

Placental zinc in normal and intra-uterine growth-retarded pregnancies

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The zinc concentration of placental tissue and cord blood in sixteen mothers who gave birth to normal babies was measured. The blood volume of each placenta was estimated from measurements of haemoglobin concentration of placental homogenate and cord blood, and, by deduction, the Zn content of blood-free placental tissue was calculated. Results were compared with eleven mothers whose fetuses showed a low biparietal diameter velocity between 17 and 28 weeks gestation and with ten mothers who gave birth to intra-uterine growth-retarded (IUGR) babies. As expected, placental weight was significantly correlated with infant birth weight. Blood-free placental tissue contained about four times more Zn (approximately 10 $\mu\text{g Zn/g}$) than cord blood (approximately 2.5 $\mu\text{g Zn/ml}$). Concentrations of Zn in blood-free placental tissue were similar in all three groups, but the cord blood Zn of mothers producing IUGR babies was significantly lower than that of the other two groups. Results of the present study suggested that fetal growth retardation in the mothers studied could not be explained by differences in blood-free placental Zn concentration, but that there may be some association between lower cord blood Zn levels and intra-uterine growth retardation.

Placental zinc: Pregnancy outcome: Intra-uterine growth retardation: Zinc

An inadequate supply of nutrients in pregnancy may prevent the normal growth and development of the fetus. For example, it has been widely demonstrated that a fairly marked deficiency of zinc affects growth in several species. Pups born to Zn-deficient rats weigh significantly less than controls and have reduced skeletal growth (Hurley, 1969). In humans, Zn deficiency has also been reported to impair fetal growth in some studies (Jameson, 1976), but not in others (McMichael *et al.* 1982; Campbell-Brown *et al.* 1985; Tuttle *et al.* 1985).

An important factor which must be taken into consideration when assessing the relationship between Zn and fetal growth is the degree of Zn depletion. Experiments with rats have clearly shown that whereas severe Zn deficiency unquestionably causes gross reduction in fetal growth, diets that are only just suboptimal with respect to Zn may, in fact, enhance fetal growth (Fairweather-Tait *et al.* 1985). In Britain, marginal Zn deficiency is a more likely possibility in pregnant women than severe Zn deficiency.

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In many studies Zn deficiency is diagnosed using maternal plasma or serum Zn levels. However, these are not generally considered to be a sensitive index of total body Zn status (Fairweather-Tait, 1988). Furthermore, the true relationship between maternal Zn status and that of the fetus cannot be determined accurately as it would require measurements of Zn in the developing infant. A good alternative is the placenta since this is the tissue involved with supplying nutrition to the fetus. It is peculiar to pregnancy, forms and develops after conception and is enzyme-rich, situated at the interface between mother and baby. During its normal growth it is in dynamic equilibrium with the surrounding blood, and with a total lifetime of several months, we believe it reflects an average exposure to particular nutrients like Zn, carried in the maternal blood, which are in turn supplied to the growing fetus. The present investigation was designed to investigate the relationship between Zn and fetal growth.

EXPERIMENTAL

Patients and methods

All mothers referred to the antenatal clinic before 17 weeks gestation were invited to attend at 17 weeks and again at 28 weeks for detailed assessment. They were selected and allocated to one of three groups in the study.

Normal control group. These patients were considered to be at low risk of having a small-for-date baby, according to the following assessments made at 28 weeks gestation: (a) diastolic blood pressure > 90 mm mercury, i.e. normotensive; (b) fasting glucose (8 h or more) 4–4.9 mmol/l (15th–97th centiles of the study population); (c) body-weight < 85 kg (< 90th centile of the study population); (d) normal increment of triceps skinfold thickness > 3 $\mu\text{m}/\text{d}$ (> 10th centile of the study population; Viegas *et al.* 1987); (e) increment in fetal biparietal diameter velocity from 17 to 28 weeks of gestation of 390–498 $\mu\text{m}/\text{d}$ (10th centile to mean + 2 SD of the study population).

Due to limited resources it was not possible to analyse the placentas of all the control mothers. Therefore a subgroup was selected as follows: (a) where a dietary assessment had been made at 28 weeks, (b) delivery between thirty-seven and forty-one completed weeks; (c) corrected birth weight between the 10th and 90th centiles (Thompson *et al.* 1968; Altman & Coles, 1980). A total of sixteen patients fulfilled these criteria, for whom placental Zn assays were performed.

Low biparietal diameter (BPD) velocity group. These patients had a BPD velocity between 17 and 28 weeks of < 390 $\mu\text{m}/\text{d}$ (< 10th centile of the study population). Apart from a reduced BPD velocity, they fulfilled all the other criteria of normality at the screening clinic defined previously. There were fourteen patients in this group, eleven of whom had placental Zn assays carried out.

Intra-uterine growth-retarded (IUGR) group. These patients were originally included in the control group described previously, i.e. were normal at the 28-week screening clinic in terms of fulfilling the previously defined criteria (a–d), but at delivery produced babies with a corrected birth weight below the 10th centile (Thompson *et al.* 1968; Altman & Coles, 1980). Ten of the thirteen mothers who were assigned to this group had placental Zn assays carried out.

Clinic assessment

All mothers attended the antenatal clinic at 17 and 28 weeks of pregnancy for a routine obstetric assessment including a fetal biparietal diameter (measured by ultrasound using a system XL2 scanner; Dynamic Imaging Ltd, Livingston, Scotland) and maternal anthropometry, namely weight (Salter spring), height (Seca metal gauge; Seca, Hamburg,

W. Germany) and triceps skinfold thickness (Holtain skinfold calliper; Holtain Ltd, Crosswell, Dyfed). All measurements were carried out by the same operator. The ultrasound scanner had a freeze frame, scale expansion, inbuilt photography unit, and linear and circumferential computerized measurements. The biparietal diameter was measured from the outer echo of one parietal bone to the inner edge of the other parietal bone of the fetal skull. The long axis was determined by locating the midline echo from the falx cerebri, taking into account the attitude of the head. Three consistent readings were taken at each scan. The BPD velocity ($\mu\text{m}/\text{d}$) was calculated for each mother by the formula (BPD at 28 weeks - BPD at 17 weeks)/time-period (d).

At 28 weeks a blood sample was taken from as many subjects as possible and fasting (> 8 h) plasma Zn was estimated. This was performed by removing a small volume of plasma, deproteinizing with trichloroacetic acid (1:3, v/v) and the Zn concentration measured by atomic absorption spectroscopy (AAS), as described below.

Dietary assessment

Women were interviewed between 28 and 30 weeks of pregnancy by a dietitian. They were all asked to recall their food intake for the 24 h ending at midnight on the previous day and these results are presented. The exact dietary recall method has been described previously (Eaton *et al.* 1984). Nutrient intakes were calculated from the 24 h recall using the food tables of Paul & Southgate (1978) on a computer program at the Dunn Nutritional Laboratory, Cambridge.

Measurements after delivery

At birth, babies were cleaned and weighed naked to the nearest 10 g on a spring balance (Howard) and placentas to the nearest 1 g on an Avery balance by the midwife responsible for the delivery. The same balances were used throughout the study. At 12-48 h after birth the following additional measurements were made: crown-heel length measured with a neonatometer (Holtain Ltd), mid-biceps and mid-triceps skinfold thickness with a Holtain calliper and mid-upper-arm circumference of the right arm with a paper tape measure. Gestational age was calculated from the mother's last menstrual period and confirmed by ultrasound. If there was a discrepancy of more than 1 week with the ultrasound reading, then the ultrasound dates were taken. Birth weights were also expressed as a standard deviation score for gestation (Thompson *et al.* 1968; Altman & Coles, 1980).

Analytical procedures

Collection of placentas. At delivery the placenta was weighed and sealed in a plastic bag and placed in a refrigerator at 4°. Within 12 h it was transferred for storage at -18° until analysed.

Estimation of blood-free tissue in placenta. Each placenta was thawed, weighed and homogenized in a stainless-steel commercial Waring blender (Jennings, Nottingham) with an equal weight of distilled water. Subsamples of the homogenate were taken and duplicate 12 ml portions centrifuged in calibrated plastic tubes at 2000 g for 15 min. The proportion of liquid to tissue was measured and the haemoglobin concentration of the supernatant fraction determined using the cyanomethaemoglobin method. The haemoglobin level of cord blood was also determined on a sample of blood collected at delivery, and from these the blood volume and, hence, by deduction the weight of blood-free tissue in the placenta was calculated.

Zn analysis. Duplicate samples of cord blood (2-3 g) and placental homogenate (8-10 g) were heated at 100° for 10 min with 10 ml concentrated nitric acid (Analar). A few drops of hydrogen peroxide were added to complete the digestion and the solution was then made

Table 1. *Details of mothers in normal, low biparietal diameter (BPD) velocity and intra-uterine growth-retarded (IUGR) groups**

(Mean values and standard deviations)

Group...	Normal (n 16)		Low BPD velocity (n 11)		IUGR (n 10)		RMS
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	24.9	—	25.1	—	27.0	—	—
Height (m)	1.61	0.06	1.60	0.07	1.60	0.05	—
Wt gain between 16 and 28 weeks gestation (kg/week)	0.604 ^a	—	0.570 ^a	—	0.405 ^b	—	0.029
Wt at 28 weeks gestation	70.0 ^a	10.3	64.9 ^a	5.0	59.8 ^b	5.2	—
Plasma zinc at 28 weeks gestation ($\mu\text{g/ml}$)	0.84 (n 15)	—	0.88 (n 8)	—	0.97 (n 5)	—	0.06

RMS, residual mean square.

^{a,b} Values with different superscript letters in each horizontal line were significantly different ($P < 0.05$).

* For details of criteria for groups, see p. 614.

up to an appropriate volume with distilled water. The Zn concentration was determined by AAS (PU 9000; Pye Unicam, Cambridge) using bovine liver as a reference standard (National Bureau of Standards, Gaithersburg, USA).

Statistical analysis

Differences between the three groups were tested using one-way analysis of variance (ANOVA). Results are presented as means and the residual mean square (RMS), where the standard error of the difference (SED) between means is equal to $\text{RMS} (1/n_1 + 1/n_2)$. Where there was a significant effect, approximate t tests were performed where $t = (\text{mean}_1 - \text{mean}_2)/\text{SED}$, with residual degrees of freedom. In the case of unequal variances a modified ANOVA program was used and standard deviations for each group are presented. The relationship between the birth weight of the baby and cord blood Zn and the Zn content of placental tissue was examined in each of the three groups by regression analysis.

RESULTS

Details of the mothers studied are presented in Table 1. There were no significant differences between the three groups in maternal age, height, or fasting plasma Zn measured at 28 weeks gestation. Mothers who fell into the IUGR group had a reduced weight velocity between 16 and 28 weeks, resulting in a lower weight at 28 weeks.

There did not appear to be a substantial difference in smoking or alcohol consumption between the three groups. In the normal group four mothers smoked (ten, ten, fifteen and twenty cigarettes/d) and one consumed 6 units alcohol/week. In the low BPD velocity group three mothers smoked (eight, ten and twelve cigarettes/d) and one consumed 16 units alcohol/week. In the IUGR group three mothers smoked (fifteen, twenty and thirty cigarettes/d) and two consumed alcohol (2 and 20 units/week).

It had not been possible to assess the food intakes of all mothers in the present study, and although dietary intake measurement was one criterion for selecting control mothers, only five of the low BPD velocity group and three of the IUGR group had been interviewed. Values for food intake are illustrated in Fig. 1. The three mothers in the IUGR

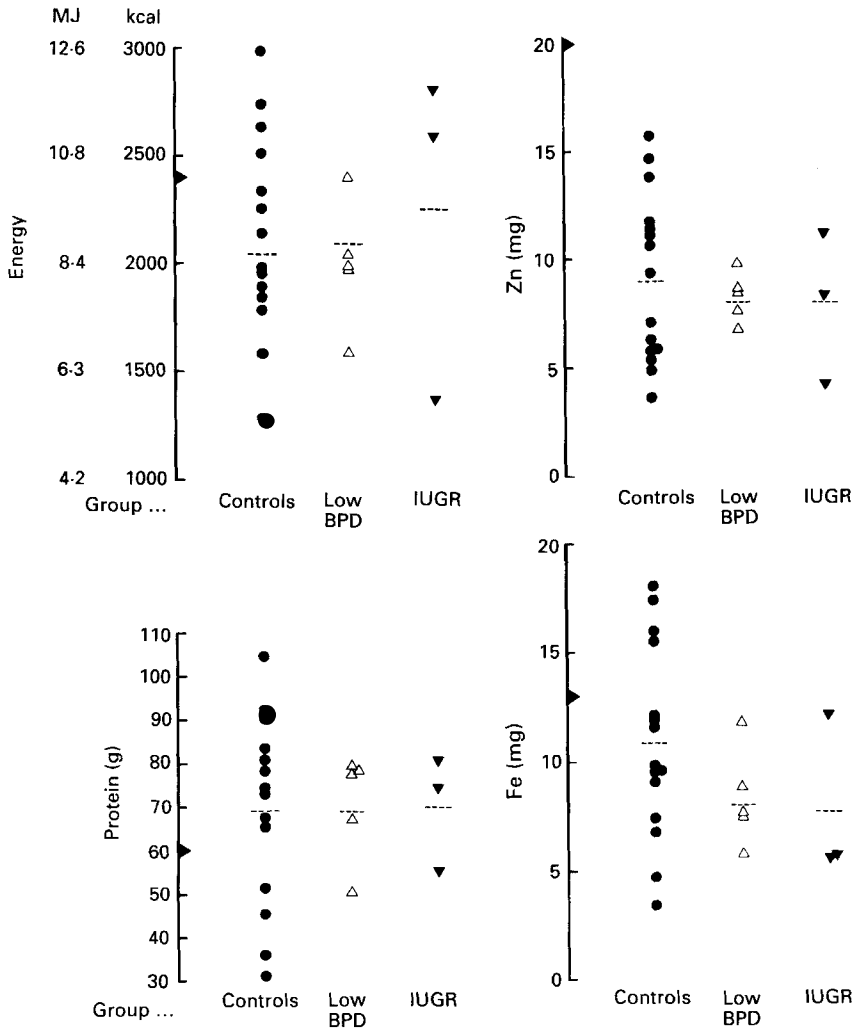


Fig. 1. Daily intakes (24 h recall) of energy, protein, zinc and iron by women at 28 weeks of pregnancy; (●) normal controls, (△) low biparietal diameter (BPD) velocity group, (▼) intra-uterine growth-retarded (IUGR) group. Individual results are presented together with group means (---). (▶) Recommended daily amount of energy, protein and Fe for pregnant women in the UK (Department of Health and Social Security, 1979) and recommended daily allowance for Zn in pregnant women in the USA (Committee on Dietary Allowance, Food and Nutrition Board, National Research Council, 1980). For details of criteria for groups, see p. 614.

group did not appear to consume less energy, protein, Zn or iron than mothers in the other two groups. Previous work (Eaton *et al.* 1984) has shown that results obtained by recall methods tend to be a little below those obtained by weighed methods. Nevertheless, all means for energy and Fe were less than the UK recommended daily amount for pregnant women (Department of Health and Social Security, 1979) and the means for Zn were less than the USA recommended daily allowance (Committee on Dietary Allowances, Food and Nutrition Board, National Research Council, 1980).

By the very nature of the grouping of the subjects there were significant differences in the outcome of pregnancy, as shown in Table 2, which could not be explained in terms of length

Table 2. *Details of babies in normal, low biparietal diameter (BPD) velocity and intra-uterine growth-retarded (IUGR) groups**

(Values are means for no. of subjects indicated)

Group...	Low BPD			RMS
	Normal (n 16)	velocity (n 11)	IUGR (n 10)	
BPD velocity 16–28 weeks ($\mu\text{m}/\text{d}$)	446.6 ^a	377.7 ^b	441.0 ^a	409.5
Gestation (weeks)	40.2	39.4	38.6	2.0
Birth wt (kg)	3.47 ^a	3.09 ^b	2.53 ^c	100.3
Head circumference (mm)	350 ^a	341 ^b	331 ^c	6.0
Length (mm)	508 ^a	494 ^a	476 ^b	35
Biceps skinfold thickness (mm)	3.01 ^a	2.70 ^b	2.42 ^b	0.11
Triceps skinfold thickness (mm)	3.60 ^a	3.26 ^a	2.60 ^b	0.27

RMS, residual mean square.

^{a,b,c} Values with different superscript letters in each horizontal line were significantly different ($P < 0.05$).

* For details of criteria for groups, see p. 614.

Table 3. *Placental measurements in normal, low biparietal diameter (BPD) velocity and intra-uterine growth-retarded (IUGR) groups**

(Mean values and standard deviations)

Group...	Normal (n 16)		Low BPD velocity (n 11)		IUGR (n 10)		RMS
	Mean	SD	Mean	SD	Mean	SD	
Placental wt (g)	682 ^a	157	595 ^a	131	473 ^b	61	—
Placental wt: birth wt	0.169	—	0.169	—	0.157	—	0.001
Zn concentration of placenta ($\mu\text{g}/\text{g}$)	7.71	1.01	7.70	0.70	7.14	0.68	—
Cord blood Hb (g/l)	160	—	148	—	155	—	60
Cord blood zinc ($\mu\text{g}/\text{ml}$)	2.4 ^a	0.4	2.6 ^{ab}	1.3	2.0 ^b	0.3	—
Blood-free (BF) placental wt (g)	453 ^a	—	413 ^a	—	302 ^b	—	5665
Zn concentration of BF placenta ($\mu\text{g}/\text{g}$)	10.33	—	9.87	—	10.16	—	1.41
Total Zn content of BF placenta (mg)	4.64 ^a	0.89	4.08 ^a	0.72	3.03 ^b	0.33	—
Total Zn in placenta (mg)	5.19 ^a	1.02	4.55 ^a	0.92	3.36 ^b	0.40	—

RMS, residual mean square; Hb, haemoglobin.

^{a,b} Values with different superscript letters in each horizontal line were significantly different ($P < 0.05$).

* For details of criteria for groups, see p. 614.

of gestation. Babies born to the low BPD velocity group had a reduced BPD velocity, but in all other aspects they were similar to the control group. However, babies born to mothers in the IUGR group weighed less, were shorter, and had reduced head circumference, and biceps and triceps skinfold thicknesses.

The placentas from mothers with IUGR babies were significantly smaller than either the normal or low BPD velocity groups, as shown in Table 3, but when expressed as a proportion of birth weight, there were no differences between the groups. The cord blood haemoglobin concentrations were similar but the Zn levels appeared to be slightly lower in

the IUGR group. When the calculated blood volume for each placenta was deducted from the weight recorded at delivery, the blood-free placental tissue again weighed less in the IUGR group.

The total Zn in the placenta was lower in the IUGR group, although the Zn concentration of placental tissue did not differ from the other two groups. There was a significant relationship between the total placental Zn level and birth weight of the infant ($R\ 0.78$, $P < 0.001$). When the Zn contributed by blood was deducted, similar results were found in blood-free placental tissue, i.e. no differences in Zn concentration. The fact that total Zn was significantly lower in the IUGR group, probably relates to differences in placental weight.

DISCUSSION

The dependence of fetal growth on adequate Zn supply via the placental tissue is indisputable. However, the required level of Zn which can be deemed 'adequate' has not yet been agreed, mainly because there is no good means of assessing the Zn status of mother and neonate. Nevertheless, a number of studies have been carried out investigating the relationship between maternal Zn nutrition and infant birth weight.

Various estimates have been made of Zn intake during pregnancy. Hambidge *et al.* (1983) used a 24 h record technique at each month of gestation and found the mean intake to be about 11 mg Zn/d. This did not change significantly at any stage of pregnancy. Simmer *et al.* (1987) found the Zn intake during the last trimester of mothers who produced IUGR babies to be less than mothers with normal-weight babies, i.e. 11.3 and 13.0 mg/d respectively. This was assessed from a 7 d diet history. A more precise estimate of Zn intake is the weighed-intake method, as used by Tuttle *et al.* (1985) in pregnant women in Scotland during the 30th week of gestation. Mean Zn intake of healthy mothers was 9.1 mg/d and of mothers at risk of delivering a growth-retarded baby was 9.4 mg/d. The dietary information presented in the present study is limited but Zn intakes were not dissimilar to those found by Tuttle *et al.* (1985), and did not show large variations between the three groups.

The results from previous studies on the relationship between Zn and intra-uterine growth are controversial. The major problem with such work is the lack of a suitable means of monitoring Zn status during pregnancy. Plasma Zn is often used, and McMichael *et al.* (1982) showed no difference between mid-pregnancy maternal serum Zn in IUGR and normal groups, which were 0.77 and 0.73 $\mu\text{g/ml}$ respectively. Our findings also showed no difference. Mean plasma Zn values at 28 weeks were 0.84, 0.88, and 0.97 $\mu\text{g/ml}$ in normal, low BPD velocity and IUGR groups respectively.

Cord blood Zn was found to be slightly reduced ($P < 0.05$) in mothers giving birth to IUGR babies. There was, however, no correlation between birth weight and cord blood Zn when the values for all the mothers were examined. This agrees with the study of Bogden *et al.* (1978) in which maternal plasma and cord blood plasma Zn levels were measured in normal- and low-birth-weight groups. When critically examining values for maternal and cord blood, it is important to remember that maternal serum Zn levels may be the outcome, rather than a determinant, of fetal growth in pregnancy. The Zn content of cord blood represents only one point in time and is, therefore, not necessarily representative of the situation throughout the 40-week gestational period.

An alternative approach to investigate the relationship between Zn and fetal growth is to study the placenta. Blood-free placental tissue reflects the long-term accumulation of Zn in the body as opposed to instantaneous measurements when blood is assayed. Ward *et al.* (1987) measured placental levels of a large number of elements, including Zn, in an attempt to correlate the concentration of each with birth weight. Mothers with babies weighing

> 3000 g at birth had mean placental Zn concentrations of 52 $\mu\text{g/g}$ dry weight, compared with 40.6 $\mu\text{g/g}$ dry weight for those with babies weighing < 3000 g. The wet weight: dry weight ratios are not given, but assuming that the water content of whole placental tissue is 83% (Lentner, 1984), these values translate to 8.8 and 6.9 $\mu\text{g/g}$ wet weight respectively. The values for whole placental tissue in the present study (Table 3) were 7.7, 7.7, and 7.1 $\mu\text{g/g}$ wet weight in normal, low BPD velocity and IUGR groups respectively. Although lower, the values for the IUGR group were not significantly different from the controls. When the contribution made by blood is deducted, the concentrations rose to 10.3, 9.9 and 10.2 $\mu\text{g/g}$ blood-free placental tissue respectively. Again there were no significant differences between the groups. Thus, the fact that total placental Zn was lower in the IUGR group reflects the differing placental weights rather than any fundamental difference in Zn content. The results of the present study show that the Zn concentration of placental tissue is about four times that of whole cord blood. Although little is known about the transport of Zn in the placenta, one study with guinea-pigs suggests active uptake at the maternal surface combined with a slow release into the fetus, down a concentration gradient (Simmer *et al.* 1985). The fact that blood-free placental tissue is relatively rich in Zn, even in placentas from mothers giving birth to IUGR babies, indicates that there is a sizeable pool of Zn, but it is not known whether or not this Zn is available to the fetus.

The results of the present study would suggest that placental Zn concentrations are not related to decreased BPD velocity or IUGR in the fetus. However, an adequate daily supply of Zn to the fetus throughout the total gestational period is a crucial factor in determining fetal nutrition and the outcome of pregnancy. The observation that cord blood Zn concentrations in mothers giving birth to IUGR babies were lower than controls may be a significant finding that warrants further research. At present there is not enough information in the literature to judge whether or not the placental Zn concentrations fell into the 'normal' range. Despite the fact that mothers in the present study had fairly low estimated Zn intakes of 8–9 mg/d, the lack of correlation between placental Zn levels and birth weight would suggest that the dietary Zn was adequate. Apart from the small difference in cord blood Zn concentrations, the results of the present study provide no real justification for the routine administration of Zn supplements to pregnant women in order to improve fetal growth or pregnancy outcome.

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