total proteins and albumin did not differ in relation to the nutritional intakes among the three groups of infants studied. Mean prealbumin concentrations were higher in the control group by the second week postpartum but were significantly different only by the fourth week. Although prealbumin concentrations were lowest in the SGA group, it was the AGA group who had the greatest number of infants with serious illnesses and the lowest calorie and protein intakes.

When the study groups were subdivided by calorie intake, protein intake, and weight gain (Table 2), only prealbumin showed differences between infants who had greater or lesser protein–calorie intake. In a previous study involving 17 premature infants (26–33 weeks of gestation), Moskowitz et al. (16) showed that mean prealbumin concentrations were significantly lower in those weighing less than 1000 g at birth and receiving less than 100 kcal/kg or less than 2 g/kg of protein per day, as compared with those who had higher intakes. The correlation coefficients were, however, weak ($r = 0.66$ for protein intake and 0.64 for calorie intake), and they found no significant correlation between the intake of calorie or protein and the prealbumin concentrations for infants weighing more than 1000 g at birth. In our study, infants of all three groups who were taking more than 120 kcal/kg or more than 2 g/kg daily were significantly older than those with low calorie and protein intakes. Thus the greater prealbumin concentrations found in those with higher nutritional intakes in all three groups may be at least in part due to age differences between the subgroups. Socha et al. (17) have demonstrated that postnatal concentrations of prealbumin in premature infants increase to full-term values within three weeks. Our values for prealbumin in the control group are similar to those reported by Moskowitz et al. (16), Socha et al. (17), and Rossi et al. (24).

In conclusion, although there are tendencies towards lower concentrations of prealbumin in premature infants with lower nutritional intakes, we found no strong correlation between calorie or protein intakes and changes in prealbumin concentrations in infants of the same postnatal age. This may be explained in part by the large interindividual variation found in prealbumin concentrations. On the other hand, the apparent correlation between intakes and prealbumin concentrations we found when infants were regrouped on the sole basis of intakes may be due in fact to the physiological increase in prealbumin concentrations in premature infants during the first few postnatal weeks (17). Furthermore, the nutritional intakes of premature infants at risk for malnutrition may still be sufficient in a neonatal intensive-care unit to avoid the occurrence of measurable metabolic deficits. We conclude that biochemical markers such as total proteins, albumin, and even prealbumin are not sufficiently sensitive to be used in the assessment of the nutritional status of premature infants.

### Table 2. Prealbumin Concentrations in the Three Groups, According to Calorie Intake, Protein Intake, and Weight Gain

<table>
<thead>
<tr>
<th>Group</th>
<th>Calorie Intake, kcal/kg per day</th>
<th>Protein Intake, g/kg per day</th>
<th>Mean weight gain, g per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;120</td>
<td>&gt;120</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>102 ± 38</td>
<td>111 ± 29</td>
<td>101 ± 39</td>
</tr>
<tr>
<td>AGA</td>
<td>94 ± 33</td>
<td>112 ± 36</td>
<td>90 ± 29</td>
</tr>
<tr>
<td>SGA</td>
<td>80 ± 24</td>
<td>109 ± 40</td>
<td>70 ± 26</td>
</tr>
<tr>
<td></td>
<td>(104)</td>
<td>(27)</td>
<td>(97)</td>
</tr>
<tr>
<td>AGA</td>
<td>86 ± 30</td>
<td>103 ± 35</td>
<td>(168)</td>
</tr>
<tr>
<td>SGA</td>
<td>72 ± 18</td>
<td>97 ± 35</td>
<td>(48)</td>
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<tr>
<td></td>
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<td><strong>b</strong></td>
</tr>
</tbody>
</table>

*Prealbumin concentration, mg/L: mean ± SD (and no. of observations). **Significantly different within groups by: *0.01 < p < 0.05, **0.001 < p < 0.01, ***p <0.001.

### Table 3. Postnatal Age (Weeks) of the Premature Infants in Relation to Calorie Intake, Protein Intake, and Weight Gain

<table>
<thead>
<tr>
<th>Group</th>
<th>Calorie Intake, kcal/kg per day</th>
<th>Protein Intake, g/kg per day</th>
<th>Mean weight gain, g per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;120</td>
<td>&gt;120</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.7 ± 0.9</td>
<td>2.5 ± 1.4</td>
<td>0.6 ± 0.9</td>
</tr>
<tr>
<td>AGA</td>
<td>2.9 ± 2.5</td>
<td>6.7 ± 3.1</td>
<td>2.6 ± 2.7</td>
</tr>
<tr>
<td>SGA</td>
<td>2.8 ± 3.0</td>
<td>5.1 ± 2.8</td>
<td>2.4 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>(138)</td>
<td>(39)</td>
<td>(123)</td>
</tr>
<tr>
<td>AGA</td>
<td>0.5 ± 0.8</td>
<td>2.2 ± 1.3</td>
<td>(51)</td>
</tr>
<tr>
<td>SGA</td>
<td>1.1 ± 1.5</td>
<td>4.8 ± 3.1</td>
<td>(105)</td>
</tr>
<tr>
<td></td>
<td>(133)</td>
<td>(64)</td>
<td>(196)</td>
</tr>
<tr>
<td>AGA</td>
<td>(136)</td>
<td>(168)</td>
<td>(168)</td>
</tr>
<tr>
<td>SGA</td>
<td>(136)</td>
<td>(168)</td>
<td>(168)</td>
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<td></td>
<td>(38)</td>
<td>(63)</td>
<td>(63)</td>
</tr>
<tr>
<td>AGA</td>
<td>(47)</td>
<td>(64)</td>
<td>(64)</td>
</tr>
<tr>
<td>SGA</td>
<td>(73)</td>
<td>(33)</td>
<td>(33)</td>
</tr>
</tbody>
</table>

*Mean ± SD (and no. of observations). All differences within groups significant at p <0.001.

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References