Cardiometabolic Risk Markers of Normal Weight and Excess Body Weight in Brazilian Adolescents

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Abstract

Excess body weight leads to a variety of metabolic changes and increases the risk for cardiovascular diseases (CVD) in adulthood. The objective of this study was to investigate the presence of risk markers for CVD among Brazilian adolescents of normal weight and with excess body weight. The markers included blood pressure, C-reactive protein, homocysteine, tumor necrosis factor alpha, fibrinogen, fasting insulin and glucose, homeostasis model assessment insulin resistance (HOMA-IR), leptin, total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and triglycerides. We calculated odds ratios (OR) using logistic regression and adjusted for the potential confounders such as age, sex, physical activity and socioeconomic background. Compared to normal weight subjects, overweight/obese adolescents were more likely to have higher systolic blood pressure (OR = 3.49, $p < 0.001$), fasting insulin (OR = 8.03, $p < 0.001$), HOMA-IR (OR = 8.03, $p < 0.001$), leptin (OR = 5.55, $p < 0.001$) and LDL-c (OR = 5.50, $p < 0.001$), and to have lower serum HDL-c concentrations (OR = 2.76, $p = 0.004$). After adjustment for confounders, the estimates did not change substantially, except for leptin for which the risk associated with overweight increased to 11.09 (95% CI: 4.05–30.35). In conclusion, excess body weight in adolescents exhibits strong associations with several markers that are established as causes of CVD in adults. This observation stresses the importance of primary prevention and of maintaining a healthy body weight throughout adolescence in order to reduce the global burden of CVD.

Key words: adolescents, cardiovascular disease, overweight, obese, leptin, cardiometabolic markers, blood pressure, cholesterol, insulin, leptin.

Introduction

The prevalence of excess body weight (overweight and obesity) in childhood and adolescence has increased globally. In Brazil, in 2010, 20.6% of adolescents were classified as having excess body weight, with 5.0% of them being obese (Brasil 2010). Excess body weight in adolescence determines the risk for excess body weight in adulthood. Excess body weight in adulthood leads to a variety of metabolic changes, to insulin resistance and inflammation, produces changes in cardiometabolic risk markers, and increases the risk for cardiovascular diseases (CVD) (Mathieu et al. 2009; Bastien et al. 2014; McGown et al. 2014).

The pathophysiological processes linking excess body weight to atherosclerosis and CVD involve factors such as visceral obesity, excess ectopic fat, insulin resistance, atherogenic dyslipidemia, and hypertension (Bastien et al. 2014). Elevated circulating inflammatory markers predict an unfavorable course of acute coronary syndromes and represent new targets for therapy (Libby et al. 2002). Furthermore, there is evidence that adipose tissue is a key organ in the production and regulation of endocrine and paracrine hormones modulating both inflammation and metabolic processes (Bastien et al. 2014). Hormones such as leptin, along with inflammatory biomarkers such as high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor alpha (TNF- α), contribute to the inflammatory profile in the process of obesity (Bastien et al. 2014), and therefore to the risk for cardiometabolic diseases (Lowe 2001).

Studies of the past decade have revealed that these pathophysiological processes are not limited to obese adults, but that, in fact, elevated cardiometabolic risk markers are increasingly common among adolescents (Lobstein et al. 2004). Most of the studies were conducted in high-income countries where the prevalence of obesity has gradually increased over the past five decades (Lobstein et al. 2004). Brazil is a country of upper middle income

that has experienced a rapid nutritional transformation and a more recent rapidly developing obesity epidemic with current obesity rates of the same magnitude as reported for North America (Monteiro et al. 1995; Monteiro et al. 2002; Lobstein et al. 2004; Ng et al. 2014). Therefore, the objective of the present study was to investigate whether excess body weight among Brazilian adolescents is paralleled by an increased cardiometabolic risk profile.

Material and Methods

Study population

The study population consisted of a cross-sectional sample of 15–17-year-old high school students living in the city of Joinville, Santa Catarina, Brazil. The first phase of the study was carried out between May and November 2007. Joinville has 28 schools with 436 classes. A total of 2,195 students agreed to participate in the study, gave written informed consent, and completed a short survey of socioeconomic and demographic characteristics. They also took home an informed consent form to be completed and signed by their parents/guardians, which was returned by 1,104 (50.3%) of them.

The second phase of data collection took place between September and November 2008. All 1,104 participants of the first phase were invited to participate in the second phase of the study. They were contacted by phone and by a personal visit to their residence, and were informed about the day and place of data collection. At the end of the study, 222 students had attended data collection. Assessments during this phase included weight and height, blood pressure, and serum levels of hs-CRP, homocysteine, TNF- α , fibrinogen, fasting insulin, fasting glucose, leptin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG).

The research was carried out in accordance with the Declaration of Helsinki and the Ethics Committee of the University of Joinville Region approved the study (Case No. 005/2007).

Data collection

The anthropometric measurements were taken early in the morning after an overnight fast. The adolescents were weighed on a Filizola® digital scale (Curitiba, PR, Brazil) with a capacity of 180 kg to the nearest 0.1 kg, using light clothes (t-shirt and short), without shoes and accessories (caps and jewelry). Height was measured with a 200-cm Cardiomed® stadiometer (Curitiba, PR, Brazil) to the nearest 0.1 cm. The anthropometric measurements were performed according to the recommendations (Gordon et al. 1988), and performed in duplicate considering the average as the final measure. We calculated body mass index (BMI) [weight (kg)/height (m²)] and used the World Health Organization's age- and sex-specific cutoffs to create two groups: >85th percentile for participants considered to be overweight/obese and ≤85th percentile for participants considered to be underweight/normal weight (de Onis et al. 2007).

Physical activity was classified according to the International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003). Income was evaluated using a Brazilian instrument that estimates the purchasing power of urban individuals and families (Associação Brasileira de Empresas de Pesquisa 2003). Information about income and physical activity was collected by interview.

Diastolic (DBP) and systolic blood pressure (SBP) was measured using the HDI/Pulse Wave™ CR-2000 Research Cardiovascular Profiling System (Hypertension Diagnostic®, Inc., Eagan, MN, USA). The examination was performed with the participants lying on a gurney after a 10-min resting period. SBP and DBP were categorized according to the criteria

proposed by the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004).

Biochemical analysis

Approximately 15 ml of venous blood was drawn from an antecubital vein of each individual in the morning after an overnight fast. Within 30 min, the remaining blood serum was separated by a 10 min centrifugation at 3500 rpm and 4°C. The sample was immediately aliquoted and frozen at -70°C until the time of analysis.

Fasting glucose, LDL-c and HDL-c analyses were performed by the colorimetric enzymatic method with a Bayer ADVIA 1650 automated analyzer, using GLUO, D-LDL and D-HDL kits, respectively (Siemens Diagnostics, Tarrytown, NY, USA). Total cholesterol and TG were analyzed with the Bayer ADVIA Centaur automated analyzer using Cholesterol and Triglycerides Liquiform kits, respectively (Labtest Diagnostica[®], Vista Alegre, MG, Brazil). Fasting glycemia was classified based on the criteria currently adopted for the diagnosis of type 2 diabetes, which are the same for adults and children (Genuth et al. 2003). Total cholesterol and fractions and TG were classified according to the recommendations of the First Brazilian Guidelines for the Prevention of Atherosclerosis during Childhood and Adolescence (Giuliano 2006). Vitamin B12, homocysteine and insulin were assayed by chemoluminescence with the Bayer ADVIA Centaur automated analyzer using the HCY (analytical sensitivity of 0.13 mg/L), VB12 (analytical sensitivity of 45 pg/mL) and IRI (analytical sensitivity of 0.5 µIU/mL) Bayer ADVIA kits, respectively (Siemens Diagnostics[®]). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the equation $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose } (\text{mmol/L}) / 22.5$. Insulin resistance was considered to be present when $\text{HOMA} \geq 2.5$ (Valerio et

al. 2006). A wide range C-reactive protein (wr-CRP) test was performed by a latex-enhanced immunoturbidimetric assay with the Bayer ADVIA 1650 automated analyzer using the Bayer ADVIA kit for wr-CRP (Siemens Diagnostics[®]) and an analytical sensitivity of 0.12 mg/L. Since no reference values for CRP exist in the international literature for adolescents, we used the values for adults (Pearson et al. 2003). Human leptin levels were determined by ELISA using an EZHL-80SK kit (Millipore Corporation[®], St. Charles, MO, USA) with 0.5 ng/mL sensitivity. Fibrinogen was estimated by the Clauss method using a commercial fibrinogen kit (Wiener lab[®], Rosário, Argentina) with an analytical sensitivity of 10 mg/L. All measurements were performed at a laboratory accredited by the Brazilian Society of Clinical Analysis.

Statistical analysis

Data were analyzed statistically using the IBM Corp. Released 2013, IBM SPSS Statistics for Macintosh[®], Version 22.0 (IBM Corp. Armonk, NY, USA.). Continuous variables are reported as mean and standard deviation (SD), or median and interquartile range. Central tendency and absolute and relative frequencies were estimated as descriptive statistics. Differences between underweight/normal weight and overweight/obese groups were performed by Mann-Whitney test. Normality was checked using the Kolmogorov-Smirnov test. Adverse levels of TC, LDL-c, triglycerides, hs-CRP, homocysteine, fibrinogen, blood pressure, leptin, TNF- α , fasting insulin, and HOMA-IR were defined as values at or above the 75th percentile. For HDL-c concentrations, the adverse level was defined as values at or below the 25th percentile. The use of the 75th and 25th percentiles to define adverse levels of CVD risk factors permitted us to conduct multivariable logistic regression analyses due to a sufficient number of participants with adverse levels (Maximova et al. 2013). These

thresholds were similar to those used in other studies with adolescents (Lambert et al. 2004; Maximova et al. 2013).

The relationships between BMI and the above cardiometabolic risk factors were assessed using the Spearman correlation coefficient. To further investigate the association of body weight status (underweight/normal weight versus overweight/obese) with each of the cardiometabolic risk factors, we calculated odds ratios (OR) and 95% confidence intervals (95%CI) using logistic regression. In these regression analyses, we adjusted for the following confounders: age, sex, family income, mother's education, and physical activity. The analysis of homocysteine was further adjusted for vitamin B12 due to the confounding influence of Vitamin B12 on the homocysteine metabolism (Jacques et al. 2001). All analyses were considered to be significant when $p < 0.05$.

Results

The characteristics of the study population are listed in Table 1. The most frequent characteristics of our study population were 16 years of age, female sex, normal weight, a moderate level of physical activity, mother's education of <9 years, and a family income of 3 to 8 times the minimum Brazilian wage (MBW). One MBW equals approximately US\$ 181.00 per month. Table 1 further shows that 20.3% of the participants had excess body weight.

Regarding the cardiometabolic characteristics listed in Table 2, 12.6% of the adolescents had high SBP, 6.6% had levels of hs-CRP within the moderate or high cardiovascular risk range, and 5.1%, 38.9% and 8.7% had elevated concentrations of homocysteine, TNF- α and fibrinogen, respectively. Most students had elevated glucose levels (62.9%; ≥ 100 mg/dL), and 46.3% of the adolescents had insulin resistance (HOMA-IR ≥ 2.5). Elevated levels of leptin (29.1%; ≥ 20 ng/dL), TC (58.3%; ≥ 170 mg/dL), LDL-c (17.9%; ≥ 130

mg/dL) and TG (14.7%; ≥ 130 mg/dL) were also observed. Twelve (5.5%) adolescents had HDL-c below the recommended value, i.e., < 45 mg/dL (Table 2). Overweight/obese participants showed significantly ($p < 0.05$) higher median values of SBP, DBP, insulin, glucose, HOMA, leptin, LDL-c and triglycerides compared to underweight/normal weight adolescents, except for HDL-c. We also observed higher frequencies of elevated values in overweight/obese participants, except for homocysteine (Table 2).

The Spearman correlation coefficients for BMI and cardiometabolic risk markers are shown in Table 3. BMI was positively and significantly ($p < 0.01$) correlated with SBP, fibrinogen, fasting insulin, HOMA-IR, leptin, LDL-c and TG, and negatively correlated with HDL-c (Table 3).

Table 4 summarizes the results of multivariable logistic regression analysis that considered cardiometabolic risk factors $\geq 75^{\text{th}}$ percentile as outcomes. In the unadjusted analysis, adolescents with excess body weight were significantly more likely to have higher values ($\geq 75^{\text{th}}$ percentile) of SBP (OR = 3.49), fasting insulin (OR = 8.03), HOMA-IR (OR = 8.03), leptin (OR = 5.55), and LDL-c (OR = 5.50) compared to those with normal weight. Lower HDL-c levels ($< 25^{\text{th}}$ percentile) were associated with excess body weight when compared to normal weight adolescents (OR = 2.76; 95%CI: 1.37–5.56). Adjustment for age, female sex, family income, mother's education or physical activity did not substantially alter the estimates, except for the risk of high leptin concentrations, which increased from 5.50 to 11.09 ($p < 0.01$) (Table 4).

Discussion

In this study, we show that Brazilian adolescents with excess body weight have several cardiometabolic risk factors. The prevalence of these risk factors may in fact be more common than previously reported (Weiss et al. 2004). Our study confirms reports of

adolescents residing in high-income countries (Freedman et al. 1999; Herder et al. 2007; Caserta et al. 2010; Maximova et al. 2013), in Brazil (Brasil et al. 2007; Silva et al. 2010; Cardoso et al. 2014; Azevedo et al. 2015), and in other upper middle-income countries (Kelishadi et al. 2007; Agirbasli et al. 2015), which have shown increases in risk markers at an early age. In addition, unlike previous studies, the present study estimated the likelihood of Brazilian adolescents with excess body weight having altered values of cardiometabolic risk markers considering 14 different markers.

Our results also show that in the presence of excess body weight and after adjusting for age, female sex, family income, mother's education and physical activity, there was an increase in the risk of insulin resistance - HOMA-IR index (OR = 8.84; $p < 0.001$). This information offers an opportunity to emphasize the importance of treating excess body weight at an early age in order to prevent future consequences. Insulin resistance is the most common metabolic alteration related to obesity and represents an important link between obesity and other cardiometabolic complications, including metabolic syndrome and type 2 diabetes mellitus (Herder et al. 2007).

Independent of body weight, the prevalence of insulin resistance observed here (46.3%) was alarming. The high prevalence of insulin resistance observed in the present study agrees with findings obtained for Brazilian and Chinese adolescents and may predict an increasing burden of metabolic disease in the near future (Rizzo et al. 2013; Yin et al. 2013). HOMA-IR may be useful for the early evaluation of insulin resistance in adolescents and could have a long-term benefit for preventive and diagnostic therapeutic interventions (Yin et al. 2013). On the other hand, there are numerous criticisms regarding the use of HOMA-IR, the most important being the lack of standardization of insulin measurements, which impairs comparisons with other studies or populations (Yin et al. 2013). Furthermore, the different cutoff points used in the literature make it difficult to compare studies.

Another point to be noted in this study is the effect of excess body weight on leptin levels. Leptin is a hormone primarily synthesized and produced by adipocytes and has been identified as a key factor in maintaining energy balance and overall body weight composition (Venner et al. 2006). Elevated circulating leptin levels are independently associated with the incidence of CVD (Koh et al. 2008), including insulin signaling, immunity, vascular function, and blood pressure regulation (Martin et al. 2008). In some contexts leptin seems to promote both atherogenesis and insulin resistance or, in contrast, may have antiatherogenic and insulin-sensitizing effects (Koh et al. 2008). These opposite actions of leptin are maintained in balance under healthy conditions but not in pathological conditions such as obesity, in which the change in leptin actions may stimulate vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy (Koh et al. 2008). These actions may contribute to the pathogenesis of hypertension, atherosclerosis, left ventricular hypertrophy, and type 2 diabetes mellitus (Koh et al. 2008).

Considering that atherosclerosis is increasingly viewed as an inflammatory disease caused by lipoproteins, metabolic signals, hemodynamic stress, and the integrated activity of immune cells and inflammatory cytokines, it is interesting to note that leptin and inflammatory pathways demonstrate reciprocal modulation and shared association with cardiovascular risk (Martin et al. 2008). Leptin is also a common mediator of the neuroendocrine and immune systems. In the immune system, leptin, in combination with CRP and interleukins 1 (IL-1) and 6 (IL-6), can act as an early acute-phase reactant produced at high levels during inflammation, which can be induced by other inflammatory mediators such as TNF- α and IL-1 (La Cava and Matarese 2004). Therefore, although leptin has well-documented proinflammatory properties, it might act as an acute-phase reactant in some conditions and not in others (La Cava and Matarese 2004). In the present study, the adolescents with excess body weight showed an 11 times higher likelihood of increased leptin

levels compared with normal participants. This finding is in agreement with the theory of a leptin-resistant state existing in most overweight/obese humans when there is a defect within the leptin signaling cascade (Venner et al. 2006).

While there is no global consensus about the definition of obesity from a biological standpoint, values used in research settings have proven useful at identifying people with risk factors for developing numerous other chronic diseases, such as type 2 diabetes, heart disease, and non-alcoholic fatty liver disease (Jean et al. 2014; McGown et al. 2014). Excess body weight must be studied as a primary condition rather than as an accidental condition derived from other diseases.

Over the last decade, important contributions have been made to the understanding of the different functions of adipose tissue. However, the interplay between the signaling cascades involved in the major outcomes of overweight/obese individuals, such as inflammation, insulin resistance and hormonal deregulation, has yet to be elucidated (McGown et al. 2014).

This study has several strengths that should be mentioned, including the collection of prospective data and the ability to adjust for several important confounding factors. All data, including anthropometric measures, were collected by the same research group, which helps reduce possible bias. In addition, we observed that 69.7% of the adolescents had TG concentration <100 mg/dL, i.e., normal values for fasting, confirming compliance with the request to fast. However, the values observed for glucose concentrations were high in both weight categories analyzed. One may speculate that some participants had fasted, but did consume some sugary drinks despite our instructions that only water consumption was allowed. This may have affected our estimates of glucose, insulin, and HOMA-IR. Other limitations may pertain to BMI as a means to classify adolescents as being of normal weight or having excess body weight. BMI allows only an indirect evaluation of adiposity and cannot

distinguish fat mass from fat-free mass. Furthermore, we acknowledge the relative low response rate and the imbalance between sexes may limit the generalizability of our findings. Lastly, the cross-sectional design does not allow conclusions related to causality.

In conclusion, excess body weight in this group of Brazilian adolescents exhibits strong associations with markers that are established as causes of CVD in adults. This underlines the importance of primary prevention early in life and effective management of adolescents with cardiometabolic risk markers. It also supports the notion of a cumulative risk for CVD throughout childhood, adolescence and adulthood, which applies globally and calls for global strategies for primary prevention.

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Conflicts of interest statement

The authors declare no conflicts of interest.

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Table 1. Characteristics of the adolescents studied.

Characteristics	n (%)	Mean (SD)
Age (years)		16.1 (0.7)
15	66 (29.7)	
16	130 (58.6)	
17	26 (11.7)	
Sex		
Male	87 (39.2)	
Female	135 (60.8)	
Body mass index (kg/m ²)		22.0 (4.1)
Underweight	17 (7.7)	
Normal weight	160 (72.1)	
Overweight	22 (9.9)	
Obese	23 (10.4)	
Physical activity		
Low	34 (15.3)	
Moderate	109 (49.1)	
High	79 (35.9)	
Mother's education (years)		7.7 (3.4)
< 9	127 (58.3)	
9–12	73 (33.5)	
≥ 12	18 (8.3)	
Family income (MBW)		US\$ 459.6 (3.8)
< 3	62 (27.9)	
3–8	141 (63.5)	
≥ 8	19 (8.6)	

Note: SD, standard deviation; MBW, minimum Brazilian wage, which in 2007 was US\$ 181.00 per month.

Table 2. Cardiometabolic profile of the adolescents studied.

Characteristics	Underweight/ Normal weight (n = 177)	Overweight/Obese (n = 45)	All (n = 222)
SBP (mmHg), median (IR)	116 (11.0)	122 (13.0)*	116 (13.0)
< 95 th percentile, n (%)	160 (90.4)	34 (75.6)	194 (87.4)
≥ 95 th percentile, n (%)	17 (9.6)	11 (24.4)	28 (12.6)
DBP (mmHg), median (IR)	61 (8.0)	63 (7.0)*	62 (8.0)
< 95 th percentile, n (%)	176 (99.4)	44 (97.8)	220 (99.1)
≥ 95 th percentile, n (%)	1 (0.6)	1 (2.2)	2 (0.9)
CRP (mg/L), median (IR)	0.1 (0.2)	0.2 (0.5)	0.1 (0.2)
< 1, n (%)	160 (93.6)	38 (92.7)	198 (93.4)
1–3, n (%)	9 (5.3)	2 (4.9)	11 (5.2)
≥ 3, n (%)	2 (1.1)	1 (2.4)	3 (1.4)
Homocysteine (μmol/L), median (IR)	7.4 (4.3)	7.6 (3.3)	7.5 (4.2)
< 5, n (%)	19 (10.9)	5 (11.6)	24 (11.0)
5–15, n (%)	146 (83.9)	36 (83.7)	182 (83.9)
≥ 15, n (%)	9 (5.2)	2 (4.7)	11 (5.1)
TNF-α (pg/mL), median (IR)	3.1 (7.0)	3.7 (6.1)	3.2 (6.4)
< 1, n (%)	16 (14.0)	4 (13.3)	20 (13.9)
1 – 5, n (%)	54 (47.4)	14 (46.7)	68 (47.2)
≥ 5, n (%)	44 (38.6)	12 (40.0)	56 (38.9)
Fibrinogen (mg/dL), median (IR)	255 (110.0)	280 (95.0)	255 (110.0)
< 200, n (%)	43 (24.6)	8 (18.6)	51 (23.4)
200 – 400, n (%)	117 (66.9)	31 (72.1)	148 (67.9)
≥ 400, n (%)	15 (8.6)	4 (9.3)	19 (8.7)
Insulin (μIU/mL), median (IR)	8.4 (5.7)	15.0 (8.6)*	9.5 (7.4)
< 15, n (%)	155 (89.1)	21 (48.7)	177 (81.2)
15 – 20, n (%)	15 (8.6)	14 (32.5)	29 (13.3)
≥ 20, n (%)	4 (2.3)	8 (18.9)	12 (5.5)
Glucose (mg/dL), median (IR)	102 (14.0)	105.0 (15.0)**	103 (13.0)
< 100, n (%)	69 (39.4)	12 (27.9)	81 (37.1)
100 – 126, n (%)	105 (60.0)	29 (67.4)	134 (61.5)
≥ 126, n (%)	1 (0.6)	2 (4.7)	3 (1.4)
HOMA-IR, median (IR)	2.1 (1.5)	3.9 (2.6)*	2.4 (1.9)
< 2.5, n (%)	106 (60.6)	11 (25.6)	117 (53.7)
≥ 2.5, n (%)	69 (39.4)	32 (74.4)	101 (46.3)
Leptin (ng/dL), median (IR)	9.9 (14.2)	26.2 (36.2)*	11.3 (19.7)
< 15, n (%)	110 (67.5)	13 (30.2)	123 (59.7)
15 – 20, n (%)	20 (12.3)	3 (7.0)	23 (11.2)
≥ 20, n (%)	33 (20.2)	27 (62.8)	60 (29.1)
TC (mg/dL), median (IR)	175 (42.0)	181 (57.0)	176 (44.3)
< 150, n (%)	39 (22.3)	9 (20.9)	48 (22.0)
150 – 169, n (%)	36 (20.6)	7 (16.3)	43 (19.7)
≥ 170, n (%)	100 (57.1)	27 (62.8)	127 (58.3)
LDL-c (mg/dL), median (IR)	110 (22.0)	127 (34.0)**	112 (26.0)
< 100, n (%)	45 (25.7)	10 (23.3)	55 (25.2)
100 – 129, n (%)	108 (61.7)	16 (37.0)	124 (56.9)

≥ 130, n (%)	22 (12.6)	17 (39.7)	39 (17.9)
HDL-c (mg/dL), median (IR)	62 (15.0)	56 (19.0)**	62 (16.0)
≥ 45, n (%)	168 (96.0)	38 (88.4)	206 (94.5)
< 45, n (%)	7 (4.0)	5 (11.6)	12 (5.5)
TG (mg/dL), median (IR)	81 (41.0)	94 (42.0)*	85 (43.3)
< 100, n (%)	129 (73.8)	23 (53.5)	152 (69.7)
100 – 129, n (%)	23 (13.1)	11 (25.6)	34 (15.6)
≥ 130, n (%)	23 (13.1)	9 (20.9)	32 (14.7)

Note: IR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; TNF- α , tumor necrosis factor alpha; HOMA-IR, homeostasis model assessment index-insulin resistance; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TG, triglycerides. *, $p < 0.01$; **, $p < 0.05$, compared to Underweight/Normal weight group, Mann-Whitney test.

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Table 3. Spearman's correlation (rho) coefficient between body mass index and cardiometabolic risk markers.

Variable	Rho	p value
Systolic blood pressure (mm Hg)	0.257	<0.001
Diastolic blood pressure (mm Hg)	0.019	0.776
CRP (mg/L)	0.102	0.139
Homocysteine (μ mol/L)	0.089	0.190
TNF- α (pg/mL)	0.058	0.491
Fibrinogen (mg/dL)	0.173	0.010
Fasting insulin (μ IU/mL)	0.407	<0.001
Fasting glucose (mg/dL)	0.066	0.331
HOMA-IR	0.386	<0.001
Leptin (ng/dL)	0.410	<0.001
Total cholesterol (mg/dL)	0.070	0.301
LDL-c (mg/dL)	0.178	0.009
HDL-c (mg/dL)	-0.199	0.003
Triglycerides (mg/dL)	0.201	0.003

Note: CRP, C-reactive protein; TNF- α , tumor necrosis factor alpha; HOMA-IR, homeostasis model assessment index-insulin resistance; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

Table 4. Multivariable logistic regression considering cardiometabolic risk factors $\geq 75^{\text{th}}$ percentile as outcome.

Cardiometabolic risk factor	Unadjusted analysis		Adjusted analysis*	
	OR (95%CI)	p value	OR (95%CI)	p value
Systolic blood pressure				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	3.49 (1.74–7.02)	<0.001	3.24 (1.42–7.39)	0.005
Diastolic blood pressure				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	1.38 (0.65–2.92)	0.407	0.98 (0.42–2.26)	0.955
CRP				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	1.52 (0.72–3.21)	0.272	1.50 (0.68–3.33)	0.321
Homocysteine [†]				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	0.90 (0.41–1.96)	0.783	0.74 (0.31–1.77)	0.503
TNF-alpha				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	0.70 (0.26–1.88)	0.479	0.71 (0.25–1.97)	0.507
Fibrinogen				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	1.16 (0.54–2.51)	0.706	1.40 (0.62–3.11)	0.419
Fasting insulin				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	8.03 (3.86–16.71)	<0.001	9.13 (4.07–20.49)	<0.001
Fasting glucose				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	1.27 (0.60–2.69)	0.540	1.39 (0.59–3.25)	0.453
HOMA-IR				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	8.03 (3.86–16.71)	<0.001	8.84 (3.98–19.64)	<0.001
Leptin				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	5.55 (2.68–11.44)	<0.001	11.09 (4.05–30.35)	<0.001
Total cholesterol				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	1.63 (0.79–3.37)	0.189	1.68 (0.77–3.66)	0.189
LDL-c				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	5.50 (2.67–11.32)	<0.001	5.98 (2.78–12.74)	<0.001
HDL-c [‡]				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	2.76 (1.37–5.56)	0.004	2.82 (1.31–6.06)	0.008
Triglycerides				
Underweight/normal weight	1.00 (reference)		1.00 (reference)	
Overweight/obese	1.63 (0.79–3.38)	0.189	1.92 (0.89–4.17)	0.098

Note: OR, odds ratio; 95%CI, 95% confidence interval; CRP, C-reactive protein; TNF- α , tumor necrosis factor alpha; HOMA-IR, homeostasis model assessment index-insulin resistance; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

All logistic regression models were adjusted for age, female sex, family income, mother's education, and physical activity. [†]Model also adjusted for vitamin B12 due to the confounding influence of Vitamin B12 on the homocysteine metabolism. ^{}Multivariable logistic regression model considering cardiometabolic risk factors (<25th percentile) as outcome.

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