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Clinical Research Article

Salivary Metabolomic Signatures and Body Mass Index in Italian Adolescents: A Pilot Study

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Abstract

Context: Obesity surveillance is scarce in adolescents, and little is known on whether salivary metabolomics data, emerging minimally invasive biomarkers, can characterize metabolic patterns associated with overweight or obesity in adolescents.

Objective: This pilot study aims to identify the salivary molecular signatures associated with body mass index (BMI) in Italian adolescents.

Methods: Saliva samples and BMI were collected in a subset of n = 74 young adolescents enrolled in the Public Health Impact of Metal Exposure study (2007-2014). A total of 217 untargeted metabolites were identified using liquid chromatography-high resolution mass spectrometry. Robust linear regression was used to cross-sectionally determine associations between metabolomic signatures and sex-specific BMI-for-age z-scores (z-BMI).

Results: Nearly 35% of the adolescents (median age: 12 years; 51% females) were either obese or overweight. A higher z-BMI was observed in males compared to females ($P = .02$). One nucleoside (deoxyadenosine) and 2 lipids (18:0-18:2 phosphatidylcholine and dipalmitoyl-phosphoethanolamine) were negatively related to z-BMI ($P < .05$), whereas 2 benzenoids (3-hydroxyanthranilic acid and a phthalate metabolite) were positively associated with z-BMI ($P < .05$). In males, several metabolites including deoxyadenosine, as well as deoxycarnitine, hyodeoxycholic acid, N-methylglutamic acid, bisphenol *P*, and trigonelline were downregulated, while 3 metabolites (3-hydroxyanthranilic acid, theobromine/theophylline/paraxanthine, and alanine) were upregulated in relation to z-BMI ($P < .05$). In females, deoxyadenosine and dipalmitoyl-phosphoethanolamine were negatively associated with z-BMI while deoxycarnitine and a phthalate metabolite were positively associated ($P < .05$). A single energy-related pathway was enriched in the identified associations in females (carnitine synthesis, $P = .04$).

Conclusion: Salivary metabolites involved in nucleotide, lipid, and energy metabolism were primarily altered in relation to BMI in adolescents.

Key Words: body mass index, BMI, obesity, metabolomics, adolescents

Obesity has tripled worldwide in the past 4 decades [1-4], becoming one of the major public health issues of the 21st century. Obesity and overweight are the most frequently diagnosed conditions in youth [5]. In 2016, 340 million children and adolescents were estimated to be either obese or overweight worldwide [2]. Obesity often begins in late childhood and either can persist or become morbid during adolescence [6]. Children who are obese or overweight before puberty are likely to continue to be obese or overweight throughout their adulthood [7], predisposing them to poor health and quality of life. Higher body mass index (BMI) from an early age is linked to increased mortality risk [8-10] through the early onset of chronic diseases, such as cardiometabolic diseases [11], cancer [12, 13], musculoskeletal disorders [14], immunological impairment [15, 16], delay and impairment of cognitive function [17, 18], or poor mental health [19]. Prevention of excess body weight and adiposity from an early age can improve long-term health and can contribute to diminishing obesity-related costs stemming from poor academic achievement [1, 20, 21] and medical treatment for chronic diseases developing later in adulthood [22, 23].

Although obesity and overweight rates in youth have decreased in Europe in the past decade, there are differences between southern and northern European countries. The prevalence of obesity in the southern Mediterranean region is higher than the rate in the northern region, with Italy being among the European countries with the highest proportion of obese or overweight youth [24]. A 2018 World Health Organization (WHO) report showed a disproportionately higher obesity prevalence among boys compared to girls in Italy and determined that the prevalence of childhood obesity and overweight in Italian boys could be as high as 21% and 42%, respectively [25]. If this trend continues in the upcoming years, obesity rates among Italian adolescents are expected to surpass the current combined obesity and

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overweight prevalence of nearly 25% in adolescent males and 14% in adolescent females [26].

Although obesity and overweight are heavily influenced by genetics [27], physical activity [28], and socioeconomic status (SES)-related factors [29], the environment, including environmental toxicants [30-32] and dietary exposures [33], play a major role in their etiology. Untargeted high-throughput metabolomics data provide a unique unified approach to measure individual exposures and their health-related effects simultaneously. The novel metabolomics approaches offer an opportunity to capture changes in endogenous metabolites that may be driven by exogenous and endogenous factors, including but not limited to environmental chemical exposures, lifestyle habits, or perceived stress [34, 35].

Metabolomics data have also been shown to be good biomarkers of environmentally induced diseases [36, 37], involving weight gain or excess body fat, as well as biomarkers of metabolic precursors for obesity-related conditions. In prior studies, plasma and serum metabolomic alterations were detected in relation to obesity in adults [38, 39] and animal models [40]. According to these studies, obese adults may be characterized by higher levels of branched and aromatic amino acids, certain carbohydrates, and fatty acid metabolites, which, in turn, may be related to disruption of glucose homeostasis and adipose tissue metabolism. However, prior studies linking metabolomics data to body weight were conducted mainly in adults [38, 39, 41] or children [42-44], and there is a scarcity of studies investigating the metabolomic profiles underlying obesity and overweight in adolescents [45-47]. In addition, although saliva can be collected through minimally invasive procedures, salivary metabolomics data are underutilized to characterize concurrent or early factors for diseases, including obesity and overweight [48]. In this study, we identified salivary metabolomic signatures associated with BMI in a subset of the Public Health Impact of Metals Exposure (PHIME) cohort of young Italian adolescents.

Materials and Methods

Study Participants

The PHIME cohort is comprised of 733 Italian adolescents recruited from 3 sites in the greater northern Italian area of Brescia [49]. These locations were initially selected for their geographic characteristics and distance from ferroalloy industrial plants, so that the cohort is comprised of individuals with a potential gradient of ambient exposure levels. From participant recruitment in 2007 to 2014 through current activity, the Bagnolo Mella location has an active ferroalloy plant, the former ferroalloy plant in Valcamonica was active from 1910 to 2001, and Garda Lake has no history of metallurgical or ferroalloy industry [50]. Data on sociodemographic factors, BMI, lifestyle, and biospecimens were collected between the ages of 11 and 21 years for each participant at enrollment [49]. For this pilot study, 74 participants provided sufficient saliva volume for untargeted metabolomics analysis and informed consent for sample use. This study was approved by the Institutional Review Boards and ethics committees from the Icahn School of Medicine at Mount Sinai (STUDY-15-00990) and the University of Brescia (PHIME II ID #0034201; June 7, 2017).

Body Mass Index

Anthropometric measures (height and weight) were collected at the enrollment visit between 2007 and 2014 through self-report. We calculated BMI (kg/m^2) z-scores (z-BMI) standardized on age and sex as indicated by the 2007 WHO growth charts using the "anthroplus" package in R (available via: https://www.who.int/tools/growth-reference-data-for-5to19-years/application-tools). We categorized individuals using the z-BMI SDs for descriptive statistics as follows: underweight (<-2 SD), normal (≥ -2 SD and ≤ 1 SD), overweight (>1 SD and ≤ 2 SD), and obese (>2 SD) [51].

Saliva Collection and Metabolomics Data Acquisition

Collection, storage, and laboratory analysis

All participants followed the same protocol for salivary sample collection (rinsed their mouths twice with Milli-Q ultrapure water), and each provided a 2 mL salivary sample after morning fasting. Samples were stored at −80 °C. Smokers were ineligible for the study. Use of dental products within the hour prior to sample collection also rendered the participant ineligible [52]. Saliva samples and quality control (QC) samples (matrix blank and multiple pooled QCs) were analyzed with liquid chromatography high resolution mass spectrometry. Prior to the analysis, all samples were reconstituted in 60 µL of 80% acetonitrile for analysis with reverse phase chromatography in negative ionization mode and hydrophobic interaction chromatography in positive and negative ionization modes, respectively [53-55]. Samples were analyzed in a randomized order and pooled QCs injected routinely throughout the run. Data acquisition was performed in a single batch per mode, and we did not identify any technical concerns across BMI groups (Supplementary Fig. S1) [56]. Metabolites were identified based upon in-house database matching considering retention time, accurate mass, and MS/MS matching (when available) with pure standards analyzed under the same conditions, providing the highest identification confidence level (Level 1 or 2) based on Metabolomics Standards Initiative criteria [57, 58]. Peaks were identified and integrated using the Personal Chemical Database Library and Profinder software (Agilent Technologies, Santa Clara, CA, USA) to provide semiquantitative measures of intensity for each metabolite. There were 434 metabolites detected by the 3 chromatography methods with different ionization modes of which 327 annotations were unique. For duplicate metabolites, only the metabolite with higher intensity values were retained for analysis. In a prior pilot evaluation, our team also conducted an internal validation indicating a lack of correlation between saliva metabolite intensity and both urine dilution and specific gravity. Thus, no correction for those measures was included in our analyses.

Data Preprocessing

We first removed any metabolites that had at least 50% of missing intensity values. Then, remaining missing values of individual metabolites (~10.8% of the data) were imputed using the individual metabolites' minimum value across subjects divided by $\sqrt{2}$. Data were $\log_2$-transformed to correct for any skewness and heteroscedasticity. Then we mean-centered the data and scaled it by using the square root of the SD for each metabolite (Pareto scaling). Lastly, to create a common

scale we normalized the individual metabolites by their mean and SD. A total of 217 metabolites were annotated and included for analyses.

Covariates

Sex, area of residence (GL, Garda Lake; VC, Valcamonica; BM, Bagnolo Mella), and SES were collected at enrollment through self-reported questionnaires. A SES index was calculated based on 2 self-reported SES variables (parental educational attainment and occupational level) [59] applying internationally defined criteria for SES evaluation in a northern Italian population (Supplementary Table S1) [56]. Briefly, educational attainment for both parents was classified into 3 levels: low (elementary and junior high school), medium (senior high school), and high (degree and post-degree). Occupations were grouped into 3 categories based on the socioeconomic situation in Italy and factors related to decision latitude and job demand: low-level professions (ie, homemaker, skilled/unskilled worker, hospital ancillaries), middle-level professions (ie, clerical workers, teachers, educators, nurses, shop assistants), and high-level professions (ie, engineer, entrepreneur, tradesman, craftsman). To obtain the final SES index, we combined the highest level of educaition and occupation level across parents (Supplementary Table S2) [56]. When 1 parent was not financially present (unemployed or deceased), we calculated their status as "low."

Statistical Analyses

To identify associations between individual metabolites and continuous z-BMI scores, we employed robust linear regression models. Due to the limited sample size of our pilot study, we selected the metabolites following the strategy proposed by Maccani et al [60]—first based on the effect size (smaller than −0.2 or greater than 0.2) and then on the nominal *P*-value. We also provided false discovery rate *P*-value correction in the Supplementary Material [56]. We adjusted all analyses for relevant sociodemographic factors based on prior literature, including continuous age (years), sex (male vs female), SES index (medium/low vs high), as well as site of recruitment (GL, VC, BM) due to potential differences in exposures. To examine sex-dimorphic patterns for the relationship between metabolites and BMI, we (1) performed sex-stratified analyses and (2) tested interaction effects by using the cross-product term of sex and the metabolites of interest. Finally, to help elucidate possible biological mechanisms through which the identified metabolites associate with BMI, we conducted pathway enrichment analyses with MetaboAnalyst (version 3.2) using the Small Molecule Pathway Database (http://www.smpdb.ca/) as a reference. Pathway enrichment analyses were implemented using a hypergeometric test evaluating whether a metabolite set is overrepresented. All data were processed and analyzed in R (version 4.1.2).

Results

PHIME adolescents were 12.1 years of age at the time of sample collection (SD = 0.86; age range: 10-14 years; 51% females) (Table 1). The majority of adolescents lived in Bagnolo Mella (57%) and had a medium/low socioeconomic status (59%). A total of 65% participants had a normal BMI, while 35% were either obese or overweight. Adolescent males had higher BMI levels (BMI mean ± SD: 20.6 ± 3.8 kg/m^2) compared to adolescent females (BMI mean ± SD: 19.9 ± 3.1 kg/m^2) (z-BMI *P*-value = .022). A suggestive higher BMI was reported in adolescents from Garda Lake, with higher proportion of overweight and obese adolescents, compared to the other 2 regions (z-BMI *P*-value = .087) (Supplementary Table S3) [56]. A descriptive summary of the 217 preprocessed metabolites is provided in Supplementary Tables S4-S6 [56].

In covariate-adjusted robust models (n = 74), we observed negative associations between z-BMI scores and levels of purine nucleoside deoxyadenosine [$\beta = -0.30$ (95% CI: −.52, −.08); $P = .008$] and 2 lipids: dipalmitoyl-phosphoethanolamine [$\beta = -0.25$ (95% CI: −.49, −.02); $P = .037$] and 18:0-18:2 phosphatidylcholine (PC) [$\beta = -0.25$ (95% CI: −.48, −.01); $P = .045$] (Figs. 1 and 2, Supplementary Fig. S2, Supplementary Table S7) [56]. For every unit increase in levels of deoxyadenosine and lipid metabolites, there was a 0.30 and 0.25 lower z-BMI score in Italian adolescents, respectively. On the other hand, a positive association was observed between several benzenoids and z-BMI, where for every unit increase in the levels of 3-hydroxyanthranilic acid and mono (5-carboxy-2-ethylpentyl) phthalate (MECPP), there was a 0.38 (95% CI: .11, .65; $P = .006$) and 0.25 (95% CI: .01, .49; $P = .047$) higher z-BMI score, respectively.

Some metabolites showed a sex-specific association with z-BMI scores. In sex-stratified analyses, levels of 9 metabolites in males and 4 metabolites in females were associated with z-BMI. Among those metabolites, deoxyadenosine—previously identified as negatively associated with z-BMI in overall analyses—was depleted in both males [$\beta = -0.37$ (95% CI: −.70, −.05), $P = .025$]) and females [$\beta = -0.38$ (95% CI: −.69, −.07), $P = .018$]) (Fig. 3, Supplementary Tables S8-S9) [56]. In males, deoxycarnitine [$\beta = -0.45$ (95% CI: −.86, −.03), $P = .035$], hyodeoxycholic acid [$\beta = -0.47$ (95% CI: −.89, −.06), $P = .026$], trigonelline [$\beta = -0.48$ (95% CI: −.78, −.18), $P = .002$], and N-methylglutamic acid [$\beta = -0.37$ (95% CI: −.71, −.02), $P = .037$] were also downregulated in the association with BMI z-scores, whereas alanine [$\beta = 0.40$ (95% CI: .05, .74), $P = .026$], 3-hydroxyanthranilic acid [$\beta = 0.38$ (95% CI: .06, .70), $P = .023$], and theobromine/theophylline/paraxanthine [$\beta = 0.42$ (95% CI: .08, .75), $P = .015$] were upregulated in relation to BMI z-scores (Fig. 3; Supplementary Table S8) [56]. Additionally, levels of bisphenol *P* were marginally negatively associated with z-BMI in males [$\beta = -0.38$ (95% CI: −.75, .00), $P = .049$] (Fig. 3; Supplementary Table S8) [56]. In females, levels of dipalmitoyl-phosphoethanolamine [$\beta = -0.38$ (95% CI: −.72, −.04), $P = .032$] were downregulated and levels of deoxycarnitine [$\beta = 0.39$ (95% CI: .03, .74), $P = .037$] and phthalate metabolite MECPP [$\beta = 0.34$ (95% CI: .03, .66), $P = .034$] were upregulated in relation to z-BMI (Fig. 3; Supplementary Table S9). Sex modified the BMI associations with theobromine/theophylline/paraxanthine (*P* for interaction = .026), deoxycarnitine (*P* for interaction = .005), and trigonelline (*P* for interaction = .016) (Supplementary Table S10) [56].

Metabolic pathways potentially involved with BMI-altered metabolites in Italian adolescents are plotted in Fig. 4. Across the overall and sex-stratified populations, several pathways were enriched consistently, though most pathways failed to reach statistical significance. Enriched pathways included a nucleotide metabolism pathway (purine metabolism) that

Table 1. Participant characteristics in the Public Health Impact of Metal Exposure subset and stratified by sex

Baseline characteristic	Overall, n = 74	Females, n = 38	Males, n = 36	*P*-value[a]
Age (years), median (IQR)	12 (12, 13)	12 (12, 13)	12 (11, 12)	.022*
Age (years), mean (SD) [range]	12.1 (0.86) [10, 14]	12.3 (0.85) [11, 14]	11.9 (0.82) [10, 14]	
Socioeconomic status, n (%)[b]				.801
Low	10 (14%)	6 (16%)	4 (11%)	
Medium	44 (59%)	21 (55%)	23 (64%)	
High	20 (27%)	11 (29%)	9 (25%)	
BMI (kg/m^2), median (IQR)	19.5 (17.8, 21.8)	19.1 (17.8, 21.6)	20.2 (18.0, 22.7)	.416
BMI (kg/m^2), mean (SD) [range]	20.3 (3.5) [15.0, 32.1]	19.9 (3.1) [15.0, 29.2]	20.6 (3.8) [15.5, 32.1]	
z-BMI, median (IQR)[c]	0.47 (−0.20, 1.41)	0.14 (−0.31, 0.87)	0.90 (0.13, 1.60)	.022*
z-BMI, mean (SD) [range][b]	0.58 (1.06) [−1.47, 3.10]	0.31 (1.02) [−1.47, 2.59]	0.87 (1.05) [−1.25, 3.10]	
BMI status, n (%)[c]				.109
Normal	48 (65%)	29 (76%)	19 (53%)	
Overweight	19 (26%)	7 (18%)	12 (33%)	
Obese	7 (9.5%)	2 (5.3%)	5 (14%)	
Site, n (%)[d]				0.763
BM	42 (57%)	21 (55%)	21 (58%)	
GL	17 (23%)	10 (26%)	7 (19%)	
VC	15 (20%)	7 (18%)	8 (22%)	

Abbreviations: BMI, body mass index; IQR, interquartile range.
[a]T-tests (age, BMI, z-BMI), Fisher test (BMI status), and chi-square (χ^2) test (socioeconomic status, site) performed to assess significant distributions of variables between male and female adolescents.
[b]"Low" and "medium" categories combined in analyses due to limited sample size of "low" category.
[c]Based on the 2007 World Health Organization BMI z-scores. No children were underweight.
[d]GL, Garda Lake; VC, Valcamonica; BM, Bagnolo Mella.
*P-value <.05.

was overrepresented in relation to z-BMI levels in the overall sample (Fig. 4a; $P = .139$) and in sex-stratified analyses (Figs. 4b, $P = .260$; and 4c, $P = .139$); an amino acid pathway (tryptophan metabolism), which was enriched in both the overall sample (Fig. 4a, $P = .114$) and in males (Fig. 4b, $P = .215$); and an energy pathway (carnitine synthesis), which was overrepresented in both sex-stratified analyses (Fig. 4b, $P = .083$; and Fig. 4c, $P = .043$). Only carnitine synthesis in females was significantly enriched where metabolites attributed to higher z-BMI scores were involved in this pathway (Fig. 4c, $P = .043$). Lastly, caffeine metabolism was uniquely enriched in males with marginal significance (Fig. 4b, $P = .091$).

Discussion

In this pilot study, we characterized salivary metabolic features linked to BMI z-scores in an Italian population to identify salivary molecular signatures associated with obesity and overweight at the early stages of adolescence. In the total sample, 1 purine nucleoside (deoxyadenosine) and 2 glycerophospholipids—a glycerophosphoethanolamine (dipalmitoylphosphoethanolamine) and a phosphatidylcholine (18:0-18:2 PC) —were negatively associated with BMI z-scores, while 2 benzenoids (3-hydroxyanthranilic acid and phthalate metabolite MECPP) were upregulated in relation to BMI z-scores. In addition, several additional metabolites were dysregulated in relation to BMI z-scores in sex-stratified analyses: these metabolites were related to energy metabolism—including lipid deoxycarnitine found dysregulated in adolescent males and females in opposite directions, a downregulated bile acid (hyodeoxycholic acid), and 2 amino acid derivatives (alanine and N-methylglutamic acid) that were dysregulated in males in opposite directions—or were potential dietary compounds—theobromine/theophylline/paraxanthine and trigonelline found in males. In general, a larger proportion of metabolites were altered in males compared to females and a higher z-BMI was also found in males.

Our results aligned with previous studies showing associations between plasma and serum metabolomics data and metabolic outcomes. Epidemiological studies have suggested that levels of nucleotide metabolites are altered in obese adult populations [61, 62] and could play a role in adipocyte functioning [63-65]. Plasma levels of nucleosides—pyrimidines and purines—were also found to be dysregulated in relation to obesity in children [44]. Furthermore, in experimental studies, supplementation with deoxyadenosine-derivatives (purine nucleosides) has been linked to weight reduction in obese mice through microbiome changes [66] and reduction of lipid levels and insulin resistance [67].

We further identified a disruption of multiple metabolites related to fatty acid oxidation and pathways involved in energy metabolism. We observed negative associations between BMI and glycerophospholipids, namely a glycerophosphoethanolamine (GPE) and a PC—the most common phospholipids in mammalian cell membranes [68]. Changes in PCs may be involved in insulin resistance and obesity through alteration in oxidative capacity and energy metabolism in the mitochondria, as well as via disruption of membrane fluidity and decreased lipid regulation [69]. Although findings from epidemiological studies linking GPEs or PCs with obesity and metabolic disorders are mixed [70-74], several prior studies indicated a downregulation of PCs among children

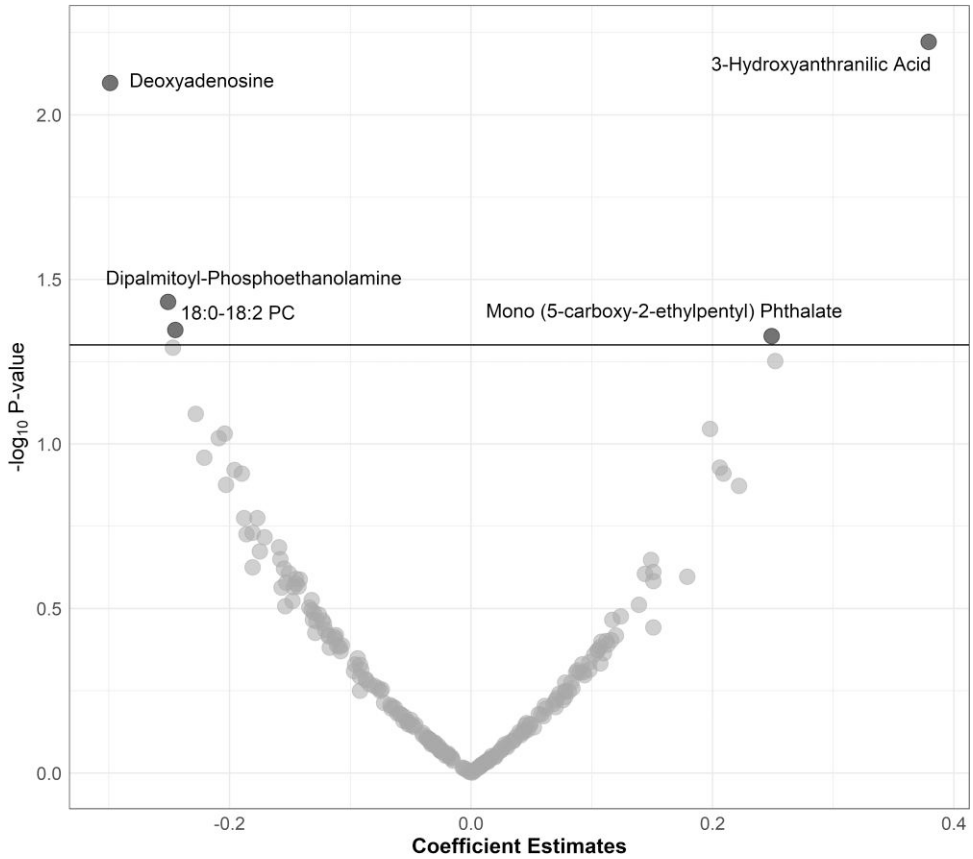

Figure 1. Volcano plot showing coefficient estimates (*x*-axis) and $\log_{10}$-transformed *P*-values (*y*-axis) of the association between individual metabolites and body mass index z-score (z-BMI) in the Public Health Impact of Metal Exposure cohort (n = 74). Robust linear regressions were adjusted for age, sex, site, and socioeconomic status. A solid black line indicates a *P*-value of .05, and values above that threshold were nominally statistically significant.

with obesity [43, 75] and of GPEs among women with obesity-related conditions, such as polycystic ovary syndrome [74]. In addition, a bile acid—hyodeoxycholic acid—showed a negative association with BMI z-scores in males in our study. Bile acids are critical for lipid absorption and proper energy homeostasis via micellar functions and receptor ligands [76, 77]. These nutrient-signaling metabolites can contribute to the dysregulation of hepatic lipid and glucose metabolism by modulating the energy balance through cell surface and nuclear receptors [78]. Previous studies indicated a decrease in several bile acids in nonfasting and fasting plasma of obese children [44], adolescents [79], and adults [80], consistent with our findings in males. We also identified a sex-specific association between BMI and levels of deoxycarnitine, a precursor of carnitine, which was upregulated in females but downregulated in males in the association with BMI. Similarly, we identified in females a substantial enrichment of carnitine synthesis, a pathway involved in energy metabolism. A dysregulation of carnitine-related pathways suggests a potential dysregulation in energy metabolism [81] and disruption of fatty acid β-oxidation and transport into the mitochondria [82].

We also observed that several salivary amino acid derivatives involved in endocrine and neurological function were altered in relation to BMI in males. Previous studies using plasma or salivary metabolomics showed increased levels of branched amino acids in obese children, adolescents, and adults [39, 47, 48, 70, 83-86]. An alteration of circulating plasma levels of amino acids, which are influenced by diet and exercise, may indicate lower muscle utilization [71, 87]. In our study, salivary alanine was upregulated in males, whereas N-methylglutamic acid was downregulated. While N-methylglutamic acid is a derivative of glutamic acid, a neurotransmitter that plays a role in cognitive function [88, 89], alanine, commonly present in human saliva [90], has been suggested to play a role in glucose metabolism via the hypothalamic-pituitary-adrenal axis [91]. Also, emerging literature points at alanine as a novel biomarker for endocrine conditions in humans [92-94].

We also found that some lifestyle chemical exposures, such as benzenoids and dietary factors, influenced BMI in this pilot study. For instance, 3-hydroxyanthranilic acid is an aminobenzoic acid involved in tryptophan metabolism that has been suggested to play a role in cardiometabolic disease [95, 96]. MECPP, a metabolite of di-2-ethylhexyl phthalate, was upregulated in the overall sample and in females, consistent with the potential endocrine disruptive role of phthalates shown in studies linking di-2-ethylhexyl phthalate metabolites and obesity in adult females [97] and adolescent females [98]. Furthermore, several patterns of dietary exposures were also observed in our sample. An upregulated metabolite related to caffeine metabolism (either theobromine, theophylline, or paraxanthine) and a likely predictor of chocolate ingestion [99-101] and contributing factor to obesity could be indicative of an unhealthy lifestyle in males. Interestingly, trigonelline, a plant hormone with anti-inflammatory properties

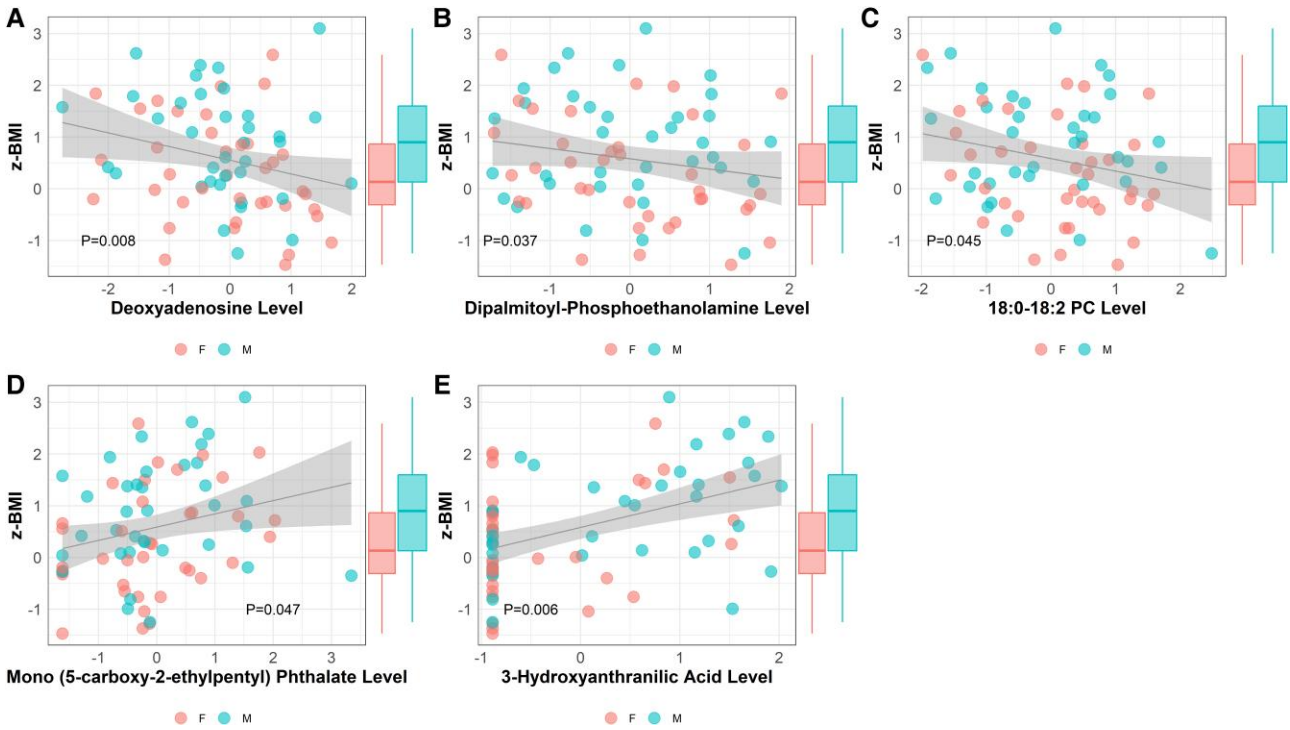

Figure 2. Scatterplots of the association between body mass index z-score (z-BMI) (*y*-axis) and levels (*x*-axis) of deoxyadenosine (a), dipalmitoyl-phosphoethanolamine (b), 18:0-18:2 phosphatidylcholine (c), mono (5-carboxy-2-ethylpentyl) phthalate (d), 3-hydroxyanthranilic acid (e) in the Public Health Impact of Metal Exposure cohort (n = 74). Box plots adjacent to each figure describe z-BMI median and interquartile range. Dots indicate individual observations and are color-coded with the sex of the participant: females (F) in red and males (M) in blue. *P*-value of the association was reported at the bottom of each plot.

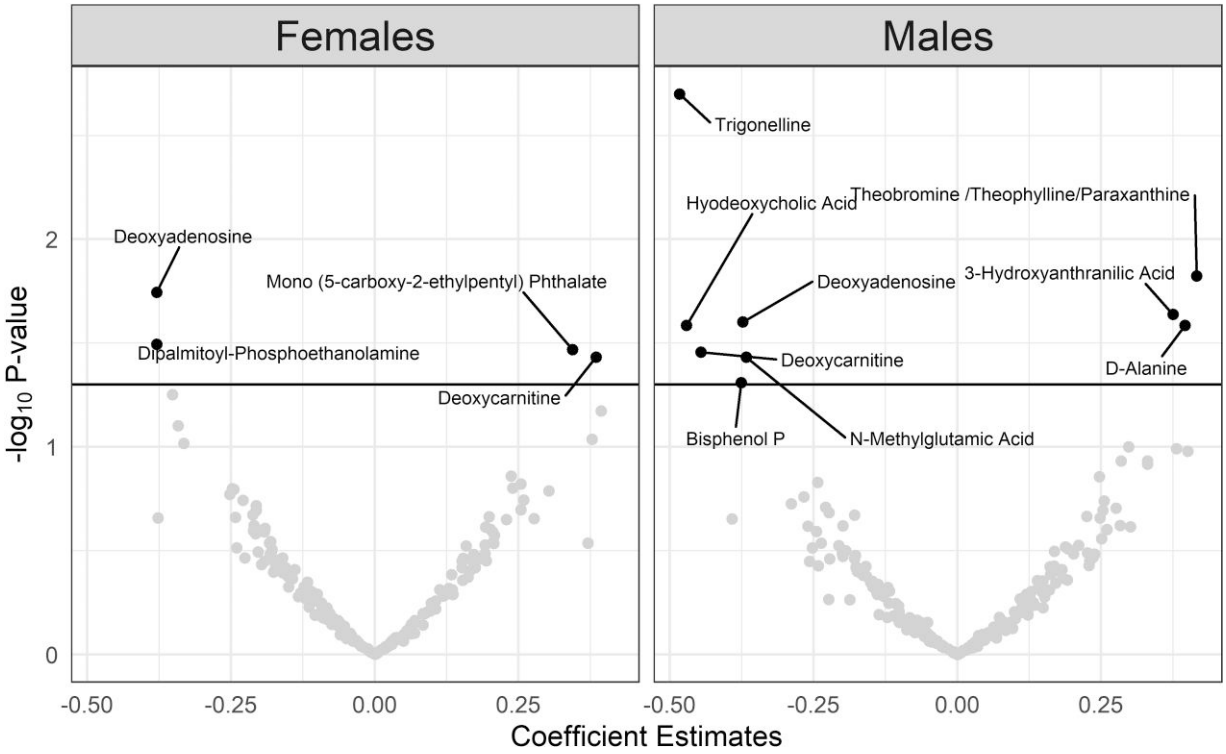

Figure 3. Volcano plots showing coefficient estimates (*x*-axis) and $\log_{10}$-transformed *P*-values (*y*-axis) of the association between individual metabolites and body mass index z-score (z-BMI) in males (n = 36) and females (n = 38). Robust linear regressions were adjusted for age, site, and socioeconomic status. A solid black line indicates a *P*-value of .05, and values above that threshold were nominally statistically significant.

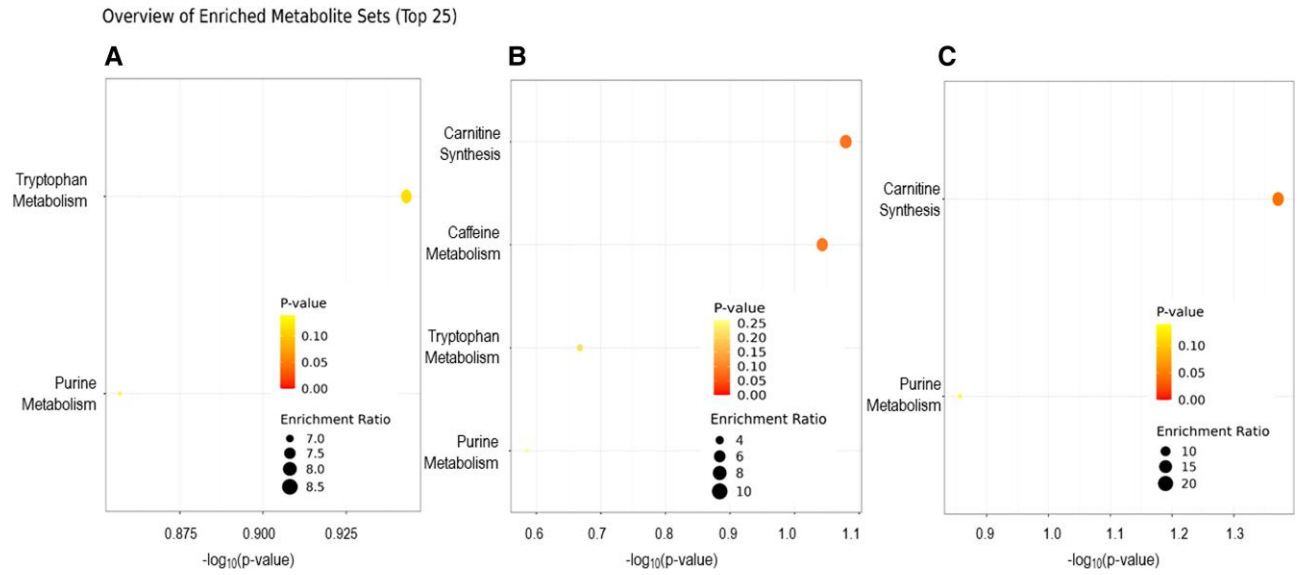

Figure 4. MetaboAnalyst pathway enrichment results in the Public Health Impact of Metal Exposure subset (n = 74). *x*-axis denotes the $-\log_{10}$-transformed *P*-value, where a higher level of significance is denoted by a red color indicating a lower *P*-value (statistically significant at $P < .05$). Higher enrichment ratio is indicated by the size of the bubble. Enrichment analyses were performed in MetaboAnalyst (v3.2). (A) Bubble plot showing enriched pathways for metabolites associated with z-BMI in robust linear regression in the overall cohort (n = 74). (B) Bubble plot showing enriched pathways for metabolites associated with z-BMI in robust linear regression in males (n = 36). (C) Bubble plot showing enriched pathways for metabolites associated with z-BMI in robust linear regression in females (n = 38).

[102] found in legumes and seeds [103-105], was negatively associated with BMI in males in this study. Altogether, environmental lifestyle exposures and a high-caloric diet may trigger increased oxidative stress and promote inflammation. Overall, these findings support the idea that an obesogenic environment, which we are able to capture through saliva, may predispose adolescents to higher risk for obesity and related diseases.

To our knowledge, this is the first study identifying salivary markers in relation to BMI in adolescents, a population that is understudied. One of the major advantages of metabolomics data is that they are early biomarkers for health conditions, such as metabolic conditions. In addition, the salivary metabolome may provide diagnostically relevant markers for lifestyle dietary habits and both oral and metabolic health [106, 107]. Several salivary metabolites have been correlated with plasma levels previously [106, 108-111], suggesting the potential use of salivary markers without the need for blood collection. Salivary metabolomics data can become a promising tool for screening obesity-related phenotypes due to the minimally invasive collection procedures of saliva [48, 106, 111, 112].

Our results should be interpreted with caution and may only be generalized to adolescents at early stages of puberty with similar lifestyles. Possible study limitations include a small sample size, a cross-sectional design, self-reported information on height and weight, which is prone to measurement error (though likely exerting nondifferential bias towards the null), or potential for false positives. To accommodate for the small sample size, we included only annotated metabolites with high confidence levels (1 or 2), and we then used the Maccani et al's approach to identify our major results. However, we acknowledge that our findings did not survive the false discovery rate correction of the type I error, as reported in the supplemental material, but they can serve as a rationale for future studies leveraging salivary metabolomics and metabolic data. We also recognize that obesity could be an important factor for early onset of puberty, particularly in females [113, 114] . Puberty stage could be in the causal pathway of such associations and was not accounted for in our analyses given that adolescents were 12 years old on average (range: males 10-14, females: 11-14) and the majority of them had already initiated puberty. In addition, although our study lacked information about antibiotic intake at the time of sample collection, we controlled for several important sociodemographic factors related to antibiotic use, such as SES [115, 116]. Lastly, our findings may also be affected by oral diseases such as periodontal and gum disorders, but these tend to co-occur with obesity-related conditions [106, 112]. Future studies with salivary samples may not only provide insights on the human metabolome in adolescents but also may be useful to interpreting in conjunction with the oral microbiome, which altogether can inform on potential mechanisms and lifestyle exposures underlying obesity in teenagers. Overall, detection of altered nucleoside levels, glycerophospholipids, and benzenoids in saliva could be relevant biomarkers for early diagnosis of developmental or obesity-related conditions in young adolescents, but this hypothesis warrants further investigation in larger prospective cohorts.

Conclusion

We identified associations between salivary metabolomic signatures and BMI in Italian adolescents. Salivary metabolites related to nucleotide, lipid, or energy metabolism were primarily altered in relation to BMI, as well as lifestyle-related chemicals. These metabolic signatures —including a nucleoside, glycerophospholipids, benzenoids, amino acids, a carnitine, a bile acid, and dietary derivatives— could be implicated particularly in endocrine functions and in biological processes involved in the etiology of obesity.

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Author Contributions

M.N., E.C.: Conceptualization; A.J., S.I.A.: Data curation, Formal analysis, Investigation; M.N., E.C.: Funding acquisition; A.J., S.I.A.: Software, Visualization; S.I.A., L.P., E.C.: Methodology; S.I.A.: Roles/Writing–original draft; D.V., R.G.L., D.P., L.P., M.H., M.N., E.C.: Writing—review & editing.

Disclosure

The authors declared no conflict of interest.

Data Availability

Data is available upon reasonable request from the authors.

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