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Reference values for leptin/adiponectin ratio in healthy children and adolescents

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ABSTRACT

Background: Leptin and adiponectin are two key adipocyte secreted hormones and both are involved in several essential physiological mechanisms. Due to their central role in energy homeostasis their ratio, the leptin/adiponectin ratio, is believed to be a marker of metabolic derangement. Pediatric reference values are needed for the risk stratification of individual-measured ratios.

Methods: Blood samples were drawn from healthy, Danish schoolchildren following an overnight fast. A ratio was calculated from serum leptin and adiponectin quantifications done using commercially available ELISA Kits.

Results: Nine hundred eighty-three participants (583 girls) aged 6–18 years were included. Smoothed percentile curves and age-group specific percentiles were calculated. A correlation with age was demonstrated, with a gradual increase with age in girls and a negative parabolic relation, with a peak in age group 10–14, in boys. The leptin/adiponectin ratio was positively correlated to the body mass index standard deviation score for both girls and boys ($p < .001$).

Conclusion: The leptin/adiponectin ratio is correlated to age and differs between the sexes in healthy children and adolescents.

1. Introduction

1.1. Adipokines

An increasing realization of the adipose tissue as an important and dynamic endocrine organ has developed in recent decades. Adipocytes synthesize and secrete several low-molecular weight peptides, or adipokines, that participate in the regulation of a variety of complex biological functions [1].

1.2. Leptin

Leptin is a key adipokine predominately secreted by adipocytes. It is an important pleiotropic protein that has long been recognized to mediate several vital physiological functions [2,3]. Notably, leptin plays a fundamental role in the regulation of feeding and energy balance [4,5]. By influencing the expression of various neuropeptides leptin regulates energy expenditure through neuroendocrinological adaptation to fasting [3,6]. Furthermore, leptin is implicated in numerous other physiological processes including fatty acid metabolism [7], the reproductive system [8] and is a critical regulator of immunity capable of modulating both innate and adaptive immune responses [9].

1.3. Adiponectin

Adiponectin is the most abundant adipokine and is almost

Abbreviations: BMI, Body Mass Index; GAMLSS, General Additive Model for Location Scale and Shape; L/A, ratio Leptin/Adiponectin ratio; SDS, Standard Deviation Score

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exclusively adipocyte-secreted [10]. The adiponectin plasma concentration is inversely correlated to adipose tissue mass [11].

Adiponectin modulates several central metabolic processes, particularly glucose homeostasis and fatty-acid oxidation, furthermore, adiponectin receptors are found in various tissues and cell types, including liver, heart and pancreatic β-cells [10,12]. Finally, adiponectin exerts potent anti-inflammatory and anti-apoptotic effects through its modulation of cytokine, chemokine, and adhesion molecule expression [12,13].

1.4. Leptin/Adiponectin ratio as a marker for illness

Due to their central role in metabolism and energy expenditure both leptin and adiponectin have gained interest as potential markers of metabolic derangement and insulin resistance [14–16].

Recently, the leptin/adiponectin radio (L/A ratio), has been proposed as a marker of metabolic derangement in adults [17,18] as well as children and adolescents [19,20]. It has also been reported to be a marker for gestational diabetes during pregnancy [21] and for endometrial cancer [22].

A remarkable increase in the incidence of childhood obesity and subsequently of the associated comorbidities has been reported globally over the last few decades [23]. With this increase, the need for cardiometabolic risk stratification has increased, creating a demand for relevant biomarkers, such as the L/A ratio. However, use of the L/A ratio as a risk marker in a pediatric clinical setting is hindered by the lack of reference values from a broad pediatric population.

1.5. Study aim

It this study, we examine the naturally occurring L/A ratio in a population-based cohort of healthy Danish school children and subsequently we calculate and provide reference materials for children and adolescents across age and sex.

2. Methods

2.1. Subjects

Danish schoolchildren (n = 1145) from several municipalities in the region of Zealand, Denmark were invited to participate between October 2010 and February 2015, as previously described by our group [24]. The exclusion criteria were age younger than 6.0 or older than 18.9 years (n = 20), > 30 days between anthropometric assessment and blood sampling (n = 11), known genetic aberrations (n = 4) or thyroid diseases requiring medication (n = 5).

To adjust for variation as a result of genetic ancestry, ethnicity (self-reported) other that Danish/North European white were excluded (n = 122). Informed (signed) consent was obtained from all participants and/or parents. The Danish Data Protection Agency and the Regional Scientific Ethics Committee approved the study (protocol no. SJ-104). The study is part of The Danish Childhood Obesity Biobank and is registered at ClinicalTrials.gov (ID no.: NCT00928473).

2.2. Measurements and data collection

Immediately prior to blood sampling, the participants were examined by trained research assistants. Height and weight were measured while wearing light indoor clothes and without shoes, using a stadiometer to the nearest millimeter and a BC-418 Segmental Body Composition Analyzer (Tanita, Tokyo, Japan) to the nearest 100 g, respectively.

Using the LMS method, a body mass index (BMI) standard deviation score (SDS) was calculated by converting BMI into a normal distribution by age and sex using the median, the coefficient of variation, and a measure of the skewness [25] based on the Box-Cox power plot from Danish BMI charts [26].

We obtained additional data on health status, including previous diagnoses, treatment, and use of medications through a structured family-based questionnaire. Any discrepancies/ambiguous information was cross-referenced through the Regional Hospital Charts.

2.3. Biochemical analyses

Blood samples were obtained from venipuncture of the antecubital vein between 7 and 9 AM following an overnight fast. If requested, the venipuncture was performed following the application of a local anesthetic (lidocaine/prilocaine mixture, EMLA®, AstraZeneca, Sweden). The samples were processed immediately and stored at −80 °C until analysis.

Adipokine concentration measurements were performed in our laboratory at the Statens Serum Institut [24,27]. Evaluation of pre-analytical variables demonstrated that both leptin and adiponectin were stable in serum for 48 h and that neither 10 freeze–thaw cycles nor three months of storage at −20 °C significantly influenced the measured values.

As described previously [24,27], serum leptin and adiponectin concentrations were quantitated using commercial ELISA assays (DY398 and DY1065, respectively), optimized in-house following the manufacturer's recommendations (R&D Systems, Minneapolis, MN, USA). The detection limit of the leptin assay was 0.0312 μg/L and the adiponectin assay range was 62.5 pg/mL–4000 pg/mL.

2.4. Statistical analyses

Statistical analyses were performed in R statistical software (v.3.5.1) [28].

The L/A ratio was calculated as [leptin]/[adiponectin]. Normality was evaluated using histograms, qq-plots and Shapiro-Wilk's test and the data was log-transformed when appropriate. Each sex was analyzed separately as previously published data has shown age- and sex dependent differences [24,27]. The Mann-Whitney *U* test or *t*-test was used to test for differences between the sexes.

To investigate the effects of age within each sex, the participants were allocated into the following age groups: 6.0–9.9, 10.0–14.9, and 15.0–18.9 years of age. These age intervals were chosen to approximate periods of pre-puberty, puberty, and post-puberty, respectively [Juul 2006]. Differences in L/A ratio values between age groups were examined using Kruskal-Wallis Rank Sum test or one-way ANOVA.

Smoothed age- and sex specific percentiles curves were calculated using the Generalized Additive Models for Locations Scale and Shape (GAMLSS) software package [29], with the penalized cubic spline function and the Box-Cox *t*-distribution family (best fit determined by the Akaike Information Criterion). Percentile values for each age group are exactly at the midpoint of each age group. A multivariate linear regression model was used to evaluate associations between log (L/A ratio) and BMI SDS. In a sub-analysis further adjustment was made for absolute serum leptin and serum adiponectin concentrations.

3. Results

3.1. Demography

Nine hundred eighty-three healthy children (583 girls), aged between 6 and 18 years (median 11.9) were included in the study. The descriptive information is presented in Table 1. The L/A ratio was non-normally distributed in the entire population (Shapiro-Wilks test, $p < .0001$) as well as in boys and girls when analyzed separately ($p < .0001$ and $p < .0001$, respectively). The girls were on average 6 months older ($p = .015$), but did not differ in terms of overall anthropometrics when compared to boys.

Table 1
Descriptive information and biomarkers in a population-based cohort of children and adolescents.

	All	Girls	Boys	P
No. of Subjects	983	583	400	
Age (years)	11.9 [9.5, 14.6]	12.1 [9.7, 15.0]	11.6 [9.1, 14.2]	**0.015**
Height (cm)	154 [139.0, 167.0]	156 [140, 166]	151 [138, 171]	0.524
Weight (kg)	43.3 [32.2, 57.0]	45.0 [32.5, 57.0]	41.4 [32.2, 57.1]	0.384
BMI SDS	0.3 (1.1)	0.3 (1.1)	0.4 (1.0)	0.371
Leptin (ng/L)	6622 [3152, 12,535]	9554 [5490, 16,885]	3255 [1853, 6356]	< **0.001**
Adiponectin (μg/L)	4429 [2968, 6662]	4502 [2868, 6569]	4405 [3059, 6723]	0.564
Leptin:Adiponectin-ratio	1.4 [0.6, 3.2]	2.0 [1.0, 4.4]	0.7 [0.4, 1.5]	< **0.001**

Data are medians and [IQR], except BMI SDS which is mean and (sd). All biomarkers are fasting serum measurements. BMI SDS: Body mass index standard deviation score. *P* values are calculated for differences between girls and boys by Mann-Whitney U test or *t*-test.
Significant results are in bold.

Table 2
Descriptive information and biomarkers in a population-based cohort of children and adolescents by age and sex.

Age (years)	6.0–9.9	10.0–14.9	15.0–18.9	P
No. of Subjects				
F	171	264	148	
M	129	206	65	
Age (years)				
F	8.5 [7.6, 9.3]	12.4 [11.2, 13.5]	16.8 [15.9, 17.7]	
M	8.3 [7.5, 9.1]	12.4 [11.1, 13.7]	16.8 [15.9, 17.8]	
BMI SDS				
F	0.2 (1.2)	0.3 (1.1)	0.4 (1.0)	0.328
M	0.4 (1.1)	0.3 (1.0)	0.4 (1.0)	0.913
Leptin (ng/L)				
F	5571 [3292, 9715]	9355 [6062, 15498]	15610 [11100, 22615]	< **0.001**
M	3161 [1853, 6353]	3672 [2183, 6774]	2010 [1206, 3585]	< **0.001**
Adiponectin (μg/L)				
F	5060 [3307, 7182]	4676 [3309, 6519]	3452.5 [2189, 5676]	< **0.001**
M	4429 [3148, 6735]	4532 [3291, 6845]	3418 [2373, 5648]	**0.011**
Leptin:Adiponectin-ratio				
F	1.1 [0.6, 2.3]	2.0 [1.1, 3.9]	4.7 [2.3, 9.8]	< **0.001**
M	0.6 [0.3, 1.5]	0.8 [0.4, 1.8]	0.7 [0.3, 1.2]	**0.018**

Data are medians and [IQR], except BMI SDS which is mean and (sd). All biomarkers are fasting serum concentrations. BMI SDS = Body mass index standard deviation score. P values are calculated for differences across age groups for each sex by Kruskal-Wallis Rank Sum Test or one-way ANOVA.
Significant results are in bold.

3.2. Relation to sex

The overall L/R ratio was significantly higher in girls (2.0 vs 0.7, $p < .0001$) reflecting the significantly higher leptin concentration (9.55 μg/L vs 3.26 μg/L, $p < .0001$). This difference was present and increased throughout the age groups (see Table 2).

3.3. Relation to age

The age-specific percentile values for both sexes are presented in Table 3. A correlation between the L/A ratio and age was found, with a steady and gradual increase in girls and a negative parabolic shape in boys, as presented in Fig. 1.

In a generalized linear model, the log-transformed L/A ratio was positively correlated to age in girls ($p < .0001$). When adjusting for BMI SDS, i.e. the degree of obesity, this age-dependent correlation

Table 3
Percentile values for Leptin:Adiponection-ratio calculated with General Additive Model for Location Scale and Shape (GAMLSS). See text for details.

	Percentiles for girls							Percentiles for boys						
Age (years)	2.5	5	10	50	90	95	97.5	2.5	5	10	50	90	95	97.5
6	0.11	0.16	0.42	0.80	1.51	3.85	5.28	0.10	0.13	0.32	0.63	1.27	3.62	5.16
7	0.14	0.20	0.52	0.99	1.87	4.77	6.55	0.09	0.12	0.30	0.58	1.17	3.33	4.74
8	0.17	0.24	0.62	1.18	2.23	5.68	7.79	0.09	0.12	0.31	0.60	1.20	3.44	4.89
9	0.20	0.28	0.73	1.38	2.61	6.65	9.12	0.11	0.14	0.36	0.70	1.40	4.01	5.71
10	0.23	0.32	0.83	1.58	2.98	7.59	10.42	0.13	0.18	0.45	0.88	1.77	5.07	7.22
11	0.26	0.36	0.95	1.80	3.40	8.67	11.90	0.16	0.21	0.53	1.04	2.09	5.96	8.49
12	0.30	0.42	1.11	2.11	3.99	10.16	13.95	0.15	0.20	0.50	0.98	1.97	5.62	8.00
13	0.36	0.50	1.32	2.51	4.75	12.10	16.61	0.12	0.15	0.39	0.76	1.52	4.35	6.20
14	0.43	0.60	1.58	2.99	5.65	14.41	19.78	0.08	0.11	0.28	0.54	1.09	3.12	4.44
15	0.51	0.71	1.86	3.54	6.68	17.03	23.36	0.07	0.10	0.24	0.48	0.95	2.72	3.88
16	0.59	0.83	2.18	4.13	7.81	19.90	27.31	0.09	0.11	0.29	0.56	1.13	3.23	4.60
17	0.68	0.95	2.51	4.76	8.99	22.92	31.45	0.10	0.13	0.33	0.65	1.31	3.74	5.32
18	0.77	1.08	2.84	5.40	10.19	25.99	35.66	0.10	0.14	0.35	0.68	1.35	3.87	5.51

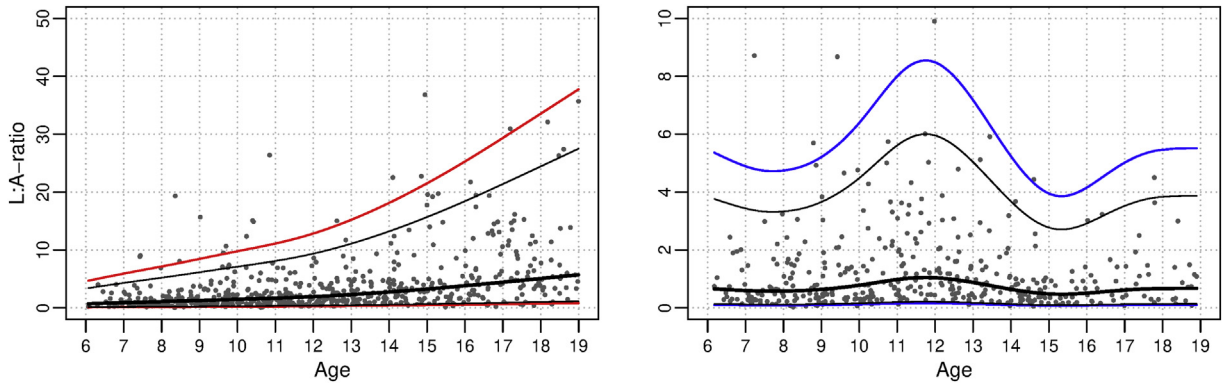

Fig. 1. Leptin/Adoponectin ratio as a function of age in healthy girls (left) and boys (right).

persisted ($p < .0001$). For boys, the log-transformed L/A ratio was not correlated with age ($p = .57$). Adjusting for BMI SDS did not change this relationship ($p = .45$).

3.4. Relation to relative weight

In a multivariate linear model adjusting for age, the log-transformed L/A ratio was positively correlated to BMI SDS for both girls and boys ($p < .001$). These relationships persisted after further adjusting for absolute serum leptin and serum adiponectin concentrations.

4. Discussion

4.1. Pediatric reference curves and this study

Reference intervals are the basis for the interpretation of laboratory results for any individual patient and can allow differentiation between healthy and unhealthy individuals [30]. The availability of precise, comprehensive reference intervals are essential for the clinical applicability of any marker. However, as is the case with the L/A ratio, robust reference intervals do not always exist -particularly in pediatric populations, where practical and ethical considerations often preclude the blood sampling of healthy children, hampering the possibility of constructing robust reference materials. Therefore, our study on 983 children is important, since data on the L/A ratio in healthy children is limited

Furthermore, the measurement of leptin in blood can be significantly affected by pre-analytical, analytical, and post-analytical sources of variation, which makes direct comparisons of leptin-dependent reference materials difficult [31].

4.2. Differences between the sexes

In accordance with well-described differences in total fat mass and fat mass percentage between the sexes; we found a sex-dependent difference in the L/A ratio with girls having higher ratios, which largely reflects the well-known phenomenon in which leptin concentrations are higher in girls than boys [27,32].

4.3. Relationship to age and pubertal development

We also demonstrated an sex-dependent correlation with age for the L/A ratio with a gradual increase in girls though all ages, and a more temporary increase in the 10–14 year age group in boys. For boys, this increase in the L/A ratio appears to be mainly driven by a rise in leptin for this age group–a rise that is in concordance with the existing literature [32]. It is therefore likely that the observed increase in the L/A ratio reflects the influence on leptin production caused by puberty-induced rising serum sex steroid concentrations [33,34].

4.4. The L/A ratio as a marker of metabolic derangement

Cross-sectional studies comparing the L/A ratio with leptin or adiponectin alone, in adults, have produced conflicting results [35], however, most support the view that L/A ratio is a superior biomarker for metabolic derangement [36–38]. Very little, and also conflicting, data in children exist. The L/A ratio has been reported to be either better [39] or equal [19] to leptin as a biomarker for metabolic derangement in children. In this study, the L/A ratio was correlated to BMI SDS even when adjusted for serum leptin and adiponectin concentrations, which indicates that the ratio per se has a role as a marker for metabolic derangement. This finding warrants further analyses of the L/A ratio in children with obesity and obesity-related morbidities.

4.5. Genetic differences

The discrepancies in published data may be due not only to age- and phenotype heterogeneity and sample size limitations in the study populations, but might also reflect that underlying genetic variations between ethnic groups influence adiponectin and leptin concentrations. Several studies have suggested that the concentration of circulating leptin is influenced by single nucleotide polymorphisms (SNPs) [40,41]. Similarly, genome-wide association studies have found that the concentration of adiponectin is influenced by SNPs [42] and highly heritable [43]. While this underlines the need for “local” reference material, it is possible that, despite the relative genetic homogeneity of our study population, several genetic subtypes exist within our study population; each with their own reference values.

4.6. Strengths and weaknesses

The lack of genetic data regarding leptin- and adiponectin-associated SNPs is therefore a potential weakness of the present study, since genetic variants may have a measurable impact on the results. Yet, the biochemical methodology was rigorous, and all samples were collected in a narrow time frame in the morning after a strict overnight fast. This minimizes the influence from the circadian and ultracircadian leptin- [44] and adiponectin [45] cycling. Furthermore, our previous study of the pre-analytic variability demonstrated stability of leptin and adiponectin in the blood sample as well as in the freezing/storage/thawing-process [24,27] and the pre-analytic conditions are unlikely to have influenced the results.

The secondary analysis was done in-house, uniformly with ongoing measurement of analytic variation, subsequently, the presented data can be considered valid regarding potential analytic biases, i.e. methodological variation. The structured family-based questionnaire concerning general health data was self-reported and therefore subject to potential bias. However, as discrepancies or ambiguous information was cross-referenced through the medical charts, it is unlikely that any

systematized bias has influenced the data.

4.7. Conclusion

This study offers important reference values for L/A ratio in a large, genetically homogeneous, healthy, cohort of Danish/North-European white schoolchildren. The study reports age- and sex dependent variations in the L/A ratio in children and adolescents and therefore demonstrates the need for age- and sex specific reference curves.

Due to the high prevalence of pediatric obesity and its associated metabolic derangement, as well as a variety of cardiovascular morbidities, it is likely that the need for metabolic risk stratification will increase in the near future, and the data presented in this study may aid therein.

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Trial registration

The study is part of The Danish Childhood Obesity Biobank; ClinicalTrials.gov ID-no.: NCT00928473, retrospectively registered June 25 2009.

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