**Age- and sex-related percentiles of liver enzyme serum levels (ALT, AST, and GGT):**

**effects of age, sex, BMI and pubertal stage**

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**Abstract**

The present study aims to clarify the effect of sex, age, BMI and puberty on transaminase serum levels in children and adolescents and to provide new age- and sex-related percentiles for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) which are unaffected by confounders such as infections, metabolic syndrome or hepatotoxic medications.Venous blood and anthropometric data were collected from 4,278 cases. Excluded were cases of participants with potential hepatotoxic medication, with metabolic syndrome or evidence of potential illness at the time of blood sampling. Age- and gender-related reference intervals were established by using an LMS-type method. Serum levels of transaminases follow age-specific patterns and relate to the onset of puberty. This observation is more pronounced in girls than in boys. The ALT percentiles showed similar w-shaped patterns in both sexes. Multivariate regression confirmed significant positive effects of age and BMI-SDS (β = 1.62) on ALT. Surprisingly, AST serum levels were significantly negatively influenced by age (β = -1.29) and BMI-SDS (β = -0.55). The GGT percentiles revealed significant sex-specific differences, correlated positively with age (β = 0.35) and showed significant association with BMI-SDS (β = 0.78). **Conclusion:** Current reference values of ALT, AST and GGT serum levels were calculated for children between 1 and 17.5 years, using modern analytical and statistical methods. This is the first and only study that revealed influences of age, gender, BMI, and puberty on the serum concentrations of all three parameters. Two sets of percentiles are available. Percentiles derived from normal weight children and adolescents and derived from children and adolescents from a general population. We suggest that these data can be used as reference values.

**Introduction**The high prevalence of obesity and non-alcoholic fatty liver disease as well as the early onset of other liver diseases, require the availability and accuracy of diagnostic instruments for detection of liver damage during early life. Transaminases are usually present in the blood at low levels and an increase in their concentrations may indicate damage of liver cells [1]. Furthermore, hepatic enzyme serum concentrations are associated with obesity, insulin resistance and type 2 diabetes in adults [2]. The World Health Organisation (WHO) recommends in children the transformation of measurement values into standard deviation scores as the best way to assess their meaning in the context of age and sex for anthropometric parameters. This approach has been progressively applied and extended to other parameters. In the past seven years, several approaches have been made to establish new reference intervals or thresholds for liver enzymes in children [3–9], particularly for alanine aminotransferase (ALT). Schwimmer et al [4] were able to show that the upper limit of ALT used in children’s hospitals varies widely and is set too high to detect chronic liver disease reliably. This could be due to the fact that many of the reference values in current use have been derived from small cohorts of healthy or hospitalized individuals or focus on a limited age range with arbitrary partitions [5]. Discrete age groups, however, do not adequately reflect the continuous changes during biological development and thus cannot always represent the exact extent and onset of age-dependent dynamics. Partition into discrete age groups for both males and females is commonly performed to describe the age and sex dependence of laboratory parameters. A continuous approach with age- and sex-related percentiles seems to be the appropriate method for laboratory analytes and will, therefore, be applied in this study [9]. Such an approach requires a large number of samples from healthy children and adolescents.

Objectives  
We aim to provide new age- and sex-related percentiles for ALT, aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) based on data from a large cohort of healthy children and adolescents between 1 and 17.5 years of age (the LIFE Child cohort, a representative population-based cohort in Germany) [10, 11]. We aim to study potential effects of sex, age, BMI and puberty on transaminase serum levels during early life.

**Methods**This article is structured according to the STROBE Statement checklist for cohort studies [12].

Study design, clinical study registration and ethnical review  
LIFE Child is a prospective longitudinal population-based cohort study with a life course approach to health and disease. The Leipzig Research Centre for Civilization Diseases (LIFE) Child study has been designed to understand how and through which mechanisms and mediators (epi) genetic, metabolic and environmental factors influence health and development in children and adolescents in modern society [10, 11]. The LIFE Child study was designed pursuant to the Declaration of Helsinki [13] and is registered under the clinical trial number NCT02550236. The ethical committee of the University of Leipzig (Reg. No. 264-10-19042010) had no objection.

Setting  
Informed and written consent was obtained from all participants and their parents. Data was pseudonymized according to German data protection law. The primary program carried out at each study centre visit includes clinical history, clinical examination, blood collection, hair and urine samples, anthropometry, and different age-dependent questionnaires [10, 11]. In this study, we only included cases with complete datasets of ALT, AST, GGT, age, and BMI (body mass index = body weight/(height2) in kg/m²). Samples with too little sample material for analysis were excluded.

Participants  
Children residing in Leipzig or neighbouring municipalities between 1 and 17.5 years were eligible for participation. The participants of this study are primarily healthy children and adolescents. Thus, their data are well suited to calculation of reference values.

Variables  
ALT, AST and GGT serum levels were determined in context of sex, age, pubertal stage, and BMI.

Data sources/measurements  
At the beginning of every visit all children participating in the LIFE Child study were asked to provide fasting, morning venous blood. The blood was collected by venipuncture (serum monovettes, Sarstedt AG&Co, Nümbrecht, Germany). The analysis was done immediately in the Central Laboratory of the University Hospital Leipzig. ALT and AST (UV tests), glucose (enzymatic UV test based on hexokinase, IDMS-traceable reference method), as well as GGT, HDL-cholesterol and triglycerides (colorimetric tests) and high sensitive C-reactive protein (latex-enhanced immunoturbidimetric test) were measured with cobas® analyzer series (photometric measuring unit, c-module, Roche Diagnostics GmbH, Mannheim, Germany). Only CE-IVD-certified laboratory tests approved for diagnostic use were applied, and all analytical procedures were performed according to the manufacturer’s instruction [10]. Systolic and diastolic blood pressure and resting pulse were measured three times automatically by a VSM 6000-monitor (WelchAllyn, Skaneateles Falls, NY, USA) with suitable FlexiPort cuff according to arm circumference (only for children older than 2.5 years). Height was measured with the stadiometer (“Prof. Keller”, Längenmesstechnik GmbH Limbach, Limbach-Oberfrohna, Germany) with a measurement accuracy of 0.10 cm. The participants were weighed with the “Seca 701” scale (seca GmbH & Co. KG, Hamburg, Germany) which is accurate to 50 g. For the measurements of circumferences and distances between osseous measuring points, a flexible, not dilatable roller tape (150 cm Picco Décor, Das Maßband, Driedorf, Germany) was used. The pubertal stage was assessed according to Tanner stages [14, 15] by specially trained and regularly instructed investigators.

Study size  
4,662 complete cases of 2,471 individuals between the ages of 0 and 18 years from 1,819 families were available (LIFE Child cohort). To assure to work with a healthy cohort, we needed to carry out several steps of exclusion (Figure 1).   
1.) Isolated extreme values of ALT, AST or GGT, identified by visual inspection, were considered as outliers and excluded, N = 9 cases.   
2.) 218 cases were excluded because of the intake of one of 92 potentially hepatotoxic drugs at the time of measurement (List of 92 hepatotoxic medications available in the Supplementary Table 1). In 48 cases, children suffered from the metabolic syndrome according to the IDF (International Diabetes Federation [16]) definition of pediatric metabolic syndrome for children between 10 and 16 years. Thus, these children presented at least 3 risk factors of metabolic syndrome (waist circumference ≥ 90th percentile, triglycerides ≥ 1,69 mmol/l, HDL-cholesterol ≤ 1,03 mmol/l, systolic blood pressure ≥ 130mmHg, diastolic blood pressure ≥ 85mmHg or fasting glucose ≥ 5,55 mmol/l). This approach was justified by several studies which have shown strong correlations between elevated liver enzymes (ALT, AST and GGT) and metabolic risk factors [2, 17–19]. Children with elevated high sensitive C – reactive protein serum levels (> 10 mg/l) were ruled out, N = 74 cases.   
3.) Due to the small numbers of very young subjects (younger than 1 year) and older adolescents (older than 17.5 years), the age range was reduced to 1 to 17.5 years by the exclusion of N = 45 cases. After these exclusions, our study population consisted of 4,278 cases (2,043 measurements of girls and 2,253 measurements of boys) from healthy children between 1 and 17.5 years. The resulting data were used for the calculations of ALT, AST, and GGT percentiles.

Statistical methods  
Percentile curves for ALT, AST and GGT were estimated as functions of the covariate age stratified by sex using a LMS-type method (LMST) implemented in the package gamlss [20]. Assuming a Box-Cox t (BCT) distribution the LMS method of Cole [21] models four parameters µ, σ, ν and τ as a function of age. These may be interpreted as relating to location (median), scale (centile-based coefficient of variation), skewness (power transformation to symmetry) and kurtosis (degrees of freedom) [21]. Estimation of parameters as continuous functions of age seems to be a more appropriate approach to reflect the physiological development of laboratory analytes [9, 22].   
Since LIFE Child has a longitudinal study design and recruits families participated with more than one child, our sampled data contains multiple measurements per child as well as measurements of siblings. Therefore, percentile calculations had to follow an adapted approach. To maintain the independence of all measurements, but also consider all measurements for the calculation, we calculated each model 1000 times on distinct subsamples of 600 independent values (one per family). The weighting was defined to maintain the same probability for each value to be chosen. The mean estimated parameter (location, scale, shape) built the basis for the calculation of the reference values. Finally, percentile curves for the 3rd (P3), 10th (P10), 50th P (50), 90th (P90) and 97th (P97) centile were calculated. The 3rd and 97th percentiles were defined as lower and upper limit of the reference interval. To determine the strength of the relationship of the different parameters, a hierarchical linear regression analysis was performed. All regression analyses were carried out using multivariate linear mixed models with sex, age, BMI-SDS and pubertal stage as predictors (Table 1). By adding random effects on the intercept for the individual nested within its family, we accounted for possible correlations between measurements and, therefore, for multiple measurements per person/family. First, non-parametric fitting was performed to identify the general trends and probable turning points. If turning points exist, the age span was subdivided into intervals of linear trends. Linear modelling was preferred due to interpretability. While partitioning age into subintervals, the intercept was shifted to the left limit of the respective interval. The Tanner stages [14, 15] were separated into two groups (Tanner stages 1 – 2 and Tanner stages 3 – 5) to emphasize on puberty-specific changes in the transaminases serum levels. Analyses were performed using the package lme4 [23] (version 1.1.10) in R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria) [24].

**Results**  
Description of the study population  
The reference population used to calculate percentiles is composed of 4,278 cases (52.2% male cases) from 2,320 individuals out of 1,722 families. Excluded were cases of participants with potential hepatotoxic medication, with metabolic syndrome, or potential illness at the time of measurement.   
Overweight and obesity were defined by using the 90th and 97th percentile of the BMI-SDS (BMI – standard deviation score) as cut-offs [25]. The average proportion of obese participants in our study was 2.6% in children younger than 7 years, and 10.9% in children older than 7 years. In total 994 cases of underweight (below the 10th percentile) (7.7%), overweight (7.1%), and obese children and adolescents (8.4%), respectively, were assessed, as well as 3,284 cases of normal weight (76.8%) children and adolescents. The mean BMI-SDS was 0.18 for girls and 0.11 for boys; this slightly elevated mean can be explained by the rising number of overweight and obese children and adolescents in Germany over the past decades [26]. Characteristics of this population are represented as mean (± s.d.) and median values in Supplementary Table 2.

Percentiles of liver enzyme serum levels derived from a healthy pediatric cohort  
The smoothed percentile curves (Figures 2 – 4) for ALT, AST and GGT are presented for girls and boys. The corresponding reference intervals are represented as values of P3 and P97 and tabulated with the median (M = 50th percentile), the coefficient of variation (S), and the skewness (L) in half-year age groups and separated for boys and girls (Supplementary Tables 3-5).   
The ALT percentiles show similar w-shaped pattern in boys and girls, starting with a peak in infancy, followed by a sharp drop until the age of 4. After that decline we found rising values, which resulted in an ALT peak at 9 years in girls and at 11 years in boys. During early adolescence serum concentrations fall in girls and start to rise again slightly with progressing adolescence. In contrast, boys show only a mild decrease between 11 and 14 years, but a notable increase in ALT serum levels between 14 and 17.5 years. The median ALT serum concentration varies between 14.9 U/l and 20.2 U/l in girls and between 17.1 U/l and 21.5 U/l in boys. The 97th percentile, which is commonly used as cut-off, spans from 24.0 U/l to 33.4 U/l in girls and from 25.1 U/l to 78.8 U/l in boys. The range between the 3rd and the 97th percentile is wider in older children and more apparent in boys than in girls.   
The AST percentiles follow a continuous downwards trend with increasing age, which differs considerably from the patterns shown by ALT and GGT serum levels. Before the age of 11, no significant gender-specific differences can be found, but between 11 and 15 years, AST serum concentrations stronger decrease in girls and between 16 and 17.5 years, girls present increasing serum concentrations, whereas the male percentiles reach a plateau. The median spans from 22.7 U/l to 45.7 U/L in girls and from 26.8 U/l to 47.0 U/l in boys. The upper limit (97th percentile) ranges from 33.6 U/l to 62.6 U/l in females and from 40.0 U/l to 62.0 U/l in males. In boys, we found an enlarging range between P3 and P97 with increasing age, it varies from 18.4 U/l to 62.0 U/l, in girls this range spans from 17.3 U/l to 62.6 U/l.   
The GGT pattern revealed sex-specific differences. While there is a continuous increase of P50 in boys over the whole age span, girls’ GGT serum levels rise until they reach a maximum of 12.5 U/l at the age of 10. After that peak the serum concentrations fall to a minimum of 11.1 U/l at 13.5 years and then slightly increase to stabilise at about 11.3 U/l. The median serum concentrations vary from 9.7 U/l to 12.5 U/l in girls and from 9.4 U/l to 16.1 U/l in boys. The upper limit (97th percentile) ranges from 13.6 U/l to 21.9 U/l in girls and 13.7 U/l to 41.2 U/l in boys. The range between P3 and P97 increases with age in both sexes and spans from 6.8 U/l to 21.9 U/L in girls and from 6.3 U/l to 41.2 U/l in boys.

Influencing factors on liver enzyme serum levels: Effects of age, sex, puberty, and BMI   
Detailed results from the multivariate regression analysis are summarized in Table 1. Due to a smaller number of subjects older than 16 years combined with partition of age into subintervals, the age range for the regression analysis was reduced to 1 – 16 years, N = 3,208 cases.

Influence of age  
All three liver enzymes are significantly associated with age. ALT serum levels of children younger than 3 years or older than 11 years were significantly negatively associated with age, between the ages 3 and 11 years, a positive link was found (β = 0.4 for both sexes). With increasing age AST serum levels significantly decrease in boys (β = -1.2) and even stronger in girls (β = -1.4). GGT serum levels are significantly positively influenced by age (β = 0.5 for boys and β = 0.3 for girls < 10 years), but differ significantly between the sexes in children above the age of 10 (β = 0.4 for boys and β = -0.2 for girls).

Influence of sex  
In children above the age of 11, gender differences for ALT serum levels were revealed. 11-year-old boys exhibit significantly higher mean ALT serum concentrations than girls at this age (intercept = 22.9 U/l, β = -3.1). Similar to ALT, girls at the age of 10 showed significantly lower mean GGT serum levels (β = -2.2) compared to boys. Boys present significantly higher mean AST serum levels than girls at the age of 1 (intercept = 43.7 U/l, β = -1.6), this sex-specific difference increases with age. Consideration of age and sex interaction in children revealed different strengths of the associations between boys and girls with age. Compared to boys of the same age, girls at 11 years have a higher trend in ALT (β = 0.1), a lower trend in AST (β = -0.2) and a lower trend in GGT (β = -0.2 below the age of 10 years) and even stronger above 10 (β = -0.6).

Influence of puberty  
All three investigated transaminases are negatively influenced by puberty independently of age. Children at Tanner stage 3 – 5 and age above the of 11 years exhibit significantly lower ALT serum levels (β = -3.1) compared to children at Tanner stages 1 – 2. Similar effects were found for AST (β = -4.6) and GGT (β = -1.3). By each year, ALT serum levels increase about 0.4 U/l in children between 3 and 11 years but drop when reaching Tanner stages 3 – 5 by 1.9 and, in addition, the age trend changes its direction (interaction term: -1.4). The slopes of AST and GGT serum levels both increase about 0.3 U/l when reaching puberty (AST: age 1 year, intercept = 43.7 U/l, GGT: age > 10 years, intercept = 14.6 U/l).

Influence of BMI  
The ALT percentile curves presented a widened span between P3 and P97 with increasing age. This effect might be mainly due to a substantial rise of P90 and P97 in adolescents, which could be influenced by a percentage increase of overweight in older children. A significantly positive correlation between BMI-SDS and ALT serum concentrations was found, even increasing with rising age (β = 1.3 < 3 years, β = 1.5 between 3 and 11 years, and β = 1.7 > 11 years). Similar to ALT we found a significant, but a slightly weaker association between GGT and BMI-SDS (β = 0.8), but surprisingly, a significantly negative association for AST and BMI-SDS (β = -0.4).

Impact of underweight, overweight and obesity on percentile curves  
Regarding clinical practice and considering the strong influence of the BMI on transaminases we calculated further percentiles of ALT, AST and GGT using only cases of normal weight children (BMI-SDS ≤ ± 1.28) as the reference population (N = 3,284 cases). This might help paediatricians to evaluate changes in transaminases levels in underweight, overweight or obese children with a focus on BMI as an influencing factor. Supplementary Figure 1 - 3 compare percentiles derived from all healthy children (study population) with those obtained from normal weight children. This allows us to depict the influence of underweight, overweight and obesity on transaminases. As expected, the range between P3 and P97 is considerably smaller in the percentiles derived from the normal weight reference group. The normal weight ALT percentiles curves are slightly lower in children older than 5 years. This effect is present in both sexes and more pronounced in P90 and P97. The GGT percentiles show the same effect throughout the entire age interval in boys and for the 90th and 97th centile in girls. The patterns of the normal weight ALT and the normal weight GGT percentiles show fewer fluctuations. The AST percentiles do not seem to be influenced by underweight, overweight, or obese conditions. In comparison with the normal weight percentiles we found only minor differences in the patterns. Influence of the BMI on AST was only seen in P90 and P97 in girls older than 15 years.

**Discussion**  
Our study presented age- and sex-specific percentiles of ALT, AST and GGT derived from the LIFE Child cohort in Germany, which consists of 4,278 cases of children and adolescents between 1 and 17.5 years. This study is the first to present percentiles for three transaminases derived from healthy children and adolescents, carried out within a standardised protocol of a cohort study and is one of the largest cohort studies on this topic. In the past seven years, various studies have been conducted to examine ALT in children and adolescents, while far fewer have been performed for AST or GGT. Characteristics of studies that published reference intervals or percentiles for transaminases are available in Supplementary Table 6. The primary studies are KiGGS [8] (nationwide health survey in children and adolescents carried out by the Robert Koch Institute, Germany), Estey et al [5] (results from the CALIPER cohort study, Canada), Zierk et al [9], Schwimmer et al [4] (SAFETY study, USA), England et al [6], Poustchi et al [3] and Dehghani et al [7].

Comparison of our new percentiles with previous cut-offs and reference intervals   
The aforementioned studies differ markedly regarding the country of origin, the ethnical structure of the reference populations, the age groups applied, inclusion and exclusion criteria, sample size, laboratory devices, statistical methods, and the serum levels proposed as the cut-off. While most of the studies used P95 as upper limit, Zierk et al [9], KiGGS [8] and LIFE Child propose P97/P97.5 as a threshold. Only KiGGS [8] and England et al [6] estimated percentiles, Zierk et al [9] applied a mixture of healthy and pathologic samples and a complex statistical approach to calculate percentiles. Dehghani et al [7] presented reference values for three distinct age intervals. This explains substantial differences in the serum levels proposed as cut-off values.   
The initial decrease in ALT has also been described by England et al [6] and apart from the missing ALT peak in early puberty in boys, Zierk et al [9] presented similar patterns for boys and girls. However, their reference values are higher than our 97th percentile values. Estey et al [5], Schwimmer et al [4], Poustchi et al [3], and Dehghani et al [7] published reference values that are markedly below ours. Looking at the trends shown by reference intervals of distinct age groups, we found noticeable changes during the onset of puberty. According to Siest et al [27] the most commonly used cut-off values for ALT and AST in adults are 40 U/l (30 – 50 U/l). Kim et al [28] recently estimated male thresholds of 30 U/l for ALT and 31 U/l for AST to be best cut-offs for the prediction of liver disease. Compared to our P97 in boys, these cut-offs are lower. The 97th percentile of LIFE Child reaches 30 U/l already at the age of 9 and continues its increasing trend throughout our observed age span. The same pattern was found for AST; our threshold significantly exceeds 31 U/l throughout the observed age span. Apart from Kim et al [28], only a few studies exist that examined AST. Estey et al [5] and Zierk et al [9] both found similar dynamics to our percentiles for boys and girls, whereas Dehghani et al [7] presented increasing reference values over age for both sexes. The proposed cut-off values from Estey et al [5] and Dehghani et al [7] are below our 97th percentiles, but those published by Zierk et al [9] are in good accordance. GGT percentiles were published by KiGGS [8] in 2009 and by Zierk et al [9] in 2015. Their percentiles correspond quite well with ours. The LIFE Child percentiles slightly exceed KiGGS’ [8] serum levels during adolescence. Compared to Zierk et al [9], our reference values are slightly lower during childhood and slightly higher in girls during adolescence.   
Comparing all mentioned reference values, we found large differences in the described trends, particularly during the onset of puberty. The previously published patterns showed varying changes during that period, such as dips or rises. However, variations have also been found within our percentiles. Most studies described gender-specific differences in serum level concentrations [4, 6, 8, 9]. In addition, we showed that gender disparity with regard to transaminases appears to increase with age. Boys exceed girls with their serum concentration regarding all three transaminases. Moreover, we found enlarging ranges between P3 and P97 with growing age for ALT, AST and, GGT, which are more pronounced in boys than in girls. Obviously, age and/or puberty influence the level of transaminases.

Strengths and limitations  
The main strengths of our study are the composition and large sample size of the reference population, the standardised assessment, and the usage of novel statistical and laboratory methods. LIFE Child is one of the largest cohorts of healthy children and adolescents in Europe and therefore very well suited for the calculation of reference values.  
Regarding limitations, others have already reported [29] that the composition of the LIFE Child cohort differs from the general distribution in the city of Leipzig, especially regarding the social background of the participants. Children from socially disadvantaged families were underrepresented in the LIFE Child study, possibly due to a less pronounced health awareness [30]. A higher proportion of obesity in older children might also cause bias. It may have influenced the level of the upper percentiles of ALT and GGT. This would be consistent with the fact that these two transaminases are known to be strongly positively correlated with the BMI [2, 18].  
Due to the ethnic composition of the city of Leipzig and underrepresentation of Non-Caucasians, this study does not describe the potential influence of ethnicity on transaminases.

**Conclusion**  
Current reference values of ALT, AST and GGT serum concentrations in children and adolescents between 1 and 17.5 years were established. A new methodological approach permitted a more detailed description of the age-dependency than in former studies. In addition, percentiles derived from normal weight children and adolescents are available, which are helpful for paediatricians in clinical practice.   
We found that age, gender, BMI, and puberty status influence the patterns of the three transaminases considered here and highlight the need for sex- and age-specific reference values. We suggest using the percentiles derived from this study as the current reference values for children and adolescents.

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**List of Abbreviations:**

WHO World Health Organisation

ALT alanine aminotransferase

AST aspartate aminotransferase

GGT gamma glutamyltransferase

LIFE Leipzig Research Centre for Civilization Diseases

BMI body mass index

STROBE STrengthening the Reporting of OBservational studies in Epidemiology

UV ultraviolet

IDMS Isotope dilution mass spectrometry

HDL high density lipoprotein

CE-IVD CE in vitro diagnostic

VSM vital sign monitor

IDF International Diabetes Federation

LMS/LMST LMS method of Cole, LMS-type

BCT Box-Cox t distribution

SDS standard deviation score

s.d. standard deviation

M median

S coefficient of variation

L skewness

KiGGS nationwide health survey in children and adolescents carried out by the Robert Koch Institute, Germany

CALIPER Canadian Laboratory Initiative in Pediatric Reference Intervals

SAFETY Screening ALT For Elevation in Today’s Youth

ERDF European regional development fund

SMWK Saxonian Ministry of Science and Arts

DFG German Research Foundation

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