Full Length Article

Pediatric reference values of alkaline phosphatase: Analysis from a German population-based cohort and influence of anthropometric and blood parameters

Jacqueline-Michéle Strauch [a,b], Mandy Vogel [a,c], Christof Meigen [a,c], Uta Ceglarek [a,d], Jürgen Kratzsch [a,d], Anja Willenberg [a,d], Wieland Kiess [a,b,c,e,*]

[a] *Leipzig Research Center for Civilization Diseases – LIFE, University of Leipzig, Philipp-Rosenthal-Str. 27, 04103 Leipzig, Germany*
[b] *Faculty of Medicine, University of Leipzig, Augustusplatz 10, 04109 Leipzig*
[c] *Center of Pediatric Research (CPL), University of Leipzig, Liebigstr. 20a, 04103 Leipzig, Germany*
[d] *Institute of Laboratory, Medicine, Clinical Chemistry and Molecular Diagnostics (ILM), University of Leipzig, Paul-List-Str. 13-15, 04103 Leipzig, Germany*
[e] *Hospital for Children and Adolescents, University of Leipzig, Leipzig, Liebigstr. 20a, 04103 Leipzig, Germany*

ARTICLE INFO

ABSTRACT

Background: Due to different growth and metabolic processes, reference values of alkaline phosphatase (AP) for children aged 3 month to 18 years are dependent on age and sex. They are not constant and differ from those of adults due to the growth processes taking place. Accordingly, reference levels of AP continuous across these ages were generated for boys and girls based on of a large German health- and population-based study, LIFE Child. We considered AP at different growth and Tanner stages and additionally its association with other anthropometric parameters. The association between AP and BMI was of particulary great interest due to controversial literature on this topic. The role of AP in liver metabolism was investigated by examining ALAT, ASAT, and GGT.
Methods: 3976 healthy children (12,093 visits) were included from the LIFE Child study from 2011 to 2020. The subjects´ age ranged from 3 months to 18 years. Serum samples from 3704 subjects (10,272 cases, 1952 boys and 1753 girls) were analysed for AP after applying specific exclusion criteria. After calculating of reference percentiles, associations between AP and height-SDS, growth velocity, BMI-SDS, Tanner stage and the liver enzymes ALAT, ASAT and GGT were examined via linear regression models.
Results: In the continuous reference levels, AP showed a first peak during the first year of life, followed by a plateau at a lower level until the start of puberty. In girls, AP increased beginning at the age 8, with a peak around 11 years, in boys beginning at the age 9, with a peak around age 13. Afterwards, AP values decreased continuously until age 18. In Tanner stages 1 and 2, AP levels did not differ between the two sexes. We found a strong positive association between AP-SDS and BMI-SDS. We also observed a significantly positive association between AP-SDS and height-SDS, which was stronger in boys than in girls. We found different intensities in the associations of AP with growth velocity depending on age group and sex. Furthermore, we found a significantly positive association between ALAT and AP in girls but not in boys, whereas ASAT-SDS and GGT-SDS were significantly positively associated with AP-SDS in both sexes.
Conclusion: Sex and age, but also BMI may act as confounding factors for AP reference ranges. Our data confirm the remarkable association between AP and growth velocity (or height-SDS, respectively) during infancy and puberty. In addition, we were able to specify the associations between AP and ALAT, ASAT, and GGT and their differences in both sexes. These relations should be considered when evaluating liver and bone metabolism markers, especially in infancy.

---

1. Introduction

Alkaline phosphatase (AP) is a ubiquitous human enzyme that exists in 4 isoforms. There are only three tissue-specific isoenzymes: intestinal alkaline phosphatase, AP of germ cells and the placenta. Tissue specificity of the 4th isoform, which has only about 50 % genetic homology to the others, is achieved via post-translational modifications. These processes result in characteristic enzymes in the liver and bone, among other areas. The total AP circulating freely in the blood is composed of approximately 50 % each of liver and bone alkaline phosphatase. In children under the age of 15, especially during periods of higher growth velocity (GV) the proportion of bone-specific alkaline phosphatase increases to up to 80 % [1,2]. The exact function of AP has not yet been clarified. However, it is suspected that AP catalyses the hydrolytic cleavage of phosphate monoesters, so that the local phosphate concentration increases, which thus contributes to bone mineralisation [1,3].

In most clinical laboratories, AP is typically determined according to the IFCC method [4]. Indications for AP determination would be primarily diseases of the hepatobiliary system (e.g. cholestasis) and bone diseases (e.g. Paget's disease, rickets or bone metastases). In children, lower AP levels are of great importance for diagnosing hypophosphatasia [5].

Serum concentrations of AP are higher in healthy children than in healthy adults due to growth and increased bone metabolism in childhood [6–8].

The establishment of reference values proceeds from the analysis of samples from a representative healthy cohort between the 2.5th and 97.5th percentile [9,10]. Estimating reference values for laboratory measures in children is difficult because research on healthy participants under 18 years is subject to strict ethical standards, while the blood withdrawal necessary for such research represents a non-essential invasive procedure [10,11]. Therefore, available reference intervals of alkaline phosphatase for children are often restricted to certain age ranges. Moreover, they rely on the partition into age intervals and therefore cannot adequately capture age dynamics [12–15]. This procedure is recommended in the CLSI guidelines [9]. We sought to establish continuous reference levels because they reflect biological variation across age better than any classification into age groups. It is conceivable that inaccuracies arise at the boundaries between age groups. Some continuous reference values have already be published [8,12,16–19]. We aimed to establish new continuous, age- and sex-dependent reference values of a healthy cohort with a large age range.

The dependence of the serum concentration of alkaline phosphatase on age and sex seems to be largely undisputed in the literature. We assumed peak values during infancy and puberty because of increased physiological growth. And indeed, the increase in AP during pubertal years, in particular, is described as steeper in boys than in girls, but the timing of the increase is delayed in boys compared to girls of the same age [20]. Rauchenzauner et al. [21] described this for BAP. Moreover, the literature shows that the peak of AP is higher in male participants than in female participants [16,20,22]. To specify these findings, we analysed the association between AP and Tanner stage in more detail.

An association between AP and growth rate or body size can be assumed, since BAP is increasingly released by osteoblasts during growth phases [1]. Stanik et al. [23] described a significant association between height-SDS and BAP, and Fischer et al. [24] found an association between BAP and growth rate. We suspected a potential association of the tissue non-specific AP with growth state, and therefore analysed the association between AP and body height-SDS or growth velocity in more detail.

Due to controversies in the literature, it is difficult to make statements about the association between alkaline phosphate and body mass index (BMI) [23,25–27]. Furthermore, due to the increasing prevalence of overweight children and the current lack of understanding about the influence of BMI on AP, we investigated this relationship in more detail. In addition, we assessed the associations of AP with other enzymes of liver metabolism.

2. Material and methods

2.1. Study design and population

The data collection took place within the population-based longitudinal study "LIFE -Child" (international trial number: NCT02550236), a study focussing on the development of civilisation diseases, but primarily investigating health determinants. The cohort is made up of healthy children between 3 months and 20 years living in the greater Leipzig metropolitan area (Germany) [28,29].

The study was designed in compliance with the "Helsinki Declaration for Human Research" and was approved by the Ethics Committee of the Medical Faculty, University of Leipzig (registration number: 264-10-19042010) [11]. Informed consent for participation in the study was given by the parents of each child.

The test procedures were quality-assured based on Standard Operating Procedures (SOP) written by certified personnel. At each visit, a medical history of the subject and the family, a clinical examination, blood sampling, anthropometric measurements and measurement of vital signs, and various fitness and developmental tests and questionnaires were conducted [28–30]. The subjects included in this study were aged between 0.2 and 18 years during the relevant visits.

Exclusion criteria were liver or tumor diseases. To greater precision, we also exclude children with a history of scoliosis or other bone diseases. We further excluded children with elevated GGT and children taking AP-affecting drugs (e.g. systemic steroids, anticontraceptives, somatotropin, testoterone). Furthermore, subjects whose AP was below −3.2 SDS or above 3.2 SDS were excluded. Exclusion criteria are shown in Fig. 1. The number of subjects per age group after exclusion can be seen in Supplement 1.

2.2. Pre-analysis and laboratory assessment

Fasting blood was taken in the morning by venipuncture using a serum monovette (Sarstedt AG&Co, Germany). The analysis of AP was carried out immediately according to the manufacturer's instructions by the Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (ILM), University Hospital Leipzig, by using the Cobas C analyzer (Roche, Germany). AP was measured with a colour test according to the IFCC standard. *p*-Nitrophenyl phosphate is separated by AP into phosphate and p-nitrophenol. The resulting p-nitrophenol is directly proportional to catalytic AP activity. It is determined by measuring the increase in extinction [4].

From 2011 to 2017, the AP was determined based on the reference method by Tietz. From 2018 to 2020, the new reference method by Schumann was used [4]. As a result, the measurement results were 4.8 % higher. In the data analysis, it was decided by consensus to multiply the measurement data before 2018 by a factor of 1.048 and then include them in the calculation.

Alanine aminotransferase (ALAT, μkat/l) and aspartate aminotransferase (ASAT) were analysed using the Cobas C analyzer with a photometric UV test. Gamma-glutamyltransferase (GGT, μkat/l) was detected with the enzymatic colour test.

2.3. Anthropometric assessment

Height was routinely determined with a stadiometer ("Prof. Keller", Längenmesstechnik GmbH Limbach, Germany) accurate to 0.1 cm. Weight was measured with the "Seca 701" scale (Seca GmbH & Co., Germany, accurate to 0.05 kg). The growth rate was calculated by dividing the difference in body size by the age difference between the current study visit and previous visit. Only age differences from 0.2 to 1.5 years and growth rates from −0.01 to 35 cm/y were included. This generated 5442 dataset on GV. The body mass index (BMI) was
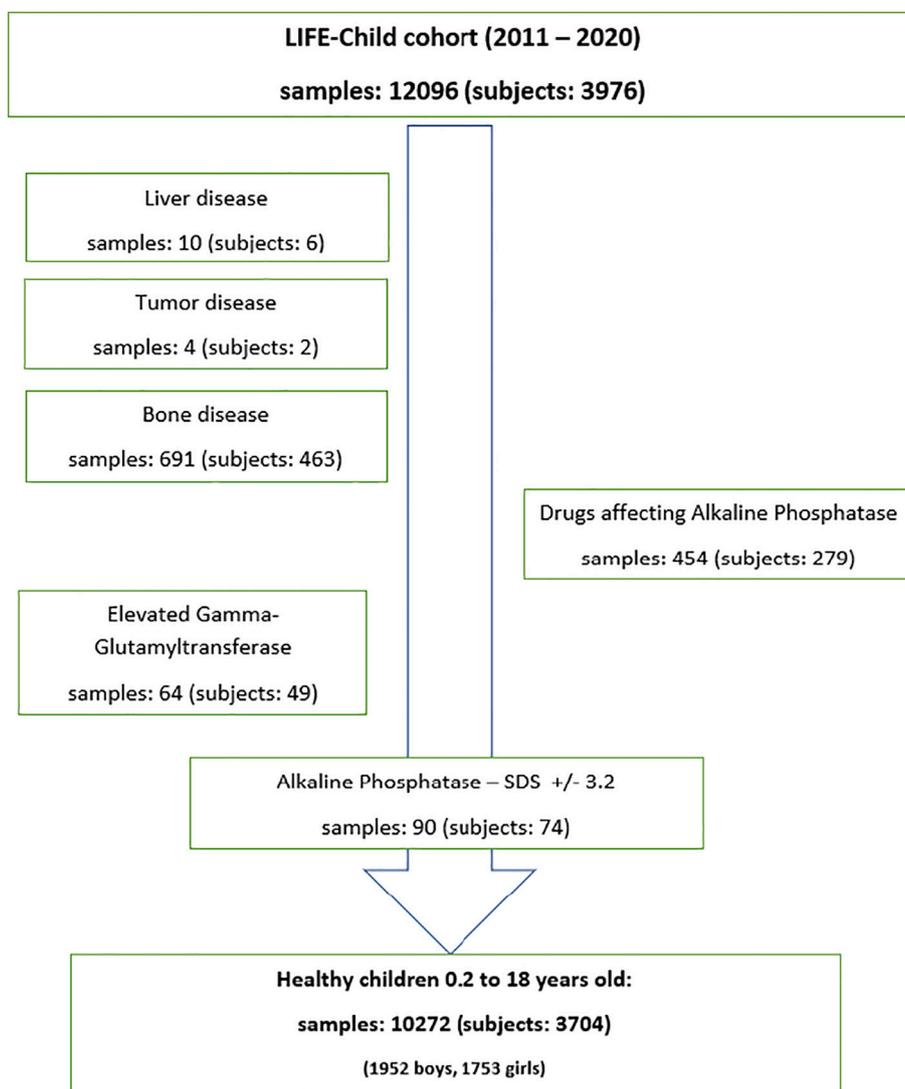
Fig. 1. Exclusion criteria in flow chart; 3976 children were included in the study between 2011 and 2020, after applying exclusion criteria we analyse a cohort of 10272 samples and 3704 subjects (1952 boys and 1753 girls).

calculated using the formula BMI = weight / height^2 [31]. Subsequently, BMI values were transformed info standard deviation scores according to the guidelines of the German Obesity Association [32]. The subjects were classified according to their pubertal status after clinical examination on the basis of sexual characteristics according to Tanner [33,34].

2.4. Statistical analyses

The estimation of age- and sex-adjusted reference values for AP was performed using generalized additive models for location scale and shape assuming a Box-Cox Power Exponential distribution [30,35,36]. We assessed the goodness of fit by visual inspection using wormplots. A resampling procedure was used, as this survey is a combined longitudinal and cross-sectional study [30]. We graphically and tabularly depicted the 2.5th, 10th, 50th, 90th and 97.5th percentiles (Fig. 2 and Supplement 2). Subsequently, AP values were transformed into age- and sex-adjusted SDS. SDS values of AP were calculated using the formula for the original LMS method as approximation: AP-SDS = $((AP/\mu)^\lambda - 1) / (\sigma \times \lambda)$ for $\lambda \neq 0$ and AP-SDS = $(1/\sigma) \times \ln(AP/\mu)$ for $\lambda = 0$ [35]. The parameters λ (L, skewness, nu), μ (M, median, mu) and σ (S, variation) depend on age and sex (Supplement 2/Table 2, AP = measured AP value). For distribution-based calculation, we provide the functions and reference values (with monthly grid points) within the R package

childsds. To assess the associations between AP and the suspected covariates, hierarchical linear regression was applied using AP-SDS as the dependent variable. The subject was added as a random effect to account for multiple measurements per subject. The assumption for linear models (linearity, normality of residuals, homoskedasticity, influential observations) was checked using diagnostic plots. We represented the strength of an association between the AP and the suspected covariates by calculating the conditional pseudo-R^2 (Supplement 3) [37]. To examine differences in AP between pubertal stages, AP values were used as outcome (without transformation into SDS because of the strong dependency between age and puberty). Because Tanner stage one comprises a relatively long time span, we divided the study population younger than 6 years into the age groups 0.2–1 years, 1–2 years, 2–6 years and older than 6 years (Fig. 4). The association between BMI-SDS, height-SDS, GV and Tanner stage with AP were tested for dependence on age and sex and the associations of ALAT-SDS, ASAT-SDS, GGT-SDS and age groups below 6 years with AP were tested for sex dependence.

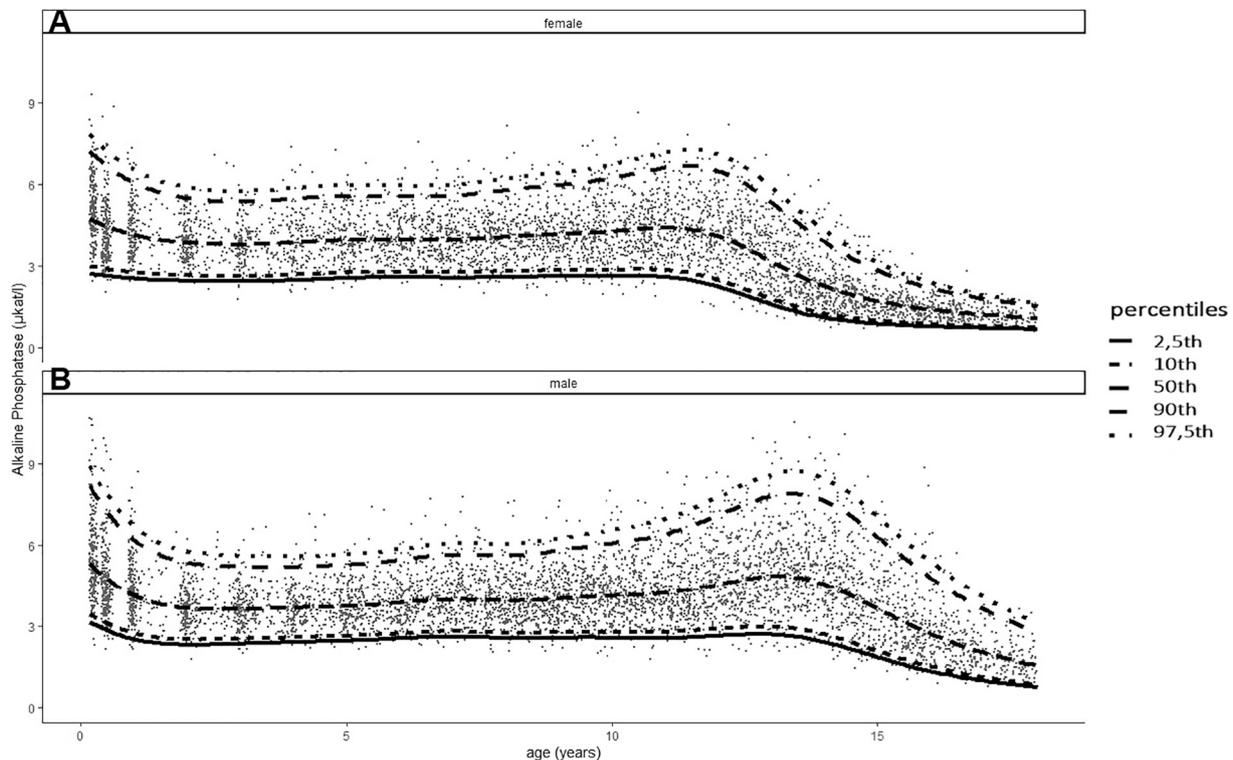Results with a p-value below 0.05 were considered statistically significant.

Fig. 2. Reference values for 3 month to 18 year old girls (A) and boys (B), 2.5th, 10th, 50th, 90th and 97.5th percentiles were shown.

3. Results

3.1. Reference intervals

Fig. 2 shows the reference values separately for boys and girls. The percentiles 2.5 and 97.5 are defined as threshold values according to the CLSI guidelines [9]. After a first peak during the first year of life, the values decreased steadily until about 2.5 years of age. From then, the levels remained fairly constant until early puberty. An increase in AP could be observed in girls beginning at age 8, with a second peak around 11 years of age (median 4.41 μkat/l). In boys, the second rise started around 9 years of age. The percentile curves reached their maximum at
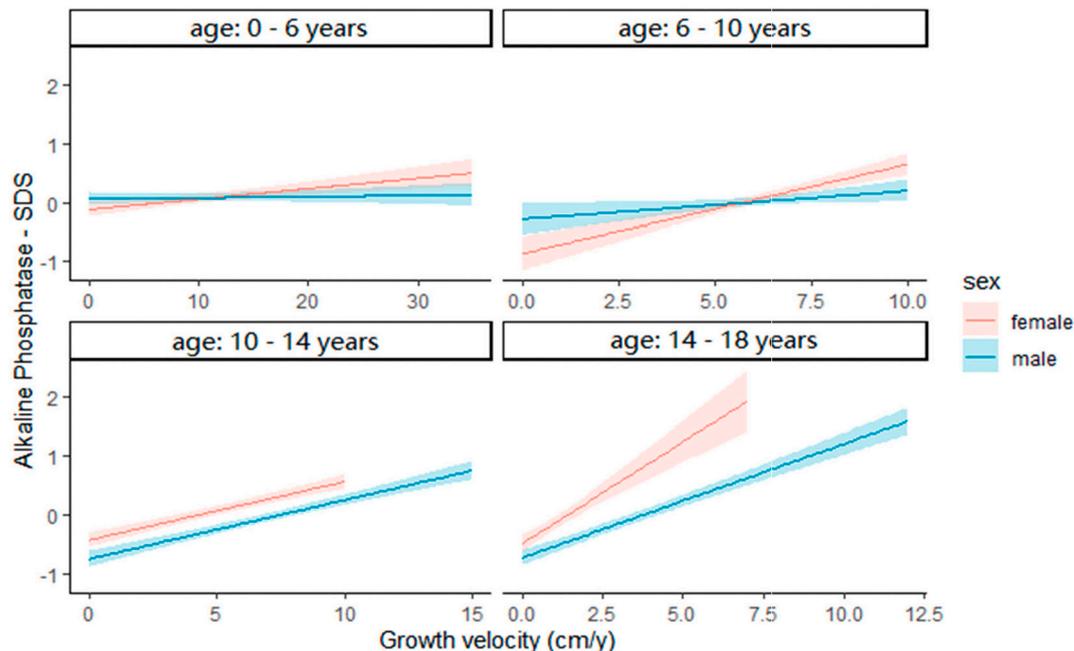

Fig. 3. Association of Alkaline Phosphatase-SDS and growth velocity in different age groups for girls (red) and boys (green): toddlers (0–6 years): no significant association was found in boys (β = 0.02, p = 0.57) but in girl (β = 0.02, p < 0.01); age group 6–10 years: a significant positive association was found in girls (β = 0.13, p < 0.01) and in boys (β = 0.05, p = 0.04); age group 10–14 years: a significant positive association was found in girls (β = 0.08, p < 0.01) and in boys (β = 0.01, p < 0.01); age group 14–18 years: a significant positive association was found in girls (β = 0.33, p < 0.01) and in boys (β = 0.19, p < 0.01); The shaded area represents the 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

age 13 (median 4.84 μkat/l). As hypothesized, the AP levels of girls in the pubertal years were lower than those of boys and the curve was flatter. For girls, the 97.5th percentile reached a maximum value of 7.19 μkat/l at 11 years of age. The highest value of the upper reference limit (97.5th percentile) in boys was reached at 13 years of age with 8.61 μkat/l. This is 19.7 % higher in boys compared to girls. After reaching their maxima, there was a constant decline in AP values in both boys and girls. The upper and lower limits reached their lowest levels around age 18 (boys 0.75–3.14 μkat/l, girls 0.69–1.65 μkat/l). The absolute values of the 2.5th, 10th, 50th, 90th, and 97.5th percentiles and the LMS parameters mu (median), sigma (variation), nu (skewness) and tau (kurtosis) are listed in Supplement 2.

3.2. Growth velocity and body size

Based on the assumption that the serum concentration of AP is also higher in phases of accelerated growth, we investigated the association with of growth rate separately for both sexes and different age groups (Fig. 3). In infants and toddlers (0–6 years), no significant association of AP with GV was found in boys ($\beta = 0.02$, $p = 0.57$). In girls, however, despite the similar effect size, this correlation was statistically significant ($\beta = 0.02$, $p < 0.01$). A stronger association was found in the age group 6–10 years in girls ($\beta = 0.13$, $p < 0.01$). In girls, β was 0.08 ($p < 0.01$) between 10 and 14 years of age and β was 0.33 ($p < 0.01$) between 14 and 18 years of age. In boys, the association of AP with GV was lower than in girls in the age group 6–10 years ($\beta = 0.05$, $p = 0.04$) and higher in age group 10–14 years ($\beta = 0.1$, $p < 0.01$). There was also a significant association of AP with GV in 14–18-year-old boys ($\beta = 0.19$, $p < 0.01$). As can be seen in Supplement 3, there was a strong and significant association between AP-SDS and height-SDS. Indeed, taller children in each respective age group also had a higher AP. Interestingly, the effect was stronger in girls ($\beta = 0.19$, $p < 0.01$) than in boys ($\beta = 0.13$, $p < 0.01$). The sex difference was statistically significant ($p < 0.01$).

3.3. Puberty

AP increased during pubertal stages. The highest AP values in girls were found in Tanner stage 2, whereas in boys, the peak occurred in stage 3 (Fig. 4). From stage 3 to stage 5, the values were higher in boys than in girls ($p < 0.01$, Fig. 4). In stages 1 and 2, there was no significant difference between the sexes. Interestingly, the values for girls fell to values below those of stage 1 in stage 4 ($\beta = -0.56$, $p < 0.01$) and further decreased in stage 5 (stage 1 vs. 5: $\beta = -1.24$, $p < 0.01$). In boys, the values in stages 2 to 4 were higher than the values in prepubertal stage 1 (e.g. stage 1 vs. 3: $\beta = 1.82$, $p < 0.01$). Only in stage 5 were AP levels similar to those from stage 1 reached ($\beta = -0.01$, $p = 0.89$). Within Tanner stage 1, levels decreased consistently across the 3 age groups (0–1, 1–2 and 2–6 years). All effects were highly significant ($p < 0.01$) (Fig. 4).

3.4. Obesity

The AP-SDS correlated positively with BMI-SDS in both sexes ($\beta = 0.10$, $p < 0.01$ in boys; $\beta = 0.07$, $p < 0.01$ in girls, Supplement 4). The effect did not differ significantly between sexes ($p = 0.19$).

3.5. Liver enzymes

Fig. 5 shows the association between AP and liver enzymes. We found higher AP-SDS to be associated with higher GGT-SDS in boys ($\beta = 0.08$, $p < 0.01$) and girls ($\beta = 0.12$, $p < 0.01$). The effect did not differ significantly between sexes ($p = 0.15$). Furthermore, there was a positive association between AP-SDS and ASAT-SDS ($\beta = 0.1$, $p < 0.01$) in both sexes. Whereas we found no significant association between AP and ALAT-SDS in boys ($\beta = 0.02$, $p = 0.42$), there was a significantly positive association in girls ($\beta = 0.11$, $p < 0.01$).
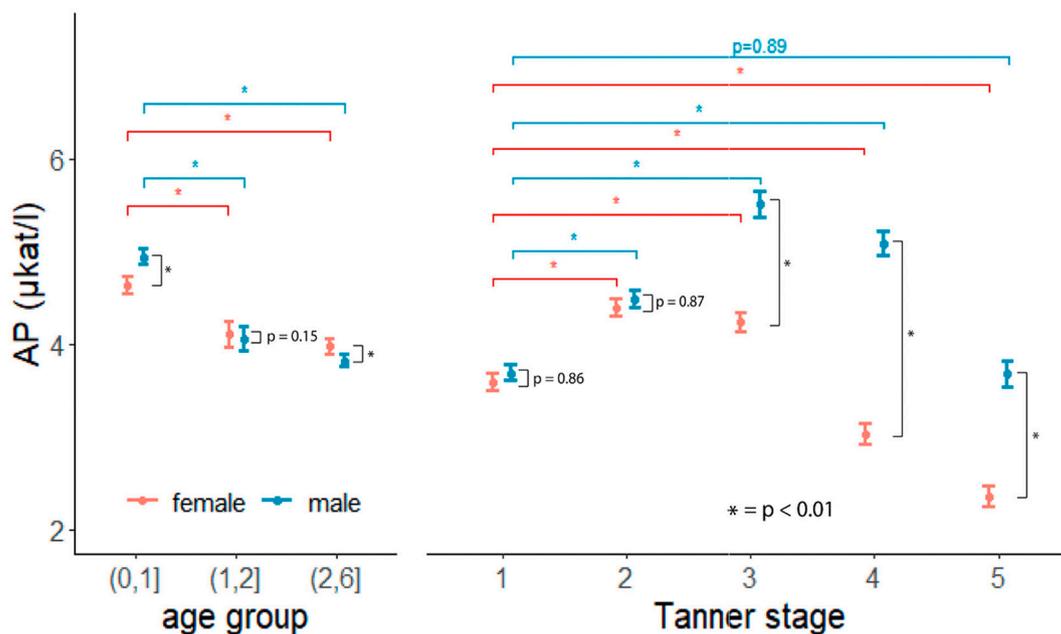

Fig. 4. Association of Alkaline Phosphatase according to age group or Tanner stage:
Left: age groups 0–1 years, 1–2 years and 2–6 years with their confidence intervals, consistently lower values of AP for older children; girls (red) and boys (green), the square brackets show the significance level p, the asterisk (*) means $p < 0.01$.
Right: children aged 6–18 years; confidence interval is shown, starting at stage 3 values for boys (green) were constantly higher than in girls (red), the peak for boys can be found in stage 3, for girls the peak is earlier in stage 2, the square brackets show the significance level p, the asterisk (*) means $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
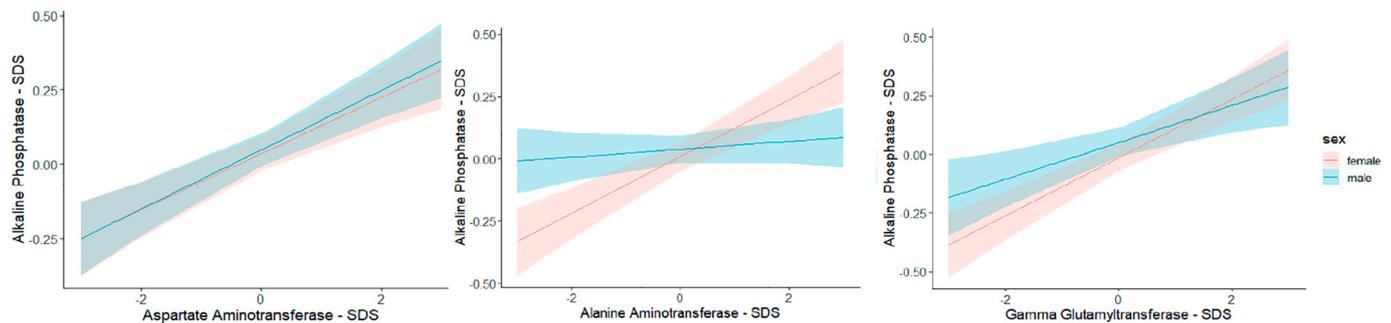
Fig. 5. Enzymes of liver metabolism (Aspartate Aminotransferase - SDS, Alanine Aminotransferase - SDS, Gamma Glutamyltransferase - SDS) and their association with Alkaline Phosphatase – SDS for boys (green) and girls (red); We found a positive association between AP-SDS and ASAT-SDS ($\beta = 0.1$, $p < 0.01$) in both sexes. We found no significant association between AP and ALAT-SDS in boys ($\beta = 0.02$, $p = 0.42$), but there was a significant positive association in girls ($\beta = 0.11$, $p < 0.01$). We found higher AP-SDS associated with higher GGT-SDS in boys ($\beta = 0.08$, $p < 0.01$) and girls ($\beta = 0.12$, $p < 0.01$), without no significant sex difference ($p = 0.15$).

The shaded area represents the 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

We conducted our study on a large number of subjects (n = 3704), with 10,272 study visits. All participants were healthy and were examined by qualified personnel. With this cohort, we covered a large age range from about 3 months to 18 years and established new continuous, age- and sex-dependent reference values for AP levels during childhood and adolescence.

Unlike for adults, uniform reference values for children between 0 and 18 years of age cannot be defined because of the wavelike progression in AP values. This specific progression, especially the two different peaks, has also found support in examinations of Asian [8,19], Turkish [13], Canadian [12], and German children [16,17,20]. A similar study among healthy Caucasian subjects from Sweden showed comparable sex- and age-specific percentile trajectories [38]. Some studies with healthy subjects supported our results [8,12,13]. However, in some studies, hospitalised children were included in the survey [7,14,17,18,20,22,39]. In contrast, we considered the establishment of reference values for healthy subjects to be more valid according to CLSI guidelines and to facilitate the detection of abnormalities more accurately in the context of clinical diagnostics. Furthermore, published reference curves for the Bonespecific Alkaline Phosphatase are similar in terms of sex differences and percentile graph trajectories and additionally support our results [21,24,40–42].

As hypothesized, AP was positively associated with height-SDS and, in some age groups, growth velocity as well. The association between AP and growth velocity could explain the course of the percentile graphs. In phases of accelerated growth, enzymes appear to be released from the bone at elevated rates. The significant positive association between AP and growth velocity or height-SDS supports this hypothesis.

Sex differences in AP appear to be largely uncontroversial in the literature [8,13,16,18,20]. One explanation could be an increased expression of androgen receptors in bone cell metabolism in male subjects [43,44] or a reduction in BAP due to the influence of estrogen [45]. Such differences are further confirmed by our analysis of AP during the pubertal stages according to Tanner.

The association between BMI and AP is controversially discussed in the literature. For example, Ali et al. [25] found increased AP in overweight adults, and Gajewska et al. [26] found a positive association between AP and BMI in children. On the other hand, Stanik et al. [23] found no significant association between BAP and BMI in children and adolescents, and Huang et al. [27] found no significant association between AP and BMI in children. One reason for significantly higher AP levels in obese children could be altered growth characteristics [46]. Kempf et al. [47] showed that obese children (especially at prepubertal ages) were taller than lean children of the same age and had an increased

GV. A potential influence of elevated leptin levels on bone cell metabolism would be conceivable [48] or the presence of AP activity within human preadipocytes would be conceivable [49]. An other possible reason could be that fat is accumulated in obese children's liver cells. Based on this hypothesis, we investigated the relationship between AP and liver metabolism parameters in more detail. Zhu et al. [8] previously studied the association of AP with other liver parameters in healthy Chinese children. Our study revealed an impressive sex difference in the association between AP-SDS and ALAT-SDS. In boys, there seemed to be no association between the two enzymes, whereas there was a clear positive association between the two enzymes in girls. A significant correlation of AP with ALAT was described in the literature [15] and a difference between both sexes in ALAT was also reported [50,51]. The specific reason for the sex difference between AP-SDS and ALAT-SDS has not yet been conclusively clarified.

In clinical practise and to avoid overdiagnosis of patients with elevated AP, it makes sense to first classify the blood values according to age and gender. After a thorough physical examination, a diagnosis of the liver parameters GGT, ASAT, ALAT, calcium and phosphate balance (possibly including 25-OH vitamin D) would be advisable. Because of the radiation exposure, an X-ray examination should be considered only under strict conditions. If the results are unremarkable, a control examination after a few weeks would be recommended; afterwards, the diagnosis with regard to transient or persistent hyperphosphatasemia should be extended or completed [52–54]. This diagnostic procedure was similarly justified in Kruse et al. [55]. In the context of our study, subjects were noticed who had a very high AP (up to 72.65 μkat/l), but did not fulfil any exclusion criteria and were not ill. We plan to investigate this anomaly further, because an elevated AP need not be pathologic. Some studies have a transient increase in AP (or the bone-specific fraction) of infectious origin in toddlers [52,53,56–58].

The most important unique feature of our study is the large number of subjects (n = 3704) with 10,272 clinical visits. With this cohort, we included a large age range, from about 3 months to 18 years. The other major benefit of our study is that all participants were healthy and were examined by qualified personnel. Certain other pediatric reference values have already been established using the blood samples from the LIFE -Child study. Examples include liver enzymes such as ALAT, ASAT and gamma-GT [51], parameters of iron metabolism [59] and cystatin C [60].

We were able to establish the age and sex dependence of AP and prove a significant association of AP with growth velocity, height-SDS, pubertal stage according to Tanner, BMI-SDS and liver parameters such as ALAT (GPT), ASAT (GOT) and GGT.

One limitation of our study is the fact that the LIFE -Child study

mainly included the Caucasian population of Central Germany. Other ethnic groups were only slightly represented. However, Colantonio et al. did not describe any influence of ethnicity on AP [12]. It should also be borne in mind that health studies usually do not reflect the full range of the social structure in terms of socio-economic status. This is true of the LIFE -Child study as well [61]. Children from families with lower incomes or lower educational attainment are less likely to participate in such studies. An other limitation was that we didn't include children younger than 3 month. Unfortunately, an analysis of BAP in such a high number of subjects was not possible. However, it can be assumed that the included subjects were liver-healthy children and so AP was not hepatically influenced.

5. Conclusion

In our study, we showed that the establishment of continuous reference values for alkaline phosphatase is possible and indispensable in the clinical context due to its strong age and sex dependency. Different growth phases in infancy and puberty and associated increases in AP should be noted. Our cohort is characterised by a large number of healthy Caucasian subjects in all pediatric age groups. We analysed numerous variables influencing the serum concentration of AP and hope this work can serve as a basis for avoiding overdiagnosis in healthy children and for better understanding physiological growth.

CRediT authorship contribution statement

Jacqueline-Michéle Strauch: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Mandy Vogel:** Data curation, Formal analysis, Methodology, Resources, Software, Validation, Writing – review & editing. **Christof Meigen:** Data curation, Formal analysis, Resources, Software. **Uta Ceglarek:** Investigation, Resources. **Jürgen Kratzsch:** Resources, Writing – review & editing. **Anja Willenberg:** Investigation, Resources. **Wieland Kiess:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

There is no conflict of interest among the authors.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2023.116809.

References

[1] L. Thomas (Ed.), Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik, 7th ed., TH-Books Verl.-Ges, Frankfurt/Main, 2008.

[2] K.P. Kohse, K.P. Kohse, K. Dörner (Eds.), Taschenlehrbuch Klinische Chemie und Hämatologie, 9th ed., Thieme, Stuttgart, 2019.

[3] E.E. Golub, G. Harrison, A.G. Taylor, S. Camper, I.M. Shapiro, The role of alkaline phosphatase in cartilage mineralization, Bone Miner. 17 (1992) 273–278.

[4] G. Schumann, R. Klauke, F. Canalias, S. Bossert-Reuther, P.F.H. Franck, F.-J. Gella, P.J. Jørgensen, D. Kang, J.-M. Lessinger, M. Panteghini, F. Ceriotti, IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 9: reference procedure for the measurement of catalytic concentration of alkaline phosphatase International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) (1), Clin. Chem. Lab. Med. 49 (2011) 1439–1446.

[5] M.J. Weiss, D.E. Cole, K. Ray, M.P. Whyte, M.A. Lafferty, R.A. Mulivor, H. Harris, A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 7666–7669.

[6] K. Kish, Y. Mezil, W.E. Ward, P. Klentrou, B. Falk, Effects of plyometric exercise session on markers of bone turnover in boys and young men, Eur. J. Appl. Physiol. 115 (2015) 2115–2124.

[7] B. Shine, Use of routine clinical laboratory data to define reference intervals, Ann. Clin. Biochem. 45 (2008) 467–475.

[8] X. Zhu, K. Wang, Q. Zhou, J. Xu, Establishment of age- and sex-specific reference intervals for serum liver function tests in pediatric population aged 1–<18 years: a prospective study, J. Clin. Lab. Anal. 35 (2021), e23708.

[9] G.L. Horowitz, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline 3rd ed., vol. 28, no. 30, Clinical and Laboratory Standards Institute, Wayne, Pa, 2010. C28-A3 =.

[10] R.L. Bertholf, Statistical methods for establishing and validating reference intervals, Lab. Med. 37 (2006) 306–310.

[11] The World Medical Association, INC, Decaration of Helsinki 2008: ethical principles for medical research involving human subjects. https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/doh-oct2008/, 2008.

[12] D.A. Colantonio, L. Kyriakopoulou, M.K. Chan, C.H. Daly, D. Brinc, A.A. Venner, M. D. Pasic, D. Armbruster, K. Adeli, Closing the gaps in pediatric laboratory reference intervals: a **CALIPER** database of 40 biochemical markers in a healthy and multiethnic population of children, Clin. Chem. 58 (2012) 854–868.

[13] S. Turan, B. Topcu, İ. Gökçe, T. Güran, Z. Atay, A. Omar, T. Akçay, A. Bereket, Serum alkaline phosphatase levels in healthy children and evaluation of alkaline phosphatase z-scores in different types of rickets, J. Clin. Res. Pediatr. Endocrinol. 3 (2011) 7–11.

[14] G. Wanjian, H. Jie, G. Liang, W. Cheng, X. Tian, S. Jianjiang, Z. Chunni, Establishment of reference interval for alkaline phosphatase in healthy children of various ethnicities, aged 0–12 years, Lab. Med. 48 (2017) 166–171.

[15] M. Gholami Bahnemiri, S. Mirabedini, P. Mohammadi, H. Barmaki, Z. Qaffaripour, M. Rezapour, M. Alijanpour, Determination of serum alkaline phosphatase reference in healthy children aged 1–18 years, Caspian, J. Intern. Med. 13 (2022) 749–756.

[16] Robert-Koch-Institut (Ed.), Bevölkerungsbezogene Verteilungswerte ausgewählter Laborparameter aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS), Robert-Koch-Institut, Berlin, 2009.

[17] J. Zierk, F. Arzideh, R. Haeckel, H. Cario, M.C. Fruhwald, H.-J. Gross, T. Gscheidmeier, R. Hoffmann, A. Krebs, R. Lichtinghagen, M. Neumann, H.-G. Ruf, U. Steigerwald, T. Streichert, W. Rascher, M. Metzler, M. Rauh, Pediatric reference intervals for alkaline phosphatase, Clin. Chem. Lab. Med. 55 (2017) 102–110.

[18] K.-L. Lü, S.-S. Xie, E. Liu, X.-M. Yu, L. Wang, Z.-Y. Yang, Q. Xiong, X.-G. Luo, W. Yang, W. Liao, Y.-P. Zhang, Age-wise trends in alkaline phosphatase activity in 167,625 Chinese children aged 0–18 years, Clin. Biochem. 79 (2020) 34–40.

[19] X. Li, C. Di Wang, Q. Yang, S.-L. Zhou, L.-Q. Zhuoga, H.-X. Wang, Q. Yao, Q. Ai Zhang, C.-X. Yang, J.-C. Xu, Establishment of age- and gender-specific pediatric reference intervals for liver function tests in healthy Han children, World J. Pediatr. 14 (2018) 151–159.

[20] J. Zierk, F. Arzideh, T. Rechenauer, R. Haeckel, W. Rascher, M. Metzler, M. Rauh, Age- and sex-specific dynamics in 22 hematologic and biochemical analytes from birth to adolescence, Clin. Chem. 61 (2015) 964–973.

[21] M. Rauchenzauner, A. Schmid, P. Heinz-Erian, K. Kapelari, G. Falkensammer, A. Griesmacher, G. Finkenstedt, W. Högler, Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years, J. Clin. Endocrinol. Metab. 92 (2007) 443–449.

[22] S. Ahmed, J. Zierk, A.H. Khan, Establishment of reference intervals for alkaline phosphatase in Pakistani children using a data mining approach, Lab. Med. 51 (5) (2020) 484–490.

[23] J. Stanik, J. Kratzsch, K. Landgraf, M. Vogel, J. Thiery, W. Kiess, A. Korner, The bone markers sclerostin, osteoprotegerin, and bone-specific alkaline phosphatase

are related to insulin resistance in children and adolescents, independent of their association with growth and obesity, Horm. Res. Paediatr. 91 (2019) 1–8.

[24] D.-C. Fischer, A. Mischek, S. Wolf, A. Rahn, B. Salweski, G. Kundt, D. Haffner, Paediatric reference values for the C-terminal fragment of fibroblast-growth factor-23, sclerostin, bone-specific alkaline phosphatase and isoform 5b of tartrate-resistant acid phosphatase, Ann. Clin. Biochem. 49 (2012) 546–553.

[25] A.T. Ali, J.E. Paiker, N.J. Crowther, The relationship between anthropometry and serum concentrations of alkaline phosphatase isoenzymes, liver-enzymes, albumin, and bilirubin, Am. J. Clin. Pathol. 126 (2006) 437–442.

[26] J. Gajewska, H. Weker, J. Ambroszkiewicz, K. Szamotulska, M. Chełchowska, E. Franek, T. Laskowska-Klita, Alterations in markers of bone metabolism and adipokines following a 3-month lifestyle intervention induced weight loss in obese prepubertal children, Exp. Clin. Endocrinol. Diabetes 121 (2013) 498–504.

[27] Y. Huang, E. Eapen, S. Steele, V. Grey, Establishment of reference intervals for bone markers in children and adolescents, Clin. Biochem. 44 (2011) 771–778.

[28] T. Poulain, R. Baber, M. Vogel, D. Pietzner, T. Kirsten, A. Jurkutat, A. Hiemisch, A. Hilbert, J. Kratzsch, J. Thiery, M. Fuchs, C. Hirsch, F.G. Rauscher, M. Loeffler, A. Körner, M. Nüchter, W. Kiess, The LIFE Child study: a population-based perinatal and pediatric cohort in Germany, Eur. J. Epidemiol. 32 (2017) 145–158.

[29] M. Quante, M. Hesse, M. Döhnert, M. Fuchs, C. Hirsch, E. Sergeyev, N. Casprzig, M. Geserick, S. Naumann, C. Koch, M.A. Sabin, A. Hiemisch, A. Körner, W. Kiess, The LIFE child study: a life course approach to disease and health, BMC Public Health 12 (2012) 1021, https://doi.org/10.1186/1471-2458-12-1021.

[30] M. Vogel, T. Kirsten, J. Kratzsch, C. Engel, W. Kiess, A combined approach to generate laboratory reference intervals using unbalanced longitudinal data, J. Pediatr. Endocrinol. Metab. 30 (2017) 767–773.

[31] K. Kromeyer-Hauschild, M. Wabitsch, D. Kunze, F. Geller, H.C. Geiß, V. Hesse, A. von Hippel, U. Jaeger, D. Johnsen, W. Korte, K. Menner, G. Müller, J.M. Müller, A. Niemann-Pilatus, T. Remer, F. Schaefer, H.-U. Wittchen, S. Zabransky, K. Zellner, A. Ziegler, J. Hebebrand, Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben, Monatsschr Kinderheilkd 149 (2001) 807–818.

[32] M. Wabitsch, A. Moss, Evidence-based (S3) guideline of the Working Group on Childhood and Adolescent Obesity (AGA) of the German Obesity Society (DAG) and the German Society of Pediatrics and Adolescent Medicine (DGKJ)]: Therapie und Prävention der Adipositas im Kindes- und Jugendalter, 2019. https://www.awmf.org/leitlinien/detail/ll/050-002.html. AWMF online.

[33] W.A. Marshall, J.M. Tanner, Variations in pattern of pubertal changes in girls, Arch. Dis. Child. 44 (1969) 291–303.

[34] W.A. Marshall, J.M. Tanner, Variations in the pattern of pubertal changes in boys, Arch. Dis. Child. 45 (1970) 13–23.

[35] T.J. Cole, P.J. Green, Smoothing reference centile curves: the LMS method and penalized likelihood, Stat. Med. 11 (1992) 1305–1319.

[36] E. Borghi, M. de Onis, C. Garza, J. van den Broeck, E.A. Frongillo, L. Grummer-Strawn, S. van Buuren, H. Pan, L. Molinari, R. Martorell, A.W. Onyango, J. C. Martines, Construction of the World Health Organization child growth standards: selection of methods for attained growth curves, Stat. Med. 25 (2006) 247–265.

[37] S. Nakagawa, H. Schielzeth, A general and simple method for obtaining R2 from generalized linear mixed-effects models, Methods Ecol. Evol. 4 (2013) 133–142.

[38] P. Ridefelt, J. Gustafsson, M. Aldrimer, D. Hellberg, Alkaline phosphatase in healthy children: reference intervals and prevalence of elevated levels, Horm. Res. Paediatr. 82 (2014) 399–404.

[39] J. Zierk, F. Arzideh, R. Haeckel, W. Rascher, M. Rauh, M. Metzler, Indirect determination of pediatric blood count reference intervals, Clin. Chem. Lab. Med. 51 (2013) 863–872.

[40] I. Gennai, N. Di Iorgi, G. Reggiardo, C. Gatti, E. Bertelli, A.E.M. Allegri, S. Barco, M. Maghnie, G. Tripodi, G. Cangemi, Age- and sex-matched reference curves for serum collagen type I C-telopeptides and bone alkaline phosphatase in children and adolescents: an alternative multivariate statistical analysis approach, Clin. Biochem. 49 (2016) 802–807.

[41] S. Mora, L. Cafarelli, P. Erba, M. Puzzovio, I. Zamproni, V. Giacomet, A. Viganò, Differential effect of age, gender and puberty on bone formation rate assessed by measurement of bone-specific alkaline phosphatase in healthy Italian children and adolescents, J. Bone Miner. Metab. 27 (2009) 721–726.

[42] K.S. Tsai, M.H. Jang, S.H. Hsu, W.C. Cheng, M.H. Chang, Bone alkaline phosphatase isoenzyme and carboxy-terminal propeptide of type-I procollagen in healthy Chinese girls and boys, Clin. Chem. 45 (1999) 136–138.

[43] B. van der Eerden, N.P. van Til, A.O. Brinkmann, C. Lowik, J.M. Wit, M. Karperien, Gender differences in expression of androgen receptor in tibial growth plate and metaphyseal bone of the rat, Bone 30 (2002) 891–896.

[44] K.M. Wiren, Androgens and bone growth: it's location, location, location, Curr. Opin. Pharmacol. 5 (2005) 626–632.

[45] A.d.C.B. Rizzo, T.B.L. Goldberg, T.P. Biason, C.S. Kurokawa, C.C.d. Silva, J. E. Corrente, H.R.C. Nunes, One-year adolescent bone mineral density and bone formation marker changes through the use or lack of use of combined hormonal contraceptives, J. Pediatr. 95 (2019) 567–574.

[46] S. Shalitin, W. Kiess, Putative effects of obesity on linear growth and puberty, Horm. Res. Paediatr. 88 (2017) 101–110.

[47] E. Kempf, M. Vogel, T. Vogel, J. Kratzsch, K. Landgraf, A. Kühnapfel, R. Gausche, D. Gräfe, E. Sergeyev, R. Pfäffle, W. Kiess, J. Stanik, A. Körner, Dynamic alterations in linear growth and endocrine parameters in children with obesity and height reference values, EClinicalMedicine 37 (2021), 100977.

[48] G. Maor, M. Rochwerger, Y. Segev, M. Phillip, Leptin acts as a growth factor on the chondrocytes of skeletal growth centers, J. Bone Miner. Res. 17 (2002) 1034–1043.

[49] A.T. Ali, C.B. Penny, J.E. Paiker, G. Psaras, F. Ikram, N.J. Crowther, The effect of alkaline phosphatase inhibitors on intracellular lipid accumulation in preadipocytes isolated from human mammary tissue, Ann. Clin. Biochem. 43 (2006) 207–213.

[50] B. Buday, P.F. Pach, B. Literati-Nagy, M. Vitai, G. Kovacs, Z. Vecsei, L. Koranyi, C. Lengyel, Sex influenced association of directly measured insulin sensitivity and serum transaminase levels: why alanine aminotransferase only predicts cardiovascular risk in men? Cardiovasc. Diabetol. 14 (2015) 55.

[51] S. Bussler, M. Vogel, D. Pietzner, K. Harms, T. Buzek, M. Penke, N. Händel, A. Körner, U. Baumann, W. Kiess, G. Flemming, New pediatric percentiles of liver enzyme serum levels (alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase): effects of age, sex, body mass index, and pubertal stage, Hepatology (Baltimore, Md.) 68 (2018) 1319–1330.

[52] V. Abbassi, A.R. Colon, R.H. Schwartz, Benign elevation of serum alkaline phosphatase, transient and persistent variety, Clin. Pediatr. 23 (1984) 336–337.

[53] S. Posen, C. Lee, R. Vines, H. Kilham, S. Latham, J.F. Keefe, Transient hyperphosphatasemia of infancy—an insufficiently recognized syndrome, Clin. Chem. 23 (1977) 292–294.

[54] E. Schönau, K.H. Herzog, H.J. Böhles, Transient hyperphosphatasaemia of infancy, Eur. J. Pediatr. 148 (1988) 264–266.

[55] K. Kruse, U. Kracht, Isolierte Erhöhung der alkalischen Serum-Phosphatase, Dtsch. Med. Wochenschr. 110 (1985) 669–674.

[56] M.P. Whyte, N.S. Ma, S. Mumm, G.S. Gottesman, W.H. McAlister, A.R. Nenninger, V.N. Bijanki, K.L. Ericson, P. Magnusson, Persistent idiopathic hyperphosphatasemia from bone alkaline phosphatase in a healthy boy, Bone 138 (2020), 115459.

[57] U. Frank, K. Kruse, Evidence for infectious origin of isolated transient hyperphosphatasemia, Eur. J. Pediatr. 143 (1985) 323–324.

[58] T. Erat, M. Atar, T. Kontbay, Transient benign hyperphosphatasemia due to COVID-19: the first case report, J. Pediatr. Endocrinol. Metab. 34 (2021) 385–387.

[59] K. Rieger, M. Vogel, C. Engel, U. Ceglarek, J. Thiery, J. Kratzsch, K. Harms, F. Glock, A. Hiemisch, W. Kiess, Reference intervals for iron-related blood parameters: results from a population-based cohort study (LIFE Child), LaboratoriumsMedizin 40 (2016) 559.

[60] N. Ziegelasch, M. Vogel, E. Müller, N. Tremel, A. Jurkutat, M. Löffler, N. Terliesner, J. Thiery, A. Willenberg, W. Kiess, K. Dittrich, Cystatin C serum levels in healthy children are related to age, gender, and pubertal stage, Pediatr. Nephrol. (Berlin, Germany) 34 (2019) 449–457.

[61] A. Dathan-Stumpf, M. Vogel, K. Rieger, J. Thiery, A. Hiemisch, W. Kiess, Serum lipid levels were related to socio-demographic characteristics in a German population-based child cohort, Acta Paediatr. (Oslo, Norway 1992) 105 (2016) e360–e367.