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Metabolites as Novel Biomarkers for Childhood Obesity-Related Traits in Mexican American Children

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Abstract

Aims—Although newer approaches have identified several metabolites associated with obesity, there is paucity of such information in pediatric populations, especially among Mexican Americans (MAs) who are at high risk of obesity. Therefore, we performed a global serum metabolite screening in MA children to identify biomarkers of childhood obesity.

Materials and methods—We selected 15 normal-weight, 13 overweight and 14 obese MA children (6–17 years), and performed global serum metabolite screening using UPLC system with Q-Tof-Micromass-spectrometer. Metabolite values were analyzed to assess mean differences among groups using one-way ANOVA, test for linear trend across groups, and examine Pearson's

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Contribution:

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Lavanya Reddivari: conducting the work in the lab, and contributed to the manuscript preparation
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correlations between them and seven cardiometabolic traits (CMTs): body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), insulin resistance (HOMA-IR), triglycerides (TG), and HDL-cholesterol (HDL-C).

Results—We identified 14 metabolites exhibiting differences between groups as well as linear trend across groups with nominal statistical significance. After adjustment for multiple testing mean differences and linear trends across groups remained significant ($P < 5.9 \times 10^{-5}$) for L-thyronine, bradykinin, and naringenin. Of the examined metabolite-CMT trait pairs, all metabolites except for 2-methylbutyroylcarnitine were nominally associated with two or more CMTs, some exhibiting significance even after accounting for multiple testing($P < 3.6 \times 10^{-3}$).

Conclusions—To our knowledge, this study - albeit pilot in nature - is the first study to identify these metabolites as novel biomarkers of childhood obesity and its correlates. These findings signify the need for future systematic investigations of metabolic pathways underlying childhood obesity.

Keywords

childhood obesity; metabolic syndrome; Mexican Americans; metabolites; biomarkers

Introduction

Childhood obesity has become worldwide epidemic with disproportionate ethnic differences in its prevalence (1–2). Based on 2009–2010 National Health and Nutrition Examination Survey (NHANES) data the prevalence of obesity was estimated to be 16.9% among children and adolescents (ages 2–19 years), with significant ethnic disparities in its prevalence: 14.0% in European Americans, 21.2% in Mexican Americans, and 24.3% in African Americans (2). Obesity and overweight are major risk factors for various chronic diseases, including type 2 diabetes (T2DM), cardiovascular disease (CVD), hypertension and metabolic syndrome (MS) (3–6). Increasing occurrence of obesity also parallels increased prevalence of MS and its component risk factors in children and adolescents, and such conditions may track into adulthood (7). Although it is well established that obesity and related traits have complex etiologies involving metabolic changes of the entire body, the actual disturbances in the metabolism leading to the progression of obesity are not clearly understood (8).

There have been continued efforts, including a range of emerging “omics” technologies, to understand the factors underlying the phenotypic expression of obesity and its correlates. The advent of advanced high-throughput metabolite profiling techniques has provided a unique opportunity to identify novel metabolites, and endophenotypes (i.e., measurable and heritable biomarkers that are genetically correlated, at least partly, with a disease/outcome). Such new information has great potential for contributing to a better understanding of the pathophysiology of obesity and related diseases, because variations in metabolite levels could reflect the integrated effects of genomic, transcriptomic and proteomic variations (9–11).

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Recently, we conducted a genetic epidemiologic investigation of cardiometabolic traits in Mexican American children and adolescents (ages 6–17 years), and found that these children bear substantial cardiometabolic burdens including overweight/obesity (53%), obesity (34%), prediabetes (13%), and MS (19%) [12]. Given the paucity of metabolomic data for young Mexican Americans (MAs), and youth from other US ethnic minorities, in the current study, we used the global metabolite approach 1) to examine metabolic differences among normal-weight, overweight and obese MA children who had participated in this study; and, 2) to assess phenotypic correlations between metabolites and 7 cardiometabolic traits.

Materials and Methods

Study participants

For this pilot study of serum global metabolite screening as it relates to childhood obesity, 15 normal-weight, 15 overweight, and 15 obese non-diabetic children and adolescents were selected based on weight-category, who had previously participated in our San Antonio Family Assessment of Metabolic Risk Indicators in Youth (SAFARI) Study (12). The selection of this subset of SAFARI children also required equal representation of girls and boys in each group (girls = 8 and boys =7; age: 6–17 years). Briefly, SAFARI study enrolled a total of 673 children aged 6–17 years from predominantly low-income extended MA families. Written informed consent was obtained from a parent or legal guardian for each child prior to his or her participation in SAFARI; written assent was also obtained from all children ≥ 7 years old. Study procedures were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Phenotype Data

As described previously (12), family history, demographic, phenotypic, and environmental data were obtained for SAFARI participants. Also, blood samples from them were obtained after a 10-hour overnight fast, were used to measure the levels of various metabolic parameters using standard protocols as described previously (12), which included fasting plasma glucose (FPG), specific serum insulin (FI), HDL cholesterol (HDL-C), and triglycerides (TG). Anthropometric measurements, including height, weight, waist circumference [WC], and blood pressure (systolic [SBP] and diastolic blood pressure [DBP]) were collected using standardized protocols, as described previously (12). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Weight categories were defined using BMI percentiles by sex and age obtained from the National Health and Nutrition Examination Survey (NHANES) III. Children and adolescents below the 85^{th} percentile were classified as normal-weight; those ≥85^{th} percentile and <95^{th} percentile as overweight; and, those ≥95^{th} percentile as obese. Using FPG and FI values, homeostasis model of assessment insulin resistance (HOMA-IR) was derived (http://www.dtu.ox.ac.uk/homacalculator).

Global metabolite profiling

Untargeted global metabolite profiling was performed in the fasting serum samples using Waters ultra-performance liquid chromatography/quadrupole orthogonal acceleration time of flight tandem micro mass spectrometer (UPLC/Q-Tof micro MS) according to the

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manufacturer's protocol (Waters, Milford, MA; http://www.waters.com/waters/home.htm). Briefly, chromatographic separation of the samples was performed on a Waters Acquity UPLC C8 column (1.8 μM, 1.0 × 100 mm) using a gradient from solvent A (95% water, 5% methanol, 0.1% formic acid) to solvent B (95% methanol, 5% water, 0.1% formic acid) at 140 μL/min flow rate and 50°C column temperature. Column eluent was infused into a Waters Q-Tof Micro MS fitted with an electrospray source. Data were collected in positive ion mode, scanning from 50–1000 at a rate of 2 scans per second. Files containing all information of micro MS data including retention times, *m*/*z*, and ion intensities in MassLynx software (Waters) was converted to cdf format using Databridge software. Raw peak areas were normalized to total ion signal in R and subjected to statistical analyses. Thus, the area under the curve for each feature represents relative intensity of that feature. Identification and alignment of peaks for metabolites was performed based on accurate mass, isotopic pattern and MS/MS information obtained from Metlin (http://metlin.scripps.edu), Human Metabolome (www.hmdb.ca) and ChemSpider (www.chemspider.com). Of the 45 samples chosen for this study, three samples were lost during extraction or UPLC/Q-Tof micro MS metabolomics analysis. Thus, the sample size of 45 was reduced to 42: normal-weight = 15, overweight = 13 and obese = 14.

Statistical Analysis

Each metabolite was treated as a quantitative trait for the analyses. The mean differences among the three groups (i.e., normal-weight/overweight/obese) were tested (2 df) using a one-way Analysis of Variance (ANOVA) technique. As an initial screening step, a $P<0.01$ was used to select peak areas from the identified total pool of peak areas unadjusted for the covariate effects of age and sex. Subsequently, the normalized peak-intensity values of the selected metabolites were transformed using inverse normalization, and adjusted for the covariate effects of age and sex. The residuals obtained after adjustment for age and sex effects were analyzed to assess differences among the three weight classes using one-way ANOVA (SPSS 19). In addition, we performed testing for linear trend across the groups and group order (1 df). As a conservative approach, we have derived a P value adjusted for multiple testing by using the number of metabolites in the data set, and the required significance threshold was 5.9×10^{-5} (0.05/850). The correlations between the selected metabolites and 7CMTs were assessed using Pearson correlation coefficients (SPSS 19). All traits were transformed using inverse normalization and adjusted for the covariate effects of age and sex. In regard to the correlations between the selected metabolites and CMTs, the required significance level was determined to be 3.6×10^{-3} per trait after accounting for multiple testing (0.05/14). No additional attempts to correct for multiple testing were made, given the substantial correlations among the examined phenotypes (12).

Results

Characteristics of the subset of 42 SAFARI children chosen for this study by weight category are given in Table 1. Untargeted, global metabolite profiling was performed in the fasting serum samples of these children using Waters's UPLC/Q-Tof micro MS. The raw data obtained as chromatograms of spectral peaks' intensities were analyzed using MassLynx software (Supplementary Figure 1). A total of 4,216 peaks representing

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metabolite fragments were obtained after eliminating background peaks; these peaks corresponded to ~850 metabolites with an average of 5 fragments per metabolite. The normalized ion intensities for each of these 4,216 peak areas were used for initial analyses. Ten peaks did not match with any known metabolites. Given that several of the remaining peaks represented the same metabolite, fourteen metabolites chosen based on initial $P<0.01$ with differences among the three groups were considered for this report. The identity and the characteristics of these metabolites are presented in Table 2. Subsequently, we repeated the ANOVA of each of the fourteen metabolites after adjusting for the covariate influences of age and sex, and the findings with nominal P values uncorrected for multiple testing are shown in Table 3. In addition, we performed testing for linear trend between the means and group order and the findings are also reported in Table 3. The significant findings that met the required significance threshold of 5.9×10^{-5} are highlighted in the table.

All 14 metabolites were found to exhibit differences between the examined groups as well as linear trend across them with nominal significance (P range: 4.8×10^{-7} [L-thyronine] - 0.018 [lysoPC (18:1)]; trend P range = 1.4×10^{-6} [naringenin] – 7.7×10^{-3} [vitamin D3]). Of the 14 metabolites, three of them including L-thyronine ($P = 4.8 \times 10^{-7}$; trend $P = 2.6 \times 10^{-5}$), naringenin ($P= 6.8 \times 10^{-6}$; trend $P = 1.4 \times 10^{-6}$) and bradykinin ($P= 5.4 \times 10^{-5}$; trend $P = 1.1 \times 10^{-5}$) showed significant differences among the three groups even after accounting for multiple testing (i.e., $P = 5.9 \times 10^{-5}$). As shown in the Table 3, for the purpose of discussion, the n-fold change in each metabolite, derived as a ratio through comparison between normal-weight and obese group means, ranged from 1.18 (malvidin3 [6-acetyl glucoside]) to 18.16 (L-thyronine). The metabolites that were found in higher concentrations in obese children, compared with normal-weight children, were bradykinin (4.17 fold), phosphocholine [16:1] (1.31 fold), and phosphotidylethanol-amine (1.28 fold). Metabolites that were found to be lower in obese children, compared with normal-weight children were L-thyronine (18.16 fold), indole-3-propionic acid (2.09 fold), naringenin (1.83 fold), 3-hydroxyquinone (1.73 fold), 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3 (1.40), 2-methylbutyroylcarnitine (1.38 fold), diglyceride (1.34 fold), calicoferol B (1.23 fold), linoleic acid (1.21 fold), lysoPC [18:1] (1.20 fold), and malvidin3-[6-acetyl glucoside] (1.18 fold). Such a trend with change in the levels of metabolites was also observed between overweight and normal-weight children.

The correlations were subsequently examined between these 14 metabolites and 7childhood obesity-related CMTs namely BMI, WC, SBP, DBP, HOMA-IR, TG and HDL-C were examined. The results of these association analyses are reported in Table 4, including the findings with nominal P-values uncorrected for multiple testing as well as the significant ones that met the required significance threshold of 3.6×10^{-3} which are highlighted in the table. Of the examined trait pairs, all the metabolites except for 2-methylbutyroylcarnitine were nominally associated with two or more of the CMTs (Table 4). Thirteen metabolites were associated with BMI and WC, twelve with TG, eight with HDL-C, six with SBP, five with DBP, and three with HOMA-IR. Notably, naringenin, a citrus flavonoid was associated with all the seven CMTs. Among the 14 metabolites, four (L-thyronine, 3-hydroxyquinine, 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3, and phosphotidylethanolamine) were associated with six of the CMTs, two (bradykinin, and phosphocholine) with five CMTs, two (diglyceride and linoleic acid) with four of the CMTs, and three(Indole-3-propionic

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acid, malvidin3-(6-acetylglucoside) and lysoPC) with three of the CMTs and one (calicoferol B) with two of the CMTs. The metabolites found to be lower among obese children showed negative correlations with SBP, DBP, HOMA-IR, TG and WC and positive correlations with HDL-C. The metabolites that were found to be higher in obese children, however showed positive correlations with BMI, WC, TG, SBP, DBP, HOMAIR, and negative correlations with HDL-C (Tables 3 and 4). As can be seen from Table 4, several of the associations continue to be significant even after accounting for multiple testing.

Discussion

In an attempt to understand the metabolic differences or metabolic dysfunction associated with childhood obesity, we screened for circulating serum metabolites in the normal-weight, overweight and obese MA children who had previously participated in SAFARI. Using the untargeted, global approach, we detected 14 metabolites with nominal or significant differences in concentration among normal-weight, overweight and obese children. Overweight and obese children, had decreased levels of 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3, 3-hydroxyquinone, diglyceride, indole-3-propionic acid, L-thyronine, lysoPC (18:1), linoleic acid, calicoferol B, malvidin3-(6-acetyl glucoside) 2-methylbutyroylcarnitine and naringenin and had increased levels of bradykinin, phosphocholine and phosphotidylethanol-amine were found in the overweight and obese children when compared with normal weight children.

Through our analyses we were able to identify – perhaps for the first time – 14 metabolites as novel biomarkers of childhood obesity and its clinical correlates; although three metabolites including L-thyronine, naringenin, and bradykinin continued to be significant even after adjusting for multiple testing. Recently, Wahl *et al.* (13) using a metabolomics approach targeting 163 metabolites from a small sample of normal-weight and obese children (ages 6–15 years) from Germany, reported an association between 14 metabolites and childhood obesity. However, only lysoPC (18:1) was found to be associated with childhood obesity in both of our studies. It may be that the differential observations between these studies can be attributed to the different metabolomics approaches utilized. Our present findings, however, together with the report by Wahl *et al*. (13), highlight that metabolic disturbances are occurring at very early stages in children and adolescents, which could contribute to the development of several diseases including obesity, T2DM and CVD later in life. Furthermore, our metabolomic data also highlight certain patterns that have not previously been shown to be associated with metabolic diseases in adults (9–11).

We found a striking 18 fold reduction in the levels of L-thyronine in obese children compared to those with normal weight. L-thyronine is derived from L-thyroxine (T4) and 3,5,3′-triiodo-L-thyronine (T3), which are essential for normal metabolism, growth and development. Thyroid hormone is a well-known modulator of lipid metabolism, which promotes lipolysis and increases fatty acid utilization and metabolic rate thereby reducing fat accumulation (14–15). Also, thyroid hormone plays a role in glucose homeostasis by increasing insulin-stimulated glucose transport and/or phosphorylation by increasing the expression of glucose transporter GLUT4 and glycolysis in the muscle (16–18). The observed negative correlation of L-thyronine with SBP, DBP, TG, BMI, WC and a positive

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correlation with HDL-C are consistent with the previous studies that demonstrated a negative influence of thyroid hormone on adiposity (14–15). It is well known that thyroid dysfunction is associated with weight change in both children and adults.

The presence of increased levels of bradykinin in overweight and obese children is of interest, given its role in vasodilation and inflammation. The release of bradykinin, a small vasoactive peptide, is probably a distal event in the inflammatory cascade and could be triggered by various mechanisms (19–20). In our study, we found a remarkable 4-fold increase in the levels of bradykininin obese children compared with normal-weight children and was positively correlated with SBP, TG, BMI and WC and negatively with HDL-C. In this context, it should be noted that: a) ACE inhibitors used for reducing blood pressure in hypertensive patients act by increasing bradykinin levels (21); and, b) overweight, obesity and excess adiposity regardless of its distribution have all been associated with impaired endothelial function in adults [measured in response to bradykinin] (22). While elevated bradykinin levels have been observed in overweight and obese children, it is unclear whether these have evolved as a potential compensatory mechanism to overcome slowly developing vascular dysfunction in these children, and/or whether they represent – even at these very early ages – an end product of inflammatory cascade itself. Bradykinin acts on the blood vessels through the release of nitric oxide, a potent inflammation suppressor, which exerts cardio protective effects and acts on other vasoactive agents. HDL-C has also been shown to increase nitric oxide (23). Obesity is associated with low HDL-C, which is a risk factor for T2DM, CVD and MS. Thus, bradykinin levels may increase in response to reduced levels of HDL-C in overweight and obese children as a compensatory mechanism to protect against blood vessel damage and future risk for CVD.

In addition to the above functions, bradykinin also helps in the insulin-dependent transportation and metabolism of glucose (24–25). Pretorius *et al.* (25) found that administration of bradykinin improved glucose uptake in overweight/obese adults. As reported by Iozzo *et al.* (26), adipose tissue blood flow ATBF resistance in obese individuals is compensated by increased insulin and bradykinin levels, although glucose uptake remained markedly impaired in obese individuals. Accordingly, in our study, increased levels of bradykinin in obese children could be triggered due to a variety of mechanisms.

We also observed that, among obese children, levels of naringenin, an exogenous dietary flavonoid found in grapefruit, orange and tomato skin were only half those of normal-weight children. It is tempting to speculate that the normal-children may be consuming more citric juices than overweight and obese children; further studies are warranted to investigate this finding. Naringenin and flavonoids have been shown to have lipid lowering, insulin-like, anti-inflammatory, anti-oxidant and anti-hypertensive properties (reviewed in 27). Not surprisingly, our data showed a negative association between naringenin and SBP, DBP, HOMAIR, BMI, WC, and TG and a positive correlation with HDL-C.

The observation of reduced levels of LysoPC (18:1), a phospholipid, in obese SAFARI children is consistent with the findings of [10] Kim *et al.* (2010) in obese adults. Recently, Ha *et al.* (28) reported that levels of several LysoPC species, including (18:1), differed between diabetic and nondiabetic men. In addition, indole-3-propionic acid, an antioxidant

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associated with cardiovascular disease was found to be lower in obese children (29). In agreement with Kim *et al.* (10), we observed a tendency towards lower levels of linoleic acid in obese children. Lower levels of 3-hydroxyquinine, 2-methylbutyroylcarnitine and higher levels of phosphocholine, and phosphotidylethanol-amine are indicative of disturbances in lipid and fatty acid metabolism; such patterns are potential risk factors for CVD. Phosphocholine, for example, has been shown to promote inflammation, and has been associated with CVD (30). Vitamin D insufficiency is also known to be associated with T2DM and obesity, and our findings of lower levels of vitamin D metabolites 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3 and calicoferol B in obese children are in support of such previous findings (31). Kabadi *et al.* (31) recently reported that abdominal obesity coupled with vitamin D insufficiency influence insulin resistance in adults.

Some limitations of our study includes its deductions based on a small sample size and its cross-sectional design as a limitation to infer causal relationships among metabolites, obesity and other cardiometabolic traits examined in this study. Since puberty can influence the metabolism, we repeated our analyses by considering pubertal status as an additional covariate, although information on pubertal status was available for only 34 of the 42 children considered for this study. The findings exhibited similar trends and a majority of the associations continued to be nominally significant (data not shown).

In conclusion, our findings highlight that metabolic disturbances have already been set in motion among overweight and obese MA children, and that certain metabolites can be considered as novel biomarkers of childhood obesity and its correlates. Biomarkers of risk, available early in life, could lead to targeted early-life screening and interventions among high-risk children, to prevent the development of serious health problems later in life. Our current plans include increasing our sample size to confirm our findings; in addition, given the nature of our SAFARI data, future validations of our findings in other populations would be very helpful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CMTs	cardiometabolic traits
BMI	body mass index

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WC	waist circumference
SBP	systolic blood pressure
DBP	diastolic blood pressure
HOMA-IR	insulin resistance
TG	triglycerides
HDL-C	HDL-cholesterol
T2DM	type 2 diabetes
CVD	cardiovascular disease
MS	metabolic syndrome
MA	Mexican Americans
NHANES	National Health and Nutrition Examination Survey
SAFARI	San Antonio Family Assessment of Metabolic Risk Indicators in Youth

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Table 1

Characteristics of the normal-weight (n = 15), overweight (n =13) and obese (n = 14) children

Variable*	**Normal weight# (Mean ± SD or %)**	**Overweight# (Mean ± SD or %)**	**Obese# (Mean ± SD or %)**
Females	53	54	57
Age (years) Range (years)	11.7 ± 2.9 8 – 17	12.2 ± 3.8 6 – 17	10.5 ± 2.8 7 – 16
Waist circumference (mm)	663.0 ± 98.8	804.0 ± 141.4	891.43 ± 99.5
BMI (kg/m^2)	18.1 ± 2.8	23.0 ± 3.8	27.5 ± 4.1
SBP (mm Hg)	100.2 ± 6.3	106.0 ± 11.2	104.7 ± 10.25
DBP (mm Hg)	60.2 ± 5.8	64.3 ± 7.4	62.6 ± 7.8
HDL-C (mg/dL)	55.3 ± 14.3	42.0 ± 8.0	41.4 ±10.7
TG (mg/dL)	51.7 ± 25.4	74.4 ± 38.7	91.8 ± 45.1
HOMA-IR	1.5 ± 0.6	1.8 ± 0.4	2.1 ± 0.5

*BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high density lipoprotein cholesterol; TG: triglyceride; HOMA-IR: Homeostasis Model of Assessment - Insulin Resistance;

#Weight categories were defined using BMI percentiles by sex and age; normal weight: <85th percentile for sex and age; overweight: ≥85th and <95th percentile for sex and age; and obese: BMI ≥95th percentile for sex and age.

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Table 2

Characteristics of the metabolites identified in this study

Identity	Formula[a]	HMDB/METLIN ID	Pathway/reaction	Retention time (min)	Actual mass	Exact mass	Tolerance (ppm)
L-Thyronine	$C_{15}H_{15}NO_4$	HMDB00667	thyroid hormone metabolism	3.42	274.1001	274.1074	26
Bradykinin	$C_{50}H_{73}N_{15}O_{11}$	HMDB04246	not in pathway	1.75	1059.5414	1059.5610	15
Indole-3-propionic acid	$C_{11}H_{11}NO_2$	HMDB02302	tryptophan metabolism	2.38	190.0838	190.0863	12
Naringenin	$C_{15}H_{15}O_5$	HMDB02670	flavanoid - exogenous	3.18	273.0788	273.0757	11
2-Methylbutyroylcarnitine	$C_{12}H_{23}NO_4$	HMDB00378	carnitine metabolism	3.52	246.1641	246.1700	23
3-Hydroxyquinine	$C_{20}H_{24}N_2O_3$	HMDB01091	metabolite of quinine	3.85	341.1819	341.1860	11
Vitamin D3*	$C_{22}H_{34}O_3$	METLIN ID: LMST03020011	vitamin D3 derivatives	4.48	347.2519	347.2581	17
LysoPC (18:1)	$C_{26}H_{52}NO_7P$	HMDB10385	lipid metabolism	5.55	522.3554	522.3554	0
Calicoferol B	$C_{27}H_{42}O_3$	METLIN ID: LMST03020180	vitamin D metabolism	7.75	415.3224	415.3207	4
Diglyceride (DG)	$C_{47}H_{76}O_5$	HMDB07672	fatty acid metabolism	10.12	721.5684	721.5616	9
Malvidin3-(6-acetyl glucoside)	$C_{25}H_{26}O_{13}$	METLIN ID: LMPK12010381	anthocyanin-exogenous	10.35	535.1547	535.1446	18
Linoleic acid	$C_{18}H_{32}O_2$	HMDB06270	fatty acid metabolism	10.37	281.2530	281.2475	19
Phosphotidylethanol-amine	$C_{42}H_{78}NO_8P$	METLIN ID: LMGP02010502	lipid metabolism	10.45	756.5684	756.5538	19
Phosphocholine (16:1)	$C_{24}H_{48}NO_7P$	METLIN ID: LMGP01050023	lipid metabolism	5.22	494.3236	494.3241	1

[a] Formulas and masses are based on $[M+H]^+$ or $[M+2H]^{2+}$;

* 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3 / 1α,22-dihydroxy-23,24,25,26,27-pentanorcholecalciferol

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Table 3

Characteristics of the fourteen metabolites in normal weight, overweight, and obese children

Identity	Metabolic Pathway	Normalized peak intensity (mean ± SD)[a]			ANOVA P-Value (2 df)[b]	Trend P-Value (1 df)[b]	Fold change[c]	Correlation with obesity	No. of CMTs correlated[d]
		Normal	Overweight	Obese					
L-Thyronine	thyroid hormone	8.2 ± 10.9	0.3 ± 0.12	0.5 ± 0.3	***4.8 × 10⁻⁷***	***2.6 × 10⁻⁵***	18.16	negative ↓	6
Bradykinin	not in pathway	29.3 ± 23.1	71.2 ± 52.3	122.2 ± 71.8	***5.4 × 10⁻⁵***	***1.1 × 10⁻⁵***	4.17	positive↑	5
Indole-3-propionic acid	tryptophan	6.0 ± 3.6	3.9 ± 3.1	2.9 ± 1.9	4.9×10^{-3}	1.3×10^{-3}	2.09	negative ↓	3
Naringenin	flavanoid[e]	44.4 ± 11.2	32.0 ± 7.9	24.3 ± 8.6	***6.8 × 10⁻⁶***	***1.4 × 10⁻⁶***	1.83	negative ↓	7
2-Methylbutyroyl-carnitine	carnitine	40.5 ± 13.9	29.6 ± 10.0	29.3 ± 8.0	0.0153	2.6×10^{-3}	1.38	negative ↓	0
3-Hydroxyquinine	quinine	21.9 ± 8.3	13.8 ± 5.2	12.7 ± 3.6	6.6×10^{-4}	7.6×10^{-4}	1.73	negative ↓	6
Vitamin D3[f]	vitamin D3	72.6 ± 19.8	58. 4 ± 22.5	52.0 ± 14.6	0.0161	7.7×10^{-3}	1.40	negative ↓	6
LysoPC (18:1)	lipid	1139.0 ± 198.8	1027.6 ± 133.6	948.6 ± 158.8	0.0176	4.8×10^{-3}	1.20	negative ↓	3
Calicoferol B	vitamin D	183.9 ± 23.9	178.8 ± 31.8	149. 7 ± 21.8	3.8×10^{-3}	2.1×10^{-3}	1.23	negative ↓	2
Diglyceride (DG)	fatty acid	148.4 ± 33.8	116.6 ± 34.5	110.6 ± 22.2	6.2×10^{-4}	2.2×10^{-4}	1.34	negative ↓	4
Malvidin3 (6-acetyl glucoside)	Anthocyanin[e]	550.1 ± 63.1	463.2 ± 68.6	465.9 ± 84.8	2.9×10^{-3}	4.4×10^{-3}	1.18	negative ↓	3
Linoleic acid	fatty acid	196.4 ± 22.3	159.8 ± 24.6	162.9 ± 29.0	1.0×10^{-3}	2.4×10^{-3}	1.21	negative ↓	4
Phosphotidylethan-olamine	lipid	3878.5 ± 858.6	4355.9 ± 816.6	4950.2 ± 910.1	9.0×10^{-3}	2.4×10^{-3}	1.28	positive↑	6
Phosphocholine (16:1)	lipid	271.5 ± 70.9	370.3 ± 75.5	354.5 ± 76.5	1.9×10^{-3}	2.4×10^{-3}	1.31	positive↑	5

[a] Normalized peak intensities were based on peak areas. Raw peak areas were normalized to total ion signal in R;

[b] Significant after accounting for multiple testing are shown in bold/italic;

[c] Comparison between normal-weight and obese groups are shown for the purpose of comparison;

[d] number of cardio-metabolic traits (CMTs) nominally associated ($P \leq 0.05$) with the specific metabolites;

[e] exogenous;

[f] 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3 / 1α,22-dihydroxy-23,24,25,26,27-pentanorcholecalciferol.

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Table 4

Association of metabolites with childhood obesity–related cardiometabolic traits (*r*= correlation between a given pair of traits)

Trait	BMI	WC	HDL	TRIG	SBP	DBP	HOMA
	***r* (P-Value)[a]**	***r* (P-Value)[a]**	***r* (P-Value)[a]**	***r* (P-Value)[a]**	***r* (P-Value)[a]**	***r* (P-Value)[a]**	***r* (P-Value)[a]**
L-Thyronine	***−0.471 (0.002)***	***−0.35 (0.023)***	***0.39 (0.010)***	***−0.32 (0.036)***	***−0.31 (0.049)***	***−0.32 (0.041)***	−0.17 (0.277)
Bradykinin	***0.565 (<0.001)***	***0.53 (<0.001)***	***−0.51 (0.001)***	***0.45 (0.003)***	***0.31 (0.045)***	0.24 (0.124)	0.29 (0.061)
Indole-3-propionic acid	**−0.518(<0.001)**	**−0.46 (0.002)**	**0.33 (0.035)**	−0.09 (0.569)	−0.003 (0.984)	−0.03 (0.835)	0.13 (0.399)
Naringenin	***−0.652 (<0.001)***	***−0.62 (<0.001)***	***0.34 (0.028)***	***−0.51 (0.001)***	***−0.31 (0.043)***	***−0.34 (0.030)***	***−0.42 (0.006)***
2-Methylbutyroylcarnitine	−0.177(0.2621)	−0.22 (0.168)	0.11 (0.474)	−0.05 (0.754)	0.09 (0.568)	0.22 (0.157)	0.15 (0.331)
3-Hydroxyquinine	**−0.510 (<0.001)**	**−0.59 (<0.001)**	**0.39 (0.011)**	**−0.58 (<0.001)**	**0.32 (0.041)**	−0.23 (0.153)	**−0.33 (0.034)**
Vitamin D3[b]	**−0.452 (0.003)**	**−0.44 (0.004)**	0.26 (0.102)	**−0.48 (0.001)**	**−0.45 (0.003)**	**−0.44 (0.003)**	**−0.47 (0.002)**
Lyso-PC	**−0.403 (0.008)**	**−0.42 (0.006)**	0.24 (0.133)	**−0.31 (0.049)**	−0.17 (0.281)	−0.01 (0.977)	0.11 (0.493)
Calicoferol B	**−0.398 (0.009)**	**−0.50 (0.001)**	0.08 (0.628)	−0.29 (0.059)	−0.16 (0.300)	−0.09 (0.536)	−0.26 (0.102)
Diglyceride (DG)	**−0.468 (002)**	**−0.36 (0.020)**	**0.48 (0.001)**	**−0.41 (0.008)**	−0.19 (0.231)	−0.24 (0.122)	0.004 (0.979)
Malvidin3-(6-acetylglucoside)	**−0.515 (<0.001)**	**−0.52 (<0.001)**	0.10 (0.545)	**−0.36 (0.020)**	−0.26 (0.092)	−0.27 (0.090)	−0.22 (0.159)
Linoleic acid	**−0.529 (<0.001)**	**−0.52 (<0.001)**	0.13 (0.413)	**−0.35 (0.024)**	**−0.31 (0.049)**	−0.29 (0.0610	−0.18 (0.252)
Phosphotidylethanolamine	**0.459 (0.002)**	**0.45 (0.003)**	**−0.35 (0.021)**	**0.64 (<0.001)**	**0.48 (0.001)**	**0.63 (<0.001)**	0.27 (0.079)
Phosphocholine (16:1)	**0.473 (0.002)**	**0.47 (0.002)**	**−0.56 (<0.001)**	**0.60 (<0.001)**	0.24 (0.122)	**0.44 (0.004)**	0.19 (0.237)

[a] Significant after accounting for multiple testing are shown in bold/italic and correlations with nominal significance are shown in bold;

[b] 1α,22-dihydroxy-23,24,25,26,27-pentanorvitamin D3 / 1α,22-dihydroxy-23,24,25,26,27-pentanorcholecalciferol.

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