The effect of combined dietary iron, calcium and folic acid supplementation on apparent ^{65}Zn absorption and zinc status in pregnant rats

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In the present study the effect of combined iron, calcium and folic acid supplementation of the diet on ^{65}Zn retention and zinc status was studied in the pregnant rat. Female Wistar rats were fed on a low- (8 µg/g) or high- (60 µg/g) Zn diet for 14 d and then mated overnight. After mating, half the rats were fed on the low- or high-Zn diet as before, whilst the other half were fed on similar diets supplemented with Fe, Ca and folic acid. The level of supplementation was chosen to reflect proportionately the possible increase in daily intakes of these nutrients by pregnant women. Rats which did not mate successfully were used as non-pregnant controls. On day 18 of pregnancy, each animal was given a meal of the appropriate diet labelled extrinsically with ^{65}Zn, and on day 20 rats were killed. Carcass ^{65}Zn retention was lower in pregnant and non-pregnant rats fed on the supplemented diets compared with those fed on the unsupplemented diets. Rats which consumed the supplemented diets throughout pregnancy had reduced plasma Zn concentrations but femur and fetal Zn concentrations were unaffected. Maternal femur Ca and fetal Fe concentrations were lower in the high-Zn groups compared with rats fed on low-Zn diets. It was concluded that the risk of inducing fetal Zn depletion as a consequence of Fe, Ca and folic acid supplementation during pregnancy appeared to be slight. However, significant differences in ^{65}Zn retention and maternal plasma Zn concentration in the supplemented groups, and reduced maternal bone Ca deposition and fetal Fe accretion in the high-Zn groups, indicated that it would seem wise to adopt a cautious approach to routine supplementation with individual minerals during pregnancy.

Dietary supplementation: Pregnancy: Zinc: Rat

High intakes of iron and folic acid have been reported to reduce zinc absorption from the diet, particularly if the level of Zn is marginal (Milne *et al.* 1984, Solomons, 1986). These findings have caused concern with regard to Fe–folate supplementation during pregnancy and possible effects on maternal and fetal Zn status, poor maternal Zn status having been demonstrated to cause congenital abnormalities in the offspring of laboratory animals (Hurley, 1969) and to be strongly associated with inter-uterine growth retardation in humans (Simmer & Thompson, 1985). It has also been shown that high intakes of calcium may reduce Zn absorption (Huber & Gershoff, 1970). The Department of Health and Social Security (1979) recommend that the daily intake of Ca should be increased from 500 to 1200 mg in the third trimester of pregnancy, which, together with routine Fe–folate supplementation, may have a deleterious effect on Zn absorption during this period of high Zn requirement.

There are a number of reports in the literature on the influence of Fe, folic acid or combined Fe–folate supplementation on Zn absorption and metabolism in rats and humans. Findings of human studies on the effect of folate supplementation alone are contradictory, one study showed significantly increased Zn excretion when physiological doses of folate were given orally (Milne *et al.* 1984), another study, however, found no effect on Zn status even after prolonged pharmacological folate therapy (Krebs *et al.* 1989).

Several studies in pregnant women, however, have demonstrated that Fe or Fe–folate supplementation results in a significant reduction in Zn absorption and maternal serum Zn status (Hambidge *et al.* 1983; Simmer *et al.* 1987; McKenzie-Parnell *et al.* 1988), but such differences are rarely associated with any apparent detrimental consequences for the outcome of pregnancy (McKenzie-Parnell *et al.* 1988). In human studies it is extremely difficult to examine the relation between maternal and fetal Zn status, and at present we have little knowledge of the consequences of reduced fetal Zn status for future development and well-being of the child. The effect of increased Ca intake, coupled with routine Fe–folate supplementation, on both maternal and fetal Zn status during pregnancy is also not known.

In the present study, [65]Zn retention and Zn status were determined in pregnant and non-pregnant rats fed on a diet supplemented with Fe, Ca and folic acid and compared with rats fed on an unsupplemented diet. The additional amounts of Fe, Ca and folic acid in the supplemented diet were calculated to be similar to the proportional increase in intake of women who increase their daily Ca intake from 500 to 1200 mg, and who routinely take a commonly prescribed Fe–folate supplement throughout pregnancy. In addition, since the level of dietary Zn may be important, the effect of supplementation on [65]Zn retention in rats fed on a diet containing two-thirds or five times the dietary concentration of 12 mg Zn/kg recommended by the American Institute of Nutrition *ad hoc* Committee on Standards for Nutritional Studies (AIN) (1977) was investigated.

MATERIALS AND METHODS
Animals and diets

Virgin, female, Wistar rats weighing approximately 230 g were randomly allocated to one of two groups and caged individually in polypropylene cages with stainless-steel grids. Rats were fed on a semi-synthetic diet *ad lib.* containing (/kg diet; analysed values) 37·7 mg Fe, 5·8 g Ca, 2·8 mg pteroylmonoglutamic acid (folic acid) and 60 mg Zn (high-Zn diet), or a similar diet containing only 8 mg Zn/kg (low-Zn diet), for 14 d. The general composition of the diet was as described previously (Fairweather-Tait & Wright, 1984) with the addition of 2·5 g methionine/kg diet. On day 15, rats were mated and on the following day the presence of a mating plug in the cage was assumed to indicate a successful mating (designated day 0 of pregnancy). Half the pregnant rats in each group were fed on the high- or low-Zn diet as before (designated the unsupplemented high- and low-Zn pregnant groups). The remaining rats were fed on a similar diet with additional Fe, Ca and folic acid to give dietary concentrations of (/kg; analysed values) 308 mg Fe, 11·6 g Ca, 6 mg folic acid (designated the supplemented high- and low-Zn pregnant groups).

The Fe, Ca and folic acid concentrations in the supplemented diet were increased to concentrations that were approximately 10, 1·2 and 2 times respectively, the AIN (1977) recommended levels for laboratory rats. These additional amounts were chosen with reference to the possible increase in daily intakes of these nutrients by pregnant women taking routine Fe–folate supplements such as Pregaday® (100 mg Fe, 360 μg folic acid per tablet; recommended daily allowance (RDA) for non-pregnant adult women 12 mg Fe (Department of Health and Social Security, 1979) and 400 μg folic acid ((US) National Research Council, 1980), and additional dietary Ca to provide 1200 mg/d (RDA for non-pregnant adult women, 500 mg (Department of Health and Social Security, 1979)). It was recognized that the recommendations for rats were designed to meet known requirements not only for growth but also for pregnancy and lactation, and are based on dietary concentrations rather than daily intakes. They are, therefore, not strictly analogous to the recommended amounts for non-pregnant adult women. However, these values provided a

useful baseline to ensure that the levels of supplementation used in the present study were not excessive.

Rats which did not mate successfully were assigned to the non-pregnant groups and fed on similar diets to the pregnant animals. It was found at the end of the experiment that several rats allocated to the pregnant groups were not in fact pregnant; the final number of animals in each group was nine for each pregnant group and nineteen to twenty-one for the non-pregnant groups. The exact number of observations is given in the tables of results.

Food intake and body-weight gain were recorded throughout the experiment.

Experimental

On day 17 of pregnancy all rats were fasted overnight and on the following morning (day 18 of pregnancy) each animal was given 5 g of the semi-synthetic diet they had been consuming during the preceding 17 d. Each meal contained 37 kBq ^{65}Zn (ZnCl2, 3·7–92·5 MBq/mg Zn; Amersham International plc, Amersham, Bucks) which was thoroughly mixed into the diet in a small volume of distilled water. Immediately after consuming the meal, each rat was counted in a NE8112 small-animal whole-body counter (NE Technology Ltd, Beenham, Berks) and, not less than 4 h after this initial count, they were offered the appropriate diet *ad lib.*

The rats were killed 48 h later (day 20 of pregnancy) with a lethal dose of sodium pentabarbitone (Euthatal; May & Baker, Dagenham, Essex) administered intra-peritoneally. The time-period of 48 h was considered to be long enough to allow transit of the radiolabelled meal through the small intestine and complete transfer of ^{65}Zn from the mucosa into the carcass, but short enough to minimize possible differences between groups due to losses of absorbed ^{65}Zn. Blood was removed by cardiac puncture for haemoglobin (Hb) estimation measured as haemoglobincyanide (Van Kampen & Zilstra, 1961) and determination of plasma Zn concentration. The stomach and the whole of the intestine were removed and the carcass plus blood counted in the whole-body counter. The right femur and liver were removed, dried, ground and analysed for Zn and Ca, and Zn and Fe respectively. Fetuses were counted, weighed, dried, ground to an homogenous powder and samples taken for Zn, Fe and Ca analysis.

Dried tissue samples were dry ashed at 480°, dissolved in warm, concentrated hydrochloric acid (11·5 M-HCl), diluted with distilled water, filtered, and mineral analysis performed using a Pye Unicam PU 9000 atomic absorption spectrometer (Pye Unicam, Cambridge). Plasma was deproteinized with trichloroacetic acid (50 g/l), centrifuged (600 *g*, 10 min) and the supernatant fraction diluted with distilled water before Zn analysis by atomic absorption spectroscopy.

Percentage ^{65}Zn retention (apparent absorption), 48 h after consuming the radiolabelled test meal, was calculated from the difference between the initial whole-body count and that obtained from carcass minus intestine. Corrections were made for counting efficiency, background and isotope decay.

Statistical analysis

Results were subjected to two-way or three-way analysis of variance. All data were $\log_{10}$ transformed because of unequal variances. Geometric means (GM) are tabulated for each measurement, with significant differences ($P < 0.05$) for pregnancy, diet and supplementation and any statistically significant pregnancy × diet, pregnancy × supplementation, diet × supplementation and pregnancy × diet × supplementation interactions. The residual mean square (RMS) and residual degrees of freedom (residual df) for $\log_{10}$ GM are also tabulated to allow calculation of the standard error (SEM) and standard error of differences (SED) of the $\log_{10}$ GM. SEM for $\log_{10}$ GM $= \sqrt{(RMS/n)}$; SED $= \sqrt{(RMS((1/n1)+(1/n2)))}$; $t = (\log_{10} GM1 - \log_{10} GM2)/SED$ with residual df.

Table 1. *Body-weight, food intake, iron, calcium and zinc status, and whole-body* ^{65}*Zn retention for pregnant (P) and non-pregnant (NP) rats fed on a supplemented (+S) or unsupplemented (−S) diet at two concentrations of Zn (8 or 60 mg/kg)* from days 0 to 20 of pregnancy*

(Values are geometric means for *n* replicates)

	P... NP...	High-Zn diet −S	High-Zn diet +S	Low-Zn diet −S	Low-Zn diet +S	Pregnancy	Diet	Supplement	Pregnancy × diet	Pregnancy × supplement	RMS (10^{-3})	Residual df
Number of replicates (*n*):	P / NP	9 / 20	9 / 19	9 / 19	9 / 21							
Body-wt‡ (g)	P	428	414	404	408	<0·001					1·33	107
	NP	304	300	306	301							
Food intake‡ (g)	P	431	434	409	414	<0·001					2·12	107
	NP	359	351	351	351							
Hb (g/l)	P	112	113	118	113	<0·001					0·47	107
	NP	145	146	147	147							
Plasma Zn (µg/ml)	P	0·95	0·85	0·39	0·31	<0·001	<0·001	<0·05	<0·001		6·04	106
	NP	1·36	1·36	1·22	1·44							
Liver:												
Dry wt (g)	P	5·61	5·32	5·08	4·77	<0·001		<0·01	<0·05		0·31	106
	NP	4·12	3·98	4·27	3·84							
Fe (mg)	P	1·61	2·60	1·67	2·58	<0·001		<0·001		<0·001	10·76	107
	NP	2·57	3·13	2·83	2·82							
Zn (µg)	P	419	421	389	373	<0·001	<0·05				2·53	107
	NP	313	309	308	292							
Femur:												
Zn (µg/g)	P	231	228	194	207	<0·001	<0·001				0·97	107
	NP	241	238	216	210							
Ca (mg/g)	P	173	195	211	217				<0·001		3·05	104
	NP	199	201	201	190							
Whole-body ^{65}Zn retention§ (% dose)	P	17·2	13·8	68·8	53·7	<0·01	<0·001	<0·001	<0·001		7·91	107
	NP	17·8	14·4	51·0	42·3							

RMS, residual mean square of $\log_{10}$ geometric means.

* For details of composition of diets and dietary treatment, see p. 416.

† *P* values (<0·05) obtained from three-way analysis of variance.

‡ Body-weight on day 20 of pregnancy; total food intake from days 0 to 20 of pregnancy.

§ ^{65}Zn retention 48 h after dose given on day 18 of pregnancy, see p. 417.

Table 2. *Litter sizes and iron, calcium and zinc concentrations of pooled fetuses from rats fed on a supplemented (+ S) or unsupplemented (− S) diet at two concentrations of Zn (8 or 60 mg/kg)* from days 0 to 20 of pregnancy*

(Values are geometric means for nine replicates)

| | High-Zn diet | | Low-Zn diet | | Statistical significance of variance ratio (F)†, effects of: | | | |
	−S	+S	−S	+S	Diet	Supplement	RMS (10^{-3})	Residual df
No. of fetus	15·5	14·8	11·9	13·7			18·22	32
Litter dry wt (g)	6·97	6·65	5·52	6·34			15·43	32
Fe (μg/g)	351	403	397	421	< 0·05	< 0·01	1·73	32
Ca (mg/g)	19·2	18·7	21·7	19·2			7·76	32
Zn (μg/g)	126	117	102	99	< 0·001		1·08	32

RMS, residual mean square of $\log_{10}$ geometric means.
* For details of composition of diets and dietary treatments, see p. 416.
† P values (< 0·05) obtained from two-way analysis of variance.

RESULTS

Body-weight gain, food intake, number of fetuses and total fetal dry weight on the 20th day of gestation were unaffected by dietary Zn concentration or supplementation of the diet with additional amounts of Fe, Ca and folic acid (Tables 1 and 2).

^{65}Zn retention

Carcass ^{65}Zn retention, 48 h after consuming the test meal, was significantly lower in all groups fed on the supplemented diet compared with the unsupplemented animals (Table 1). Retention was markedly higher in rats fed on the low-Zn diet compared with rats fed on the high-Zn diet, with the low-Zn pregnant rats having a significantly greater ^{65}Zn retention than their non-pregnant counterparts (Table 1). This difference was not observed in high-Zn groups where carcass retention of the radiolabel was similar in pregnant and non-pregnant animals.

Maternal Zn, Fe and Ca status

Femur Zn and Ca concentrations, plasma Zn concentration, total liver Fe and Zn, and whole blood Hb concentration were used to assess maternal status.

There were significant effects of pregnancy, diet and supplementation on plasma Zn levels (Table 1). Pregnant animals had a lower plasma Zn concentration than non-pregnant rats, and rats fed on the low-Zn diets had a reduced plasma Zn level compared with the high-Zn groups. All pregnant, and low-Zn non-pregnant, rats fed on the supplemented diet had depressed plasma Zn concentrations compared with the unsupplemented controls.

Femur Zn concentration was reduced in pregnant compared with non-pregnant rats and in low-Zn groups compared with their high-Zn counterparts. There was no effect of supplementation on bone Zn levels (Table 1).

Total liver Zn increased with pregnancy and decreased with the consumption of a low-Zn diet. This was largely due to changes in liver weight. There was no effect of supplementation (Table 1).

Femur Ca concentration was unaffected by pregnancy or supplementation. There was, however, a significant pregnancy × diet interaction resulting from the higher femur Ca

concentrations in the pregnant low-Zn rats compared with pregnant high-Zn animals (Table 1).

Total liver Fe was decreased by pregnancy and increased with Fe supplementation in all but the low-Zn non-pregnant group (Table 1). Differences in total liver dry weight were also observed. Pregnant rats had characteristically larger livers than non-pregnant animals, although a significant pregnancy × diet interaction indicated that this increase was less marked in animals fed on the low-Zn diet (Table 1). In addition, it was found that both pregnant and non-pregnant animals consuming the supplemented diets had reduced liver weights compared with unsupplemented groups (Table 1).

There was no effect of supplementation or dietary Zn concentration on Hb concentration but, as expected, pregnancy resulted in a significant fall in Hb level (Table 1).

Fetal Zn, Fe and Ca status

Fetuses from rats fed on the low-Zn diets had significantly lower Zn concentrations than those from rats fed on the higher-Zn diets (Table 2). There was an indication that supplementation may have influenced fetal Zn accretion since mean values for fetal Zn concentration were lower for the supplemented groups, although this did not quite reach statistical significance ($P = 0.06$).

Fetal Ca concentration was unaffected by supplementation or dietary Zn concentration (Table 2). However, the Fe concentration in fetuses from supplemented rats was significantly increased. Fe concentration was also significantly higher in fetuses from both supplemented and unsupplemented rats fed on the low-Zn diet compared with those fed on the higher level of Zn (Table 2).

Maternal and fetal folate status was not determined in the present investigation.

DISCUSSION

Adequate maternal Zn nutrition during pregnancy is essential for normal embryonic and fetal development (Simmer & Thompson, 1985). It is, therefore, important to identify both dietary and physiological factors which may reduce Zn absorption during this period of high requirement. In the present investigation the effect of combined Fe, folic acid and Ca supplementation of the diet on ^{65}Zn retention was studied in late pregnancy in the rat, when the fetal demand for Zn is high. In view of the ability of animals to adapt to reduced Zn absorption via decreased whole-body Zn turnover (Fairweather-Tait et al. 1985) and the likelihood that the response to supplementation may be dependent on dietary Zn concentration, the influence of supplementation throughout pregnancy on maternal and fetal Zn status at two levels of dietary Zn was also investigated.

The retention of ^{65}Zn was markedly higher in all animals fed on the low-Zn diet, as previously shown (Fairweather-Tait et al. 1985). Pregnancy resulted in increased retention only in those groups fed on the low-Zn diet, pregnant rats given the high-Zn diet having similar values to their non-pregnant counterparts. Thus, it appears that in the pregnant rat the efficiency of Zn absorption is elevated only when dietary Zn is limited. This contrasts with the finding of Kirchgessner et al. (1982) who found a substantially higher apparent Zn absorption towards term in sows fed on both high and low levels of Zn.

The present study demonstrated that both pregnant and non-pregnant rats fed on a meal of a semi-synthetic diet supplemented with Fe (273 μg/g), Ca (5·8 mg/g) and folic acid (3·2 μg/g), extrinsically labelled with ^{65}Zn, had a significantly lower ^{65}Zn retention than rats given an unsupplemented meal. The additional amounts of Fe, Ca and folic acid were chosen to reflect proportionately the increase in daily intakes of these nutrients by pregnant women, as discussed previously (see p. 416). The retention of ^{65}Zn was reduced by approximately 20 % in all groups given the supplemented meal, irrespective of dietary Zn

concentration or pregnancy state. However, the effect of long-term consumption of the diets on the Zn status of the animals was more complex.

Pregnancy and reduced dietary Zn intake significantly decreased plasma and femur Zn concentrations. High- and low-Zn groups of pregnant rats given the unsupplemented diet had a 30 and 68 % lower plasma Zn concentration respectively than non-pregnant counterparts. The reduction in plasma Zn values in pregnant animals given the higher level of dietary Zn is a normal response to pregnancy, probably arising from increased blood volume, decreased serum albumin and redistribution to the fetus and placenta (Hambidge et al. 1983). An additional decrease in plasma Zn was observed in both groups of rats fed on the supplemented diet throughout pregnancy. The lower ^{65}Zn retention from the supplemented semi-synthetic diet given on day 18 of pregnancy was, therefore, reflected in lower maternal plasma Zn concentrations. However, femur Zn concentration was unaffected by supplementation, which supports the argument that the Zn associated with bone cannot be readily mobilized to buffer metabolically active pools (Fairweather-Tait et al. 1985). The observed reduction in femur Zn concentration in pregnant v. non-pregnant rats and high-Zn v. low-Zn rats was probably the result of reduced transfer of Zn to the bone rather than increased mobilization. Non-pregnant supplemented and unsupplemented groups fed on the higher level of dietary Zn had similar plasma Zn concentrations. However, a significant reduction in plasma Zn was observed in the non-pregnant supplemented animals when dietary Zn was limited, confirming that a proportion of the Zn in the supplemented diet was unavailable for absorption. It appears, therefore, that whilst non-pregnant animals were at risk of reduced Zn status only when the supplement was consumed with a low-Zn diet, pregnant rats were susceptible even when the dietary Zn concentration was well in excess of the AIN (1977) recommendation.

It has been suggested that the pregnant rat can mobilize endogenous Zn from metabolically active pools and transfer it to the fetus, thus protecting the fetus from maternal Zn depletion (Masters et al. 1983, 1986). In the present study any such redistribution was insufficient to prevent the reduction in fetal Zn concentration in the low-Zn group of rats and, although there was no significant effect of supplementation on fetal Zn levels, mean values were lower for both the high- and low-Zn supplemented groups ($P = 0.06$) suggesting a marginal effect. It would appear, therefore, that the size of the mobilizable pool of Zn is extremely small. Reductions in fetal Zn concentration were not associated with a decrease in litter size or fetal weight. However, the relation between fetal Zn status and future growth and development requires consideration.

There was no effect of Ca supplementation on femur Ca concentration in either pregnant or non-pregnant rats, or on fetal Ca accretion. A significant pregnancy × diet interaction indicated that the deposition of bone Ca was less in the pregnant rats fed on the higher level of Zn than in those fed on the low-Zn diet, suggesting that an antagonistic effect of Zn on Ca utilization may be detectable in pregnancy at a lower dietary Zn concentration than that required to produce a similar response in non-pregnant animals (Magee & Chang Fu, 1979).

The additional dietary Fe had no effect on maternal haemoglobin concentration, and a similar fall in Hb concentration was observed in both supplemented and unsupplemented pregnant animals, showing that the apparent decline in Hb level was not influenced by a substantial increase in Fe intake. Liver Fe stores, however, were generally increased by supplementation, but remained slightly lower in pregnant compared with non-pregnant rats. Fetal Fe accretion was increased by dietary Fe supplementation. There was also a significantly greater Fe concentration in fetuses from both supplemented and un-supplemented low-Zn rats compared with their high-Zn counterparts indicating that, as wtih Ca, higher levels of Zn intake may reduce Fe utilization in pregnancy.

Despite the additional deposition of Fe in the liver of supplemented animals, total liver

weight was significantly decreased compared with unsupplemented groups. The reason for this is unclear, but it was noted that the reduction in liver weight was greatest in the low-Zn supplemented groups and that there was a significant pregnancy × diet interaction, indicating that liver weight in pregnant rats was influenced by low dietary Zn per se, as shown previously (Fairweather-Tait et al. 1985). In addition, the smallest decrease in liver weight was observed in the supplemented, high-Zn non-pregnant animals, which was the only group to show no decline in plasma Zn concentration compared with unsupplemented controls. Reductions in liver weight, however, were not consistently associated with a lower liver Zn content or concentration. The metabolic changes resulting in these observed differences require further investigation.

Many reports have suggested a deleterious effect of Fe or folic acid supplementation, or both, on Zn absorption in human subjects, when administered together in solution (Solomons, 1986). In the presence of food, however, the inhibitory effect of Fe is absent or very much reduced in both animals (Fairweather-Tait & Southon, 1989) and man (Sandstrom et al. 1987) even at high Fe:Zn molar ratios. The situation with regard to Ca is equally confusing. O'Dell (1969) and Forbes (1964) concluded from rat studies that an effect of dietary Ca in reducing Zn absorption does not exist, or is minimal, unless the diet contains phytic acid, whilst Heth & Hoekstra (1965) and Huber & Gershoff (1970) found an effect of Ca in the absence of phytate, although this appeared to be influenced by the dietary concentration of phosphorus and Zn. Generally, in the adult rat, a direct Ca antagonism of Zn absorption from semi-synthetic diets is difficult to demonstrate unless the diet is marginal with respect to Zn. The present study, however, demonstrated that supplementation of a semi-synthetic diet with Fe, Ca and folic acid resulted in reduced radiolabelled Zn retention, accompanied by a significant fall in maternal plasma Zn concentration, even when the dietary Zn concentration was five times higher than that recommended for growth, pregnancy and lactation in the rat. Thus, although individual components of the supplement may not have been expected to influence the Zn status of rats fed on more than adequate amounts of Zn, there was clearly an effect when they were administered in combination. In view of a recent report by Krebs et al. (1989), showing that subjects with Fragile X syndrome who received 10–20 mg folic acid/d for at least 1 year had apparently normal Zn status, it seems probable that the additional amounts of Fe and Ca, rather than folic acid, were responsible for the decrease in Zn utilization observed in the present investigation.

It is very unlikely that a pregnant woman would commence routine Fe supplementation, or rapidly increase her Ca intake, from the time of conception. Pregnancy would not normally be confirmed until approximately the 12th week of gestation, Fe supplements may not be taken on a daily basis and additional dietary Ca intake is recommended only in the third trimester (Department of Health and Social Security, 1979). The experimental model used in the present study, therefore, represents an extreme situation. Furthermore, the results of the present study indicate that, despite significant reductions in radiolabelled Zn retention, differences in maternal Zn status due to supplementation were small and the fetus was largely protected. It may be argued, therefore, that the risk of inducing either maternal or fetal Zn depletion as a consequence of increased intakes of these minerals is very slight, unless dietary Zn intake, both before and during pregnancy, is consistently low. Nevertheless, in view of the essential role of Zn in cellular metabolism, and hence fetal development, and the uncertainty about the consequences of marginal Zn depletion, it would seem wise to adopt a cautious approach to excessive Fe and Ca supplementation during pregnancy unless there are clear indications that such supplementation is necessary. In addition, since the present findings suggest that high levels of Zn intake reduced maternal bone Ca deposition and fetal Fe accretion, it would also seem inappropriate to consider offsetting any potential deleterious effect of increased Fe and Ca intakes by the

administration of routine Zn supplements. The results of the present study are an illustration of the complexity of interactions that may occur between Zn, Fe and Ca, as modified by pregnancy, and illustrate the importance of moderation with respect to dietary supplementation with individual mineral elements.

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