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Clinical Research Article

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Childhood Adiposity Associated With Expanded Effector Memory CD8$^+$ and Vδ2$^+$Vγ9$^+$ T Cells

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Abbreviations: BMI, body mass index; DXA, dual-energy x-ray absorptiometry; FMI, fat mass index; IL, interleukin; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; T_{CM}, T central memory cells; T_{EM}, T effector memory; T_{EMRA}, T effector memory RA-positive; T_{EMRO}, T effector memory RO-positive; Th, T helper cells; TNF-α, tumor necrosis factor-α; Treg, T regulatory cells.

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Abstract

Context: Adult obesity is associated with chronic low-grade inflammation and may give rise to future chronic disease. However, it is unclear whether adiposity-related inflammation is already apparent in childhood.

Objective: To study associations between child adiposity measures with circulating monocytes and naive and memory subsets in CD4, CD8, and $\gamma\delta$ T cell lineages.

Methods: Ten-year-old children (n = 890) from the Generation R Cohort underwent dual-energy x-ray absorptiometry and magnetic resonance imaging for body composition (body mass index [BMI], fat mass index [FMI], android-to-gynoid fat mass ratio, visceral fat index, liver fat fraction). Blood samples were taken for detailed immunophenotyping of leukocytes by 11-color flow cytometry.

Results: Several statistically significant associations were observed. A 1-SD increase in total FMI was associated with +8.4% (95% CI 2.0, 15.2) Vδ2$^+$Vγ9$^+$ and +7.4% (95% CI 2.4, 12.5) CD8$^+_{TEMRO}$ cell numbers. A 1-SD increase in visceral fat index was associated with +10.7% (95% CI 3.3, 18.7) Vδ2$^+$Vγ9$^+$ and +8.3% (95% CI 2.6, 14.4) CD8$^+_{TEMRO}$ cell numbers.

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Higher android-to-gynoid fat mass ratio was only associated with higher Vδ2$^+$Vγ9$^+$T cells. Liver fat was associated with higher CD8$^+_{TEMRO}$ cells but not with Vδ2$^+$Vγ9$^+$ T cells. Only liver fat was associated with lower Th17 cell numbers: a 1-SD increase was associated with –8.9% (95% CI –13.7, –3.7) Th17 cells. No associations for total CD8$^+$, CD4$^+$T cells, or monocytes were observed. BMI was not associated with immune cells.

Conclusion: Higher Vδ2$^+$Vγ9$^+$ and CD8$^+_{TEMRO}$ cell numbers in children with higher visceral fat index could reflect presence of adiposity-related inflammation in children with adiposity of a general population.

Key Words: child, effector memory T cell, gamma delta T cell, fat mass, inflammation, monocyte

Childhood overweight and obesity are major public health concerns (1). High childhood body mass index (BMI) predisposes to high BMI in adulthood (2-4). In school-aged children and adolescents, high BMI increases the risk of adverse health outcomes associated with overweight and obesity in adulthood (3, 4). These adverse health outcomes include asthma, insulin resistance, coronary heart disease, and metabolic syndrome (3, 5, 6). In addition, obesity might increase the risk of respiratory tract infections (7) and it increases the morbidity and mortality in COVID-19 (8).

Adiposity is associated with chronic low-grade inflammation, which predominantly originates in visceral adipose tissue (9-12). Fat biopsies of adults with overweight or obesity have been shown to be infiltrated with Th1, Th17, and CD8$^+$ T cells, which might be a reflection of this chronic low-grade inflammation (12). Positive associations have been observed between human fat mass and intermediate and nonclassical monocytes in blood (13, 14). In adolescents with overweight or obesity, increased circulating effector memory CD4$^+$ and CD8$^+$ T cells have been observed compared with adolescents without overweight or obesity (15).

Adiposity-related inflammation is thought to contribute to the onset of obesity-related morbidity such as insulin resistance (13, 16). CD8$^+$ T cells are probably contributors to this adiposity-related inflammation in an early phase by production of pro-inflammatory cytokines, such as interleukin 2 (IL-2), which promote T cell proliferation and adipogenesis (9). In a later phase, the intermediate and nonclassical monocytes are thought to contribute to the adiposity-related inflammation through increased tumor necrosis factor-α (TNF-α) production (13, 17, 18). Th1 and Th17 are producers of various pro-inflammatory cytokines, potentially contributing to the pro-inflammatory state in overweight and obesity, as the presence of these pro-inflammatory cytokines, such as TNF-α, has previously been associated with the onset of obesity-related morbidity (12, 16, 19, 20). Regarding γδ T cells, fewer studies are present, but increased differentiation into effector memory γδ T with a decreased antiviral response has been observed in adults with overweight or obesity (21, 22). Within the γδ T cells, the Vδ2$^+$Vγ9$^+$ subset is the most dominant in human blood (23-25).

The majority of studies on adiposity-associated inflammation have been performed in mouse models or human adults. Reports on the effects of adipose tissue on the immune system in school-aged children and studies with detailed immune phenotyping and information on fat mass distribution in children are not present. Previous studies on the associations between immune cell numbers and adiposity in adults did not account for confounders such as sex, ethnicity, lifestyle factors and socio-economic status (24).

We hypothesized that higher fat mass in childhood is associated with higher numbers of pro-inflammatory monocytes and higher T effector memory cells in peripheral blood. We here related body composition determinants (BMI, fat mass index [FMI]) and body fat distribution determinants (android-to-gynoid fat mass ratio, visceral fat index and liver fat fraction)as determined by dual-energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) with a detailed set of monocyte and T cell subsets in 890 10-year-old children of a population-based cohort.

Methods

Study Design

This cross-sectional study was performed within the Generation R Study, a population-based cohort study from early pregnancy onwards located in Rotterdam, the Netherlands. The design of the study has been previously described in detail (26). The medical ethical committee of Erasmus University Medical Center approved the study (MEC-2012–165). Written informed consent was collected from all parents or legal guardians of the children. We selected children of whom information on BMI or fat mass measurements by DXA and immune cell numbers at 10 years of age were available. The number of children included in the individual analyses is dependent on the

availability of information on both exposure and outcome and ranges from 535 to 881 children (Table 1).

Body Composition Measurements

BMI (kg/m^2) was calculated from height and weight measurements at our research center. Next, age- and sex-adjusted BMI standard deviation scores were calculated based on Dutch reference growth charts (27). Children were categorized into BMI categories as follows: underweight (n = 44, 4.8%), healthy weight (n = 719, 80.9%), overweight (n = 107, 12.0%) and obesity (n = 20, 2.3%), based on cutoffs of the International Obesity Task Force (28). Total fat mass (grams), android fat mass, and gynoid fat mass were determined with DXA measurements (GE Lunar iDXA, enCORE software version 12.6; GE Healthcare) (29, 30). A fat mass index (FMI, kg/m^4) was calculated (31). Android-to-gynoid fat mass ratio was determined by the division of android fat by gynoid fat. The android fat is the central fat mass in the abdomen whereas the gynoid fat mass reflects the fat mass distribution within the hip (29). MRI was performed to determine visceral fat mass and liver fat fraction. From these measurements, a visceral fat index was calculated by division of visceral fat mass by height^3 (31).

Immune Cell Measurements

Peripheral blood samples were obtained and stained to obtain absolute numbers of peripheral blood CD45$^+$ and CD3$^+$ T cells using a diagnostic lyse-no-wash protocol (BD Biosciences) (24). Next, 11-color flow cytometry was performed on LSRII Fortessa (BD Biosciences) with a standardized configuration according to Euroflow protocols for detailed immunophenotyping of monocyte and T cell subset cell numbers (32, 33).

Monocytes and T cells were defined within the SSC^{low}CD45^{dim} population of CD45$^+$ leukocytes (Figs. 1 and 2). For monocyte gating, CD3$^+$ T cells and CD19$^+$ B cells were excluded prior to selection for CD123$^-$IREM-2$^+$HLA-DR$^+$ cells. Within CD123$^-$IREM-2$^+$HLA-DR$^+$ cells, classical (CD14$^+$CD16$^-$), intermediate (CD14$^+$CD16$^+$), and nonclassical monocytes (CD14$^-$CD16$^+$) were defined (Fig. 1) (18).

Table 1. Number of children included in the individual analyses

	BMI	FMI	Android-to-gynoid fat mass ratio	Visceral fat index	Liver fat fraction
Monocyte subsets					
Monocytes	881	872	872	576	651
Classical monocytes	878	869	869	575	649
Intermediate monocytes	878	869	869	575	649
Nonclassical monocytes	878	869	869	575	649
T cell subsets					
TCR γδ$^+$ T cells	855	846	846	557	630
Vδ2$^+$Vγ9$^+$ T cells	879	870	870	575	651
Vδ2$^+$Vγ9$^+$ T naive	874	866	866	574	649
Vδ2$^+$Vγ9$^+$ T_{CM}	874	866	866	574	649
Vδ2$^+$Vγ9$^+$ T_{EM}	874	866	866	574	649
Vδ2$^+$Vγ9$^+$ T_{EMRA}	874	866	866	574	649
CD4$^+$ T cells	834	825	825	541	614
CD4$^+$ T naive	852	843	843	555	628
CD4$^+$ T_{CM}	852	843	843	555	628
CD4$^+$ T_{EMRO}	852	843	843	555	628
CD4$^+$ T_{EMRA}	852	843	843	555	628
Th1 cells	829	821	821	538	611
Th2 cells	828	820	820	537	610
Th17 cells	824	816	816	535	607
Treg cells	818	809	809	537	609
CD8$^+$ T cells	828	820	820	539	612
CD8$^+$ T naive	848	840	840	554	626
CD8$^+$ T_{CM}	848	840	840	554	626
CD8$^+$ T_{EMRO}	848	840	840	554	626
CD8$^+$ T_{EMRA}	848	840	840	554	626

The numbers represent the number of children included in the individual analyses.

Abbreviations: BMI, body mass index; FMI, fat mass index; $T_{CM,}$ T central memory; TCR; T cell receptor; $T_{EM,}$ T effector memory; $T_{EMRA,}$ T effector memory RA-positive; $T_{EMRO,}$ T effector memory RO-positive; Th, T helper; Treg, T regulatory.

Vδ2$^+$Vγ9$^+$ T cells were defined within the CD3$^+$ TCRαβ$^-$ cells on the basis of Vδ2 and Vγ9 positivity (Fig. 2). Within total Vδ2$^+$Vγ9$^+$ T cells, naive (CD45RA$^+$CD27$^+$), central memory (T_{CM}; CD45RA$^-$CD27$^+$) cells, effector memory (T_{EMRO}; CD45RA$^-$CD27$^-$), and effector memory RA-positive (T_{EMRA}; CD45RA$^+$CD27$^-$) cell subsets were defined (34).

Within TCRαβ$^+$CD3$^+$ T cells, CD4$^+$ and CD8$^+$ T cell numbers were determined. For each lineage, T naive (CD45RO$^-$CCR7$^+$) cells, T central memory (T_{CM}; CD45RO$^+$CCR7$^+$) cells, T effector memory RO-positive (T_{EMRO}; CD45RO$^+$CCR7$^-$) cells and T effector memory RA-positive (T_{EMRA}; CD45RO$^-$CCR7$^-$) were defined. Within the CD4$^+$ lineage, T regulatory (Treg; CD4$^+$CD127^{dim}CD25^{high}), T helper (Th) 1 (CCR6$^-$CXCR3$^+$CCR4$^-$), Th2 (CCR6$^-$CXCR3$^-$CCR4$^+$) cells, and Th17 (CCR6$^+$CXCR3$^-$CCR4$^+$) cell numbers were determined (33). The antibodies used for the flow cytometry analyses are presented in Table 2.

FacsDIVA software v8 (BD Biosciences, San Jose, USA), Infinicyt software (Cytognos, Salamanca, Spain), and FlowJo software v10 (FlowJo LLC, USA) were used for data analyses. The gating strategies are presented in Figs. 1 and 2 and in our previous study (33). Absolute cells per microliter of blood (cells/μL) were calculated by multiplying the percentage of cells of interest by the total leukocyte and T cell numbers obtained from the Trucount analyses.

Covariates

Information on maternal characteristics including pre-pregnancy BMI (kg/m^2) and educational level (higher vs secondary and primary) was obtained by means of questionnaires obtained during pregnancy (5, 24). Information on each child's sex was obtained from midwife and hospital registries. Postpartum questionnaires retrieved information on ever breastfeeding during the first 4 months (33). The child's ethnic background (Western vs non-Western) was defined based on each parent's country of birth (5, 24, 26, 35). Information on diet quality score and sports activity (>2 vs <2 hours per week) was obtained from questionnaires at, respectively, ages 8 and 10 years (35, 36). The food-based diet quality score is the sum of 10 diet components ("fruit," "vegetables," "whole grains," "fish," "legumes," "nuts, "dairy," "oils and soft or liquid margarines," "sugar-containing beverages," "high fat and processed meat") obtained from food frequency questionnaires. The score ranges from 0 to 10 in which a higher score refers to a healthier diet (36).

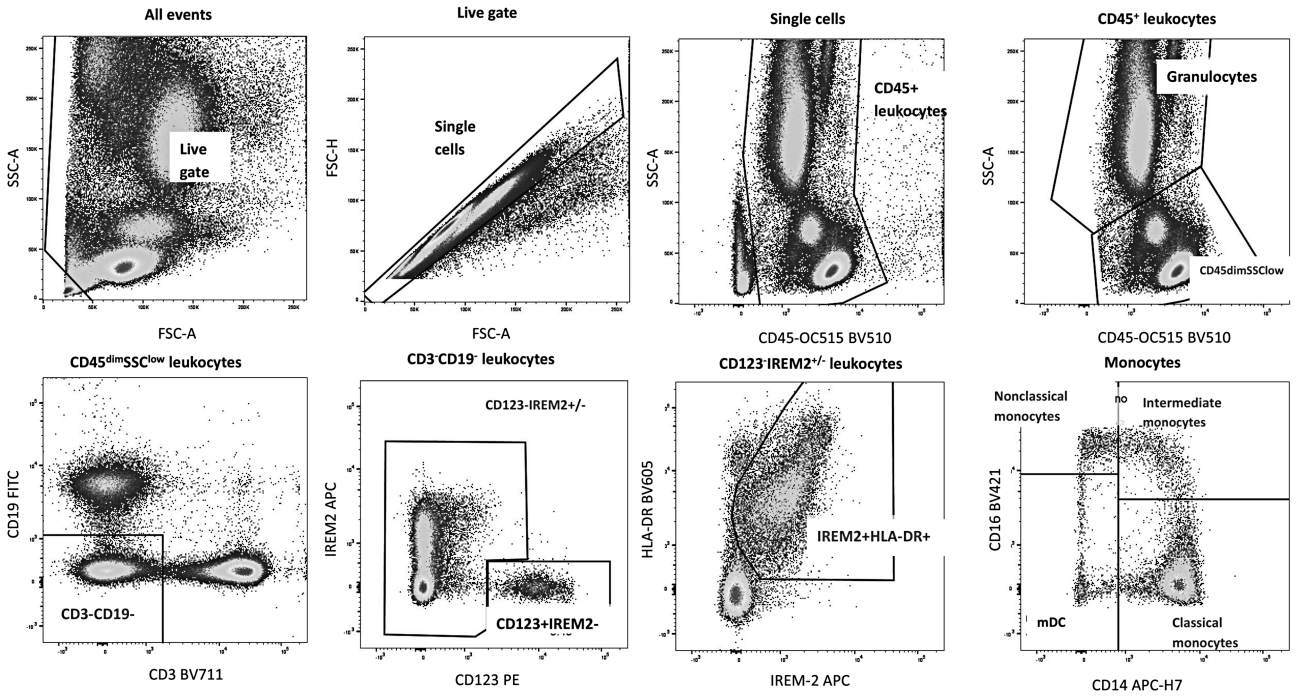

Figure 1. Representative sample illustrating the gating strategy used to identify monocyte subsets in peripheral blood using 11-color flow cytometry. Within CD45$^+$ leukocytes SSC^{low}CD45^{dim} populations were defined. After exclusion of CD3$^+$ T cells and CD19$^+$ B cells, CD123$^-$IREM-2$^{+/-}$ cells and later IREM-2$^+$HLA-DR$^+$ cells were defined. IREM-2$^+$HLA-DR$^+$ cells included the following monocytes: classical monocytes (CD14$^+$CD16$^-$), intermediate monocytes (CD14$^+$CD16$^+$), and nonclassical monocytes (CD14$^-$CD16$^+$). Abbreviations: dim, dimmer; FSC-A, forward scatter area; FSC-H, forward scatter height; IREM, immune receptor expressed on myeloid cells; mDC, myeloid dendritic cells; SSC-A, side scatter area.

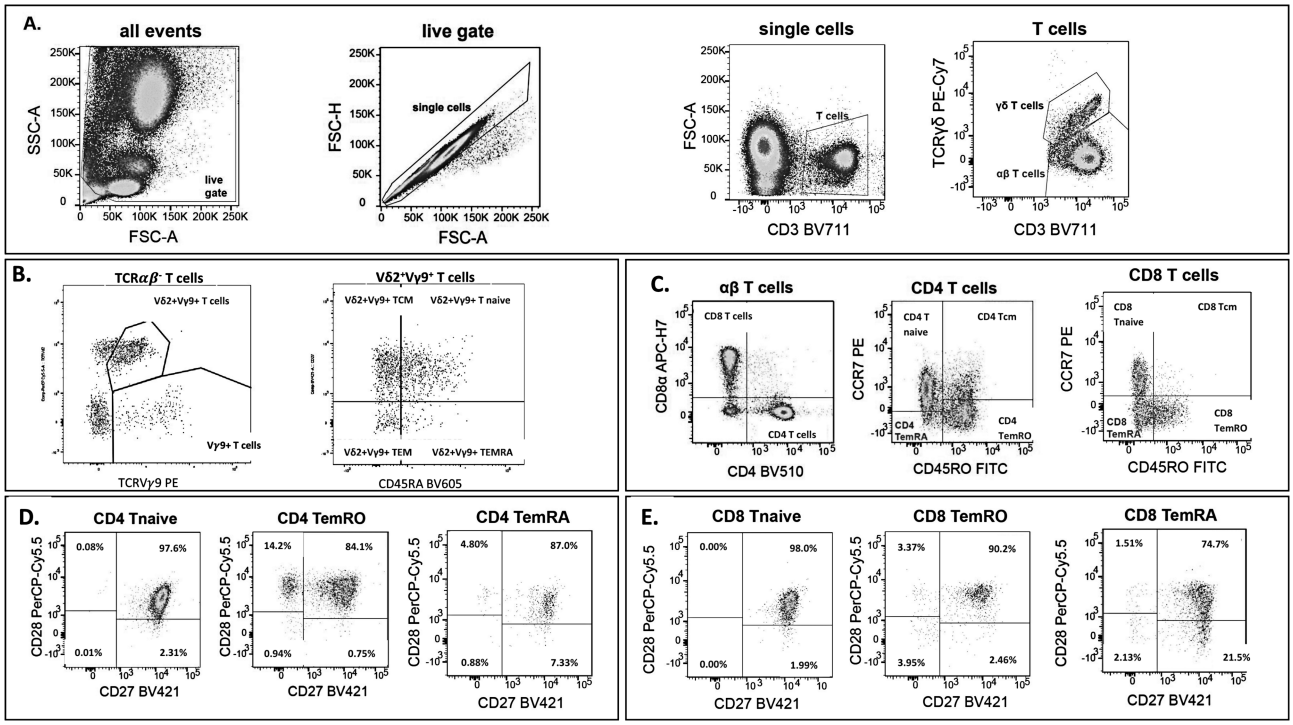

Figure 2. Representative sample illustrating the gating strategy used to identify Vδ2$^+$Vγ9$^+$ T cell and CD4$^+$ and CD8$^+$ memory subsets in peripheral blood using 11-color flow cytometry. After exclusion of αβTCRαβ$^+$ T cells, TCRαβ$^-$ Vδ2$^+$Vγ9$^+$ T cells were defined. Within TCRαβ$^-$ Vδ2$^+$Vγ9$^+$ T cells, T naive (CD45RA$^+$CD27$^+$) cells, T central memory (T_{CM}; CD45RA$^-$CD27$^+$) cells, T effector memory (T_{EM}; CD45RA$^-$CD27$^-$) cells, and T effector memory RA-positive (T_{EMRA}; CD45RA$^+$CD27$^-$) cells were defined. After exclusion of TCRαβ$^-$ T cells, the CD4$^+$ and CD8$^+$ memory T_{CM} and T_{EMRO} and T_{EMRA} cells were defined. Abbreviations: FSC-A, forward scatter area; FSC-H, forward scatter height; SSC-A, side scatter area; Tcm, central memory T cell; Tem, effector memory T cell.

Statistical Analyses

Characteristics of the study population were determined and represented as median with interquartile range (IQR, 25%-75% range) for continuous nonnormally distributed variables and mean with SD for continuous normally distributed variables. To gain more insight in the distribution of the characteristics between children without overweight or obesity vs children with overweight or obesity, we presented the data for the total group and for those 2 specific weight groups. For standardization and interpretation purposes, z-scores for the body composition determinants (BMI, FMI) and body fat distribution determinants (android-to-gynoid fat mass ratio, visceral fat index, and liver fat fraction) were used. All children had the same age and therefore standardization by age was not necessary. Immune cell numbers were recalculated by adding 1 because of values equaling zero and natural log-transformed afterwards to obtain a normal distribution.

Multivariable linear regression analyses were conducted to determine associations between body composition and fat distribution and immune cell numbers adjusted for child and maternal confounders. Potential confounders were added if they were associated with immune cells based on our previous study and if they met the epidemiological criteria for confounders in our study, and finally, if the effect estimate changed at least by 10% after addition of the potential confounder (24). The models were adjusted for maternal pre-pregnancy BMI, maternal educational level, child's sex, child's ethnicity, breastfeeding during the first 4 months, food-based diet quality score at 8 years, and doing sports. We tested but did not include the variables of birth weight and gestational age because these did not affect the effect estimates of the models:.

Multiple imputation (n = 20) using predictive mean matching was performed for missing values on confounders. All measures of association are presented as pooled estimates from the imputed data sets and represent the percentage cell increase or decrease. No differences in value distribution or direction of associations were observed between the nonimputed and imputed dataset. To account for potential multiple testing, a 2-sided *P* value lower than 0.0125 was considered significant using the Bonferroni method (*P* = 0.05 divided by the 4 immune cell categories: monocytes, TCRγδ T, CD4$^+$, and CD8$^+$ T cells). The fat mass and immune cell

Table 2. Antibodies used in flow cytometric analyses

Antibody	Clone	Fluorochrome	Supplier	Identifier RRID
CD3	OKT3	BV711	BioLegend, San Diego, CA	AB_2875052
CD4	OKT4	BV510	BioLegend, San Diego, CA	AB_2561866
CD8	SK1	APC-H7	BD Biosciences	AB_1645482
CD14	MO-P9	APC-H7	BD Biosciences	AB_1645464
CD16	3G8	BV421	Biolegend, San Diego, CA	AB_2561578
CD19	HIB19	FITC	BD Biosciences	AB_395812
CD21	B-ly4	APC	BD Biosciences	AB_2085309
CD25	BC96	BV421	BioLegend, San Diego, CA	AB_11126749
CD27	O323	BV421	BioLegend, San Diego, CA	AB_11150782
CD28	CD28.2	PerCP-Cy5.5	BioLegend, San Diego, CA	AB_2073718
CD38	HIT2	BV605	BioLegend, San Diego, CA	AB_2562915
CD45	GA90	OC515	Cytognos	AB_2848147
CD45RA	HI100	BV605	BioLegend, San Diego, CA	AB_2563814
CD45RO	UCHL1	FITC	DAKO, Glostrup, Denmark	AB_578677
CD127	A019D5	APC	BioLegend, San Diego, CA	AB_10900804
CCR4	TG6/CCR4	PECy7	BioLegend, San Diego, CA	AB_2244410
CCR6	G034E3	PerCP-Cy5.5	BioLegend, San Diego, CA	AB_10918437
CCR7	3D13	PE	ThermoFisher Scientific	AB_10670625
CCR10	69036	PE	R&D systems, Minneapolis, MN	AB_2204787
CXCR3	G025H7	FITC	BioLegend, San Diego, CA	AB_10983066
CXCR5	51505	APC	R&D systems, Minneapolis, MN	AB_357109
HLA-DR	L243	BV605	BioLegend San Diego, CA	AB_11219187
IREM2	UP-H2	APC	Immunostep	AB_11140615
TCRαβ	IP26	PE-Cy7	BioLegend San Diego, CA	AB_10639947
TCRγδ	11F2	PECy7	BD Biosciences	AB_2870377
TCRVδ2	B6	PerCP-Cy5.5	BioLegend, San Diego, CA	AB_1877263
TCRVγ9	B3	PE	BioLegend, San Diego, CA	AB_1236408

measures are highly correlated and therefore no additional correction for multiple testing was performed. Effect modification by sex and BMI was tested by adding interaction terms to the regression models but were not statistically significant ($P > 0.05$) and therefore stratification by sex or BMI was not performed. Statistical analyses were performed using SPSS version 25.0 for Windows (IBM Corp., Armonk, NY, USA) and R version 3.6.1.

Results

Study Population

Children had a median age of 9.8 [IQR 9.7, 10.0] years. Children with overweight or obesity were more often girls (63.8% vs 52.6%), had lower food-based diet quality scores (4.4 ± 1.1 SD vs 4.64 ± 1.1 SD), performed more often <2 hours sports/week (38.5% vs 27.2%) and had less often a Western ethnicity (66.9% vs 86.2%) (all statistically significant with $P < 0.05$) compared with children without overweight or obesity. Mothers of children with overweight or obesity had a higher median pre-pregnancy BMI (kg/m^2) compared with mothers of children without overweight or obesity (25.2 [IQR 22.1, 30.1] vs 22.5 [IQR 20.7, 24.4] and had more often a lower educational level (62.2% vs 39.1%). Percentages of missing values within the covariates ranged from 0% to 24.7%. The study population characteristics are presented in Table 3.

Adiposity Measures Do Not Correlate With Changes in Monocyte Subsets

No associations were observed between BMI, FMI, visceral fat index, android-to-gynoid fat mass ratio, or liver fat fraction with total monocyte numbers. When studying the monocyte subsets, namely classical, intermediate, and nonclassical monocytes, no associations were observed with the adiposity measures (Tables 4-6).

Vδ2$^+$Vγ9$^+$T Cell Subsets Were Higher in Children With Higher Fat Measures

A 1-SD higher FMI, android-to-gynoid fat mass ratio, or visceral fat index were statistically significantly associated with 8.4% (95% CI 2.0, 15.2), 8.2% (95% CI 2.4, 14.4) and 10.7% (95% CI 3.3, 18.7) higher total Vδ2$^+$Vγ9$^+$T

cell numbers, respectively. No associations for BMI and liver fat mass ratio with Vδ2$^+$Vγ9$^+$ T cells were observed. When studying Vδ2$^+$Vγ9$^+$ T cells, the same directions of associations were observed, but the associations for total Vδ2$^+$Vγ9$^+$ T cells were not driven by a specific subgroup (Tables 4-6).

CD8$^+_{TEMRO}$ Cells Are Increased in Children With Higher FMI, Visceral Fat Index, and Liver Fat Fraction

None of the fat mass measures were correlated with total CD8$^+$ T cells. Yet, higher FMI, visceral fat index, and liver fat fraction were all statistically significantly associated with higher CD8$^+_{TEMRO}$ cells: 7.4% (95% CI 2.4, 12.5), 8.3% (95% CI 2.6, 14.4) and 6.6% (95% CI 1.6, 11.8) higher for each SD increase in the respective fat measure. Android-to-gynoid fat mass ratio was not associated with CD8$^+$ T cell numbers. A 1-SD increase in liver fat fraction was associated with 8.8% (95% CI −13.8, −3.7) lower Th17 cell numbers. Other adiposity measures were not associated with Th17. No associations were observed between memory CD4$^+$ T cell numbers and adiposity measures (Tables 4-6).

Discussion

The present study determined the association between adiposity and immune cell numbers in a general population of school-aged children with adjustment for several confounders. In this population, we showed that higher total FMI, visceral fat index, and android-to-gynoid fat mass ratio were associated with higher numbers of circulating Vδ2$^+$Vγ9$^+$ T cells. Higher total FMI, visceral fat index, and liver fat fraction were associated with higher CD8$^+$ T$_{EMRO}$ cell numbers. Only higher liver fat fraction was associated with lower peripherally circulating Th17 cell numbers. No associations were observed between adiposity and monocytes or memory CD4$^+$ T cell numbers in children of the general population.

Table 3. Details of the study population

	Total group (N = 890)	Healthy weight (N = 719)[a]	Overweight/obese (N = 127)*	Missing (%)
Birth and infant characteristics				
Female sex, N (%)	481 (54.0)	378 (52.6)	81 (63.8)	0.0
Western ethnicity, N (%)	729 (83.3)	610 (86.2)	83 (66.9)	1.7
Breastfeeding during first 4 months, N (%)	631 (90.3)	516 (90.5)	80 (87.0)	21.5
Child characteristics				
Age at follow-up measurements (y)	9.8 [9.7, 10.0]	9.8 [9.7, 10.0]	9.9 [9.7, 10.0]	0.0
Height (cm)	142.4 [138.4, 146.9]	142.2 [138.2, 146.5]	145.7 [140.9, 150.2]	0.0
Food-based diet quality score at 8 years (36)	4.6 (1.1)	4.6 (1.1)	4.4 (1.1)	24.6
Doing sports ≥2 h/wk, N (%)	526 (71.4)	444 (72.8)	56 (61.5)	17.2
Child's body composition				
BMI (kg/m^2)	17.0 [15.8, 18.5]	16.8 [15.8, 17.8]	21.2 [20.5, 23.3]	0.0
Weight status (N, %)				0.0
Underweight[a]	44 (4.9)	-	-	
Healthy weight[a]	719 (80.8)	-	-	
Overweight[a]	107 (12.0)	-	-	
Obese[a]	20 (2.2)	-	-	
Total fat mass (g)	8464 [6812, 11 325]	8162 [6749, 9925]	16 386 [14 147, 19 381]	1.0
Fat mass index (kg/m^4)	2.1 [1.7, 2.7]	2.0 [1.7, 2.4]	3.7 [3.3, 4.2]	1.0
Visceral fat mass (g)	386.7 [306.4, 516.2]	373.2 [294.4, 477.2]	709.1 [540.1, 872.6]	34.7
Liver fat fraction (%)	1.97 [1.69, 2.39]	1.94 [1.68, 2.33]	2.54 [2.01, 3.14]	26.1
Android-to-gynoid fat ratio	0.24 [0.20, 0.29]	0.23 [0.20, 0.27]	0.38 [0.33, 0.44]	1.0
Maternal characteristics				
Pre-pregnancy BMI (kg/m^2)	22.6 [20.7, 24.9]	22.5 [20.7, 24.4]	25.2 [22.1, 30.1]	24.4
Higher educational level (N, %)	500 (59.4)	426 (62.2)	45 (39.1)	5.4

Values are based on the nonimputed dataset and presented as mean (SD) for normally distributed numerical characteristics and as median [25%-75% range] for nonnormally distributed numerical characteristics or as numbers (%) for categorical characteristics.

Abbreviations: BMI, body mass index; wk, week.

[a]Clinical categories based on the International Obesity Task Force age and sex specific BMI cutoffs (28). Children with underweight were excluded.

Table 4. Associations between body composition measurements and monocyte and T cell subsets

	Body mass index[a]		Fat mass index		Android-to-gynoid fat mass ratio	
	% cell change (95% CI)	*P* value	% cell change (95% CI)	*P* value	% cell change (95% CI)	*P* value
Monocyte subsets						
Monocytes	0.66 (−0.54, 1.87)	0.28	2.26 (−1.00, 5.63)	0.18	0.34 (−2.56, 3.32)	0.82
Classical monocytes	0.66 (−0.60, 1.93)	0.31	2.45 (−0.99, 6.00)	0.17	0.26 (−2.79, 3.41)	0.87
Intermediate monocytes	0.48 (−1.19, 2.19)	0.57	0.74 (−3.75, 5.43)	0.75	−1.31 (−5.28, 2.82)	0.53
Nonclassical monocytes	1.33 (−0.62, 3.33)	0.18	0.72 (−4.45, 6.17)	0.79	1.83 (−2.93, 6.83)	0.46
T cell subsets						
TCRγδ$^+$ T cells	0.88 (−0.77, 2.55)	0.30	5.58 (1.10, 10.26)	0.014	4.51 (−0.48, 8.71)	0.03
Vδ2$^+$Vγ9$^+$ T cells	1.14 (−1.17, 3.51)	0.34	**8.41 (1.99, 15.23)**	**0.010**[b]	**8.23 (2.42, 14.37)**	**0.005**[b]
Vδ2$^+$Vγ9$^+$ T naive cells	2.67 (−0.20, 5.62)	0.07	7.94 (0.16, 16.31)	0.046	8.49 (1.35, 16.13)	0.02
Vδ2$^+$Vγ9$^+$ T_{CM} cells	−0.081 (−2.96, 2.88)	0.96	7.50 (−0.42, 16.06)	0.06	5.03 (−2.04, 12.61)	0.17
Vδ2$^+$Vγ9$^+$ T_{EM} cells	−0.63 (−2.62, 1.40)	0.54	2.28 (−3.01, 7.86)	0.41	3.10 (−1.76, 8.20)	0.22
Vδ2$^+$Vγ9$^+$ T_{EMRA} cells	1.36 (−0.53, 3.28)	0.16	4.22 (−0.83, 9.52)	0.10	5.05 (0.40, 9.90)	0.03
CD4$^+$ T cells	0.04 (−0.99, 1.07)	0.94	0.97 (−1.74, 3.76)	0.49	−0.23 (−2.65, 2.25)	0.86
CD4$^+$ T naive cells	−0.58 (−1.82, 0.67)	0.36	−1.19 (−4.43, 2.17)	0.48	−1.84 (−4.77, 1.18)	0.23
CD4$^+$ T_{CM} cells	0.12 (−1.56, 1.84)	0.89	2.68 (−1.85, 7.43)	0.25	1.79 (−2.28, 6.04)	0.39
CD4$^+$ T_{EMRO} cells	0.74 (−1.01, 2.53)	0.41	3.06 (−1.64, 7.97)	0.21	−0.05 (−4.11, 4.18)	0.98
CD4$^+$ T_{EMRA} cells	2.31 (−0.73, 5.45)	0.14	6.49 (−1.69, 15.36)	0.12	2.61 (−4.49, 10.24)	0.48
Th1 cells	1.49 (−0.74, 3.78)	0.19	5.28 (−0.70, 11.63)	0.09	3.31 (−2.03, 8.94)	0.23
Th2 cells	0.62 (−1.10, 2.36)	0.48	3.24 (−1.32, 8.00)	0.17	−1.28 (−5.23, 2.83)	0.54
Th17 cells	0.70 (−1.38, 2.83)	0.51	2.67 (−2.82, 8.47)	0.35	−2.01 (−6.77, 2.99)	0.42
Treg cells	−0.12 (−1.31, 1.09)	0.85	1.02 (−2.13, 4.28)	0.53	0.07 (−2.74, 2.97)	0.96
CD8$^+$ T cells	0.98 (−0.13, 2.10)	0.09	3.75 (0.70, 6.89)	0.02	1.24 (−1.46, 4.02)	0.37
CD8$^+$ T naive cells	0.62 (−0.81, 2.07)	0.40	2.84 (−1.10, 6.94)	0.16	0.32 (−3.19, 3.95)	0.86
CD8$^+$ T_{CM} cells	−0.59 (−1.85, 3.10)	0.63	3.99 (−2.69, 1.12)	0.25	2.38 (−3.56, 8.68)	0.44
CD8$^+$ T_{EMRO} cells	1.79 (−0.13, 3.76)	0.07	**7.35 (2.40, 12.54)**	**0.003**[b]	3.95 (−0.42, 8.52)	0.08
CD8$^+$ T_{EMRA} cells	2.31 (0.22, 4.45)	0.03	5.91 (0.26, 11.89)	0.04	3.15 (−1.90, 8.46)	0.23

The numbers represent the percentage increase or decrease in absolute cell number per microliter of blood (95% CI) per SD increase in body composition measurements. Numbers are based on multivariable linear regressions and adjusted for the following covariates: maternal pre-pregnancy BMI, maternal educational level, child's sex, child's ethnicity, breastfeeding during the first 4 months, food-based diet quality score at 8 y and playing sports.

Abbreviations: BMI, body mass index; $T_{CM,}$ T central memory; TCR; T cell receptor; $T_{EM,}$ T effector memory; $T_{EMRA,}$ T effector memory RA-positive; $T_{EMRO,}$ T effector memory RO-positiw.

[a]Clinical categories based on the International Obesity Task Force age and sex specific BMI cutoffs (28). Children with underweight were excluded.

[b]Statistically significant after correction for multiple testing (4 independent tests), $P < 0.0125$.

Previous Literature and Interpretation

Previous studies on fat mass and CD8$^+$ T cells showed that mice with obesity had higher CD8$^+$ T cells in adipose tissue (9, 37). This increase in CD8$^+$ T cell numbers in adipose tissue and blood has also been observed in adults and adolescents who are overweight or obese (12, 15, 37-39). However, we did not observe associations between BMI and immune cell numbers. We extended previous literature by determining fat mass by DXA and MRI, as these are more accurate in determining fat mass composition and fat mass distribution compared with anthropometric measures (31). Indeed, we did observe associations for fat mass: positive associations were observed for FMI, visceral fat index, and liver fat fraction with total CD8$^+$ and $CD8^+_{TEMRO}$ cell numbers. The observed higher number of CD8$^+$ T_{EM} cells in our study and previous studies could be a direct effect of the increased fat mass. A previous study in adipose tissue of adults showed that CD8$^+$ T cell infiltration, and especially the effector memory subset, might be the first immune cell appearance in adiposity-induced inflammation (9, 38). The effector CD8$^+$ T cells lack CCR7 expression and are able to directly migrate toward infected or inflamed tissues to execute their effector function (40). It is thought that this effector function of the CD8$^+$ T_{EMRO} cells further triggers the inflammatory cascade that is observed in adipose tissue by production of pro-inflammatory cytokines and the subsequent attraction of monocytes (38). Based on mice studies, this initiation and maintenance of inflammation by effector CD8$^+$ T cell is thought to play a role in obesity-related morbidity such as insulin resistance (38, 41, 42). In adults with metabolic syndrome, increased differentiation of

Table 5. Associations between visceral fat mass and liver fat fraction and monocyte and T cell subsets

	Visceral fat index		Liver fat fraction	
	% cell change (95% CI)	*P* value	% cell change (95% CI)	*P* value
Monocyte subsets				
Monocytes	3.54 (−0.07, 7.27)	0.06	0.41 (−2.80, 3.73)	0.81
Classical monocytes	3.63 (−0.17, 7.58)	0.06	0.50 (−2.89, 4.01)	0.78
Intermediate monocytes	3.82 (−1.17, 9.06)	0.14	−2.55 (−6.80, 1.89)	0.26
Nonclassical monocytes	4.24 (−1.63, 10.45)	0.16	0.80 (−4.35, 6.21)	0.77
T cell subsets				
TCRγδ$^+$ T cells	5.31 (0.21, 10.68)	0.04	4.89 (0.35, 9.64)	0.04
Vδ2$^+$Vγ9$^+$ T cells	**10.72 (3.29, 18.69)**	**0.004**[a]	7.51 (1.07, 14.36)	0.02
Vδ2$^+$Vγ9$^+$ T naive cells	11.13 (2.19, 20.86)	0.014	8.42 (0.66, 16.78)	0.03
Vδ2$^+$Vγ9$^+$ T_{CM} cells	10.21 (1.03, 20.22)	0.03	3.16 (−4.46, 11.38)	0.43
Vδ2$^+$Vγ9$^+$ T_{EM} cells	3.64 (−2.38, 10.03)	0.24	1.50 (−3.77, 7.05)	0.59
Vδ2$^+$Vγ9$^+$ T_{EMRA} cells	6.78 (0.89, 13.01)	0.02	3.97 (−1.16, 9.38)	0.13
CD4$^+$ T cells	0.80 (−2.26, 3.96)	0.61	1.73 (−1.07, 4.61)	0.23
CD4$^+$ T naive cells	−0.33 (−4.08, 3.57)	0.87	0.41 (−3.00, 3.93)	0.82
CD4$^+$ T_{CM} cells	0.46 (−4.44, 5.61)	0.86	1.21 (−3.30, 5.94)	0.61
CD4$^+$ T_{EMRO} cells	4.33 (−0.82, 9.74)	0.10	2.55 (−2.04, 7.35)	0.28
CD4$^+$ T_{EMRA} cells	2.30 (−6.26, 11.65)	0.61	0.92 (−6.84, 9.33)	0.82
Th1 cells	6.07 (−0.38, 12.93)	0.07	−0.31 (−5.92, 5.64)	0.92
Th2 cells	0.19 (−4.72, 5.35)	0.94	1.53 (−3.03, 6.31)	0.52
Th17 cells	−0.70 (−6.51, 5.47)	0.82	**−8.88 (−13.75, −3.73)**	**0.001**[a]
Treg cells	2.24 (−1.22, 5.83)	0.21	1.15 (−2.04, 4.45)	0.48
CD8$^+$ T cells	4.42 (0.87, 8.09)	0.014	1.24 (−1.84, 4.41)	0.44
CD8$^+$ T naive cells	3.79 (−0.72, 8.49)	0.10	1.57 (−2.46, −2.46)	0.45
CD8$^+$ T_{CM} cells	5.54 (−1.91, 13.55)	0.15	0.94 (−5.48, 7.79)	0.78
CD8$^+$ T_{EMRO} cells	**8.33 (2.59, 14.38)**	**0.004**[a]	**6.60 (1.63, 11.82)**	**0.009**[a]
CD8$^+$ T_{EMRA} cells	2.59 (−3.45, 9.00)	0.41	3.39 (−2.14, 9.23)	0.24

The numbers represent the percentage increase or decrease in absolute cell number per microliter of blood (95% CI) per SD increase in fat mass measurements. Numbers are based on multivariable linear regressions and adjusted for the following covariates: maternal pre-pregnancy BMI, maternal educational level, child's sex, child's ethnicity, breastfeeding during the first 4 months, food-based diet quality score at 8 y and playing sports.

Abbreviations: T_{CM}, T central memory; TCR; T cell receptor; T_{EM}, T effector memory; T_{EMRA}, T effector memory RA-positive; T_{EMRO}, T effector memory RO-positive; Th, T helper; Treg, T regulatory

[a]Statistically significant after correction for multiple testing (4 independent tests), $P < 0.0125$

CD8$^+$ T cells has been observed (43). Yet, the persistency and dynamics of the observed immune profile over time in relation to adiposity in children and the long-term effects of these immune alterations are topics for future study.

In contrast to previous studies, we did not observe associations between BMI or other fat mass measures and monocytes (9, 13, 14, 16, 38, 44-46). Previous studies showed that monocyte infiltration and macrophage accumulation in adipose tissue occurred after the CD8$^+$ T cell recruitment in adipose tissue (9, 38). Therefore, it could be hypothesized that we here observed an earlier phase of adiposity-related inflammation, as the population of this study comprises school-aged children with only 2.3% children with obesity. However, some studies also observed monocyte increase in children (14, 45, 46). Age could explain this difference, as 2 studies included children up to 16 years old and 1 study children up to 18 years old, whereas our study included 10-year-old children. The relatively low number of children with obesity might also be an explanation as 1 study only observed the higher monocyte number in children with obesity (46).

In addition to the classical αβT cells, we examined γδ$^+$ T cell numbers and the predominant Vδ2$^+$Vγ9$^+$ subset (25). These Vδ2$^+$Vγ9$^+$ T cells have innate-like features and are activated by phosphoantigens through butyrophilin 3A, independent of major histocompatibility complex (MHC) (25). Following activation, Vδ2$^+$Vγ9$^+$ cells contribute to the host's defense against pathogens by the production of IFN-γ. However, increased numbers of γδ$^+$ T cells in adipose tissue have been associated with inflammation and insulin resistance in mice (21). Still, insights into γδ$^+$ T cells, specifically the Vδ2$^+$Vγ9$^+$ subset in relation to adiposity is scarce. A study in 15 adults with obesity showed a lower percentage of Vδ2$^+$Vγ9$^+$ T cells than a nonobese control group (22). We observed in contrast to this previous study higher numbers of Vδ2$^+$Vγ9$^+$ T cells in adipose children of

Table 6. Monocyte and T cell subsets associations in children with overweight and obesity compared with children with normal weight (REF)

	Healthy weight[a] (N = 719)	Overweight and obese[a] (N = 127)	
	% cell change (95% CI)	% cell change (95% CI)	*P* value
Monocyte subsets			
Monocytes	REF	5.20 (–3.16, 14.27)	0.23
Classical monocytes	REF	5.52 (–3.31, 15.15)	0.23
Intermediate monocytes	REF	3.48 (–7.95, 16.20)	0.56
Nonclassical monocytes	REF	5.48 (–7.85, 20.73)	0.44
T cell subsets			
TCRγδ$^+$ T cells	REF	6.83 (–4.43, 19.41)	0.25
Vδ2$^+$Vγ9$^+$ T cells	REF	14.51 (–2.15, 33.99)	0.09
Vδ2$^+$Vγ9$^+$ T naive cells	REF	13.51 (–6.52, 37.69)	0.20
Vδ2$^+$Vγ9$^+$ T_{CM} cells	REF	14.07 (–6.38, 38.97)	0.19
Vδ2$^+$Vγ9$^+$ T_{EM} cells	REF	6.10 (–7.44, 21.62)	0.40
Vδ2$^+$Vγ9$^+$ T_{EMRA} cells	REF	12.36 (–1.20, 27.79)	0.08
CD4$^+$ T cells	REF	–4.11 (–10.53, 2.77)	0.24
CD4$^+$ T naive cells	REF	–7.52 (–15.11, 0.75)	0.07
CD4$^+$ T_{CM} cells	REF	1.49 (–9.63, 13.98)	0.80
CD4$^+$ T_{EMRO} cells	REF	–5.13 (–15.50, 6.52)	0.37
CD4$^+$ T_{EMRA} cells	REF	–3.44 (–21.00, 18.03)	0.73
Th1 cells	REF	0.031 (–14.02, 16.38)	1.00
Th2 cells	REF	0.52 (–10.53, 12.93)	0.93
Th17 cells	REF	3.70 (–9.96, 19.43)	0.61
Treg cells	REF	–3.11 (–10.71, 5.13)	0.45
CD8$^+$ T cells	REF	1.99 (–5.49, 10.08)	0.61
CD8$^+$ T naive cells	REF	–0.76 (–10.23, 9.71)	0.88
CD8$^+$ T_{CM} cells	REF	10.97 (–6.11, 31.16)	0.22
CD8$^+$ T_{EMRO} cells	REF	5.20 (–7.10, 19.13)	0.42
CD8$^+$ T_{EMRA} cells	REF	–1.00 (–13.98, 13.92)	0.89

The numbers represent the percentage increase or decrease in absolute cell number per microliter of blood (95% CI) when comparing children with overweight or obesity to children with normal weight. Numbers are based on multivariable linear regressions and adjusted for the following covariates: maternal pre-pregnancy body mass index (BMI), maternal educational level, child's sex, child's ethnicity, breastfeeding during the first 4 months, food-based diet quality score at 8 years, and playing sports.

Abbreviations: $T_{CM,}$ T central memory; TCR; T cell receptor; $T_{EM,}$ T effector memory; $T_{EMRA,}$ T effector memory RA-positive; $T_{EMRO,}$ T effector memory RO-positive; Th, T helper; Treg, T regulatory.

[a]Clinical categories based on the International Obesity Task Force age and sex specific BMI cutoffs (28). Children with underweight were excluded.

the general population (22). This can possibly be explained by the fact that this previous study studied relative immune cell numbers in contrast to absolute numbers as was used in our study. Yet, this previous study did observe higher numbers of effector memory Vδ2$^+$Vγ9$^+$ T cells (22). The exact role of Vδ2$^+$Vγ9$^+$ T in metabolic diseases remains to be studied (47). Increased differentiation of Vδ2$^+$Vγ9$^+$ T cells has been associated with decreased IFN-γ responses and thereby a reduced host defense against viral antigens (22, 48). This might contribute to the increased susceptibility of overweight individuals to severe or persistent viral infection, for example, with SARS-CoV2 in COVID-19 patients (8).

Overall, we observed the strongest associations for visceral fat index with immune cells and no associations between BMI and immune cells. These observations are similar to previous literature that stresses the importance of determining fat mass distribution rather than body mass composition in defining populations at risk for adverse health effects of high fat mass (12, 49, 50). Namely, it has been shown that adipose tissue is actively involved in various metabolic processes which vary upon the location of the tissue (10, 51). In particular, the viscerally located white adipose tissue has been associated with systemic low-grade inflammation (12, 51). Measures of fat mass distribution that we used in this study were android-to-gynoid fat mass ratio, visceral fat index, and liver fat fraction, all indicators of centrally located fat mass. Previously, higher android-to-gynoid fat mass ratio in children was shown to be a risk for developing metabolic syndrome independent of BMI status (52). Likewise, both visceral and liver fat have been associated with adverse metabolic outcomes in children (53, 54). Therefore, measuring fat mass quantity and distribution might be more specific indicators for low-grade inflammation in adiposity.

Overall, we observed consistent results for $V\delta2^+V\gamma9^+$ T and $CD8^+_{TEMRO}$ across different fat measurements. However, specifically for liver fat, decreased Th17 cells were observed. This might reflect an increased recruitment of Th17 toward liver fat, which has been observed previously in nonalcoholic fatty liver disease (NAFLD) (55, 56). Possibly, excess liver fat mainly causes local inflammation in an early stage, ultimately leading to systemic inflammation (55). In NAFLD, it has been shown that especially Th17 cells cause liver damage and fibrosis progression (57). Further studies are needed because conflicting results about Th17 are present, as there are also studies that have shown increased circulating Th17 in adult patients with NAFLD (56). It is currently unknown what the clinical relevance of our Th17 observation in relation to adiposity could be in children.

The pathophysiology of adiposity-related inflammation likely comprises a combination of factors: initiation of adipocyte cell death caused by hypertrophy, hypoxia due to insufficient vascularization of expanded adipose tissue, and oxidative stress of the adipocytes leading to production of pro-inflammatory adipokines such as leptin (58). It is thought that these chronic inflammatory triggers result in T cell exhaustion and premature immunosenescence (59). However, this is an area that needs further study, especially in children with adiposity. The reduced functionality of exhausted T cells could underlie the increased incidence of infection and cancer observed in patients with obesity (48, 59). Importantly, weight loss following bariatric surgery was observed to reverse the premature immunosenescence in patients with obesity (43). This T cell plasticity following reversibility of adiposity-related inflammation underscores the importance of early detection of adiposity in children with appropriate lifestyle intervention.

Methodological Considerations

This study included a large cohort of school-aged children with comprehensive datasets of body composition measures and immune cell numbers and detailed information on important confounders such as socio-economic status and lifestyle factors. Adiposity was studied in detail by both BMI and fat mass measures determined by DXA and MRI, both accepted as accurate measures for determining body fat distribution (31, 60).

Despite this unique setting, we still encountered several limitations and considerations. First, we did not study fat mass and immune cells over time, which prevented us from drawing conclusions about the direction of the associations or about the effect of fat mass change over time on immune cell numbers. Second, validation is needed of MRI and DXA as fat mass composition and fat distribution measures in large populations of children (60). Although both MRI and DXA are increasingly used and generally accepted in determining fat mass distribution in children, most validation studies have been performed in adults (60). Third, although we used extensive multiparameter flow cytometry, we were unable to include the surface marker CCR7 into the γδT cell subset tube to more accurately distinguish between naive and T_{EMRA} subsets of $V\delta2^+V\gamma9^+$ T cells (61, 62). Fourth, we could not study the adiposity measures in children with obesity specifically because of the limited number of children within this group. Fifth, the variability of fat mass amounts and BMI was limited due to the small number of children with obesity within our cohort. The low variability of BMI is relatively unique and could explain the lack of observations for BMI within our study. Lastly, because this is an observational study, there is a chance of residual confounding for example by unmeasured dietary or lifestyle factors.

Conclusion

Adiposity in school-aged children of a general population was associated with higher $V\delta2^+V\gamma9^+$ and $CD8^+$ T cells, whereas specifically higher liver fat was associated with lower Th17 cell numbers. These results might indicate that fat-associated inflammation is already present at a young age.

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Author Contributions: M.Z. designed and supervised the flow cytometry experiments; C.L., C.G., and M.Z. performed the flow cytometry analyses. T.V. and V.J. designed the fat mass measurements. K.L., H.M., M.Z., and J.K. designed the study. K.L. had full access to the data and performed data analyses. S.S. provided advice regarding the fat mass analyses. K.L., C.L., J.K., M.Z., and H.M. interpreted the data. K.L., H.M., M.Z., and J.K. wrote the manuscript and all authors critically revised and commented on the manuscript. J.K. supervised the study.

Additional Information

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Data Availability: Some or all datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet* 2017; **390**:2627-2642.
2. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. The relation of childhood BMI to adult adiposity: the Bogalusa Heart Study. *Pediatrics*. 2005;**115**(1):22-27.
3. Biro FM, Wien M. Childhood obesity and adult morbidities. *Am J Clin Nutr*. 2010;**91**(5):1499S-1505S.
4. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med*. 1997;**337**(13):869-873.
5. Mensink-Bout SM, Santos S, van Meel ER, et al. General and organ fat assessed by magnetic resonance imaging and respiratory outcomes in childhood. *Am J Respir Crit Care Med*. 2020;**201**(3):348-355.
6. Toubal A, Kiaf B, Beaudoin L, et al. Mucosal-associated invariant T cells promote inflammation and intestinal dysbiosis leading to metabolic dysfunction during obesity. *Nat Commun*. 2020;**11**(1):3755.
7. Maccioni L, Weber S, Elgizouli M, et al. Obesity and risk of respiratory tract infections: results of an infection-diary based cohort study. *BMC Public Health*. 2018;**18**(1):271.
8. de Frel DL, Atsma DE, Pijl H, et al. The impact of obesity and lifestyle on the immune system and susceptibility to infections such as COVID-19. *Frontiers Nutrition* Published November 19, 2020;**7**:597600. doi:10.3389/fnut.2020.597600
9. Yuan Y, Li H, Liao Y, Feng C. CD8+ T cells are involved in early inflammation before macrophages in a rat adipose tissue engineering chamber model. *J Tissue Eng Regen Med*. 2019;**13**(9):1499-1506.
10. Grant RW, Dixit VD. Adipose tissue as an immunological organ. *Obesity (Silver Spring)*. 2015;**23**(3):512-518.
11. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;**444**(7121):860-867.
12. McLaughlin T, Liu LF, Lamendola C, et al. T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. *Arterioscler Thromb Vasc Biol*. 2014;**34**(12):2637-2643.
13. Poitou C, Dalmas E, Renovato M, et al. CD14dimCD16+ and CD14+CD16+ monocytes in obesity and during weight loss: relationships with fat mass and subclinical atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2011;**31**(10):2322-2330.
14. Schipper HS, Nuboer R, Prop S, et al. Systemic inflammation in childhood obesity: circulating inflammatory mediators and activated CD14++ monocytes. *Diabetologia*. 2012;**55**(10):2800-2810.
15. Spielmann G, Johnston CA, O'Connor DP, Foreyt JP, Simpson RJ. Excess body mass is associated with T cell differentiation indicative of immune ageing in children. *Clin Exp Immunol*. 2014;**176**(2):246-254.
16. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;**112**(12):1796-1808.
17. Devêvre EF, Renovato-Martins M, Clément K, Sautès-Fridman C, Cremer I, Poitou C. Profiling of the three circulating monocyte subpopulations in human obesity. *J Immunol*. 2015;**194**(8):3917-3923.
18. Wong KL, Tai JJ, Wong WC, et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood*. 2011;**118**(5):e16-e31.
19. McLaughlin T, Ackerman SE, Shen L, Engleman E. Role of innate and adaptive immunity in obesity-associated metabolic disease. *J Clin Invest*. 2017;**127**(1):5-13.
20. Chehimi M, Vidal H, Eljaafari A. Pathogenic role of IL-17-producing immune cells in obesity, and related inflammatory diseases. *J Clin Med*. Published July 14, 2017;**6**(7):68. doi:10.3390/jcm6070068
21. Mehta P, Nuotio-Antar AM, Smith CW. γδ T cells promote inflammation and insulin resistance during high fat diet-induced obesity in mice. *J Leukoc Biol*. 2015;**97**(1):121-134.
22. Costanzo AE, Taylor KR, Dutt S, Han PP, Fujioka K, Jameson JM. Obesity impairs γδ T cell homeostasis and antiviral function in humans. *Plos One*. 2015;**10**(3):e0120918.
23. Hoeres T, Smetak M, Pretscher D, Wilhelm M. Improving the efficiency of Vγ9Vδ2 T-cell immunotherapy in cancer. *Front Immunol*. 2018;**9**:800.
24. van den Heuvel D, Jansen MAE, Nasserinejad K, et al. Effects of nongenetic factors on immune cell dynamics in early childhood: the Generation R Study. *J Allergy Clin Immunol*. 2017; **139**:1923-1934.e1917.
25. Davey MS, Willcox CR, Hunter S, et al. The human Vδ2+ T-cell compartment comprises distinct innate-like Vγ9+ and adaptive Vγ9- subsets. *Nat Commun*. 2018;**9**(1):1760.
26. Kooijman MN, Kruithof CJ, van Duijn CM, et al. The Generation R Study: design and cohort update 2017. *Eur J Epidemiol*. 2016; **31**:1243-1264.
27. Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP. Body index measurements in 1996-7 compared with 1980. *Arch Dis Child*. 2000;**82**(2):107-112.
28. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes*. 2012;**7**(4):284-294.
29. Gishti O, Gaillard R, Manniesing R, et al. Fetal and infant growth patterns associated with total and abdominal fat distribution in school-age children. *J Clin Endocrinol Metab*. 2014;**99**(7):2557-2566.
30. Helba M, Binkovitz LA. Pediatric body composition analysis with dual-energy x-ray absorptiometry. *Pediatr Radiol*. 2009;**39**(7):647-656.

31. Santos S, Monnereau C, Felix JF, Duijts L, Gaillard R, Jaddoe VWV. Maternal body mass index, gestational weight gain, and childhood abdominal, pericardial, and liver fat assessed by magnetic resonance imaging. *Int J Obes (Lond)*. 2019;**43**(3):581-593.
32. Kalina T, Flores-Montero J, van der Velden VH, et al.; EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia*. 2012;**26**(9):1986-2010.
33. Looman KIM, van Meel ER, Grosserichter-Wagener C, et al. Associations of Th2, Th17, Treg cells, and IgA+ memory B cells with atopic disease in children: The Generation R Study. *Allergy*. 2020;**75**(1):178-187.
34. Dieli F, Poccia F, Lipp M, et al. Differentiation of effector/memory Vdelta2 T cells and migratory routes in lymph nodes or inflammatory sites. *J Exp Med*. 2003;**198**(3):391-397.
35. Voortman T, van den Hooven EH, Heijboer AC, Hofman A, Jaddoe VW, Franco OH. Vitamin D deficiency in school-age children is associated with sociodemographic and lifestyle factors. *J Nutr*. 2015;**145**(4):791-798.
36. van der Velde LA, Nguyen AN, Schoufour JD, et al. Diet quality in childhood: the Generation R Study. *Eur J Nutr*. 2019;**58**(3):1259-1269.
37. Wang L, Sun P, Wu Y, Wang L. Metabolic tissue-resident CD8(+) T cells: a key player in obesity-related diseases. *Obes Rev*. 2021;22(3):e13133. doi:10.1111/obr.13133
38. Nishimura S, Manabe I, Nagasaki M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med*. 2009;**15**(8):914-920.
39. Liu R, Nikolajczyk BS. Tissue immune cells fuel obesity-associated inflammation in adipose tissue and beyond. *Front Immunol*. 2019;**10**:1587.
40. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;**401**(6754) :708-712.
41. Winer S, Winer DA. The adaptive immune system as a fundamental regulator of adipose tissue inflammation and insulin resistance. *Immunol Cell Biol*. 2012;**90**(8):755-762.
42. Lumeng CN, Maillard I, Saltiel AR. T-ing up inflammation in fat. *Nat Med*. 2009;**15**(8):846-847.
43. Jongbloed F, Meijers RWJ, IJzermans JNM, et al. Effects of bariatric surgery on telomere length and T-cell aging. *Int J Obes (Lond)*. 2019;**43**(11):2189-2199.
44. Ying W, Fu W, Lee YS, Olefsky JM. The role of macrophages in obesity-associated islet inflammation and β-cell abnormalities. *Nat Rev Endocrinol*. 2020;**16**(2):81-90.
45. Mattos RT, Medeiros NI, Menezes CA, et al. Chronic low-grade inflammation in childhood obesity is associated with decreased IL-10 expression by monocyte subsets. *Plos One*. 2016;**11**(12):e0168610.
46. Breslin WL, Johnston CA, Strohacker K, et al. Obese Mexican American children have elevated MCP-1, TNF-α, monocyte concentration, and dyslipidemia. *Pediatrics*. 2012;**129**(5):e1180-e1186.
47. van der Woude H, Krebs J, Filoche S, Gasser O. Innate-like T cells in the context of metabolic disease and novel therapeutic targets. *Immunometabolism* 2020; **2**:e200031.
48. Kallemeijn MJ, Boots AMH, van der Klift MY, et al. Ageing and latent CMV infection impact on maturation, differentiation and exhaustion profiles of T-cell receptor gammadelta T-cells. *Sci Rep*. 2017;**7**(1):5509.
49. Ardesch FH, Ruiter R, Mulder M, Lahousse L, Stricker BHC, Kiefte-de Jong JC. The obesity paradox in lung cancer: associations with body size versus body shape. *Frontiers Oncol*. Published online November 10, 2020;**10**:591110. doi:10.3389/fonc.2020.591110
50. Piché ME, Poirier P, Lemieux I, Després JP. Overview of epidemiology and contribution of obesity and body fat distribution to cardiovascular disease: an update. *Prog Cardiovasc Dis*. 2018;**61**(2):103-113.
51. Zoico E, Rubele S, De Caro A, et al. Brown and beige adipose tissue and aging. *Front Endocrinol (Lausanne)*. 2019;**10**:368.
52. Samsell L, Regier M, Walton C, Cottrell L. Importance of android/gynoid fat ratio in predicting metabolic and cardiovascular disease risk in normal weight as well as overweight and obese children. *J Obes*. 2014;**2014**:846578.
53. Geurtsen ML, Santos S, Felix JF, et al. Liver Fat and Cardiometabolic Risk Factors Among School-Age Children. *Hepatology*. 2020;**72**(1):119-129.
54. Yan Y, Liu J, Zhao X, Cheng H, Huang G, Mi J; China Child and Adolescent Cardiovascular Health study (CCACH) research group. Abdominal visceral and subcutaneous adipose tissues in association with cardiometabolic risk in children and adolescents: the China Child and Adolescent Cardiovascular Health (CCACH) study. *BMJ Open Diabetes Res Care*. 2019;**7**(1):e000824.
55. Chackelevicius CM, Gambaro SE, Tiribelli C, Rosso N. Th17 involvement in nonalcoholic fatty liver disease progression to non-alcoholic steatohepatitis. *World J Gastroenterol*. 2016;**22**(41):9096-9103.
56. Rau M, Schilling AK, Meertens J, et al. Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. *J Immunol*. 2016;**196**(1):97-105.
57. Van Herck MA, Weyler J, Kwanten WJ, et al. The differential roles of T cells in non-alcoholic fatty liver disease and obesity. *Front Immunol*. 2019;**10**:82.
58. Fuster JJ, Ouchi N, Gokce N, Walsh K. Obesity-induced changes in adipose tissue microenvironment and their impact on cardiovascular disease. *Circ Res*. 2016;**118**(11):1786-1807.
59. Kado T, Nawaz A, Takikawa A, Usui I, Tobe K. Linkage of CD8+ T cell exhaustion with high-fat diet-induced tumourigenesis. *Sci Rep*. 2019;**9**(1):12284.
60. Simoni P, Guglielmi R, Aparisi Gómez MP. Imaging of body composition in children. *Quant Imaging Med Surg*. 2020;**10**(8):1661-1671.
61. Bromley SK, Thomas SY, Luster AD. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol*. 2005;**6**(9):895-901.
62. Rufer N, Zippelius A, Batard P, et al. Ex vivo characterization of human CD8+ T subsets with distinct replicative history and partial effector functions. *Blood*. 2003;**102**(5):1779-1787.