



Reference values for serum total adiponectin in healthy non-obese children and adolescents



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ABSTRACT

Background: Adiponectin is an abundant adipocyte-secreted hormone that modulates a number of metabolic processes and is correlated to various metabolic disorders. Pediatric reference levels are needed for the risk stratification and interpretation of individual serum adiponectin levels.

Methods: A total of 1193 healthy, non-obese Danish schoolchildren (730 girls, 463 boys) aged 6–18 years (median 11.9) were examined by trained medical staff. Total serum adiponectin concentrations in venous fasting blood samples were quantitated by a DuoSet® ELISA human Adiponectin/Acrp30 (R&D Systems) following optimization.

Results: In a generalized linear model adjusted for BMI SDS, total serum adiponectin concentrations were correlated to age in girls ($p < 0.0001$) and boys ($p = < 0.0001$) and for both sexes combined ($p < 0.0001$). No significant difference between sexes was found. Reference intervals were calculated using age as a continuous variable. The best fitted reference curve for both sexes was: 50th percentile: $Y = -0.1478 * X + 6.046$; 2.5th percentile: $Y = -0.06256 * X + 2.34$; 97.5th percentile: $Y = -0.4086 * X + 22.39$, where $Y =$ adiponectin in $\mu\text{g/mL}$ and $X =$ years of age (from 6 to 18 years).

Conclusion: We developed a pediatric reference levels for total serum adiponectin in a sample of 1193 Danish children and adolescents aged 6–18 years. A correlation with age was demonstrated in children, but no significant difference was seen between the sexes.

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1. Introduction

Adiponectin is an adipocyte-secreted hormone that modulates a number of metabolic processes, including glucose homeostasis and fatty-acid oxidation [1]. It is the most abundant adipokine in serum and interestingly, studies in adults have demonstrated an inverse correlation between hormone secretion and adipose tissue hypertrophy as the serum adiponectin concentration decreases with increasing fat tissue [2,3]. Recently, adiponectin has gained increasing attention in pediatrics as a serum biomarker due to its correlation to various metabolic risk factors such as insulin resistance, dyslipidemia, metabolic syndrome, and also cardiovascular disease [4–7].

Childhood obesity has increased dramatically across ethnic groups in recent decades, and with that the obesity-associated comorbidities have risen in importance [8]. Consequently, the need for metabolic risk stratification has increased and created a demand for pediatric reference values for relevant biomarkers, including adiponectin. However, the interpretation of serum values in children, and thus an assessment of the clinical utility of the analyte, is hampered by the lack of reference values from a pediatric setting. Contrary to adults, only a few studies on children and adolescents have been published [9–14]. Furthermore, the available data is largely obtained in small and heterogeneous study materials containing a limited number of healthy, non-obese children [9,15].

The objective of the present study is to further elucidate the naturally occurring serum concentrations of adiponectin in a large un-biased cohort of healthy, non-obese Danish schoolchildren aged 6 to 18 years by quantitating serum adiponectin concentrations and subsequently to establish reference ranges for children and adolescents across sex and age groups.

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2. Material

2.1. Subjects

Danish schoolchildren from several municipalities in the region of Zealand, Denmark were included between October 2010 and November 2013. Exclusion criteria were obesity defined as a body mass index (BMI) above the 95th percentile for sex and age [16] at the time of inclusion, and known diseases requiring regular medication. Furthermore, twins were excluded. All participants and/or parents signed informed consent. The study was approved by the Danish Data Protection Agency and the Regional Scientific Ethics Committee (protocol no. SJ-104).

2.2. Anthropometric measurements

The participants were examined by skilled research assistants immediately prior to blood sampling. Height and weight were measured while wearing light indoor clothes and no shoes using respectively a stadiometer to the nearest millimeter and a BC-418 Segmental Body Composition Analyzer (Tanita, Tokyo, Japan) to the nearest 100 g. BMI standard deviation score (SDS) was calculated according to the Danish BMI charts and thereby taking growth and development into consideration [17]. The degree of baseline BMI is expressed in SDS in order to adjust for age- and sex related variation in reference intervals. Additional data on socioeconomic status and health status were obtained through a structured family-based questionnaire. Similarly, the pubertal developmental stage was assessed by a questionnaire where standardized pictures of the various pubertal developmental stages are depicted and thereby aiding the participants and their parents in estimating the appropriate pubertal stage ad modum Tanner [18,19].

2.3. Biochemical analyses

After an overnight fast, venous blood samples were collected from each child between 7:00 and 9:00 am. The samples were processed immediately and the serum was stored at -80°C until further analysis. Total serum adiponectin concentrations were quantitated in singlo by the DuoSet® ELISA Development System for human adiponectin/Acrp30 (Catalog no.: DY1065, R&D Systems, Texas, USA) following in-house optimization of the assay procedure recommended by the manufacturer. The assay quantitates adiponectin of low, intermediate and high molecular weight. The assay range was 62.5 pg/mL–4000 pg/mL. With each plate was run two controls at 977 pg/mL (S.E.M.: 17 pg/mL, $n = 55$) and 92.0 pg/mL (S.E.M.: 1.5 pg/mL, $n = 55$), respectively. An analysis of pre-analytical variation demonstrated that storage at -20°C for three months as well as 10 freeze–thaw cycles did not significantly influence adiponectin concentration measurements.

2.4. Statistical analyses

As studies have demonstrated that healthy girls experience a gradual increase in body fat percentage throughout childhood and adolescence whereas healthy boys have a more stable body composition and often even decreasing fat percentage [20], the data was analyzed separately for each sex and subsequently adjusted for age and BMI-SDS.

Statistical modeling was performed using the R software version 3.1.0. Skewness was estimated by D'Agostino test and log transformation of data was performed when appropriate. Differences between sexes were evaluated non-parametrically for each age interval using the Wilcoxon–Mann–Whitney test.

The correlation between adiponectin and age was evaluated using linear regression and in a generalized linear model, adjusted for BMI SDS. Reference intervals were defined by non-parametric 95-percentile intervals, and band reference intervals derived from the log-normal distribution were also calculated. Best fitting equations for age-dependent adiponectin concentrations were calculated, from

linear to the 3rd degree polynomial regression for the 2.5th, 5th, 50th, 95th, and 97.5th percentile and subsequently compared.

3. Results

A total of 1193 healthy children (730 girls and 463 boys) aged 6–18 (median 11.9) years old were included in the study. The children had normal weight (median 42.5 kg, range 18.4–85.7 kg) for height (median 154.0 cm, range 111.6–195.0 cm) yielding a median BMI SDS of 0.34 (range -1.95 – $+1.95$).

The empirically observed data points and the corresponding reference curves including the calculated 95% confidence intervals are presented in Fig. 1.

In a generalized linear model, total serum adiponectin concentration was found to be correlated to age in girls ($p < 0.0001$) and boys ($p = 0.0015$), as well as for both sexes combined ($p < 0.0001$). This age-dependent association was augmented when adjusting for BMI SDS i.e. the degree of obesity ($p < 0.0001$ for girls, boys and all children). There were no statistically significant associations with social class or BMI SDS between sex and age groups (data not shown).

The reference values for serum adiponectin concentration in the different age intervals for both sexes and combined are presented in Table 1. There was no significant difference between sexes when compared within age groups using non-parametric statistics (see Table 1).

The calculation of reference intervals was performed using age as a continuous variable. The best fitted curve from the first to the 3rd degree polynomial for the 2.5th, the 50th and the 97.5th percentile was calculated for both sexes independently and compared with no indication of a higher order of polynomial being statistically superior. The best fitted curve, a 1st degree polynomial, was calculated for both sexes as 50th percentile: $Y: -0.1478 * X + 6.046$, $R^2 = 0.4113$; 2.5th percentile: $Y: -0.06256 * X + 2.34$, $R^2 = 0.3979$; 5th percentile: $Y: -0.05867 * X + 2.518$, $R^2 = 0.4029$; 95th percentile: $Y: -0.5178 * X + 19.91$, $R^2 = 0.2909$, 97.5th percentile: $Y: -0.4086 * X + 22.39$, $R^2 = 0.1426$, where $Y =$ adiponectin in and $X =$ years of age (range 6–18) for both sexes. The calculated distributions of median, 2.5th and 97.5th percentiles for both sexes in the respective age groups are presented in Table 2.

Of the total of 1193 included children the pubertal stages were obtained 841 cases (322 boys, 518 girls). We performed a selective analysis of adiponectin levels of the individual pubertal stages in boys and girls and both genders combined (Fig. 2). We found a borderline significant negative correlation between adiponectin and pubertal stage in boys ($p = 0.06$) but not in girls ($p = 0.52$) nor in the combined data ($p = 0.25$).

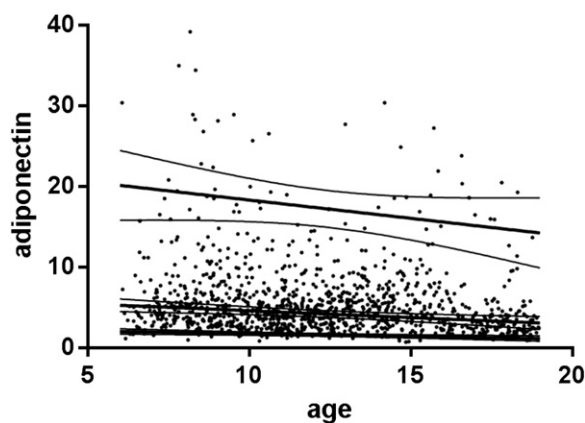


Fig. 1. Empirical observations (dots) with imposed calculated reference curves (lines) for adiponectin ($\mu\text{g/mL}$) in healthy adolescents showing in descending order the 97.5th percentile, 50th percentile, and 2.5th percentile with 95% confidence intervals (thin lines).

Table 1Empiric reference values (50th, 2.5th, and 97.5th percentile) in $\mu\text{g/mL}$ of total adiponectin in males and females from 6 to 18 years. WMW: Wilcoxon–Mann–Whitney test; P: p-value.

Age	6	7	8	9	10	11	12	13	14	15	16	17	18
Boys n	16	32	49	49	49	53	55	43	45	25	17	14	12
Median	4.076	4.743	5.049	4.728	4.318	3.900	4.233	5.051	4.520	5.307	3.322	2.722	2.368
2.5th	1.507	3.014	1.887	2.236	2.342	1.447	1.781	1.571	1.395	2.110	1.643	1.947	1.097
97.5th	10.313	16.937	28.478	25.176	19.568	10.001	13.391	10.070	16.883	17.672	13.008	5.223	10.444
Girls n	19	47	67	71	75	77	69	59	54	46	49	48	32
Median	5.109	5.156	5.248	4.610	4.075	4.151	4.414	5.427	4.834	5.061	2.890	3.333	3.438
2.5th	1.666	1.651	1.205	1.910	1.388	1.813	1.668	1.964	0.770	1.378	1.433	1.501	1.147
97.5th	23.758	20.567	24.425	11.823	17.562	13.447	13.804	12.026	18.597	21.018	19.982	15.934	12.380
WMW	127	727.5	1562	1898.5	2014.5	1873	1808	1106	1284	636	414	280.5	166
P	0.4172	0.8108	0.6588	0.4247	0.367	0.4288	0.6544	0.2198	0.6303	0.4664	0.9766	0.3544	0.5016
Total n	35	79	116	120	124	130	124	102	99	71	66	62	44
Median	4.438	5.096	5.210	4.638	4.140	4.146	4.310	5.150	4.746	5.103	2.920	3.135	2.510
2.5th	1.561	2.439	1.555	2.079	1.829	1.447	1.735	1.666	0.927	1.528	1.444	1.509	1.028
97.5th	17.898	19.488	28.358	16.882	19.174	13.309	14.417	12.022	18.585	19.635	19.243	15.918	13.044

4. Discussion

Many laboratory variables in pediatrics vary with sex, age, growth, and development and consequently the reference values for a given variable should ideally be established in the pediatric age ranges for both sexes. Various methods for establishing reference intervals have been proposed [21] and large study groups of “normal” subjects are necessary to enable an age and sex stratification. However, for practical reasons large groups of normal children are rarely studied, and furthermore, ethical considerations may preclude the blood sampling in healthy children. These factors reduce the possibility of collecting large samples from a standard population of normal children. As an example hereof, the studies of serum adiponectin concentrations by Cangemi et al. [9] and Papaioannou et al. [14] had a healthy control group of 172 and 170 individuals respectively.

In the present study the serum concentrations of adiponectin in a large cohort of 1193 healthy, non-obese Danish schoolchildren were investigated and the presented data fills a relative gap in the literature, as data on serum total adiponectin concentrations in children and adolescents are currently sparse when compared to adults. Furthermore, very little of the currently available data are derived from healthy, non-obese children and accordingly many conveniently available pediatric study materials that have been used as reference groups may occasionally harbor biases, that should be identified and adjusted. In contrast the group of children that was examined in the present study is less likely to be biased as the children were unselected healthy schoolchildren.

We demonstrated an age dependent correlation with serum adiponectin in both boys and girls which is in accordance with previously published data [9,22]. Interestingly, this correlation persisted even when adjusted for BMI SDS, the latter being a surrogate marker for the amount of adipose tissue. This suggests that the serum adiponectin concentrations were independent of the degree of fat mass in the children and adolescents. Accordingly there appears to be an age-dependent development in the naturally occurring concentration of serum adiponectin in children and adolescents, irrespective of the relative amount of adipose tissue. Similar observations were done by Cangemi et al. [9]. Equally, this is in concordance with a recent meta-analysis on data from healthy adults, in which no association between BMI and adiponectin levels was demonstrated [23].

Interestingly, we found no statistical difference between sexes when compared in age groups. However, the 95th percentile appears to be non-significantly elevated in girls. The latter tendency has previously been reported as only significant from birth to the age of nine years [9] and our findings are supportive hereof, although the lack of children younger than 6 in our study precludes a direct comparison with other studies. A smaller study of 160 children reported decreasing adiponectin levels in boys at mid puberty to become significantly lower than in girls [13] yet with only 41 girls and 39 boys studied, the difference to our data may be due to small simple sizes. However, our data is in concordance with data from adults where the adiponectin levels did not differ between men and women [23]. We did find an overall lower median range of serum adiponectin than previously reported in plasma by Cangemi et al. [9]. Although this difference may reflect genetic and/or metabolic differences in the study populations it may also reflect technical differences as plasma concentrations of hormones generally are higher than serum concentration [24]. Finally, the possibility that the observed differences in the various studies can be due to a relative low number of studied individuals in the different age groups cannot be ruled out.

A previous study by Böttner et al. [25] has demonstrated a significantly negative correlation between adiponectin and pubertal stage in boys albeit not in girls. We here report similar although not significant decrease in adiponectin in boys, but not in girls. This difference in our data may reflect once again reflect the number of studied individuals as the study by Böttner et al. included 200 normal weight children as compared to our 841 normal weight children. A potential weakness in the sub-analyses of pubertal stage in our study is the self reported pubertal stages. Yet we have no reason to believe that the self reported data contain any systematic error and our overall findings is also in concordance with the finding done by Böttner et al. [25].

The circulating adiponectin concentration has been demonstrated to be highly heritable and is therefore likely to be under strong genetic control [26]. The independence of BMI SDS in adiponectin concentrations observed in our study may reflect such a genetic component in the regulation of adiponectin concentrations.

As all measurements and analyses in this study were done in-house, the presented data can be considered valid regarding a major potential bias in this subject, i.e. the degree of childhood obesity. The health questionnaire concerning general health data was self-reported and

Table 2Calculated reference values (50th, 2.5th, and 97.5th percentile) in $\mu\text{g/mL}$ of total adiponectin in children and adolescents aged 6 to 18 years.

Age	6	7	8	9	10	11	12	13	14	15	16	17	18
50th	5.159	5.011	4.864	4.716	4.568	4.420	4.272	4.125	3.977	3.829	3.681	3.533	3.386
2.5th	1.965	1.902	1.839	1.777	1.714	1.652	1.589	1.527	1.464	1.402	1.339	1.277	1.214
97.5th	19.938	19.530	19.121	18.713	18.304	17.895	17.487	17.078	16.670	16.261	15.852	15.444	15.035

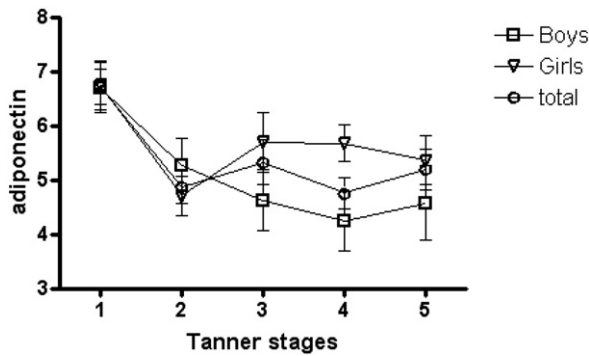


Fig. 2. Mean adiponectin levels in boys, girls, and both genders combined as a function of pubertal stages, expressed as Tanner stages. Data are given as mean \pm SEM.

therefore subject to well-known potential bias. Still, it is less likely for any systematized bias to have influenced the data.

Similarly, an analysis of the pre-analytic variability demonstrated stability of adiponectin in the blood samples. There was no indication of influence by 10 freeze–thaw cycles, nor by storage at $-20\text{ }^{\circ}\text{C}$ for three months. Given that the samples were stored at $-80\text{ }^{\circ}\text{C}$ and only subjected to 1 freeze–thaw cycle no pre-analytic condition is likely to have influenced the results.

Hypoadiponectinemia has been associated with increased risk of the metabolic syndrome and cardiovascular disease [27–29] and it has been proposed that alterations in plasma adiponectin concentrations may help evaluating phenotypically non-obese children, detecting those with more unfavorable risk profiles independent of BMI status [30]. The data from healthy non-obese Danish children presented in this study may therefore potentially help in metabolic risk stratification of children; a task that is likely to gain in importance in the near future as a consequence of the high prevalence of childhood obesity and its associated complications.

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