INTRODUCTION

The human brain develops intensely during foetal life and matures further during early childhood. The hormone osteocalcin is known to have an impact on bone formation and on metabolic functions, like insulin sensitivity and glucose homoeostasis.¹⁻³ Research has shown that osteocalcin passed through the placenta, and the blood–brain barrier, in mice, but its exact functions in utero or in brain development are still unknown.⁴ One animal study found that mice without the ability to produce osteocalcin expressed behavioural traits as-sociated with depression and anxiety and showed signs of impaired memory formation and spatial learning. However, when they were...
injected with osteocalcin, their spatial learning and memory formation were enhanced, and their behaviour normalised. The same study also found that maternal osteocalcin transferred to foetal mice pups through the placenta and prevented neuronal apoptosis in the hippocampus. This is interesting, because human studies imply that the hippocampus is involved in spatial cognition, memory and anxiety. These findings indicate that osteocalcin plays a part in neurodevelopment and mental health in mice, but it is unclear whether these findings apply to humans. A study of 24 adults showed that low serum osteocalcin levels were associated with a higher body mass index and lower cognitive performance, as well as micro structural changes in the basal ganglia and hypothalamus of the brain. Another study of 196 adult males with type 2 diabetes showed a similar association between low serum osteocalcin levels and impaired cognition. The hypothalamus and the hippocampus are important brain areas for memory, behaviour and appetite regulation, whereas the basal ganglia coordinates smooth movements. Therefore, these brain areas are possible targets when it comes to how osteocalcin may play a role in neurodevelopment. If osteocalcin has a direct effect on the hypothalamus, hippocampus or the basal ganglia, it is theoretically possible that it might have an impact on human memory, spatial cognition or the maturation of motor development. Because large parts of brain development occur before birth, and during infancy, the possible effects of osteocalcin on the human brain are interesting from a developmental perspective.

Osteocalcin is a non-collagenous hormone that is mainly produced by the osteoblasts. Small amounts are released into the circulation, and these levels differ with age. There seems to be a specific pattern. Infants have high osteocalcin at birth, and the level rises during the first months of life and then declines at some point between 3 months and 3 years. Then, it remains fairly stable until puberty, when it rises again. However, to date, there have been no set paediatric reference values for osteocalcin, and researchers have not established what factors have an impact on osteocalcin levels in the early lives of healthy children.

The aim of this explorative study was to provide further knowledge on the potential associations between serum osteocalcin levels during infancy and early childhood and later cognitive and psycho-motor development. To do this, we specifically focused on full-scale intelligence quotient (IQ), motor development and behaviour at 4 years of age.

2 | PATIENTS AND METHODS

2.1 | Cohort

The cohort was composed of subjects who took part in two birth cohorts at the Halland Hospital Halmstad, Sweden, from 2008 to 2009. The first was the Cord Clamping Study, which was a randomised clinical trial that focused on neurodevelopment in relation to early and respectively late cord clamping. The study enrolled 382 healthy children (53% girls), who were born vaginally to Swedish-speaking parents at the hospital from 16 April 2008 to 22 May 2009. Only full-term births, defined as 37 + 0 to 41 + 6 weeks of gestation, were included. Some of these children were also enrolled in the Growth Study, which was held at the same time at the same hospital. The Growth Study is an ongoing birth cohort that focuses on early factors that influence childhood obesity and metabolic syndrome, including hormones like osteocalcin, and 396 infants were enrolled at birth. The population for this study was 158 children (51% girls) who had taken part in at least one test in the Cord Clamping Study at 4 years of age and had completed the 36-month follow-up for the Growth Study (Figure 1). These 158 represented 61% of the 258 who had enrolled in both birth cohorts.

2.2 | Data collection

Data were extracted from both studies. These included parental weight, height and educational level and the child neurodevelopmental assessment, blood tests and food diary entries at 4 months of age. If children were exclusively or partially breastfed, it was coded as any breastfeeding compared with no breastfeeding.

Three areas were explored for the neurodevelopmental assessment.

The first was cognitive function, including full-scale IQ. At 48–51 months of age, the children were assessed by a psychologist, using the older age band of the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III), covering 4–7 years of age. The WPPSI-III assesses verbal and cognitive performance in five different domains: full-scale IQ, verbal IQ, performance IQ, the processing speed quotient and the basal language quotient. Full-scale IQ is based on verbal IQ, performance IQ and processing speed, and the scores are standardised to a mean of 100. A score of <85, namely, −1 standard deviation (SD), is subnormal. We also used the pertinent parts of the Ages & Stages Questionnaire, Third Edition (ASQ-III) for the cognitive assessment, namely, the communication, problem-solving and social development domains. The ASQ-III was translated into Swedish and completed by the parents when the children were 48 months old. The test comprises a total

Key Notes

- This novel study explored associations between total serum osteocalcin levels in 158 healthy, vaginal term-born infants and neurodevelopment at 4 years of age.
- Serum osteocalcin at 4 months of age was associated with higher intelligence quotient and better motor control at 4 years, whereas cord levels were negatively associated with processing speed and fine motor control.
- These results supported an association between osteocalcin and neurodevelopment, but more research is needed.
score and five subdomains: communication, gross motor, fine motor, problem-solving and personal social development. It is standardised on an interval scale and is designed to find difficulties.

The second area was motor development, which was explored with the remaining subdomains of the ASQ-III, namely, gross motor and fine motor domains. In addition, the manual dexterity area from the Movement Assessment Battery for Children, Second Edition (MABC-2) was tested at the same time as the cognitive assessment and by the same psychologist. The tests included posting coins, threading beads and the drawing trail test. All the tests were scored in an interval manner and normally distributed.

The third area, behaviour, was assessed by the Strengths and Difficulties Questionnaire (SDQ). Completed by the parents at the 48-month follow-up, it covered the period when the children were 3–4 years of age. The SDQ scores covered behaviour in five different subdomains: emotional difficulties, conduct difficulties, hyperactivity difficulties, peer problems and the prosocial score. It is an ordinal scale that is not centred to the mean.

### 2.3 Blood analyses

The results for non-fasting serum osteocalcin were obtained from the Growth Study. These were taken from cord blood and following EMLA local anaesthetic (AstraZeneca, Cambridgeshire, UK) at 4, 12 and 36 months of age. Additional oral glucose was administered as a distraction at 4 months if needed. The samples were frozen immediately, due to the instability of serum osteocalcin, and then analysed in duplicate at the Gothenburg Pediatric Growth Research Center laboratory in Sweden (accredited number 1899), after all the children had reached 36 months of age in 2012. Samples from all the children were in the same assay batch. Total serum osteocalcin was measured using the iSYS technique (Immunodiagnostic Systems Holdings PLC, Tyne and Wear, UK) and expressed as ng/mL. The intra-assay coefficient of variation for the serum osteocalcin was 1.4%, and the inter-assay coefficient of variation was 4.0%. The proportion of undercarboxylated osteocalcin was not analysed.

### 2.4 Statistics

Linear regression analyses were performed for serum osteocalcin at all four time points: cord and 4, 12 and 36 months of age. They were also performed for the WPPSI-III, ASQ-III and MABC-2 tools at 4 years of age. Spearman correlation analysis and graphical scatter plots were performed to investigate potential linearity. The linear regression model, based on the osteocalcin level at 4 months and the full-scale IQ, was complemented by a multivariable linear regression model and adjusted for factors known to influence IQ during childhood. These included sex and parental educational levels of <12 years of schooling or 12 or more years, which was equivalent to graduation. Tests for non-linearity was not performed.

Then, we formed quartile groups, based on the serum osteocalcin level at 4 months of age, and compared the 72/144 (50%)

---

**FIGURE 1** Flow chart depicting the selection of children and the attrition of study participants for both original studies until the 4-year follow-up. Of the 158 children, blood samples were not complete for 8 children at cord, 14 at 4 months, 15 at 12 months and 7 at 36 months due to technical difficulties with the analysis in the test situation; 158 children answered both Ages & Stages Questionnaire (ASQ) and Strengths and Difficulties Questionnaire (SDQ), 154 children answered Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III), and 153 children answered Movement Assessment Battery for Children, Second Edition (MABC-2). Note: This is not a complete list of the follow-up programme for the original studies. Abbreviations: Anth, anthropometrics; Blood, blood sampling; Charts, information retrieved from charts; Diary, food diary.
The following analyses were performed. WPPSI-III: full-scale IQ, verbal IQ, performance IQ, processing speed quotient and basal language quotient. ASQ-III: total score, communication, gross motor, fine motor, problem-solving and personal social. The manual dexterity subtest from the Assessment Battery for Children, Second Edition; WPPSI-III, Wechsler Preschool and Primary Scale of Intelligence, Third Edition.


### Results

The mean serum osteocalcin value varied with the children's age in this cohort of 158 children (51% girls). The mean and SD serum osteocalcin were 46.7 ± 28.2 ng/mL for cord blood, 98.7 ± 36.0 ng/mL at 4 months, 78.2 ± 24.2 ng/mL at 12 months and 67.9 ± 22.2 ng/mL at 36 months.

No association was found between osteocalcin and the timing of cord clamping (p > 0.05) with linear regression modelling for the exact timing. The same was true when early versus late clamping was analysed with the independent t-test (p > 0.2) for all osteocalcin time points.

#### 3.1 Osteocalcin and cognitive function

An association between full-scale IQ and serum osteocalcin at 4 months of age was found, with a coefficient of determination of 3.1% (Table 1). Serum osteocalcin remained a significant factor for full-scale IQ when it was adjusted for the child’s sex and parental educational level (Table 2).

When we compared the first and fourth osteocalcin quartile groups with regard to full-scale IQ, children with a serum osteocalcin level in the top quartile scored on average 5.6 points higher (95% confidence interval [1.3, 9.9]), compared with the lowest quartile at 4 months of age. The mean IQ figures were 118.8 ± 8.8 versus 113.2 ± 9.2 (Table 3).

Furthermore, performance IQ, a subdomain of the WPPSI-III, showed an association with linear regression modelling (p < 0.05; Table 1) to serum osteocalcin at 4 months of age. However, the Spearman correlation coefficient was not significant (p 0.089), and the scatter dot was inconclusive. No associations were seen for verbal IQ, processing speed or basal language quotient and the 4-month osteocalcin sample with linear regression analyses (data not shown). Thereafter, mean scores for verbal IQ, performance IQ, processing speed and basal language quotient were compared between the first and fourth osteocalcin quartile groups. There was a higher mean score in the highest, than lowest, serum osteocalcin quartile for performance IQ (Table 3).

The cord blood sample did not show any significant associations with full-scale IQ, but a significant negative association was seen for the subtest of processing speed (Table 1). No associations for any of the other subdomains in the WPPSI-III and cord blood were found (data not shown).

No associations were found for WPPSI-III and serum osteocalcin at 12 or 36 months, and there were no associations for the cognitive parts of the ASQ-III and serum osteocalcin at any age.

### Table 1

<table>
<thead>
<tr>
<th>Test variable</th>
<th>Time for osteocalcin test</th>
<th>N</th>
<th>Intercept</th>
<th>R²</th>
<th>Unstandardised B coefficient</th>
<th>95% CI</th>
<th>p value</th>
<th>Spearman correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed (WPPSI-III) Cord</td>
<td>Cord</td>
<td>137</td>
<td>103.1</td>
<td>0.039</td>
<td>-0.066</td>
<td>[-0.121, -0.010]</td>
<td>&lt;0.05</td>
<td>-0.182</td>
</tr>
<tr>
<td>Fine motor (ASQ-III) Cord</td>
<td>Cord</td>
<td>147</td>
<td>56.7</td>
<td>0.042</td>
<td>-0.062</td>
<td>[-0.102, -0.014]</td>
<td>&lt;0.05</td>
<td>-0.199</td>
</tr>
<tr>
<td>Threading beads (M-ABC) Cord</td>
<td>Cord</td>
<td>143</td>
<td>9.1</td>
<td>0.046</td>
<td>-0.025</td>
<td>[-0.044, -0.006]</td>
<td>&lt;0.05</td>
<td>-0.224</td>
</tr>
<tr>
<td>Full-scale IQ (WPPSI-III) 4 months</td>
<td>Cord</td>
<td>139</td>
<td>112.1</td>
<td>0.031</td>
<td>0.049</td>
<td>[0.003, 0.095]</td>
<td>&lt;0.05</td>
<td>0.171</td>
</tr>
<tr>
<td>Drawing trail test (MABC-2) 4 months</td>
<td>Cord</td>
<td>137</td>
<td>8.0</td>
<td>0.054</td>
<td>0.012</td>
<td>[0.004, 0.060]</td>
<td>&lt;0.05</td>
<td>0.266</td>
</tr>
<tr>
<td>Performance IQ (WPPSI-III) 4 months</td>
<td>Cord</td>
<td>138</td>
<td>110.8</td>
<td>0.036</td>
<td>0.041</td>
<td>[0.005, 0.077]</td>
<td>&lt;0.05</td>
<td>0.145</td>
</tr>
<tr>
<td>Gross motor (ASQ-III) 4 months</td>
<td>Cord</td>
<td>141</td>
<td>52.5</td>
<td>0.029</td>
<td>0.034</td>
<td>[0.001, 0.066]</td>
<td>&lt;0.05</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Note: The following analyses were performed. WPPSI-III: full-scale IQ, verbal IQ, performance IQ, processing speed quotient and basal language quotient. ASQ-III: total score, communication, gross motor, fine motor, problem-solving and personal social. The manual dexterity subtest from the MABC-2: manual dexterity score, posting coins, threading beads and drawing trail. Regression analyses not reported in Table 1 were not significant. Bold style indicates significant correlations (p < 0.05).

Subjects in the highest (n = 36) and lowest quartiles (n = 36) with regard to cognitive function, motor development and behaviour. At 4 months of age, 14 children did not have complete blood tests due to technical difficulties in the test situation. The neurodevelopmental assessment was not complete in eight children because they were tired or unwilling during testing.

Means were used to compare the WPPSI-III, ASQ-III and MABC-2 for continuous standardised test scores, and the independent samples t-test was used for group comparisons. The Mann-Whitney U test was used for group comparisons for any SDQ ordinal scale variables that were not standardised. For background characteristics, the independent samples t-test was used for group comparisons, with numeric variables, and the χ² test was used for categorical variables.

The statistical analyses were performed using SPSS statistics for Windows, version 26.0 (IBM Corp, New York, USA), and p < 0.05 was considered significant.

### Osteocalcin and motor development

Linear regression modelling showed an association between serum osteocalcin at 4 months and the ASQ-III gross motor...
subdomain at 4 years of age, but not for the ASQ-III score in total (Table 1). At this age, children with an osteocalcin level in the highest quartile scored 4.3 points higher for gross motor skills than children in the lowest quartile \( p < 0.05 \); Table 3). There were nine (26%) children with serum osteocalcin levels in the lowest quartile who scored below −1 SD (±45) on the gross motor subdomain. No children scored below −1 SD in the highest osteocalcin quartile \( p < 0.001 \). Motor development was assessed by the MABC-2 and serum osteocalcin at 4 months, and this showed that the drawing trail subtest was significant \( p < 0.05 \), with a coefficient of determination of 6.1% and Spearman correlation of 0.266 (Table 1). Linear regression modelling did not find any associations between serum osteocalcin at 4 months and the other subtests in the MABC-2.

Linear regression modelling showed a negative association between serum osteocalcin in cord blood samples and the fine motor subtest in the ASQ-III and threading beads in the MABC-2. The respective coefficients of determination were 4.2% and 4.6% \( p < 0.05 \). No other associations were found for cord samples and motor development.

There were no associations between any of the motor developmental assessments and serum osteocalcin at 12 or 36 months.

### 3.3 Osteocalcin and behaviour

When high and low serum osteocalcin levels were analysed in relation to the SDQ scores for the total as well as the individual subdomains, there were no differences between the quartile groups for any of the four time points: from birth to 36 months of age.

### 3.4 Representativeness of the population

The study subjects were compared with all the children born vaginally at full term in the region during the study period. They were similar to the general population in all aspects but birth weight, the mothers’ ages and parental educational level. The children we studied were an average of 100 g heavier at birth, their mothers were 1.6 years older and the educational level of the parents was higher than the general population \( p < 0.05 \); Table 4).\(^4\) However, when we compared the first and fourth quartiles, there was no difference in birth weight \( p = 0.259 \), mothers’ age \( p = 0.665 \) or parental educational level \( p = 0.506 \) between the groups.

### 4 DISCUSSION

High levels of serum osteocalcin at 4 months of age were associated with higher IQ at 4 years of age, and this association remained after the data were adjusted for the child’s sex and parental educational level. The 4-month serum osteocalcin level also showed a significant association with improved gross motor control, but no association was found for behaviour at 4 years of age. In addition, serum osteocalcin in cord blood showed negative associations with processing speed and fine motor development at 4 years of age. This finding suggests that if osteocalcin has any impact on neurodevelopment in humans, the impact may vary during different critical periods of development.

Full-scale IQ is based on verbal IQ, performance IQ and processing speed; although processing speed and verbal IQ were not significant individually, the overall full-scale IQ was significant. We suggest that full-scale IQ together with verbal IQ, performance IQ, processing speed but not basal language quotient may be considered to reflect spatial visualisation, as well as short-term and long-term memory. All these abilities are related to the hippocampal brain structures. With full-scale IQ and performance IQ showing an association to osteocalcin at 4 months would mean that our results were in line with the earlier mouse study.\(^4\)

Children with a high serum osteocalcin level at 4 months scored higher for the drawing trail test at 4 years. This subtest requires high levels of coordination, as smooth movement is required. Smooth movements are directed by the basal ganglia, the same structure that

---

**Note:** Bold style indicates significant \( p \) values.

**Abbreviations:** CI, confidence interval; IQ, intelligence quotient.

**Note:** The mean and standard deviation serum osteocalcin at 4 months of age: 98.7 ± 36.0 ng/mL.

**Table 2** Univariate and multivariate linear regression model with full-scale IQ at 4 years of age as the dependent variable in relation to total serum osteocalcin at 4 months of age as well as the child’s sex and parental educational level

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full-scale IQ (95% CI)</td>
<td>Unstandardised B coefficient (95% CI)</td>
</tr>
<tr>
<td>Serum osteocalcin at 4 months(^a)</td>
<td>112.2 [107.4, 117.0] 0.05 [0.0, 0.1]</td>
<td>0.038</td>
</tr>
<tr>
<td>Female sex</td>
<td>115.3 [112.1, 116.5] 4.8 [1.8, 7.8]</td>
<td>0.002</td>
</tr>
<tr>
<td>Both parents with a high educational level</td>
<td>114.6 [112.4, 116.8] 5.5 [2.2, 8.9]</td>
<td>0.001</td>
</tr>
</tbody>
</table>
has demonstrated microstructural changes due to serum osteocalcin levels in humans. In contrast, we found that a high serum osteocalcin level in cord blood was associated with significantly decreased fine motor control at 4 years of age. However, both the fine motor subdomain for the ASQ-III and the MABC-2 threading beads tests were significantly lower ($p < 0.05$). There is some uncertainty around cord blood, as it could be influenced by child, mother and placental factors. In the current study, serum was extracted from the venous part of the umbilical cord, which mostly contains infant blood. If there is causality between serum osteocalcin levels and neurodevelopment, this contradiction might imply that osteocalcin plays different roles during different developmental periods. This pattern is not unusual in human medicine. For instance, one study showed that hormone adiponectin levels in cord blood were positively associated with birth weight, but the association was negative at a later age. Another aspect worth highlighting is that no associations were found for later osteocalcin levels, namely, at 12 and 36 months, and with later neurodevelopment. The reason for this is unknown. However, we could speculate that these time points were too late in relation to the brain growth spurt to show an association, if there is a causality between osteocalcin and later neurodevelopment.

Serum osteocalcin levels did not seem to be associated with later behaviour, as assessed by the SDQ. The mice model seemed to indicate that deprivation of osteocalcin induced depression and anxiety-like behaviour. If the results were comparable, we could have expected to see some tendencies in the SDQ emotional difficulties subdomain for children with the lowest osteocalcin levels in our study. However, no differences were seen based on serum osteocalcin levels. This raises questions about whether we can compare animal and human studies with regard to behaviour and osteocalcin. Was the behaviour anomaly in the mice model due to a complete lack of osteocalcin and not just low levels? Other questions included whether the screening tools we used, which were to designed to find children who needed further assessment, were too basic to answer the research question. We could also speculate about whether our cohort was too small and too healthy.

### TABLE 3 Comparison of mean scores from the neurodevelopment assessment at 48 months and children in the first and fourth quartiles for osteocalcin levels at 4 months of age

<table>
<thead>
<tr>
<th>Test score</th>
<th>Low osteocalcin (1st quartile $n = 36$)</th>
<th>Mean (SD) No.</th>
<th>High osteocalcin (4th quartile $n = 36$)</th>
<th>Mean (SD) No.</th>
<th>Mean difference (95% CI)</th>
<th>$p$ value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPPSI-III$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-scale IQ</td>
<td>113.2 (9.2) 35</td>
<td>118.8 (8.8) 34</td>
<td>5.6 [1.3, 9.9]</td>
<td><strong>&lt;0.05</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>117.4 (10.9) 35</td>
<td>122.7 (12.5) 34</td>
<td>5.3 [−0.3, 11.0]</td>
<td>0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance IQ</td>
<td>112.1 (7.5) 35</td>
<td>116.2 (7.0) 34</td>
<td>4.1 [0.6, 7.6]</td>
<td><strong>&lt;0.05</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed quotient</td>
<td>97.8 (7.4) 33</td>
<td>101.7 (9.3) 34</td>
<td>3.9 [−0.2, 8.0]</td>
<td>0.061</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal language quotient</td>
<td>112.9 (10.8) 33</td>
<td>113.1 (10.7) 32</td>
<td>0.2 [−5.2, 5.5]</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASQ-III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>273.0 (28.8) 36</td>
<td>282.7 (17.5) 35</td>
<td>9.7 [−1.6, 21.0]</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>56.0 (7.4) 36</td>
<td>58.2 (4.2) 36</td>
<td>2.2 [−0.6, 5.1]</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross motor</td>
<td>53.3 (10.1) 36</td>
<td>57.6 (3.5) 36</td>
<td>4.3 [0.7, 7.9]</td>
<td><strong>&lt;0.05</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine motor</td>
<td>52.6 (8.5) 36</td>
<td>55.1 (8.2) 35</td>
<td>2.5 [−1.4, 6.5]</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problem-solving</td>
<td>55.8 (6.6) 36</td>
<td>56.9 (6.0) 35</td>
<td>1.0 [−2.0, 4.0]</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal social</td>
<td>55.3 (7.4) 36</td>
<td>54.8 (7.4) 36</td>
<td>−0.4 [−3.9, 3.0]</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABC-2$^d$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manual dexterity score</td>
<td>8.0 (2.1) 35</td>
<td>8.6 (2.9) 34</td>
<td>0.6 [−0.6, 1.8]</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posting coins:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preferred hand</td>
<td>7.7 (2.6) 35</td>
<td>7.8 (3.3) 34</td>
<td>0.1 [−1.3, 1.5]</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-preferred hand</td>
<td>8.3 (3.0) 35</td>
<td>8.3 (3.6) 34</td>
<td>−0.0 [−1.6, 1.5]</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threading beads</td>
<td>7.8 (2.9) 35</td>
<td>7.8 (3.9) 34</td>
<td>−0.0 [−1.7, 1.6]</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drawing trail</td>
<td>8.7 (1.7) 35</td>
<td>9.8 (1.5) 34</td>
<td>1.1 [0.4, 1.9]</td>
<td><strong>&lt;0.005</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


$^a$Note that quartile groups were based on total serum osteocalcin, and the number of participants differed due to some tests not being completed.

$^b$p values were calculated using the independent samples $t$-test, and significant values are indicated in bold style.

$^c$WPPSI-III Swedish edition.

$^d$The manual dexterity area for the MABC-2.
Our study had a number of limitations. Due to the novelty of this topic, we designed this study to examine early osteocalcin levels and its potential associations to neurodevelopment in a broad manner. Multiple testing always involves risks of type I errors. The impact or presence of this being difficult to establish, but the results for the 4-month osteocalcin levels and later neurodevelopment were similar to what we expected, based on earlier studies. 4–6,7

To date, little is known about the natural pattern and influencing factors of osteocalcin during childhood. Blood sugar was not measured in this study, and the children did not fast before testing. Neither was information about potential maternal diabetes gathered. This might be considered a limitation because of the involvement that osteocalcin has in glucose metabolism. However, it is not known whether these factors directly influence osteocalcin levels in children. Furthermore, serum osteocalcin can be measured as total osteocalcin, as seen in this study, or undercarboxylated osteocalcin. Recent studies have suggested that it is undercarboxylated osteocalcin, also called uncarboxylated osteocalcin, which plays an active role in the brain, but this has not been proven. 4 However, there is no set relationship between these two forms of osteocalcin, and it is not possible to calculate the amount of undercarboxylated osteocalcin from total serum osteocalcin measurements. That is why the study design could not be changed afterwards. 19 Therefore, the potential impact that using total serum osteocalcin would have had was difficult to estimate. In addition, the lack of serum osteocalcin reference values in children influenced the choice of study design and the comparison between children with the highest and lowest quartiles. This resulted in the original sample size of 158 to 72 children, because 14 did not have complete osteocalcin levels. Nevertheless, weak but significant associations were found for osteocalcin at 4 months of age and full-scale IQ at 4 years of age. The full-scale IQ was 5.6 points higher in the top serum osteocalcin quartile than in the lowest quartile. This difference would be of potential clinical significance if serum osteocalcin was shown to cause differences in IQ levels. Also, nine of the 36 children in the lowest quartile group for osteocalcin levels scored below −1 SD would be of potential clinical significance if serum osteocalcin was shown to cause differences in IQ levels. Also, nine of the 36 children in the lowest quartile group for osteocalcin levels scored below −1 SD for gross motor control, compared with none of the 36 children in the highest quartile group. This was a significant difference.

Families with high socio-economic status are more likely to participate in studies than those with a low socio-economic status. 20 This, together with the inclusion criteria, provided a selection bias that may explain the higher than expected result for full-scale IQ. The number of subjects that took part in this study was 61% of the 258 who had been enrolled in both of the birth cohorts. This gives a higher drop-out rate than the drop-out rates for the Growth Study (19%) and the Cord Clamping Study (31%). This suggests that some parents chose to continue with just one study because of the rigorous follow-up programmes for both studies. This resulted in a higher educational level and older mothers than the general population, which is why the representativeness of the population and the external validity may be questioned. Parental IQ was not assessed in this study, and educational level was used as a proxy. Although the representativeness of this approach may be questioned, there was no difference in the educational levels between the quartile groups that were used in this study. This selection bias is unlikely to have had an impact on the linearity or difference between quartile groups.

5 | CONCLUSION

This human study, based on two Swedish birth cohorts, showed associations between osteocalcin levels in serum and later
neurodevelopment. Because this is a new research area, it is difficult to know exactly what these findings imply, and the results should be carefully interpreted. Even though the study had some limitations, we believe that the results presented are interesting enough to warrant further research, as they were in line with earlier animal and human studies. We suggest that it would be useful to carry out future research on potential associations between osteocalcin and IQ as well as osteocalcin and motor function.

ACKNOWLEDGEMENTS

We would like to thank the families who took part in this study, research nurses Eivor Kjellberg and Monika Nygren for their important work during the clinical follow-up visits and Barbro Lindquist for carrying out the neurocognitive testing. This research project received funding from the Swedish Medical Research Council (2013-3013 and 522-7238), the Samariten Independent Foundation, the Research and Development Board, Halland Regional Council, the Halland Regional Development Council and the Västra Götaland Regional Council.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Sara Berggren https://orcid.org/0000-0002-8310-0220
Ola Andersson https://orcid.org/0000-0002-3972-0457
Lena Hellström-Westas https://orcid.org/0000-0003-3498-6069
Jovanna Dahlgren https://orcid.org/0000-0002-9637-3439
Josefine Roswall https://orcid.org/0000-0001-7269-648X

REFERENCES


How to cite this article: Berggren S, Andersson O, Hellström-Westas L, Dahlgren J, Roswall J. Serum osteocalcin levels at 4 months of age were associated with neurodevelopment at 4 years of age in term-born children. Acta Paediatr. 2022;111:338–345. doi:10.1111/apa.16151