Pediatric Diabetes

ISPAD International Society for Pediatric and Adolescent Diabetes

Pediatric Diabetes 2015
doi: 10.1111/pedi.12292

Original Article

Cardiometabolic risk: leg fat is protective during childhood

Samouda H, De Beaufort C, Stranges S, Hirsch M, Van Nieuwenhuyse J-P, Dooms G, Gilson G, Keunen O, Leite S, Vaillant M, Lair M-L, Dadoun F. Cardiometabolic risk: leg fat is protective during childhood. Pediatric Diabetes 2015.

Background: Childhood obesity is associated with early cardiometabolic risk (CMR), increased risk of adulthood obesity, and worse health outcomes. Leg fat mass (LFM) is protective beyond total fat mass (TFM) in adults. However, the limited evidence in children remains controversial.
Objective: We investigated the relationship between LFM and CMR factors in youth.
Subjects: A total of 203 overweight/obese children, 7–17-yr-old, followed in the Pediatric Clinic, Luxembourg.
Methods: TFM and LFM by dual energy x-ray absorptiometry and a detailed set of CMR markers were analyzed.
Results: After TFM, age, sex, body mass index (BMI) Z-score, sexual maturity status, and physical activity adjustments, negative significant partial correlations were shown between LFM and homeostasis model assessment of insulin resistance (HOMA) (variance explained: 6.05% by LFM*; 7.18% by TFM**), fasting insulin (variance explained: 5.71% by LFM*; 6.97% by TFM**), triglycerides (variance explained: 3.96% by LFM*; 2.76% by TFM*), systolic blood pressure (variance explained: 2.68% by LFM*; 4.33% by TFM*), C-reactive protein (variance explained: 2.31% by LFM*; 4.28% by TFM*), and resistin (variance explained: 2.16% by LFM*; 3.57% by TFM*). Significant positive partial correlations were observed between LFM and high-density lipoprotein (HDL) cholesterol (variance explained: 4.16% by LFM*) and adiponectin (variance explained: 3.09% by LFM*) (*p-value < 0.05 and **p-value < 0.001). In order to adjust for multiple testing, Benjamini–Hochberg method was applied and the adjusted significance level was determined for each analysis. LFM remained significant in the aforementioned models predicting HOMA, fasting insulin, triglycerides, and HDL cholesterol (Benjamini and Hochberg corrected p-value < 0.01).
Conclusions: LFM is protective against CMR in children, at least in terms of insulin resistance and adverse blood lipid profiles.

Hanen Samouda[a], Carine De Beaufort[b], Saverio Stranges[a], Marco Hirsch[c], Jean-Paul Van Nieuwenhuyse[d], Georges Dooms[d], Georges Gilson[e], Olivier Keunen[f], Sonia Leite[g], Michel Vaillant[h], Marie-Lise Lair[h,a] and Frédéric Dadoun[i,a]

[a]Population Health Department, Center for Health Studies, Luxembourg Institute of Health, Strassen, Luxembourg; [b]Diabetes & Endocrinology Care Clinique Pédiatrique (DECCP), Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg; [c]Rheumatology Department, ZithaKlinik, Luxembourg, Luxembourg; [d]Radiology Department, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg; [e]Department of Clinical Biology, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg; [f]Norlux Neuro-Oncology Laboratory, Luxembourg Institute of Health, Strassen, Luxembourg; [g]Centre of Competence for Methodology and Statistics (CCMS), Luxembourg Institute of Health, Strassen, Luxembourg; [h]Santé et Prospectives, Sanem, Luxembourg; and [i]Endocrinology and Diabetology Department, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg

Key words: cardiometabolic risk – DXA – fat mass – leg fat – visceral fat

Corresponding author: Hanen Samouda, PhD,
Population Health Department,
Luxembourg Institute of Health,
1A-B, rue Thomas Edison,
L1445 Strassen,
Luxembourg.
Tel: +352 26 970 745;

fax: +352 26 970 717;
e-mail: hanene.samouda@lih.lu

Submitted 30 December 2014.
Accepted for publication 26 May 2015

A total of 250 million children are overweight or obese in the world (1). This is a major public health concern because child overweight/obesity, specifically trunk, abdominal, and visceral excess adipose tissues, associate with early metabolic and cardiovascular (CV) risk factors and diseases in young people (2–7). Pediatric obesity and cardiometabolic risk (CMR) are associated with an increased risk for adulthood obesity and related health outcomes as well (8–11). In adults, leg fat seems to be protective beyond total fat mass (TFM) and trunk fat as assessed by dual energy x-ray absorptiometry (DXA) (12). Indeed, some studies have found leg fat to be protective beyond TFM and trunk fat against impaired glucose and lipid metabolism, insulin action, arterial pressure regulation, a less favorable inflammatory profile, and even vascular damages and coronary artery risk and diseases (13–20). Actually, gluteofemoral adipose tissue seems to have a specific entrapment, storage, and low rate release of fatty acids in adults, protecting from excessive ectopic fat exposure and accumulation, in particular at the level of visceral organs and muscle (21). Furthermore, a favorable leptin and adiponectin profile is associated with the lower body fat depots in adults independent of total adiposity (19). In children, however, only scarce body composition studies have been performed in order to analyze the relationship between CMR factors and lower limbs fat as assessed by DXA. Three teams have reported on the DXA trunk vs. lower limbs fat distribution effect by means of the android to gynoid fat ratios [(L1–L4 fat mass area/lower limbs fat)] (22), (fat mass area from the ilium to the mandible/lower body fat from the ilium) (23), and/or of the [(subscapular + waist)/(hip + thigh) fat mass] body fat distribution index (24). A fourth study assessed the relationship between the CMR factors and the (DXA-leg fat/total body fat) percentage (25). However, these studies produced inconsistent results with regard to the relationship between the aforementioned adiposity indices and the CMR factors. Furthermore, android to gynoid body fat ratios describe general body fat distribution and therefore cannot differentiate between trunk and leg fat effects. Finally, except for the study conducted by Teixeira et al. (26), none of these studies have assessed the protective role of leg fat beyond overall adiposity in childhood, and/or beyond visceral adipose tissue (VAT), also recognized as an independent metabolic and CV risk factor (6). Teixeira et al. (26) attempted to define the role of leg fat independent of general adiposity in children, but the models developed by the authors were likely invalid because of a high multicollinearity observed between total and regional body fat measurements. Previous studies are also limited to American, Indian, or French populations, and conclusions may not be extrapolated to other populations (20, 22–26).

In this study, we aim to analyze the relationship between DXA leg fat measurements and CMR factors in Luxembourg children and adolescents, independent of overweight, obesity, and visceral adiposity.

Methods

Subjects

A total of 203 overweight and obese Caucasian children, 7–17 yr old and frequenting the *Diabetes & Endocrinology Care Pediatric Clinic* (Centre Hospitalier de Luxembourg), agreed to participate, with personal and parental informed consent, between September 2006 and June 2008. Exclusion criteria were diseases able to affect body composition such as Prader Willi syndrome, hypoparathyroidism, leptin deficiency, and/or Laurence Moon Biedl syndrome. The study was approved by the CNER (National Ethics Committee of Research) and authorized by the National Commission for Data Protection. The study was performed according to the Declaration of Helsinki.

DXA measurements

Conventionally predefined TFM and leg fat mass (LFM) were assessed by DXA, using a Hologic® QDR4500W densitometer (Hologic Inc., Waltham, MA, USA). Leg fat area was delineated by three standard DXA cut lines: the angled line bisecting the femoral neck below the pelvis, the vertical line lateral to the leg and going on until the heel (until the floor), and a second vertical line linking the pubic symphysis to the toes (to the floor) (27).

Magnetic resonance imaging analysis

VAT was assessed by magnetic resonance imaging (MRI) using a 1.5-T magnet (GE Signa HDXT system, GE Medical Systems, Milwaukee, WI, USA). Eight L4–L5-level centered and contiguous images,

slice thickness = 10 mm, were performed by the two-dimensional T1-weighted gradient echo pulse sequence (repetition time = 120 ms, echo time = 4 ms, flip angle = 90°, number of excitations = 1, field of view = 48 cm, time of acquisition = 13 s, matrix = 512 × 224, eight channels phased array body coil). A semi-quantitative method developed in ImageJ (U. S. National Institutes of Health, Bethesda, MD, USA) (28), shown to provide good intra- and inter-raters reproducibility (29), was used to calculate the VAT area. After loading the L4–L5-MRI-axial-section into ImageJ, a macro was executed to (i) automatically delineate the total body section using binary conversion and holes filling, (ii) define total adipose tissue by rater-defined threshold, (iii) delineate manually the visceral and non-subcutaneous adipose tissue sections using adjustable predefined masks, and (iv) automatically quantify the VAT area. Only abdominal MRI measures were used for the analysis. Leg fat measures by MRI were not available for comparison.

Anthropometric and clinical measurements

Height, weight, and waist circumference (measured midway between the lower rib and the iliac crest) were assessed by one trained examiner, according to the Lohmann recommendations (30). Body mass index (BMI) Z-scores were calculated using the Dutch L, M, and S values (31) (National LMS data are unavailable) and the free LMS Growth software and method developed by Tim Cole (32). Established overweight and obesity thresholds correspond to the 91th (91.496) and 99th (99.350) percentiles in boys, respectively, to the 89th (89.083) and 98th (98.644) percentiles in girls (31, 32). Systolic (SBP) and diastolic (DBP) blood pressures were measured in the sitting position with an aneroid sphygmomanometer (Welch AL, Milwaukie, OR, USA). The average of three readings was used. BP Z-score was determined (33). The Tanner stages were used to assess the sexual maturity status. We also investigated the weekly number of hours which every participant spends practicing physical activities.

Biological assessment

Fasting glucose, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured with Roche reagents on a P module of Roche Modular (Basel, Switzerland). For the determination of C-reactive protein (CRP), an Olympus latex reagent was used on the same P module of a Roche Modular. Fasting insulin was measured by chemiluminescent assay on Siemens Immulite 2000 (Deerfield, IL, USA), whereas fibrinogen was determined on Stago Compact (Asnières sur Seine, France). Leptin, adiponectin, and resistin were measured with enzyme-linked immunosorbent assay kits provided by Mediagnost (Reutlingen, Germany). Homeostasis model assessment of insulin resistance [HOMA IR = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5] was calculated. All blood samples were taken after 12 h fasting.

Statistical analyses

Statistical analyses were performed using SPSS® for Windows (17.0). Normal distribution of data was checked using the Kolmogorov–Smirnov test and confirmed by a visual inspection of the frequency histograms and Q–Q plots. Skewed variables (i.e., triglycerides, HDL cholesterol, fasting insulin, HOMA IR, CRP, fibrinogen, adiponectin, leptin, and resistin) were log transformed. Descriptive data were provided as mean ± SD and/or percentages. Student's *t* and chi-squared tests were used to compare sex differences in means and percentages. To assess the distinctive LFM, TFM, and VAT aptitudes to predict CMR factors, univariate regressions were performed between LFM, TFM, and/or VAT, and each laboratory biological measurement. Partial correlations (r partial) were assessed. To determine the potential protective effect of LFM beyond TFM, we performed multivariable linear regressions between fasting glucose, fasting insulin, HOMA IR, triglycerides, HDL cholesterol, LDL cholesterol, CRP, adiponectin, leptin, resistin, SBP, DBP, and fibrinogen as dependent and LFM and TFM as independent variables. Comparative models were applied to assess the relationship between VAT and the aforementioned biological parameters, as well as the additional effect of LFM to both VAT and TFM. Each univariate and/or multivariable model was age-, sex-, sexual maturity status-, BMI Z-score-, and physical activity-adjusted. Results with a p-value < 0.05 were initially considered statistically significant. In order to evaluate the effect of multiple testing, the Benjamini–Hochberg adjusted p-values were calculated, and the adjusted significance level was determined for each set of analyses (34). The tolerance of each variable was tested in order to assess inter-variables multicollinearity and allow the inclusion of the variables in the models. Tolerance is the proportion of the variance of a variable in the multivariable models that is not accounted for by other independent variables. A minimal tolerance of 0.1 was set for the variables to be included in the models, as recommended by SPSS® for Windows Version 17.0.

Results

General characteristics are detailed in Table 1.

Table 1. Participants characteristics

	Girls Mean ± SD	Boys Mean ± SD	All children Mean ± SD
Age (yr)	12.2 ± 2.5	11.8 ± 2.4	12.0 ± 2.4
BMI (kg/m^2)	28.5 ± 5.6	28.2 ± 4.9	28.3 ± 5.3
BMI Z-score	2.42 ± 0.58	2.68 ± 0.53*	2.54 ± 0.57
Waist circumference (cm)	83.8 ± 12.4	86.5 ± 11.5	85.1 ± 12.0
TFM (kg)	32.5 ± 14.3	30.1 ± 10.9	31.4 ± 12.8
LFM (kg)	12.7 ± 6.2	11.2 ± 4.2	12.0 ± 5.4
VAT (cm^2)	35.0 ± 18.0	40.2 ± 19.1	37.4 ± 18.7
Fasting glucose (mg/dL)	86.2 ± 6.8	86.9 ± 6.2	86.5 ± 6.5
Fasting insulin (mUI/L)	17.5 ± 8.5	14.8 ± 8.3*	16.2 ± 8.5
HOMA IR	3.76 ± 1.98	3.21 ± 1.87*	3.50 ± 1.94
Triglycerides (mg/dL)	98.4 ± 58.4	90.0 ± 51.1	94.3 ± 55.1
HDL cholesterol (mg/dL)	54.4 ± 12.7	52.9 ± 12.1	53.7 ± 12.4
cLDL cholesterol (mg/dL)	92.3 ± 29.0	93.0 ± 28.2	92.6 ± 28.6
SBP (mmHg)	117 ± 12	118 ± 14	117 ± 13
SBP Z-score	0.99 ± 1.04	0.91 ± 1.10	0.95 ± 1.07
DBP (mmHg)	71 ± 9	72 ± 8	72 ± 9
DBP Z-score	0.75 ± 0.78	0.81 ± 0.64	0.78 ± 0.71
CRP (mg/L)	2.9 ± 4.1	3.2 ± 3.8	3.1 ± 4.0
Fibrinogen (g/L)	3.7 ± 0.7	3.6 ± 0.6	3.6 ± 0.7
Adiponectin (μg/mL)	8.0 ± 4.7	7.8 ± 4.5	7.9 ± 4.6
Leptin (ng/mL)	38.7 ± 23.1	27.4 ± 16.1**	33.3 ± 20.8
Resistin (ng/mL)	5.3 ± 2.2	5.1 ± 2.0	5.2 ± 2.1
	Percentages	Percentages	Percentages
Overweight	36.8	35.1	36.0
Obese	63.2	64.9	64.0

BMI, body mass index; cLDL, low-density lipoprotein cholesterol; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; LFM, leg fat mass; SBP, systolic blood pressure; SD, standard deviation; TFM, total fat mass; VAT, visceral adipose tissue. *p-value < 0.05 and **p-value < 0.001.

Prediction of CMR factors using leg fat only

After age, sex, BMI Z-score, sexual maturity status, and physical activity adjustments, and considering the Benjamini–Hochberg adjusted p-values, significant positive partial correlations were observed between LFM and leptin (p-value < 0.004), VAT and triglycerides, as well as VAT and CRP (p-value < 0.04), TFM and leptin (p-value < 0.004). Significant negative partial correlations were observed between VAT and HDL cholesterol as well as between VAT and adiponectin (p-value < 0.04) (Table 2).

Prediction of CMR factors using models associating LFM with TFM and/or with VAT

After combination of LFM and TFM, and adjustments by age, sex, BMI Z-score, sexual maturity status, and physical activity, significant negative partial correlations were noticed in the multivariable models between LFM and fasting insulin, HOMA, triglycerides, CRP, resistin concentrations, and SBP Z-score. Variances (V) explained by LFM ranged between 2.16 and 6.05% in these cases: resistin (V = 2.16%), CRP (V = 2.31%), SBP Z-score (V = 2.68%), triglycerides (V = 3.96%), fasting insulin (V = 5.71%), and HOMA (V = 6.05%) (p-values < 0.05).

When significant positive partial correlations were observed by LFM in the multivariable models, variance explained by LFM was 3.09% for adiponectin and 4.16% for HDL cholesterol concentrations (p-values < 0.05). Within this framework, in the same multivariable models, partial correlations linking up TFM to the aforementioned biological variables were significantly positive for fasting insulin, HOMA, triglycerides, SBP Z-score, CRP, and resistin but not significant in relation to HDL cholesterol and adiponectin. TFM partially explained variance was of the order of 7.18% to predict HOMA IR (p-value < 0.001), 6.97% to predict fasting insulin (p-value < 0.001), 4.33% for SBP Z-score (p-value < 0.05), 4.28% for CRP (p-value < 0.05), 3.57% for resistin (p-value < 0.05), and 2.76% for triglycerides (p-value < 0.05) prediction.

The global explained variances of the models ranged from 50.5 (fasting insulin) to 10.9% (adiponectin) (Table 3). After adjusting for the Benjamini–Hochberg's multiple testing method, LFM remained significant in the models predicting fasting insulin, HOMA, triglycerides, and HDL cholesterol

Table 2. Pearson's correlation coefficients (R) between leg fat mass, respectively visceral fat, total fat mass, and cardiometabolic risk factors (age, sex, BMI Z-score, sexual maturity status, and physical activity adjustments)

	LFM		VAT		TFM	
	R^2 model	r partial LFM	R^2 model	r partial VAT	R^2 model	r partial TFM
Fasting glucose	0.054	−0.057	0.059	−0.081	0.051	−0.003
Fasting insulin†	0.468**‡	0.003	0.459**‡	0.080	0.475**§	0.116
HOMA IR†	0.460**‡	−0.005	0.449**‡	0.059	0.467**§	0.110
Triglycerides†	0.148**‡	−0.118	0.174**‡	0.233*¶	0.137**§	−0.036
HDL cholesterol†	0.125**‡	0.193*	0.109*‡	−0.228*¶	0.104*§	0.117
LDL cholesterol	0.021	−0.116	0.011	0.017	0.017	−0.094
SBP Z-score	0.207**‡	0.060	0.221**‡	0.120	0.220**§	0.142
DBP Z-score	0.166**‡	−0.034	0.170**‡	0.177*	0.165**§	0.017
CRP†	0.151**‡	0.086	0.236**‡	0.266**§	0.169**§	0.166*
Fibrinogen†	0.160**‡	0.172*	0.176**‡	0.042	0.157**§	0.162*
Adiponectin†	0.098*‡	0.175*	0.153**‡	−0.240*¶	0.081*	0.111
Leptin†	0.612**‡	0.392**§	0.569**‡	−0.071	0.614**§	0.398**§
Resistin†	0.087*‡	0.057	0.086*‡	0.055	0.100*	0.132

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; LFM, leg fat mass; SBP, systolic blood pressure; TFM, total fat mass; VAT, visceral adipose tissue.
*p-value < 0.05 and **p-value < 0.001.
†Log-transformed variables.
‡Benjamini and Hochberg corrected p-value < 0.01.
§Benjamini and Hochberg corrected p-value < 0.04.
¶Benjamini and Hochberg corrected p-value < 0.004.

(Benjamini and Hochberg corrected p-value < 0.01). TFM remained significant in the models predicting fasting insulin, HOMA, SBP Z-score, CRP, and resistin (Benjamini and Hochberg corrected p-value < 0.02) (Table 3). No lower tolerance than 0.1 was observed in the models 1 and 2 combining LFM and TFM, excluding the collinearity between the variables (Table 3).

When associated at the same time with VAT and TFM (after age, sex, BMI Z-score, sexual maturity status, and physical activity adjustments) and according to the Benjamini–Hochberg adjustment method, LFM was significantly correlated with fasting insulin and HOMA (negative r partial) ($p < 0.007$). On the other hand, VAT did not show any significant contribution in the tripartite model associating LFM, TFM, and VAT. Finally, a lower tolerance than 0.1 was observed in this third model, suggesting a certain multicollinearity between VAT, LFM, and TFM (Table 4).

Discussion

This study suggests a protective effect of LFM, beyond overall adiposity and from childhood onwards, against potential CMR development because of overweight and obesity. Indeed, LFM as assessed by DXA showed statistically significant and inverse relationships with fasting insulin, HOMA IR, triglycerides, SBP, CRP, and resistin; significant positive relationship with HDL cholesterol and adiponectin in 7- to 17-yr-old overweight and obese Luxembourg youths; and a significant protective relationship against insulin resistance and adverse blood lipid profiles, even after adjusting the significance levels according to the Benjamini–Hochberg method.

Moreover, variances explained by LFM were rather substantial in the multivariable models and could reach 6.05% in some cases, for instance to predict HOMA IR, with 7.18% variance explained by TFM. We also noticed that variances explained by both TFM and LFM were rather close in the multivariable models, suggesting in case of high values of TFM that LFM may be the key player protecting against CMR.

To our knowledge, none of the children-related studies have shown such associations, except for Staiano et al. (25) who observed an inverse association between leg fat and triglycerides concentrations only, after TFM adjustment. The authors however examined the associations with leg fat percentages (leg fat divided by whole body fat), whereas we investigated the relationships between the absolute values of LFM and CMR factors (after TFM adjustment in both cases). This may be the reason why Staiano et al. (25) did not observe significant relationships between leg fat and the other CMR factors tested: blood pressure, fasting triglycerides, HDL cholesterol, glucose, insulin, and CRP. On the other hand, we observed similar relationships to those highlighted in the adult studies. Beyond the implication of TFM, large LFMs were linked up to low triglycerides, LDL

Table 3. Cardiometabolic risk prediction using models combining leg and total fat masses (age, sex, BMI Z-score, sexual maturity status, and physical activity adjustments)

	Model 1: LFM, TFM			Model 2: VAT, TFM		
	R^2 Model 1	r partial LFM	r partial TFM	R^2 Model 2	r partial VAT	r partial TFM
Fasting glucose	0.067	−0.127	0.114	0.056	−0.074	0.005
Fasting insulin†	0.505**‡	−0.239*§	0.264**¶	0.462**‡	0.078	0.095
HOMA IR†	0.499**‡	−0.246*§	0.268**¶	0.452**‡	0.059	0.091
Triglycerides†	0.171**‡	−0.199*§	0.166*	0.164**‡	0.217*§	−0.047
HDL cholesterol†	0.141**‡	0.204*§	−0.136	0.121*‡	−0.224*§	0.117
LDL cholesterol	0.022	−0.073	0.025	0.014	0.018	−0.077
SBP Z-score	0.241**‡	−0.164*	0.208*¶	0.228**‡	0.112	0.146
DBP Z-score	0.177**‡	−0.117	0.113	0.164**‡	0.167*	0.021
CRP†	0.188**‡	−0.152*	0.207*¶	0.278**‡	0.267**§	0.225*§
Fibrinogen†	0.160**‡	0.059	0.016	0.199**‡	0.029	0.195*
Adiponectin†	0.109*‡	0.176*	−0.113	0.146*‡	0.243*§	0.091
Leptin†	0.617**‡	0.083	0.110	0.632**‡	−0.103	0.378**§
Resistin†	0.119*‡	−0.147*	0.189*¶	0.097*‡	0.040	0.150*§
Tolerance		0.060	0.077			

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; LFM, leg fat mass; SBP, systolic blood pressure; TFM, total fat mass; VAT, visceral adipose tissue.
*p-value < 0.05 and **p-value < 0.001.
†Log-transformed variables.
‡Benjamini and Hochberg corrected p-value < 0.04.
§Benjamini and Hochberg corrected p-value < 0.01.
¶Benjamini and Hochberg corrected p-value < 0.02.

cholesterol, and fasting insulin levels, as well as to high HDL cholesterol values in healthy European men and women (14). LFM was also inversely associated with blood pressure, plasma lipids, and glucose in severely obese premenopausal women (13), with insulin resistance and dyslipidaemia in postmenopausal older women (16), with metabolic syndrome and inflammatory markers (CRP, interleukin 6, tumor necrosis factor alpha, and plasminogen activator inhibitor-1) in obese older adults (17), and with fasting and 2-h post-load glucose levels in old men and women (18).

A positive relationship between high adiponectin levels and LFM, insulin sensitivity and better glycaemic, lipid, and inflammatory profiles has also been shown in adults, but not yet in youth. Recent pediatric studies show that adiponectin DNA methylation is reduced by the combined presence of obesity and insulin resistance (19, 35). This certainly highlights the potentially very important role of high LFM levels in the adiponectin expression and also in the regulation of the adiponectin-associated complications in childhood overweight/obesity.

In our study, no significant associations were observed with LFM after adjusting by both VAT and TFM, except for fasting insulin and HOMA IR. This is probably because of the multicollinearity observed between the three fat's compartments and/or to the population's sample which might be too limited to provide answers to this question. These issues probably need to be raised with larger samples.

In addition, it is important to mention that when we consider LFM as a single body composition predictor, it appears significantly associated only with leptin (after age, sex, BMI Z-score, sexual maturity status, and physical activity adjustments). Therefore, in our pediatric sample, the single LFM acts as the TFM. This being said, previous pediatric studies did not really investigate the implication of the single LFM. Therefore, we have no element of comparison with the literature, except for the Teixeira et al. (26) study which showed that only apolipoprotein B was significantly positively associated with LFM. No other significant univariate partial correlations were indeed observed between LFM and serum triglycerides, total, LDL and HDL cholesterol, and apolipoproteins A-I in this study (26). In fact, in the framework of univariate analysis, the latest children-related studies have examined the implication of at least a 2-DXA parameter combination in the form of adiposity indices. Aucouturier et al. (22) showed a positive relationship between insulin resistance and an android to gynoid fat ratio [(L1–L4 fat mass area/lower limbs fat)] in French obese children and adolescents. Daniels et al.(24) established unfavorable plasma lipid and lipoprotein concentrations, blood pressure, and left ventricular mass associations with a higher body fat

Table 4. Prediction of cardiometabolic risk factors using models combining leg, visceral and total fat masses (age, sex, BMI Z-score, sexual maturity status, and physical activity adjustments)

	Model 3: LFM, VAT, TFM			
	R^2 model 3	r partial LFM	r partial VAT	r partial TFM
Fasting glucose	0.087	−0.179	−0.130	−0.166*
Fasting insulin†	0.490**‡	−0.229*§	−0.002	0.247*¶
HOMA IR†	0.485**‡	−0.246*§	−0.026	0.261**¶
Triglycerides†	0.191**‡	0.178*	0.151	0.145
HDL cholesterol†	0.138*‡	0.138	−0.170*	−0.079
LDL cholesterol	0.015	−0.004	0.016	−0.027
SBP Z-score	0.233**‡	−0.075	0.081	0.127
DBP Z-score	0.167**‡	−0.066	0.136	0.068
CRP†	0.287**‡	−0.113	0.219*	0.195*
Fibrinogen†	0.201**‡	0.050	0.044	0.033
Adiponectin†	0.166*‡	0.155	−0.184*	−0.105
Leptin†	0.633**‡	0.053	−0.081	0.117
Resistin†	0.134*‡	0.201*	−0.030	0.243*¶
Tolerance		0.054	0.028	0.033

BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; LFM, leg fat mass; SBP, systolic blood pressure; TFM, total fat mass; VAT, visceral adipose tissue.
*p-value < 0.05 and **p-value < 0.001.
†Log-transformed variables.
‡Benjamini and Hochberg corrected p-value < 0.04.
§Benjamini and Hochberg corrected p-value < 0.01.
¶Benjamini and Hochberg corrected p-value < 0.007.

distribution index [(subscapular + waist)/(hip + thigh) fat mass], in 9- to 17-yr-old Ohioan boys and girls (24). Another DXA-android to gynoid fat ratio (fat mass area from the ilium to the mandible/lower body fat from the ilium) was positively associated with CMR factors (lipid and insulin biomarkers) in 6- to 18-yr-old Indian boys, in the study carried out by Jahagirdar et al. (23). In adults, positive associations were observed when authors investigated the impact of the single LFM on CMR factors, as indicated in particular in the Van Pelt et al. study (16). Also, authors showed a protective effect of LFM (negative significant relationship) after TFM adjustment (16). These findings in adults are in several points similar to ours in children and adolescents. Consequently, this study probably adds a new dimension to the understanding of the relationship between metabolically healthy obesity and body composition in youth, through the protective role of the LFM. This concept is well-established in adults (36), but still to be defined among the youngest (37).

This study leads to the conclusion that, after taking TFM into account, LFM may protect against CMR factors in children and adolescents, at least in terms of insulin resistance and hyperlipidaemia. This means that different levels of LFM must be considered within the framework of obesity prevention and therapeutic processes in childhood.

Acknowledgement

We thank Julien Jacobs (Luxembourg Institute of Health) for the data management and Patrick Kastler (Centre Hospitalier de Luxembourg) to have performed the magnetic resonance imaging analyses.

Conflict of interest

The authors have not declared any conflicts of interest.

Author contributions

HS, CDB, FD, and MLL designed the research study. CDB recruited the participants. MH implemented the dual energy x-ray absorptiometry (DXA) protocol. JPVN implemented the magnetic resonance imaging (MRI) protocol and wrote the *MRI acquisition* part. GD managed the MRI measurements. GG managed the blood samples analysis and wrote the *biological measurements* part. OK implemented and wrote the *MRI image analysis* protocol. SL and HS performed statistics and interpreted data. MV managed the statistics analysis. HS collected anthropometric data, analyzed MRI and DXA images, and wrote the manuscript. HS, CDB, SS, MH, JPVN, GD, GG, OK, SL, MV, MLL, and FD have critically revised the article and approved the several versions.

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