Dietary Nucleotide Supplementation Raises Erythrocyte 2,3-Diphosphoglycerate Concentration in Neonatal Rats1,2

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ABSTRACT The present study was designed to test if dietary intake of nucleotides increases erythrocyte 2,3-diphosphoglycerate (2,3-DPG) in neonatal rats. To this end, rat pups were fed a nucleotide-supplemented formula (S, n = 14) from d 9 until d 16 after birth. The results were compared with those obtained from a group of breast-fed pups (C, n = 14) and a group of pups artificially fed with nucleotide-free formula (NS, n = 14). Neonatal weight, 2,3-DPG concentration, hematocrit (Hct) and hemoglobin concentration (Hb) were determined before the experiment (d 9) and after treatment (d 16). In all groups, 2,3-DPG concentration was greater at d 16 than at d 9, and the increase was greater in the S group than in the NS group. Alterations in neonatal weight, Hct and Hb concentration did not differ among the groups. On d 16 the 2,3-DPG/Hb ratio, reflecting the affinity of hemoglobin for oxygen, was significantly higher in the C and S groups than in the NS group. We conclude that in neonatal rats, dietary nucleotides increase erythrocyte 2,3-DPG concentration. Studies need to be conducted in humans to assess the effect of this increase on both neonatal peripheral hemodynamics and metabolism in this species. J. Nutr. 129: 662–665, 1999.

KEY WORDS: • artificial feeding • nucleotides • erythrocyte • oxygen affinity • rats

The higher affinity of hemoglobin for 2,3-diphosphoglycerate (2,3-DPG),4 compared to oxygen, shifts the oxygen disso-

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2 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.
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4 Abbreviations used: C, breast-fed rat pups; 2,3-DPG, 2,3-diphosphoglycerate; HbF, fetal hemoglobin; Hct, hematocrit; NS, rat pups fed a nucleotide-free formula; P50, O2 pressure at which 50% of blood is saturated; S, rat pups fed a nucleotide-supplemented formula.

MATERIALS AND METHODS

The composition of nucleotide supplemented (S) and nonsupplemented (NS) formula (Abbott laboratories B. V., Zwolle, NL) is shown in Table 1. Sprague-Dawley rat pups (n = 42), and their dams (n = 4), were supplied by a commercial breeder (Charles-River Laboratories, Zurich, Switzerland) 5 d after birth. All facilities and procedures were approved by the Institutional Animal Care and Use

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Committee of the University of Genova. Pups taken from the same litter were numbered and progressively assigned to three groups, group C (n = 14), NS (n = 14) and S (n = 14). The back of each animal was marked with colored dye to distinguish the various groups and to allow putting the pups with their own mothers during the night throughout the experimental period. Afterward the animals were allowed acclimation to the centralized experimental animal facilities until d 9 postpartum with a 12 h/12 h light/dark cycle. The dams were given free access to standard nonpurified diet.

The experimental period (d 9–d 16) was chosen after conducting several preliminary studies and allowed us to avoid inherent problems caused by artificial feeding, overly young pups, and rat weaning that occurs approximately at d 20 of life. On the basis of previous in vitro evidence, this period was also determined to be the best to observe the proposed increase in erythrocyte 2,3-DPG synthesis Dawson et al. (1971).

On d 9, seven pups from each group were randomly weighed and killed by decapitation. Blood was collected from the heart (1 mL) to measure 2,3-DPG, hematocrit (Hct) (microcapillary method) and hemoglobin (Hb) (OSM; Radiometer, Copenhagen, Denmark) concentrations. In some pups the amount of blood that was obtained from the heart after decapitation was too small to perform analysis on Hct and Hb. Until d 16 after birth, group C was breast-fed. Groups NS and S were separated from their mothers every day at 0900 h until d 16. They were fed every 3 h by slow intragastric injection with formulas, which was either S or NS. The amount of injected fluid was calculated following neonatal artificial rat feeding curves Messer et al. (1969) and was gradually increased during the experimental period from 0.75 to 1.5 mL per injection. This depended on the neonatal weight gain and the age of the pups and was decided to avoid gastric overdistension. Studies based on solely artificial intake were previously paralleled by an increased risk of death by overdistension and increased intestinal gas formation (Dymsza et al. 1964, Miller and Dymza 1963). Therefore, to obtain a high survival rate, the pups were placed with their dams at 2000 h to allow suckling. On d 16 all pups were weighed and killed by decapitation. Blood was collected from the heart (1 mL) to measure 2,3-DPG, Hct and Hb concentrations. The 2,3-DPG/Hb ratio, reflecting the affinity of Hb for oxygen, was calculated for each rat pup.

**Assays.** Erythrocyte 2,3-DPG concentration was measured by quantitative enzymatic determination with Diagnostics (St. Louis, MO) 2,3-

![FIGURE 1](https://academic.oup.com/jn/article-abstract/129/3/662/4722173 by guest on 03 May 2018)

**TABLE 1**

<table>
<thead>
<tr>
<th>Amount/L formula</th>
<th>NS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates, g</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Protein, g</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Lipids, g</td>
<td>37</td>
<td>3.7</td>
</tr>
<tr>
<td>Linoleic acid, g</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Linolenic acid, g</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Energetic content, kJ</td>
<td>680</td>
<td>680</td>
</tr>
<tr>
<td>Cytidine 5’ monophosphate, mg</td>
<td>—</td>
<td>1.15</td>
</tr>
<tr>
<td>Uridine 5’ monophosphate, mg</td>
<td>—</td>
<td>4.50</td>
</tr>
<tr>
<td>Guanosine 5’ monophosphate, mg</td>
<td>—</td>
<td>2.00</td>
</tr>
<tr>
<td>Adenosine 5’ monophosphate, mg</td>
<td>—</td>
<td>1.70</td>
</tr>
<tr>
<td>Inosine 5’ monophosphate, mg</td>
<td>—</td>
<td>0.59</td>
</tr>
</tbody>
</table>

1 Standard diet was provided by a commercial supplier (Mucedola, Milano, Italy).
DPG acid reagents. Freshly obtained heparinized blood (1 mL) was mixed with cold trichloroacetic acid (3 mL) and was shaken vigorously for 3 s then centrifuged (10 min, 3000 g). The supernatant (2,3-DPG in trichloroacetic acid) was enzymatically hydrolyzed to 3-phosphoglycerate and inorganic phosphorus by the 2,3-DPG phosphatase activity associated with the enzyme phosphoglycerate mutase. As previously described (Keitt 1971), liberated phosphorus, the oxidation of NADH to NAD reflects the concentration of DPG originally present. Determination of 2,3-DPG as described above had a 2.2% CV.

Statistics. The results are presented as median, 75th and 25th percentile throughout the text unless otherwise stated. Differences between d 9 and d 16 in each group were evaluated by Wilcoxon-Ranked-Sum-Test. Differences among groups C, NS and S were evaluated using the Mann-Whitney-U-test. A $P$ value of $<0.05$ (two-sided) was considered significant.

RESULTS

Neonatal weight, 2,3-DPG concentration, Hct and Hb for all groups are shown in Figure 1. Erythrocyte 2,3-DPG concentration and neonatal weight increased in all groups by d 16 compared to d 9. The 2,3-DPG concentration at d 9 did not differ among the groups while at d 16 it was significantly lower in the NS group than in the C- and S groups ($P < 0.05$). In C and NS groups both Hct and Hb were greater at d 16 than at d 9 ($P < 0.05$). The difference was not significant in the S group, though this may be related to the relatively limited number of observations. The change in erythrocyte 2,3-DPG concentration was significantly greater in the S group than in the NS group ($P < 0.05$) (Fig. 2). In the NS rat pups the 2,3-DPG/Hb ratio was significantly lower at d 16 than at 9 and was lower than in the C and S pups (Fig. 3; $P < 0.05$).

DISCUSSION

The present study was designed to determine if neonatal erythrocyte 2,3-DPG concentration can be increased by exogenous dietary nucleotide administration. In the S group the 2,3-DPG concentration was 25% higher at d 16 compared to d 9. In the C and NS groups, a 15% increase in 2,3-DPG concentration was observed between d 9 and 16. Since a neonatal rise in 2,3-DPG confirmed by others in different species (Bartels et al. 1979, Baumann et al. 1973, Blunt et al. 1971, Noble et al. 1983), the 15% rise in 2,3-DPG observed in the C and NS groups was considered to be a normal physiological phenomenon. Taking this physiological 2,3-DPG postnatal increase into account, the additional 10% increase in erythrocyte 2,3-DPG observed in the S group likely can be attributed to nucleotide supplementation. Therefore, since no data concerning the nucleotide profile of rat milk are available, we can hypothesize that it is low in nucleotides.

The rises in Hct and Hb were also considered physiological since the pattern of changes between d 9 and 16 were comparable among the three groups and were observed by others in different species (Menendez-Patterson et al. 1987, Mortola et al. 1986, Styka and Penney 1977). Supplementation of nucleotides to the neonatal rat pups did not cause additional effects on the postnatal rise of Hct and Hb.

In the first month of life, human neonate Hb levels and Hct decrease, while DPG erythrocyte concentration remains stable or slightly increases (Delivoria-Papadopoulou et al. 1971). As in adults (Hielm 1969, Torrance et al. 1970), this “physiological neonatal anemia” is probably compensated for by an increased DPG/Hb ratio that maintains an adequate oxygen delivery to the tissues by increasing the pO₂ at which the blood reaches the 50% saturation ($P_{50}$). Therefore, Hb concentration is negatively correlated with $P_{50}$ values (Koizumi 1991, Samaja et al. 1990).

Unfortunately, the aggressive sampling procedure and the small amount of blood that was obtained from each pup did not allow us to measure other variables (pH, pCO₂, HbF%), that may affect the position of the oxygen dissociation curve.

FIGURE 2. Percentage change in erythrocyte 2,3-diphosphoglycerate (2,3-DPG) concentration on d 16 after birth as compared to d 9 after birth in normal breast-fed (C), nonsupplemented (NS) and nucleotide-supplemented (S) neonatal rat pups. Data are presented as medians and 75th and 25th percentiles: *$P < 0.05$ vs. NS, Mann-Whitney-U-Test.

FIGURE 3. 2,3-diphosphoglycerate (2,3-DPG)/Hb-ratio (mmol/g), 9 and 16 d after birth of breast-fed (C), nonsupplemented (NS) and nucleotide-supplemented (S) neonatal rat pups. Data are presented as medians and 75th and 25th percentile: *$P < 0.05$ vs. d 9, Wilcoxon-Ranked-Sum-Test; $P < 0.05$ Mann-Whitney-U-Test vs. C or S.
However, in these rat pups, despite a physiological Hb and Hct increase, the additional increase in 2,3-DPG obtained in the S group prevented the drop in the 2,3-DPG/Hb ratio, which was observed in the NS group. This suggests that in the S group, artificial feeding with nucleotide-supplemented formula is associated with a higher peripheral oxygen supply.

Not surprisingly, in this particular experiment, the increases in the erythrocyte 2,3-DPG concentration and the 2,3-DPG/Hb ratio were not paralleled by a greater increase in neonatal weight in the S group. Theoretically, a rise in erythrocyte 2,3-DPG would have a more profound effect on neonatal weight gain and on the metabolic performance of neonates having a compromised peripheral metabolic environment and decreased erythrocyte 2,3-DPG values as a result of either an unpaired intrauterine growth and/or altered lung function subsequent to neonatal respiratory distress syndrome (Farquharson 1983, Siegel et al. 1979). This is confirmed by other results (Cosgrove et al. 1996) describing improved catch-up growth after nucleotide administration, in small for gestational age infants whose intestinal mucosa was hypothesized to be functionally impaired as a result of undernutrition.

Currently the effect of an increase in 2,3-DPG, particularly on the peripheral hemodynamic and metabolic environment in neonates, cannot be deduced from either the present study or from other studies (Cosgrove et al. 1996). However our experimental data may provide essential background for performing clinical investigations in human newborns.

From the present study we can conclude that erythrocyte 2,3-DPG concentration can be increased by dietary supplementation of nucleotides in neonatal rats. As hypothesized above, it remains to be seen whether artificial formulas enriched with nucleotides might provide positive effects on the neonatal pathological conditions (respiratory distress syndrome, bronchopulmonary dysplasia) in which more oxygen release to peripheral tissues is required. Further experimental and clinical nutritional investigations are needed to achieve this goal.

LITERATURE CITED


