Research Article

Age-Related Association of Calcitonin with Parameters of Anthropometry, Bone and Calcium Metabolism during Childhood

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Abstract

Introduction: The thyroid parafollicular hormone calcitonin (CT) shows particularly high blood levels in early childhood, a period of high bone turnover, which decrease with increasing age. Data about the physiological role of CT during infancy, childhood, and adolescence are contradictory or lacking. ***Objective:*** We hypothesize that CT demonstrates age-related correlations with parameters of bone growth and turnover as well as with parameters of calcium homeostasis. ***Methods:*** 5,410 measurements of anthropometric data and venous blood samples were collected from 2,636 participants of the LIFE Child study, aged 2 months–18 years. Univariate correlations and multiple regression analysis were performed between serum CT and anthropometric indicators (height standard deviation scores [SDS] and BMI-SDS), markers of calcium (Ca) homeostasis (Ca, parathyroid hormone, 25-OH vitamin D, and phosphate [P]), bone formation (procollagen type 1 N-terminal propeptide [P1NP], osteocalcin), and bone resorption (β-CrossLaps). ***Results:*** CT was significantly associated with Ca ($\beta = 0.26$, $p < 0.05$) and P1NP/100 ($\beta = 0.005$, $p < 0.05$) in children aged 2 months–1.1 years. These relations were independent of age and sex and could not be confirmed in children aged 1.1–8 years. Independent of age, sex, puberty, P, and height SDS CT showed a significant positive relation to Ca ($\beta = 0.26$; $p < 0.001$) in children aged 8–18 years. ***Conclusions:*** Our findings suggest a unique association between CT and Ca in periods of rapid bone growth and point to a possible involvement of CT in promoting bone formation during the first year of life.

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Introduction

Calcitonin (CT) is a peptide hormone which is produced by the C-cells of the thyroid gland. The release of CT is stimulated by an acute rise in serum calcium (Ca) levels [1, 2].

The biological effect of CT is to maintain Ca homeostasis together with parathyroid hormone (PTH) and vitamin D as it lowers the elevated blood Ca concentration [2] due to its inhibitory effect on bone resorption medi-

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ated by osteoclasts [3–6]. Friedman and Raisz [5] who cultured bone shafts of rat embryos in a medium with CT observed a suppression of bone resorption after adding PTH. Furthermore, CT acts on kidney by not only increasing Ca and phosphate (P) excretion [7] but also by stimulating 1,25-dihydroxyvitamin D_3 production [8]. Davey and Findlay [9] suggest 2 roles for CT: regulation of first bone turnover to protect the skeleton and second of Ca homeostasis.

There is only poor evidence for the assumption that CT could act strongly age-dependent. Cooper et al. [10] discovered a more pronounced effect of CT on serum Ca in rats 5 weeks old compared to older rats. Moreover, an indirect clue for an age-dependent functional role of CT was the observation of a 5-fold decrease in serum CT from the neonatal period to adolescence [11].

Concomitant with the age dependency, CT effects appear to be dependent on bone turnover rate: In healthy human adults who have physiologically distinctly lower rates of bone turnover than children no effect of CT injection on serum Ca level was found [7]. Interestingly, neither thyroidectomized patients without detectable CT nor patients with medullary thyroid carcinoma (MTC) and high CT blood levels revealed a disturbance of their skeletal homeostasis [9, 12]. However, in states of high rates of bone turnover we hypothesize that CT responds to changes of serum biomarkers for bone formation as osteocalcin (OC) and procollagen type 1 N-terminal propeptide (P1NP) as well as to changes of β-CrossLaps (BCL) that reflects bone resorption. In adults with an increased bone turnover derived from a thyrotoxicosis or Paget's disease the administration of CT leads to the reduction of bone resorption and lower plasma Ca level [7, 13].

Infants and children, especially within the first years of life and during puberty demonstrated a higher bone metabolism than in later life [14] and therefore we assume that their CT values were functionally regulated. Data about the biological effect of CT and about the underlying mechanism for its upregulation during infancy were contradictory or lacking so far. Furthermore, most of the studies which had a focus on the physiology and pathophysiology of CT in childhood were published in the 1970s and 1980s with only a small number of subjects. Previous findings should be reevaluated given the availability of improved immunoassays for measuring CT.

Accordingly, we hypothesize that CT is age-dependently involved in (1) growth, (2) Ca homeostasis, and (3) bone turnover of infants and children. Therefore, we investigated if the specific biomarkers (1) height standard deviation scores (SDS) and BMI-SDS; (2) Ca, PTH, 25-OH vitamin D (25OHD), and P as well as (3) OC and P1NP and BCL are correlated to serum CT levels in different age groups.

Materials and Methods

Research Design and Participants
Infants, children, and adolescents of the LIFE Child study, a population-based longitudinal cohort study at the Leipzig Research Center for Civilization Diseases, were included into our investigations between 2011 and 2017. Additional information can be found in Poulain et al. [15] and Quante et al. [16].

Exclusion criteria were endocrine disorders; diseases of bone and muscles, of kidney and liver, of the cardiovascular system, of the gastrointestinal tract; coagulation disorders, and mental illnesses. Subjects taking drugs interfering with hormonal or bone metabolism were also excluded. As a result, the study cohort consisted of 2,636 participants aged approximately 2 months–18 years (1,236 females and 1,400 males) with 5,410 measurements (2,486 females and 2,924 males). The cohort was divided into 4 prepubertal age groups: (1) 2 months–1.1 years; (2) 1.1–4 years; (3) 4–6 years; (4) 6–8 years and into 5 pubertal stage-dependent subgroups (aged 8–18 years). Detailed numbers of subjects are shown within tables and figures. The first age group was extended to the age limit of 1.1 instead of 1 year due to the allowed age range of the 1-year visit date within our study (1 year ± 1 month).

Anthropometric Measurements
Anthropometric data were collected by trained and certified study assistants following a standardized protocol. For the calculation of BMI, the following formula was used: bodyweight (kg) divided by height (m^2). Age- and sex-specific SDS values of height and BMI were calculated according to tables of Kromeyer-Hauschild et al. [17]. Pubertal status was evaluated under the terms of Tanner stages (I–V) [18, 19].

Laboratory Parameter
CT, P1NP, OC, 25OHD, BCL, and PTH were measured using the Cobas ECLIA system (electrochemiluminescence immunoassay method, Cobas, Roche, Mannheim) method. Levels of total Ca were quantified by photometric color tests, P was measured by a photometric UV test by the Cobas clinical chemistry unit. Coefficients of variations for measurements of the same patients' sera were published recently [20, 21].

Statistical Analysis
All data were processed using R [22] version 3.3.3 (2017-03-06). Descriptive statistics were given as mean and standard deviation. Due to considerable skewness, CT was log-transformed (logCT). Univariate relationships were estimated using Spearman's rank correlation coefficient. In correlation analyses only the first measurement per child was considered.

Multivariable relationships were estimated applying linear mixed effect models as implemented in the lme4 package [23]. To account for multiple measurements per subject, the subject was included as random intercept. All models were adjusted for age and sex. The *p* values were estimated using normal approximation

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Table 1. Characteristics of anthropometric and biochemical data on the study cohort (mean values±standard deviation) in age-related subgroups

	Age range				
	0.17–1.1 years	>1.1–4 years	>4–6 years	>6–8 years	>8–18 years
Boys					
Age, years	0.56±0.31	2.66±0.72	5.00±0.60	7.02±0.60	12.2±2.58
Height, cm	68.8±6.42	93.2±7.49	111±6.01	126±6.27	157±16.6
Height SDS	0.34±1.00	−0.11±0.97	−0.01±0.96	0.25±0.95	0.28±1.01
BMI, kg/m^2	16.4±1.28	16.2±1.29	15.5±1.21	16.0±2.42	19.5±4.32
BMI-SDS	−0.07±0.95	0.23±0.85	−0.05±0.79	−0.04±1.04	0.19±1.14
CT, ng/L	13.8±7.64	6.13±3.50	5.01±2.75	4.69±2.85	3.17±2.46
logCT, ng/L	1.07±0.25	0.72±0.25	0.64±0.24	0.59±0.27	0.36±0.38
Calcium, mmol/L	2.59±0.09	2.46±0.08	2.45±0.08	2.45±0.08	2.46±0.09
Phosphate, mmol/L	1.84±0.17	1.65±0.15	1.57±0.12	1.54±0.13	1.47±0.18
PTH, pmol/L	2.61±1.27	2.99±1.00	3.03±1.00	3.02±0.96	3.45±1.26
25OHD, ng/mL	43.6±16.2	26.3±12.2	23.4±10.6	24.0±9.44	22.6±10.3
P1NP, ng/mL	1,724±747	651±186	599±152	567±144	627±295
OC, ng/mL	123±42.1	73.4±20.7	81.8±19.4	86.7±19.5	113±45.7
BCL, pg/mL	1,027±206	1,126±335	1,458±356	1,517±344	1,778±548
Girls					
Age, years	0.56±0.30	2.72±0.72	5.04±0.62	7.04±0.56	12.4±2.53
Height, cm	66.9±6.43	92.4±7.31	110±6.28	125±6.68	154±13.2
Height SDS	0.16±1.02	−0.17±0.93	−0.21±0.87	0.14±1.01	0.17±0.95
BMI, kg/m^2	15.8±1.23	16.0±1.22	15.4±1.40	16.1±2.64	20.2±4.89
BMI-SDS	−0.14±0.92	0.16±0.81	−0.08±0.83	0.03±1.04	0.29±1.21
CT, ng/L	12.6±7.22	4.58±2.69	3.38±2.16	3.10±1.91	1.72±1.73
logCT, ng/L	1.03±0.26	0.59±0.26	0.44±0.30	0.40±0.31	0.06±0.38
Calcium, mmol/L	2.62±0.08	2.49±0.08	2.48±0.08	2.47±0.08	2.45±0.09
Phosphate, mmol/L	1.85±0.16	1.64±0.15	1.60±0.11	1.54±0.12	1.41±0.18
PTH, pmol/L	2.79±1.38	3.31±1.33	3.35±1.10	3.45±1.14	3.67±1.27
25OHD, ng/mL	43.3±16.7	25.1±.11.9	23.7±10.8	23.0±8.97	21.5±9.73
P1NP, ng/mL	1,649±607	655±179	620±145	607±165	485±291
OC, ng/mL	116±39.6	78.8±21.5	89.0±22.4	96.7±24.5	93.4±43.5
BCL, pg/mL	1,026±220	1,097±328	1,494±386	1,534±407	1,430±544

N (boys, aged 0.17–8 years): ≥241; *n* (boys, aged >8–18 years): ≥1,516; *n* (girls, aged 0.17–8 years): ≥156; *n* (girls, aged >8–18 years): ≥1,306. CT, calcitonin; SDS, standard deviation score; log, logarithmized; PTH, parathyroid hormone; 25OHD, 25-hydroxyvitamin D; P1NP, procollagen type 1 N-terminal propeptide; OC, osteocalcin; BCL, β-CrossLaps.

as implemented in the multcomp package [24]. All *p* values were corrected for multiple testing using the method of controlling for the false discovery rate [25]. Statistical tests with *p* values <0.05 were considered as statistically significant.

Results

Characteristics of the Study Population (Age: 2 months–18 years)

We included laboratory and anthropometric parameters of a total amount of 5,410 measurements (2,924 boys and 2,486 girls) (Table 1). As expected, the mean CT values (and mean logCT values) were higher in boys compared to the girls in all age groups. Overall, the boys were on average 0.3 years younger than the girls (boys = 8.78 years, girls = 9.08 years); height SDS was +0.22 in boys versus +0.10 in girls and BMI-SDS +0.12 in boys versus +0.18 in girls.

Univariate Correlation Analysis
Anthropometric Parameter versus CT

As hypothesized, height SDS was positively associated with CT in children aged 2 months–1.1 years but reached

Table 2. Univariate correlations of CT with markers of anthropometry, calcium homeostasis, and bone metabolism within the study cohort in age-related subgroups

	Age range[x]			
	rho[a] (0.17–1.1 years; b/g)	rho[a] (1.1–4 years; b/g)	rho[a] (>4–6 years; b/g)	rho[a] (>6–8 years; b/g)
Anthropometry				
Age	**−0.53***/−0.65***	**−0.21**/−0.30***	0.07/−0.08	−0.02/−0.01
Height SDS	**0.16***/0.09	−0.11/−0.05	**−0.20**/−0.10	**−0.16***/−0.12
BMI-SDS	**0.23**/0.09	0.13/0.09	−0.08/0.11	−0.13/0.02
Calcium homeostasis				
Calcium	**0.25**/0.13	0.04/0.04	−0.02/−0.07	−0.03/−0.00
Phosphate	0.15/0.17	−0.03/0.15	**−0.18***/−0.07	−0.03/**−0.15***
PTH	**−0.22***/−0.37***	−0.03/0.08	0.02/0.06	0.13/0.02
25OHD	−0.17/0.04	−0.00/0.02	0.04/0.15	−0.00/0.08
Bone markers				
P1NP	**0.50***/0.42***	−0.02/−0.08	−0.10/−0.05	−0.14/0.03
OC	**0.36***/0.35***	−0.10/−0.07	**−0.16***/−0.03	−0.12/−0.02
BCL	0.00/0.06	−0.13/−0.07	−0.15/−0.02	−0.08/−0.09

Results of boys and girls were shown separately (b/g). For all subgroups, $n = 106$–263. All significant correlation coefficients are marked in bold. CT, calcitonin; SDS, standard deviation score; PTH, parathyroid hormone; 25OHD, 25-hydroxyvitamin D; P1NP, procollagen type 1 N-terminal propeptide; OC, osteocalcin; BCL, β-CrossLaps. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. [a] Indicates Spearman's rank coefficient. [x] The univariate correlations of the age group 8–18 years can be found in the supplement.

significance only in boys. In contrast, a significantly negative association was found in boys aged 4–6 years and 6–8 years (Table 2) and in boys and girls aged 8–18 years (online suppl. Table 5; for all online suppl. material, see www.karger.com/doi/10.1159/000512107). BMI-SDS was significantly positively correlated with CT in boys aged 2 months–1.1 years. In contrast, 8- to 18-year-old children and adolescents showed a significantly negative correlation.

Calcium Homeostasis versus CT

In boys aged 2 months–1.1 years we detected a positive and significant correlation between Ca and CT (Table 2). In girls of the same age the correlation was also positive but did not reach significance ($p = 0.17$). Likewise, girls aged 8–18 years Ca showed a positive and significant correlation with CT while this correlation was significantly negative in same-aged boys (online suppl. Table 5). P was significantly negatively associated with CT in boys aged 4–6 years and girls aged 6–8 years and significantly positively in girls aged 8–18 years. In the age group 2 month–1.1 years PTH was also significantly negatively correlated with CT and Ca in both sexes but we did not

find any significant correlations in older children. In the pubertal group 25OHD showed a positive and significant correlation with CT in both sexes.

Bone Markers versus CT

We found significantly positive correlations of P1NP and OC with CT in boys and girls aged 2 months–1.1 years (Table 2) and a significantly negative correlation between OC and CT in boys aged 4–6 years. In the pubertal group there was a switch to significantly negative correlations of P1NP, OC, and BCL with CT in boys while these correlations were significantly positive in girls (online suppl. Table 5).

Multiple Regression Analysis

After adjustment for age and sex, from the anthropometric parameters, height-SDS remained independently negatively correlated in age groups 4–6 years and 6–8 years. BMI-SDS was significantly positively correlated in children aged 1.1–4 years and significantly negatively correlated in the age group 6–8 years (Table 3).

In the group of parameters of the Ca homeostasis the positive correlation between CT and Ca in the group 2

Table 3. Multiple linear regression of CT levels of the study cohort with markers of anthropometry, calcium homeostasis, and bone metabolism adjusted for age and sex (and for the age group 8–18 also for puberty) in age-related subgroups

	Age range				
	β^b 0.17–1.1 years	β^b >1.1–4 years	β^b >4–6 years	β^b >6–8 years	β^b >8–18 years
Anthropometry					
Height SDS	−0.02	−0.01	**−0.05****	**−0.04****	**−0.06****
BMI-SDS	0.01	**0.03***	0.01	**−0.02***	**−0.04****
Calciumhomeostasis					
Calcium	**0.24***	0.13	0.12	0.08	**0.17***
Phosphate	0.08	−0.08	−0.17	**−0.18***	**−0.22****
PTH	−0.01	0.01	−0.01	0.003	**−0.01***
25OHD	−0.0004	−0.0002	0.001	0.001	**0.002****
Bone markers					
P1NP/100	**0.004***	**−0.01***	**−0.02***	−0.01	**−0.01****
OC	**0.001***	−0.001	−0.0005	−0.0004	**−0.0009****
BCL	-4×10^{-5}	-3×10^{-5}	-4×10^{-5}	-3×10^{-7}	**-7×10^{-5}****

CT, calcitonin; SDS, standard deviation score; PTH, parathyroid hormone; 25OHD, 25-hydroxyvitamin D; P1NP, procollagen type 1 N-terminal propeptide; OC, osteocalcin; BCL, β-CrossLaps. n for the subgroups 0.17–8 years = 402–635; n for those >8–18 years = 2,072–2,368. All significant regression coefficients are marked in bold. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. [b] Indicates the regression coefficients (regression coefficients are adjusted for age and sex (for the age group 8–18 also for pubertal stages).

months–1.1 years was still significant (Fig. 1). In 6–8 years old children the correlation between P and CT was significantly negative.

From the bone markers the positive correlation of OC and P1NP with CT continued to be significant in children aged 2 months–1.1 years. In children aged 1.1–6 years there was a significantly negative correlation between CT and P1NP.

In the age group 8–18 years CT was significantly positively associated with calcium and 25OHD and significantly negatively with height-SDS, BMI-SDS, P, PTH, P1NP, osteocalcin, and BCL after adjusting for age, sex, and puberty.

Combined Multiple Linear Regression Model

After including all significantly associated parameters (age- and sex-adjusted models) in a final model, we found significantly positive correlations of Ca and P1NP with CT in the age group 2 months–1.1 years (Table 4).

For the age group 6–8 years negative correlations remained significant for height SDS (β = −0.04**) and P (β = −0.18*) after the inclusion of age, sex, height SDS, and P as independent variables.

For the group of 8–18-year-old subjects, the final model included age, sex, Ca, P, height SDS, and pubertal stag-
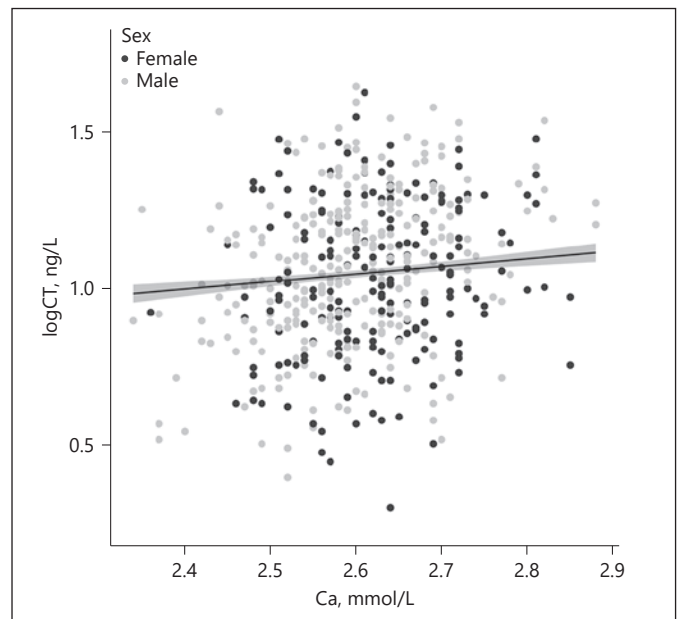
Fig. 1. Data of logCT versus calcium (Ca) in the age group of 2 months–1.1 years adjusted for age and sex (multiple measurements per subject were considered). The solid line characterizes the logCT versus calcium relationship: β = 0.24*($p < 0.05$). The grey area defines the standard error. n (boys) = 280 and n (girls) = 195. CT, calcitonin; log, logarithmized.
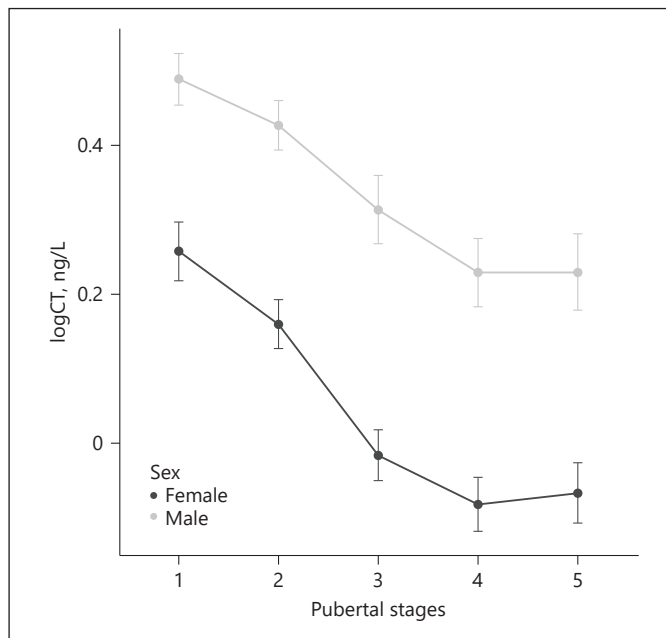
Fig. 2. Mean logCT levels during puberty adjusted for age, phosphate, calcium, height SDS, pubertal stages and stratified by sex (8–18 years). Shown are the 95% confidence intervals. Pubertal stages were evaluated according to Tanner. n (boys) = 1,054 and n (girls) = 1,263. CT, calcitonin; log, logarithmized; SDS, standard deviation scores.

Table 4. Combined multiple linear regression model for children in the age between 2 months and 1.1 years

	β^b ($n = 462$)
Age	**−0.36*****
Sex (male[c])	0.04
Calcium	**0.26***
P1NP/100	**0.005***

Age, sex, calcium, and P1NP were included as independent variables for CT as dependent variable. P1NP, procollagen type 1 N-terminal propeptide. All significant regression coefficients are marked in bold. * $p < 0.05$. *** $p \leq 0.001$. [b] Indicates the regression coefficient. [c] Girls = reference.

es as predictors. CT levels significantly decreased with advancing Tanner stages until stage 4 in both sexes and with higher levels in boys than in girls during all Tanner stages (Fig. 2). Independent of age, sex, and puberty, there was a significant negative association of CT with P ($\beta = -0.26$***) and height SDS ($\beta = -0.06$***) and a significant positive association with Ca ($\beta = 0.26$***).

Discussion

To the best of our knowledge, this is the first study to investigate correlations between CT and potential CT-associated hormones and biomarkers in an age-, sex- and puberty-dependent manner, evaluating the associations over a broad age range from 2 month to 18 years, with a large number of observations ($n = 5,410$) and the use of a high-sensitive immunoassay for measuring CT developed after 2010.

Our results present a positive relationship between Ca and CT in periods of rapid bone growth in the first year of life and during puberty. Therefore, this relationship is clearly age-related. Our data also indicate that CT could be associated with processes of bone formation but only in children aged 2 months–1.1 years. As published recently [21], CT levels showed a clear age and gender dependence with maximal levels in the first year of life and significantly higher values in boys ($p < 0.01$). An accelerated decline of CT levels from newborns to children at the age of 4 and 5 years was observed for both sexes. Therefore, we examined age group data separately, first by univariate correlations and then by multiple regression analysis to enable indirect assumptions from statistical correlations to potential physiological processes.

C-Cell Hyperplasia and CT

In general, CT may be elevated because of an increased number of C-cell in the thyroid. The higher CT concentration observed in boys may be explained by the higher C-cell density similar to that seen in men with higher CT levels [26] and higher C-cell density than in women [27]. There are only few references that estimate the C-cell density in children. In normal neonatal human thyroid glands the number of C-cells and the CT content were 10 times those observed in normal adult thyroid gland [28]. This might be a clue to the higher levels of CT in children and the sex-related differences. An increase in size and number of C cells is called C-cell hyperplasia, seen in different clinical conditions: neoplastic C-cell hyperplasia precedes and accompanies MTC in MEN2 and is associated with increased CT level; physiological C-cell hyperplasia has been described in association with a great variety of pathological conditions, for example, autoimmune thyroiditis, thyroid non-Hodgkin lymphoma, and calcium metabolism disorders [29]. The cause of C-cell hyperplasia and/or the elevated CT levels in physiological C-cell hyperplasia is unknown.

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Anthropometric Parameters and CT

The finding that premature or newborn babies have elevated serum CT levels [30–33] suggests an association of CT with anthropometric parameters of bone growth, especially in the age group of 2 months–1.1 years. This hypothesis is supported by a positive correlation between height SDS but also BMI-SDS and CT. However, after adjusting the data for age and sex, this association was no longer significant.

Calcium Homeostasis and CT

Ca and CT were positively and significantly correlated even after adjusting for age and sex in the age group 2 months–1.1 years. For this reason, we hypothesize that in periods of growth and rapid bone turnover there is a higher "CT responsiveness" [32] to changes of Ca concentration; thus, an acute increase of Ca level by osteolytic activity of PTH or absorption from the gut leads to an elevation of CT, which in turn decreases the Ca level by inhibition of osteoclasts. By this tight oscillation of Ca level by PTH and CT, the maintaining of Ca homeostasis is ensured.

In periods of high demands for Ca, for example, pregnancy, lactation, and growth, CT contributes to Ca homeostasis [34] supporting the concept of a CT-related osteoprotective role [9]. Because the bone mineral content is particularly vulnerable in these growth periods there might be a strong connection between an increase of Ca level and an elevation of CT to protect the bones against resorption.

In contrast, several authors described a significant negative correlation between Ca and CT in neonates at about 24 h of age [31, 35–37]. Others observed either no or only a poor correlation between both parameters in the newborns [38, 39]. Within these case studies, CT was measured directly after birth or in postconceptional days whereas the youngest participant of our study was approximately 2 months old. After birth when the placental transfer of Ca stops, a new balance of Ca homeostasis has to be found since the Ca level diminishes for the first 24–48 h before it increases slightly and then eventually levels off [40]. So, our data can barely be compared to the results described above. Studies that deal with older infants are almost completely lacking. Additionally, a missing correlation between Ca and CT in 6–12-year-old children ($n = 37$) has been stated already in the literature [41] and supports our findings in 6–8-year-old children. Another study that measured CT in children from birth to 16 years of age found a positive correlation ($r = 0.40$) with Ca, but they did not appear to have considered the role of age as

confounder of neither the association nor CT levels itself [11]. Our negative association between P and CT in children aged 6–8 years or 8–18 years was similar as already known from 6- to 12-year-old children [41].

In contrast to CT, PTH is released into blood circulation in states of relative hypocalcemia, and it stimulates bone to deliver Ca into the circulation. Consequently, PTH should be negatively correlated to Ca as well as to CT. Our univariate correlations in the age group 2 months–1.1 years revealed these relationships. Nevertheless, we could not confirm this effect between CT and PTH in the multiple regression analysis and in older children. However, 6–12-year-old children demonstrated this relationship in a paper published approximately 40 years ago [41]. To represent vitamin D metabolism, we measured 25OHD, the precursor of the fully active 1,25-dihydroxyvitamin D (1,25OH2D). We expected to find a negative relationship between CT and 25OHD since, contrary to CT, 1,25OH2D generally increases blood calcium level via promotion of calcium absorption in the intestine and the calcium reabsorption in the kidneys.

However, as this relationship is complex because of interrelated feedback loops the correlation between these 2 parameters could be masked. In contrast, another study described a positive correlation ($r = 0.80$ $p < 0.01$), but only in cord blood of term infants, so one can barely compare the results [30].

Bone Markers and CT

The simple correlations in children aged 2 months–1.1 years showed a positive association for the bone formation markers OC and P1NP and CT (Table 2) which was confirmed in particular after adjusting for age and sex. As the relationships of height SDS, BMI-SDS, Ca, and PTH with CT were, as mentioned above, almost exclusively detected in 2 month–1.1-year-old infants, it is not astonishing that we found comparable data also for bone markers. In general, our findings were supported by Castagna et al. [42] and some last century studies that assumed a role of CT in early bone growth and mineralization [30, 42–45] notably in the first years of life when the bone growth rate is particularly high. Others only suppose a temporal relation between skeletal growth and serum CT concentrations [33] or indicate that CT does not influence bone formation as young patients with MTC and constant high blood levels of CT do not present an abnormal bone growth [46]. The effect of CT on bone is lost after constant high levels (MTC) or prolonged administration (pharmacotherapy) by downregulation of the CT recep-

tor, called "escape" phenomenon [47]. But this is not the case in acute changes of CT stimulated by acute Ca increase in blood.

Interestingly, animal models appear to be unsuited to describe the human state as they deliver contrary data: CT appears to activate a signaling pathway that inhibits bone formation [48]. Accordingly, an increased bone formation was found in mice with ablated CT and CT gene-related peptide.

Previously, it was described that CT decreased bone resorption in periods of rapid growth [3, 4]. However, we did not find a significant correlation between CT and BCL. An explanation for this finding is that CT only heads off peaks of bone resorption.

Puberty and CT

The second important period of accelerated bone growth and therefore a potential period of a physiological CT action is puberty when bone formation rate increases approximately five-fold [49]. The peak growth velocity usually happens 2 years later in males (12–16 years) than in females (10–14 years) [50]. We found that CT levels decreased with advancing Tanner stages. Silva et al. [44] could support our findings by detecting a decrease of CT during puberty in a study of the early 1980s.

Due to heterogeneity of the puberty group their univariate correlations have to be interpreted with caution. Accordingly, we formed a combined model and adjusted our model for age, sex, puberty, P, and height SDS. By this tool and in line with the results of the first year of life, we found a significant positive relation between Ca and CT. In contrast, a recent paper with a distinctly lower number of 60 healthy adolescent girls demonstrated no relationship between Ca and CT [49]. We did not find positive associations between CT and bone formation markers in puberty; the correlations were even negative. This could possibly be due to puberty-related confounders (e.g., sex steroids) which may influence the correlation between CT and P1NP and OC.

Limitation of the Study

The major limitation of our study emerges from general restrictions of our statistical analysis. Our findings revealed only the absence or the presence of a statistical significance in regression or correlation analyses; accordingly, the study cannot prove any causal relationships between the investigated biomarkers. Although we were able to include >100 measurements per age group, we recommend confirming our results with a larger study cohort and children with growth or bone disorders.

Conclusion

Periods of rapid bone growth are an extremely vulnerable period for maintaining bone density and therefore they have to be strongly regulated. We found a significant positive relationship between CT and Ca which suggests an age-dependent osteoprotective role of CT. The positive correlation between CT and bone formation markers in the first year of life indicates a possible involvement in growth-stimulating processes.

Statement of Ethics

The study was designed in accordance with the Declaration of Helsinki (1964) and its later amendments. It was approved by the Ethics Committee of the University of Leipzig under registration No. 264-10-19042010. The LIFE Child study is registered with the trial number NCT02550236. All parents and subjects older than 12 years have given their informed written consent.

Conflict of Interest Statement

The authors have nothing to disclose.

Author Contributions

Design of the study: J.K. and J.S. Data collection: F.E., M.G., and J.S. Data calculation: M.V. and J.S. Interpretation of the results: J.K. and J.S. Writing of the manuscript: J.S., supported by J.K. Revision of the manuscript: M.V., M.G., F.E., A.K., F.R., W.K., and J.K. Supervision of the project: W.K.

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