ORIGINAL RESEARCH

Prevalence of prediabetes in children and adolescents by class of obesity

Stefania Pedicelli[1] | Danilo Fintini[1] | Lucilla Ravà[2] | Elena Inzaghi[1] | Annalisa Deodati[1] | Maria Rita Spreghini[1] | Carla Bizzarri[1] | Michela Mariani[1] | Stefano Cianfarani[1,3] | Marco Cappa[1] | Melania Manco[4]

[1]Unit of Endocrinology, Dipartimento Pediatrico Universitario, Università di Tor Vergata, Rome, Italy

[2]Clinical Epidemiology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

[3]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

[4]Research Area for Multifactorial Diseases and Complex Phenotypes, Bambino Gesù Children's Hospital, Rome, Italy

Correspondence
Melania Manco, Research Area for Multifactorial Diseases and Complex Phenotypes, Bambino Gesù Children's Hospital, IRCCS, Via F. Baldelli 38, 00146 Rome, Italy.
Email: melania.manco@opbg.net

Summary

Background: To evaluate prevalence of prediabetes (impaired fasting glucose, IFG; impaired glucose tolerance, IGT; and high glycated haemoglobin, h-HbA1c) in children and adolescents in relation to class of age and obesity; to appraise association with estimates of insulin metabolism, cardiovascular risk factors and alanine aminotransferase (ALT) levels.

Methods: Study of marginal prevalence (i.e., as function of sex, age and obesity class) of isolated and combined IFG, IGT and h-HbA1c in children (age 4–9.9 years) and adolescents (age 10–17.9 years) and association to blood pressure (BP), total, HDL and non-HDL cholesterol, triglycerides, ALT and insulin sensitivity/secretion indexes.

Results: Data of 3110 participants (51% males, 33% children; 33% overweight, 39% obesity class I, 20.5% class II, 7.5% class III) were available. Unadjusted prevalence of prediabetes was 13.9% in children (2.1% IFG, 6.7% IGT, 3.9% h-HbA1c, IFG-IGT 0.06%) and 24.6% in adolescents (3.4% IFG, 9.4% IGT, 5.5% h-HbA1c, IFG-IGT 0.09%). Combined h-HBA1c was found in very few adolescents. Prevalence of prediabetes increased significantly by class of obesity up to 20.5% in children and 31.6% in adolescents. Phenotypes of prediabetes were differently but significantly associated with increased systolic and diastolic BP (by 2–7.3 and ~8 mmHg, respectively), triglycerides (by 23–66 mg/dl), and ALT levels (by 10–22 UI/L) depending on the prediabetes phenotype.

Conclusion and Relevance: It is worth screening prediabetes in children aged <10 years old with obesity classes II and III and in adolescents. In those with prediabetes, monitoring of blood pressure, triglycerides and ALT levels must be encouraged.

Abbreviations: 1 and 2HG, 1 and 2 h glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressures; FG, fasting glucose; FLD, fatty liver disease; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic assessment model algorithm of insulin resistance; HT, hypertension; IFG, impaired fasting glucose; IGI, insulinogenic index; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; ISSI-2, insulin secretion-sensitivity index-2; NGT, normal glucose tolerance; OPBG, "Bambino Gesù" Children's Hospital; SBP, systolic blood pressures; T2D, type 2 diabetes.

1 | INTRODUCTION

With epidemic obesity in childhood, the incidence of prediabetes is worldwide rising in youth.[1] Haemoglobin A1c (HbA1c), fasting plasma glucose (FPG) and 2-h plasma glucose are all recommended for screening of prediabetes in youth.[2] Even so, the three tests reflect different features of impaired glucose metabolism and therefore do not identify in most of the cases the same individuals, particularly in younger age groups.[3]

Impaired fasting glucose (IFG) is associated prevalently with altered hepatic and kidney insulin resistance and with reduced fasting insulin secretion.[4] Impaired glucose tolerance (IGT) is mostly associated with reduced first and second phase insulin secretions, increased insulin resistance and, thus, reduced disposition index (DI), that is, the estimate of beta-cell function relative to insulin sensitivity.[4] HbA1c is not associated with severity of hampered insulin sensitivity and/or secretion, but reflects overall glucose levels and particularly postprandial ones.[4]

In 2018 the American Diabetes Association (ADA) made the recommendation to screen for prediabetes all youth with overweight at 10 years of age or above with one or more of risk factors among nonwhite race, family history of type 2 diabetes (T2D) in first or second degree relatives, maternal gestational diabetes, or signs of insulin resistance, specifically acanthosis nigricans, hypertension, dyslipidemia, polycystic ovarian syndrome or small-for-gestational age birth weight.[5] Indeed, the prevalence of dysglycemia, escalated from ~23% in youth with overweight and one risk factor to up to ~45% in youth with ≥4 risk factors.[6]

In line with current guidelines for screening,[5] most of the studies have investigated prevalence of dysglycemia in children older than 10 years old. Analysis of data from the National Health and Nutrition Examination Survey from 1999 through 2012 demonstrated that prevalence of IFG and prediabetic HbA1c increased with worsening of obesity from overweight to class III obesity in adolescents older than 12 years old. Data on younger children were not available. Noteworthy, in the studied population, prevalence of other obesity comorbities such as high blood pressure and dyslipidemia increased with worsening obesity in both children and adolescents.[7]

To date, statistics on prevalence of prediabetes in children below 10 years of age are scarce with no recommendation for screening in this age-group.

Since the severity of obesity influences as said the rate of other comorbidities which occur significantly at higher rate also in children affected by obesity classes II and III as it does in grown up teens,[7] and obesity and associated cardiovascular morbidities during childhood increase the risk of substantial complication and death in adulthood,[8,9] we speculate that prediabetes occurs already in children with severe obesity.

To test the hypothesis, we investigated prevalence of prediabetes in children below age 10 years old as compared to adolescents in relation to the severity of their obesity. We investigated also the association between the different phenotypes of isolated and combined prediabetes with metabolic features such as oral glucose tolerance test (OGTT) derived measures of insulin sensitivity and secretion, cardiovascular risk factors and alanine aminotransferase (ALT) levels in respect to age and obesity class.

2 | METHODS

2.1 | The 'Bambino' meta-cohort

The 'Bambino' meta-cohort ($N = 4887$) includes data of children and adolescents who were normal-weight ($N = 937$, 19.2%) or presented with overweight or obesity ($N = 3950$) at the enrolment. They were all referred by general practitioners from the metropolitan area of Rome to participate in 'The Bambino Study: Profiling the genetic risk of complex diseases in the Italian population'[10] or in other research studies on the risk of obesity and metabolic abnormalities associated to it. Studies were run between 2006 and 2016 at the Bambino Gesù Children's Hospital (OPBG, Ospedale Pediatrico Bambino Gesù).[11–16]

At the time of the enrolment, participants, aged 12 months to 25 years old were healthy; that is with no diagnosis of chronic illness, genetic, renal or endocrine disease. None was following a weight loss or an intensive exercise program or was prescribed any medication including oral contraceptives.

For the purposes of this study, we evaluated data of individuals with overweight and obesity, aged 4–18 years old who had complete dataset of anthropometrics, lipid profile, liver function test and 5-point OGTT glucose data. In a group of participants, data on HbA1c were available. Participants' medical history was recalled by the hospital electronic medical record (EMR). Race/ethnicity was classified as White (W), Black African, Hispanic and Asian.

2.2 | Ethics

The study was approved by the OPBG Ethics Committee (#2050/2020). Written informed consent in accordance with the Helsinki declaration was obtained from the child's parents or legal guardians. Children over age 6 and adolescents provided written absent.

2.3 | Anthropometric measurements and biochemical assays

Anthropometry was evaluated and laboratory tests were performed in all the participants according to established protocols at OPBG. Participants were asked to refrain from intensive physical activity in the 3 days prior to any study. Weight and height were measured using standard

procedure. Up to 3 blood pressure measurements were taken by a physician after a 5-min rest and the mean of 3 measurements was recorded.[13]

Blood samples were drawn after 8–12 h fast. A standard OGTT (1.75 g/kg body weight up to a maximum of 75 g) was performed with flavoured glucose (Glucosio Sclavo Diagnostics, 75 g/150 ml). One anti-cubital i.v. catheter was inserted for blood sampling and was maintained patent by a normal saline drip during the test. Glucose level was measured by glucose oxidase technique (Cobas Integra, Roche) and insulin by a chemiluminescent immunoassay method (ADVIA Centaur analyser; Bayer Diagnostics). HbA1c was measured using an automated high performance liquid

chromatography VARIANT II TURBO Haemoglobin Testing System (Bio Rad, Hercules, CA).

2.4 | Calculation and case definition

Body mass index (BMI), and sex- and age-specific standard deviation scores (SDS) of BMI were calculated.[17] Obesity was graded as recommend by the Centers for Disease Control as class I obesity (BMI ≥95th percentile), class II obesity (BMI ≥120% of the 95th percentile) and class III obesity (BMI ≥140% of the 95th percentile).[18]

TABLE 1 Clinical and biochemical characteristics of children and adolescents ($n = 3110$)

	Children N = 1027	Adolescents N = 2083	p
Age (years)	8.2 (8.1–8.3)	12.7 (12.6–12.8)	<0.0001
Males % n (%)	567 (55)	1029 (49)	0.2
Overweight n (%)	87 (8.5)	942 (45.2)	
Obesity class I n (%)	356 (34.6)	855 (41)	
Obesity class II n (%)	416 (40.5)	219 (10.5)	
Obesity class III n (%)	168 (16.4)	67 (3.3)	
BMI (kg/m^2)	29.4 (29–29.7)	29.2 (29–29.4)	0.8
BMI z score (SDS)	2.63 (2.59–2.68)	2.6 (2.55–2.62)	0.4
Waist circumference (cm)	87.2 (86–88.5)	87 (86–87-9)	0.8
Systolic blood pressure (mmHg)	116 (114.8–117.3)	116 (115–116.9)	0.9
Diastolic blood pressure (mmHg)	66 (65–66.7)	66 (65.7–67)	0.3
Total cholesterol (mg/dl)	154 (152.3–156.5)	158 (156.7–159.5)	0.004
HDL cholesterol (mg/dl)	47 (46.1–47.6)	47 (46.6–47.7)	0.5
Non-HDL cholesterol (mg/dl)	106 (104.3–108.6)	110 (108.6–111.6)	0.006
Triglycerides (mg/dl)	87 (83.4–90.8)	92 (88.8–94.5)	0.06
ALT (UI/L)	28.6 (26.2–28.4)	33.3 (28.6–30.5)	0.004
Fasting glucose (mg/dl)	81(80.3–81.5)	81 (80.4–81.2)	0.8
1 h plasma glucose (mg/dl)	123 (121.3–125.5)	129 (127.3–130.2)	<0.0001
2 h plasma glucose (mg/dl)	107 (105.4–108.2)	110 (109.1–111)	<0.0001
Fasting insulin (μUI/ml)	16.2(15.5–16.9)	16.8 (16.2–17.4)	0.2
Glycated haemoglobin (mmol/mol)	34 (33.3–34.0)	34 (33.8–34.4)	0.06
HOMA-IR (dimensionless)	3.3 (3.15–3.47)	3.43 (3.30–3.56)	0.3
HOMA-B (dimensionless)	375 (344–406)	364 (344.6–384.7)	0.6
Insulin Sensitivity index (μmol/kg/pM)	4.9 (4.7–4.4)	4.6 (4.4–4.7)	0.03
Insulinogenic Index (pmol/μmol)	0.48 (0.45–0.51)	0.53 (0.51–0.55)	0.008
AUC$_G$ (mg/dl/min)	115.6 (114–117)	118 (117–119)	0.001
AUC$_I$ (μUI/ml/min)	95 570 (59116–132 024)	93 883 (78292–109 475)	0.9
Ratio AUC$_G$/AUC$_I$ (dimensionless)	2.7 (2.30–3.22)	2.6 (2.47–2.79)	0.4
ISS-2 (dimensionless)	5.5 (4.40–6.70)	5.5 (4.37–6.72)	0.9
Disposition index (dimensionless)	1.7 (1.61–1.75)	1.7 (1.645–1.74)	0.8

Note: Data are expressed as mean and 95% CI.

Abbreviations: ALT, alanine aminotransferases; AUC, area under the curve; BMI, body mass index; G, glucose; HDL, high density lipoproteins; HOMA-IR, homeostasis model assessment of insulin resistance; I, insulin; IGI, insulinogenic index; ISI, insulin sensitivity index; ISS-2, Insulin Secretion-Sensitivity Index-2; TG, triglycerides.

Glucose tolerance was classified according to the ADA criteria.[5] Prediabetes was defined either as IFG (FG >100 mg/dl and <126 mg/dl); IGT (2HG ≥140 and <200 mg/dl); or high HbA1c (h-HBA1c, HbA1c ≥5.7 and <6.4%).

Hypertension (HT) was defined as systolic (SBP) or diastolic blood pressure (DBP) >95th percentile for age, height and gender in patients below 13 years of age and as ≥120 mmHg in those older.[19]

Dyslipidemia was defined as total cholesterol at or above 200 mg/dl, HDL-C at or below 35 mg/dl, and triglycerides ≥100 (0–9 years), and ≥130 (10–19 years).[20]

As endophenotypes of insulin metabolism, we computed the following parameters: the Homeostasis Model Assessments of fasting Insulin Resistance,[21] the whole-body Insulin Sensitivity Index (ISI),[22] the areas under the glucose and insulin curve (AUC_G and AUC_I) by using the trapezoidal rule, the insulinogenic index (IGI),[23] and the Insulin Secretion-Sensitivity Index-2, ISSI-2.[24] The DI was calculated as product of ISI and IGI.

High levels of ALTs were defined according to the 'Screening ALT for Elevation in Today's Youth' cutoffs (ALT ≥26UI/L in boys and ≥22 UI/L in girls).[25]

2.5 | Statistical analysis

Categorical data were presented as count and percentage, while continuous data as mean and 95% CI. Between-group comparisons of categorical variables were performed by Chi-square test or Fisher exact test as appropriate, while between-group comparisons of continuous variables were performed by using the Student t test. To compare the prevalence of each glycaemic status category or prediabetes across groups within the sample, we fitted logistic regression models with the different prediabetes phenotypes as dependent variables and age group, sex and class of obesity as independent variables. Therefore, we calculated the predictive margins, that is, the probability of each prediabetes phenotype by level of each independent variable and adjusted by the remaining independent variables included in the models.

The *Cohen's kappa* index was used to assess the agreement between IFG, IGT and h-HBA1c.

Multivariable linear regression analyses were performed in order to assess the simultaneous effect of prediabetes phenotype (isolated and combined IFG, IGHT and high HbA1c, and any prediabetes), age class (children as reference) and class of obesity (overweight as reference) on cardiovascular risk factors (SBP; DBP, total and HDL-cholesterol, triglycerides), ALT levels and parameters of insulin metabolism (ISI; IGI, DI and ISSI-2).

The ROC (receiver operating characteristic) analysis was used to test the diagnostic accuracy of ALT levels to identify children and adolescents with prediabetes. The area under the ROC curve (AUC) was used to determine the best ALT cut-off based on combination of sensitivity and specificity levels.

Analyses were performed with Stata 17 (StataCorp LLC, College Station, TX). A p value <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Description of the population

From the sample of 3950 participants with overweight or obesity, we excluded 611 participants being below 4 years old or having incomplete dataset. Participants belonging to the groups of young adults, non-white race and with diabetes were excluded owing to the low number. In detail, 41 participants being above 18 years of age, and 78 being not of white race; 10 patients were excluded having T2D (3 patients with isolated FG, 6 with isolated 2HG in the diabetic range, and 2 with both FG and 2HG in the diabetic range; marginal prevalence of T2D 0.3%, 95% CI 0.18–0.56).

Data of the residual 3110 patients (1596 males, 51%) were used for the analysis: 1029 with overweight (33%), 1211 (39%) with obesity class I, 635 (20.5%) with obesity class II and 235 (7.5%) with obesity class III. Sex distribution was not different between groups. Table 1 shows clinical and biochemical characteristics of children (N = 1027, 33%) and adolescents (N = 2083, 67%). We found significant differences between children and adolescents in ISI ($p = 0.03$), IGI ($p = 0.008$), and AUC_G ($p = 0.001$). Adolescents showed values of 1HG and 2HG significantly higher than children ($p < 0.0001$ for both).

TABLE 2 Prevalence of obesity co-morbidities in children and adolescents by class of obesity

		High blood pressure	High total cholesterol	Low high density lipoproteins cholesterol	High triglycerides	High alanine aminotransferases
Children (n = 1027)	Overweight (n = 87)	10.7%[a]	23%[b]	8.21%[b]	12.9%	33%[b]
	Obesity class I (n = 357)	21.6%[b]	29%[b]	6.6%[d]	13.7%	40.2%
	Obesity classes II and III (n = 584)	41.8%	27.6%[b]	10%[e]	15.6%	46%
Adolescents (N = 2083)	Overweight (n = 942)	24.7%[a]	9%	12.7%	9%	46.4%
	Obesity class I (n = 855)	42.0%	6.6%	11%	11.1%	46.5%
	Obesity classes II and III (n = 286)	48.6%	7%	15%	14.4%	46.1%

Note: Comparison of comorbidity prevalence by class of obesity within each age group ([a]$p < 0.0001$) and by age within classes of obesity (i.e., overweight versus overweight) in children versus adolescents ([b]$p < 0.0001$; [c]$p = 0.01$; [d]$p = 0.02$; [e]$p = 0.05$).

TABLE 3 Marginal prevalence of isolated and combined prediabetes in children and adolescents

Prediabetes	IFG	IGT	h-HbA1c	IFG and IGT	IFG and h-HbA1c	IGT and h-HBA1c	IFG and IGT and h-HBA1c	Any prediabetes
Whole sample (n = 3110)								
Children	2.18 (0.02–4.09)	6.71 (3.26–10.16)	3.87 (1.04–6.72)	0.059 (0.013–0.106)	Empty	Empty	Empty	13.9 (9.21–18.6)
Adolescents	3.44 (1.42–5.57)	9.42 (6.26–12.58)	5.56 (3.11–8.0)	0.09 (0.05–1.43)	-	-	-	24.6 (19.9–29.2)[a]
Males	3.26 (1.36–5.16)	8.09 (5.16–11.01)	4.21 (2.04–6.37)	0.081 (0.039–1.247)	-	-	-	19.7 (15.4–23.9)
Females	2.60 (0.08–4.38)	8.67 (5.55–118.80)	5.75 (3.12–8.34)	0.085 (0.037–1.338)	-	-	-	21.3 (16.8–25.8)
Overweight	1.85 (1.48^-05–3.71)	5.21 (2.10–8.31)	4.70 (1.69–7.71)	0.04 (0.005–0.082)	-	-	-	13.8 (9.18–18.6)
Obesity class I	2.88 (0.09–4.86)	9.40 (5.93–12.87)	4.67 (2.17–7.18)	1.13 (0.054–0.173)	-	-	-	21 (16.2–25.8)[b]
Obesity classes II–III	4.58 (0.06–8.50)	10.72 (5.11–16.33)	5.81 (1.47–10.15)	0.09 (0.019–0.170)	-	-	-	28.7 (20.47–37)[c]
Children (n = 1027)								
Males	2.92 (−0.03–6.17)	11.52 (5.39–17.65)	2.93 (−0.32–6.20)	0.07 (0.0018–0.147)	-	-	-	20.33 (12.6–28.1)
Females	2.96 (−0.003–6.25)	5.0 (0.73–9.28)	5.89 (1.34–10.4)	0.07 (−0.009–0.157)	-	-	-	15.9 (8.76–23.0)
Overweight	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
Obesity class I	1.49 (−0.14–4.39)	8.76 (2.10–15.42)	1.52 (−1.43–4.47)	1.12 (0.003–0.221)	-	-	-	13.33 (5.22–21.4)
Obesity classes II–III	3.64 (0.50–6.78)	8.11 (3.55–12.68)	5.78 (1.90–9.66)	0.051 (−0.0066–0.109)	-	-	-	20.5 (13.7–27.3)
Adolescents (n = 2083)								
Males	3.55 (1.12–5.98)	6.55 (3.36–9.78)	5.09 (2.16–8.03)	0.081 (0.033–1.42)	1.77 (0.0004–3.51)	3.85 (1.37–6.33)	1.77 (0.0004–3.51)	19.9 (14.7–25.1)
Females	2.43 (0.31–4.54)	10.85 (6.56–15.14)	5.57 (2.50–8.64)	0.094 (0.033–1.56)	1.45 (−0.018–3.10)	4.55 (1.63–7.47)	1.45 (−0.018–3.10)	24.4 (18.5–30.2)
Overweight	2.20 (0.06–4.33)	5.95 (2.54–9.36)	5.45 (2.17–8.74)	0.0529 (0.007–0.099)	1.64 (−0.02–3.49)	2.72 (0.03–5.07)	1.64 (−0.02–3.49)	16.9 (11.5–22.3)
Obesity class I	3.50 (0.09–6.05)	9.75 (5.70–13.81)	5.92 (2.67–9.17)	0.11 (0.044 0.189)	1.49 (−0.01–3.17)	4.43 (1.59–7.26)	1.49 (−0.01–3.17)	24.6 (18.7–30.5)
Obesity classes II–III	4.04 (−0.15–9.59)	14.41 (3.73–25.11)	2.21 (−2.09–6.52)	0.140 (0.003 0.277)	2.09 (−0.20–6.19)	8.98 (0.4–7.5)	2.09 (−0.20–6.19)	31.6 (17.9–45.3)[d]

Note: Data are expressed as mean and 95% CI. Unadjusted marginal prevalence in children versus adolescents and adjusted by sex and obesity class in children and adolescents are reported. *p* refers to the statistical significance at the model.

Abbreviations: h-HBA1c, high glycated haemoglobin; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

[a]OR 2.04 (95% CI 1.21–3.44), *p* = 0.007.

[b]OR 1.65 (95% CI 1.01–2.70), *p* = 0.042.

[c]OR 2.54 (95% CI 1.36–4.72), *p* = 0.003.

[d]OR 2.28 (95% CI 1.08–4.8), *p* = 0.03.

TABLE 4 Determinants associations between cardiometabolic outcomes and prediabetes phenotypes in multivariate models adjusted for classes of age and obesity

	SBP (mmHg)	DBP (mmHg)	Triglycerides (mg/dl)	HDL-C (mg/dl)	Total-C (mg/dl)	Non HDL-C (mg/dl)	Alanine Amino Transferase (UI/L)	Insulin-sensitivity Index (μmol/kg/pM)	Disposition index (dimensionless)	Insulino-genic Index (pmol/μmol)	ISSI-2 (dimensionless)
IFG	2.07	1.16	18.3[d]	−3.61[e]	2.87	5.44	10.49[e]	−0.94[e]	−0.89[a]	−0.11[f]	−3.07
Adolescents	4.24[a]	2.52[a]	7.01[e]	−1.70[d]	0.51	2.28	4.92[d]	−0.95[a]	−0.04	0.09[a]	−0.31
Obesity	5.01[a]	2.41[a]	5.76[c]	−2.29[a]	−0.28	1.83[g]	0.55	−0.77[a]	−0.06[g]	0.06[a]	−0.38
IGT	2.95[e]	2.76[c]	25.3[a]	−2.19[f]	4.58[g]	5.49[f]	21.7[a]	−2.10[a]	−0.66[a]	0.04	−2.29
Adolescents	4.0[a]	2.29[c]	5.67[g]	−1.64[e]	0.32	2.09	4.07[e]	−0.86[a]	−0.02	0.096[a]	−0.22
Obesity	4.87[a]	2.27[a]	5.41[c]	−2.29[a]	−0.35	1.77[g]	0.31	−0.73[a]	−0.05	0.057[a]	−0.36
h-HbA1c	2.90	1.60	27.5[a]	−2.7[g]	2.85	5.79	4.10	−1.27[f]	−0.37[e]	0.13	1.59
Adolescents	4.67[a]	1.85	12.1[f]	−2.14[g]	0.14	2.14	6.93[d]	0.76	−0.09	0.10[g]	0.21
Obesity	4.85[a]	1.53[f]	6.41[g]	−2.28[a]	−2.82	−0.58	2.89[g]	0.87[c]	−0.08	0.08[d]	−1.93
IFG and IGT	2.72	1.80	22.9[g]	−7.4[e]	−6.96	−0.31	21.3[d]	−2.25[d]	−1.08[a]	−0.06	−3.5
Adolescents	4.24[a]	2.52[a]	7.06[e]	−1.69[d]	0.57	2.3	4.88[d]	−0.95[a]	−0.044	0.09a	−0.31
Obesity	5.02[a]	2.41[a]	6.02[a]	−2.32[a]	−0.21	1.92[g]	0.72	−0.78[a]	−0.07[e]	0.05[g]	−0.41
IFG and h-HbA1c	7.27	8.26[g]	51.6[e]	−5.18	−21.4[f]	−16.2	12.8	−0.93	−0.79[f]	−0.09	−3.8
Adolescents	4.73[a]	1.82	13.7[e]	−2.29[f]	0.46	2.6	7.03[d]	−0.84[f]	−0.10	0.11[f]	0.38
Obesity	4.88[a]	1.55[f]	7.13[g]	−2.35[a]	−2.83	−0.52	2.95[g]	−0.89[e]	−0.08	0.09[e]	−1.89
IGT and h-HbA1c	6.78[g]	5.29[g]	54.3[a]	−3.42	−1.87	1.62	14.06[d]	−1.90	−0.45	0.43[a]	−2.67
Adolescents	4.55[a]	1.72	11.1[g]	−2.14[g]	0.42	2.43	6.4[d]	−0.73	−0.089	0.08	0.48
Obesity	4.83[a]	1.51[f]	6.23[g]	−2.29[a]	−2.74	−0.5	2.7[g]	−0.85[d]	−0.07	0.08[e]	−1.85
IFG and h-HbA1c and IGT	7.27	8.26[g]	14.3[d]	−0.73	5.09	5.89[f]	6.75[d]	−0.59	−0.51[a]	−0.00	−1.52
Adolescents	4.73[a]	1.82	12.3[f]	−2.24[f]	−0.26	1.83	6.44[d]	0.79	−0.06	0.11[g]	0.50
Obesity	4.88[a]	1.55[f]	5.94	−2.28[a]	−3.13	−0.90	2.48	−0.86[d]	−0.05	0.09[e]	−1.78
Any prediabetes	7.27	8.26[g]	66.2[d]	−6.59	−22.9[g]	−16.24	15.4	−1.23	−0.75[f]	−0.039	−3.8
Adolescents	4.73[a]	1.82	13.4	−2.26[f]	0.55	2.68	6.94[d]	−0.83	−0.10	0.11[g]	0.39
Obesity	4.88[a]	1.55[f]	7.28	−2.3[a]	−2.87	−0.55	2.96[g]	−0.89[c]	−0.08	0.09[c]	−1.89

Note: Children and overweight categories were used as references in the models. Statistical significance: [a] $p < 0.0001$; [b] $p \leq 0.0005$; [c] $p \leq 0.001$; [d] $p \leq 0.005$; [e] $p \leq 0.01$; [f] $p \leq 0.02$; [g] $p \leq 0.05$.

Abbreviations: DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; ISSI-2, insulin secretion index-2; SBP, systolic blood pressure; Total-C, total cholesterol.

Table 2 informs prevalence of high blood pressure, high total cholesterol, triglycerides and ALT, and low HDL-cholesterol in children and adolescents with different class of obesity. Prevalence of high blood pressure increased by worsening of obesity in children as well as in adolescents ($p < 0.0001$ for both age groups) and it was significantly more prevalent in adolescents with obesity class I than in children matched for class of obesity ($p < 0.0001$). When matching for class of obesity, high total cholesterol ($p < 0.0001$ for all the comparisons) was found more frequently in children than in adolescents and, vice versa, low HDL-cholesterol more frequently in adolescents (p between 0.01 and 0.05). Children with overweight had lower prevalence of high ALT as compared to adolescents belonging to the overweight class of obesity ($p < 0.0001$).

3.2 | Phenotypes of prediabetes

Table 3 reports unadjusted and adjusted prevalence of isolated and combined phenotypes of prediabetes and overall prevalence of prediabetes in the two groups. No child with overweight had prediabetes (empty rows in Table 2). Adolescents had significantly higher risk of prediabetes than children (OR 2.04; 95% CI 1.21–3.44; $p = 0.007$).

Prevalence of prediabetes increased significantly with the worsening of the obesity status from overweight to obesity classes I–III and the highest prevalence was seen in adolescents suffering obesity classes II–III. Indeed, in the whole population of children and adolescents, being affected by obesity class I (OR 1.65; 95% CI 1.01–2.70, $p = 0.042$) and class II or III (OR 2.54, 95% CI 1.36–4.72, $p = 0.003$) was associated with higher risk of prediabetes as compared to presenting with overweight. In the group of adolescents, obesity classes II–III was associated with an OR for prediabetes of 2.28 (95% CI 1.08–4.8, $p = 0.03$).

Cases of isolated IFG, IGT and high HbA1c were not statistically different between the two groups. Categories of combined high HbA1c with either IFG or IGT were represented only in adolescents while combined IFG and IGT were present in both children and adolescents.

Cohen's Kappa coefficient, an estimate of inter-rater reliability, was 0.117 between IFG and IGT ($p < 0.0001$); 0.13 ($p = 0.0004$) between IFG and high HbA1c; 0.197 ($p < 0.0001$) between IGT and high HbA1c; and 0.085 ($p < 0.0001$) among the three phenotypes.

3.3 | Association of prediabetes phenotypes and cardiometabolic outcomes

Table 4 reports the effect of prediabetes phenotypes on cardiometabolic outcomes, adjusted for age group (children as reference category) and class of obesity (overweight as reference category) as estimated by multivariate linear regression models. Depending on the phenotype of prediabetes analysed in the model, there was an average increase of SBP ranging from 2 to 7.8 mmHg and of 8 mmHg increased DBP in IFG and h-HbA1c phenotypes. IFG, IGT and h-HbA1c were associated with a dramatic increase of triglycerides

levels between 23 and 27.5 mg/dl and any prediabetes with an increase up to 66 mg/dl. As to the association of prediabetes with ALT levels, we found an increase between 10 UI/L in cases with IFG and 22 UI/L in those with IGT. h-HbA1c was associated with a statistically significant increase of ALT levels only when the phenotype was combined with IGT alone or in association to IFG and, suggesting that it is worth screening and monitoring ALT levels in cases of prediabetes as defined by IFG and/or IGT.

On the other hand, the accuracy of ALT levels to diagnose prediabetes was fair for the combination of IFG and IGT (AUROC 0.76; 95% CI 0.68–0.84); good for isolated IGT (AUROC 0.67; 95% CI 0.62–0.71); combined IGT and h-HBA1c (AUROC 0.66, 95% CI 0.54–0.79); combined IFG and h-HBA1c (AUROC 0.64, 95% CI 0.46–0.83) and fail for any prediabetes (AUROC 0.61, 95% CI 0.55–0.66), isolated IFG (AUROC 0.60, 95% CI 0.53–0.67) and isolated h-HbA1c (AUROC 0.54, 95% CI 0.46–0.63).

4 | DISCUSSION

We report prevalence of overall prediabetes as high as ~14% in children and ~25% in adolescents with overweight and obesity from the 'Bambino' meta-cohort.

In both age groups, most of the prediabetes cases were represented by people with isolated IGT followed by cases with isolated high HbA1c, and lastly by those with isolated IFG. Rates of isolated IFG, IGT and high HbA1c were not significantly different between age groups.

Among cases of combined prediabetes, the combo IFG and IGT was found as the most frequent in both children and adolescents. Very few cases had combined high HbA1c with IFG or IGT or both; and they were exclusively adolescents.

4.1 | Prediabetes in children and adolescents

The finding that prediabetes is a highly prevalent condition in adolescents with obesity is consistent with studies in other large populations, including the latest report from the 2005 to 2016 National Health and Nutrition Examination Survey (NHANES).[26] On top, and worth noting, we extend this observation to children aged <10 years old, demonstrating that children with obesity classes II and III have increased risk of certain prediabetes phenotypes as compared to overweight peers. To the best of our knowledge no previous study investigated prevalence of different prediabetes phenotypes by class of obesity in such a large population of children with obesity.

In adolescents with obesity, we found lower prevalence of IFG and higher prevalence of IGT as compared to the latest NHANES report.[26] A lower prevalence of IFG was noted also in the group of normal weight children and adolescents belonging to the 'Bambino' meta-cohort (~0.8%, unpublished data). Our population was made exclusively of white race individuals and we believe that genetics and ethnicity mix might have played a significant role in conjunction with

under investigated environmental factors (food and lifestyle habits, exposure to chemicals, etc.) to magnify differences in the prevalence of the three phenotypes in our population as compared to the multiracial NHANES people. The core of the 'Bambino' meta-cohort was a study aimed at disentangling genetic contribution to values of fasting glucose in Italian children and adolescents. In a group of 1660 individuals with BMI ranging from normal weight to obesity class III, we observed that some genetic variants known to influence the individual's risk of T2D risk, contributed to higher fasting glucose.[10]

Rates of prediabetes increased significantly with growing older from childhood to adolescence but also with worsening of obesity from overweight to obesity class III. Therefore, our findings highlight the importance to grade severity of obesity as recommend by the CDC and to screen for prediabetes before age 10 years-old those children with classes II and III obesity (as diagnosed by BMI ≥120% and 140% of the 95th percentile, respectively).

4.2 | Association between prediabetes phenotypes and cardiovascular risk factors

Findings underline also the need for close monitoring of cardiovascular risk factors, that is, in the first place triglycerides levels and blood pressure, in children and adolescents with prediabetes. Any prediabetes was associated with an average increase of fasting triglycerides as high as 66 mg/dl which is clinically relevant, while there was a graded and significant rise of triglycerides levels from IFG to IGT. Nevertheless high HbA1c, isolated or combined with IFG or IGT, contributed to triglycerides upsurge by 27–54.3 mg/dl.

Combined h-HbA1c and any prediabetes were also associated with the highest increase of systolic and diastolic blood pressure.

The most dramatic decrease of HDL-C values was seen in cases with combined IFG and IGT, any prediabetes and combined IFG and h-HbA1c.

Association between prediabetes phenotypes and values of total and non-HDL-cholesterol were not clinically consistent in our models.

Growing older and severity of obesity contributed significantly in some cases to the variability of triglycerides, HDL-cholesterol and blood pressure values in our population. The extent of their contribution varied depending on the phenotype of prediabetes analysed in the model (Table 4).

4.3 | Prediabetes and raised levels of ALT

In a general population of children with normal weight and obesity, those who were diagnosed with obesity had values 10 U/L higher than normal-weight children and values higher of 21–25 UI/L were suggestive of fatty liver disease.[25] In our population, the worsening of obesity from overweight to obesity classes I to III was not associated with any significant increase of ALT. In models of IFG, IGT and their combo, the most influential contributor was the altered glucose phenotype suggesting an association of IFG and IGT with liver damage which is

unrelated to the severity of obesity. IFG, IGT and their combination were significantly associated with an increase of ALT levels by ~10 to 21 UI/L. Consistently with our findings, the large German 'Adipositas Patienten Verlaufsbeobachtung' study found significantly increased risk of prediabetes and T2D in children and adolescents with mild (25–50 UI/L) and advanced (>50 UI/L) increase of ALT levels.[26]

Nevertheless, in our population, accuracy of ALT in the diagnosis of these phenotypes of prediabetes was between fair (combined IFG and IGT) and good (isolated IGT). Values of serum ALT between 23 and 25 UI/L were diagnostic of these conditions with sensitivity 70%–80% and specificity 50%–60% suggesting that current upper threshold of normal is too high in youth with obesity. Having any prediabetes trended to be associated with an increase of ALT levels in average by 15 UI/L which is clinically unneglectable. On the contrary, there was no association of ALT levels with the prediabetes phenotype as diagnosed by high HbA1c values.

Thus far, there are no guidelines for ALT screening in children and adolescents with obesity. The serum alanine aminotransferase level is a critical parameter for evaluating liver injury in non-alcoholic fatty liver disease. However, the currently accepted upper limits of normal for serum ALT are debated, as they may be excessively high.[27] Our findings demonstrated that a substantial number of children and adolescents with prediabetes have normal serum ALT levels, defined by current thresholds, and we were unsuccessful in the attempt at identifying accurate cutoffs of ALT to diagnose prediabetes. Nevertheless, close monitoring of serum ALT fluctuation and liver ultrasound evaluation should be recommended especially in youth with obesity and particularly in girls who have IFG and/or IGT in view of the high prevalence of prediabetes in young patients with fatty liver disease. The Non-Alcoholic Steatohepatitis Clinical Research Network found an estimated prevalence of prediabetes in youth with fatty liver as high as ~23% with girls having 1.6 greater odds of having prediabetes than boys.[28]

4.4 | Strengths and limitations

Although we investigated prevalence of prediabetes in a large population of exclusively white non-Hispanic children and adolescents, we could not investigate sexual dimorphism of prediabetes. Owing to retrospective design of the study, we could not assess the association between prediabetes and pubertal stage in the two sexes since the information was not consistently annotated in the EMRs.

Issues related to prediabetes in youth such as sexual dimorphism, test reproducibility, rate of persistence and progression to diabetes in adulthood of each phenotype remain important gaps in knowledge as well as socioeconomic, cultural and environmental factors that may influence prevalence and progression.

5 | CONCLUSIONS

While overt T2D is still less frequent than in other countries,[29] the prevalence of prediabetes in the Italian youth is worrying and its scale

is not less big than in the United States. Of note, we found that prediabetes is highly prevalent also in children who suffer obesity classes II and III and this condition is not exclusive of the teenage. In both age groups, we found many cases with HbA1c within the pre-diabetic range suggesting that HbA1c is worth testing in the screening of children with severe obesity and adolescents with excess body weight. Combined phenotypes of h-HBA1c with IFG or IGT were found only in the group of adolescents. Combined IFG and IGT were in both groups.

The very little overlap of the three prediabetes phenotypes suggests the need of testing all the three in youths with overweight and obesity while the optimal approach to screen and diagnose prediabetes and diabetes in youth remains to be defined in longitudinal studies.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

All authors declare no conflict of interest related to the manuscript.

AUTHOR CONTRIBUTIONS

Melania Manco had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design; acquisition, analysis, or interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; supervision. Stefania Pedicelli, Danilo Fintini, Elena Inzaghi, Annalisa Deodati, Maria Rita Spreghini, Carla Bizzarri, Michela Mariani performed acquisition, analysis, or interpretation of data. Stefania Pedicelli, Danilo Fintini, Elena Inzaghi, Annalisa Deodati, Maria Rita Spreghini, Carla Bizzarri, Michela Mariani performed critical revision of the manuscript for important intellectual content. Lucilla Ravà performed acquisition, analysis, or interpretation of data; critical revision of the manuscript for important intellectual content; statistical analysis. Marco Cappa performed acquisition, analysis, or interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding, supervision. Stefano Cianfarani performed acquisition, analysis, or interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding, supervision.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are not publicly available for reasons related to privacy and participants' consent but are available from the corresponding author on reasonable request.

ORCID

Danilo Fintini https://orcid.org/0000-0002-0103-7951
Melania Manco https://orcid.org/0000-0002-6581-975X

REFERENCES

1. International Diabetes Federation facts and figures. The IDF Diabetes Atlas Tenth edition 2021. https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html
2. American Diabetes Association. Standards of medical care in diabetes—2010. *Diabetes Care*. 2010;33((Supplement 1)):S11-S61. doi:10.2337/dc10-S011
3. Vajravelu ME, Lee JM. Identifying Prediabetes and type 2 diabetes in asymptomatic youth: should HbA1c be used as a diagnostic approach? *Curr Diab Rep*. 2018;18(7):43.
4. Bergman M, Abdul-Ghani M, DeFronzo RA, et al. Review of methods for detecting glycemic disorders. *Diabetes Res Clin Pract*. 2020;165:108233.
5. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. *Diabetes Care*. 2018;41(Suppl 1):S13-S27.
6. Saleh M, Kim JY, March C, Gebara N, Arslanian S. Youth prediabetes and type 2 diabetes: risk factors and prevalence of dysglycaemia. *Pediatr Obes*. 2022;17(1):e12841.
7. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med*. 2015;373(14):1307-1317.
8. Guo SS, Wu W, Chumlea WC, Roche AF. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. *Am J Clin Nutr*. 2002;76:653-658.
9. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. *N Engl J Med*. 2010;362:485-493.
10. Balkhiyarova Z, Luciano R, Kaakinen M, et al. Relationship between glucose homeostasis and obesity in early life-a study of Italian children and adolescents. *Hum Mol Genet*. 2021;30:ddab287.
11. Brufani C, Ciampalini P, Grossi A, et al. Glucose tolerance status in 510 children and adolescents attending an obesity clinic in Central Italy. *Pediatr Diabetes*. 2010;11(1):47-54.
12. Shashaj B, Luciano R, Contoli B, et al. Reference ranges of HOMA-IR in normal-weight and obese young Caucasians. *Acta Diabetol*. 2016;53(2):251-260.
13. Shashaj B, Bedogni G, Graziani MP, et al. Origin of cardiovascular risk in overweight preschool children: a cohort study of cardiometabolic risk factors at the onset of obesity. *JAMA Pediatr*. 2014;168(10):917-924.
14. Del Chierico F, Manco M, Gardini S, et al. Fecal microbiota signatures of insulin resistance, inflammation, and metabolic syndrome in youth with obesity: a pilot study. *Acta Diabetol*. 2021;58(8):1009-1022.
15. Manco M, Marcellini M, Devito R, Comparcola D, Sartorelli MR, Nobili V. Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. *Int J Obes*. 2008;32(2):381-387.
16. Manco M, Bedogni G, Marcellini M, et al. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. *Gut*. 2008;57(9):1283-1287.
17. Flegal KM, Wei R, Ogden CL, Freedman DS, Johnson CL, Curtin LR. Characterizing extreme values of body mass index-for-age by using the 2000 Centers for Disease Control and Prevention growth charts. *Am J Clin Nutr*. 2009;90(5):1314-1320.
18. Freedman DS, Berenson GS. Tracking of BMI z scores for severe obesity. *Pediatrics*. 2017;140(3):e20171072.

19. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics*. 2011;128((Suppl 5)):S213-S256.

20. Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics*. 2017;140(3):e20171904.

21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-419.

22. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9):1462-1470.

23. Phillips DI, Clark PM, Hales CN, Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabet Med*. 1994;11(3):286-292.

24. Retnakaran R, Shen S, Hanley AJ, Vuksan V, Hamilton JK, Zinman B. Hyperbolic relationship between insulin secretion and sensitivity on oral glucose tolerance test. *Obesity (Silver Spring)*. 2008;16(8):1901-1907.

25. Schwimmer JB, Dunn W, Norman GJ, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology*. 2010;138(4):1357-1364.

26. Koutny F, Weghuber D, Bollow E, et al. Prevalence of prediabetes and type 2 diabetes in children with obesity and increased transaminases in European German-speaking countries. Analysis of the APV initiative. *Pediatr Obes*. 2020;15(4):e12601.

27. Andes LJ, Cheng YJ, Rolka DB, Gregg EW, Imperatore G. Prevalence of Prediabetes among adolescents and young adults in the United States, 2005-2016. *JAMA Pediatr*. 2020;174(2):e194498.

28. Newton KP, Hou J, Crimmins NA, et al. Nonalcoholic steatohepatitis clinical research network. Prevalence of prediabetes and type 2 diabetes in children with nonalcoholic fatty liver disease. *JAMA Pediatr*. 2016;170(10):e161971.

29. SEARCH for Diabetes in Youth Study Group, Liese AD, D'Agostino RB Jr, et al. The burden of diabetes mellitus among US youth: prevalence estimates from the SEARCH for diabetes in youth study. *Pediatrics*. 2006 Oct;118(4):1510-1518.