BMI, total and abdominal fat distribution, and cardiovascular risk factors in school-age children

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BACKGROUND: More specific total body and abdominal fat mass measures might be stronger associated with cardiovascular risk factors in childhood, than BMI. We examined the independent associations of total and abdominal fat measures with cardiovascular risk factors in school age children.

METHODS: We performed a population-based cohort study among 6,523 children. At the age of 6 y, we measured childhood BMI, and general and abdominal fat mass, using dual-energy X-ray absorptiometry, and ultrasound and cardiovascular risk factors.

RESULTS: Conditional on BMI, higher fat mass percentage and abdominal fat mass were associated with higher blood pressure, total- and low-density lipoprotein (LDL)-cholesterol, insulin and c-peptide levels, but with lower left ventricular mass and high-density lipoprotein (HDL)-cholesterol (P values < 0.05). These associations differed between underweight, normal weight, overweight, and obese children. Higher childhood adiposity measures were associated with increased odds of cardiovascular risk factors clustering, with the strongest effect for fat mass percentage (odds ratios: 3.01 (95% confidence interval: 2.67, 3.9).

CONCLUSION: Our results suggest that general and abdominal fat measures are associated with cardiovascular risk factors in childhood, independent from BMI. These measures may provide additional information for identification of children with an adverse cardiovascular profile.

Childhood obesity is a major public health problem (1). It seems that not only overweight and obesity, but also higher body mass across the full range is associated with risk factors for cardiovascular and metabolic diseases in childhood and adulthood (2–5). BMI does not distinguish lean mass from fat mass (6). Among adults and children, total body fat mass assessed by dual-energy X-ray absorptiometry (DXA) seems to be, independent from BMI, associated with cardiovascular risk factors (3,7). Also, waist circumference, as proxy for abdominal fat mass, was independent of BMI related to the risk of mortality in adults, suggesting that central or abdominal adiposity is

more strongly associated with adverse health outcomes (4,8). Abdominal fat mass is an accumulation of both subcutaneous and visceral adipose tissues. In adults, visceral adipose tissue accumulation is stronger related with an adverse glucose and lipid profile than subcutaneous adipose tissue accumulation (8). Thus far, population-based studies focused on the associations of different detailed total body and abdominal fat mass measures with cardiovascular risk factors in children show inconsistent results. Abdominal fat mass in children has been identified as a stronger predictor of cardiovascular risk factors, as compared to BMI, though results are not consistent (2,3,9). These inconsistent findings may be explained by different measures of fat mass and variation of children's age included in these studies.

Therefore, we examined in a population-based cohort study among 6,523 school-age children, the independent associations of BMI and total body and abdominal fat mass measures with risk factors for cardiovascular disease.

RESULTS

Subject Characteristics

Table 1 presents children characteristics. The correlation coefficients between childhood adiposity measures and cardiovascular risk factors are given in the **Supplementary Tables S1 and S2** (online).

Total and Abdominal Adiposity Measures and Cardiovascular Risk Factors

Higher childhood BMI was associated with higher blood pressure, higher left ventricular mass, and higher blood levels of total- and low-density lipoprotein (LDL)-cholesterol, triglycerides, insulin and c-peptide, and with lower blood levels of high-density lipoprotein (HDL)-cholesterol levels (**Table 2**, all P values < 0.05). As compared to associations of BMI, the associations of fat mass percentage and abdominal fat measures with systolic blood pressure, left ventricular mass and blood levels of insulin and c-peptide were weaker, whereas those with blood levels of lipids were stronger. No differences in the strength of these associations were present between

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Table 1. Subject characteristics

Characteristics	Underweight N = 333	Normal weight N = 5,027	Overweight N = 858	Obese N = 294	P value
Childhood characteristics					
Age (years), mean (SD)	6.1 (0.5)	6.2 (0.5)	6.3 (0.6)	6.4 (0.7)	<0.001
Sex, number (%)					
Boys	179 (53.8)	2,608 (51.9)	372 (43.4)	110 (37.4)	<0.001
Girls	154 (46.2)	2,419 (48.1)	486 (56.6)	184 (62.6)	
Ethnicity, Number (%)					
European	210 (64)	334 (68.2)	421 (50.5)	184 (65.2)	<0.001
Non-European	118 (36)	1,560 (31.8)	412 (49.5)	98 (34.8)	
Gestational age at birth, median (95% range)	39.9 (32.8, 42.1)	40.1 (35.7, 42.3)	40.1 (36.4, 40.4)	39.9 (36, 42.1)	<0.001
Birth weight (g), mean (SD)	3,132 (558)	3,428 (548)	3,495 (546)	3,468 (560)	<0.001
Anthropometrics					
Height (m), mean (SD)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	<0.001
Weight (kg), mean (SD)	18.8 (2.0)	22.2 (2.7)	27.4 (3.7)	34.1 (5.7)	<0.001
BMI (kg/m ²), mean (SD)	13.5 (0.4)	15.7 (0.9)	18.5 (0.8)	21.9 (1.9)	<0.001
Body fat distribution					
Fat mass percentage (%), mean (SD)	20.6 (3.4)	23.4 (4.1)	30.7 (4.6)	38.3 (4.6)	<0.001
Android/ gynoid fat mass ratio, mean (SD)	0.2 (0.0)	0.2 (0.1)	0.3 (0.1)	0.4 (0.1)	<0.001
Abdominal fat mass distribution					
Subcutaneous area (cm ²), median (95% range)	0.3 (0.1, 0.8)	0.4 (0.2, 1.1)	0.9 (0.4, 2.1)	1.7 (0.8, 3.2)	<0.001
Preperitoneal area (cm ²), median (95% range)	0.3 (0.1, 0.6)	0.4 (0.2, 0.8)	0.6 (0.2, 1.4)	1.0 (0.3, 1.5)	<0.001
Cardiovascular risk factors					
Systolic blood pressure (mm/Hg), mean (SD)	99.8 (7.5)	102.2 (7.9)	105.4 (8.1)	108.4 (9.2)	<0.001
Diastolic blood pressure (mm/Hg), mean (SD)	59.7 (6.5)	60.5 (6.8)	61.5 (6.9)	63.3 (6.9)	<0.001
Left ventricular mass, (g/m ²), mean (SD)	46.7 (10.3)	52.5 (11.0)	58.1 (12.1)	64.3 (12.6)	<0.001
Total-cholesterol (mmol/l), mean (SD)	4.1 (0.6)	4.2 (0.6)	4.3 (0.7)	4.4 (0.6)	<0.001
LDL-cholesterol (mmol/l), mean (SD)	2.3 (0.5)	2.3 (0.6)	2.0 (0.6)	2.6 (0.6)	<0.001
HDL-cholesterol (mmol/l), mean (SD)	1.4 (0.3)	1.4 (0.3)	1.3 (0.3)	1.3 (0.30)	0.001
Triglycerides (mmol/l), median (95% range)	0.9 (0.4, 2.5)	0.9 (0.4, 2.3)	1.0 (0.4, 2.4)	1.1 (0.4, 3.1)	<0.001
Insulin (U/l), median (95% range)	116 (14, 391)	108 (16, 372)	139 (23, 448)	148 (29, 537)	<0.001
C-Peptide (ng/ml), median (95% range)	0.97 (0.28, 2.25)	0.94 (0.29, 2.07)	1.02 (0.38, 2.33)	1.08 (0.39, 2.60)	<0.01
Clustering of cardiovascular risk factors (%)	2.1 (0.1)	6.7 (0.3)	26.2 (0.4)	42.2 (0.5)	<0.01

Values are means (SD), percentages or median (95% range). P value was estimated by using one-way ANOVA test for continuous variables and χ^2 tests for categorical variables. N, number.

subcutaneous and preperitoneal abdominal fat mass measures. Additionally, adjusting these associations for BMI attenuated the associations of fat mass percentage and abdominal fat mass measures with blood pressure and blood levels of insulin and c-peptide into nonsignificant (**Supplementary Table S3** online). **Supplementary Table S4** online shows that adding fat mass percentage and abdominal fat mass measures to the models slightly increased the portion of variance already explained by BMI.

Figure 1 shows that conditional on BMI, fat mass percentage and subcutaneous fat mass area, but not android/gynoid fat mass ratio and preperitoneal abdominal fat mass area, were positively associated with blood pressure, and inversely with

left ventricular mass. Higher fat mass percentage and abdominal fat mass measures were associated with higher total-cholesterol, LDL-cholesterol, triglycerides, insulin and c-peptide blood levels, and lower HDL-cholesterol blood levels. For blood pressure, left ventricular mass, and total and LDL-cholesterol levels, the strongest effect estimates were observed for fat mass percentage, whereas for triglycerides the strongest effect estimates were observed for android/gynoid fat mass ratio.

Table 3 shows that among normal weight and overweight children, higher fat mass percentage was associated with higher blood pressure, whereas among obese children higher fat mass percentage was stronger associated with HDL-cholesterol and insulin. Higher android/gynoid fat mass ratio was most

Table 2. Associations of childhood total body and abdominal fat mass measures with cardiovascular risk factors

Cardiovascular risk factors	General adiposity		Body fat distribution			Abdominal fat mass distribution		
	N	BMI (SDS)	N	Fat mass percentage (SDS)	Android/Gynoid fat mass (SDS)	N	Subcutaneous fat mass area (SDS)	Preperitoneal fat mass area (SDS)
Systolic blood pressure (SDS)	6,069	0.22 (0.19, 0.24)**	5,958	0.16 (0.14, 0.19)**	0.11 (0.09, 0.14)**	5,020	0.16 (0.13, 0.18)**	0.09 (0.06, 0.12)**
Diastolic blood pressure (SDS)	6,069	0.07 (0.04, 0.09)**	5,958	0.08 (0.06, 0.11)**	0.05 (0.03, 0.08)**	4,971	0.06 (0.03, 0.09)**	0.04 (0.01, 0.07)*
Left ventricular mass (SDS)	5,966	0.35 (0.33, 0.37)**	5,825	0.09 (0.06, 0.11)*	0.10 (0.08, 0.12)**	5,072	0.04 (0.01, 0.07)*	−0.01 (−0.04, 0.01)
Total-cholesterol (SDS)	4,321	0.08 (0.05, 0.11)**	4,140	0.13 (0.10, 0.17)**	0.09 (0.06, 0.12)**	3,445	0.12 (0.09, 0.16)**	0.14 (0.10, 0.18)**
LDL-cholesterol (SDS)	4,216	0.08 (0.05, 0.12)**	4,143	0.15 (0.11, 0.18)**	0.10 (0.06, 0.13)**	3,448	0.14 (0.10, 0.17)**	0.12 (0.09, 0.16)**
HDL-cholesterol (SDS)	4,217	−0.08 (−0.11, −0.05)**	4,144	−0.10 (−0.13, −0.06)**	−0.12 (−0.15, −0.09)**	4,352	−0.07 (−0.11, −0.03)**	−0.04 (−0.06, −0.00)†
Triglycerides (SDS)	4,202	0.11 (0.07, 0.14)**	4,130	0.13 (0.09, 0.16)**	0.18 (0.15, 0.21)**	3,438	0.11 (0.07, 0.15)**	0.13 (0.09, 0.16)**
Insulin (SDS)	4,173	0.17 (0.14, 0.20)**	4,093	0.13 (0.09, 0.17)**	0.11 (0.07, 0.14)**	3,410	0.10 (0.06, 0.14)**	0.09 (0.06, 0.13)**
C-peptide (SDS)	4,182	0.14 (0.11, 0.17)**	4,102	0.11 (0.07, 0.14)**	0.11 (0.08, 0.14)**	3,419	0.07 (0.03, 0.11)**	0.07 (0.04, 0.11)**

Values are linear regression coefficients (95% confidence interval). The estimates represent differences in cardiovascular risk factors (outcomes) per SDS of childhood anthropometrics, total body and abdominal fat mass measures (determinants). Models are adjusted for age, sex and ethnicity. Models with body fat and abdominal fat mass measures are additionally adjusted for height. Models adjusted for BMI are given in are given in **Supplementary Table S3** online. † < 0.05; *P value < 0.01; **P value < 0.001. N, number; SDS, SD scores.

strongly associated with higher blood pressure among obese children, but not consistently with other cardiovascular risk factors. We observed the strongest associations of higher fat mass percentage and abdominal fat mass measures with lower left ventricular mass among underweight children. The associations of abdominal fat mass measures with cardiovascular risk factors were higher among obese children. Interaction terms were constructed by using BMI in four categories and adiposity measures as continuous variables. The interaction terms between BMI categories and adiposity measures for total-cholesterol were not significant. Associations of childhood total body and abdominal fat mass measures with c-peptide levels among different obesity categories are shown in **Supplementary Table S5** online. For all analyses, sensitivity analyses were performed among boys and girls separately and no consistent sex differences were present (results not shown).

Risks of Hypertension, Hypercholesterolemia, and Clustering of Cardiovascular Risk Factors

Figure 2 shows that higher BMI and fat mass percentage and abdominal fat mass tended to be associated with higher risks of hypertension, hypercholesterolemia, and clustering of cardiovascular risk factors, with similar effect estimates for the associations of the different fat measures with the risks of hypertension and hypercholesterolemia. For clustering of cardiovascular risk factors, we observed the strongest effect estimate for fat mass percentage (odds ratio: 3.01; 95% confidence

interval: 2.67, 3.39) per SDS total body fat mass. After excluding the android fat mass percentage as a component from the definition of clustering of cardiovascular risk factors, similar results were observed for the associations of body fat distribution measurements with clustering of cardiovascular risk factors (**Supplementary Figure S1** online)

DISCUSSION

This large-scale population-based study among school-age children showed that both fat mass percentage and abdominal fat mass measures were associated with cardiovascular risk factors, independent from BMI. Higher childhood body fat distribution measures were strongly associated with increased risks of childhood hypertension, hypercholesterolemia, and clustering of cardiovascular risk factors.

Methodological Considerations

We performed a cross-sectional study within a population-based cohort with a large number of subjects. The response rate at baseline was 61%. Of all children participating at the age of 6 y, 78% (6,523) participated in the adiposity and cardiovascular follow-up studies. The nonresponse could lead to biased effect estimates if the associations of different obesity measures with cardiovascular risk factors would be different between children included and not included in the analyses (10). Assuming that children with a higher BMI are less likely to participate in the detailed adiposity and cardiovascular

Figure 1. Associations of total body and abdominal fat mass measures with cardiovascular risk factors, conditional on BMI. (a) Systolic blood pressure. (b) Diastolic blood pressure. (c) Left ventricular mass. (d) Total-cholesterol. (e) Low-density lipoprotein-cholesterol. (f) High-density lipoprotein-cholesterol. (g) Triglycerides. (h) Insulin. Values are standardized regression coefficients (95% confidence interval) from conditional analyses. The estimates represent differences in systolic and diastolic blood pressure, left ventricular mass, different lipid levels and insulin per standardized residual change of total, and abdominal fat mass measures. Models are adjusted for age, sex, and ethnicity. AVGFM, android/gynoid fat mass ratio; FMP, fat mass percentage; PPAFM, preperitoneal fat mass area; SCAFM, subcutaneous fat mass area; SDS, standard deviation score.

follow-up studies, our estimates may be underestimated. Birth weight was lower in those who were included in the current analyses than in those who were not included. However, it is hard to speculate whether this difference would affect the observed associations materially, but we consider this unlikely. We obtained detailed measures of childhood adiposity. DXA quantifies fat content with high precision, but cannot differentiate between abdominal visceral and subcutaneous fat compartments. However, we used abdominal ultrasound, a valid method for measuring both subcutaneous abdominal fat mass and preperitoneal fat mass (11). Both DXA and abdominal ultrasound have been validated against CT (12,13).

Use of 30-min fasting blood samples may have led to underestimation of the observed associations. However, it has been shown in adults that nonfasting lipid levels can accurately predict increased risks of cardiovascular events in later life (14). The different adiposity measures were correlated, which may explain why the associations are difficult to interpret when are included in one regression models. The main advantage of the conditional analyses is that the effect estimates are completely statistically independent when combined in one model. Due to the cross-sectional analyses, we were not able to explore directions and causality of the observed associations. Therefore, it is of interest to perform further longitudinal analyses to examine

Table 3. Associations of childhood total body and abdominal fat mass measures with cardiovascular risk factors among underweight, normal weight, overweight, and obese children

	Cardiovascular risk factors						
	Systolic blood pressure (SDS)	Diastolic blood pressure (SDS)	Left ventricular mass (SDS)	LDL-cholesterol (SDS)	HDL-cholesterol (SDS)	Triglycerides (SDS)	Insulin (SDS)
Fat mass percentage (SDS)							
Underweight	−0.10 (−0.27, 0.07)	—	−0.30 (−0.46, −0.13)**	—	−0.13 (−0.36, 0.11)	—	0.07 (−0.16, 0.30)
Normal weight	0.13 (0.09, 0.17)**	—	−0.21 (−0.25, −0.18)**	—	−0.07 (−0.12, −0.02)*	—	0.05 (0.00, 0.10)†
Overweight	0.09 (0.00, 0.17)†	—	−0.27 (−0.36, −0.18)**	—	−0.13 (−0.23, −0.03)*	—	0.18 (0.06, 0.30)*
Obese	0.04 (−0.13, 0.22)	—	0.07 (−0.09, 0.24)	—	−0.24 (−0.42, −0.06)*	—	0.33 (0.12, 0.59)**
<i>P</i> value for interaction	<0.01	0.68	<0.01	0.07	0.05	0.10	<0.01
Android/Gynoid (SDS)							
Underweight	0.03 (−0.14, 0.20)	0.00 (−0.17, 0.18)	0.12 (−0.04, 0.28)	−0.20 (−0.41, −0.02)†	−0.32 (−0.55, −0.09)*	—	0.05 (−0.17, 0.28)
Normal weight	0.04 (0.01, 0.08)†	0.02 (−0.02, 0.05)	−0.02 (−0.05, 0.02)	0.06 (0.02, 0.11)†	−0.06 (−0.11, −0.01)†	—	0.02 (−0.03, 0.07)
Overweight	0.01 (−0.06, 0.09)	0.04 (−0.04, 0.11)	−0.12 (−0.19, −0.05)**	0.14 (0.05, 0.22)*	−0.15 (−0.23, −0.07)**	—	0.14 (0.05, 0.23)*
Obese	0.13 (0.01, 0.24)†	0.15 (0.04, 0.25)*	0.09 (−0.03, 0.20)	0.09 (−0.04, 0.23)	−0.19 (−0.31, −0.08)*	—	0.10 (−0.05, 0.25)
<i>P</i> value for interaction	<0.01	<0.01	<0.01	<0.01	0.02	0.62	<0.01
Subcutaneous area (SDS)							
Underweight	−0.08 (−0.24, 0.07)	—	−0.12 (−0.26, 0.00)	—	−0.08 (−0.27, 0.12)	0.12 (−0.09, 0.33)	0.00 (−0.20, 0.20)
Normal weight	0.11 (0.07, 0.15)**	—	−0.10 (−0.14, −0.06)**	—	−0.03 (−0.08, 0.02)	0.06 (0.01, 0.11)†	0.05 (0.01, 0.10)†
Overweight	0.09 (−0.02, 0.19)	—	−0.16 (−0.26, −0.05)*	—	−0.12 (−0.24, 0.01)	0.16 (0.03, 0.29)*	0.09 (−0.06, 0.24)
Obese	0.24 (0.02, 0.47)†	—	0.05 (−0.18, 0.27)	—	−0.23 (−0.48, 0.02)	0.36 (0.10, 0.62)*	0.21 (−0.08, 0.50)
<i>P</i> value for interaction	<0.01	0.10	<0.01	0.52	<0.01	<0.01	<0.01
Preperitoneal area (SDS)							
Underweight	−0.08 (−0.23, 0.08)	−0.13 (−0.28, 0.03)	−0.17 (−0.30, −0.04)	0.01 (−0.18, 0.19)	0.05 (−0.15, 0.24)	0.01 (−0.20, 0.21)	−0.03 (−0.22, 0.16)
Normal weight	0.06 (0.02, 0.09)*	0.04 (−0.00, 0.07)	−0.13 (−0.16, −0.10)**	0.13 (0.08, 0.17)**	0.02 (−0.02, 0.07)	0.08 (0.03, 0.12)*	0.07 (0.03, 0.12)*
Overweight	0.04 (−0.04, 0.13)	0.01 (−0.08, 0.10)	−0.15 (−0.23, −0.06)*	0.20 (0.10, 0.30)**	−0.05 (−0.15, 0.05)	0.18 (0.08, 0.29)*	0.06 (−0.06, 0.18)
Obese	0.12 (−0.02, 0.25)	0.08 (−0.04, 0.21)	0.03 (−0.11, 0.16)	0.14 (−0.02, 0.30)	−0.14 (−0.30, 0.01)	0.23 (0.07, 0.40)*	0.08 (−0.10, 0.25)
<i>P</i> value for interaction	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01

Values are linear regression coefficients (95% confidence interval). The estimates represent differences in cardiovascular risk factors per SDS change of total body and abdominal fat mass measures among different obesity categories. Analyses are adjusted for age, sex and ethnicity. Results for c-peptide are similar as insulin, and are given in **Supplementary Table S5** online. Interaction terms were constructed using BMI in four categories and adiposity measures as continuous variables. † < 0.05; **P* value < 0.01; ***P* value < 0.001.

SDS, SD scores.

Figure 2. Associations of total body and abdominal fat mass measures with the risk of hypertension, hypercholesterolemia and clustering of cardiovascular risk factors in children. (a) Hypertension. (b) Hypercholesterolemia. (c) Clustering of cardiovascular risk factors. Values are OR (95% CI) that reflect the risk of hypertension, hypercholesterolemia, and clustering cardiovascular risk factors per SD change in BMI, total body, and abdominal fat mass measures in children. Models are adjusted for age, sex, and ethnicity. AVGFM, android/gynoid fat mass ratio; CI, confidence interval; FMP, fat mass percentage; OR, odds ratios; PPAFM, preperitoneal fat mass area; SCAFM, subcutaneous fat mass area.

the associations of these adiposity measures with cardiovascular risk factors in adolescence and adulthood.

Interpretation of Main Findings

Many studies have shown associations of different adiposity measures with cardiovascular risk factors (1–4,8). BMI may be a suboptimal measure in children, as it is unable to distinguish lean mass from fat mass. Detailed total body and abdominal fat mass measures may be useful to identify children with an adverse cardiovascular profile. Thus far, not much is known about these associations in school-age children. In our study, BMI, fat mass percentage, and abdominal fat measures were strongly positively correlated. These observations suggest that the correlations between BMI, fat mass percentage, and waist circumference previously shown in both adults and older aged children are also present in school-aged children (3,15,16). The relatively weaker correlation between BMI and preperitoneal fat mass suggests that BMI is only weakly related to visceral fat mass.

We observed that fat mass percentage, was independent from BMI, associated with various cardiovascular risk factors. Also, the associations of body fat mass measures with lipid levels tended to be stronger than the associations for BMI. Similarly, a study among 5,235 English children aged 9–12 y observed that BMI and total fat mass in childhood were associated with cardiovascular risk factors in adolescents, with slightly stronger effect estimates for total fat mass measures (15). Surprisingly, we observed that independent from BMI, fat mass percentage was inversely associated with left ventricular mass. Another study among 201 children aged 6–17 y old reported a similar associations (17), suggesting muscle mass is the major determinant of left ventricular mass in childhood.

Both android/gynoid fat mass ratio and subcutaneous abdominal fat mass area, which reflect waist to hip ratio and waist circumference, respectively, were associated with cardiovascular risk factors, independent from BMI. Multiple studies in both adults and older aged children have reported similar effect estimates (18,19). However, waist to hip ratio has not

consistently been identified as a strong predictor of cardiovascular risk factors. These inconsistencies may be due to the large variations in the level of total body and abdominal fat mass; therefore, both lean and obese individuals may have the same waist to hip ratio. We measured subcutaneous and preperitoneal fat mass using ultrasound, and used preperitoneal fat mass as a measure of visceral fat mass (11,12). In adults and adolescents, both subcutaneous abdominal fat mass and visceral abdominal fat mass are associated with cardiovascular risk factors and visceral fat mass tends to be stronger related with HDL-cholesterol, triglycerides, and insulin resistance (8, 20, 21). As compared to associations of preperitoneal fat mass, we observed stronger associations for subcutaneous abdominal fat mass area with most cardiovascular risk factors. Thus, in children, visceral fat mass may not be strongly associated with cardiovascular risk factors, which may be explained by less pathogenic and only a small accumulation of visceral adipose tissue at younger ages. In line with our findings, a study among 783 young men (22) showed that visceral abdominal fat mass is not stronger associated with cardiovascular risk factors than subcutaneous abdominal fat mass.

The effects of specific fat measures on health outcomes may differ between normal, overweight, and obese children. A study among 359,387 European adults showed that the associations of waist circumference with risk of death were stronger among subjects with a lower BMI (4). Similarly, a study among 2,003 adolescents showed that males with a normal BMI and elevated waist circumference were more likely to have elevated levels of cardiovascular risk factors (23). We observed that the associations of higher abdominal fat mass measures with cardiovascular risk factors were stronger among obese children. Another study among adults from 18 to 80 y old showed that subjects with body fat percentage within the obesity range had higher levels of cardiovascular risk factors (24). Therefore, also in school-age children, the associations of general and abdominal fat mass may differ between the BMI groups.

Our results suggest that children with higher levels of general and abdominal fat mass are independent of their BMI,

an adverse cardiovascular risk profile. Whether and to what extent detailed fat mass percentage and abdominal fat measures should be used in clinical practice is not known yet. The additional clinical value of detailed fat measures as compared to BMI may be only limited. The additional variance explained in cardiovascular risk factors by more direct measures of adiposity in our models was small. Also, taking into account greater comfort, feasibility and lower costs of measuring BMI in children, BMI alone might be an appropriate measure for clinical practice in children. Although clinical practice may not be in direct need for detailed measures of total and abdominal fat measures at this age, our findings strongly suggest that detailed body fat distribution measurements are important tools in etiological studies focused on the early origins of cardio-metabolic diseases.

Conclusions

Fat mass percentage and abdominal fat mass measures are associated with cardiovascular risk factors in school-age children, independent from BMI. These measures may provide additional information for identification of children with an adverse cardiovascular profile, and may be important measures for etiological research focused on development of cardiovascular and metabolic diseases. Further studies are needed to examine the longitudinal associations of these specific fat mass measures with development of cardiovascular risk factors and disease in later life.

METHODS

Study Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands (10). The study was conducted according to the guidelines of the Helsinki Declaration and approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (MEC 198.782/2001/31). In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Response rate at baseline was 61%. Written consent was obtained from parents. 8,305 children participated in follow-up studies at the age of 6 y (median: 6.0 y (95% range 5.6, 7.9 y)), of whom 6,523 (78%) children participated in detailed cardiovascular follow-up measurements and 4,294 (66%) had blood samples available. This lower number for blood samples is mainly due to nonconsent for venous puncture. Children who did not participate in the follow-up measures at 6 y had a lower gestational age at birth and lower birth weight (**Supplementary Table S6** online).

Childhood Assessments

At the age of 6 y, we measured height and weight without shoes and heavy clothing. Weight was measured to the nearest gram using an electronic scale (SECA 888, Almere, The Netherlands). Height was measured to the nearest 0.1 cm by a stadiometer (Holtain Limited, Crosswell, Crymch, UK). BMI (kg/m^2) was calculated. Childhood underweight, normal weight, overweight, and obesity were defined by the International Obesity Task Force cut offs (25).

Total body and regional fat mass percentages were measured using DXA (iDXA, GE-Lunar, 2008, Madison, WI), and analyzed with the enCORE software v.12.6 (7). iDXA can accurately detect whole-body fat mass within less than 0.25% coefficient of variation. Children were placed without shoes, heavy clothing, and metal objects in supine position on the DXA table. Fat mass percentage (%) was calculated as percentage of total body weight (kg) measured by DXA. We calculated the ratio of android and gynoid fat mass. The android/gynoid

fat ratio reflects the central body fat distribution in the abdomen (android) and hip (gynoid) regions (7).

Abdominal examinations were performed with ultrasound, as described in detail before (12). Briefly, preperitoneal and subcutaneous fat thicknesses were measured with a linear (L12-5 MHz) transducer (11), which was placed perpendicular to the skin surface on the median upper abdomen. We scanned longitudinally just below the xiphoid process to the navel along the midline (linea alba). All measurements were performed off-line. Subcutaneous fat mass distance (SC-distance) was measured as distance of the inner surface of subcutaneous tissue to the linea alba. Preperitoneal fat mass distance (PP-distance) was measured as distance of the linea alba to the peritoneum on top of the liver. Subcutaneous and preperitoneal fat mass areas were measured as areas of 2 cm length along the midline starting from the maximum preperitoneal distance in direction of the navel (SC-area, PP-area). We measured three times the areas of 2 cm length along midline, and we used the mean value of these measures. The intraobserver reproducibility and the intraclass correlation coefficients ranged from 0.93 to 0.97.

Blood pressure was measured at the right brachial artery four times with 1-min intervals, using the validated automatic sphygmomanometer Datascope Accutor Plus (Paramus, NJ) (26). We calculated the mean value for systolic and diastolic blood pressure using the last three blood pressure measurement of each participant. Echocardiography measurements were performed using methods recommended by the American Society of Echocardiography, and used to calculate the left ventricular mass (27,28).

Thirty-minutes fasting, blood samples were collected to measure total-, HDL-, and LDL-cholesterol, triglycerides, insulin, and C-peptide concentrations, using Cobas 8000 analyser (Roche, Almere, The Netherlands). Quality control samples demonstrated intra- and interassay coefficients of variation ranging from 0.77 to 1.39%, and 0.87 to 2.40%, respectively.

We defined hypertension as systolic and diastolic blood pressure above the 95th percentile, using age- and height-specific cut-points (29). Recommendations from National Cholesterol Education Program for children age 2–9 y were used to define adverse levels of cholesterol; high cholesterol ($>5.1 \text{ mmol/l}$) (30). For defining children with clustering of cardiovascular risk factors, we used the previously described definition of childhood metabolic syndrome phenotype (31), which means having three or more of the following components: android fat mass $\geq 75^{\text{th}}$ percentile; systolic or diastolic blood pressure $\geq 75^{\text{th}}$ percentile; HDL-cholesterol $\leq 25^{\text{th}}$ percentile or triglycerides $\geq 75^{\text{th}}$ percentile; and insulin level $\geq 75^{\text{th}}$ percentile. We used android fat mass as percentage of total body fat mass, as proxy for waist circumference.

Statistical Analysis

First, we compared childhood characteristics between different childhood obesity categories using one-way ANOVA tests. We examined the correlations between all childhood adiposity and cardiovascular outcomes using Pearson or Spearman rank correlation coefficients. Second, we assessed the associations of childhood fat measures with cardiovascular risk factors using different linear regression models. Model 1 was adjusted for child's age at measurements, sex, ethnicity, and height. Model 2 was additionally adjusted for child's current BMI. For these analyses, we log-transformed not normally distributed abdominal fat mass measures and cardiovascular risk factors. We constructed standard deviation scores ((observed value-mean)/SD) for all variables to enable comparison in effect size of different outcome measures. We explored whether adding specific fat mass measures to the model with BMI explained more of the variance for each outcome. To take account for the correlation between different adiposity measures, we also examined the associations of detailed childhood fat mass measures with cardiovascular risk factors, independent from BMI by performing linear regression analyses to assess the associations of fat mass measures conditional on BMI (32). We constructed fat mass variables, which are statistically independent of BMI, allowing simultaneous inclusion in multiple regression models. Details of these models are given in the **Supplementary Methods** online. Third, we tested potential interactions between childhood

BMI categories and childhood adiposity measures. Subsequently, we performed linear regression analyses to examine the associations of childhood adiposity measures with cardiovascular risk factors in different BMI categories. Finally, we used logistic regression models to examine the associations of childhood BMI, fat mass percentage, and abdominal fat mass measures with the risks of hypertension, hypercholesterolemia, and clustering of cardiovascular risk factors. These models were adjusted for child's age at measurements, sex, and ethnicity. All analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS, Chicago, IL).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

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AUTHOR CONTRIBUTIONS

O.G., R.G., and V.W.V.J. designed and conducted the research and wrote the paper. O.G. analyzed the data. B.D., M.A., E.M.vdB., A.H., O.H.F., and L.d.J. provided comments and consultation regarding the analyses and manuscript. O.G., R.G., and V.W.V.J. had primary responsibility for final content. All authors gave final approval of the version to be published.

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