Longitudinal Study of Concentrations of Plasma, Erythrocyte and Urine Magnesium, Calcium, Phosphorus, Potassium, Zinc and Copper during Insulin-Dependent Diabetic Pregnancy

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Zusammenfassung

In Plasma (Pl), Erythrozyten (Erc) und 24h-Urin (U) wurden die Konzentrationen von Magnesium (Pl-Mg, Erc-Mg, U-Mg), Gesamt-Kalzium (Pl-Ca, U-Ca), Phosphor (Pl-P, U-P), Kalium (Plasma-K, Erc-K, U-K), Zink (Pl-Zn, Erc-Zn) und Kupfer (Pl-Cu) bei Insulin-abhängigen Diabetikerinnen bestimmt; die Messungen begannen bereits vor der Konzeption und erstreckten sich bis einen Monat nach der Geburt. Die Kationen wurden mittels AAS bestimmt. Hochsignifikante Änderungen ergaben sich für Pl-Cu (μmol/l) vom ersten Trimester (16,3 ± 4,3) bis zu einem Monat nach der Geburt (19,2 ± 2,88) und einem zwischenzeitlichen Anstieg auf 34,4 ± 7,95 im dritten Trimester. Für Pl-Mg, Pl-Ca und Pl-Zn ergaben sich nicht signifikante Tendenzen zu erniedrigten Spiegeln während der Schwangerschaft und einem Anstieg auf die Ausgangswerte nach der Geburt; die Werte im dritten Trimester bzw. ein Monat nach der Schwangerschaft betrugen 0,64 ± 0,05 bzw. 0,71 ± 0,08 mmol Mg/l, 1,66 ± 0,15 bzw. 1,79 ± 0,15 mmol Ca/l und 8,92 ± 2,31 bzw. 9,87 ± 2,01 μmol Zn/l. Andererseits war das Erc-Zn noch einen Monat nach der Geburt mit 217 ± 34,2 μmol/l leicht erhöht ($p > 0,05$). Die Gesamt-Proteine nahmen während der Schwangerschaft ab (63,5 ± 4,54 g/l) und stiegen nach der Geburt an (71,5 ± 3,67 g/l). Die Daten zeigen, daß sich bei gut eingestellten Diabetikerinnen im Vergleich zu stoffwechselgesunden Schwangeren nur einige wenige, schwach ausgeprägte Abweichungen im Mg, Ca, P, K, Zn und Cu-Haushalt ergeben.

Summary

Plasma (Pl), erythrocyte (Erc) and 24-h urine (dU) concentrations of magnesium (Pl-Mg, Erc-Mg, dU-Mg), calcium (total Pl-Ca, dU-Ca), phosphorus (Pl-P, dU-P), potassium (Pl-K, Erc-K, dU-K), zinc (Pl-Zn, Erc-Zn), and copper (Pl-Cu) were investigated in insulin-dependent diabetic (IDD) women from before conception until one month after delivery. Cations were measured by atomic absorption spectrometry. Changes in Pl-Cu from the first trimester of gestation (16.3 ± 4.30 μmol/l) to one month after delivery (19.2 ± 2.88 μmol/l), with an intermediary result of 34.4 ± 7.95 μmol/l in the third trimester, were highly significant ($P < 0.0001$). For Pl-Mg, Pl-Ca and Pl-Zn, there was a non-significant tendency to decreasing values during gestation and a return to prepregnancy concentrations after delivery (respective values 0.64 ± 0.05 vs 0.71 ± 0.08 mmol/l, 1.66 ± 0.15 vs 1.79 ± 0,15 mmol/l and 8.92 ± 2.31 vs 9.87 ± 2.01 μmol/l in the third trimester of gestation compared to one month after delivery). On the contrary, there was a non-significant increase of Erc-Zn which was still apparent one month after birth (217 ± 34.2 μmol/l). Total proteins decreased during pregnancy (63.5 ± 4.54 g/l during the third trimester) and increased after delivery (71.5 ± 3.67 g/l). This study indicates that with good glycemic control there were only a few slight changes in Mg-, Ca-, P-, K-, Zn-, and Cu metabolism in pregnant IDD women relative to normal pregnant women cited in the literature.

Résumé

Les concentrations plasmatiques (Pl), érythrocytaires (Erc), urinaires des 24 heures (dU) de magnésium (Pl-Mg, Erc-Mg, dU-Mg), calcium (Pl-Ca total, dU-Ca), phosphore (Pl-P, dU-P), potassium (Pl-K, Erc-K, dU-K), zinc (Pl-Zn, Erc-Zn) et cuivre (Pl-Cu) ont été déterminées chez des femmes diabétiques insulino-dépendantes (DID) depuis avant la conception jusqu'à un mois après l'accouchement. Les cations furent analysés par spectrométrie d'absorption atomique. L'évolution de Pl-Cu du premier trimestre de gestation (16,3 ± 4,30 μmol/l) jusqu'à un mois après l'accouchement (19,2 ± 2,88 μmol/l), avec un résultat intermédiaire de 34,4 ± 7,95 μmol/l au troisième trimestre, était très significative. Pour Pl-Mg, Pl-Ca et Pl-Zn, il y avait une diminution non significative des valeurs pendant la grossesse et un retour à des concentrations d'avant la grossesse après l'accouchement (valeurs respectives de 0,64 ± 0,05 vs 0,71 ± 0,08 mmol/l, 1,66 ± 0,15 vs 1,79 ± 0,15 mmol/l et 8,92 ± 2,31 vs 9,87 ± 2,01 μmol/l au troisième trimestre de grossesse par rapport à un mois après l'accouchement). Au contraire, il y avait une augmentation non significative de Erc-Zn qui était encore observée un mois après la naissance (217 ± 34,2 μmol/l). Les protéines totales diminuaient pendant la grossesse (63,5 ± 4,54 g/l pendant le troisième trimestre) et augmentaient après l'accouchement (71,5 ± 3,67 g/l). Cette étude indique qu'avec un contrôle glycémique satisfaisant, il y avait seulement quelques changements légers dans le métabolisme des Mg, Ca, P, K, Zn et Cu de femmes enceintes DID par rapport aux femmes enceintes normales citées dans la littérature.

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Introduction

Although several studies have reviewed the data about magnesium, calcium, phosphorus, copper and zinc during pregnancy [1–6] or diabetes mellitus [7–9], little research has been devoted to the combined effects of pregnancy and diabetes mellitus on maternal mineral metabolism [5, 10, 11]. However, maternal metabolic abnormalities relative to certain trace elements may amplify the effects of energy substrate deficits involved in the genesis of fetal malformations [12–15].

The purpose of this study was to determine plasma (Pl), erythrocyte (Erc) and 24-h urine (dU) concentrations of magnesium (Pl-Mg, Erc-Mg, dU-Mg), calcium (total Pl-Ca, dU-Ca), phosphorus (Pl-P, dU-P), potassium (Pl-K, Erc-K, dU-K), zinc (Pl-Zn, Erc-Zn), copper (Pl-Cu), total glycohemoglobin (gHb), fructosamine and total proteins in insulin-dependent diabetic (IDD) women before conception, when possible, to one month after delivery (non-lactating women).

Materials and Methods

Analyses were conducted from April 1990 to May 1992.

Subjects

After giving their informed consent, 17 Caucasian IDD women (22 to 40 years of age) who had received insulin for at least 3 years were recruited at the Nantes Diabetes Center. Pregnancies were planned, and intensive treatment (multiinjection of insulin) was initiated before conception since good glycemic control was required prior to pregnancy. Each woman was seen every two to four weeks depending on whether close diabetological monitoring was needed or not. Specimens for blood and urine analysis were collected before conception, once in the middle of each trimester of gestation (7 ± 1, 20 ± 1 and 33 ± 1 weeks of gestation), at delivery and one month post partum. For the 5 women included from the start of the protocol, this represented 6 specimens of 5 ml of blood each. For the 12 women included in the study from the second trimester or third trimester of gestation, 5 ml specimens were collected in the middle of each trimester, at delivery and one month post partum. No maternal complications occurred, and none of the newborns presented congenital malformations.

Assay techniques

Venous blood from fasting diabetic women was drawn into 5 ml Venoject tubes containing lithium heparin (Terumo France) and then immediately centrifuged at 3 500 x g for 8 min at 10 °C. Directly afterwards, Mg, Ca and Zn concentrations were measured in plasma, erythrocytes and urine by the specific (and reference) method of flame atomic absorption spectrometry (Philips Pye Unicam SP9, Philips). Potassium was measured in plasma, erythrocytes and urine by emission spectrometry using the same apparatus. Copper was analyzed by flameless atomic absorption spectrometry (the reference method) with Zeeman effect in plasma diluted 50-fold with demineralized water (Model 3030; Perkin Elmer). Phosphorus was determined in plasma and urine by ultraviolet detection (340 nm) of ammonium phosphomolybdate (cat. no. A 02477; Biotrol). Biotrol-33 Plus (cat. no. A 02270, lot 573), a multiconstituent bovine serum specially adjusted for accuracy control of electrolytes, was used for quality control. Target values and our intraassay coefficients of variation (CV, indicated in parentheses) in this bovine serum are as follows: P 1.97 mmol/l (1.63 %), Mg 0.95 mmol/l (1.25 %), Ca 2.49 mmol/l (1.30 %), Cu 13.4 µmol/l (1.36 %), Zn 10.7 µmol/l (2.64 %), K 5.15 mmol/l (1.14 %). Accuracy for P was 99.2 %, Mg 100.2 %, Ca 100.3 %, Cu 101.4 %, Zn 99.0 % and K100.6 %. Maternal gHb (chromatography on cation exchange resin; CV 1.55 % for a value of 6.25 %), fructosamine (reduction of nitroblue tetrazolium in alkaline medium and conversion to formazan; CV 2.03 % for a concentration of 1.40 mmol/l) and plasma total proteins were determined in the Nantes University Hospital Biology Institute.

Statistical Analysis

The Kruskal-Wallis test was used to search for significant changes in variables from the first trimester of gestation to one month after delivery [16]. A Spearman correlation calculation was performed between total gHb and the other variables at each analysis. Both statistical procedures were implemented by Systat software.

Results

Our results are summarized in tab. 1. The Kruskal-Wallis test indicated that Pl-Cu changes from the first trimester of gestation to one month after delivery were significant ($P < 0.0001$); Pl-Cu increased in the second trimester and was decreased after delivery.

For Pl-Mg, Pl-Ca and Pl-Zn, there was a non-significant tendency toward decreasing values during gestation and a rapid return to prepregnancy concentrations after delivery. On the contrary, there was a non-significant increase of Erc-Zn which persisted for one month after birth. Urinary Mg, -Ca, -K and -P were in the normal ranges [17]. Total proteins decreased during pregnancy and increased after delivery. Total gHb was above normal value (N < 7 %) [17], but the results for fructosamine were normal [18].

No significant Spearman correlation was found between gHb and the other variables at each analysis time.

Discussion

Alterations occur in the functioning of most maternal organ systems during pregnancy [3]. Metabolic control during the pregnancy of our diabetic women was satisfactory, and no obvious differences in mineral results were noted between our well-controlled diabetic pregnancies and normal ones described in the literature [11, 19–39]. Marked differences in the number of subjects in our report make longitudinal comparisons difficult, which may explain why decreases in Pl-Mg and Pl-Ca were significant in some studies [2, 11, 39, 40] but not in ours. Although the organism in most circumstances maintains Pl-Mg levels within narrow limits, the normal pregnant woman tends to develop lower than normal Pl-Mg [1, 34, 35, 39–41]. The drop in Pl-Mg during the third trimester may contribute to increased uterine irritability [34]. Moreover, as homeostasis of Ca is closely related to that of Mg, there is an evident tendency toward a Pl-Ca decrease [1, 2, 21, 22]. Simultaneously, the concentration of total proteins lowers as a result of hemodilution [34]. It is very likely that in our IDD mothers there was a decrease in Mg and Ca bound to pro-

Tab. 1: Changes of Plasma, Erythrocyte and Urine Values in Insulin-dependent Diabetic Women (Mean and SD).

	Units	Before pregnancy	First trimester	Second trimester	Third trimester	At delivery	One month after delivery non-lactating mothers
Pl-Mg	mmol/l	0.78 (0.02) n=5	0.70 (0.08) n=5	0.68 (0.06) n=10	0.64 (0.05) n=17	0.67 (0.08) n=16	0.71 (0.08) n=12
Erc-Mg	mmol/l	1.86 (0.20) n=5	1.78 (0.24) n=5	1.89 (0.17) n=10	1.82 (0.17) n=17	1.91 (0.21) n=16	2.01 (0.24) n=12
Pl-Ca	mmol/l	1.77 (0.16) n=5	1.73 (0.18) n=5	1.74 (0.15) n=10	1.66 (0.15) n=17	1.64 (0.20) n=16	1.79 (0.15) n=12
Pl-P	mmol/l	1.23 (0.16) n=5	1.07 (0.21) n=5	1.07 (0.18) n=10	1.16 (0.16) n=17	1.22 (0.42) n=16	1.13 (0.21) n=12
Pl-K	mmol/l	4.15 (0.60) n=5	3.99 (0.74) n=5	3.66 (0.31) n=8	3.92 (0.34) n=17	4.09 (0.59) n=12	4.24 (0.70) n=9
Erc-K	mmol/l	80.1 (39.3) n=5	97.6 (4.77) n=5	94.6 (5.65) n=8	97.7 (5.78) n=17	97.4 (4.86) n=12	98.9 (6.63) n=9
Pl-Zn	μmol/l	10.7 (0.36) n=5	10.3 (3.72) n=5	8.91 (2.04) n=10	8.92 (2.31) n=17	9.43 (2.66) n=16	9.87 (2.01) n=12
Erc-Zn	μmol/l	171 (40.8) n=5	178 (44.3) n=5	181 (26.5) n=10	215 (45.8) n=17	211 (43.7) n=16	217 (34.2) n=12
Pl-Cu	μmol/l	17.0 (1.06) n=3	16.3 (4.30) n=3	34.3 (6.42) n=6	34.4 (7.95) n=12	33.4 (9.41) n=12	19.2 (2.88)[a] n=10
dU Mg	mmol/24 h	3.75 (1.37) n=5	3.71 (0.70) n=5	3.77 (1.05) n=10	4.32 (1.18) n=17	3.20 (1.66) n=13	3.69 (2.07) n=11
dU Ca	mmol/24 h	3.74 (1.73) n=5	4.51 (0.79) n=5	5.27 (1.85) n=10	5.15 (2.86) n=17	3.08 (2.34) n=13	3.21 (1.70) n=11
dU P	mmol/24 h	24.7 (11.1) n=5	23.0 (6.47) n=5	26.5 (7.84) n=10	22.9 (6.34) n=17	23.8 (10.4) n=13	26.6 (8.52) n=11
dU K	mmol/24 h	49.0 (22.5) n=5	56.6 (15.4) n=5	62.7 (23.9) n=10	66.8 (26.9) n=17	75.6 (61.2) n=13	61.5 (22.1) n=11
Total gHb	%	9.15 (1.01) n=4	8.01 (1.24) n=4	8.98 (2.35) n=9	9.21 (0.90) n=13	8.68 (1.67) n=13	9.40 (2.02) n=6
Fructosamine	mmol/l	1.50 (0.24) n=3	1.54 (0.24) n=4	1.54 (0.31) n=9	1.32 (0.21) n=15	1.22 (0.17) n=14	1.52 (0.23) n=6
Total proteins	g/l	70.0 (3.46) n=3	73.9 (3.12) n=4	67.8 (2.80) n=9	63.5 (4.54) n=15	64.1 (6.70) n=12	71.5 (3.67) n=6

[a] Kruskal-Wallis test from first trimester to one month after delivery: $P < 0.0001$.

teins, particularly albumin [3, 22]. *Kuoppala* [42] confirmed a decrease of Ca values in IDD pregnant women but found no differences for serum-Mg or serum-P, whereas *Gödény* et al. [10] noted that Pl-P and Erc-P were significantly higher in diabetic pregnant women than in controls.

Insulin-dependent diabetic patients are known to develop hypomagnesemia, which is apparently related mainly to urinary Mg losses and true Mg depletion [6, 13, 21]. However, it is noteworthy that with satisfactory glycemic control in our IDD-mothers there was no abnormal urinary loss [43], contrary to the results reported by *Spätling* et al. for normal pregnancy [41]. Therefore, we consider that the decrease of Pl-Mg in IDD-pregnancy was normal and essentially physiologic [39]. *Borella* et al. [11] and *Kurzel* [34] have also indicated that the significance of a relatively small deviation of plasma or serum Mg from control concentrations should not be overestimated in the case of well-regulated diabetic women. Our results for Erc-Mg in IDD mothers were low, in agreement with our previous findings for non-pregnant IDD women [44]. However, low results have also been reported during normal pregnancy [45].

Plasma-K and Erc-K change non-significantly, remaining within narrow limits during diabetic pregnancy [3, 10].

Few studies have dealt with Pl-Zn, Pl-Cu and Erc-Zn in IDD-pregnancy, although there have been numerous investigations in normal pregnancy [10, 11]. Generally, Pl-Zn is decreased in diabetic patients and Pl-Cu is higher than in control subjects [7–9]. Similarly, Pl-Zn is generally lower and Pl-Cu higher in normal pregnancy at term [35]. Copper and Zn are considered fundamental for maternal and fetal metabolism because of their multiple biological roles [3–5,46,47]. In our group of IDD-pregnant women with good glycemic control, changes in Pl-Zn and Pl-Cu during gestation are in agreement with most values reported by other authors for normal pregnancy [4,5,19–33,35–37,48,49].

Our results during gestation showed a slight non-significant drop in Pl-Zn concentrations and a significant increase in Pl-Cu concentrations during the second and third trimesters, followed by a drop after delivery. The exact mechanism of Pl-Zn reduction during pregnancy remains unknown. Because albumin is the principal transport protein for Zn, any reduction in Zn might be secondary to reduced albumin concentration [25,30,49–51]. In our study, we noted a total protein decrease. Differences in circulating Pl-Zn may also be related to increased plasma volume associated with hormonal influences that direct Pl-Zn to the liver, erythrocytes and placenta; to increased maternal Zn requirements; and to inadequate Zn intake during pregnancy [25,26,35,37,49,51].

Concerning Erc-Zn, we noted an elevation of concentrations, particularly during the third trimester, which remained high one month after delivery. Erythrocyte-Zn is predominantly associated with carbonic anhydrase (EC 1.4.2.1). An increase in maternal Erc-Zn might thus compensate for low levels of carbonic anhydrase in the fetus during pregnancy [25,52]. Moreover, Erc-Zn has generally been reported to increase when Pl-Zn decreases by redistribution from one compartment to another [25]. *Gödény* et al. [10] reported higher Erc-Zn and lower Pl-Zn concentrations during diabetic than reference pregnancy, whereas *Wibell* et al. [21] found no significant differences in serum-Zn concentrations between well-treated IDD-pregnant women and control women at term. However, *Borella* et al. [11] noted significantly higher maternal Pl-Zn concentrations in the third trimester in diabetic pregnancy, probably due to reduced plasma expansion and/or reduced Zn transfer to the fetus.

The significant increase of Pl-Cu during pregnancy could result in part from the inverse relationship between Zn and Cu [32,36], and the increased plasma ceruloplasmin (90% of Pl-Cu) induced by elevated estrogen secretion during pregnancy could partially account for increased Pl-Cu concentrations [5,25,28,35,36,49]. Hypercupremia during pregnancy may also be due to a redistribution of Cu from maternal tissues, especially from the liver, and/or to an increase in gastrointestinal Cu absorption or a decrease in hepatobiliary Cu secretion [33,36,49]. Moreover, low free Cu concentrations similar to those in non-pregnant women have been found in maternal serum [37]. Plasma-Cu seems to increase similarly in diabetic and normal pregnancy [20,25,29,31], although *Borella* et al. [11] reported significantly higher Pl-Cu results in IDD mothers than in control pregnant women, thus suggesting that high Pl-Cu could be characteristic of women with hyperactive placenta.

It may be concluded that in diabetic pregnancy with good glycemic control there are only minor deviations in Mg-, Ca-, P-, K-, Zn- and Cu metabolism relative to normal pregnant women [34, 53].

References

[1] *Seelig, M. S.:* Magnesium deficiency in the pathogenesis of disease. Early roots of cardiovascular, skeletal, and renal abnormalities. Plenum Medical Book Company, New York 1980, p. 488.

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

[3] *Gödény, S.; Borbély-Kiss, I.; Koltay, E.; László, S.; Szabó, G.:* Determination of trace and bulk element in plasma and erythrocytes of healthy pregnant women by Pixe method. Int. J. Gynaecol. Obstet. **24** (1986) 191–199.

[4] *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.

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

[6] *Mimouni, F.; Tsang, R. C.:* Perinatal magnesium metabolism: personal data and challenges for the 1990s. Magnes. Res. **4** (1991) 109–117.

[7] *Mooradian, A. D.; Morley, J. E.:* Micronutrient status in diabetes mellitus. Am. J. Clin. Nutr. **45** (1987) 877–895.

[8] *Schlienger, J. L.; Grunenberger, F.; Maier, E. A.; Simon, C.; Chabrier, G.; Leroy, M. J. F.:* Perturbation des oligoéléments plasmatiques dans le diabète. Relation avec l'équilibre glycémique. Presse Med. **17** (1988) 1076–1079.

[9] *Manser, W. W. T.:* Diabetes mellitus. The trace element connection. J. Pak. Med. Assoc. **39** (1989) 91–93.

[10] *Gödény, S.; Borbély-Kiss, I.; Koltay, E.; László, S.; Szabó, G.:* Determination of trace and bulk elements in plasma and erythrocytes of diabetic pregnant women by Pixe method. Int. J. Gynaecol. Obstet. **24** (1986) 201–207.

[11] *Borella, P.; Szilagyi, A.; Than, G.; Csaba, I.; Giardino, A.; Facchinetti, F.:* Maternal plasma concentrations of magnesium, calcium, zinc and copper in normal and pathological pregnancies. Sci. Total. Environ. **99** (1990) 67–76.

[12] *Garrel, D.:* La femme diabétique: Grossesse et contraception. In: 9ème Symposium International de Diabétologie, Venice, Italy. Geneva (Switzerland): Excerpta Medica (1986) 148–156.

[13] *Mimouni, F.; Miodovnik, M.; Tsang, R. C.; Holroyde, J.; Dignan, P. S.; Siddiqi, T. A.:* Decreased maternal serum magnesium concentration and adverse fetal outcome in insulin-dependent diabetic women. Obstet. Gynecol. **70** (1987) 85–88.

[14] *Tchobroutsky, C.:* La grossesse chez la femme diabétique. Rev. Prat. **39** (1989) 2705–2706.

[15] *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.

[16] *Snodgrass, J. G.:* The numbers game. Statistics for psychology. Oxford University Press, New York 1977, p. 467.

[17] *Borel, J.; Caron, J.; Chanard, J.* et al.: Comment prescrire et interpréter un examen de biochimie. 2th ed., Maloine, Paris 1984, p. 850.

[18] *Georges, P.; Bellanné, C.:* Fructosamines. Act. Pharm. Biol. Clin. **4** (1987) 82–84.

[19] *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.

[20] *Tuttle, S.; Aggett, P. J.; Campbell, D.; Mac Gillivray, I.:* Zinc and copper nutrition in human pregnancy: a longitudi-

nal study in normal primigravidae and in primigravidae at risk of delivering a growth retarded baby. Am. J. Clin. Nutr. **41** (1985) 1032–1041.
[21] *Wibell, L.; Gebre-Medhin, M.; Lindmark, G.:* Magnesium and zinc in diabetic pregnancy. Acta Paediatr. Scand. [Suppl.] **320** (1985) 100–106.
[22] *Habib, Z.; Abdulla, M.:* Plasma levels of zinc, copper, magnesium and calcium during early weeks of gestation. Acta Pharmacol. Toxicol. **59** (1986) 602–605.
[23] *Singh, V. K.; Sharma, M.; Gupta, A.; Rohatgi, P.:* Serum copper level in pregnancy, labour and puerperium. J. Indian. Med. Assoc. **84** (1986) 206–208.
[24] *Kaira, V. B.; Kaira, R.; Khandelwal, R.:* Serum copper and ceruloplasmin in normal and pregnant women. Indian J. Pathol. Microbiol. **30** (1987) 327–332.
[25] *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.
[26] *Argemi, J.; Serrano, J.; Gutiérrez, M. C.; Ruiz, M. S.; Gil, A.:* Serum zinc binding capacity in pregnant women. Ann. Nutr. Metab. **32** (1988) 121–126.
[27] *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) 805–811.
[28] *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.
[29] *Lockitch, G.; Jacobson, B.; Quigley, G.; Butcher, L.:* Effect of normal gestation on essential and toxic trace elements (Abstract). Clin. Chem. **36** (1990) 973.
[30] *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) 678–684.
[31] *Noubah, A. M.; Al-Awqati, M. A.:* Ultrafiltrable copper and related analytes in maternal and cord blood. Clin. Chem. **36** (1990) 860–864.
[32] *Okonofua, F. E.; Isinkaye, A.; Onwudiegwu, U.; Amole, F. A., Emofurieta, W. A.; Ugwu, N. C.:* Plasma zinc and copper in pregnant Nigerian women at term and their newborn babies. Int. J. Gynecol. Obstet. **32** (1990) 243–245.
[33] *Raina, V.; 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) 186–187.
[34] *Kurzel, R. B.:* Serum magnesium levels in pregnancy and preterm labor. Am. J. Perinatol. **8** (1991) 119–127.
[35] *Makinde, O. O.; Amole, F.; Ogunniyi, S. O.:* Serum copper, zinc and magnesium in maternal and cord blood at delivery. West Afr. J. Med. **10** (1991) 168–170.
[36] *Veena, R.; Narang, A. P. S.; Banday, A. W.; Bhan, V. K.:* Copper and zinc levels in maternal and fetal cord blood. Int. J. Gynecol. Obstet. **35** (1991) 47–49.
[37] *Yasodhara, P.; Ramaraju, L. A.; Raman, L.:* Trace minerals in pregnancy. 1. Copper and zinc. Nutr. Res. **11** (1991) 15–21.
[38] *Lombargo, G.; Di Pasquale, G.; Manganoro, R.* et al.: Magnesium content of mononuclear blood cells in newborn infants and its correlation with maternal magnesium status. Magnes. Trace Elem. **10** (1991–1992) 30–33.
[39] *Weissberg, N.; Schwartz, G.; Shemesh, O.* et al.: Serum and mononuclear cell potassium, magnesium, sodium and calcium in pregnancy and labour and their relation to uterine muscle contraction. Magnesium Res. **5** (1992) 173–177.
[40] *Hurley, L. S.:* Magnesium deficiency in pregnancy and its effects on the offspring. In: *Durlach, J.:* 1st International symposium on magnesium deficit in human pathology. Edition du Symposium, Vittel (France) 1971, pp. 481–492.
[41] *Spätling, L.; Disch, G.; Classen, H. G.:* Magnesium in pregnant women and the newborn. Magnes. Res. **2** (1989) 271–280.
[42] *Kuoppala, T.:* Alterations in Vitamin D metabolites and minerals in diabetic pregnancy. Gynecol. Obstet. Invest. **25** (1988) 99–105.
[43] *Nicoll, G. W.; Struthers, A. D.; Fraser, C. G.:* Biological variation of urinary magnesium. Clin. Chem. **37** (1991) 1794–1795.
[44] *Speich, M.; Gelot, S.; Arnaud, P.; Murat, A.:* Plasma and erythrocyte magnesium in insulin-dependent and non-insulin dependent-diabetics: correlations between twelve variables. Mag. Bull. 7 (1985) 140–145.
[45] *Tsang, R. C.; Strub, R.; Brown, D. R.; Steichen, J.; Hartman, C.; Chen, I. W.:* Hypomagnesemia in infants of diabetic mothers: Perinatal studies. J. Pediatr. **89** (1976) 115–119.
[46] *Arnaud, J.; Favier, A.:* Le zinc. In: *Chappuis, P.:* Les oligoéléments en médecine et biologie. Editions Médicales Internationales, Cachan 1991, pp. 347–397.
[47] *Houot, O.; Tarallo, P.:* Le cuivre. In: *Chappuis, P.:* Les oligoéléments en médecine et biologie. Editions Médicales Internationales, Cachan 1991, pp. 459–498.
[48] *Swanson, C. A.; King, J. C.:* Zinc and pregnancy outcome. Am. J. Clin. Nutr. **46** (1987) 763–771.
[49] *Versieck, J.; Cornelis, R.:* Trace elements in human plasma or serum. CRC Press, Boca Raton, FL 1989, p. 224.
[50] *Smith, M. A.; Moser-Veillon, P. B.; Nagey, D. A.; Douglas, L. W.; Smith, J. C.:* Blood and urinary zinc changes after a glucose challenge in early and late pregnancies. Am. J. Clin. Nutr. **48** (1988) 664–670.
[51] *Jeswani, R. M.; Vani, S. N.:* A study of serum zinc levels in cord blood of neonates and their mothers. Indian J. Pediatr. **58** (1991) 683–687.
[52] *Speich, M.; Murat, A.; Auget, J. L.; Bousquet, B.; Arnaud, P.:* Magnesium, total calcium, phosphorus, copper, and zinc in plasma and erythrocytes of venous cord blood from infants of diabetic mothers: Comparison with a reference group by logistic discriminant analysis. Clin. Chem. **38** (1992) 2002–2007.
[53] *Nagra, S. A.; Answar T.; Anwar, J.; Farooqi, M. I.:* Longitudinal study in the concentration of serum calcium, copper, magnesium, zinc and hemoglobin during the last trimester of pregnancy in pakistani women. Nutr. Res. **11** (1991) 1341–1348.

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