Reference Values for Magnesium, Calcium, Phosphorus, Copper and Zinc in Umbilical Cord Plasma and Erythrocytes of Newborns and Their Statistical Interpretation

M. Speich[1], A. Pineau[2], S. Gelot[2], O. Laborde[3], H.-G. Classen[4])

Zusammenfassung

Intrauterines Wachstum und fötale Entwicklung werden maßgeblich durch eine adäquate Versorgung mit Nährstoffen, speziell mit Mineralstoffen und Spurenelementen, bestimmt. Es ist bekannt, daß eine inadäquate alimentäre Versorgung der Mutter oder Krankheiten, welche den Mineralstoff-Metabolismus beeinträchtigen, mutagene und teratogene Effekte verursachen können oder in späteren Abschnitten der Schwangerschaft unspezifische foetotoxische Folgen wie retardiertes Wachstum haben können. Daher schien es nötig, Referenzwerte für diese Parameter in neonatalem Gewebe zu bestimmen.

Magnesium (Mg), Gesamt-Calcium (Ca), Phosphor (P), Kupfer (Cu) und Zink (Zn) wurden in Nabelschnur-Plasma (Pl) und Erythrocyten (E) gemessen; anschließend wurden Korrelationen und schrittweise Regressionsgleichungen für 66 kaukasische, termingerecht Geborene (35 Knaben, 31 Mädchen) errechnet.

Da keine geschlechtsspezifischen Unterschiede statistisch nachweisbar waren, wurden Jungen und Mädchen zu einer Gruppe zusammengefaßt und gemeinsam betrachtet. Korrelations- und schrittweise Regressionsberechnungen ergaben, daß Pl-Cu und E-Zn, die zudem miteinander positiv korrelieren, diejenigen Variablen darstellen, die den höchsten Bezug zu Schwangerschaftsdauer, Geburtsgewicht und Körperlänge aufweisen. Die wichtigsten Bezüge für Pl-Mg waren — in absteigender Rangfolge — Pl-P, Pl-Ca, E-Cu und der Kopfumfang. Keine Beziehung ergab sich für das E-Mg.

Pl-Mg =– 0,015 + 0,127 Pl-P + 0,130 Pl-Ca –0,006 E-Cu + 0,012 Kopfumfang (p < 0,001).

Summary

Intrauterine growth and development largely depend on an adequate supply of nutrients, especially of minerals and trace elements. Insufficient dietary intake or diseases adversely affecting mineral metabolism are known to exert mutagenic and teratogenic effects, or can result in unspecific fetal damage such as retarded growth at later stages of pregnancy. In view of these data it seemed useful to establish reference values for these parameters in neonatal tissue samples.

Magnesium (Mg), total calcium (Ca), phosphorus (P), copper (Cu) and zinc (Zn) were determined in umbilical cord plasma (Pl) and erythrocytes (E), and correlations and stepwise regression equations were then calculated for 66 white full-term newborns (35 boys, 31 girls).

No statistically significant sex-related differences could be proven; therefore both groups of newborns were combined and considered as a homogeneous population. Correlation and stepwise regression calculations revealed that Pl-Cu and E-Zn were the variables most related to gestational age, birth weight and body length; in addition, there was a positive correlation between Pl-Cu and E-Zn. The most significant regressors for Pl-Mg were, in decreasing order of importance, Pl-P, Pl-Ca, E-Cu and head circumference. No regressor was found to be significant for E-Mg.

Pl-Mg = – 0.015 + 0.127 Pl-P + 0.130 Pl-Ca – 0.006 E-Cu + 0.012 head circumference ($P < 0.001$).

Résumé

La croissance intra-utérine et le développement dépendent largement d'apports adéquats en nutriments, notamment en minéraux et éléments trace. Une alimentation insuffisante de la mère ou des maladies, affectant le métabolisme minéral, peuvent être mutagènes et tératogènes ou induire des troubles fétaux non spécifiques comme des retards de croissance aux stades ultérieurs de la grossesse. En conséquence, il est apparu nécessaire d'établir les valeurs de référence de ces paramètres dans le sang de nouveaunés.

Le magnésium (Mg), le calcium total (Ca), le phosphore (P), le cuivre (Cu) et le zinc (Zn) ont été déterminés dans le plasma (Pl) et les érythrocytes (E) du sang du cordon ombilical. Les corrélations et les équations de régression pas-à-pas ont ensuite été calculées chez 66 nouveau-nés blancs, à terme (35 garçons, 31 filles).

Aucune différence significative en fonction du sexe n'ayant été mise en évidence, les deux groupes d'enfants furent réunis et considérés comme une seule population. Les corrélations et les équations de régression pas-à-pas ont mis en évidence que Pl-Cu et E-Zn étaient les variables les plus liées à l'âge gestationnel, au poids et à la taille de naissance. De plus, il y avait une corrélation positive entre Pl-Cu et E-Zn. Les régresseurs les plus significatifs de Pl-Mg étaient par ordre d'importance décroissante Pl-P, Pl-Ca, E-Cu et le périmètre crânien. Il n'y avait aucune variable explicative significative de E-Mg.

Pl-Mg = – 0,015 + 0,127 Pl-P + 0,130 Pl-Ca – 0,006 E-Cu + 0,012 périmètre crânien ($P < 0,001$).

Introduction

During the past decade, analytic advances have made it possible to determine the diverse micro- and macroelements of the human organism with precision. Most trace elements play an important role as enzyme activators or as constituents of metalloenzymes [1,2]. The lack of any element can lead to major impairment of physiological and biological functions [3,4]. In the embryo, mineral supply and metabolism disorders are potentially mutagenic, teratogenic or fetotoxic and may also induce abortion or premature birth. In fetal life, mineral element disorders may produce relatively ste-

[1]) Laboratoire de Biochimie

[2]) Laboratoire de Toxicologie et d'Hygiène Industrielle, Faculté de Pharmacie, 1, rue Gaston Veil, F-44035 Nantes Cedex 01

[3]) Service de Gynécologie A, Hôpital Femme-Enfant CHU, F-44035 Nantes Cedex

[4]) Universität Hohenheim, Fachgebiet: Pharmakologie und Toxikologie am Institut für Biologische Chemie und Ernährungswissenschaft, D-7000 Stuttgart 70

reotyped and nonspecific effects such as fetal growth retardation and various abnormalities [3, 5]. Other effects may be latent and become overt much later in life in the form of neurological and psychological disorders or even increased susceptibility to carcinogenesis and atherogenesis. Teratogenesis may even occur in subsequent generations [2, 5–9].

The purpose of this study was to determine reference values in umbilical cord plasma (Pl) and erythocytes (E) for magnesium (Pl-Mg, E-Mg), calcium (Pl-Ca), phosphorus (Pl-P), copper (Pl-Cu, E-Cu) and zinc (Pl-Zn, E-Zn). In addition, correlations and stepwise regression equations were calculated to detect possible relationships between these variables and gestational age, birth weight, birth length and head circumference of newborns selected according to optimal criteria.

Subjects and methods

Subjects

From February to April 1990, 66 white full-term babies (35 boys, 31 girls) were studied in the maternity ward of the University Hospital in Nantes, France. All pregnancies were uncomplicated and resulted in term vaginal delivery. Mothers (19 to 40 years) were healthy and had no history of serious disease. The Apgar score at 1 and 5 min was always 10. None of the infants had congenital malformation.

Assay Techniques

Within 5 min of delivery, cord blood specimens were drawn into Venoject tubes containing lithium heparin (Ref. T206 LH, Code VT 050 HL1, Terumo France, 78181 Saint-Quentin-en-Yvelines Cedex, France) and then, without delay, centrifuged at 3.500 x g for 8 min at 10 °C. Immediately afterwards, Mg, Ca and Zn concentrations were measured by flame atomic absorption spectrometry (Philips Pye Unicam SP9 model, Philips, 93002 Bobigny Cedex, France) according to previously described protocols [10]. Copper was analyzed by flameless atomic absorption spectrometry with Zeeman effect in plasma and erythrocytes diluted 50-fold with demineralized water (Perkin Elmer model 3030, Perkin Elmer, 78054 Saint-Quentin-en-Yvelines Cedex, France). Phosphorus was determined by ultraviolet detection (340 nm) of ammonium phosphomolybdate (Biotrol kit, ref. A02477, Biotrol, 75140 Paris Cedex 03, France).

Statistical Analysis

We used the Student *t*-test for comparisons of means according to sex, the Pearson correlation and stepwise regression analysis [11]. All statistical procedures were implemented using Systat software (Systat Inc., Evanston, IL, U.S.A.).

Results

Since no statistically significant sex-related differences could be proven (Tab. 1), the two groups of newborns were combined and considered as a homogeneous population. The mineral parameters were all normal-distributed and the respective reference ranges ($\overline{X} \pm 2$ SD) were: Pl-Mg (0.85 ± 0.16 mmol/l), E-Mg (1.76 ± 0.30 mmol/l), Pl-Ca (2.48 ± 0.44 mmol/l), Pl-P

Tab. 1: Results in umbilical cord plasma and erythrocytes of male and female newborns (mean and SD).

	Pl-Mg (1)	E-Mg (2)	Pl-Ca (3)	Pl-P (4)	Pl-Cu (5)	E-Cu (6)	Pl-Zn (7)	E-Zn (8)	Mother's age (9)	Weeks of gestation (10)	Birth weight (11)	Birth height (12)	Head circumference (13)
	(mmol/l)				(µmol/l)				y	w	kg	cm	cm
- Male, n = 35	0.86 (0.09)	1.77 (0.14)	2.46 (0.24)	1.52 (0.24)	5.45 (2.17)	12.7 (2.75)	16.4 (3.60)	43.2 (13.0)	27.3 (5.26)	39.4 (1.44)	3.34 (0.42)	49.3 (2.00)	34.6 (1.10)
- Female, n = 31	0.84 (0.08)	1.74 (0.16)	2.50 (0.19)	1.63 (0.25)	5.73 (2.13)	13.1 (3.29)	15.6 (2.21)	37.2 (13.7)	28.4 (4.50)	39.7 (1.35)	3.30 (0.41)	49.5 (1.90)	34.1 (1.43)
- Comparison of means	NS[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
- Male and Female, n = 66	0.85 (0.08)	1.76 (0.15)	2.48 (0.22)	1.57 (0.25)	5.58 (2.14)	12.9 (3.00)	16.0 (3.02)	40.4 (13.6)	27.8 (4.91)	39.5 (1.39)	3.32 (0.41)	49.4 (1.94)	34.4 (1.28)
- Pearson correlation for the 66 infants $P < 0.05$	(+) 1,3 (+) 1,4 (+) 1,7		(+) 3,5 (+) 3,6[b] (+) 3,8	(+) 4,7 (-) 4,8	(+) 5,6 (+) 5,8 (+) 5,10 (+) 5,11 (+) 5,12			(+) 8,10[b]		(+) 10,11 (+) 10,12 (+) 10,13	(+) 11,12 (+) 11,13	(+) 12,13	

[a] NS = not significant : $P > 0.05$ (Student's *t*-test)
[b] $P < 0.001$

(1.57 ± 0.50 mmol/l), Pl-Cu (5.58 ± 4.28 μmol/l), E-Cu (12.9 ± 6.00 μmol/l), Pl-Zn (16.0 ± 6.04 μmol/l), E-Zn (40.4 ± 27.2 μmol/l). Among the many significant correlations ($P < 0.05$) determined, it is noteworthy that Pl-Cu was correlated with the number of weeks of gestation and with birth weight and birth length.

The stepwise regression equations for the 66 newborns were:

- birth weight = 3.460 + 0.009 E-Zn – 0.031 Pl-Zn ($P < 0.01$);
- birth height = 48.265 + 0.201 Pl-Cu ($P = 0.074$);
- Pl-Mg = – 0.015 + 0.127 Pl-P + 0.130 Pl-Ca – 0.006 E-Cu + 0.012 head circumference ($P < 0.001$).

No regressor was found to be significant for head circumference and E-Mg.

Discussion

Several recent studies have reported data concerning the levels of electrolytes and trace elements in plasma or serum of newborns [9, 12–20], with results similar to those for the reference values reported here. However, very few data have been published on erythrocytes, and these, to our knowledge, have concerned only E-Zn [21–23]. As in our study, no sex-related differences were found, for magnesium [18], copper and zinc [24].

Unlike *Nelson* et al. [25], who reported that Mg values were not correlated with Ca or P values in different groups of infants, or *Brown* [26] who noted a negative correlation between Pl-Mg and Pl-Ca, we found that Pl-Mg was positively correlated with Pl-Ca and Pl-P as well as Pl-Zn. Moreover, the positive correlation between Pl-Cu and birth weight shown in our study confirms previous results [24].

The most significant regressors accounting for birth weight were, in decreasing order of importance, E-Zn, which was positively correlated with Pl-Cu and gestational age, and Pl-Zn. Except for *Arumanayagam* et al. [27] who found no significant correlations between cord Pl-Zn and birth weight, most authors have reported significant positive correlations between birth weight and Pl-Zn [24, 28] or E-Zn [23]. Since Zn is an essential constituent of many enzyme systems involved in normal cell division and is also required for protein synthesis, impairment of these processes may retard fetal growth and thus reduce birth weight [8, 29–31].

Fetal growth and basic functions are strongly dependent on exchange processes across the placenta, which is also the site of metabolic activity and hormone production. The placenta transports Ca ions actively, rendering the fetus hypercalcemic relative to the mother. This results in calcitonin release and may inhibit parathyroid hormone secretion conducive to fetal skeletal formation [12]. Fetal P and Mg concentrations are also higher than those of the mother [4, 12, 32]. The Mg gradient is sufficient to protect the fetus from severe maternal Mg deprivation, and the existence of a hypothetical active transport system from the mother to the fetus has been suggested [4].

Physiological changes during pregnancy include decreased Pl-Zn and increased Pl-Cu concentrations. The decrease in Zn reflects a maternal-fetal transfer, an expansion of maternal plasma volume, decreased Zn binding affinities or transport protein concentrations (albumin, aplha-2 macroglobulin), and increased Zn requirement or inadequate Zn intake [22, 33, 34]. The increase in Cu can be attributed to increased ceruloplasmin as a result of elevated estrogen concentrations [19, 20]. At birth, Pl-Zn values in infants are higher and Pl-Cu values lower than those of the mother when pregnant and even when not pregnant. The high Pl-Zn concentration may reflect an active transfer of Zn across the placenta [22, 27, 34]. The low Pl-Cu concentration may be associated with a low fetal ceruloplasmin rate [19, 20]. However, this rate increases with gestational age, probably accounting for the positive correlation between Pl-Cu and the number of weeks of gestation found by us and *Bouglé* et al. [15]. *Noubah* and *Al-Awqati* [19] showed that the contribution of fetal ceruloplasmin to total Cu concentration may be less than in pregnant women or normal controls. Copper does not easily diffuse accross the placenta but accumulates in the layers of the placenta, and from there it is transferred to the fetus by an active process of diffusion according to the needs of the fetus [35]. Very little investigation has been devoted to the determination of the erythrocyte concentration of these variables in newborn infants. Fetal E-Zn concentrations have been found to be much lower than in adults [10]. The fact that E-Zn is predominantly associated with the carbonic anhydrase enzyme system (EC 1.4.2.1) may indicate that lower E-Zn values in neonates correspond to relatively low concentrations of carbonic anhydrase [5, 8, 22].

It may be concluded that at birth there are no significant sex-related differences among the variables studied. The variables most related to gestational age, birth weight and birth length, as shown by our correlation and stepwise regression calculations, are Pl-Cu and E-Zn, which themselves were positively correlated.

The most significant regressors for Pl-Mg were, in decreasing order of importance, Pl-P, Pl-Ca, E-Cu and head circumference. No regressor was found to be significant for E-Mg.

The neonatal reference values reported here are based on an optimal, selected reference population.

In further investigations, sick and abnormal newborns as well as babies of mothers suffering from different diseases will be studied, and electrolyte levels measured in these patients will be related to the reference values reported here.

Acknowledgements

We thank *Laure Leroy* for her excellent secretarial assistance.

References

[1] *Kruse-Jarres, J. D.:* Clinical indications for trace element analyses. J. Trace Elem. Electrolytes Health Dis. **1** (1987) 5–19.

[2] *Vallee, B. L.:* Zinc: biochemistry, physiology, toxicology and clinical pathology. Biofactors **1** (1988) 31–36.

[3] *Doyle, W.; Crawford, M. A.; Wynn, A. H. A.; Wynn, S. W.:* Maternal nutrient intake and birth-weight. J. Hum. Nutr. Dietetics **2** (1989) 415–22.

[4] *Spätling, L.; Disch, G.; Classen, H.-G.:* Magnesium in pregnant women and the newborn. Magnesium Res. **2** (1989) 271–80.

[5] *Perlman, M.:* Perinatal aspects of trace metal metabolism. In: *Rennert, O. M.; Chan, W.-Y.* (eds): Metabolism of trace metals in man. Volume I: Developmental aspects. CRC Press, Boca Raton, Florida 1984, pp. 51–62.

[6] *Hurley, L. S.:* Magnesium deficiency in pregnancy and its effects on the fetus. Mag.-Bull. **3**, 1a (1981) 202–08.

[7] *Laitinen, R.; Vuori, E.; Viikari, J.:* Serum zinc and copper: associations with cholesterol and triglyceride levels in children and adolescents. Cardiovascular risk in young Finns. J. Am. Coll. Nutr. **8** (1989) 400–06.

[8] *Favier, A.; Favier, M.:* Conséquences des déficits en zinc durant la grossesse pour la mère et le nouveau-né. Rev. Fr. Gynecol. Obstet. **85** (1990) 13–27.

[9] *Favier, A.; Favier, M.:* Place de la carence en zinc dans l'étiologie des malformations du tube neural. Rev. Fr. Gynecol. Obstet. **85** (1990) 49–55.

[10] *Speich, M.; Gelot, S.; Arnaud, P.; Van Goc, N.; Robinet, N.; Pineau, A.:* Multiple and simple correlations between magnesium, calcium, zinc, potassium, total- and HDL-cholesterol in 111 reference subjects. Mag.-Bull. **6** (1984) 137–41.

[11] *Sprent, P.:* Applied non parametic statistical methods. Chapman and Hall, London, New York 1989, pp. 1–259.

[12] *Pitkin, R. M.:* Calcium metabolism in pregnancy and the perinatal period: a review. Am. J. Obstet. Gynecol. **151** (1985) 99–109.

[13] *Colombo, M. L.; Grillo, C.; Ricci, B. M.; Erbeia, M.; Buschini, M. P.:* Valori sierici di zinco nel neonato a termine e pretermine. Minerva Pediatr. **39** (1987) 153–57.

[14] *Lockitch, G.; Halstead, A. C.; Wadsworth, L.; Quigley, G.; Reston, L.; Jacobson, B.:* Age-and sex- specific pediatric reference intervals and correlations for zinc, copper, selenium, iron, vitamins A and E, and related proteins. Clin. Chem. **34** (1988) 1625–28.

[15] *Bouglé, D.; Foucault, P.; Voirin, J. et al.:* Taux du molybdène, du sélénium et du cuivre plasmatiques à l'accouchement. Arch. Fr. Pediatr. **46** (1989) 95–98.

[16] *Clemente Yago, F.; Cortes Castell, E.; Moya Benavent, M.; Ballester Galindo, I.; Herero Galiana, A.; Campello Oltra, M. J.:* Metabolismo mineral de calcio, magnesio y fósforo en el recién nacido normal. An Esp. Pediatr. **31** (1989) 533–36.

[17] *Meites, S.* (ed): Pediatric clinical chemistry. Reference (normal values). AACC Press, 3rd ed. Washington 1989, pp. 1–329.

[18] *Geven, W. B.; Vogels-Mentink, G. M.; Willems, J. L.; De Boo, T.; Lemmens, W.; Monnens L. A. H.:* Reference values of magnesium and potassium in mononuclear cells and erythrocytes of children. Clin. Chem. **36** (1990) 1323–27.

[19] *Noubah, A. M.; Al-Awqati, M. A.:* Ultrafiltrable copper and related analytes in maternal and cord blood. Clin. Chem. **36** (1990) 860–64.

[20] *Speich, M.:* Le cuivre dans la grossesse normale, diabétique et en périnatologie. Rev. Fr. Gynecol. Obstet. **85** (1990) 34–39.

[21] *Iolascon, A.; Perrone, L.:* Erythrocytic zinc content during childhood. Acta Haematol. **73** (1985) 114–16.

[22] *Powell-Beard, L.; Lei, K. Y.; Shenker, L.:* Effect of long-term oral contraceptive therapy before pregnancy on maternal and fetal zinc and copper status. Obstet. Gynecol. **69** (1987) 26–32.

[23] *Kuhnert, B. R.; Kuhnert, P. M.; Lazebnik, N.; Erhard, P.:* The effect of maternal smoking on the relationship between maternal and fetal zinc status and infant birth weight. J. Am. Coll. Nutr. **7** (1988) 309–16.

[24] *Ette, A.; Adi Ibeziako, P.:* Plasma zinc and copper concentrations in pregnant Nigerian women and newborn. Afr. J. Med. Med. Sci. **14** (1985) 99–103.

[25] *Nelson, N.; Finnström, O.; Larsson, L.:* Plasma ionized calcium, phosphate and magnesium in preterm and small for gestational age infants. Acta Poediatr. Scand. **78** (1989) 351–57.

[26] *Brown, I. R. R.:* More on the relation between magnesium and calcium in serum and plasma. Clin. Chem. **32** (1986) 221.

[27] *Arumanayagam, M.; Wong, F. W. S.; Chang, A. M. Z.; Swaminathan, R.:* Zinc concentration in umbilical cord tissue and cord plasma in appropriate-for-gestational-age babies. Eur. J. Obstet. Gynecol. Reprod. Biol. **23** (1986) 121–26.

[28] *Singh, P. P.; Khushlani, K.; Veerwal, P. C.; Gupta, R. C.:* Relationship between birth weight and zinc status in newly born infants and their mothers. Indian J. Physiol. Pharmacol. **33** (1989) 134–35.

[29] *Dreosti, I. E.:* Zinc in prenatal development. In: Prasad, A. S.; Dreosti, I. E.; Hetzel, B. S. (eds): Clinical applications of recent advances in zinc metabolism. AR Liss, New York 1982, pp. 19–38.

[30] *Singh, P. P.; Khushlani, K.; Veerwal, P. C.; Gupta, R. C.:* Maternal hypozincemia and low-birth-weight infants. Clin. Chem. **33** (1987) 1950.

[31] *Neggers, Y. H.; Cutter, G. R.; Acton, R. T. et al:* A positive association between maternal serum zinc concentration and birth weight. Am. J. Clin. Nutr. **51** (1990) 678–84.

[32] *Kuoppala, T.; Tuimala, R.; Parviainen, M.; Koskinen, T.; Ala-Houhala, M.:* Serum levels of vitamin D metabolites, calcium, phosphorus, magnesium and alkaline phosphatase in Finnish women throughout pregnancy and in cord serum at delivery. Hum. Nutr. Clin. Nutr. **40 C** (1986) 287–93.

[33] *Fehily, D.; Kisvani, A. S.; Jenkins, D. M.; Cremin, F. M.:* Redistribution of plasma zinc between albumin and α_2-macroglobulin in pregnancy. J. Trace. Elem. Electrolytes Health. Dis. **1** (1987) 63–68.

[34] *Bro, S.; Berendtsen, H.; Nørgaard, J.; Høst, A.; Jørgensen, P. J.:* Serum zinc and copper concentrations in maternal and umbilical cord blood. Relation to course and outcome of pregnancy. Scand. J. Clin. Lab. Invest. **48** (1988) 805–11.

[35] *Raina, P.; Narang, A. P. S.; Banday, A. W.; Bhan, V. K.:* Copper and zinc levels in maternal and fetal cord blood. Trace Elem. Med. **7** (1990) 186–87.

(Correspondence to: Dr. *M. Speich*, Laboratoire de Biochimie, Faculté de Pharmacie, 1, rue Gaston Veil, B.P 1024, 44035 Nantes Cedex 01, France)