Maternal calcium supplementation and cardiovascular risk factors in twin offspring

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Accepted 10 June 2004

Background There is evidence that maternal calcium supplementation may result in lower offspring blood pressure. We hypothesized that maternal calcium supplementation also influences other cardiovascular risk factors.

Methods In the Tasmanian Infant Health Study, supplements reportedly taken in pregnancy were recorded. Twin children of 147 participating mothers were seen at mean age 9 years. Blood pressure was measured in all 294 children and fasting blood samples taken from 230 (78%) for glucose and insulin, triacylglycerol, total cholesterol (T-C) and HDL cholesterol (HDL-C). LDL cholesterol (LDL-C) was calculated.

Results Children of supplemented mothers (n = 110, 77 had venipuncture) had lower geometric mean triacylglycerol, T-C, and LDL-C than other children. After adjustment for potential confounding factors, geometric mean ratios were 0.86 (95% CI: 0.75, 0.98), 0.94 (95% CI: 0.90, 0.99) and 0.90 (95% CI: 0.83, 0.98) respectively. The association with T-C and LDL-C was seen principally among children with BMI > 17.5: estimated ratios 0.85 (95% CI: 0.79, 0.92) for total cholesterol and 0.79 (95% CI: 0.70, 0.90) for LDL cholesterol (P for interaction 0.001 and 0.009 respectively). There was no significant association between maternal calcium supplementation and child size at birth and follow up, blood pressure, fasting glucose or insulin or HDL-C.

Conclusions Maternal calcium supplementation may confer health benefits on twin offspring, especially if they are relatively fat. Calcium availability could permanently programme lipid metabolism during fetal life, directly or by influencing maternal lipid profile. Our findings need to be replicated in other studies and in singletons. If confirmed, our findings could have important implications for population health.

Keywords Calcium, pregnancy, lipids, children, cardiovascular disease

There has been much interest in the observed association between size at birth and risk of cardiovascular disease in adult life,1,2 but from a public health perspective we need to study factors that are potentially modifiable, rather than fetal growth or birth size.3 A negative association between maternal calcium intake and infant blood pressure was noted in an observational study.4 Subsequently, in an experimental study, Belizan et al. found that systolic blood pressure in children of women given calcium supplements in pregnancy was lower than in children of controls.5 This association was significantly stronger with increasing body mass index (BMI) (P = 0.0001 for interaction) and strongest in children with BMI > 17.5. Preliminary data from a cross-sectional study of 712 6 month olds suggested a weak negative association between maternal calcium intake and infant systolic blood pressure, and no interaction with infant fatness.6 Adult offspring of rat dams with experimental calcium deficiency had higher blood pressure than offspring of control dams.7 In a cross-fostering study, spontaneously hypertensive rat pups suckled by calcium deficient mothers had higher mean arterial pressure, regardless of maternal calcium intake during pregnancy.8

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In 147 twin pairs in Tasmania, we tested the hypothesis that maternal calcium supplementation might also influence other risk factors for cardiovascular disease in their offspring.

We studied a cohort of twins to examine aspects of the fetal origins of adult disease hypothesis. We have indicated previously that twin pregnancies offer a number of opportunities. Among these, we suggested study of twins may provide an opportunity to examine the relationship between maternal nutrition and offspring outcome more efficiently, because in a twin pregnancy the mother’s reserves and the ‘supply line’ to each fetus will be more stretched than in a singleton pregnancy.

### Methods

#### Subjects

During the years 1988–1995, children were recruited into the Tasmanian Infant Health Study (TIHS) soon after birth, to investigate sudden infant death syndrome. Mothers of all live-born twins in Tasmania were approached soon after birth, and socio-demographic data, including maternal age at recruitment (in the neonatal period) and details of maternal education, were collected from women who agreed to participate. Mothers were coded as having tertiary education if they had undertaken formal education after leaving high school at age 18 years. They were asked whether they had taken any nutritional supplements during pregnancy, and this information was recorded. Routine obstetric data, including gestation length and infant birthweights, were copied from hospital records.

We obtained funding to study twin pairs recruited into TIHS during 1991, 1992 or 1993. Those still living in Tasmania were approached soon after birth, and infant birthweights, were copied from hospital records. We also calculated maternal BMI in early pregnancy from mid-upper arm girth measurements of anthropometry and blood pressure, and had a 5-ml fasting blood sample taken.

#### Ethics

This current study and the TIHS were approved by the University of Tasmania Human Research Ethics Committee. Procedures followed were in accordance with institutional guidelines and with the Helsinki Declaration of 1975, as revised in 1983. We obtained both written parental consent and verbal child agreement.

#### Measurements at follow up

Children were seen in 2000, 2001, or 2002 and underwent measurements of anthropometry and blood pressure, and had a 5-ml fasting blood sample taken.

Height was measured, with bare feet, using a stadiometer. Weight in light clothing was measured using bathroom scales, calibrated daily. Child body mass index (BMI, weight in kg/height in m^2) was calculated as a measure of body fatness. On the basis of Belizan’s findings we also categorized children according to whether child BMI was ≤17.5 versus >17.5. We also calculated maternal BMI in early pregnancy from reported height and weight.

Child blood pressure was measured three times using a Critikon Dinamap Adult/Pediatric Vital Signs Monitor with cuff size selected in accordance with mid-upper arm girth measurement. Children were seated with their left arm resting on a pillow and elbow at approximately heart level. There are no published guidelines on whether to include or omit the first of serial systolic blood pressure measurements (generally higher than subsequent readings) when calculating mean blood pressure for an individual. As suggested by others, we present data using the average of the last two of three systolic and diastolic blood pressure measurements, though we also undertook analyses using the mean of all three measurements, to check that this decision did not influence the results.

Blood samples were analysed by an accredited laboratory. Plasma glucose was measured using the Ortho Clinical Diagnostics Vitros Analyser and triglycerides by Vitros Ektachem Test. Insulin was measured by double antibody solid phase radioimmunoassay, using the Phadebas Insulin kit. Standard enzymatic methods were used to measure serum cholesterol and serum high density lipoprotein cholesterol (HDL-cholesterol), the latter after precipitation of very low density lipoprotein and low density lipoprotein with dextran sulphate.

Low density lipoprotein cholesterol (LDL-cholesterol) was calculated from total cholesterol, HDL-cholesterol and triglycerides.

#### Statistical analyses

Data on most of the laboratory measures were skewed, so we used natural logarithms of these measures in analyses and present geometric means, with ratios for comparisons between groups. We used a mixed effects regression approach (with random intercept for each twin pair) to estimate the effect of maternal calcium supplementation on child outcomes of interest, allowing for the lack of independence between co-twins. Regression analyses were performed using the ‘xtreg’ command in Stata (StataCorp 2003). Stata Software Release 8.1, College Station, Texas). We tested for effect modification using appropriate interaction terms.

### Results

Altogether 463 children from twin pregnancies were recruited into TIHS in 1991, 1992 or 1993. Eleven had co-twins who were not available for recruitment. Of the 226 recruited pairs, 23 had left Tasmania, so there were 203 eligible twin pairs.

Altogether 147/203 pairs (72%) agreed to participate: 14 pairs could not be traced and 42 pairs declined. Both children from 113/147 pairs agreed to provide a blood sample, and in a further 4 pairs only one twin agreed, so blood samples were collected from 230/294 children (78% of those seen).

Mothers of participants were older when their twins were born (mean 30.4 years [SD 4.6] versus 28.3 [5.4], 95% CI for difference -0.6, -3.6) and more likely to have had tertiary education than non-participants (16.3% versus 7.4%). Mean gestation length and birthweight differed little between participants and non-participants, as did the ratio of male to female children and the proportion of women who took calcium supplements (35.4% of participants versus 37.5% non-participants).

Characteristics of mothers of participating children who did not take calcium supplements versus those who did are shown in Table 1. Mean (SD, range) age of children at follow up was 9.0 (0.9, 7.5–10.6) years in the unsupplemented and 9.1 (0.8, 7.4–11) years in the supplemented group. Of the 110 children of women who took calcium supplements, 52 (47%) were male, compared with 97/184 (53%) children whose mothers did not.
Table 1  Maternal characteristics according to whether mothers took calcium supplements during pregnancy

<table>
<thead>
<tr>
<th></th>
<th>No calcium supplement</th>
<th>Took calcium supplement</th>
<th>95% CI for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N mothers</td>
<td>92</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>% mothers with tertiary education</td>
<td>19.6%</td>
<td>25.5%</td>
<td>−8.2%, 12.0%</td>
</tr>
<tr>
<td>Mean (SD) maternal age in years</td>
<td>30.0 (4.6)</td>
<td>31.1 (4.7)</td>
<td>−0.03, 2.2</td>
</tr>
<tr>
<td>Mean (SD) first maternal weight during this pregnancy, in kg</td>
<td>62.6 (10.8)</td>
<td>64.2 (12.3)</td>
<td>−1.2, 4.5</td>
</tr>
<tr>
<td>% first pregnancy</td>
<td>32.6%</td>
<td>38.2%</td>
<td>−10.4%, 21.6%</td>
</tr>
<tr>
<td>Mean (SD) gestation length in weeks</td>
<td>36.3 (2.9)</td>
<td>36.3 (2.8)</td>
<td>−0.7, 0.6</td>
</tr>
</tbody>
</table>

Table 2 shows birthweight and size at follow up of the children, as well blood pressure and measures of glucose homeostasis and blood lipid profile. Ninety-nine (34%) children had BMI > 17.5: 32/110 (29%) of those whose mothers took calcium supplements and 67/184 (36%) of those whose mothers did not.

We found no evidence of an association between maternal calcium supplementation and child birthweight, current size or fatness, nor with either systolic or diastolic blood pressure, or with fasting insulin or glucose levels (Table 2). However, children whose mothers took calcium supplements had lower plasma triacylglycerol, total cholesterol and low density lipoprotein (LDL) cholesterol levels.

Most of the outcome measures were strongly related to child age and/or sex, and some of the measures to whether the mother had a tertiary education or the twins’ birth order in the family. After adjustment in regression models for these factors only slight confounding effects were apparent, but some precision was gained—as reflected in some strengthening of the associations with lipid levels.

In regression models, we looked for evidence of interaction between maternal calcium supplementation and child BMI (as a continuous variable), in terms of outcome measures in Table 2. After adjustment as above, there was weak evidence of interaction for total cholesterol (P = 0.02), but no such evidence for any of the other measures. However, when children were categorized according to whether their BMI was <17.5 versus ≥17.5, there was stronger evidence of interaction between this BMI classification and the association between maternal calcium supplementation and total and LDL-cholesterol (P for interaction 0.001 and 0.009 respectively, after adjustment for potential confounding factors, Table 2). Among children with BMI <17.5, there was no difference in these measures, according to maternal calcium supplementation, but in those with BMI ≥17.5, estimated geometric mean ratios were 0.85 (95% CI: 0.79, 0.92) for total cholesterol and 0.79 (95% CI: 0.70, 0.90) for LDL cholesterol.

We considered the possibility that among children in the fatter group, BMI was higher in children whose mothers did not take supplements but this proved not to be the case. Among children with BMI ≥17.5, mean child BMI was 21.1 in those whose mothers took calcium, versus 19.9 in those whose mothers did not.

Pearson correlation coefficient for the relationship between maternal and child BMI was 0.32, P < 0.001, so we considered the possibility that child BMI could be acting as a proxy for maternal BMI during pregnancy. We looked for evidence of interaction between maternal BMI and maternal calcium supplementation, for each of the outcomes shown in Table 2, but no such evidence was found (data not shown).

Discussion

Twin children whose mothers reported taking calcium supplements during pregnancy had lower levels of total cholesterol, LDL-cholesterol and triacylglycerol than other children, despite the two groups of children having similar BMI. On the basis of Belizan’s findings with respect to blood pressure we tested for interaction between maternal calcium supplementation and child BMI. For both total and LDL-cholesterol, the association with maternal calcium supplementation reflected a strong association in children with BMI > 17.5 (the fattest third of this population), with no apparent association in the lighter children.

We found no evidence of an association between maternal calcium supplementation and child size at birth or at follow up, in agreement with the findings of Belizan et al.5

Previous studies4–6 suggest that maternal calcium intake can programme offspring blood pressure, though the effect size is small. In Belizan’s study5 a strong effect of maternal calcium supplementation was seen in fatter children and there was significant interaction with child BMI, but in Gillman’s study of infants6 the association was weak and there was no evidence of interaction. We found no overall association with either systolic or diastolic pressure and no evidence of interaction with child BMI. Our findings were the same whether we used mean of the last two blood pressure measurements, as presented, or mean of all three. We do not know why offspring of women who took calcium supplements should have a more favourable lipid profile. Intracellular calcium plays a key role in the regulation of adipocyte lipid metabolism14,15 and calcium intake influences dietary fat absorption from the gastrointestinal tract.16–18 It is possible that maternal calcium supplementation altered programming of lipid metabolism in the fetus. Alternatively any effect of maternal calcium supplementation on offspring lipid
### Table 2: Child birthweight and size and cardiovascular risk factors in childhood, according to whether the mother took calcium supplements during pregnancy

<table>
<thead>
<tr>
<th></th>
<th>No calcium supplement</th>
<th>Took calcium supplement</th>
<th>Difference</th>
<th>95% CI for difference</th>
<th>P</th>
<th>Adjusted(^{a}) estimated difference</th>
<th>95% CI for adjusted(^{a}) difference</th>
<th>P(^{a})</th>
<th>P(^{b}) for interaction difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N children</td>
<td>184</td>
<td>110</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean (SD) birthweight in kg</td>
<td>2.44 (0.59)</td>
<td>2.50 (0.59)</td>
<td>0.06</td>
<td>–1.0, 0.2</td>
<td>0.5</td>
<td>0.07</td>
<td>–1.0, 0.3</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>Mean (SD) height at follow up in cm</td>
<td>133.5 (8.2)</td>
<td>135.5 (7.2)</td>
<td>2.0</td>
<td>–0.5, 4.4</td>
<td>0.2</td>
<td>1.5</td>
<td>–0.2, 3.3</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>Mean (SD) weight at follow up in kg</td>
<td>31.2 (7.4)</td>
<td>32.3 (8.0)</td>
<td>1.1</td>
<td>–1.3, 3.5</td>
<td>0.4</td>
<td>0.8</td>
<td>–1.3, 2.9</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>Mean (SD) body mass index at follow up</td>
<td>17.3 (2.6)</td>
<td>17.4 (3.1)</td>
<td>0.1</td>
<td>–0.7, 1.0</td>
<td>0.8</td>
<td>0.08</td>
<td>–0.8, 0.9</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td>Mean (SD) systolic blood pressure in mmHg</td>
<td>96.4 (13.3)</td>
<td>95.6 (10.8)</td>
<td>–0.8</td>
<td>–4.2, 2.6</td>
<td>0.6</td>
<td>–0.7</td>
<td>–4.1, 2.7</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean (SD) diastolic blood pressure in mmHg</td>
<td>52.6 (7.4)</td>
<td>51.8 (6.8)</td>
<td>–0.8</td>
<td>–2.8, 1.2</td>
<td>0.4</td>
<td>–0.9</td>
<td>–2.9, 1.1</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>N children who gave a blood sample</td>
<td>153</td>
<td>77</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Geometric mean fasting insulin, mU/l</td>
<td>6.8</td>
<td>7.1</td>
<td>1.03</td>
<td>0.87, 1.23</td>
<td>0.7</td>
<td>1.0</td>
<td>0.8, 1.2</td>
<td>0.95</td>
<td>0.1</td>
</tr>
<tr>
<td>Geometric mean fasting glucose, mmol/l</td>
<td>4.9</td>
<td>4.9</td>
<td>1.02</td>
<td>0.99, 1.04</td>
<td>0.2</td>
<td>1.0</td>
<td>0.99, 1.04</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Geometric mean triacyglycerol, mmol/l</td>
<td>0.87</td>
<td>0.76</td>
<td>0.88</td>
<td>0.76, 1.01</td>
<td>0.07</td>
<td>0.86</td>
<td>0.75, 0.98</td>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>Geometric mean total cholesterol, mmol/l</td>
<td>4.71</td>
<td>4.46</td>
<td>0.95</td>
<td>0.90, 0.99</td>
<td>0.049</td>
<td>0.94</td>
<td>0.90, 0.99</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Geometric mean HDL(^{c}) cholesterol, mmol/l</td>
<td>1.42</td>
<td>1.48</td>
<td>1.04</td>
<td>0.97, 1.12</td>
<td>0.3</td>
<td>1.04</td>
<td>0.96, 1.12</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Geometric mean LDL(^{d}) cholesterol, mmol/l</td>
<td>2.83</td>
<td>2.55</td>
<td>0.91</td>
<td>0.84, 0.99</td>
<td>0.02</td>
<td>0.90</td>
<td>0.83, 0.98</td>
<td>0.02</td>
<td>0.009</td>
</tr>
</tbody>
</table>

\(^{a}\) Adjusted for maternal education, pair birth order (in family) and child age and sex.

\(^{b}\) P-value for interaction effect comparing calcium difference between twins with BMI >17.5 and those with BMI ≤17.5.

\(^{c}\) High density lipoprotein cholesterol.

\(^{d}\) Low density lipoprotein cholesterol.
profile could have been mediated through a change in lipid profile of their mothers. In a study of hypercholesterolaemic men, those randomly allocated a high calcium diet had lower total cholesterol and LDL cholesterol than those allocated a low calcium diet, and a higher percentage of dietary fat excreted in faeces.\textsuperscript{17} There are no published data on calcium supplementation and lipid profile in pregnant women.

There are alternative explanations. Firstly, these may be chance findings, even though some associations and interactions were similar to those seen in Belizan’s study for blood pressure.\textsuperscript{5}

Secondly, there may be unidentified confounding. Mothers who took calcium supplements during pregnancy may also have given their children more dairy products or even calcium supplements than other women. Increased calcium in the children’s diet might have affected their lipid profile. In the CARDIA study,\textsuperscript{18} dairy consumption was inversely associated with the incidence of dyslipidaemia among young adults who were overweight (BMI $\geq 25$ kg/m$^2$) at baseline but not among leaner individuals (BMI $< 25$ kg/m$^2$).

However, many women probably took calcium because they were advised to do so while pregnant. Obstetricians, midwives and dieticians commonly recommend that women with multiple pregnancies should take calcium supplements, on the grounds that their requirements are greater. Furthermore, at the time these women were pregnant with twins (early 1990s) there was increasing interest among obstetricians in the possibility that calcium supplements could reduce the risk of pregnancy hypertension and preterm delivery.\textsuperscript{19}

Many of the women taking calcium supplements were also taking other micronutrient supplements, but we found no evidence that these were associated with child lipid profile (data not shown).

It is not clear why the association between maternal calcium intake and child total and LDL-cholesterol should be stronger in the latter group of children. We hypothesized that child fatness could have been acting as a proxy for maternal fatness in pregnancy, and that the underlying interactions were with maternal fatness. The influence of maternal calcium supplementation could have been restricted to offspring of fatter mothers and few studies have reported that fatter people have lower calcium intake.\textsuperscript{15} We found no evidence of interaction between maternal calcium supplementation and maternal BMI, in terms of child lipid profile. However, maternal weight in early pregnancy was measured at varying times during gestation on a variety of scales and recalled by the mother, so maternal BMI was based on poor quality anthropometric data.

Our study has weaknesses. We do not have consistent information on dosage, with no information about the period over which supplements were taken nor how regularly. We acknowledge that data obtained via recall in an observational study provides weaker evidence than a study with experimental design. Nevertheless our findings raise an important hypothesis that needs to be tested in a prospective randomized trial.

**Conclusion**

Twins comprise a small, albeit increasing proportion of the general population, but the population health implications of our findings will depend on whether an effect of maternal calcium supplementation on offspring lipid profile is confirmed in experimental studies and seen in singletons. This could be investigated in children born to mothers participating in completed or on-going randomized trials of calcium supplementation in pregnancy.

**Acknowledgements**

This study was funded by the National Heart Foundation of Australia and Ruth Morley is supported by VicHealth (the Victorian Health Promotion Foundation). The Tasmanian Infant Health Study was funded by the National Health and Medical Research Council of Australia, US National Institutes of Health, Tasmanian State Government, Australian Rotary Health Research Fund, Sudden Infant Death Research Foundation, National Sudden Infant Death Syndrome Council of Australia, Community Organisations’ Support Program of the Department of Human Services and Health, Zonta International, Wyeth Pharmaceuticals and Tasmanian Sanatoria After-Care Association. Kristen Hynes co-ordinated this study and with Jennifer Cochrane managed and prepared the data. Anne-Louise Ponsonby, Jennifer Cochrane and Malcolm Riley made important contributions to the Australian Rotary Health Study. We are very grateful to the nurses who undertook fieldwork and to the children who participated, and their families.

### KEY MESSAGES

- Twin children whose mothers took calcium supplements in pregnancy had lower triacylglycerol, total cholesterol, and low density lipoprotein (LDL)-cholesterol levels than other children.
- The associations between maternal calcium and both total and LDL-cholesterol were seen only in children with body mass index $> 17.5$, not in thinner children.
- If confirmed in other populations our findings would have major public health implications.

### References


Commentary: Maternal calcium intake and offspring cardiovascular risk factors

Eduardo Bergel and José M Belizán*

In this issue of the Journal, Morley, Carlin and Dwyer1 look for associations between maternal calcium supplementation and offspring blood pressure, lipid profile and markers of glucose homeostasis. They found that children whose mothers took calcium supplements during pregnancy have a better lipid profile at nine years of age and suggest that calcium availability could permanently programme lipid metabolism during foetal life. Systematic reviews provide rather strong evidences of inverse associations between birthweight and hypertension2 and glucose intolerance3 in later life. It has been proposed that adverse conditions in utero, such as foetal under-nutrition, can result in metabolic and physiological ‘programming’ of functions of the body with lifelong effects on disease risk. But can lipid metabolism be programmed? A recent systematic review of studies in humans does not support a link between birthweight and blood lipid levels.4 However, the authors stated that more data is needed to make any definitive conclusions. The evidence in animals is much stronger, showing that under-nutrition during gestation permanently changes lipid metabolism.

The focus of most studies on programming in humans is on birthweight, although numerous experiments have shown that minor alterations to the diets of pregnant animals, which may not even change their offspring’s body size at birth, can produce lasting changes in their physiology and metabolism.5