Prevalence and Cluster of Cardiometabolic Biomarkers in Overweight and Obese Schoolchildren: Results from a Large Survey in Southwest Germany

Gabriele Nagel,1* Kilian Rapp,1 Martin Wabitsch,2 Gisela Büchele,1 Anja Kroke,3 Iris Zöllner,4 Stephan K. Weiland,1† and Wolfgang Koenig5

BACKGROUND: Obesity is associated with substantial metabolic changes and subclinical inflammation. We explored associations between body mass index (BMI) and cardiometabolic biomarkers and their clustering in overweight and obese schoolchildren.

METHODS: In this population-based, cross-sectional study among 450 children 10 years old, we measured adiponectin, leptin, inflammatory markers, apolipoprotein (apo) A1 and B, and lipoprotein-associated phospholipase A2 (Lp-PLA2). Except for adiponectin and apoAI (10th percentile) the 90th percentile was used as cutoff point. Body weight was categorized in age- and sex-specific BMI percentiles and overweight and obesity according to International Obesity Task Force definitions.

RESULTS: In linear regression models, all cardiometabolic markers except apoB were statistically significantly associated with overweight. In logistic regression models, compared with the reference category (25th–75th percentile of BMI), overweight was associated with increased concentrations of leptin [odds ratio (OR) 59.80; 95% CI 16.68–214.39], C-reactive protein (6.30; 2.95–13.45), fibrinogen (2.82; 1.33–6.01), and low apoAI (2.62; 1.19–5.75). Overweight was positively associated with interleukin-6, Lp-PLA2, and apoB concentrations and inversely with adiponectin concentrations. Most importantly, in obese children 35% showed one, 20% two, 10% three, and 15% four or more abnormal cardiometabolic biomarkers. The number of abnormal cardiometabolic markers increased in overweight (p_trend < 0.001) and obese (p_trend < 0.001) children.

CONCLUSIONS: Overweight and obesity in children are associated with complex metabolic changes and a low-grade inflammatory response, and thus might not only accelerate cardiovascular disease later on, but may also be associated with the initiation of atherosclerosis in early life.

© 2007 American Association for Clinical Chemistry

Childhood obesity is associated with a range of health problems, which may last until adult life and cause premature morbidity and mortality (1). In adults, the relation between the metabolic syndrome comprising insulin resistance, dyslipidemia (low HDL cholesterol and high triglycerides), hypertension, obesity, and atherosclerotic cardiovascular disease is well established (2). There is growing evidence that overweight and obesity are related to other cardiovascular risk factors even in childhood (3–5). Moreover, the presence of cardiovascular risk factors in childhood was found to be predictive for increased risk of cardiovascular disease in later life (6).

Based on our current understanding suggesting that white adipose tissue acts as an endocrine organ and plays a crucial role in the regulation of insulin sensitivity and lipid metabolism, various obesity-related biomarkers, so-called adipocytokines, have been proposed (7–9). Particularly in adults, biomarkers for low-grade inflammation, insulin sensitivity, and lipid...
metabolism have been assessed in relation to cardiovascular disease (9, 10). Leptin correlates with body mass index (BMI) by regulating food intake and basal metabolism and is linked to coronary artery disease (11). Adiponectin is inversely related to BMI and plays a role in the regulation of insulin sensitivity and fatty acid metabolism (12). In a prospective study, adiponectin predicted myocardial infarction in men (13); however, more recent studies revealed mixed results (14, 15). Inflammatory markers such as interleukin (IL)-6 (16) and in particular C-reactive protein (CRP) are considered risk factors for the metabolic syndrome (17) and cardiovascular disease (7). Fibrinogen, an acute-phase reactant that also plays a central role in the coagulation cascade, has been linked to obesity in adults (18) and children (19).

As biomarkers of lipid metabolism, apolipoprotein (apo) A1 and apoB have been shown to be good predictors for cardiovascular disease (9). In addition, lipoprotein-associated phospholipase A2 (Lp-PLA2), which is mainly produced by monocytes/macrophages and primarily bound to LDL cholesterol in the peripheral circulation, was found to be predictive for cardiovascular events in adults (8).

 Childhood obesity tends to persist into adulthood and causes chronic conditions (1, 6). Knowledge about pathomechanisms and early markers of disease may facilitate refined primary prevention strategies such as healthy diet or physical activity and thus represents an important public health issue (1). The purposes of this study were (a) to explore associations between BMI and cardiometabolic biomarkers and (b) to investigate their clustering among overweight and obese children in a representative large group of 10-year-old children.

Materials and Methods

STUDY POPULATION
Within the framework of a health surveillance program in children, we carried out a cross-sectional study on body weight. The investigation was coordinated by the Department of Internal Medicine II—Cardiology, University Medical Center. We measured leptin (ng/L), adiponectin (mg/L), and IL-6 (ng/L) by use of ELISA (R&D Systems) in EDTA plasma samples. The lower detection limits were approximately 7.8 ng/L for leptin, 0.25 mg/L for adiponectin, and 0.11 ng/L for IL-6. Interassay imprecision (CV) was 3.9% for leptin at 9530 ng/L (n = 7), 5.8% for adiponectin at 4.4 mg/L (n = 7), and 7.7% for IL-6 at 1.77 ng/L (n = 7). We also measured Lp-PLA2 by use of ELISA (PLAC test, diaDexus). The detection limit was 1.3 μg/L, and the interassay CV was 8.8% at 204 μg/L (n = 7) and 4.0% at 376 μg/L (n = 7). We measured CRP, fibrinogen, apoAI, and apoB by use of immunonephelometry on a BN II analyzer (Dade Behring). Detection limits were 0.16 mg/L for CRP and 0.15 g/L for fibrinogen. The interassay CVs were 4.7% for CRP at 1.25 mg/L and 1.1% for fibrinogen at 2.2 g/L. The interassay CV for apoAI at 1.73 g/L was 6.7% (n = 6), and the corresponding CV for apoB at 0.92 g/L was 4.6% (n = 6). For most of the analyzed biomarkers, no accepted external cutoff points were available to define increased concentrations in children. Therefore, we used values above the 90th percentile of the biomarker distribution in our population, except for apoAI and adiponectin, for which the 10th percentile was a biologically plausible cutoff point. We calculated sex-specific increased concentrations: CRP (≥1.65 mg/L for boys and ≥1.99 mg/L for girls), IL-6 (≥2.82 ng/L for boys and ≥3.14 ng/L for girls), fibrinogen (≥2.85 g/L for boys and ≥2.93 g/L for girls), adiponectin (≥0.88 g/L for boys and ≥0.80 g/L for girls), leptin (≥13 918 ng/L for boys and ≥20 292 ng/L for girls).
for girls), and Lp-PLA₂ (≥193 ng/L for boys and ≥192 ng/L for girls). For adiponectin (≥4.63 mg/L for boys and ≤5.17 mg/L for girls) and apoAI (≤1.29 g/L for boys and ≤1.26 g/L for girls), abnormal results were defined as values below the 10th percentile.

**STATISTICAL ANALYSIS**

We determined median and interquartile range (IQR, 25th–75th percentile) of the biomarkers in the 90th BMI percentile and in overweight and obese children. Spearman rank correlation coefficient (ρ) was calculated between cardiometabolic markers. Statistical significance was determined on the basis of 2-sided P-values of <0.05.

Linear regression models with continuous values of the explanatory variables (log-transformed if not normally distributed) were calculated using the 25th–75th BMI percentile as a reference group to examine the relationship with BMI by groups (0–10th, 10th–25th, 75th–90th, and ≥90th percentile). In addition, we calculated the associations of these BMI categories with concentrations of biomarkers compared to the reference BMI category using logistic regression models adjusted for age. Because sex-specific cut points were applied, no further adjustment for sex was performed. Clustering of abnormal biomarker concentrations was determined by BMI categories and in overweight and obese children according to IOTF definitions. Because of the strong correlation between leptin concentrations and BMI (ρ = 0.801, P <0.001), we did not include leptin concentrations in the clustering. Tests for trend across clusters were performed by including the ordered variable as continuous in the logistic regression model. All analyses were carried out with the statistical software package SAS release 9.1 (SAS Institute).

**Results**

Overall, 450 schoolchildren (52.7% girls), mean age 10 years (SD 0.6) and mean BMI 17.1 kg/m² (SD 3.1), were included in this analysis. About 20% of the children had at least one non-German parent. Using IOTF cut points according to Cole et al. (22), the prevalence for overweight was 21.6% and for obesity 4.4%. Between obese children and children in the 25th–75th BMI percentile, distributions of the plasma concentrations differed significantly in boys and girls for CRP (P <0.001) and leptin (P <0.001). For fibrinogen and apoAI, significant associations were found only for boys (P <0.001 and 0.025, respectively), and for IL-6, only for girls (P = 0.027).

Table 1 shows correlation coefficients of the cardiometabolic markers. There were correlations between plasma leptin concentrations and CRP (ρ = 0.46), fibrinogen (ρ = 0.26), IL-6 (ρ = 0.22), and apoAI (ρ = −0.25). The inflammatory markers were correlated: CRP with fibrinogen (ρ = 0.55), CRP with IL-6 (ρ = 0.42), and IL-6 with fibrinogen (ρ = 0.32).

Table 2 shows the linear associations of cardiometabolic markers with BMI percentiles, overweight, and obesity. Compared with the reference category (25th–75th percentile of BMI), overweight children...
tended to have higher concentrations of leptin (β-coefficient 1.65; 95% CI 1.46–1.85), CRP (1.07; 0.82–1.32), fibrinogen (0.35; 0.22–0.47), IL-6 (0.38; 0.02–0.74), and Lp-PLA₂ (15.46; 7.62–23.30). In contrast, lower apoAI (β-coefficient −0.11; 95% CI 0.15 to −0.06) and adiponectin (−1.54; −2.54 to −0.54) concentrations were associated with overweight. ApoB concentrations changed slightly (β-coefficient 0.04; 95% CI 0.01–0.08). Cardiometabolic markers clearly increased across the upper BMI categories as well as in the range from overweight to obesity (see Supplemental Data Figures).

Table 3 presents odds ratios (ORs) for abnormal concentrations of various biomarkers in children with different BMI categories, overweight, and obesity, using the 25th–75th percentile as a reference category. Plasma leptin concentrations showed the strongest association with increased BMI categories and with overweight and obesity. Low adiponectin concentrations were associated with obesity, whereas the association with overweight was not significant.

In contrast, high concentrations of the inflammatory markers CRP, IL-6, and fibrinogen were strongly related to obesity. For CRP and fibrinogen, the association with overweight was also significant. Among markers of lipid metabolism, high plasma apoB and Lp-PLA₂ concentrations did not show consistent associations with overweight and obesity. Decreased apoAI concentrations were associated with overweight but not with obesity.
The clustering of abnormal cardiometabolic biomarker concentrations with increasing BMI categories (except for leptin) and with overweight and obesity is illustrated in Fig. 1. Among overweight children, 71% showed at least one abnormal biomarker (36% had one, 21% had two, 10% had three, and 4% had four or more). In obese children, similar numbers were found for one, two, or three markers, whereas 15% of these children had four or more abnormal biomarkers. Thus, 80% of obese children had at least one abnormal value. The number of abnormal cardiometabolic markers increased with increasing categories of BMI. Compared to the reference category (25th–75th percentile), the number of abnormal cardiometabolic markers increased in overweight ($P_{\text{trend}} < 0.001$) and obese ($P_{\text{trend}} < 0.001$) children.

### Discussion

In this study, cardiometabolic biomarkers in general were associated with overweight and obesity in young children, with a clear clustering particularly in obese children. Consistent with published reports, we observed a strong positive association between plasma leptin concentrations and increasing BMI (23). Leptin may be linked to cardiovascular disease by enhanced platelet aggregation and promotion of a prothrombotic state, and by angiogenesis and impairment of vas-
cular function in adolescents (11). Our data for leptin (24), adiponectin, CRP (25), fibrinogen (25), IL-6 (25), apoAI (26), and apoB (26) are also consistent with the literature. For Lp-PLA₂, no data among children exist so far.

In line with the results of most previous studies, we found an inverse relationship between adiponectin concentrations and BMI (3, 12, 27). In experimental studies, high adiponectin concentrations have been shown to exert antiatherogenic, antidiabetic, and anti-inflammatory effects. Low plasma concentrations may indicate impaired insulin sensitivity and thus increased risk of type 2 diabetes, but little is known about the underlying biological mechanisms. Results from prospective studies regarding the association between low adiponectin and cardiovascular disease end points are controversial (14, 15); thus, further research is required to understand the role of adiponectin in atherogenesis.

Our observation of positive associations of plasma IL-6 and CRP concentrations with BMI is consistent with earlier reports (13, 28). Several authors have shown that high CRP concentrations are associated with obesity in children (3, 17, 28) and in adults (13, 18). In addition, correlations between CRP concentrations and high blood pressure or dyslipidemia were found in children and adolescents (29). Finally, various in vitro and in vivo studies have suggested a direct role of CRP in atherogenesis, but this issue is discussed controversially (30).

In the young children from our study, high fibrinogen concentrations were also associated with overweight and obesity. This observation is consistent with findings from a study among German children 12 years old (31) and with a small case-control study from Spain (19). Fibrinogen correlates with inflammatory markers and plays a central role in the coagulation cascade (16). Because IL-6 represents the main trigger for
the hepatic production of CRP and fibrinogen, these inflammatory markers are clearly interrelated. In our data, however, IL-6 was only moderately correlated with both CRP and fibrinogen, which is also consistent with the literature (16).

We further found overweight to be associated with low apoAI concentrations, whereas no clear association was found with plasma apoB concentrations. This is also discussed controversially in the literature. No associations between plasma apoAI and apoB concentrations with obesity were found in cross-sectional studies among German (31) and South Korean (32) children. In another cross-sectional study among 13-year-old children in Portugal, apoB concentrations were higher in obese than nonobese children (33). In a small study including Japanese children ages 5–14 years, no association between apoAI concentrations and obesity was found, whereas apoB concentrations were positively related to obesity and obesity was found, whereas apoB concentrations were higher in obese than nonobese children (33). In a small study including Japanese children ages 5–14 years, no association between apoAI concentrations and obesity was found, whereas apoB concentrations were positively related to obesity (34). In a recent case-control study on premature coronary artery disease among adolescents and children in India, higher apoB and lower apoAI concentrations were found in cases compared to controls (35). These different results may be due to differences in age range, ethnicity, or study design.

Oxidized LDL represents the substrate for Lp-PLA2, which in turn generates proatherogenic compounds like lysophosphatidylcholine and oxidized fatty acids (10). We found an association between increased Lp-PLA2 concentrations and higher BMI percentiles, which is consistent with the correlation of plasma Lp-PLA2 with apoB concentrations in our study. To date, a large number of prospective studies in initially healthy subjects and in patients with manifest atherosclerosis have documented that increased Lp-PLA2 activity or mass is associated with increased cardiovascular risk, suggesting a proatherogenic activity (8, 10). However, little is known about the effects of increased Lp-PLA2 in children.

Our finding of a clustering of abnormal cardiometabolic biomarkers in obese children is in line with previous reports concerning traditional cardiovascular risk factors in relation to obesity (4, 5, 36). Classic cardiovascular risk factors such as high blood pressure, hyperglycemia, and dyslipidemia were investigated among children and adolescents in Finland (3–18 years old) (36), in Taiwan (12–16 years old) (4), and in the US (5–17 years old) (5), Freedman et al. (5) found that 26% of their study population had at least one risk factor and 4% had three or more. However, we observed at least one abnormal cardiometabolic marker in 71% of overweight and in 80% of obese children. Compared to overweight children, we observed a higher burden of abnormal cardiometabolic markers in obese children, although the number of obese children per cluster was small; but abnormal cardiometabolic markers also clustered in increasing BMI categories with larger numbers. Our observations, that none of the investigated markers was increased in 20% of obese children and that among children with BMI below the 90th percentile abnormal biomarker concentrations were observed, are consistent with findings on traditional cardiovascular risk factors (4). In our study, obesity was associated with abnormal plasma concentrations of inflammatory markers (CRP, fibrinogen, and IL-6) and adiponectin. However, some of the cardiometabolic markers, such as IL-6 and adiponectin, could be more predictive for obesity (Tables 2 and 3, Supplemental Data).

Pubertal state may have distorted the associations between biomarkers and overweight or obesity. Leptin is discussed as a factor linked to the timing of puberty (37), which is associated with changes of body fat distribution and rapid growth. Our study sample was homogeneous in age, which may have minimized the relevance of pubertal state as a modifying factor. Because cardiometabolic markers are biologically interrelated, correlations between these biomarkers and obesity may not be independent. In a cross-sectional study among adults, however, adiponectin concentrations were not significantly correlated with most immunological parameters, suggesting that adiponectin and inflammatory markers may act independently (38). Except for apoAI, adiponectin was not correlated with other laboratory markers in this study.

The definition of overweight by means of BMI remains somewhat arbitrary in children, and its limitation regarding the distinction between fat and fat-free mass is well known (39). In 1991, childhood obesity was defined as having a BMI above the age-specific 95th BMI percentile in the US. Cole et al. (22) suggested age- and sex-specific BMI cutoff points for international comparisons of overweight and obesity in children, which are based on retrograde extrapolations in adults on childhood BMI. Compared to the IOTF definitions, we found similar proportions for obesity using national cut points (4.9%), whereas on the international scale (22), the proportion of overweight children was higher than using national cutoff values (16.2%) (21). In linear and logistic regression models, markedly stronger associations with obesity than with overweight were found in our study sample for low plasma adiponectin and high IL-6, CRP, fibrinogen, Lp-PLA2, and leptin concentrations. These associations suggest that unfavorable concentrations of cardiometabolic biomarkers may indicate the burden of metabolic changes caused by overweight and obesity. Particularly strong associations with overweight and obesity were seen for inflammatory markers.
So far, risk factors associated with increased BMI in early childhood are not clear, and risk-associated cutoff values for BMI have not been established for children. An increased BMI is currently defined by percentiles or standard deviation score (SDS) values obtained by reference values from a population. Several guidelines for diagnostic procedures and treatment are based on such cutoff values, e.g., for German children (21, 40). Only few studies have tried to relate cardiovascular risk factors to BMI in children (4, 5, 36). The present study adds information on the associations of the biochemical markers adiponectin, leptin, CRP, IL-6, fibrinogen, apoAI, apoB, and LP-PLA2, with overweight and obesity among 10-year-old children living in Germany. Results demonstrate that cardiometabolic risk factors cluster in higher BMI categories and in overweight and obese children.

In summary, cardiometabolic biomarkers in these children are strongly associated with overweight and obesity, suggesting an adverse effect on the vascular wall very early in life. In particular, the clustering of multiple unfavorable biomarkers strongly supports the need for early intervention.

Grant/funding Support: None declared.

Financial Disclosures: None declared.

Acknowledgments: The authors thank Gerlinde Trischler for excellent technical assistance, Bernhard Link for study coordination, Holger Knebel for documentation and data management, and Anne Katrin Kersten for assisting with data analysis. Finally, we thank all study participants.

References

23. Lambert M, Delvin EE, Paradis G, O’Loughlin J, Hanley JA, Levy E. C-reactive protein and features of the metabolic syndrome in a population-based...


