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Author Contributions:

C.F.B., M.A.V.L., U.L.T., P.L.H., M.C., T.H. and J.H. designed research. C.F.B., U.L.T., and J.H. conducted the data collection. C.F.B. and M.A.V.L. performed literature search. M.A.V.L. analyzed data and generated the tables and figures. C.F.B., M.A.V.L. and U.L.T. wrote the paper draft. All authors contributed to interpretation of data, critical revision of the manuscript, and approved the final manuscript.

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Abbreviations:

BMI: Body mass index

DXA: Dual-energy X-ray absorptiometry

HOMA-IR: Homeostasis model of assessment - insulin resistance

L/A ratio: Leptin/adiponectin ratio

SDS: Standard deviation score

Abstract

Background: It is imperative to develop markers for risk stratification and detection of cardiometabolic comorbidities in children with obesity. The adipokines leptin and adiponectin are both involved in fat mass regulation and the development of obesity-related disorders, furthermore, their ratio (L/A ratio) is suggested to be associated with insulin resistance and cardiometabolic risk.

Objective: To evaluate associations between fasting serum concentrations of the adipokines (total leptin and adiponectin as well as the L/A ratio) and cardiometabolic comorbidities in children with overweight/obesity.

Methods: 2,258 children with overweight/obesity or normal weight aged 6-18 years were studied. Differences in anthropometrics and adipokine concentrations were tested using Wilcoxon rank-sum test. Associations between the adipokines and cardiometabolic risk were tested using Spearman's correlation and logistic regression, adjusted for age and BMI-SDS.

Results: Compared to normal weight children; children with overweight/obesity exhibited higher leptin concentrations, lower adiponectin concentrations and higher L/A ratios. After adjusting for age and degree of obesity, girls with overweight/obesity in the upper quartile range for the L/A ratio, when compared with girls in the lower quartile range, were more likely to have insulin resistance (OR: 7.78 [95% CI, 3.78-16.65]), dysglycemia (OR: 3.08 [95% CI, 1.35-7.31]), and dyslipidemia (OR: 2.53 [95% CI, 1.18-5.59]); while boys were more likely to have insulin resistance (OR: 4.45 [95% CI, 2.03-10.10]).

Conclusions: Independent of the degree of obesity, leptin, adiponectin, and the L/A ratio were associated with insulin resistance and other cardiometabolic comorbidities in children with overweight/obesity, but the L/A ratio exhibited stronger associations than the respective adipokines.

Keywords: Adiponectin, Biomarkers, Insulin Resistance, Leptin, Pediatric Obesity

1. Introduction

Childhood obesity remains a major worldwide health challenge,[1] and is accompanied by numerous complications including subclinical disturbances in glucose metabolism, collectively known as prediabetes.[2] Without intervention 30-40% of adults with prediabetes will develop type 2 diabetes within a few years.[3] However, in children, similar large-scale studies have not yet been conducted. But disease progression in children and adolescents has been reported to be different and may occur more rapidly than in adults[4] and life expectancy is estimated to be reduced by as much as 15 years in adolescent patients with type 2 diabetes.[5] Consequently, it is important to identify individuals within the population of children with obesity, who are at an increased risk of developing obesity related complications, in order to offer preventive interventions earlier.

Adipose tissue secretes multiple hormones (adipokines), that exhibit important roles in metabolic regulation and physiological homeostasis.[6] Two central adipokines, leptin and adiponectin, are known to affect multiple metabolic processes, including the regulation of body weight and energy expenditure.[7,8] Furthermore, obesity-related hyperleptinemia and hypoadiponectinemia associate with metabolic complications including insulin resistance, type 2 diabetes and cardiovascular disease.[9-11]

Since leptin and adiponectin change inversely in relation to BMI, their ratio – the leptin/adiponectin (L/A ratio) – has been suggested as a more sensitive marker of metabolic syndrome in children and adolescents than serum concentrations of total leptin or adiponectin respectively.[12,13] In adults, the L/A ratio has been studied as a predictor of various metabolic conditions.[14-17] As these adipokines change during growth and development, including during

puberty,[18-20] it is essential to determine their clinical utility as markers of metabolic derangement in the pediatric population, especially in children and adolescents with overweight/obesity.

In this study, we investigate the concentrations of fasting serum total leptin and adiponectin as well as the L/A ratio in a large group of children and adolescents, classified as either overweight/obese or normal-weight.

In the group with overweight/obesity we further examine the associations between these biomarkers and various cardiometabolic risk factors, as well as the utility of leptin, adiponectin, and the L/A ratio to risk stratify individuals with dysglycemia, insulin resistance, dyslipidemia, and hypertension, independent of their degree of obesity. Finally, we investigate whether varying degrees of obesity affect the accuracy of the L/A ratio in classifying individuals with cardiometabolic comorbidities.

2. Methods

2.1 Subjects

This study included two cohorts of children and adolescents (N=2.727) enrolled into The Danish Childhood Obesity Data and Biobank from January 2009 to June 2014: an obesity clinic cohort (N=1.592) who was included in a multidisciplinary obesity treatment program,[21,22] and a population-based cohort (N=1.135) recruited by voluntary enrollment following informative meetings at elementary-, middle- and high schools in 11 municipalities in the region of Zealand and the Capital region in Denmark.[23]

Exclusion criteria were: 1) age younger than 6.0 or older than 18.9 years (N=85); 2) underweight defined as BMI < 10th percentile of Danish age- and sex-specific references[24] (N=57); 3) more than 30 days between the clinical examination and the blood sample (N=327).

2.2 Phenotyping

Trained medical staff performed the phenotyping involving a clinical examination, including height and weight, and collected a venous blood sample after an overnight fast. Additionally, each child and family were asked to complete a standardized questionnaire of personal and family medical history; for the obesity clinic cohort the questionnaire was completed during the first consultation in the clinic, whereas the children in the population-based cohort were asked to complete the questionnaire at home prior to collection of the blood sample and clinical examination.

Height was measured by stadiometer to the nearest 1 mm and weight was measured to the nearest 0.1 kg on a Tanita medical scale, WB-110 (Tanita Corp., Tokyo, Japan). BMI SDS was calculated by the LMS method[25] based on a national reference population[24]. Waist circumference was measured at the umbilical level to the nearest 5 mm in a standing position and post-exhalation. Blood pressure was measured with an electronic sphygmomanometer validated in children, Omron 705IT® (Omron Corporation, Kyoto, Japan). After five minutes of rest in a supine position, the blood pressure was measured three times on the right upper arm and average values were calculated from the last two measurements.

Whole body dual-energy X-ray absorptiometry (DXA) scans were performed on the children enrolled at The Children's Obesity Clinic, on a GE Lunar iDXA (ME+200179, GE

Healthcare, Madison, Wisconsin, USA).[26] Total fat mass percentage was calculated as (total body fat mass/(total body mass-total bone mass)*100).

Puberty was evaluated by a pediatrician in the obesity clinic cohort, while the children in the population-based cohort self-reported pubertal stage based on a questionnaire with pictures of the five Tanner puberty stages. Self-reported pubertal staging has been validated to distinguish between prepubertal (Tanner 1) and pubertal (Tanner 2-5) stages,[27] and we classified individuals in both cohorts into one of these two stages for further analysis.

2.3 Definitions of cardiometabolic comorbidities

Dysglycemia was defined as fasting plasma glucose between 5.6 mmol/L –6.9 mmol/L or HbA1c between 39-47 mmol/mol as recommended by the American Diabetes Association as these are two out of three components used to diagnose prediabetes.[28] The third component, a 2-hour standard oral glucose tolerance test was not available in our data material.

Insulin resistance was defined as a Homeostatic Model Assessment: Insulin Resistance (HOMA-IR) > the 90th percentile for age and sex in the Danish population, published from our own center.[29] The HOMA-IR was calculated as (serum insulin (mU/L) x plasma glucose (mmol/L) / 22.5).[30]

Dyslipidemia was defined as concentrations of total cholesterol > 5.2 mmol/L (200 mg/dL), LDL > 3.4 mmol/L (130 mg/dL), HDL < 0.9 mmol/L (35 mg/dL), or triglycerides > 1.7 mmol/L (150 mg/dL), equivalent to concentrations above the 95th percentile according to the American Heart Association.[31]

Hypertension was defined as a systolic blood pressure and/or diastolic blood pressure ≥ 95th percentile for age, sex, and height according to the American Academy of Pediatrics.[32]

2.4 Biochemical analyses

The blood samples were collected between 7 and 9 AM after an overnight fast and obtained from venipuncture of the antecubital vein. If requested a local anesthetic was applied prior to venipuncture (lidocaine/prilocaine mixture, EMLA®, AstraZeneca, Sweden). The samples were processed immediately and stored at -80°C until further analysis.

Following appropriate sample dilution, total serum leptin and adiponectin concentrations were quantitated *in singlo* using optimized versions of commercially available ELISA kits (DuoSet, R&D Systems, Minneapolis, MN, USA Catalog no.: DY398 and DY1065, respectively). The detection limit of the leptin assay was 0.0312 µg/L. The assay calibrator was highly purified *Escherichia coli* expressed recombinant human leptin provided by the manufacturer (Catalog no.: AF398, R&D Systems, Minneapolis, MN, USA). The intra- and inter-assay coefficients of variations were <5% and <10%, respectively. The adiponectin assay quantitates adiponectin as low, intermediate and high molecular weight. The assay range was 62.5 pg/mL–4000 pg/mL. Each plate was run with two controls, a high concentration control measuring 977 pg/mL (S.E.M.: 17 pg/mL, n = 55) and a low control measuring 92.0 pg/mL (S.E.M.: 1.5 pg/mL, n = 55), respectively. An assessment of possible sources of pre-analytical variation demonstrated that both leptin and adiponectin were stable for three months at -20 °C, and for 10 freeze-thaw cycles.[19,20] At room temperature, we and others found both analytes to be stable for 48 hours.

Plasma glucose concentrations were determined on a Dimension Vista® 1500 Analyser (Siemens, Germany). Serum insulin concentrations were analyzed on a Cobas 6000 Analyzer (Roche Diagnostics, Denmark). Whole-blood HbA1c was analyzed on a Tosoh high-

performance liquid chromatography G8 analyser (Tosoh Corporation, Japan). The samples were stored at −80°C before analysis in a period of 3 months to 6 years in the cohort with overweight/obesity and in a period of 3 months to 2.5 years in the population-based cohort. The samples of glucose and insulin were analyzed during the summer 2015 from the same batch number by technicians blinded in this design as described in another publication from our group.[33]

Concentrations of plasma total cholesterol, triglycerides, and HDL cholesterol were determined on a Cobas 6000 Analyzer (Roche Diagnostics, Mannheim, Germany) until May 2013 and on a Dimension Vista 1500 Analyzer (Siemens Healthcare, Erlangen, Germany) hereafter. LDL cholesterol was calculated using the Friedewald formula (LDL-C = total cholesterol−HDL-C−TG/5).[34]

2.5 Ethical considerations

All participants gave informed consent. Written informed consent was obtained from parents of participants younger than 18 years old, and from the participants when 18 years or older. The study was approved by the Scientific Ethics Committee of Region Zealand, Denmark (protocol no. SJ-104) and by the Danish Data Protection Agency.

2.6 Statistical analyses

Statistical analyses were performed in R statistical software (v.3.5.2).[35] The normal weight group and the group with overweight/obesity were defined as a BMI SDS from the 10-90th percentile and above the 90th percentile, respectively. We chose the 90th percentile cut-off as proposed by Cole et *al.*[36] to correspond to a BMI of 25 at age 18, based on International

Obesity Task Force pediatric data from six countries (P90.5 in boys and P89.3 in girls). Normality of data was evaluated using histograms and qq-plots. Differences in baseline data and biomarkers between the groups were examined using the Wilcoxon rank-sum test. Age- and sex specific percentile curves were calculated for individuals with overweight/obesity, using the Generalized Additive Models for Locations Scale and Shape (GAMLSS) software package,[37] as previously described.[29,38] Spearman's partial correlation coefficients between the biomarkers and cardiometabolic risk factors in the group with overweight/obesity, adjusted for age and BMI SDS, were calculated using the R package 'ppcor'[39] and the unadjusted P-values are provided in Table 2. The group with overweight/obesity was divided into quartiles based on biomarker concentrations, and for each biomarker odds ratios and 95% confidence intervals of exhibiting cardiometabolic comorbidities in the upper quartile compared with the lower quartile, were examined using a multivariable logistic regression model controlling for the effect of age and BMI SDS. Puberty is normally associated with a transient insulin resistance.[40] However, we have recently shown that in the presence of overweight/obesity insulin resistance continues to increase after the end of puberty, resulting in an almost linear relationship between insulin resistance and age in the pediatric age range.[29] In this study of cardiometabolic risk, we found that additionally controlling for puberty in the group with overweight/obesity did not change the pattern of correlations or odds ratios, and as pubertal status was only available in 75% of participants, puberty was not included in the analysis. To compare the accuracy of the L/A ratio for identifying individuals with overweight/obesity with and without cardiometabolic comorbidities with the accuracy of leptin and adiponectin alone, receiver operating characteristic (ROC) analyses and the area under the curve (AUC) were calculated using the R package 'pROC'.[41] AUC's were

compared by bootstrapping, as the biomarkers' associations showed different directions.[41] Finally, to test the relationship between comorbidities and the L/A ratio across varying degrees of obesity, the increase in odds ratios of exhibiting insulin resistance was calculated. An increment of one L/A ratio standard deviation (SD) in the normal weight group was used. Degree of obesity was classified in three classes, as suggested by Skinner *et al.*[42] Class I (corresponding to 100-120% of the 95th Center for Disease Control and Prevention growth charts), percentile for age and sex), class II (120-140%), and class III (>140%).

3. Results

3.1 Baseline data

The study included 2,258 children and adolescents (1,279 girls) for analysis: 1,425 with overweight/obesity (784 girls) and 833 with normal weight (495 girls). The distribution by ethnic origin was 91.1% North-European white, 6.4% Middle Eastern, 1.1% Asian, 1.2% African, 0.04% Inuit, and 0.2% Hispanic. Descriptive data are shown in Table 1. Age and height were similar between the groups, and as expected, the group with overweight/obesity exhibited a higher weight, waist and body mass index standard deviation score (BMI SDS) (all P<0.001).

3.2 Relation to overweight/obesity

Girls and boys with overweight/obesity exhibited higher leptin and L/A ratio concentrations and lower adiponectin concentrations compared with their normal weight peers (P<0.001). Figure 1 further illustrates how L/A-ratio changes with age in girls and boys with

overweight/obesity compared with published population-based reference values from our group.[38]

3.3 Correlations with cardiometabolic risk factors

For girls and boys with overweight/obesity, age- and BMI SDS adjusted correlation coefficients between leptin, adiponectin, and L/A ratio with cardiometabolic risk factors are presented in Table 2. For markers of body composition, waist circumference and total fat mass correlated significantly with leptin and L/A ratio in both girls and boys, whereas only waist circumference correlated with adiponectin in girls. For markers of glucose metabolism, only insulin and HOMA-IR correlated significantly with leptin, adiponectin, and L/A ratio in both girls and boys. For the lipids, the strongest correlations were observed between triglycerides and high L/A ratios and between HDL cholesterol and low adiponectin values in girls and boys. For blood pressure, the strongest correlation was observed between diastolic blood pressure and high values of leptin and L/A ratio in boys.

3.4 Risk of cardiometabolic comorbidities

Among the group with overweight/obesity, the adjusted odds ratios of exhibiting cardiometabolic comorbidities in the upper quartile vs. the lower quartile of leptin, adiponectin and L/A ratio are shown in table 3. In girls, adjusted for age and BMI SDS, a L/A ratio in the upper quartile was associated with a 3.1-fold higher odds of exhibiting dysglycemia, a 7.8-fold higher odds of exhibiting insulin resistance, and a 2.5-fold higher odds of exhibiting dyslipidemia compared with the lower quartile. Similarly, in boys, a L/A

ratio in the upper quartile was associated with a 4.5-fold higher odds of exhibiting insulin resistance.

For leptin, we observed similar adjusted odds ratios of exhibiting insulin resistance for both girls and boys, but with lower absolute values than for the L/A ratio. For adiponectin, values in the upper quartile were associated with ~2.0-fold lower odds of exhibiting insulin resistance and dyslipidemia in both girls and boys (Table 3).

The accuracy of the L/A ratio to identify subjects with cardiometabolic comorbidities was compared with the accuracy of leptin and adiponectin alone, by calculating and comparing the AUC's (Table 3). This demonstrated significant differences between the accuracy of L/A ratio and adiponectin in both girls and boys for almost all measured comorbidities. There were no significant differences between the accuracy of L/A ratio and leptin, but the absolute odd ratios were higher for L/A ratio for almost all measured comorbidities.

3.5 L/A ratio as a marker of insulin resistance across obesity classes

After subdividing the children and adolescents with obesity into three obesity classes (class I, class II and class III), we examined the increase in the odds ratio of exhibiting insulin resistance with a 1 SD increase in L/A ratio, adjusted for age and sex. We found a 1.2-fold higher odds of exhibiting insulin resistance for both class I and class II obesity (Figure 2). In contrast, we did not observe significant increases for the normal weight group or class III obesity.

4. Discussion

Childhood obesity and its related complications represent an alarming and increasing public health challenge. Exploring the associated deranged adipokine state is important in defining the severity of associated morbidity, yet there exist few markers hereof. Understanding the pathophysiological links between obesity, concentrations of adipokines and cardiometabolic comorbidities could allow for more accurate identification of high-risk patients. In this study, we examined the concentrations of fasting serum leptin, adiponectin, and the L/A ratio in a large group of children and adolescents with and without overweight/obesity, as well as their associations with insulin resistance and other markers of metabolic derangements, in order to examine if the stratification of children with overweight/obesity on the basis of their L/A ratio adds additional information than their degree of obesity alone.

4.1 Main findings

Importantly, we found that among children with overweight/obesity when adjusting for age and degree of obesity, mainly the markers of glucose metabolism, insulin and HOMA-IR correlated significantly with leptin, adiponectin, and L/A ratio in both sexes. Similarly, we demonstrated that, independent of their degree of obesity, children with overweight/obesity had 4-8 times higher odds of exhibiting insulin resistance if their L/A ratio was in the upper quartile compared with children whose L/A ratio was in the lower quartile.

Several studies have examined these adipokines and their ratio in relation to cardiometabolic risk in children; however, in most cases, the populations were small and collectively they have resulted in contradictory findings. A study of 175 Hispanic adolescents aged 8-13 years concluded that the L/A ratio was not a better predictor of insulin sensitivity than the additive effect of leptin and adiponectin levels.[43] An American study investigated the L/A ratio in 39

children with and without obesity and found a strong correlation of the L/A ratio to auxological parameters and percent body fat.[13] Other studies in children have investigated the association between leptin and adiponectin and cardiometabolic risk[44] and anthropometric variables and body composition,[45] without including the L/A ratio.

Our results demonstrate the correlation between adipokine imbalance and various markers of dysglycemia, insulin resistance, and other cardiometabolic comorbidities. This study adds considerably to the notion of the L/A ratio as a better marker of obesity-related comorbidities, as other studies have indicated. Notably, our results suggest that L/A ratio, as a marker, is superior to both adiponectin and leptin. Still, on an individual basis there is a certain degree of overlap between the children with overweight/obesity and normal weight, indicating that a single measurement in any individual is insufficient to risk stratify, but that the L/A ratio may be part of such a risk stratification.

It is noteworthy that the correlation appeared to be weakened in the cohort of children with the highest degree of obesity, as indicated in Figure 2. Whereas it may seem counter-intuitive, we hypothesize that it may reflect a homeostatic regulation that is disintegrating in severe obesity; i.e. that above a certain critical level, the adipokines cannot physiologically adjust appropriately and the association is subsequently weakened.

We also demonstrate that girls and boys with overweight/obesity exhibit higher leptin concentrations and L/A ratios as well as lower adiponectin concentrations compared to their normal weight counterparts. This result is illustrated clearly in Figure 1 and is in accordance with the literature and has been reported in other pediatric populations.[12,13,45,46]

4.2 Strengths and limitations

A major strength of the present study is that it is based on a large group of carefully phenotyped children and adolescents with either normal weight or overweight/obesity. Furthermore, as strict and identical blood sampling protocols were followed, and as all samples were analyzed in the same laboratory in a standardized manner, pre-analytical or analytical errors can be considered negligible. Therefore, this large study aids considerably to interpret the existing conflicting data.

A limitation of the present study is that 2-hour glucose tolerance tests were not available for a full evaluation of prediabetes. Instead, fasting plasma glucose and serum insulin concentration as well as the HOMA-IR were used, as these measures have been shown to correlate with the gold standard of insulin sensitivity from clamp studies.[30]

This study is by nature explorative and an obvious limitation to the cross-sectional design is that it cannot describe causality. However, the aim of the present study was to investigate the associations between leptin, adiponectin, and the L/A ratio and markers of cardiometabolic risk in order to aid clinicians in evaluation of children and adolescents with overweight and obesity, for which the cross-sectional design is applicable.

4.3 Future directions

There is an ongoing and increasing interest in understanding the complex relationship between the homeostatic regulation of adipokines in children and adolescents with obesity and related comorbidities. This question is not only interesting from a scientific point of view in order to further understand the pathophysiology of childhood obesity, but equally important for clinicians in daily clinical care despite a challenged and burdened public health

sector. Future studies should focus on identifying children that are particularly susceptible to develop severe obesity-related complications, and on elucidating existing causal links in the relationships between adipokines and cardiometabolic comorbidities, including the glucose metabolism.

4.4 Conclusion

With the alarmingly high prevalence of obesity in the pediatric population and the concomitant cardiometabolic comorbidities, the need for efficient markers for risk stratification and early detection is imperative. The present study supports that the L/A ratio is superior to leptin and adiponectin concentrations, respectively and may be a useful marker for clinicians in the classification of cardiometabolic risk and insulin resistance in pediatric patients with overweight or obesity.

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Tables

Table 1. Anthropometric characteristics and adipokine concentrations of study cohorts.

	Normal weight	**Overweight/obesity**	**P-value**
N	833	1.425	
Age (years)	11.9 [9.5, 14.6]	11.8 [9.6, 14.0]	0.125
Height (cm)	154 [139, 166]	154 [142, 165]	0.124
Weight (kg)	41.0 [31.1, 54.5]	62.1 [47.4, 78.9]	**<0.001**
Waist (cm)	66 [60, 72]	89 [80, 101]	**<0.001**
BMI SDS	0.13 [-0.40, 0.65]	2.79 [2.22, 3.26]	**<0.001**
Leptin (ng/L)	5.612 [3.001, 10.670]	27.940 [16.420, 46.660]	**<0.001**
Adiponectin (µg/L)	4.499 [3.076, 6.762]	3.500 [2.581, 4.990]	**<0.001**
Leptin/adiponectin ratio	1.1 [0.5, 2.6]	7.7 [3.8, 14.5]	**<0.001**

Table 1. Data are medians and IQR. All biomarkers are from fasting serum measurements. BMI SDS: Body mass index standard deviation score. P values are calculated by Wilcoxon rank-sum test. Significant P values are bold.

Table 2 – Correlation coefficients between leptin, adiponectin and leptin/adiponectin ratio and cardiometabolic risk factors in children and adolescents with overweight/obesity.

	Leptin	**Adiponectin**	**L/A ratio**
	rho (P-value)	*rho (P-value)*	*rho (P-value)*
Girls			
Age	**0.54** (<.0001)	**-0.20** (<.0001)	**0.52** (<.0001)
BMI SDS	**0.64** (<.0001)	**-0.15** (<.0001)	**0.55** (<.0001)
Waist	**0.23** (<.0001)	**-0.07** (0.0325)	**0.20** (<.0001)
Total fat mass% (DXA)	**0.40** (<.0001)	0.07 (0.1416)	**0.19** (<.0001)
Glucose (mmol/L)	0.00 (1.0000)	**-0.09** (0.0080)	0.06 (0.1651)
HbA1c (mmol/mol)	-0.01 (0.6043)	**-0.10** (0.0021)	**0.09** (0.0192)
Insulin (pmol/L)	**0.23** (<.0001)	**-0.19** (<.0001)	**0.32** (<.0001)
HOMA-IR	**0.22** (<.0001)	**-0.20** (<.0001)	**0.31** (<.0001)
Cholesterol (mmol/L)	**0.11** (0.0030)	-0.04 (0.1508)	**0.09** (0.0162)
LDL-C (mmol/L)	0.07 (0.1014)	**-0.06** (0.0445)	**0.08** (0.0419)
HDL-C (mmol/L)	0.07 (0.0748)	**0.20** (<.0001)	**-0.10** (0.0026)
Triglycerides (mmol/L)	**0.14** (0.0002)	**-0.16** (<.0001)	**0.21** (<.0001)
Systolic BP SDS	**-0.07** (0.0422)	-0.01 (0.7092)	-0.04 (0.2281)
Diastolic BP SDS	0.01 (0.9452)	0.04 (0.4181)	0.00 (1.0000)
Boys			
Age	**0.22** (<.0001)	**-0.31** (<.0001)	**0.33** (<.0001)
BMI SDS	**0.64** (<.0001)	**-0.16** (<.0001)	**0.58** (<.0001)
Waist	**0.35** (<.0001)	-0.03 (0.3836)	**0.30** (<.0001)
Total fat mass% (DXA)	**0.54** (<.0001)	0.09 (0.0695)	**0.29** (<.0001)
Glucose (mmol/L)	0.04 (0.4902)	-0.05 (0.1347)	0.07 (0.1347)
HbA1c (mmol/mol)	0.02 (0.8764)	0.04 (0.4251)	-0.02 (0.5231)
Insulin (pmol/L)	**0.36** (<.0001)	**-0.12** (0.0008)	**0.42** (<.0001)
HOMA-IR	**0.34** (<.0001)	**-0.13** (0.0007)	**0.41** (<.0001)
Cholesterol (mmol/L)	**0.23** (<.0001)	0.03 (0.6777)	**0.16** (0.0002)
LDL-C (mmol/L)	**0.18** (<.0001)	-0.01 (0.5749)	**0.14** (0.0012)
HDL-C (mmol/L)	0.09 (0.0507)	**0.22** (<.0001)	-0.07 (0.0536)
Triglycerides (mmol/L)	**0.21** (<.0001)	**-0.13** (0.0004)	**0.27** (<.0001)
Systolic BP SDS	0.01 (1.0000)	0.01 (0.9889)	0.02 (0.8638)

Diastolic BP SDS	**0.17** (0.0002)	-0.05 (0.1781)	**0.15** (0.0009)

Table 2. Values are age and BMI SDS-adjusted Spearman correlation coefficients and P values for correlations of adiponectin, leptin and their ratio with cardiometabolic risk factors. The age correlation is unadjusted, while the BMI SDS correlation is adjusted only for age. Abbreviations: BMI SDS = Body mass index standard deviation score. HbA1c = Hemoglobin A1c. HOMA-IR = Homeostasis Model of Assessment Insulin Resistance. Significant results are bold.

Table 3 – Odds ratios (95% CI) and ROC analysis for the association between leptin, adiponectin, and leptin/adiponectin ratio and cardiometabolic comorbidities in children and adolescents with overweight/obesity.

		Leptin		**Adiponectin**		**L/A**	
	Total group prevalence	*OR[a] (95% CI) Q4 vs. Q1*	*AUC (SE)*	*OR[a] (95% CI) Q4 vs. Q1*	*AUC (SE)*	*OR[a] (95% CI) Q4 vs. Q1*	*AUC (SE)*
Girls							
Dysglycemia	22.2 %	2.31 (0.96-5.80)	0.571 (0.025)	0.81 (0.46-1.42)	0.562 (0.024)	**3.08 (1.35-7.31)**	0.587 (0.024)
Insulin resistance	52.5 %	**6.26 (2.95-13.84)**	0.660 (0.020)	**0.49 (0.31-0.78)**	0.596 (0.021)	**7.78 (3.78-16.65)**	0.669 (0.020)***
Dyslipidemia	24.2 %	2.06 (0.89-5.02)	0.621 (0.023)	**0.50 (0.29-0.83)**	0.604 (0.240)	**2.53 (1.18-5.59)**	0.645 (0.022)*
Hypertension	15.9 %	0.83 (0.32-2.21)	0.552 (0.029)	1.34 (0.71-2.53)	0.529 (0.030)	0.66 (0.26-1.71)	0.550 (0.028)
Boys							
Dysglycemia	27.4 %	1.93 (0.86-4.41)	0.606 (0.024)	1.11 (0.64-1.93)	0.538 (0.028)	1.86 (0.87-4.01)	0.601 (0.025)**
Insulin resistance	58.5 %	**3.41 (1.60-7.49)**	0.726 (0.021)	**0.53 (0.30-0.94)**	0.587 (0.023)	**4.45 (2.03-10.10)**	0.729 (0.020)***
Dyslipidemia	22.0 %	1.64 (0.70-3.92)	0.664 (0.025)	**0.54 (0.29-0.99)**	0.613 (0.028)	2.63 (0.90-4.79)	0.692 (0.026)***
Hypertension	15.0 %	1.41 (0.53-3.87)	0.597 (0.035)	0.89 (0.44-1.80)	0.530 (0.036)	1.29 (0.49-3.46)	0.596 (0.035)*

Table 3. Abbreviations: OR = odd ratios. Q4 and Q1 = upper and lower quartile for leptin, adiponectin, and L/A ratio. AUC = Area Under the Curve of ROC analysis, based on all children and adolescents with overweight/obesity. SE = Standard Error. See text for definitions of each of the cardiometabolic comorbidities. [a] Adjusted for age and body mass index standard deviation score. Significant odds ratios are bold. * = $p<0.05$,

** = $p<0.01$, and *** = $p<0.001$ between AUC for adiponectin and L/A ratio. No significant differences were found between AUC for leptin and L/A ratio.

Figure Legends

Figure 1. Smoothed 5th, 50th, and 95th percentile curves for leptin/adiponectin ratio for girls (top panels, red) and boys (bottom panels, blue). Leftmost panels with full lines represent population-based reference values from [38] and rightmost panels with dotted lines represent the children and adolescents with overweight/obesity. (Six very high values from the children and adolescents with overweight/obesity were outside the depicted range: Girls, L/A ratio: 117 (16 years), 76 (18 years), 69 (18 years), 69 (15 years); Boys, L/A ratio: 86 (17 years), 70 (13 years).)

Figure 2. Odds ratios for exhibiting insulin resistance per 1 SD increase in L/A ratio according to weight classes. Analyses were adjusted for age and sex. SD was calculated from the population-based cohort included in this study. Normal weight was classified according to Danish reference values [24], obesity classes according to American Center for Disease Control and Prevention growth charts [42], see text for details.