ORIGINAL ARTICLE

Intrauterine programming of urinary calcium and magnesium excretion in children born to mothers with insulin dependent diabetes mellitus

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Background: Offspring of diabetic rats have reduced urinary calcium and magnesium excretion compared with offspring of controls; these differences persist up to16 weeks after birth, a time equivalent to young adulthood in humans.

Objectives: To test the hypothesis that urinary calcium and magnesium excretion would be lower in children born to mothers with insulin dependent diabetes mellitus (ChMIDDM) than those born to non-diabetic mothers.

Methods: Concentrations of calcium, magnesium, sodium, and creatinine were measured in first void spot urine samples collected from 45 (28 male; median age 9.6 years) ChMIDDM and 127 (58 male; median age 11.3 years) controls. Analysis of covariance was used to test for differences in urinary calcium to creatinine ratios (UCa/Cr), magnesium to creatinine ratios (UMg/Cr), and log sodium to creatinine ratios (logUNa/Cr) between controls and ChMIDDM after allowing for the effects of sex and age.

Results: UCa/Cr (difference −0.10, 95% confidence interval (CI) −0.19 to −0.01; p = 0.03) and UMg/Cr (difference −0.15, 95% CI −0.22 to −0.08; p<0.0001) were lower in ChMIDDM than controls. However, logUNa/Cr did not differ between ChMIDDM and controls (difference −0.14, 95% CI −0.33 to 0.05; p = 0.1). The daily estimated intake of magnesium, sodium, and protein were significantly higher and that of calcium non-significantly higher in ChMIDDM than controls. In ChMIDDM, UCa/Cr and UMg/Cr were not related to diabetic control of mothers.

Conclusions: Results of this study provide the first evidence that in humans, as in rats, there is modification of renal Ca and Mg handling in ChMIDDM, which persists well into childhood.

Perturbations of bone mineral metabolism have been reported in infants born to mothers with insulin dependent diabetes mellitus (IDDM). Such infants have a higher risk of developing neonatal hypocalcaemia and hypomagnesaemia,[1] especially when the maternal glycaemic control is poor during pregnancy.[2] Mimouni *et al*[3] reported a 10% reduction in the radial bone mineral content, measured by single photon absorptiometry, in infants born to mothers with IDDM compared with controls. However, the whole body bone mineral content measured by dual *x* ray absorptiometry was not found to be impaired in infants born to mothers with IDDM.[4] We have previously shown that the maternofetal transport of calcium and magnesium across in situ perfused rat placentas of streptozotocin induced diabetic rats was significantly lower than in control animals.[5] These alterations in placental transport of bone mineral ions were prevented by control of the maternal diabetes with insulin.[5] More recently we have shown that offspring of diabetic rats have reduced urinary calcium and magnesium excretion compared with offspring of controls, and these differences persisted up to16 weeks after birth, a time equivalent to young adulthood in humans.[6 7] These results suggest that renal tubular reabsorption of calcium and magnesium may be programmed in utero, probably in response to impaired placental transport of these ions. The precise mechanisms responsible for in utero programming of these bone mineral ions are not clear. However, in the offspring of diabetic rats, there is evidence for upregulation of transport proteins (calbindin-D_{28K} and plasma membrane Ca^{2+}-ATPase) that are responsible for reabsorption of filtered calcium from the distal renal tubule.[8]

There have been no previous studies in humans of urinary calcium and magnesium excretion in children born to mothers with insulin dependent diabetes mellitus (ChMIDDM). Therefore, in this cross sectional study, we investigated urinary excretion of calcium, magnesium, and sodium in ChMIDDM and in controls who were born to non-diabetic mothers. We hypothesised that urinary calcium and magnesium excretion would be lower in ChMIDDM than those born to non-diabetic mothers.We also studied urinary sodium excretion to determine whether any observed changes were restricted to bivalent bone mineral ions. The effect of dietary intake of calcium, magnesium, and other nutrients known to influence urinary excretion of calcium, such as sodium[9] and protein,[10] was also studied.

METHODS

The study was approved by Central Manchester local research ethics committee, and written consent was obtained from parents and from older subjects. Sixty seven (35 male) 5–18 year old white children born to mothers with insulin dependent diabetes mellitus (ChMIDDM) agreed to take part in the study. These children were born to mothers whose IDDM was managed at our tertiary perinatal centre by a dedicated team comprising an obstetrician, a physician, a dietician, and diabetes specialist midwives. Pregnant women

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Abbreviations: ChMIDDM, children born to mothers with insulin dependent diabetes mellitus; Cr, creatinine; HbA_{1c}, haemoglobin A_{1c}; IDDM, insulin dependent diabetes mellitus; SDS, standard deviation scores; UCa/Cr, urinary calcium to creatinine ratio; UMg/Cr, urinary magnesium to creatinine ratio; UNa/Cr, urinary sodium to creatinine ratio

Table 1 Age, height standard deviation scores (SDS), weight SDS, and body mass index (BMI) SDS for 45 children born to mothers with insulin dependent diabetes mellitus (ChMIDDM) and 127 controls

	Controls		ChMIDDM		
	Mean	SD	Mean	SD	p Value
Age (years)	11.7	3.8	10.1	3.3	0.008
Weight SDS	0.37	0.91	0.77	1.11	0.03
Height SDS	0.19	0.84	0.67	0.94	0.004
BMI SDS	0.37	1.00	0.57	1.24	0.3

The mean values in the two groups were compared using a *t* test.

with IDDM were treated using standardised protocols with set series of monitoring investigations carried out at regular intervals. The haemoglobin A_{1c} (HbA_{1c}) assay changed four times during the period when ChMIDDM were born; it was not possible to pool the results from the assays, for example, after expressing them as standard deviation scores (SDS). The subjects were classified into two categories: poor control, at least one HbA_{1c} value above the upper limit for the assay reference range; good control, HbA_{1c} concentrations within the assay reference range. The gestational age at delivery and birth weight of infants born to mothers with IDDM were obtained from case notes, and transformed into weight SDS for gestation using the reference neonatal growth data (TJ Cole; software from the Child Growth Foundation, London W4 1PW, UK). Macrosomia in infants born to mothers with IDDM is thought to result from fetal hyperinsulinism secondary to maternal and fetal hyperglycaemia resulting from poor glycaemic control during pregnancy.[11 12] We therefore used the infant birth weight SDS as a proxy measure for the glycaemic control during pregnancy. We also divided the ChMIDDM into four groups (B to E) according to their mother's classification as described by White,[13 14] which is based on the age of onset of diabetes, its duration, and presence of vascular or other complications of the disease. Children born to mothers with gestational diabetes, type 2 diabetes mellitus, and those with congenital malformation were excluded from the study. A group of 127 (58 male) healthy white children with a similar age distribution, recruited for a study of bone mass acquisition during childhood, served as controls.

All foods eaten by the subjects during a three day period (two weekdays and one weekend day) were recorded in food diaries. The daily intake of Mg, Ca, Na, and protein were estimated using the CompEat version 5 for Windows Nutritional Software (CompEat Nutrition Systems, Closterworth, Grantham, UK), which incorporates the 6th edition of McCance and Widdowson's tables of food values. These daily intakes were expressed as ratios to body weight. In ChMIDDM the dietary assessment was about 12 months before collection of urine samples. In the control group, the dietary assessment was within one week of the urine collections. Owing to unexpected problems with mineral assays on urine samples in the original cohort of 67 ChMIDDM that were collected at the time when dietary assessments were undertaken, 45 (28 male) children from this group provided a second urine sample about 12 months after the dietary assessments, and it is this sample that is used in the analysis presented here. Urine samples from control subjects were collected throughout the year, whereas in ChMIDDM they were collected between December and February. In children, accurate 24 hour urine collections can be difficult to obtain, and therefore molar ratios of urinary Ca to creatinine (UCa/Cr), Mg to creatinine (UMg/Cr), and Na to creatinine (UNa/Cr) in the first morning urine sample were used in the analysis, as these variables correlate well with 24 hour urinary excretion of these minerals.[15] After collection, urine samples were stored in a −40℃ freezer before analysis. A 2 ml sample of thawed urine was acidified with 30 µl 5 M HCl, and mixed and pH adjusted to a value of 1–2. Samples were centrifuged, and the supernatant analysed for Ca, Mg, P, and Cr using the Hitachi 917 autoanalyser (Hitachi, Tokyo, Japan). Urinary Na concentrations were analysed using the same autoanalyser but without prior acidification.

Statistical analyses were performed using *R* version 1.7.[16] The data are summarised as means (SD), and simple comparisons were made using *t* tests. UCa/Cr and UMg/Cr are known to vary with age,[17] and there is evidence for sex differences in UCa/Cr during puberty.[18] Thus an analysis of covariance was used to test for differences in UCa/Cr, UMg/Cr, and UNa/Cr between controls and ChMIDDM, after allowing for the effects of sex and age (using a cubic spline representation with 4 degrees of freedom) and including interaction terms—that is, a separate spline fit to age for each sex combination. Visual inspection of the fitted curves showed that the smoothed fits to age were adequate, and the main conclusions were confirmed using splines with more[6] and fewer[3] degrees of freedom. Tests for interactions between age, sex, and subject group were conducted to confirm that there was no significant heterogeneity in the response between sexes or with age, although these tests have limited statistical power. Similarly, we considered more

Table 2 Estimated daily intake (mg) of calcium, magnesium, sodium, and protein for 43 children born to mothers with insulin dependent diabetes mellitus (ChMIDDM) and 98 controls

	Controls	ChMIDDM	Difference (95% CI)	p Value
Calcium	884 (454)	967 (403)	118 (−46 to 282)	0.2
Magnesium	233 (72)	259 (65)	28 (2 to 55)	0.04
Sodium	2580 (720)	2940 (840)	410 (140 to 690)	0.004
Protein	64 (19)	76 (21)	14 (6 to 21)	<0.001

The data are presented as mean (SD). The mean differences between the two groups and their associated confidence intervals (CI) and significance levels are shown, after adjustment for weight and sex.

Table 3 Differences in urinary calcium to creatinine ratio (UCa/Cr), UMg/Cr, and UNa/Cr between children born to mothers with insulin dependent diabetes mellitus (ChMIDDM) and controls, with and without adjustment for sex and age

		Effect size (95% CI)	p Value
UCa/Cr	Unadjusted	−0.06 (−0.15 to 0.04)	0.2
	Adjusted	−0.10 (−0.19 to −0.01)	0.03
UMg/Cr	Unadjusted	−0.12 (−0.20 to −0.031)	0.007
	Adjusted	−0.15 (−0.22 to −0.08)	<0.0001
Log(UNa/Cr)	Unadjusted	−0.11 (−0.30 to 0.09)	0.3
	Adjusted	−0.14 (−0.33 to 0.05)	0.1
UCr (mmol/l)	Unadjusted	0.61 (−1.33 to 2.55)	0.5
	Adjusted	0.97 (−0.90 to 2.84)	0.3

Log(UNa/Cr) is presented, as the data were skewed. Age and sex adjustment was performed using the cubic spline model, with a sex specific age effect.

complex models, explicitly including pubertal stage or height and weight, and the results of these largely confirmed the results of the simpler models and so have not been included here. Effect sizes are presented as estimated differences with and without adjustment for age and sex, along with the associated 95% confidence intervals. The UNa/Cr values displayed a skewed distribution and thus were analysed after a log transformation. Dietary data were similarly compared using analysis of covariance adjusted for weight as a spline fit and sex. In ChMIDDM, we explored the correlations between birth weight SDS and UCa/Cr, UMg/Cr, and UNa/Cr, using Pearson correlations. Within this group, we also compared the median values for each of these variables between the four White's groups using one way analysis of variance and Pearson correlations (the latter as a test for trend). Those with poor or good diabetic control were compared using a *t* test and its associated estimate of the difference between groups and 95% confidence interval. In these comparisons, we used the fits to the full dataset to adjust the values of the urine variables to a common age (median age 11.1) and sex (female). $p<0.05$ was considered to be significant.

RESULTS

Table 1 shows age, anthropometric variables, and dietary intakes of Ca, Mg, Na, and protein of the subjects in the two groups. Although ChMIDDM were younger than controls, their median weight SDS and height SDS were higher than that of the controls. The body mass index SDS did not differ in the two groups. The mean daily dietary intakes of Ca, Mg, Na, and protein were all higher in ChMIDDM than the controls (table 2). After adjustment for weight and sex, the dietary Na (mean difference 410 mg/day), Mg (28 mg/day), and protein (14 mg/day) intakes were significantly higher in ChMIDDM than controls; Ca intake was also higher (118 mg/day) but not significantly so. UCa/Cr and UMg/Cr were lower in ChMIDDM than controls after adjustment for age and sex (table 3). However, as shown in table 3, log(UNa/Cr) did not differ between ChMIDDM and controls.

As shown in table 4, in ChMIDDM, the median UCa/Cr, UMg/Cr, and log(UNa/Cr) values after adjustment for age and sex did not differ according to glycaemic control during the three trimesters categorised as "poor control" or "good control". The birthweight SDS of ChMIDDM were not related to UCa/Cr ($r = -0.03$, $p = 0.9$), UMg/Cr ($r = 0.05$, $p = 0.7$), and UNa/Cr ($r = -0.04$, $p = 0.8$). As shown in table 5, the median UCa/Cr, UMg/Cr, and UNa/Cr values, again with adjustment for age and sex, did not differ significantly between the four White's groups.

DISCUSSION

We found that urinary excretion of Ca and Mg was significantly lower in ChMIDDM than in controls born to non-diabetic mothers. In contrast, the urinary excretion of Na, although lower in the ChMIDDM group, was not significantly different between the two groups, indicating that the observed changes in renal handling were predominantly restricted to the two bivalent bone mineral ions studied. This effect was not explained by differences in diet, as daily intake of Ca, Mg, and Na per kg body weight was higher in ChMIDDM than in controls. These findings are similar to those observed in offspring of streptozotocin induced diabetic rats, who excreted significantly decreased urinary Ca and Mg than offspring of controls, an effect that persisted up to16 weeks after birth, a time equivalent to young adulthood in humans.[6, 7] The opposite is seen in insulin

Table 4 Comparison of urinary calcium to creatinine ratio (UCa/Cr), UMg/Cr, and UNa/Cr in children born to mothers with insulin dependent diabetes mellitus with good control and those with poor control during the three trimesters of pregnancy, with adjustment for age and sex

	Good control		Poor control			
	N	Mean (SD)	N	Mean (SD)	Difference (95% CI)	p Value
1st trimester	28		11			
UCa/Cr		0.30 (0.25)		0.39 (0.19)	−0.09 (−0.24 to 0.07)	0.3
UMg/Cr		0.50 (0.18)		0.55 (0.13)	−0.05 (−0.16 to 0.05)	0.3
UNa/Cr		14.6 (9.7)		12.2 (4.0)	2.4 (−2.1 to 6.8)	0.3
2nd trimester	16		22			
UCa/Cr		0.29 (0.21)		0.34 (0.26)	−0.03 (−0.20 to 0.11)	0.6
UMg/Cr		0.54 (0.14)		0.50 (0.19)	0.04 (−0.07 to 0.15)	0.4
UNa/Cr		16.8 (11.8)		13.1 (6.0)	3.7 (−3.0 to 10.4)	0.3
3rd trimester	13		25			
UCa/Cr		0.30 (0.31)		0.32 (0.20)	−0.02 (−0.22 to 0.18)	0.9
UMg/Cr		0.56 (0.18)		0.50 (0.17)	0.06 (−0.06 to 0.19)	0.3
UNa/Cr		15.7 (12.2)		14.0 (7.0)	1.7 (−6.1 to 9.4)	0.7

The values are mean (SD). Poor control defined as mothers with at least one HbA_{1c} value above the upper limit for the assay reference range; good control defined as mothers with HbA_{1c} concentrations within the assay reference range. The two groups were compared with *t* tests, and the results are presented as the associated difference in means together with 95% confidence interval (CI).

Table 5 Urinary calcium to creatinine ratio (UCa/Cr), UMg/Cr, and UNa/Cr, after adjustment for age and sex, in children born to mothers with insulin dependent diabetes mellitus classified into four groups (B–E) according to White[13 14]

	B (n = 11)	C (n = 17)	D (n = 13)	E (n = 4)	p Value	p Value (trend)
UCa/Cr	0.38 (0.30)	0.28 (0.20)	0.28 (0.25)	0.27 (0.07)	0.7	0.4
UMg/Cr	0.55 (0.15)	0.48 (0.16)	0.53 (0.19)	0.42 (0.19)	0.5	0.4
UNa/Cr	11.6 (4.1)	13.3 (7.0)	18.0 (12.2)	10.7 (5.2)	0.2	0.3

Values are mean (SD). The values for each of these variables were not different when compared using either one way analysis of variance or a trend test.

dependent diabetic mothers who show increased urinary Ca and Mg excretion.[19]

Given this difference between ChMIDDM and the control group, it might be expected that within the former group there would be an association with the severity of the diabetes in the mother or the degree of glycaemic control during pregnancy. However, we did not observe a difference between ChMIDDM whose mothers were classified as having poor control or good control. Furthermore, we did not observe an association between birth weight SDS (which could be considered a surrogate for glycaemic control) or maternal White's groups and urinary Ca and Mg excretion. However, owing to the retrospective nature of the study and the changes in HbA_{1c} assay during the course of the study, the absence of a demonstrable effect here should not be taken as definitive evidence that the urinary secretion of these minerals is not related to maternal glycaemic control during pregnancy. Further prospective studies are required to collect appropriate data to address this issue.

The mechanisms underlying the reduced urinary Ca and Mg excretion in ChMIDDM are not fully understood, but probably involve in utero programming of mechanisms that are responsible for their renal handling. The bulk of the filtered Ca is passively reabsorbed by the paracellular pathway in proximal tubules, and about 15% occurs actively via transcellular pathways in the distal convoluted tubules. The transcellular Ca^{2+} transport involves three steps: entry of Ca^{2+} across the apical membrane via Ca channels, cytosolic diffusion of Ca^{2+} bound to a vitamin D_3 sensitive, Ca binding protein (calbindin-D_{28K}), and active extrusion of Ca^{2+} across the basolateral membrane mediated by plasma membrane Ca^{2+}-ATPase and by Na^+/Ca^{2+} exchanger.[20] Studies in our laboratory have shown that the renal plasma membrane Ca^{2+}-ATPase and calbindin-D_{28K} expression are increased in neonatal offspring of diabetic rats, relative to controls.[8] The upregulation of these two proteins in the fetal nephron is probably due to some aspect of the intrauterine environment, such as impaired maternofetal Ca and Mg transport,[5] fetal hyperglycaemia, and/or hyperinsulinism. If the expression of these proteins remains permanently upregulated, then the inappropriately increased Ca reabsorption will result in persistence of relative hypocalciuria during child and young adulthood. Similar mechanisms are likely to be responsible for intrauterine programming of renal Mg reabsorption.

There has been considerable interest recently in the intrauterine "programming" of fetal metabolic and endocrine functions as an adaptive response to inadequate supply of nutrients. These permanent adaptations permit the survival of the fetus, but when nutrition becomes plentiful after birth, they are associated with an increased risk of obesity, type 2 diabetes mellitus, hypertension, and cardiovascular disease in later life.[21] In contrast with these detrimental health effects, intrauterine programming that leads to reduced urinary excretion of Ca and Mg in ChMIDDM may have long term beneficial effects. Hypercalciuria is a well known risk factor for development of renal stones and nephrocalcinosis.[22] It is therefore plausible that children and adults born to mothers with IDDM may be less prone to morbidity and mortality associated with nephrolithiasis. Ca is the most important mineral constituent of the skeleton, and urinary excretion of Ca is an important component of Ca metabolism. Thus a positive Ca balance resulting from its reduced urinary excretion may lead to higher bone mass and thus strength in children and adults born to mothers with IDDM. Indeed, Birdsey *et al*[6] showed that offspring of diabetic rats have increased cortical bone mass compared with controls. Potentially beneficial effects of such programming of urinary mineral handling in ChMIDDM require confirmation by longitudinal cohort follow up studies.

This study has a number of limitations. These include its cross sectional design and relatively small numbers of ChMIDDM. The control children were originally recruited for a study of bone mass acquisition in white children. The dietary intakes were based on food diaries completed by the subjects and/or their parents, and it is possible that the observed differences in their nutrient intakes were due to differences in recording accuracy in the two groups. Parents of the ChMIDDM group are likely to have a greater awareness of diets, therefore their recording of foods eaten by the children could be more accurate. Owing to unexpected problems, dietary assessments in ChMIDDM were undertaken about 12 months before urine samples were collected for UCa/Cr, UMg/Cr, and UNa/Cr assays. The optimum method for measurement of urinary mineral excretion is based on full 24 hour urine collection. However, collections are difficult to obtain in children and limit potential recruitment. Thus single urine samples were collected, and molar UCa/Cr, UMg/Cr and UNa/Cr ratios were used in the analysis, as these correlate well with 24 hour urinary excretion of these minerals.[15] Urine samples were collected throughout the year in controls and between December and February in ChMIDDM. Therefore the observed differences in urinary excretion of minerals in the two groups may be due to seasonal variation in calciotropic hormone concentrations. However, Hilgenfeld *et al*[23] did not find seasonal variations in UCa/Cr in a cohort of school age children. Furthermore, we did not find evidence of seasonal variation in our data. If we only include the control data measured in the same season as the ChMIDDM, we find similar differences, and these remain significant for Mg ($p = 0.03$) and very close to significant for Ca ($p = 0.05$). Owing to four changes in HbA_{1c} assays during the period when the ChMIDDM were born, we were not able explore the direct relation between maternal HbA_{1c} concentrations measured during various stages of pregnancy and UCa/Cr, UMg/Cr, and UNa/Cr. Despite these limitations, we have shown that urinary excretion of Ca and Mg was significantly lower in ChMIDDM than in controls, as previously shown experimentally in rats.

In conclusion, our results provide evidence for intrauterine programming of renal bivalent cation handling in children born to diabetic women. These findings require confirmation in larger and preferably longitudinal studies. Such studies should also explore the potentially beneficial health outcomes of reduced urinary Ca and Mg excretion in ChMIDDM, in terms of the incidence of nephrolithiasis and skeletal mineralisation.

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