Original Investigation

Origin of Cardiovascular Risk in Overweight Preschool Children

A Cohort Study of Cardiometabolic Risk Factors at the Onset of Obesity

Blegina Shashaj, MD, PhD; Giorgio Bedogni, MD; Maria P. Graziani, MD; Alberto E. Tozzi, MD; Maria L. DiCorpo, MD; Donatella Morano, MD; Ludovica Tacconi, MD; Patrizio Veronelli, MD; Benedetta Contoli, Ph.D; Melania Manco, MD, PhD

IMPORTANCE To date, the relationship among adiposity, insulin resistance, and cardiovascular risk factors at the onset of overweight or obesity has been unexplored.

OBJECTIVES To assess whether insulin resistance and metabolic abnormalities are detectable at the onset of obesity and to unravel the interplay among adiposity, insulin resistance, and other such abnormalities.

DESIGN, SETTING, AND PARTICIPANTS The Origin of Cardiovascular Risk in Overweight Preschool Children cohort study aimed to evaluate at the onset of obesity in preschool children the prevalence of metabolic abnormalities, including hypertension, dyslipidemia, impaired carbohydrate metabolism, and nonalcoholic fatty liver disease. Between July 1, 2011, and July 30, 2012, in the Rome municipality, 13 family pediatricians enrolled healthy children (age range, 2.0-5.8 years) in the study during their routine practice of growth monitoring. Clinical medical records of 5729 children were reviewed; 597 children manifested new-onset overweight or obesity as their body mass index changed from normal weight to overweight or obesity in the previous 12 months according to the International Obesity Task Force classification. Of them, 219 were studied.

MAIN OUTCOMES AND MEASURES Patients with new-onset overweight or obesity underwent clinical laboratory testing, including oral glucose tolerance test, and ultrasonographic investigations of fatty liver and intimal medial thickness of the common carotid arteries, subcutaneous adipose tissue, and visceral adipose tissue. The homeostatic assessment model algorithm–insulin resistance was calculated.

RESULTS Among the entire population (n = 5729), overweight increased from 7.0% at 2.0 years to 16.9% at 5.8 years, with corresponding figures of 1.1% to 2.9% for obesity. In total, 597 overweight or obese children (10.4%) were identified, and 219 of them (36.7%) were studied. Among the latter, 86 patients (39.3%) had at least 1 metabolic abnormality. Hypertension was diagnosed in 29 patients (13.2%), dyslipidemia in 55 patients (25.1%), impaired fasting glucose level in 7 patients (3.2%), and glucose intolerance in 6 patients (2.7%). Nonalcoholic fatty liver disease was diagnosed in 68 patients (31.1%).

CONCLUSIONS AND RELEVANCE Cardiometabolic risk factors, including fatty liver, are detectable in preschoolers at the onset of overweight or obesity, despite short-term exposure to excess weight and reduced insulin sensitivity. Our findings suggest the need to screen for cardiometabolic abnormalities at an earlier age than is now recommended.

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Author Affiliations: Bambino Gesù Children's Hospital, Istituto di Ricovero e Cura a Carattere Scientifico, Rome, Italy (Shashaj, Tozzi, Contoli, Manco); Clinical Epidemiology Unit, Liver Research Center, Area Science Park, Trieste, Italy (Bedogni); Federazione Italiana Medici Pediatri, Rome, Italy (Graziani, DiCorpo, Morano, Tacconi, Veronelli).

Corresponding Author: Melania Manco, MD, PhD, Bambino Gesù Children's Hospital, Istituto di Ricovero e Cura a Carattere Scientifico, Rome, Italy 00165 (melania.manco@opbg.net).

The prevalence of obesity in childhood has increased worldwide in recent decades, with children manifesting obesity at progressively younger ages.[1] In 2010, it was estimated that 43 million preschoolers would develop overweight and obesity.[2] A US survey,[3] the Early Childhood Longitudinal Study, Kindergarten Class of 1998-1999, recently demonstrated that children who were overweight at kindergarten entry had a 4-fold increased risk for obesity by the eighth grade. At study enrollment, almost 15% of kindergarten-age children manifested overweight, and 12.4% demonstrated obesity.

While the burden of obesity has been widely defined in school-age children and older, data on preschoolers are scarce and inconsistent. The latest systematic review of the prevalence rates in European countries reports the highest rates of overweight or obesity among preschoolers from the Mediterranean basin.[4] In Italy, the prevalence of overweight according to the International Obesity Task Force (IOTF) classification ranged from 10.2% at age 2 years to 14.4% at age 4 years, with the prevalence of obesity ranging from 3.1% at age 2 years to 7.8% at age 4 years.[4]

Obesity represents the most common cause of insulin resistance (IR) in youth.[5] Insulin resistance can be the main element in a cluster of cardiometabolic abnormalities associated with obesity, but by itself it increases adiposity in a vicious cycle.[6,7] Few researchers have examined the occurrence of IR in preschoolers apart from some investigations in children with low birth weight for gestational age or after exposure to intrauterine growth restraint. These studies began observation at age 5 years in healthy children[8] and at age 2 years in patients with severe obesity.[9] One study[10] reported on hypertension and IR as comorbidities of obesity in a sample of 75 children (age range, 3-5 years), but it provided no information on the onset of obesity.

To our knowledge, no study has systematically investigated the prevalence of the full spectrum of metabolic abnormalities involved in metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) among children with overweight or obesity aged 2 to 6 years at the onset of their weight gain, independent of predisposing risk conditions such as low birth weight and rapid catch-up growth. The Origin of Cardiovascular Risk in Overweight Preschool Children cohort study aimed to evaluate at the onset of obesity in preschool children the frequency and incidence of metabolic abnormalities, including hypertension, dyslipidemia, impaired carbohydrate metabolism, and NAFLD, and cardiovascular abnormalities, including increased intimal medial thickness (IMT) of the common carotid arteries, left ventricular hypertrophy, left atrial dilatation, and impaired cardiac function.

To accomplish these objectives, more than 5000 healthy children aged 2 to 6 years were enrolled in the study. Children who developed overweight or obesity in the 12 months before the enrollment date were identified. The prevalence of metabolic abnormalities was estimated, and IMT was measured at the onset of overweight or obesity. Thereby, we assessed whether metabolic abnormalities are detectable at the onset of obesity and attempted to unravel the interplay among adiposity, insulin resistance, and other such abnormalities.

Methods

The study was approved by the Ospedale Pediatrico Bambino Gesù, Rome, Italy, ethics committee. Written informed consent was obtained from the parents or legal guardians of study participants, and patient data were identified to guarantee confidentiality.

Study Population

Between July 1, 2011, and July 30, 2012, in the Rome municipality, 13 family pediatricians enrolled healthy children (age range, 2.0-5.8 years) in the study during their routine practice of growth monitoring (yearly follow-up growth control visits). Growth data of 5729 children from birth to the time of enrollment were collected. Using questionnaires, information was collected on socioeconomic status (ie, the parents' annual income, employment status, and education level) and on the child's lifestyle habits and medical history.

Children whose body mass index (BMI) changed from normal weight to overweight or obesity in the 12 months before the enrollment date were identified and referred to the Bambino Gesù Children's Hospital, Rome, Italy, to undergo clinical, laboratory, and ultrasonographic evaluations. Exclusion criteria were chronic illness, genetic disease, endocrine diseases, carbohydrate metabolism, consumption of drugs affecting growth, and time to diagnosis of overweight or obesity exceeding 12 months.

Clinical Evaluation

Anthropometric measurements were performed by family pediatricians according to a standardized procedure and were recorded using a software program (Infantia Studio 2000; Fimesan Spa or Junior Bit; SoSePe Srl). Weight was measured with scales certified for medical use (90/384/EEC; Seca) with a precision of ±50 g in children wearing minimal clothing and was recorded to the nearest 100 g. Height was measured with a stadiometer (Holtain) and was recorded to the nearest 0.5 cm. The mean of 2 measurements was used. Classifications of normal weight, overweight, and obesity were defined according to the IOTF criteria.[11]

Systolic and diastolic blood pressure (BP) was measured on the right arm with the participant seated using an automated oscillatory system and appropriately sized arm cuffs (Dinamap; Criticon Inc) in the quiet setting of the pediatrician's office by the family pediatrician.[12] The mean of 3 BP measurements was used.

Laboratory Evaluation

Fasting triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol levels were assessed using colorimetric kits (modular systems P/S Can 433; Roche/Hitachi). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase, and uric acid levels were evaluated using a radioimmunoassay method (ADVIA 1650; Bayer Diagnostics); the normal ranges were 10 to 37 U/L for ALT, 10 to 65 U/L for AST, and <50 U/L for γ-glutamyltransferase (to convert ALT, AST, and γ-glutamyl-

transferase levels to microkatals per liter, multiply by 0.0167). Also measured were high-sensitivity C-reactive protein (hs-CRP) level by nephelometry (BN; Dade Behring), blood glucose level by the glucose oxidase technique (Cobas Integra; Roche), and insulin level by a chemiluminescent immunoassay method (ADVIA Centaur analyzer; Bayer Diagnostics).

A standard oral glucose tolerance test (1.75 g of glucose per kilogram of body weight up to a maximum of 75 g) was performed. Also calculated were the homeostatic assessment model algorithm-insulin resistance (HOMA-IR),[13] whole-body insulin sensitivity,[14] and the Insulinogenic Index.[15]

Excluded were patients with abnormal liver function test results or increased liver brightness on ultrasonography, as well as those with hepatotropic virus infections, the use of steatogenic drugs, and other causes of fatty liver. The exclusions were according to an established protocol at the Ospedale Pediatrico Bambino Gesù.[16]

Ultrasonographic Assessments

Ultrasonographic examinations were performed in real time by 2 radiologists. An imaging system (Acuson Antares; Siemens) equipped with convex and linear transducers (3.5-14.0 MHz) and tissue harmonics was used.

Visceral and subcutaneous fat was measured according to standardized procedures.[17] The thickness of visceral adipose tissue (VAT) was defined as the thickness of fat tissue between the posterior edge of the abdominal muscles and the lumbar spine, and the thickness of subcutaneous adipose tissue (SAT) was defined as the thickness of fat tissue between the skin-fat interface and the linea alba.

Nonalcoholic fatty liver disease was suspected in the presence of ultrasonography-detected hepatic steatosis. The steatosis was scored as 0 if absent (normal liver echotexture), 1 if mild (slight and diffuse increase in fine parenchymal echoes with normal visualization of the diaphragm and portal vein borders), 2 if moderate (moderate and diffuse increase in fine echoes with slightly impaired visualization of the diaphragm and portal vein borders), and 3 if severe (fine echoes with poor or no visualization of the diaphragm, portal vein borders, and posterior portion of the right lobe).[18]

High-resolution B-mode ultrasonography of the common carotid arteries was performed. Intimal medial thickness was defined as the mean distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall, 1 cm proximal to the carotid bulb during end diastole. We measured 4 values on each side; the maximum IMT and the mean IMT were calculated separately for each side.[16]

Definition of Metabolic Abnormalities

Dyslipidemia was diagnosed in the presence of at least 1 of the following conditions: cholesterol level or triglycerides level exceeding the 95th percentile or high-density lipoprotein cholesterol level less than the 5th percentile for age and sex according to the American Academy of Pediatrics.[19] Hypertension was defined as systolic or diastolic blood pressure exceeding the 95th percentile for age, sex, and height.[20] Impaired fasting glucose level was defined as a fasting glucose level of 100 mg/dL or higher, and impaired glucose tolerance was defined as a 2-hour glucose level 140 mg/dL or higher following the oral glucose tolerance test (to convert glucose level to millimoles per liter, multiply by 0.0555).[21]

As described elsewhere,[22] IR was defined as a HOMA-IR of 1.58 or higher, which corresponded to a HOMA-IR exceeding the 95th percentile in 21 healthy age-matched control children of normal weight. Among the controls, the mean (SD) age was 4.2 (1.5) years, the mean (SD) BMI (calculated as weight in kilograms divided by height in meters squared) was 16.2 (1.3), and the mean (SD) BMI *z* score was 1.98 (1.07).

Statistical Analysis

Using the IOTF classification, we estimated the age-adjusted probability of grade 1 thinness, grade 2 thinness, grade 3 thinness, normal weight, overweight, and obesity using an ordinal generalized linear model. The model used the IOTF classification as the outcome (discrete, 1 [normal weight], 2 [overweight], or 3 [obesity]) and age as the predictor (continuous in years). Repeated measures were taken into account by specifying cluster CIs for each participant.

Continuous variables are given as the median (interquartile range) because of skewed distributions. The interquartile range was calculated as the difference between the 75th and 25th percentiles. Categorical variables are reported as the number (percentage) of participants with the characteristics of interest. Between-group comparisons were performed with unpaired *t* test or Wilcoxon signed rank test for continuous variables according to data heteroskedasticity and with Fisher exact test or χ^2 test for categorical variables. Pearson product moment correlation coefficient was used to assess significant correlations among variables. Statistical significance was set at $P \leq .05$. Statistical analyses were performed using available software (STATA 12.1; StataCorp LP).

Results

Whole Population

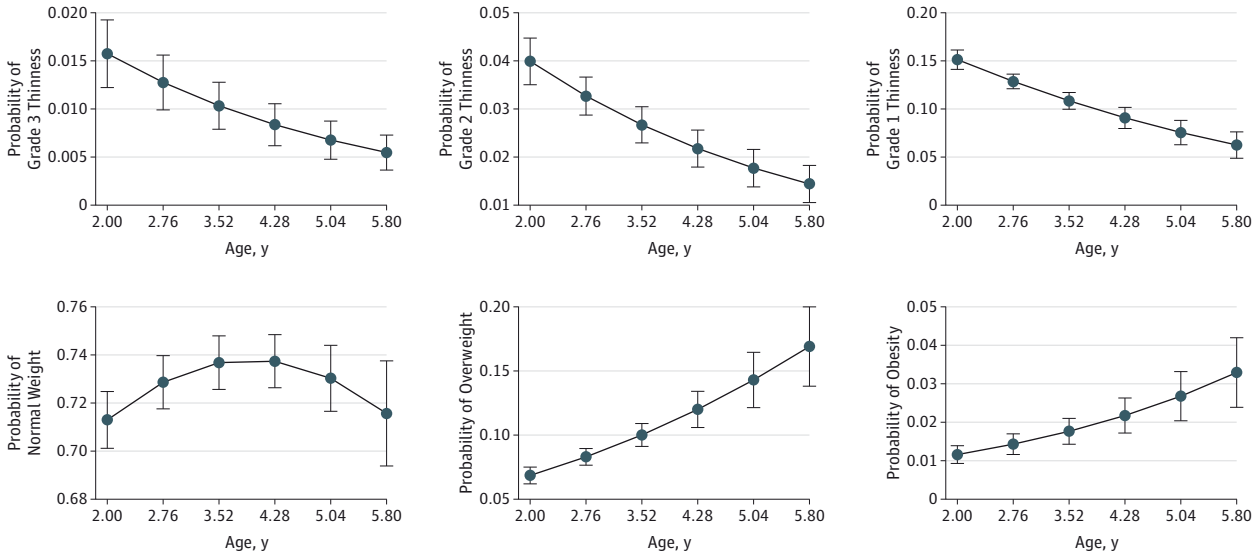
The **Figure** shows the probability of IOTF classification according to a child's age among the whole population (n = 5729) based on growth charts at study baseline; 51.1% of the sample were male. The age distributions among the entire sample were 40.2% aged 2 to 3 years, 25.9% aged 3 to 4 years, 18.9% aged 4 to 5 years, and 15.0% aged 5 to 6 years.

Cases of grade 2 or grade 3 thinness were few, but a clear decrease in grade 1 thinness was seen from age 2.0 to 5.8 years. While normal weight remained stable at approximately 72% with age, a clear increase was observed in the probability of overweight (from 7.0% at 2.0 years to 16.9% at 5.8 years) and obesity (from 1.1% at 2.0 years to 2.9% at 5.8 years).

Metabolic Abnormalities in Preschoolers With Overweight or Obesity

We identified 597 children who had developed overweight or obesity from normal weight in the 12 months before the enrollment date. We were able to contact parents and invite 397 of them to participate in the study. Parents of 178 children re-

Figure. Age-Adjusted Probability of Body Mass Index According to the International Obesity Task Force Classification



Values are point estimates (95% CIs) obtained from a proportional odds logistic regression with cluster CIs.

fused consent. Therefore, 219 children with overweight or obesity participated and underwent laboratory testing and ultrasonographic measurements. All 219 patients (123 with overweight and 96 with obesity) were of white race/ethnicity, and 46.1% were male; their mean (SD) age was 4.9 (2.1) years, their mean (SD) BMI was 18.9 (2.7), and their mean (SD) BMI *z* score was 1.89 (1.54). No significant differences in age, sex, BMI, BMI *z* score, or socioeconomic status were observed between participants and nonparticipants.

Dyslipidemia was diagnosed in 55 children with overweight or obesity (25.1%), with low high-density lipoprotein cholesterol levels accounting for 11.8%, high total cholesterol levels accounting for 8.2%, and hypertriglyceridemia accounting for 8.2%. Seven patients had both high total cholesterol level and hypertriglyceridemia, and dyslipidemia was diagnosed in the presence of at least 1 of the following conditions. Impaired fasting glucose level was diagnosed in 7 patients (3.2%), and glucose intolerance was diagnosed in 6 patients (2.7%). Hypertension was diagnosed in 29 patients (13.2%). Disturbed carbohydrate metabolism was found in 13 patients with overweight or obesity (5.9%) (7 with impaired fasting glucose level and 6 with glucose intolerance), and none had first-degree relatives with a history of diabetes mellitus. Sixty-eight patients (31.1%) had NAFLD; it was mild in 60 patients and moderate in the other 8 patients. Eighty-six patients (39.3%) manifested 1 (32.6%) or 2 (6.7%) metabolic abnormalities. Children having obesity with 1 or more metabolic abnormalities had significantly higher BMI (mean [SD], 20.3 [2.3] vs 19.5 [1.7]; $P = .04$) and SAT thickness (mean [SD], 8.2 [4.3] vs 6.4 [2.5] mm; $P < .05$) than children having obesity with no metabolic abnormalities.

Insulin resistance was observed in 77 patients (35.2%). They had significantly higher BMI, diastolic BP, fasting blood glucose level, triglycerides level, γ-glutamyltransferase level, and VAT and SAT thicknesses than patients with a HOMA-IR below the threshold (**Table 1**), while no differences were found in hs-CRP level, uric acid level, white blood cell count, heart rate, IMT, or epicardial adipose tissue.

Among patients with NAFLD, increased AST level was found in 15 patients (22.1%). They had significantly higher BMI, ALT and AST levels, VAT and SAT thicknesses, and epicardial adipose tissue than patients without NAFLD (**Table 2**).

Correlations Among BMI, Biomarkers, and Ultrasonographic Characteristics

When multiple linear regression analysis was performed, BMI was significantly related to the HOMA-IR (regression coefficient, 0.448; 95% CI, 0.142-0.754; $P = .005$), hs-CRP level (regression coefficient, 0.070; 95% CI, 0.005-0.134; $P = .03$), and γ-glutamyltransferase level (regression coefficient, 0.145; 95% CI, 0.038-0.250; $P = .008$). No significant correlation was found between BMI or BMI *z* score and systolic BP, diastolic BP, fasting and 2-hour glucose levels, uric acid level, ALT level, AST level, or lipid levels (total cholesterol, high-density lipoprotein cholesterol, and triglycerides).

Linear regressions were performed to identify significant determinants of the HOMA-IR, and only those variables that were related ($P < .20$) were modeled together into a bootstrapped regression model to identify major predictors of the HOMA-IR (**Table 3**). Univariate analyses were performed to identify the determinants of NAFLD (**Table 4**).

Discussion

The findings of our study demonstrate that metabolic abnormalities related to obesity, including NAFLD, are present from early age in a large proportion of children with overweight or

Table 1. Adiposity, Biochemical, and Ultrasonographic Characteristics Among Children Having Overweight or Obesity With vs Without Insulin Resistance

Variable	Insulin Sensitivity (n = 142)	Insulin Resistance (n = 77)	*P* Value
Sex, No. (%)			.70
Male	64 (45.1)	37 (48.1)	
Female	78 (54.9)	40 (51.9)	
Age, mean (SD), y	4.73 (2.18)	5.28 (1.50)	.01
Body mass index, mean (SD)[a]	18.69 (2.16)	20.04 (3.34)	<.001
Body mass index *z* score, mean (SD)	1.59 (1.14)	2.60 (2.19)	<.001
Overweight, No. (%)	92 (64.8)	31 (40.3)	.009
Obesity, No. (%)	50 (35.2)	46 (59.7)	.009
Blood pressure, mean (SD), mm Hg			
Systolic	90.0 (10.0)	90.0 (10.0)	.60
Diastolic	60.0 (5.0)	60.0 (5.8)	.03
Glucose level, mean (SD), mg/dL			
Fasting	84.0 (8.5)	92.0 (7.0)	<.001
2-Hour	93.0 (21.0)	97.0 (19.0)	.05
Impaired fasting glucose level, No. (%)	0	7 (9.1)	.02
Impaired glucose tolerance, No. (%)	2 (1.4)	4 (5.2)	.62
Fasting insulin level, mean (SD), μIU/mL	3.40 (1.90)	7.84 (2.81)	<.001
Homeostatic assessment model algorithm-insulin resistance, mean (SD)	0.69 (0.45)	1.73 (0.71)	<.001
Whole-body insulin sensitivity, mean (SD)	15.57 (11.73)	8.04 (4.14)	<.001
Ratio of AUCG to AUCI, mean (SD)	6.13 (5.80)	3.22 (2.43)	<.001
Cholesterol level, mean (SD), mg/dL			
Total	154.0 (28.0)	148.0 (44.5)	.45
High-density lipoprotein	51.0 (13.0)	52.0 (14.0)	.69
Triglycerides level, mean (SD), mg/dL	45.0 (22.0)	57.0 (38.5)	.01
Alanine aminotransferase level, mean (SD), U/L	28.0 (5.0)	25.0 (6.5)	.13
Aspartate aminotransferase level, mean (SD), U/L	34.0 (6.5)	34.0 (7.5)	.49
γ-Glutamyltransferase level, mean (SD), U/L	20.0 (3.0)	22.0 (4.0)	.01
Nonalcoholic fatty liver disease, No. (%)	74 (52.1)	38 (49.4)	.20
Thickness, mean (SD), mm			
Visceral adipose tissue	59.40 (15.80)	65.65 (19.10)	.02
Subcutaneous adipose tissue	5.10 (4.95)	8.15 (6.87)	<.001
Ratio of visceral adipose tissue thickness to subcutaneous adipose tissue thickness, mean (SD)	11.68 (10.26)	7.46 (5.05)	<.001

Abbreviations: AUCI, area under the curve for insulin; AUCG, area under the curve for glucose.

SI conversion factors: To convert glucose level to millimoles per liter, multiply by 0.0555. To convert insulin level to picomoles per liter, multiply by 6.945. To convert cholesterol level to millimoles per liter, multiply by 0.0259. To convert triglycerides level to millimoles per liter, multiply by 0.0113. To convert alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase levels to microkatals per liter, multiply by 0.0167.

[a] Calculated as weight in kilograms divided by height in meters squared.

obesity, even in the presence of a short history of overweight (with an onset in the prior 12 months) and a HOMA-IR deemed as normal in prepubertal or school-age children.[22,23] Our results suggest that the risk for metabolic abnormalities related to obesity begins to manifest early in the natural history of weight gain. The origin of cardiovascular risk can probably be sought in the intrauterine period of life, in keeping with a theory of the developmental origin of health and disease.[24] Evidence also points to the need to begin at a younger age the second-level screening that is now recommended for school-age children with overweight.[25]

Few studies[10,26] have assessed the prevalence of metabolic abnormalities among young children with overweight or obesity, and our study is one of the first to evaluate children at the onset of overweight and in such a large population of preschoolers. Despite the difficulties involved in sampling and studying such young children,[27] the Origin of Cardiovascular Risk in Overweight Preschool Children cohort study was made possible owing to the regular monitoring of children's growth by Italian family pediatricians in the first years of the children's lives. This makes the study unique.

Although a full diagnosis of metabolic syndrome cannot be made in children younger than 10 years,[28] risk factors related to the syndrome have been described as prevalent among this age group. The Bogalusa Heart Study[29] found that approximately 50% of children with overweight aged 5 to 10 years have a cardiovascular risk factor such as high BP, dyslipidemia, or elevated insulin level. In the present series, we did not consider hyperinsulinemia a metabolic abnormality. One metabolic risk factor was found in 32.6% of patients with overweight or obesity, and 2 or more abnormalities were present in 6.4%. Dyslipidemia and hypertension, in addition to NAFLD, were related to increased adiposity as estimated by BMI, albeit with a lower prevalence than that reported among older

Table 2. Adiposity, Biochemical, and Ultrasonographic Characteristics Among Children Having Overweight or Obesity With vs Without Nonalcoholic Fatty Liver Disease (NAFLD)

Variable	No NAFLD (n = 151; 69%)	NAFLD (n = 68; 31%)	*P* Value
Sex, No. (%)			.10
Male	67 (44.4)	40 (58.8)	
Female	84 (55.6)	28 (41.2)	
Age, mean (SD), y	4.89 (2.20)	4.86 (1.49)	.60
Body mass index, mean (SD)[a]	18.86 (2.23)	19.90 (3.24)	.03
Body mass index *z* score, mean (SD)	1.77 (1.36)	2.48 (1.70)	.08
Overweight, No. (%)	91 (60.3)	26 (38.2)	.06
Obesity, No. (%)	60 (39.7)	42 (61.8)	.06
Blood pressure, mean (SD), mm Hg			
Systolic	90.0 (10.0)	90.0 (10.0)	.15
Diastolic	60.0 (5.0)	60.0 (5.0)	.25
Glucose level, mean (SD), mg/dL			
Fasting	87.0 (11.0)	88.0 (9.0)	.07
2-Hour	94.0 (19.5)	93.0 (19.5)	.35
Impaired fasting glucose level, No. (%)	5 (3.3)	1 (1.5)	.50
Impaired glucose tolerance, No. (%)	2 (1.3)	4 (5.9)	.50
Fasting insulin level, mean (SD), µIU/mL	4.56 (3.52)	5.18 (5.40)	.22
Homeostatic assessment model algorithm–insulin resistance, mean (SD)	0.99 (0.82)	1.17 (1.23)	.17
Whole-body insulin sensitivity, mean (SD)	12.01 (8.17)	12.54 (12.10)	.70
Ratio of AUCG to AUCI, mean (SD)	4.97 (3.85)	3.64 (5.00)	.80
Cholesterol level, mean (SD), mg/dL			
Total	153.0 (33.0)	148.0 (36.5)	.90
High-density lipoprotein	52.0 (15.0)	50.0 (11.5)	.12
Triglycerides level, mean (SD), mg/dL	49.0 (30.0)	52.0 (28.0)	.61
Alanine aminotransferase level, mean (SD), U/L	26.0 (5.0)	28.0 (6.0)	.02
Aspartate aminotransferase level, mean (SD), U/L	34.0 (4.0)	41.0 (12.5)	<.001
Thickness, mean (SD), mm			
Visceral adipose tissue	59.55 (15.20)	66.50 (20.80)	<.001
Subcutaneous adipose tissue	6.10 (4.47)	8.60 (8.60)	<.001
Ratio of visceral adipose tissue thickness to subcutaneous adipose tissue thickness, mean (SD)	9.83 (6.48)	7.20 (10.58)	.60
Epicardial adipose tissue thickness, mean (SD), mm	2.80 (2.00)	3.60 (1.60)	.01
Intimal medial thickness, mean (SD), mm			
Right	0.40 (0.10)	0.50 (0.10)	.67
Left	0.50 (0.10)	0.50 (0.10)	.67
Maximum	0.50 (0.20)	0.50 (0.10)	.13

Abbreviations: AUCI, area under the curve for insulin; AUCG, area under the curve for glucose.

SI conversion factors: To convert glucose level to millimoles per liter, multiply by 0.0555. To convert insulin level to picomoles per liter, multiply by 6.945. To convert cholesterol level to millimoles per liter, multiply by 0.0259. To convert triglycerides level to millimoles per liter, multiply by 0.0113. To convert alanine aminotransferase and aspartate aminotransferase levels to microkatals per liter, multiply by 0.0167.

[a] Calculated as weight in kilograms divided by height in meters squared.

children with overweight and obesity, probably due to the short exposure to obesity.[30-32]

The degree of IR that was related to increased visceral and subcutaneous adiposity was modest compared with the

Table 3. Multivariable Analysis of Risk Factors Related to the Homeostatic Assessment Model Algorithm–Insulin Resistance

	Model 1 R^2 = 0.207		Model 2 R^2 = 0.286		Model 3 R^2 = 0.321		Model 4 R^2 = 0.357	
Predictor Variable[a]	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Subcutaneous adipose tissue thickness	0.099 (0.064-0.135)	<.001	0.100 (0.066-0.134)	<.0001	0.098 (0.064-0.131	<.0001	0.083 (0.049-0.118)	<.0001
Triglycerides level			0.007 (0.003-0.011)	<.001	0.007 (0.003-0.011)	.001	0.007 (0.003-0.011)	.001
Diastolic blood pressure					0.031 (0.006-0.056)	.02	0.033 (0.009-0.058)	.009
Visceral adipose tissue thickness							0.012 (0.003-0.021)	.01

[a] Excluded variables were age, body weight, γ-glutamyltransferase level, alanine aminotransferase level, and epicardial adipose tissue thickness.

Table 4. Univariate Analysis of Risk Factors Related to the Presence of Nonalcoholic Fatty Liver Disease

Variable	Odds Ratio (95% CI)	*P* Value
Systolic blood pressure	0.009 (−0.002 to 0.019)	.10
Visceral adipose tissue thickness	0.007 (0.000 to 0.013)	.04
Subcutaneous adipose tissue thickness	0.032 (0.003 to 0.061)	.03
Epicardial adipose tissue thickness	0.047 (−0.027 to 0.121)	.21
Homeostatic assessment model algorithm-insulin resistance	0.008 (−0.107 to 0.124)	.89
Maximum intimal medial thickness	0.792 (−0.103 to 1.686)	.08
Body mass index *z* score	0.792 (−0.103 to 1.686)	.23

HOMA-IR commonly observed in older children or adolescents with overweight and obesity.[33] It may be more accurate to say that we observed reduced insulin sensitivity, and not true IR, in the patients herein. However, they showed higher a HOMA-IR compared with preschoolers having normal weight. The EarlyBird,[8] a study of healthy children with normal weight, reports a HOMA-IR ranging from 0.36 to 0.64 in 5-year-old boys and girls.

The HOMA-IR was predicted not only by the thickness of visceral and subcutaneous fat but also by the level of circulating triglycerides and the diastolic BP (Table 3). The relationship among reduced insulin sensitivity, abdominal adiposity, and circulating triglycerides level is expected because lipolysis is increased in the presence of IR and enlarged adipose tissue depots, promoting increased free fatty acid delivery to the liver for hepatic triglyceride synthesis.[34] When the latter is unable to accommodate increased free fatty acid accumulation in hepatocytes and their export to the bloodstream because low-density lipoproteins are ineffective, hepatic steatosis develops.[35]

In our series of young patients, 1 in 3 children had NAFLD; their HOMA-IR was not statistically significantly different from that of patients without fatty liver. In the detection of fatty liver, ultrasonography seems to perform better than assay of liver enzymes in population investigations.[36] Ultrasonography has good specificity and sensitivity for mild to moderate steatosis,[18] although which range should be considered normal for liver enzymes is controversial.[36] Even defining values below the threshold of 40 U/L as normal, 20% of patients with biopsy-proven NAFLD will have normal ALT levels.[37] The relationship between NAFLD and greater VAT and SAT thicknesses reinforces the concept of enlarged abdominal adiposity as a pivotal risk factor for NAFLD.[38]

Regarding low-grade inflammation, we found a statistically significant relationship between hs-CRP level and BMI, in keeping with the results of the third National Health and Nutrition Examination Survey[39] in children aged 6 to 18 years. Conversely, no relationship was found between hs-CRP level and the HOMA-IR, which is consistent with the notion that IR and other metabolic alterations anticipate the elevation of hs-CRP level that occurs later in life, after a longer exposure to obesity, IR, and impaired lipid metabolism.[40] This may also be the case for IMT, which has been disproved as an early marker of atherosclerosis and endothelium dysfunction.[16]

Strengths of the present study are the young age of the patients and the short exposure to increased adiposity and reduced insulin sensitivity. The enrolled participants were sufficiently naive to constitute a model to study, in postnatal life, the first line of metabolic events triggered by increased adiposity. However, we are aware of certain methodological caveats owing to practicality in a population study and ethical restraints such as the use of proxy methods for measuring adiposity, IR, and hepatic steatosis. Major limitations of the study are also the lack of a large sample of control subjects with normal weight and the fact that no information was available on waist circumference (a reliable marker of metabolic syndrome). Longitudinal observation of the whole population, and especially children who developed early overweight, will provide deeper insight into the natural course of metabolic abnormalities related to increased adiposity, ruling out whether they have any clinical meaning.

Conclusions

In summary, our findings prove that metabolic abnormalities, reduced insulin sensitivity, and NAFLD are present in children aged 2 to 6 years, early after the onset of overweight or obesity. Future research should lead to a better understanding of the fine interplay among these factors and their progression into adulthood. For the present, our findings emphasize the need to start screening for cardiometabolic abnormalities using multidisciplinary strategies at an earlier age than is now recommended.

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