

## Original papers

QJM

### Size at birth, adult intestinal calcium absorption and 1,25(OH)<sub>2</sub> vitamin D

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#### Summary

**Background:** Adult bone mineral status is modified by early environmental influences, but the mechanism of this phenomenon is unknown. Intestinal calcium absorption and vitamin D metabolism are integrally involved in bone metabolism and may be programmed during early life.

**Aim:** To examine the early-life influences on calcium absorption and its control in 322 post-menopausal female twins.

**Methods:** Intestinal calcium absorption was assessed by the stable strontium (Sr) method. Serum PTH, 25(OH) and 1,25(OH)<sub>2</sub> vitamin D were measured and recalled birth weight recorded.

**Results:** Fractional intestinal Sr absorption ( $\alpha$ Sr) was correlated with serum 1,25(OH)<sub>2</sub> vitamin D ( $p < 0.001$ ), but not with 25(OH) vitamin D. Birth weight was inversely associated with serum

1,25(OH)<sub>2</sub> vitamin D ( $p = 0.04$ ), the association being independent of serum calcium, phosphate, creatinine and PTH. Birth weight was inversely correlated with  $\alpha$ Sr ( $p = 0.03$ ), this association being independent of age, season, customary calcium intake and serum 25(OH) vitamin D; however, when serum 1,25(OH)<sub>2</sub> vitamin D was added into the model, the association became non-significant, suggesting that the association was partially mediated via serum 1,25(OH)<sub>2</sub> vitamin D.

**Discussion:** We found a significant inverse association between birth weight and intestinal calcium absorption that is partially explained by an association between serum 1,25(OH)<sub>2</sub> vitamin D and birth weight. This suggests a mechanism whereby the intra-uterine environment might affect adult skeletal status.

#### Introduction

There is consistent evidence of a genetic component to adult bone mineral density (BMD), bone geometry and microarchitecture,<sup>1–3</sup> with between 50% and 85% of their variance being explained by genetic factors. However, there is emerging evidence that adult bone mass may also be

influenced by early life environmental influences. Epidemiological evidence to support the importance of early environment comes from studies documenting an association between weight in infancy and adult bone mineral content.<sup>4–6</sup> The mechanism underlying this association is believed

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to be the programming of a range of metabolic and endocrine systems. Programming is the term used for persisting changes in structure and function caused by environmental stimuli during critical periods of early development, and is now recognized to play an important part in the aetiology of other chronic diseases including ischaemic heart disease, hypertension and diabetes mellitus.<sup>7–10</sup> The calcium vitamin D axis is integrally involved in normal calcium and bone metabolism, and abnormalities of intestinal calcium absorption are believed to predispose to osteoporosis.<sup>11–14</sup> In this study, we tested the hypothesis that adult intestinal calcium absorption is permanently modified by intra-uterine environmental influences.

## Methods

### Subjects

We recruited 322 normal post-menopausal female Caucasian twins from an ongoing UK-wide twin study that was set up to investigate genetic and environmental factors in a variety of complex traits and chronic diseases. The twins were recruited from two sources 29% from an existing twin register at the Institute of Psychiatry, London, where they were initially asked to participate in research into an unspecified range of fields, the other 71% via a national media campaign asking for female twins to take part in a research project on bone and joint problems.<sup>1</sup> Ethical approval for the study was obtained from the St Thomas's Hospital Ethics Committee. From 1 August 1994 to July 1995, all twins seen in the department who were concordant for post-menopausal status, and were not currently using hormone replacement therapy or any drugs known to affect calcium metabolism, were entered into the study. Post-menopausal status was defined as a gap of 12 months since the last normal menstrual period and was confirmed by estimation of serum follicle-stimulating hormone. All subjects completed a risk factor questionnaire, and those with a serious medical illness affecting mobility or bone mass were excluded from the study. Height and weight were measured by trained research nurses using electronic scales and a wall-mounted stadiometer. Recalled birth weight was recorded at the time of the interview.

### Strontium absorption test

Both members of a twin pair attended on the same day after a minimum 6 h fast. Intestinal calcium absorption was assessed using the 60-min stable strontium technique, which correlates well with

intestinal calcium absorption (correlation coefficients >0.9).<sup>15,16</sup> Pure fresh orange juice (50 ml) containing 2.5 mmol (667 mg) of SrCl<sub>2</sub> was administered. Blood samples were drawn 60 min later, separated, and serum stored at -70 °C within 40 min of venepuncture. The serum was diluted 1:3 in 0.2% KCl in 0.1 M HCl, prior to strontium measurement using atomic absorption flame photometry at 460.7 nm (Pye Unicam). The sensitivity of this method is 1 µM. Intra- and inter-assay coefficients of variation were 5.0% and 8.0%, respectively. The fractional strontium absorption ( $\alpha$ Sr) was calculated as serum Sr (µmol/l) × (0.2 × kg body weight) divided by the test dose of Sr 2.5 mmol, expressed as a proportion.

### Serum metabolites

All assays were performed on serum samples drawn after a minimum 6-h fast, on the same visit as the strontium absorption test. 25(OH) vitamin D was extracted with acetonitrile assayed by radioimmuno assay (Incstar). The sensitivity of the assay is 5 µg/l. The intra- and inter-assay coefficients of variation were 6.1% and 15.6%, respectively. 1,25(OH)<sub>2</sub> vitamin D was measured by a radio receptor assay (Nichols Institute) after extraction using a solid-phase C18 OH cartridge. The sensitivity of the assay is 2 ng/l. Intra- and inter-assay coefficients of variation at 50 ng/l were 10.0% and 11.1%, respectively. Serum intact PTH was measured using a two-site chemiluminometric (sandwich) immunoassay (Maglicite, Ciba Corning). The sensitivity of the assay is 1.5 ng/l. Intra and inter assay coefficients of variation at 40 ng/l were 10.0% and 11.1%, respectively. Serum electrolytes, urea and creatinine were measured using a Vitros 950 analyser (Ortho Diagnostics).

Dietary calcium intake was assessed using two different validated food frequency questionnaires: a modified Oxford general food frequency questionnaire which enquired about average food intake over the previous year,<sup>17</sup> and a specific calcium frequency and amount questionnaire assessing calcium intake in the fortnight before the strontium absorption test.<sup>18</sup>

### Statistical methods

Associations between  $\alpha$ Sr, 1,25(OH)<sub>2</sub> vitamin D and other variables were initially assessed by Pearson correlation coefficients. Associations between birth weight and other variables of interest were initially assessed by univariate multi-level linear regression. The distribution of each dependent variable was assessed prior to modelling. A log transformation was taken if an improvement in the

assumption of normality was observed. Multi-level linear regression (using the generalized estimating equations) models were used in the analysis to allow for the fact that data from twins are not statistically independent. Multiple multi-level regression models were constructed using stepwise regression to adjust for potential confounders at the 5% significance level. Indicator variables were used to adjust for categorical data, such as season of blood sample. For presentational purposes, recalled birth weight was categorized into five equally sized quintiles. All analyses were performed using Stata (Stata Corporation).

## Results

The subjects were a median of 11 years post-menopause, and none was currently using hormone replacement therapy. The mean calcium intake was >1000 mg/day, alcohol consumption was low, and only 13% were current smokers (Table 1). There was no significant association between  $\alpha$ Sr and either alcohol intake or smoking.  $\alpha$ Sr was positively correlated with serum 1,25(OH)<sub>2</sub> vitamin D ( $r=0.23$ ,  $p<0.001$ ), but there was no significant correlation with serum 25(OH) vitamin D ( $r=-0.05$ ,  $p=0.36$ ), serum calcium ( $r=0.03$ ,  $p=0.66$ ) or age ( $r=0.003$ ,  $p=0.96$ ). There was no significant correlation between  $\alpha$ Sr and dietary calcium intake using the modified Oxford food frequency questionnaire ( $r=-0.09$ ,  $p=0.15$ ) or the frequency and amount calcium questionnaire ( $r=0.04$ ,  $p=0.50$ ).

**Table 1** Baseline clinical characteristics of the subjects

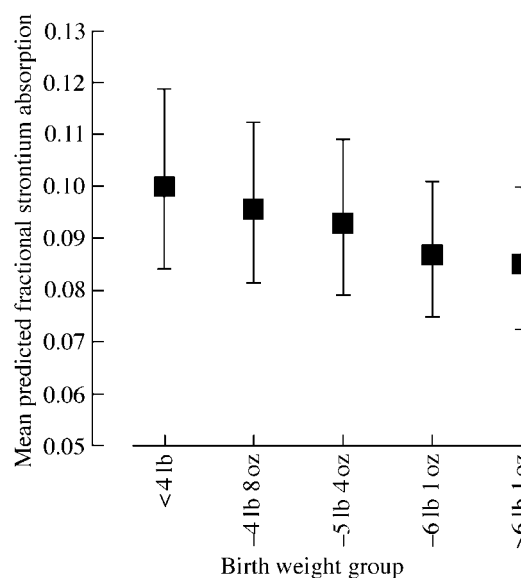
Characteristic	
Age (years)	59.3 (5.6)
Height (cm)	160.9 (6.0)
Weight (kg)	63.6 (9.84)
Years since menopause	11.0 (6–15)
Current smoking (% yes)	14.0
Smoking (pack/year/habit)	11.8 (3.9–29.8)
Alcohol (units/week)	2 (1–3)
Calcium intake (Oxford) (mg/day)	1051 (353)
Vitamin D intake	3.32 (2.07)
Recalled birth weight (kg)	2.27 (0.63)
Corrected serum calcium (mmol/l)	2.36 (0.09)
Serum PTH (ng/l)	27.1 (11.7)
Serum 25(OH) vitamin D ( $\mu$ g/l)	28.3 (12.6)
Serum 1,25(OH) <sub>2</sub> vitamin D (ng/l)	34.3 (7.7)
Fractional Sr absorption (%)	7.76 (5.84–10.8)

Data are means (SD) for normally distributed variables and medians (IQR) for non-normally distributed variables.

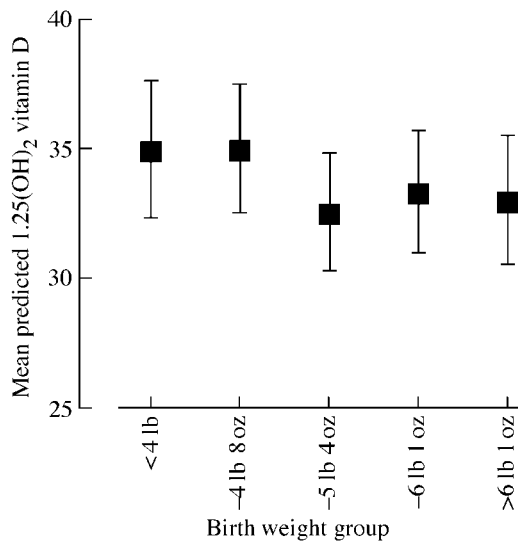
In multi-level regression analysis, there was a marked seasonal variation in  $\alpha$ Sr ( $p<0.001$ ), with absorption lowest in winter and highest in spring: 0.051 vs. 0.091, respectively (with birth weight and PTH included in the model). There were also significant seasonal variations in 1,25(OH)<sub>2</sub> vitamin D ( $p=0.04$ , including birth weight and creatinine) and 25(OH) vitamin D ( $p<0.001$ , including PTH and albumin), but not for PTH. When 1,25(OH)<sub>2</sub> vitamin D and 25(OH) vitamin D were included in a multivariable model, the association between  $\alpha$ Sr and season was virtually unchanged.

In multi-level regression analysis,  $\alpha$ Sr was inversely correlated with recalled birth weight ( $\beta=-0.048$ ,  $p=0.03$ ) (Figure 1). Thus, with birth weight in quintiles, women with a birth weight of >6 lb 1 oz ( $n=53$ ) had a 16% lower  $\alpha$ Sr than those whose birth weight was <4 lb ( $n=47$ ) (0.084 vs. 0.100, respectively). In multivariable regression analysis, this association was independent of age, height, current weight, season, calcium and vitamin D intake. The association was also independent of serum 25(OH) vitamin D, serum calcium, serum phosphate, creatinine and PTH. 1,25(OH)<sub>2</sub> vitamin D was also significantly associated with recalled birth weight ( $\beta=-0.021$ ,  $p=0.04$ ) (Figure 2). The association of  $\alpha$ Sr with birth weight became non-significant ( $\beta=-0.032$ ,  $p=0.15$ ) when serum 1,25(OH)<sub>2</sub> vitamin D was added into the model. In agreement with our previous observations,<sup>4,5</sup> adult height was significantly associated with birth weight, but no other variable was significantly associated with recalled birth weight (Table 2).

Serum 1,25(OH)<sub>2</sub> vitamin D was inversely correlated with serum creatinine ( $r=-0.20$ ,  $p<0.01$ )



**Figure 1.** Average predicted fractional strontium absorption by birth weight group.



**Figure 2.** Average predicted 1,25(OH)<sub>2</sub> vitamin D by birth weight group.

**Table 2** Regression coefficients of indices of calcium metabolism and body build with recalled birth weight

	Regression coefficient	<i>p</i>
α Strontium	-0.484	0.02
1,25(OH) <sub>2</sub> vitamin D	-0.872	0.02
25(OH) vitamin D	0.559	0.34
PTH	-0.589	0.28
Corrected serum calcium	0.003	0.41
Phosphate	-0.012	0.17
Creatinine	-0.009	0.98
Urea	-0.034	0.52
Height (cm)	1.073	<0.001
Weight (kg)	0.487	0.28

and positively correlated with serum PTH ( $r=0.12$ ,  $p=0.03$ ), and with vitamin D intake ( $r=0.12$ ,  $p=0.06$ ). There was no correlation with age, serum calcium, phosphate or serum 25(OH) vitamin D.

## Discussion

In this study of normal, post-menopausal women, we have shown a significant inverse correlation between recalled birth weight and adult intestinal calcium absorption as estimated by the stable strontium technique. This would suggest that a poor *in utero* environment leads to a permanent up-regulation or programming of adult intestinal calcium absorption. Programming refers to the phenomenon whereby an early environmental influence exerts long-term or permanent effects of

adult physiology. The best example of programming is the effect of early exposure to sex hormones on reproductive physiology. Female rats injected with testosterone propionate at 5 days post delivery, although developing normally until puberty, fail to ovulate or develop normal female sexual behaviour.<sup>19</sup> The same injection at 20 days has no effect, suggesting that there are critical periods when the sex hormone axis is sensitive to programming. Other animal experiments have demonstrated that gestational undernutrition can lead to smaller offspring, and that the earlier the nutritional insult, the more likely the effects on body weight and size are to be permanent.<sup>20</sup> The majority of case control studies have demonstrated a reduced fractional calcium absorption in patients with osteoporosis compared to age-matched controls.<sup>11–13</sup> More recently a large cohort study, the Study of Osteoporotic Fractures, has shown that women with a low fractional calcium absorption at baseline had an increased risk of hip fracture over the ensuing 4.8 years. This association was most marked in those with a low dietary calcium intake.<sup>14</sup> The mechanism whereby intestinal calcium absorption is reduced in patients with osteoporosis is uncertain: several studies have identified a reduction in circulating serum 1,25(OH)<sub>2</sub> vitamin D,<sup>11,12</sup> whereas others have suggested an increase in intestinal resistance to 1,25(OH)<sub>2</sub> vitamin D.<sup>11–13</sup> It is most likely that a combination of the two mechanisms is acting. The programming of intestinal calcium absorption would also appear to be due to a combination of factors. There is undoubtedly a reduction in serum 1,25(OH)<sub>2</sub> vitamin D concentrations with increasing birth weight, which could potentially explain the reduced intestinal calcium absorption. However, adjusting for serum 1,25(OH)<sub>2</sub> vitamin D in a multivariable model, although reducing the strength of the association between weight at birth and intestinal calcium absorption, did not remove the association completely, suggesting an element of intestinal resistance to the action of serum 1,25(OH)<sub>2</sub> vitamin D.

The association between recalled birth weight and intestinal calcium absorption in this study was substantial, with a reduction in absorption of 16% when moving from the lowest to the highest quintile of weight at birth. Applying this difference to the data from the Study of Osteoporotic Fractures, in whose subjects the mean dietary calcium intake was similar, would produce an increase in the rate/risk of hip fracture of 17%. If the analysis were restricted to those in the lowest tertile of dietary calcium intake from the SOF, in whom serum 1,25(OH)<sub>2</sub>-vitamin D-mediated active calcium absorption predominates, this would equate to a 90% increase in the risk of hip fracture.

In agreement with other groups<sup>21,22</sup> we have demonstrated a significant seasonal variation in serum 25(OH) vitamin D, serum 1,25(OH)<sub>2</sub> vitamin D and  $\alpha$ Sr, with levels lowest in the winter months. Contrary to Krall *et al.*, we did not find a significant seasonal variation in serum PTH; however, there was a non-significant trend towards higher levels in winter months. The lack of a seasonal variation in PTH is unexplained: compared to those studied by Krall *et al.*, our subjects had similar dietary vitamin D intake and were resident at higher latitudes. Our subjects did however have significantly higher dietary calcium intakes than those of Krall. In a multivariable model, the seasonal variation in  $\alpha$ Sr could not be explained by variations in serum 1,25(OH)<sub>2</sub> vitamin D and 25(OH) vitamin D.

How might the association between birth weight and intestinal calcium absorption be explained? Although the intestinal absorption of strontium occurs via both active transcellular and passive paracellular pathways, the 60 min test, with its low carrier load, is likely to reflect active rather than passive absorption. Active intestinal absorption of calcium is vitamin D dependent, regulated mainly by 1,25(OH)<sub>2</sub> vitamin D,<sup>11,23</sup> but also to a lesser extent by 25(OH) vitamin D.<sup>24</sup> The association of  $\alpha$ Sr and birth weight was independent of 25(OH) vitamin D, but not of 1,25(OH)<sub>2</sub> vitamin D, which also demonstrated a significant inverse correlation with birth weight. This suggests that the *in utero* influences on  $\alpha$ Sr are mediated in part by intra-uterine influences on adult serum concentrations of 1,25(OH)<sub>2</sub> vitamin D.

The major determinants of serum 1,25(OH)<sub>2</sub> vitamin D are the availability of the substrate, 25(OH) vitamin D and the activity of the enzyme 25 OHD 1- $\alpha$  hydroxylase. There was no significant association between serum 25(OH) vitamin D concentrations and recalled birth weight. Furthermore, the serum 25(OH) vitamin D concentrations were well within the normal range. This would argue against the hypothesis that the intra uterine effect on serum 1,25(OH)<sub>2</sub> vitamin D is due to the availability of its substrate.

The association with birth weight may therefore be mediated via renal 25 OHD 1- $\alpha$  hydroxylase activity. The control of renal 25 OHD 1- $\alpha$  hydroxylase activity is closely regulated with activity being reduced by: decreased serum PTH and growth hormone concentrations, high serum 1,25(OH)<sub>2</sub> vitamin D, phosphate and calcium concentrations and decreasing renal function.<sup>28</sup> We have confirmed a positive correlation between serum 1,25(OH)<sub>2</sub> vitamin D and PTH and an inverse correlation with renal function as assessed by serum creatinine. However, neither PTH,

calcium, creatinine or phosphate were associated with birth weight. Furthermore, the association between birth weight and 1,25(OH)<sub>2</sub> vitamin D remained statistically significant after their inclusion into the regression model. If the association is not mediated by substrate availability, or the effects of PTH, calcium, phosphate and renal function on renal 25 OHD 1- $\alpha$  hydroxylase activity, what are the alternative explanations?

It is possible that the programming of serum 1,25(OH)<sub>2</sub> vitamin D is mediated via perturbations in the growth hormone/insulin-like growth factor-1 axis (GH/IGF-1 axis). There is a considerable body of direct and indirect evidence to suggest that growth hormone (GH) and insulin-like growth factor 1 (IGF-1) can modify intestinal calcium absorption and serum 1,25(OH)<sub>2</sub> vitamin D concentrations.<sup>26-31</sup> Furthermore, there is convincing evidence that the GH/IGF-1 axis is programmed.<sup>32-34</sup>

Genetic factors undoubtedly play a role in determining intestinal calcium absorption and vitamin D metabolism. Provisional reports have suggested a genetic component to intestinal calcium absorption.<sup>35,36</sup> Several investigators have examined the role of polymorphisms of the vitamin D receptor gene in explaining the genetic variance of intestinal calcium absorption,<sup>37-39</sup> with variable results. The heterogeneity of the studies performed to date, varying in subject characteristics and the absorption techniques used make it difficult to form a firm conclusion, but it is likely that polymorphisms of the VDR are associated at least to some extent with intestinal calcium absorption. However, genetic factors seldom act in total isolation from the environment, and it is likely that early environment interacts with the genome. Interaction of the early life environment with the vitamin D receptor gene might potentially explain an effect of early environment on intestinal resistance to the actions of serum 1,25(OH)<sub>2</sub> vitamin D by altering the expression or function of the vitamin D receptor and hence active calcium absorption.

There are several potential limitations of this study that may have led to an underestimate of the size of the association. The most important of these is the ascertainment of birth weight data. The birth weight data, being recalled after approximately 60 years, will be subject to a degree of misclassification. The mean recalled birth weights were, however, similar to actual birth weights previously reported in twins, which ranged from 2290 g to 2560 g.<sup>40,41</sup> Due to the nature of the birth weight data, in particular its propensity to be rounded to the nearest half pound, it was felt that a twin-based within-pair analysis would add little to the presented individual based analysis presented here. Furthermore, data from the Nurses Health Study

confirms the validity of self-reported birth weight in middle-aged Caucasian women.<sup>42</sup> As the hypothesis tested in this study was not known to the women, we have no reason to believe there to be a differential misclassification. Any bias would therefore tend to reduce the apparent strength of any association. Vitamin D metabolites are avidly bound to vitamin-D-binding protein in the circulation, with only a small percentage being free and metabolically active. It is possible that the association of free vitamin D metabolites may differ from the total concentrations of metabolites measured in this study.

In conclusion, we have shown that low recalled birth weight is associated with an increased adult intestinal fractional absorption of strontium and serum concentrations of 1,25(OH)<sub>2</sub> vitamin D. At present the exact mechanisms of this association are unclear, but they suggest a mechanism by which early life environmental factors may influence bone metabolism and density.

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