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Clinical Research Article

Reference Values for Serum Leptin Levels in Children, Adolescents, and Adults With Normal Weight, Overweight, and Obesity

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Abstract

Background: Interpretation of blood leptin concentration in clinical practice and research is limited by a lack of comprehensive reference values. We aimed to establish reference ranges across the age and weight spectrum, taking into consideration important covariates age (0-75 years), pubertal status, and body weight status (normal to extreme obesity).

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Method: Data from 12 629 individuals across 16 European cohorts were pooled and extracted for weight, height, Tanner stage (TS), and serum leptin concentration via ELISA (Leptin ELISA kit). Generalized additive models for location, shape, and scale were used to render reference curves stratified by sex, TS, and weight status.

Results: In boys, serum leptin concentrations increased between ages 6 and 12, followed by a decline after age 12, while girls showed an increase until age 15, with body mass index (BMI) SD score (SDS) dependent trajectories thereafter. Leptin concentrations were generally higher in girls than boys, except in boys aged 9 to 15 years with a BMI-SDS of > 3. In adults, women consistently had higher leptin concentrations across all BMI categories. In men, leptin concentrations decreased until the mid-20s for a BMI of 30 kg/m^2 and until age 50 for a BMI of 40 kg/m^2, stabilizing thereafter.

Conclusion: We present the first reference curves for leptin concentrations across the entire age and weight range. An online tool and an R package for calculating leptin z-scores that are specific to age, sex, TS, and BMI (or BMI-SDS) are now available for clinical and research use at https://leptin.science.

Key Words: leptin, children, adolescent, adult, obesity, overweight

Leptin is a proteohormone primarily secreted by adipocytes and encoded by the *LEP* gene. It serves as a key component of the endocrine system because leptin connects nutritional changes to adaptive responses across nearly all physiological systems. Through binding to its widely distributed receptor, leptin exerts systemic effects, most notably in the regulation of hunger and satiety, neuroendocrine function, and energy homeostasis and in modulating inflammatory responses (1-3). Leptin crosses the blood–brain barrier to act on hypothalamic neurons, particularly in the arcuate nucleus, where it modulates 2 key pathways: the orexigenic (neuropeptide Y) and anorexigenic (POMC/α-MSH) system. Lack of activation of the melanocortin-4 receptor by α-MSH results in increased appetite and hunger and decreased energy expenditure, highlighting leptin's central role in body weight regulation (4).

Leptin can readily be measured in the serum. Hence, reference ranges are essential for interpreting serum leptin concentrations. Existing reference values are limited to children and adolescents with normal weight (5-8) or to healthy adults with body mass index (BMI) values up to 35 kg/m² (9-14). Data in populations with obesity were largely statistically approximated due to the relatively low numbers of subjects included from the upper weight range. Subsequent efforts to define reference values have addressed specific age (5-7) or BMI groups (6, 8) but have not comprehensively accounted for the key determinants of sex, BMI, and pubertal stage [Tanner stage (TS)]. In children, adolescents, and adults with overweight or obesity, reference ranges have not been established. Yet, reference ranges in this group have been of particular interest for distinguishing obesity caused by leptin-deficient states from other forms of obesity. Due to the strong relationship between leptin concentration and adipose mass, a patient with obesity may have a seemingly normal or even an elevated leptin concentration that may still be lower than expected given the degree of adiposity. This concept is akin to an "inappropriately normal" concentration seen with other hormone imbalances (eg, parathyroid, thyroid). Thus, interpretations of leptin concentrations in people—especially children—with obesity are largely determined by the subjective understanding and experiences of the treating physician or scientist.

Our aim was to establish sex-specific leptin reference curves spanning the ages of 0 to 75 years, encompassing a BMI SD score (SDS) range from −2 to +4 in children and adolescents and a BMI range up to 50 kg/m² in adults and incorporating Tanner staging where applicable.

Materials and Methods

Cohorts' Inclusion Criteria

We included cohorts of children, adolescents, and adults with a cross-sectional design if they included the following data elements: sex (male/female), age (years), pubertal development (TS, only in cohorts comprising children and adolescents), BMI (kg/m^2) or BMI-SDS who had serum leptin concentration measured with the leptin E077 SENSITIVE Leptin ELISA kit (Mediagnost, Reutlingen, Germany) (reference material: International Standard WHO NIBSC 97/594, recombinant leptin). Sixteen cohorts from Germany, Denmark, and the Netherlands (Europe) met the inclusion criteria and are described in detail in Supplemental Table S1 [study design, participants, country, population-based recruitment (yes or no), sample size, age range (15)]. Nine cohorts (cohorts 2, 3, 4, 6, 10, 11, 12, 13, 14) were recruited from the general population. Seven cohorts specifically included patients with obesity (cohorts 1, 5, 7, 8, 9, 15, 16) [Supplemental Table S1 (15)]. The data from a cohort (cohort 4, n = 713) have previously been used to establish reference values for leptin concentrations in children and adolescents (5). Descriptive statistics of patients by cohort included in the pooled analyses are listed in Supplemental Table S2 (15). The final pooled cohort comprised 12 629 subjects: 5940 children and adolescents (0.25-<18 years, 48% male) and 6689 adults (18-89 years, 46% male). Sample size and basic characteristics by age group are listed in Table 1.

Anthropometry and Puberty

As reported in the individual cohort description, weight and height data were collected with an accuracy of 0.1 cm and 0.1 kg. BMI was computed as body weight (in kg) divided by the square of height (in meters). SDS of BMI were constructed by the lambda-mu-sigma (LMS) method (16). The pubertal stage (development of breast and pubic hair) was documented according to the classification by Tanner (17). For the analysis of subgroups, the classification was based on pubic hair development. All procedures were carried out by trained and certified study personnel.

Blood Sampling and Measurement of Serum Leptin Concentration

As reported in the individual cohort description, blood samples were drawn from the vein. In the majority of cohorts (12/16), blood samples were taken in a fasting state [Supplemental Table S1 (15)]. Serum leptin concentrations were measured using the E077 SENSITIVE Leptin

Table 1. Descriptive statistics for the pooled dataset, separated into children and adolescents (< 18 years) and adults (18-75 years), and weight status. Data are shown only for patients up to 75 years of age (N = 273 adults were > 75 years of age)

	Children and adolescents with BMI < 90th percentile						Adults with BMI < 25 kg/m^2					
	0-6 (N = 1455)		6-14 yrs (N = 1961)		14-18 yrs (N = 440)		18-40 yrs (N = 537)		40-60 yrs (N = 1116)		60-75 yrs (N = 795)	
	Female n = 643	Male n = 812	Female n = 1024	Male n = 937	Female n = 202	Male n = 238	Female n = 196	Male n = 341	Female n = 455	Male n = 661	Female n = 348	Male n = 447
Age (years)												
Mean/median	2.8/2.9	2.6/2.5	9.2/8.5	9.0/8.4	16.0/16.0	15.8/15.6	32.0/35.0	28.8/28.9	49.4/50.0	50.5/51.4	67.2/67.2	67.4/67.5
[Min-max]	[0.1-6.0]	[0.1-6.0]	[6.1-14.0]	[6.1-14.0]	[14.0-18.0]	[14.0-18.0]	[18.0-40.0]	[18.0-40.0]	[40.0-60.0]	[40.1-60.0]	[60.0-75.0]	[60.1-75.0]
Weight group	n (%)											
Underweight (BMI <10th perc.)	41 (5.5%)	62 (6.9%)	100 (6.3%)	69 (4.5%)	16 (2.6%)	34 (6.1%)						
Normal weight (BMI >10th & < 90th perc.)	602 (81%)	750 (83%)	924 (58%)	868 (57%)	186 (30%)	204 (37%)						
Underweight (BMI <18.5 kg/m^2)							1 (0.2%)	4 (0.6%)	0 (0%)	10 (0.7%)	4 (0.3%)	4 (0.3%)
Normal weight (BMI > 18.5 & > 24.9 kg/m^2)							195 (44%)	337 (52%)	455 (35%)	651 (46%)	344 (29%)	443 (31%)
BMI SDS[a]/BMI (kg/m^2)	BMI SDS[a]						BMI (kg/m^2)					
Mean (SD)	0.0 (0.6)	0.0 (0.6)	−0.1 (0.7)	0.0 (0.7)	0.0 (0.6)	0.0 (0.6)	21.8 (1.8)	22.3 (1.6)	22.4 (1.8)	23.1 (1.6)	22.9 (1.7)	23.3 (1.5)
Median (Q1, Q3)	0.0(−0.5, 0.5)	0.0 (−0.5, 0.5)	−0.1 (−0.6, 0.4)	0.0 (−0.6, 0.5)	−0.1 (−0.5, 0.6)	0.0 (−0.5, 0.4)	21.7 (20.2, 23.3)	22.3 (21.0, 23.5)	22.6 (21.0, 23.9)	23.5 (22.0, 24.5)	23.1 (21.9, 24.2)	23.7 (22.6, 24.4)
[10% centile-90% centile]	[−0.8-0.8]	[−0.9-0.9]	[−0.9-0.9]	[−0.9-0.9]	[−0.9-0.9]	[−0.9-0.8]	[19.5-24.3]	[20.2-24.5]	[20.0-24.8]	[20.6-25.0]	[20.5-24.6]	[21.2-24.8]
[Min-max]	[−1.3-1.3]	[−1.3-1.3]	[−1.3-1.3]	[−1.3-1.3]	[−1.2-1.3]	[−1.2-1.3]	[17.8-25.0]	[16.1-25.0]	[16.9-25.0]	[18.9-25.0]	[15.4-25.0]	[16.9-25.0]
Leptin (ng/mL)												
Mean (SD)	2.1 (1.6)	1.7 (1.4)	4.4 (3.7)	2.7 (3.4)	8.5 (5.7)	2.0 (2.7)	9.0 (5.9)	2.0 (1.8)	9.0 (6.7)	2.4 (2.3)	9.5 (6.6)	2.7 (1.9)
Median (Q1, Q3)	1.6 (1.0, 2.7)	1.2 (0.8, 2.1)	3.2 (1.9, 5.6)	1.7 (1.0, 3.1)	7.0 (5.0, 11.0)	1.2 (0.7, 2.4)	8.0 (4.9, 11.8)	1.3 (1.0, 2.4)	7.4 (4.5, 11.9)	1.8 (1.0, 3.1)	7.6 (4.9, 12.4)	2.3 (1.3, 3.7)
[10% centile-90% centile]	[0.7-4.0]	[0.5-3.4]	[1.2-9.2]	[0.6-5.5]	[2.6-16.8]	[0.3-4.3]	[2.7-15.5]	[0.5-4.3]	[2.7-16.1]	[0.6-4.6]	[3.1-18.5]	[0.9-5.4]
[Min-max]	[0.2-12.9]	[0.2-8.6]	[0.2-31.5]	[0.1-36.9]	[1.0-35.2]	[0.1-22.6]	[1.2-43.4]	[0.1-13.0]	[0.4-63.2]	[0.0-20.8]	[0.2-44.3]	[0.1-10.4]

(*continued*)

Table 1. Continued

	Children and adolescents with BMI ≥ 90th percentile						Adults with BMI ≥ 25 kg/m^2					
	0-6 yrs (n = 191)		6-14 yrs (n = 1148)		14-18 yr (n = 744)		18-40 yrs (n = 556)		40-60 yrs (n = 1604)		60-75 yrs (n = 1809)	
	Female N = 102	Male N = 89	Female N = 558	Male N = 590	Female N = 424	Male N = 320	Female N = 243	Male N = 313	Female N = 857	Male N = 747	Female N = 846	Male N = 963
Age (years)												
Mean/Median	3.2/3.4	2.6/2.5	10.9/11.3	11.0/11.4	15.6/15.4	15.6/15.4	30.1/34.0	31.3/34.4	51.3/52.2	50.9/51.7	67.1/67.1	67.3/67.4
[Min-Max]	[0.2-6.0]	[0.2-5.9]	[6.0-14.0]	[6.0-14.0]	[14.0-18.0]	[14.0-18.0]	[18.0-40.0]	[18.0-40.0]	[40.0-60.0]	[40.0-60.0]	[60.0-75.0]	[60.0-75.0]
Weight group	n (%)											
Overweight (BMI >90th & < 97th perc.)	42 (5.6%)	41 (4.6%)	133 (8.4%)	132 (8.4%)	32 (5.1%)	31 (5.6%)						
Obesity (BMI >97th & > 99.5th perc.)	12 (1.6%)	23 (2.6%)	205 (13%)	289 (19%)	100 (16%)	112 (20%)						
Severe obesity (BMI >99.5th perc.)	48 (6.6%)	25 (2.8%)	220 (14%)	169 (11%)	292 (47%)	177 (32%)						
Overweight (BMI >25 and <30 kg/m^2)							108 (25%)	88 (13%)	568 (43%)	428 (30%)	606 (51%)	563 (40%)
Obesity grade 1 (BMI >30 and >35 kg/m^2)							41 (9.3%)	66 (10%)	186 (14%)	176 (13%)	183 (15%)	288 (20%)
Obesity grade 2 (BMI >35 kg/m^2)							94 (21%)	159 (24%)	103 (7.9%)	143 (10%)	57 (4.8%)	112 (7.9%)
Leptin (ng/mL)												
Mean (SD)	13.8 (13.0)	7.9 (9.2)	31.5 (21.2)	24.3 (15.8)	44.6 (23.2)	32.1 (25.4)	36.8 (24.2)	18.6 (25.1)	22.8 (14.8)	6.8 (6.7)	25.9 (17.5)	7.3 (6.2)
Median (Q1, Q3)	9.4 (3.6, 20.3)	3.9 (1.6, 10.2)	27.2 (16.4, 41.7)	21.4 (12.4, 32.7)	39.2 (28.5, 54.6)	26.9 (15.1, 41.4)	29.8 (20.8, 49.4)	7.8 (4.2, 18.9)	19.0 (12.8, 28.5)	5.1 (3.2, 8.4)	21.1 (14.6, 32.8)	5.6 (3.4, 8.8)
[10% Centile-90% Centile]	[2.1-31.2]	[0.6-23.2]	[10.9-56.5]	[6.7-45.0]	[21.1-73.4]	[9.0-61.1]	[12.1-68.8]	[2.3-55.6]	[9.1-40.7]	[1.9-12.7]	[10.0-45.2]	[2.0-14.1]
[Min-Max]	[0.2-58.1]	[0.2-41.8]	[0.2-183.6]	[0.7-96.9]	[0.2-153.8]	[1.1-207.3]	[0.6-150.4]	[0.7-117.7]	[1.0-171.9]	[0.6-77.5]	[3.0-183.9]	[0.4-52.5]

Abbreviations: BMI, body mass index; Q, quartile; SDS, SD score.
[a]Standard deviation score of BMI were constructed by the lambda-mu-sigma (LMS) method (16).

ELISA kit| with the detection limit of 0.01 ng/mL and an inter- and intra-assay coefficient of variability (CV) below 10% (interassay CV 7.2% at 2.04 ng/mL or 7.5% at 14.86 ng/mL; intra-assay CV 2.63% at 4.1 ng/mL or 4.35% at 22.44 ng/mL) (18).

Statement of Ethics

The individual ethics votes per cohort are as follows: cohort 1, Leipzig childhood obesity cohort (approved by the ethics committee of the University of Leipzig, reference number: 007/04; 029-2006; 265-08); cohort 2, LIFE Child study (approved by the ethics committee of the University of Leipzig, reference number: reg. no. 264-10-19042010); cohort 3, Ulm Research on Metabolism, Exercise and Lifestyle in Children (approved by the ethics committee of the University of Ulm with application no. 126/10); cohort 4, Danish cohort of children and adolescents (approved by the ethics committee of Copenhagen, reference number: V200.1996/90); cohort 5 (approved by the ethics committee of the University of Ulm); cohort 6, Ulm Birth Cohort Study (approved by the ethics committee of the University of Ulm with application no. 264/18); cohort 7, YES (approved by the ethics committee of the University of Ulm with application no. 89/12); cohort 8 (approved by the ethics committee of the University of Ulm with application no. Nr. 247/18) ; cohort 9 (approved by the ethics committee of the University of Ulm with application no. 355/16); cohort 10, COVID-19 BaWü Study (approved by the ethics committee of the University of Ulm with application no. 152/20); cohort 11 (approved by the ethics committee of the VU Medical Centre, reference number: 89/71); cohort 12, LIFE Adult Study (approved by the ethics committee of the University of Leipzig, reg. no. 263-2009-14122009); cohort 13, EPIC-Potsdam study (ethical approval was given by the ethics committee of the Federal State of Brandenburg, Germany); cohort 14, longitudinal study on nutrition and health status in a free-living elderly population in Giessen (GISELA) (approved by the ethics committee of the faculty of medicine at Justus Liebig University, reference number: 30/95); cohort 15 (approved by the ethics committee of the University of Leipzig, approval number 159-12-21052012 and 017-12-23012012); cohort 16 (approved by the ethics committee of the University of Ulm with application no. 30/20).

Statistical Analyses

Descriptive statistics

Descriptive statistics are presented as mean, SD, median, interquartile range, 10th and 90th percentiles, and minima and maxima for continuous variables and counts and percentages for categorical variables.

Modeling of leptin percentile curves

As leptin concentrations are strongly correlated with fat mass, reference curves were modeled dependent on age and BMI-SDS. BMI-SDS was chosen because it measures the deviation from the expected mean BMI by age and sex, independent of physiological age-related BMI change. Thus, BMI-SDS is the most robust and prevalent measure for assessing weight status in children and adolescents. The reference values according to pubertal stage were modeled likewise but only including children older than 6 years. Leptin concentrations below or above the limits of detection were marked as left- or right-censored. The continuous modeling of the resulting interval-censored variable depended on age and BMI-SDS (stratified by sex), and generalized additive models of location, shape, and scale were applied (19).

Children and adolescents. After goodness-of-fit tests, we assumed an underlying censored Box-Cox T distribution with varying coefficients for the location, scale, and skewness parameters. We checked for nonlinearity and for an interaction between the two predictors (age and BMI-SDS). Finally, we modeled the location parameter dependent on age and BMI-SDS (p-splines), including the interaction (penalized varying coefficient model). The scale parameter was modeled on second-degree polynomials in both predictors. The skewness parameter depended on second-degree polynomials in both predictors only for younger children. For older children, skewness did not depend on age but was modeled only on BMI-SDS (also second-degree, for girls). There was no BMI-SDS dependency for boys either. There was no evidence of an age or a BMI-SDS dependency for the kurtosis parameters. Furthermore, the same outcome was modeled on TS as a categorical variable and BMI-SDS as a continuous variable, again stratified by sex. Only observations from children older than 6 were included. The location parameter was modeled dependent on TS and, as before, BMI-SDS (p-spline), including the interaction (penalized varying coefficient model). Scale and shape parameters were also modeled dependent on TS and BMI-SDS, as done for the age-dependent models. Skewness and kurtosis were included as constant terms.

Because only a few children under the age of 6 had a BMI-SDS above 2, we restricted the BMI-SDS range accordingly. For subjects between the ages of 6 and 13 or younger, we estimated reference values up to 3 BMI-SDS. For children 14 and older, reference ranges were estimated up to 4 BMI-SDS.

Adults. For leptin concentrations in adults, models were built the same way but using BMI instead of BMI-SDS as a predictor. For adults, similar models were built with leptin as the outcome and BMI and age as predictors. BMI was chosen, as it is the most prevalent parameter for determining weight status in adults, ensuring broad applicability. BMI-SDS values are generally not used for adults, and therefore, no reliable reference values across the adult age span are available. In the final model, the location parameter was modeled dependent on age and BMI-SDS (p-splines), including the interaction. The scale parameter was modeled on third-degree polynomials in both predictors. The skewness parameter depended on age and BMI for females but not for males. Kurtosis showed no dependence and was, therefore, added as a constant term.

Model quality was assessed visually using diagnostic plots [residual vs fitted, residual against index and predictors, kernel density estimate of the residuals, QQ-normal plot of the residuals; plots are shown in the Supplementary Material (15)]. Subsequently, percentiles were calculated from the estimated distribution parameters. All analyses were computed with R version 4.3 (19, 20). The 2-sided significance level was set to $\alpha = .05$.

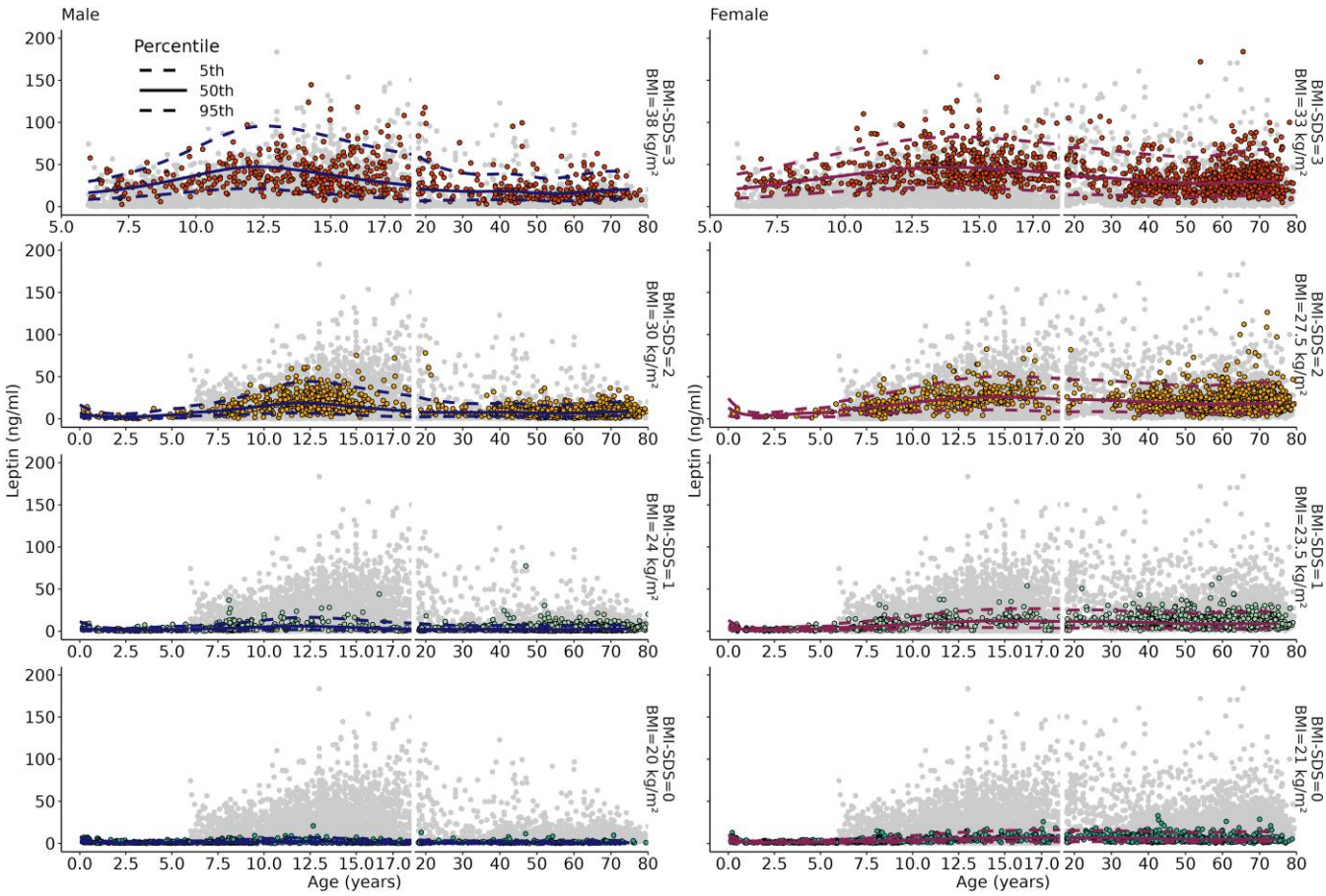

Figure 1. Colored circles represent the raw leptin values plotted across ages for the respective BMI-SDS values (0, + 1, + 2, + 3) in children, or corresponding BMI in adults [eg, a BMI-SDS of 3 corresponds to a BMI of 38 kg/m^2 at the age of 18 years in boys and a BMI of 33 kg/m^2 in girls using the German age- and sex-specific reference values (16)]. Lines represent modeled percentile curves for leptin (5th, 50th, and 95th percentiles). The models included age, sex, and BMI-SDS in children < 6 years; age, sex, BMI-SDS, and Tanner stage in children and adolescents 6 to 18 years; and age, sex, and BMI in adults (18-75 years).

Abbreviations: BMI, body mass index; SDS, SD score.

Results

Figure 1 shows raw leptin concentrations and modeled percentile curves across the entire age range from 0 to 75 years, stratified by weight status measured as BMI-SDS (children) or BMI (adults). As the reference values depended on age and sex, we considered BMI-SDS for children under 6 years, BMI-SDS plus TS for children and adolescents aged 6 to 18 years, or BMI in adults. The 5th, 50th, and 95th leptin percentiles for males and females are displayed dependent on BMI-SDS in the range of −1 to +3 (in children and adolescents) or the corresponding BMI values (in adults).

During the first year of life, serum leptin concentrations showed a marked decline across all percentiles, in both sexes and all BMI-SDS classes (Fig. 2A). This decline continued until the age of 2 years, although it was less pronounced between 1 and 2 years of age. After the age of 2 years, serum leptin concentrations at the 50th and 95th percentiles increased with advancing age, with a more substantial rise observed in higher leptin percentiles and at higher BMI-SDS. Notably, pronounced sex differences in serum leptin concentrations were evident during the first 6 months of life, with girls exhibiting higher serum leptin concentrations than boys. Overall, leptin percentiles were consistently higher in girls compared with boys.

In boys, leptin concentrations showed an increase across all percentiles between the ages of 6 and 12 years across all BMI-SDS classes (Fig. 2B). After the age of 12 years, the percentile curves for leptin gradually declined. For the lower and central percentiles, the decrease leveled off between 16 and 17 years of age. In girls, leptin concentrations increased across all percentiles up to 15 years. Between 15 and 16 years of age, the leptin percentile curves remained stable for BMI-SDS values up to +1. By contrast, in girls with a BMI-SDS greater than +2, the 50th and 95th percentile curves exhibited a decline. The pronounced sex difference in leptin concentrations, characterized by higher leptin concentrations in girls than in boys, was very strong after the age of 12 but only in adolescents with BMI-SDS values up to +2. In boys older than 9 years with a BMI-SDS of +3, the upper percentile (95th percentile) was higher than that of girls, and this difference persisted until the age of 15 years. After 15 years of age, the 95th percentile for boys fell below that of girls. By contrast, in boys older than 14 years of age with a BMI-SDS of +4, both the 95th and 50th percentiles remained higher than those of girls. The 50th percentile began to converge between boys and girls starting at age 17, while the 95th percentile for boys continued to exceed that of girls even with increasing age.

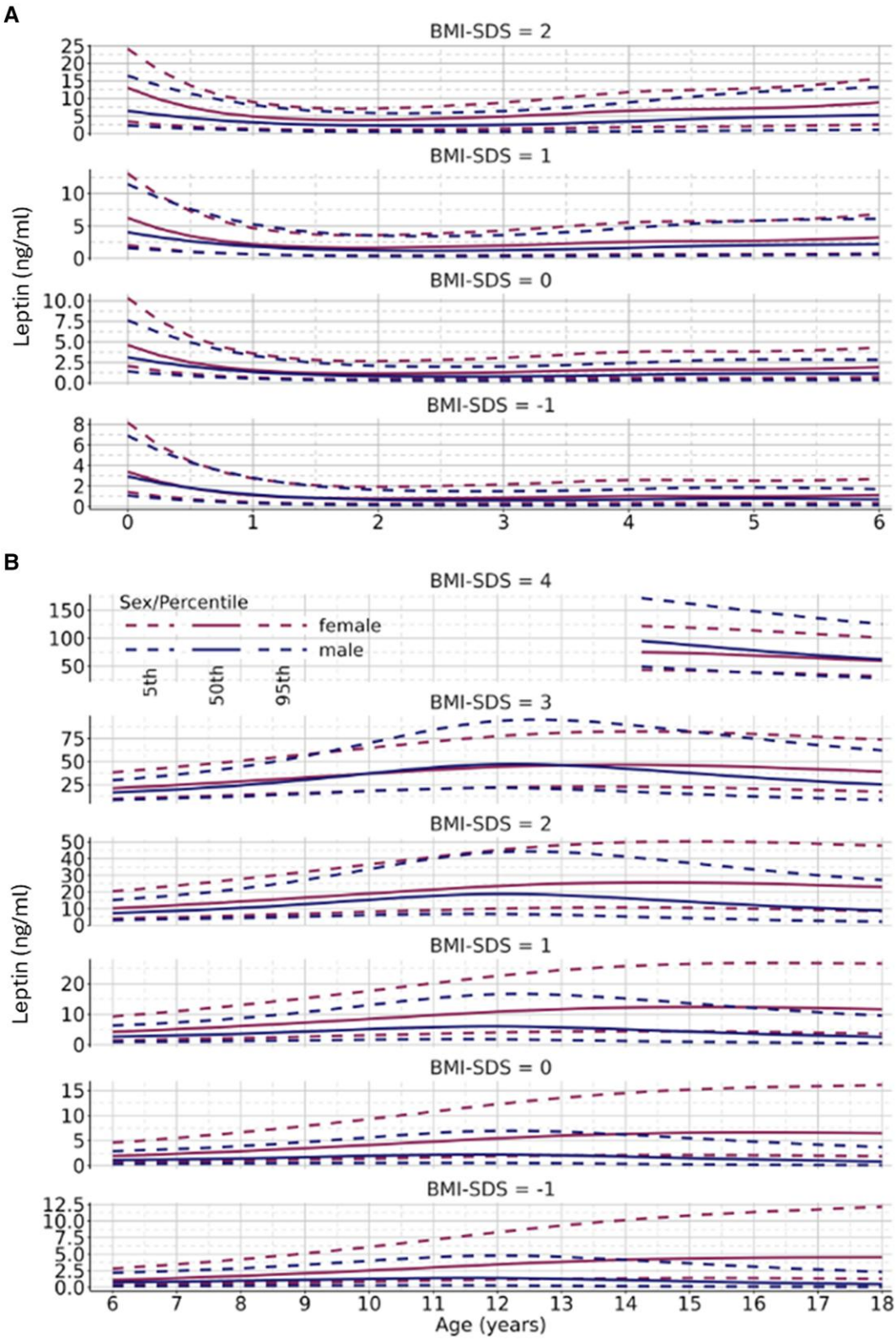

Figure 2. Percentile curves for leptin (5th, 10th, and 95th percentiles) by age and BMI-SDS values [by lambda-mu-sigma (LMS) method (16)] in (A) younger children (< 6 years) and (B) older children and adolescents (6-18 years). The y-axis is scaled differently. (A) Younger children <6 years of age (models include age, sex, and BMI-SDS). (B) Older children and adolescents (6-18 years; models include age, sex, BMI-SDS, and Tanner stage). The modeling of leptin percentile curves for a BMI-SDS of 4 was only possible from the age of 14 onwards due to a low sample size with SDS > 4 in younger children.

Abbreviations: BMI, body mass index; SDS, SD score.

In general, age dynamics in leptin concentrations were more pronounced in children and adolescents with higher BMI-SDS values than in those with lower BMI-SDS values.

When examining leptin concentrations across TS, there was an increase in leptin concentrations between TS1 and TS2 in all BMI-SDS classes in boys (Fig. 3). From TS2 to TS5, leptin

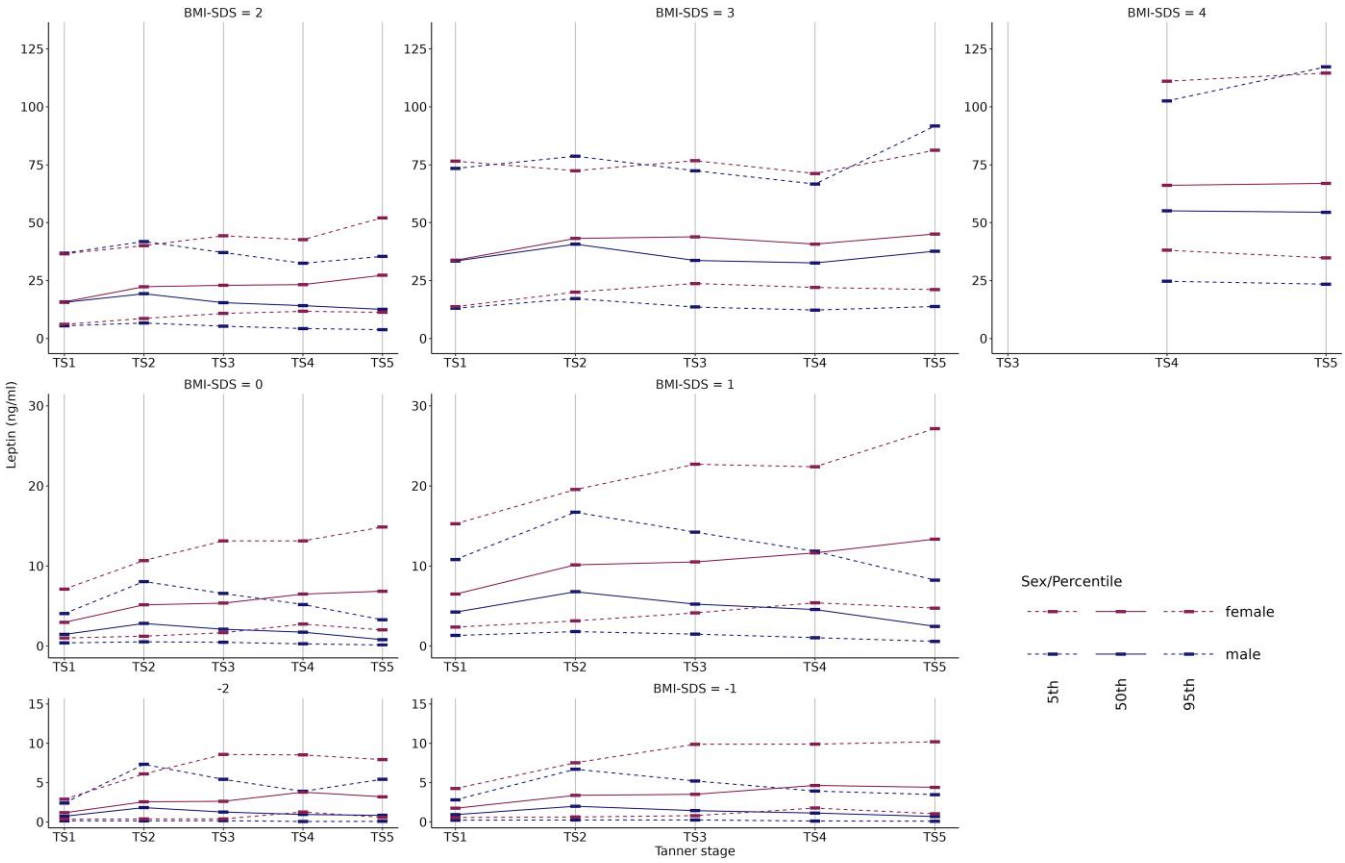

Figure 3. Serum leptin concentrations in males and females by TS and BMI-SDS values. The y-axis is scaled differently.

Abbreviations: BMI, body mass index; SDS, SD score; TS, Tanner stage.

concentrations decreased, but this decrease was observed only in boys with BMI-SDS values up to +2. For BMI-SDS 2 to 3, there was a decrease in leptin concentrations between TS2 and TS4, followed by a rise in leptin concentrations between TS4 and TS5. In boys with a BMI-SDS greater than 3, a decline in leptin concentrations was observed between TS2 and TS3, followed by a continuous increase up to TS5. In girls, the relationship between TS and leptin concentrations was dependent on the BMI-SDS. Up to a BMI-SDS of 0, there was an increase in leptin concentrations between TS1 and TS4, followed by a decline between TS4 and TS5. For BMI-SDS values between 0 and 1, leptin concentrations rose between TS1 and TS2, after which the leptin concentrations remained stable despite progressing puberty TSs. For BMI-SDS 1 to 2, there was a continuous increase in leptin concentrations with advancing TSs. In girls with a BMI-SDS greater than 2, after the increase in leptin concentrations between TS1 and TS2, a plateau phase occurred, followed by a rise in leptin concentrations between TS4 and TS5. As shown in Supplemental Fig. S1, leptin percentile curves depended more strongly on BMI-SDS values than on TS (15). In boys, only the leptin concentrations corresponding to the upper percentiles decreased slightly from T2 to T4 across all weight groups. No remarkable change in leptin concentrations across T2, T3, or T4 was observed in the lower percentiles in males or in females. From T4 to T5, in general, percentiles leveled off for both sexes, whereas leptin percentiles increased in children with BMI-SDS > 3.

In adults, women consistently maintained higher leptin concentrations throughout the entire age range, across all BMI categories and across all percentiles (Fig. 4). In men with BMI 30 kg/m^2, the decrease in leptin concentrations that had started during puberty continued until their mid-30s before plateauing. In men with a BMI of 40 kg/m^2, this decline in leptin concentrations continued until the age of 50, after which the percentiles stabilized. In women, a decrease in the 50th and 95th percentiles of leptin was observed with advancing age up to age 50, followed by stable percentiles. Only in the upper BMI range (BMI > 30 and < 40) for women was there an increase in the 95th leptin percentile with increasing age, beginning at age 50.

Online Tool for the Calculation of Leptin-SDSs in Children, Adolescents, and Adults

We developed an online tool on the website https://leptin.science to allow users to calculate individual leptin-SDS values by entering the parameters age, sex, body height, body weight or BMI, and TS (for children and adolescents) based on the leptin reference values presented here. For research purposes, an R package (https://cran.r-project.org/web/packages/childsds/index.html) is also available on the website, enabling the calculation of leptin-SDS values for entire cohorts using the same parameters. In the supplement, the use of the online tool for calculating the leptin SDS value is demonstrated using 2 examples of 2 patients (15).

Discussion

In clinical practice, serum leptin concentrations are measured in children, adolescents, and adults to evaluate health disorders that are related to body weight and hormone metabolism.

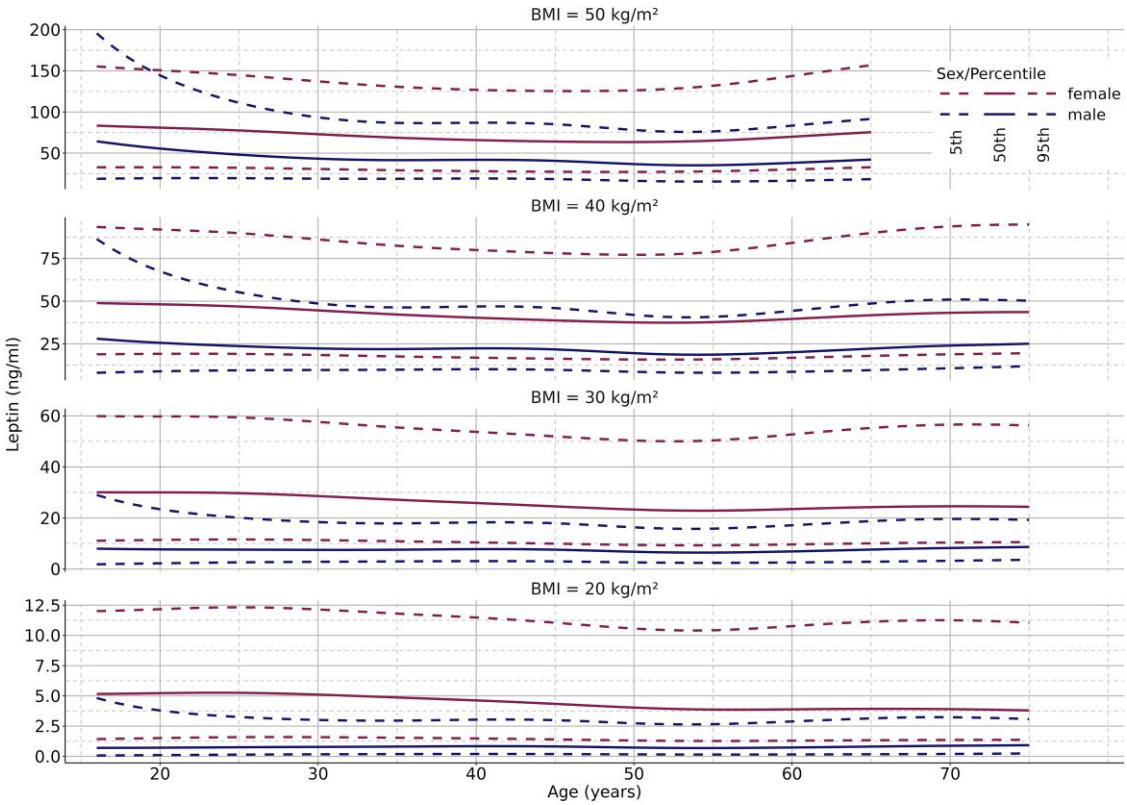

Figure 4. Percentile curves for leptin (5th, 10th, and 95th percentiles) by age and BMI (in adults 18-75 years); models include age, sex, and BMI. The y-axis is scaled differently.

Abbreviation: BMI, body mass index.

Additionally, leptin concentrations are analyzed in research studies to investigate the endocrine and metabolic functions of leptin. However, interpreting leptin concentrations has been challenging due to the absence of comprehensive reference values spanning the full age range from childhood to adulthood while accounting for key determinants such as BMI (including overweight and obesity categories) and TS in children and adolescents. To overcome this limitation, we developed sex-specific leptin reference values spanning ages 0 to 75 years, with BMI-SDS values ranging from −1 to 4 in children and adolescents, including TS, and a BMI range of 15 to 60 kg/m² in adults. Furthermore, we created a user-friendly tool to calculate leptin-SDS values for application in both clinical and research settings.

We observed a significant decline in leptin percentiles during the first 6 months of life, independent of BMI-SDS and sex, with levels stabilizing by age 2. Few studies have investigated leptin dynamics during infancy and early childhood. One study of 317 children up to 18 months observed no sex differences in leptin concentrations during early infancy and noted a slight decrease after 6 months of age (21). However, methodological limitations, such as grouping leptin values into broad fixed age intervals and comparing mean values across the entire cohort, may have obscured dynamic changes and age-dependent sex differences that we specifically addressed. A longitudinal study of 80 children (0-9 years) from Mexico reported a similar initial decline in leptin from birth to 2 years, followed by a gradual increase up to 5 years and a more pronounced rise by 9 years (22). These observations are in line with our findings. The decline in leptin concentrations observed during the first 6 months of life can be attributed to the physiological process of mini-puberty in both sexes. The activation of the hypothalamic-pituitary-gonadal (HPG) increases LH and FSH levels and stimulates estrogen production in the ovaries and testosterone production in the testes. Elevated leptin concentrations during the beginning of this phase may further amplify HPG axis activation, thereby increasing LH and FSH secretion. In boys, GnRH pulsatility decreases during the first 6 months, such that gonadotropins typically become undetectable by the end of this period. Serum testosterone levels, which resemble those observed during puberty, peak around the third month (23). Testosterone has been shown in vitro to suppresses leptin production dose dependently (24), which likely contributes to the leptin decrease observed. In girls, an initial increase in GnRH pulsatility raises LH and FSH levels, temporarily stimulating ovarian estrogen production (25). After 6 months, GnRH pulsatility declines. In some girls, physiological GnRH secretion can persist until the second year of life (23).

We found that leptin percentiles stabilized in boys after the significant decline during the first 6 months. From 9 years of age, leptin concentrations increased until approximately age 12, followed by a subsequent decrease. This pattern is linked to a resurgence in GnRH pulsatility beginning at 9 years of age, leading to increased LH and FSH secretion from the anterior pituitary. LH stimulates Leydig cells to produce testosterone, as occurs during mini-puberty (23). Testosterone suppresses leptin production dose dependently (24) and, along with increased muscle mass relative to fat mass during puberty (26), contributes to the decline in leptin concentrations.

Interestingly, we observed that boys between the ages of 9 and 14 with a BMI-SDS of 3 exhibited leptin percentiles

exceeding those of girls. Additionally, boys with a BMI-SDS of 2 showed an increase in leptin concentrations with advancing TS after the initial decline between TS1 and TS3. This phenomenon may be explained by increased aromatase activity in adipose tissue, which converts androgens into estrogens. Higher body fat percentages in boys may lead to greater conversion of testosterone into estrogen, which is positively associated with leptin concentrations (27).

In girls, we observed a stabilization of leptin percentiles, with a slight increase in the upper BMI-SDS range from the age of 2 years until approximately 15 years. This pattern can be explained by the rise in GnRH pulsatility with the onset of puberty, which stimulates estrogen production, accompanied by an accumulation of body fat. As puberty progresses, GnRH pulsatility stabilizes, resulting in a more consistent frequency and amplitude of GnRH secretion. Notably, in individuals with BMI-SDS values exceeding 2, leptin percentiles continue to rise beyond the age of 15, particularly between TS4 and TS5, likely due to enhanced aromatase activity in the adipose tissue and associated increases in estrogen levels (27).

We observed that leptin percentiles were more strongly influenced by BMI-SDS than by TS. This aligns with findings by Blum et al (5), who established leptin reference values in a cohort of children and adolescents with normal weight, accounting for age, sex, and TS. The relationship between leptin concentrations and BMI was consistent for boys in TS1 and TS2. However, during TS3, TS4, and TS5, average leptin concentrations decreased at a given BMI as pubertal development advanced. This trend was far less pronounced in girls (5). In terms of absolute values, boys and girls exhibited minimal differences in leptin concentrations during prepuberty and early puberty, whereas a significant divergence was observed in TS5. Even in that study, which primarily focused on a cohort of patients with normal weight, BMI emerged as a critical determinant of leptin concentrations. In contrast, our pooled cohort included children and adolescents with normal weight, overweight, obesity, and severe obesity. In the group of patients with obesity, the variability of leptin percentiles was more dependent on BMI-SDS than on TS, suggesting the presence of a sex-specific regulator other than fat mass (9).

In adults, we observed consistently higher leptin concentrations in women compared with men across all age groups, a finding that aligns with well-established evidence of sexual dimorphism in leptin concentrations (28, 29). Differences in body composition and circulating gonadal steroid levels significantly contribute to this phenomenon (24). Notably, in men with a BMI of 30 kg/m^2, leptin percentiles declined until the mid-20s, while in men with a BMI of 40 kg/m^2, this decline extended until approximately 50 years of age. This delayed decrease may reflect impaired gonadal function, as severe obesity is linked to increased aromatase activity and functional hypogonadism. Elevated aromatase activity enhances estrogen production, which correlates positively with leptin levels (27), and may slow the decline in leptin concentrations, extending it into later adulthood. Obesity-related hypogonadism, characterized by dysregulation of the HPG axis and reduced LH secretion and impaired testosterone production, may be exacerbated by insulin resistance and inflammation (30). While testosterone levels decline naturally with age (~1.6% per year) (31), this decline is often more pronounced in men with obesity.

In women, leptin percentiles were consistently higher across all age groups, independent of BMI. Declines in both the 50th and 95th percentile curves for leptin concentrations were observed between the ages of 20 and 55, regardless of BMI. This period coincides with significant changes in body composition, including an increase in body fat percentage and a reduction in muscle mass (32) and a shift toward a relative increase in visceral fat (33). The menopausal transition may further contribute to this decline, as reduced estrogen levels—known to stimulate leptin production (27)—can lower leptin concentrations. A modest rise in leptin percentiles after the mid-50s may reflect selection bias or survival-related changes in the study population.

Why Are Sex-, Age-, BMI-, and TS-dependent Leptin Reference Values Necessary for Children, Adolescents, and Adults?

There are open research questions about the significance of leptin, for example, in the regulation of hunger and satiety (34), in the regulation of puberty and fertility (35), and in genetic factors influencing leptin concentrations (36), as well as about the hypothesis of hyperleptinemia (37), relative leptin deficiency (38), or leptin resistance (39). For example, the hypothesis of relative leptin deficiency assumes that there are patients with low circulating leptin concentrations relative to their BMI. Akinci et al demonstrated that the treatment of adults with obesity and nonalcoholic steatohepatitis and relative leptin deficiency with metreleptin improved the global NASH score (38). We found that prepubertal children with obesity and hepatic steatosis exhibited significantly lower z-scores of circulating leptin concentrations compared with children without hepatic steatosis (40). Adults with obesity with low leptin concentrations may benefit from treatment with metreleptin in terms of weight reduction (41). These and other studies have relied on surrogate definitions—such as cohort-specific percentiles (eg, the 25th percentile)—to define relative leptin deficiency, due to the lack of leptin reference values.

Strengths

One of the primary strengths of our analysis lies in the inclusion of data from n = 12 629 children, adolescents, and adults. To our knowledge, this manuscript represents the first endeavor to establish reference values for leptin concentrations in children, adolescents, and adults considering age, sex, and BMI or BMI-SDS and TS (in children and adolescents), including the whole spectrum from normal weight to extreme obesity. Another strength is the use of the LMS method (42) for calculating reference percentiles that aligns with World Health Organization-recommended methods for age-dependent reference intervals (43). The process of pooling existing cohorts to establish reference values for a laboratory parameter is known to be challenging due to potential influences from preanalytical, analytical, and postanalytical factors. Our approach for addressing this challenge included leptin data from children and adolescents measured with a single assay method, thereby strengthening the reliability and robustness of our findings.

Limitations

The timing of blood collection significantly influences leptin concentrations, with leptin concentrations peaking around 0200 hours and reaching a nadir at 1000 to 1400 hours.

In our cohorts, the time of blood sampling was recorded, and samples were predominantly taken in the morning, as recommended by Blum et al (9). Ideally, blood samples should have been collected in the morning under fasting conditions (44) across all cohorts, but this protocol was not consistently achieved and must be acknowledged as a potential limiting factor.

In the case of females, serum leptin concentrations vary throughout the menstrual cycle, with significantly higher concentrations during the luteal phase compared with the follicular phase (45). Since we did not have information on the menstrual cycle phase at the time of blood collection, we were not able to consider this factor. The dependency of leptin concentrations in childhood and adolescence on pubertal stage was considered in the calculation of reference values using TS. However, classification according to Tanner can be inaccurate, even by trained examiners (46). Nevertheless, it is the method used in practice to determine an individual's pubertal stage.

To the best of our knowledge, no long-term stability data for leptin sera were available from the literature. To overcome this lack of data, the company Mediagnost, which developed the leptin E077 SENSITIVE Leptin ELISA kit, reported that 285 measurements of an in-house quality control serum sample of 5.22 µg/L were taken for more than 15 years and yielded a variation of 10.9% without a trend for significantly changed values. These data (unpublished) suggest a sample stability of at least 10 years when stored at −20 °C.

We present results obtained using a single assay, and application may vary by assay. The general comparability of leptin measurements with different analytical methods is supported by its calibration against the international leptin standard (WHO NIBSC 97/594). Accordingly, a number of leptin assays from different manufacturers should ensure comparability of their measurement data with the NIBSC/WHO 97/594 calibrated Mediagnost results. In the case of otherwise established immunoassays, biological variability in the antigen-antibody interaction may result in somewhat divergent results. Comparison-derived mathematical formulas or internationally accepted assay transfer procedures [e.g., CLSI EP09 (47)] may be helpful for working with data from leptin values from manufacturers other than those mentioned here. Future studies should explore cross-validation with other commonly used platforms to enhance the applicability of the reference curves across different laboratory settings.

This project aimed to establish reference values using all the data available from the pooled cohorts that met the inclusion criteria. Consequently, no separate subsample was available to validate these reference values. We opted for this comprehensive approach because a dataset comprising approximately 12 000 children, adolescents, and adults was large enough for the chosen statistical model to be applied effectively. To enhance the generalizability and clinical utility of the developed reference curves, future studies should focus on their validation in independent and demographically diverse populations. This may include external validation using datasets from cohorts not involved in the original model development, ideally encompassing different geographic regions, age groups, and ethnic backgrounds and ideally with blood samples that have been collected in the morning under fasting conditions and in females during the follicular phase of the menstrual cycle. Moreover, prospective studies could assess the prognostic value of the reference curves in identifying individuals at increased risk for leptin-related metabolic or endocrine disorders. Such efforts are essential to confirm the robustness and applicability of the reference standards in both research and clinical practice.

Conclusion

We established reference values for leptin concentrations in a sample of children, adolescents, and adults spanning a broad range of ages from 0 to 75 years. Notably, the modeling of the leptin percentile curves integrated key determinants of leptin concentrations, including age, sex, and BMI or BMI-SDS and TS in children and adolescents. Our aim was to establish comprehensive reference values that reflect leptin concentrations across a wide BMI spectrum, from normal weight to extreme obesity. These reference values provide valuable tools for both clinical practice and research, allowing for the calculation of leptin-SDS values on the basis of age, sex, and BMI or BMI-SDS and TS (for children and adolescents) using an online calculator or R package. It is recommended that, both in clinical practice and in research studies, blood sampling for leptin measurement should be performed in the morning and preferably in a fasting state. In females, leptin concentrations should be measured during the follicular phase of the menstrual cycle. Moreover, the use of these leptin reference values enables the identification of specific subgroups, which can inform the development of novel therapeutic approaches. Additionally, these reference values support the evaluation of leptin concentrations in the context of follow-up assessments. The application of these reference values in clinical and research settings will demonstrate their practical utility and enhance our understanding of leptin's role in health and disease.

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the cohorts where leptin concentrations had not yet been measured. We also thank Jane Zagorski for her excellent language editing and editorial support.

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Disclosures

M.B. received honoraria as a consultant and speaker from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi-Sankyo, Lilly, Novo Nordisk, Novartis, Pfizer, and Sanofi. M.B.R. resided on advisory boards for Mediagnost, Aeterna Zentaris, and Lumos. J.v.S. received lecture fees from Chiesi Farmaceutici. M.W. participated as principal investigator in clinical trials of Novo Nordisk and has consulted for Rhythm Pharmaceuticals, Chiesi Farmaceutici, and Novo Nordisk and received lecture fees from Mediagnost GmbH, Novo Nordisk, Rhythm Pharmaceuticals, and Chiesi Farmaceutici. L.P. is an employee, managing director, and shareholder of Mediagnost GmbH. All other authors have no potential conflicts of interest to disclose.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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