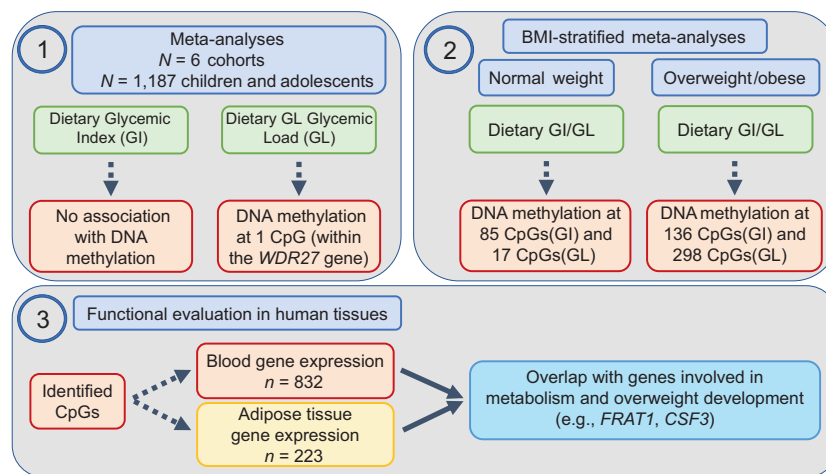


Epigenome-Wide Meta-analysis Reveals Associations Between Dietary Glycemic Index and Glycemic Load and DNA Methylation in Children and Adolescents of Different Body Sizes

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ARTICLE HIGHLIGHTS

- This study was undertaken to elucidate mechanisms underlying a diet with high glycemic index (GI) and glycemic load (GL) during childhood and increased cardiometabolic risk.
- We wanted to determine if dietary GI and GL are associated with DNA methylation at metabolic genes and if epigenetic patterns are altered in people with overweight.
- Dietary GI and GL were associated with 537 DNA methylation sites, especially in people with overweight. DNA methylation was related to expression of genes known to affect metabolism.
- A high GI and GL diet may influence epigenetic regulation in young people.



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Raffael Ott,^{1,2} Robert Stein,^{3,4}
Helena H. Hauta-alus,^{5,6,7,8}
Justiina Ronkainen,⁹
Sílvia Fernández-Barrés,^{10,11,12}
Ulrike Spielau,³ Holger Kirsten,¹³
Tanja Poulain,¹⁴ Phillip E. Melton,^{15,16}
Leanne K. Küpers,^{17,18}
Hatim Azaryah,^{19,20} Marco Colombo,³
Kathrin Landgraf,³ Elmar W. Tobì,²¹
Therese O'Sullivan,²² Rae-Chi Huang,²²
Cristina Campoy,^{12,19,20,23}
Christiane Winkler,^{1,2,24} Jesus Vioque,^{12,25}
Martine Vrijheid,^{10,11,12} Wieland Kiess,^{3,14}
Antje Körner,^{3,4,14} Sylvain Sebert,⁹
Marjo-Riitta Jarvelin,^{9,26,27,28}
Anette-G. Ziegler,^{1,2,24} and
Sandra Hummel^{1,2,24}

OBJECTIVE

Dietary glycemic index (GI) and glycemic load (GL) are associated with cardiometabolic health in children and adolescents, with potential distinct effects in people with increased BMI. DNA methylation (DNAm) may mediate these effects. Thus, we conducted meta-analyses of epigenome-wide association studies (EWAS) between dietary GI and GL and blood DNAm of children and adolescents.

RESEARCH DESIGN AND METHODS

We calculated dietary GI and GL and performed EWAS in children and adolescents (age range: 4.5–17 years) from six cohorts ($N = 1,187$). We performed stratified analyses of participants with normal weight ($n = 801$) or overweight or obesity ($n = 386$). We performed look-ups for the identified cytosine–phosphate–guanine (CpG) sites (false discovery rate [FDR] < 0.05) with tissue-specific gene expression of 832 blood and 223 subcutaneous adipose tissue samples from children and adolescents.

RESULTS

Dietary GL was positively associated with DNAm of cg20274553 (FDR < 0.05), annotated to *WDR27*. Several CpGs were identified in the normal-weight (GI: 85; GL: 17) and overweight or obese (GI: 136; GL: 298; FDR < 0.05) strata, and none overlapped between strata. In participants with overweight or obesity, identified CpGs were related to RNA expression of genes associated with impaired metabolism (e.g., *FRAT1*, *CSF3*).

CONCLUSIONS

We identified 537 associations between dietary GI and GL and blood DNAm, mainly in children and adolescents with overweight or obesity. High-GI and/or -GL diets may influence epigenetic gene regulation and thereby promote metabolic derangements in young people with increased BMI.

Over the past decades, the prevalence of obesity has increased worldwide, leading to increased morbidity and mortality (1), mainly due to emergence of obesity-related,

¹Institute of Diabetes Research, Helmholtz Munich, German Research Center for Environmental Health, Munich, Germany

²Forschergruppe Diabetes e.V. at Helmholtz Munich, German Research Center for Environmental Health, Munich, Germany

³Medical Faculty, University of Leipzig, University Hospital for Children and Adolescents, Center for Pediatric Research, Leipzig, Germany

⁴Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG) of the Helmholtz Zentrum München at the University of Leipzig and University Hospital Leipzig, Leipzig, Germany

⁵PEDEGO Research Unit, Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland

⁶Population Health Unit, Finnish Institute for Health and Welfare, Helsinki, Finland

⁷Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

⁸Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

⁹Research Unit of Population Health, Faculty of Medicine, University of Oulu, Oulu, Finland

¹⁰ISGlobal, Barcelona, Spain

¹¹Universitat Pompeu Fabra, Barcelona, Spain

¹²Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Carlos III Institute, Madrid, Spain

¹³Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany

noncommunicable diseases (NCDs) (2). There is strong evidence that obesity and obesity-related adverse health consequences have their origins in early life. Early childhood is a vulnerable phase when sustained obesity manifests (3). Aside from the prenatal and early childhood periods, late childhood and adolescence have been proposed as a second window of opportunity for establishing lasting lifestyle behaviors that affect the development of obesity and related NCDs (4). This is supported by findings from a large population-based study indicating that an increase in BMI between 7 years of age and early adulthood predicts an increased risk of type 2 diabetes (T2D) in adulthood (5). Growth during childhood and adolescence is affected by a variety of genetic and environmental factors, especially diet.

Diets high in glycemic index (GI) and glycemic load (GL) are associated with cardiovascular risk factors (6), hyperinsulinemia, and markers of insulin resistance (7) and obesity (8) in children, and with T2D, coronary heart disease, and metabolic syndrome in adults (9). The underlying (molecular) mechanisms remain largely unexplored, although they may help explain the known health benefits of a low GI and/or GL diet. DNA methylation (DNAm) is a key epigenetic mechanism involved in the regulation of gene activity. Accumulating evidence suggests that dietary factors, such as folate and resveratrol, can influence DNAm and may thus contribute to the effects of diet on long-term health (10). Recently, maternal dietary GI and GL in pregnancy have been associated with DNAm in cord blood (11). In that study, the findings differed between mothers with normal weight and those

with overweight or obesity (hereafter, overweight/obesity), and most associations were observed in the offspring of mothers with overweight/obesity, potentially indicating modification by body size (11). Consistent with these findings, some of the observed associations between GI and, particularly, GL and cardiometabolic health were more pronounced in participants with an increased BMI (9).

So far, the effects of dietary GI and GL on the epigenomes of children and adolescents remain unknown. We hypothesized that dietary GI and GL are associated with blood DNAm in children and adolescents and, therefore, could serve as markers of dietary exposure. We further hypothesized that BMI subgroup analysis reveals a different epigenetic pattern in participants with normal weight than those with overweight/obesity, and we explored its potential role in the development of NCDs by investigating metabolism-related gene expression in blood and subcutaneous adipose tissue.

RESEARCH DESIGN AND METHODS

Participants

Six cohorts that contribute to the Pregnancy and Childhood Epigenetics (PACE) consortium (12) participated in this cross-sectional meta-analysis ($N = 1,187$) (for cohort-specific information, see Supplementary Material). With the exception of the Raine Study Gen2 (Raine) in Australia, all cohorts were from Europe (Spain: Infancia y Medio Ambiente [INMA]; Germany: The LIFE Child Study [LIFE Child]; Leipzig Atherobesity Childhood Cohort [Atherobesity]; the Study in Teens of the Natural Course of Type 1 Diabetes [TEENDIAB];

and Finland: the Northern Finland Birth Cohort 1986 [NFBC1986]). The analysis only included children and adolescents (age range: 4.5–17.0 years). There were no participants with diabetes in the analysis. To avoid the potential underlying genetic effects, only one participant per family was included, based on the completeness of the data, and by equal completeness, the selection was done randomly. Participants with a GI or GL above or below 5 times the SD of the individual cohort mean were also excluded (NFBC1986: GI, $n = 2$ and GL, $n = 11$; LIFE Child: GL, $n = 1$). Further descriptions of the cohorts are provided in the Supplementary Material. Ethical approval and informed consent were obtained from each cohort according to national and international standards (Supplementary Material).

Dietary GI and GL Calculations

A food's GI is defined as the 2-hour incremental area under the curve of blood glucose after consumption of a defined portion, typically 50 g of available carbohydrate, expressed as percentage to the glycemic response of a reference food, usually glucose or white bread (13). Thus, a food's GI ranges between 0 and 100. The GL is defined as the food-specific GI multiplied by the amount of carbohydrates in that food item (13). We used a harmonized method across cohorts to calculate the dietary GI and GL from food frequency questionnaires (FFQs) or dietary records (for cohort-specific information, including validation of assessment methods, see Supplementary Material), as described previously (11). Briefly, the carbohydrate content and GI (using glucose

¹⁴LIFE Child Leipzig Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany

¹⁵Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

¹⁶University of Western Australia, School of Population and Global Health, Perth, Western Australia, Australia

¹⁷The Generation R Study Group, Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam, the Netherlands

¹⁸Department of Pediatrics, Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam, the Netherlands

¹⁹Department of Paediatrics, School of Medicine, University of Granada, Granada, Spain

²⁰EURISTIKOS Excellence Centre for Paediatric Research, Biomedical Research Centre, University of Granada, Granada, Spain

²¹Periconceptional Epidemiology, Department of Obstetrics and Gynaecology, Division of

Obstetrics and Prenatal Medicine, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

²²Nutrition and Health Innovation Research Institute, Edith Cowan University, Perth, Western Australia, Australia

²³Instituto de Investigación Biosanitaria de Granada (Ibs-Granada), Granada, Spain

²⁴Forscherguppe Diabetes, School of Medicine, Klinikum rechts der Isar, Technical University Munich, Munich, Germany

²⁵Alicante Institute for Health and Biomedical Research, University Miguel Hernandez, Alicante, Spain

²⁶Department of Epidemiology and Biostatistics, School of Public Health, Imperial College, London, U.K.

²⁷Medical Research Council–Public Health England Centre for Environment and Health, School of Public Health, Imperial College, London, U.K.

²⁸Department of Life Sciences, College of Health and Life Sciences, Brunel University London, Middlesex, U.K.

Corresponding author: Sandra Hummel, sandra.hummel@helmholtz-munich.de

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as the reference) of food items were retrieved from country-specific reference data (European countries: Diabetes, Obesity, and Genes [DIOGENES] [14]; Australia: Atkinson et al. [15]). The food-specific information was linked to individual food items consumed, based on the FFQ or dietary record data. If food items were missing from the country-specific reference data, the information was taken from those of other countries. All food items with carbohydrate contents <1 g/100 g were excluded. The individual dietary GI and GL values were calculated as the weighted average of all foods consumed according to common formulas (11).

Cohort-Specific Epigenome-Wide Association Studies Analyses

We used blood DNAm as the outcome measure, determined with either Infinium HumanMethylation450 (450 K) or MethylationEPIC (EPIC) Bead-Chip arrays (Illumina Inc., San Diego, CA). Cohorts with the 450 K array were INMA, NFBC1986, and Raine; cohorts with the EPIC array were LIFE Child, Atherobesity, and TEENDIAB. Each cohort performed their own quality control and normalization procedures (Supplementary Table 1). For all analyses, we used normalized, untransformed β values, where 0 indicated no methylation and 1 indicated complete (100%) methylation. For better interpretation, effect estimates were thus multiplied by 100 to express the change in percentage of methylation. A common prespecified analysis plan with R script for performing the epigenome-wide association studies (EWAS) was provided to all cohorts. We excluded extreme DNAm values, using the Tukey method, as described previously (11). The cohort-specific analyses between dietary GI or GL and epigenome-wide DNAm were performed using robust linear regression (R package *limma*) and were adjusted for age, sex, parental education (cohort-specific definition, see Supplementary Material), smoking, total energy intake, blood cell type estimates, and technical variables. The analyses were additionally adjusted for cohort-specific covariables, if needed (Supplementary Material). Total energy intake was unavailable in NFBC1986 and was not included in these analyses. Blood cell types were predicted using the Houseman method, implemented in the R package *minfi* (16). Because we considered BMI as a potential moderator, we analyzed the same models in the BMI

stratum normal weight, as defined by the BMI SD score (SDS) ($-2 \leq \text{BMI SDS} \leq 1$) and overweight/obese ($1 < \text{BMI SDS}$), based on World Health Organization age- and sex-specific reference charts (17,18). All EWAS results were successfully checked for quality, using the R package *QCEWAS* (19).

Meta-analyses

Before running the meta-analyses, we excluded probes on sex chromosomes, known cross-reactive probes, and probes with sequence polymorphism (20,21). All remaining probes were meta-analyzed if they were contained in at least two cohorts. Given that the 450 K and EPIC arrays differ in terms of probe numbers, the sample size for array-specific probes varied. The EPIC array probes covered 91.9% of probes on the 450 K array. To correct for potential deflation or inflation and bias in the individual-cohort EWAS results, we applied the R package *bacon* with the following modifications: $n_{\text{iter}} = 100,000$, $n_{\text{burnin}} = 25,000$ (22) (Supplementary Table 2). We then performed fixed-effects invariance-weighted meta-analyses with the *bacon*-corrected data using the R package *metafor* (23).

After each meta-analysis, we used Cochrane Q tests to evaluate the heterogeneity of the model. The cohorts Raine and Atherobesity introduced notable heterogeneity in the GL model (Supplementary Fig. 1), and these cohorts, therefore, were excluded from the GL analysis (remaining $n = 693$) to reduce the possibility of spurious findings. Because the models of the NFBC1986 cohort did not include the total energy intake, we checked the heterogeneity with and without this cohort. Because the heterogeneity did not change after inclusion of NFBC1986 (Supplementary Fig. 2), we performed all meta-analyses including NFBC1986. We report the I^2 value to indicate potential heterogeneity. To avoid possible human error, the meta-analyses were independently repeated by another analyst using the software *METAL* (24), and all results were confirmed. The final meta-analyses models showed no sign of deflation/inflation (λ) and bias (μ) (Supplementary Table 3). We used the false discovery rate (FDR) to correct for multiple testing, and P_{FDR} values < 0.05 were considered statistically significant. Annotation to genomic locations (reference genome hg19) was done with Illumina's annotation files (450K: *IlluminaHumanMethylation450kanno.ilmn12.hg19*;

EPIC: *IlluminaHumanMethylationEPICanno.ilm10b4.hg19*). The results of the meta-analyses have been uploaded to the public EWAS catalog (Zenodo; <https://doi.org/10.5281/zenodo.7220349>).

Look-ups of Findings

We checked whether our findings overlapped with significant DNAm sites in meta-EWAS of related traits: maternal dietary GI and GL (11) or glycemia (25) in pregnancy and cord-blood DNAm, and childhood BMI and blood DNAm (26). We performed look-ups within the EWAS catalog (27) and the Accessible Resource for Integrative Epigenomic Studies (ARIES) methylation quantitative trait loci (mQTL) database of blood from children aged 7 years (450 K array only) (28). For the overlapping mQTLs, we searched whether the genetic variants are linked to the following traits: childhood obesity, obesity, T2D, and cardiovascular disease. We performed mendelian randomization (MR) analysis (two-sample), using the R package *TwoSampleMR*, for the identified risk variant using the Wald ratio. We used the ARIES mQTL data as the exposure set and genome-wide association studies catalog data of these traits as the outcome set to explore potential causal effects of the mQTL-CpGs on obesity and disease risk (Supplementary Material).

To determine whether our findings were significantly related to tissue-specific gene expression, we queried the Human Early Life Exposome (HELIX) *cis*-expression quantitative trait methylation (*cis*-eQTM) database (adjusted for blood cell type) of blood from 832 children (mean age: 8.1 years; 450 K array only) (29), and checked for associations with *cis*-eQTMs in subcutaneous adipose tissue samples from 223 participants (mean age: 10.5 years) in the Leipzig Childhood Adipose Tissue Cohort (ClinicalTrials.gov identifier NCT02208141; EPIC array; Supplementary Material).

Functional Enrichment

We queried the Locus Overlap Analysis (LOLA) Core database to search for enrichments of chromosomal markers, enhancer or repressor regions, and transcription-factor binding sites (30).

RESULTS

Cohort Summary

This analysis included a total of 1,187 children and adolescents (Table 1). Across the cohorts, the mean dietary GI ranged

Table 1—Characteristics of the populations by participating cohorts

	INMA	TEENDIAB	LIFE Child	Atherobesity	NFBC1986	Raine Study
<i>N</i>	177	181	105	109	227	388
Country	Spain	Germany	Germany	Germany	Finland	Australia
Age, mean (SD), years	4.5 (0.2)	10.8 (1.3)	11.6 (2.6)	13.2 (2.7)	16.1 (0.4)	17.0 (0.3)
Female sex, %	53.7	48.9	49.5	56.9	57.7	45.9
Parental education level, %						
Low	24.9	3.7	19.0	29.4	10.6	44.8
Medium	42.9	12.6	61.9	42.2	73.1	35.5
High	32.2	84.7	19.0	28.4	16.3	19.5
Current smoker, %	0	0	0	9.2	50.2	26.5
BMI, mean (SD), kg/m ²	16.2 (1.4)	17.9 (3.2)	23.8 (6.6)	24.7 (7.3)	21.5 (3.2)	21.1 (3.9)
BMI SDS, mean (SD)	0.6 (0.9)	0.2 (1.2)	1.6 (1.6)	1.3 (1.6)	0.2 (1.0)	0.5 (1.2)
Type of dietary assessment	FFQ	DR	FFQ	DR	FFQ	DR
GI, mean (SD)	50.8 (2.6)	56.7 (3.9)	56.0 (4.5)	54.0 (4.9)	61.9 (5.8)	58.3 (3.8)
GL, mean (SD)	99.6 (29.5)	161.6 (52.8)	141.6 (61.6)	117.4 (35.4)	124.2 (47.2)	151.9 (23.5)
Total energy intake, mean, kcal/day	1,648 (353)	2,211 (577)	2,147 (860)	1,892 (488)	NA	2,306 (588)
Only participants with normal weight						
<i>N</i>	131	131	39	49	180	271
Age, mean (SD), years	4.5 (0.2)	10.8 (1.3)	11.6 (2.5)	13.5 (2.6)	16.1 (0.4)	17.0 (0.3)
Female sex, %	50.4	48.1	53.8	63.3	59.4	48.0
Parental education level, %						
Low	23.7	3.5	7.7	18.4	10.5	43.9
Medium	43.5	13.0	56.4	34.7	72.3	35.1
High	32.8	82.5	35.9	46.9	17.2	21.1
Current smoker, %	0	0	0	12.2	50.0	25.1
GI, mean (SD)	50.8 (2.6)	56.6 (3.8)	56.6 (3.9)	53.6 (4.2)	62.0 (5.7)	58.3 (3.7)
GL, mean (SD)	100.1 (29.8)	161.2 (53.7)	141.9 (46.1)	130.1 (36.8)	126.3 (46.1)	154.0 (23.0)
Total energy intake, mean (SD), kcal/day	1,654 (353)	2,204 (576)	2,076 (622)	2,043 (517)	NA	2,323 (591)
Only participants with overweight/obesity						
<i>N</i>	46	50	66	60	47	117
Age, mean (SD), years	4.5 (0.3)	10.6 (1.3)	11.6 (2.7)	13.0 (2.9)	16.0 (0.3)	17.0 (0.2)
Female sex, %	63.0	50.0	47.0	51.7	51.1	41.0
Parental education level, %						
Low	28.3	2.0	25.8	38.3	10.7	47.0
Medium	41.3	8.0	65.2	48.3	76.6	36.7
High	30.4	90.0	9.1	13.3	12.8	16.3
Current smoker, %	0	0	0	6.7	51.1	29.9
GI, mean (SD)	50.8 (2.7)	57.0 (4.3)	55.7 (4.9)	54.3 (5.4)	61.1 (6.2)	58.3 (4.1)
GL, mean (SD)	98.1 (29.1)	162.5 (50.9)	141.4 (69.5)	107.0 (30.9)	116.4 (50.9)	147.2 (24.0)
Total energy intake, mean (SD), kcal/day	1,630 (358)	2,228 (580)	2,189 (976)	1,768 (430)	NA	2,269 (581)

DR, dietary record; FFQ, food frequency questionnaire; NA, not available.

from 50.8 to 61.9, and GL ranged from 99.6 to 161.6. Similar frequencies of sex and smoking and similar ranges of GI and GL were observed after stratification into the normal weight ($n = 801$) and overweight/obese ($n = 386$) categories (Table 1).

Meta-analysis of the Whole Sample Set

In the analysis of all available samples, dietary GI showed no association with blood DNAm after FDR correction. The top five CpGs based on P_{nominal} are presented in Supplementary Table 4. Among

these, the association with the smallest SE was observed between dietary GI and cg01578632 ($\beta = -2.5 \times 10^{-2}$; SE = 5.1×10^{-3} ; $r^2 = 0$; $P_{\text{nominal}} = 8.7 \times 10^{-7}$), annotated to WW domain containing E3 ubiquitin protein ligase 2 (*WWP2*).

Dietary GL was positively associated with blood DNAm at cg20274553 ($\beta = 2.3 \times 10^{-3}$; SE = 4.2×10^{-4} ; $r^2 = 0$; $P_{\text{FDR}} = 2.2 \times 10^{-2}$) located in intron 13 of the WD repeat domain 27 (*WDR27*) gene (see Table 2 and Supplementary Table 5 for the top five CpGs based on P_{nominal}).

Meta-analysis of BMI Strata

In the normal-weight stratum, the DNAm of 85 CpGs was significantly associated with GI and the DNAm of 17 CpGs with GL (Fig. 1A and B, Supplementary Tables 6 and 7). There was considerable heterogeneity ($I^2 > 75$) in 36 CpGs of the GI analysis and six CpGs of the GL analysis (Supplementary Figs. 3 and 4).

In the overweight/obese stratum, the DNAm of 136 CpGs was significantly related to GI and the DNAm of 298 CpGs to GL (Fig. 1C and D, Supplementary

Table 2—CpGs associated with dietary GI or GL, annotated to the same genes in multiple meta-analysis models

Annotated gene*	Probe identifier (CpG)	Gene region	Relation to CpG island	Meta-analysis model	N†	Estimate‡	SE‡	P value	FDR-corrected P value	r ²	mQTL
WDR27	cg20274553	Intron	Open sea	GL (whole group)	691	0.0023	0.0004	3.26×10^{-8}	0.0224	0	No
	cg25687360	Intron	Open sea	GI (overweight/obese)	386	0.0492	0.0102	1.40×10^{-6}	0.0176	80.8	No
DNMT3B	cg08951101	Intron	Open sea	GI (normal weight)	219	−0.3736	0.0438	1.57×10^{-17}	1.09E−11	94.9	No
	cg25446789	Intron	Open sea	GI (overweight/obese)	386	0.1918	0.0419	4.81×10^{-6}	0.0364	82.1	No
OSBP2	cg03688771	Intron	Open sea	GI (normal weight)	219	0.2484	0.0468	1.16×10^{-7}	0.0053	63.2	No
	cg08036104	Intron	Open sea	GI (overweight/obese)	176	0.1141	0.0229	6.37×10^{-7}	0.0116	75.8	No
IFFO2	cg11237901	Intron	Open sea	GI (normal weight)	219	−0.5548	0.1192	3.25×10^{-6}	0.0345	91.8	No
	cg09243533	Intron	Island	GL (normal weight)	481	0.0042	0.0007	9.04×10^{-8}	0.0155	28.2	No
KPNB1	cg04057642	Exon	South shelf	GI (overweight/obese)	386	−0.2253	0.0494	5.15×10^{-6}	0.0375	54.5	Yes
	cg27491277	Exon	Open sea	GL (overweight/obese)	116	0.0102	0.0020	5.39×10^{-7}	0.0051	0	No
HAS3	cg12055844	Exon	Open sea	GI (overweight/obese)	176	0.0524	0.0115	6.18×10^{-6}	0.0401	0	No
	cg00505936	Promoter	North shore	GL (overweight/obese)	159	−0.0119	0.0027	1.11×10^{-5}	0.0322	53.3	Yes
ADGRV1	cg12332383	Intron	Open sea	GI (normal weight)	219	0.2592	0.0517	5.37×10^{-7}	0.0115	75.8	No
	cg07908697	Intron	Open sea	GL (overweight/obese)	116	0.0128	0.0029	1.19×10^{-5}	0.0334	63.3	No

*Based on Illumina annotation. †N differs depending on the number of the investigated group (whole, normal weight, or overweight/obese) and number of samples with the respective CpG (e.g., depending on the 450 K or EPIC array). ‡Effect size and SE are presented as percentage changes in DNAm per 1-point increase in the GI or GL.

Tables 8 and 9). Considerable heterogeneity ($I^2 > 75$) was observed in 40 CpGs of the GI analysis and 47 CpGs of the GL analysis (Supplementary Figs. 5 and 6).

Among the CpGs with highest heterogeneity, mainly one cohort but not always the same one, had a strong effect. Leave-one-out analysis did not show one distinct cohort driving all the associations (Supplementary Figs. 7–10).

In total, the BMI-stratified analyses identified 536 significant DNAm sites (Supplementary Table 10). Of these, 222 were only available in the cohorts with the EPIC array. We found no overlap of significant CpGs across any model (full and BMI-stratified models). However, approximately 93% of the significant CpGs in the BMI-stratified analyses ($n = 500$ of 536) had the same effect direction as in the whole-sample meta-analysis, and effect estimates showed a strong correlation between the BMI-stratified and whole-sample results (Spearman $r = 0.90$; $P < 2.2 \times 10^{-16}$, Supplementary Table 10). When comparing the effect estimates of CpGs significant in one stratum and nonsignificant in the other, 245 of 536 CpGs had different directions of effects (Supplementary Table 10).

Table 2 shows the overlapping annotated genes of the significant CpGs across all models. Like the association with GL in the whole-sample analysis, DNAm at a CpG (cg25687360) located in intron 8 of the *WDR27* gene was positively associated with GI in children and adolescents with overweight/obesity (Table 2). However, this association was largely driven by the NFBC1986 cohort, and did not remain significant without NFBC1986 ($P_{\text{nominal}} = 7.4 \times 10^{-1}$). In participants with overweight/obesity, CpGs associated with both GI and GL were annotated to the genes karyopherin $\beta 1$ (*KPNB1*) and hyaluronan synthase 3 (*HAS3*) (Table 2).

Look-ups of Findings in Published Resources

Across all significant findings ($n = 537$ CpGs with $FDR < 0.05$), we found no overlap with CpGs identified in meta-EWAS of related traits (see *Research Design and Methods*).

By querying the EWAS catalog using all 537 CpGs, we identified overlaps with 1,121 previously published associations (corresponding to 339 unique CpGs; Supplementary Table 11). Enrichment was found for the traits age, sex, tissue, and Alzheimer

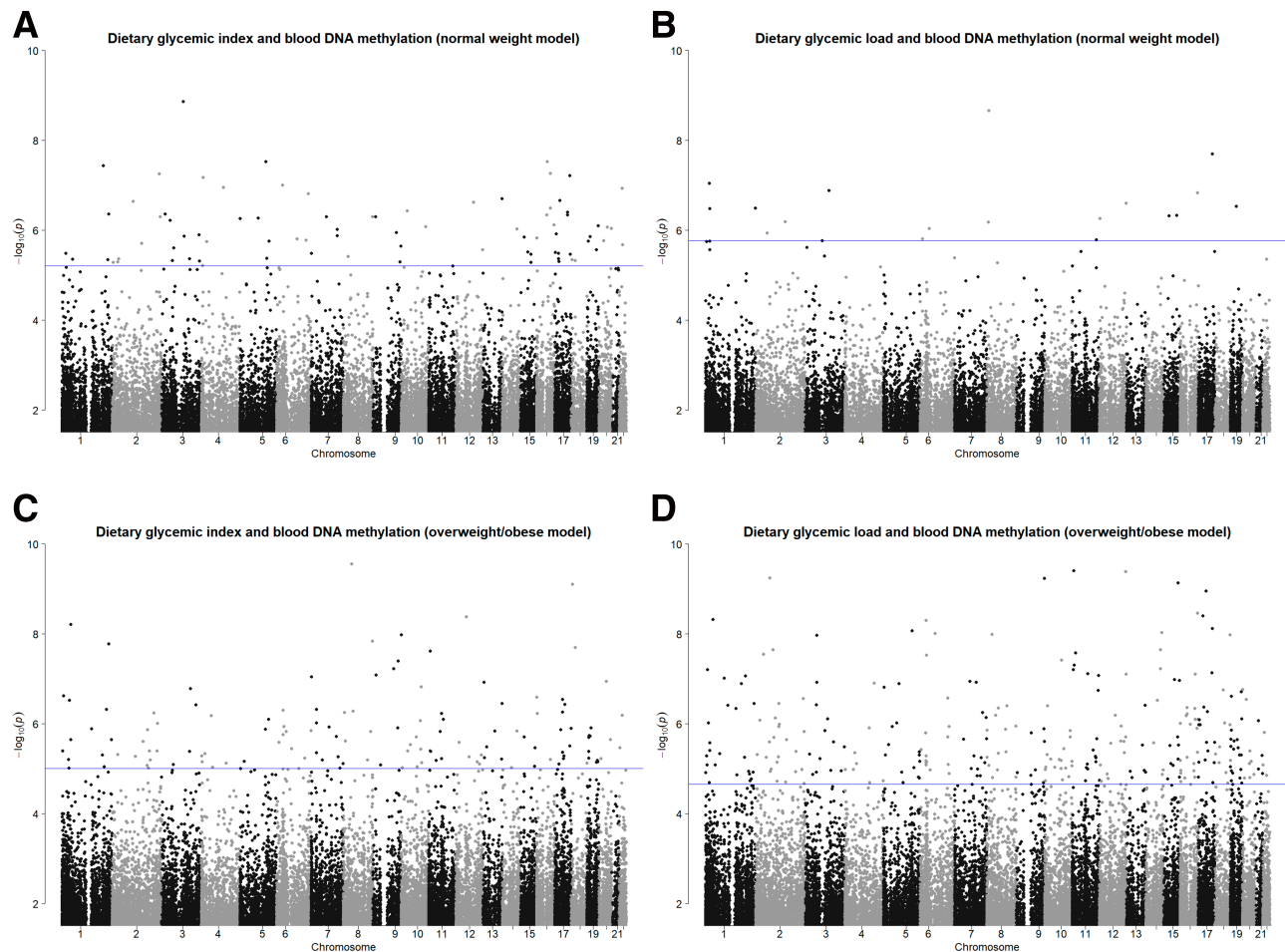


Figure 1—Manhattan plots of EWAS with dietary GI and GL in children and adolescents with normal weight or overweight/obesity. Each plot shows the nominal *P* values (as negative logarithms) of all meta-analyzed DNAm sites across the autosomal chromosomes (1–22) for their association with either the dietary GI (A and C) or GL (B and D) in people with normal weight (A and B; maximum *n* = 801) or overweight/obesity (C and D; maximum *n* = 386). The blue line indicates the threshold of the nominal *P* value at an FDR of 0.05 in each model. Dots above the blue line show significant (FDR < 0.05) DNAm sites.

disease Braak stage (hypergeometric test, FDR < 0.05). Because of the large overlap with age and sex, we evaluated whether these variables were associated with the identified CpGs in the TEENDIAB cohort. Only one CpG was significantly associated with age, and eight were associated with sex (Supplementary Table 12). Of 1,121 overlapping associations with the EWAS catalog, 77 were attributed to environmental exposures, including alcohol consumption, breastfeeding, and sedentary behavior; 95 were attributed to pregnancy-related exposures (e.g., gestational age, maternal overweight/obesity), and seven associations were attributed to glycemic traits (e.g., maternal gestational diabetes) (Supplementary Table 11). Of the 339 unique CpGs, 77 were related to multiple traits (Supplementary Table 13), for example, cg10807894, which has been associated with maternal overweight/obesity, nitrogen dioxide exposure, maternal BMI, eosinophilia, gestational age, and smoking.

Of all 537 CpGs, 99 showed an overlap with mQTLs (Supplementary Table 14). None of the mQTLs overlapped with genetic variants associated with childhood obesity, obesity, and cardiovascular disease, but one, rs9560114, has been linked to T2D. MR analysis showed that the association between this single nucleotide polymorphism and T2D is putatively caused by DNAm of CpG cg24892433. Higher DNAm at cg24892433 was associated with a decreased risk for T2D ($\beta = -6.8 \times 10^{-2}$; SE = 1.2×10^{-2} ; $P = 3.6 \times 10^{-8}$; Supplementary Table 15). Interestingly, a higher GL was associated with lower DNAm at this CpG in participants with overweight/obesity (Supplementary Table 9).

Look-ups of Tissue-Specific Gene Expression

For the look-up in the blood eQTM data of the HELIX project (450 K array), we used 315 CpGs from our findings located on the 450 K array. We observed overlaps with 76 eQTMs

(corresponding to 39 unique CpGs; Supplementary Table 16). The overlapping eQTMs were annotated to 35 genes, and the top five CpG–RNA transcript pairs corresponding to the most significant identified CpGs in the meta-analyses are shown in Table 3.

For the look-up in subcutaneous adipose tissue eQTMs from the Leipzig Childhood Adipose Tissue Cohort (EPIC array), we used 502 CpGs from our findings available in the EPIC array. We found an overlap with 89 eQTMs (corresponding to 48 unique CpGs) associated with 84 unique RNA transcripts (Supplementary Table 17). Among these, the top five most significant CpGs are shown in Table 3. When we compared the overlapping eQTMs from both tissues, eight CpGs were found in both sources; however, these were related to different genes, apart from *PLEK* (cg13060970).

Functional Enrichment

By querying the LOLA database using the significant CpGs of each BMI model separately,

Table 3—Overlapping CpGs significantly associated with dietary GI or GL in the whole group or BMI-stratified models and eQTM data of blood and subcutaneous adipose tissue

Probe identifier (CpG)	Gene region	Relation to CpG island	Meta-analysis effect*	Meta-analysis model	CpG annotated gene	Transcript annotated gene	Transcript identifier	CpG-transcript effect†	CpG-transcript SE†	CpG-transcript P value	mQTL
Child blood (HELIX database)											
cg05474605	Intron	Open sea	0.03	GI (overweight/obese)	SEPP1	CCDC152	TC05002348.hg.1	0.19	0.47	6.7×10^{-5}	Yes
cg01723892	Intergenic	Open sea	0.03	GI (overweight/obese)		NTPCR	TC01001926.hg.1	0.20	0.32	7.4×10^{-10}	No
cg07151406	Intron	North shore	0.40	GI (overweight/obese)	PGAM1	PGAM1	TC10000695.hg.1	−0.07	0.12	1.7×10^{-9}	No
cg07151406	Intron	North shore	0.40	GI (overweight/obese)	PGAM1	FRAT1	TC10000692.hg.1	0.06	0.14	3.0×10^{-5}	No
cg13060970	Exon	Open sea	0.03	GI (overweight/obese)	PLEK	PLEK	TC02000398.hg.1	−0.32	0.46	3.0×10^{-12}	No
Child/adolescent subcutaneous adipose tissue (Leipzig Childhood Adipose Tissue Cohort)											
cg05399244	Intron	Open sea	−0.15	GI (overweight/obese)	RPH3AL	GEMIN4	ILMN_1770206	0.96	0.21	1.3×10^{-5}	No
cg13060970	Exon	Open sea	0.03	GI (overweight/obese)	PLEK	PLEK	ILMN_1795762	−2.81	0.70	7.6×10^{-5}	No
cg13060970	Exon	Open sea	0.03	GI (overweight/obese)	PLEK	ARHGAP25	ILMN_1777998	−1.48	0.29	9.5×10^{-7}	No
cg13060970	Exon	Open sea	0.03	GI (overweight/obese)	PLEK	CNRIP1	ILMN_3232894	1.79	0.41	1.8×10^{-5}	No
cg07723258	Exon	Open sea	−0.02	GI (overweight/obese)	MED24	CSF3	ILMN_2322768	−2.41	0.44	1.4×10^{-7}	Yes

The table shows only the five most significant CpG-transcript pairs identified (based on FDR-corrected *P* values for CpGs in the meta-analysis). For all significant pairs, see Supplementary Tables 16 and 17. *Effect sizes are presented as percentage changes in DNAm per 1-point increase in the GI or GL. †CpG-transcript effect sizes and SEs in blood represent changes in expression as log-two-fold change per 10% DNAm and in adipose tissue change as log2 expression per % DNAm.

we found enrichments for various regulatory elements, including transcription factors (e.g., forkhead-box protein A2) and active (e.g., H3K9K14ac) or repressive (e.g., H3K9me3) histone modifications (Supplementary Table 18). Contrary to participants with normal weight, CpG sites identified in participants with overweight/obesity showed enrichments for the metabolism-related transcription factors cAMP-responsive element-binding protein 1 and GA-binding protein (Supplementary Table 18).

CONCLUSIONS

This study is the largest EWAS of dietary GI and GL and blood DNAm in children and adolescents to date, to our knowledge. Overall, we found one CpG associated with GL in the total group and 536 hits in the BMI-stratified models, particularly in the participants with overweight/obesity. Among these, many CpGs were significantly related to eQTMs in the blood and subcutaneous adipose tissue of same-age children and were located within genetic regions of various regulatory factors.

The CpG positively associated with GL in the total group (cg20274553) is annotated to *WDR27*, which is involved in cellular scaffolds for protein–protein interactions. We also found that a nearby CpG (cg25687360) in *WDR27* was positively associated with GI in participants with overweight/obesity. Greater *WDR27* gene expression was detected in a model of hepatic steatosis and, importantly, was linked to higher DNAm in the intronic region of *WDR27* (31). Both of the identified CpGs are also located in introns of *WDR27*, and higher GI or GL exposure was associated with higher DNAm of these probes. Because there was no overlap with eQTM data, confirmation is needed that these CpGs affect *WDR27* expression. In a study with patients with syndromic obesity, duplicated copy number variants of *WDR27* were observed (32), potentially indicating a pathogenic role here. Therefore, *WDR27* is an interesting candidate in the development of obesity and metabolic disease and may be regulated by intronic DNAm, which, in turn, may be susceptible to dietary exposure rich in simple carbohydrates.

Dietary GI and GL were associated with 536 CpGs in participants with normal weight or overweight/obesity, a pattern also observed previously (11). In participants with overweight/obesity, GI and GL

were related to CpGs located at the genes *KBNP1* and *HAS3*. Both genes potentially promote metabolic disorders (33,34). Greater hepatic *KBNP1* expression has been found in mice fed a high-fat diet, contributing to enhanced pro-inflammatory cytokine levels and insulin resistance (33). *HAS3* has been proposed as a potential therapeutic target for the activation of brown adipose tissue (BAT) thermogenesis. Inhibition of *HAS3* in mice improved BAT's thermogenic capacity and thereby prevented diet-induced obesity (34). However, because the respective CpGs were unavailable in the eQTM data, it remains open whether these CpGs, indeed, affect *KBNP1* and *HAS3* expression.

To shed more light on CpG–gene relations, we interrogated the overlap with eQTMs from blood and adipose tissues from participants of similar ages—an important aspect because many eQTMs differ between young people and adults (29). We observed 165 CpG–transcript pairs possibly affected by GI or GL exposure, such as *FRAT1* regulator of WNT signaling pathway 1 (*FRAT1*), which prevents glycogen–synthase kinase 3 (*GSK3*) from inhibiting mammalian target of rapamycin complex 1 (*mTORC1*) (35). We found that higher GI intake was associated with higher CpG methylation, which was related to higher gene expression of *FRAT1*. This may lead to reduction in *GSK3* activity, allowing increased *mTORC1* action (35). The nutrient-sensor *mTORC1* is involved in hepatic lipid metabolism promoting, for example, de novo lipogenesis, and inhibiting lipophagy (36). Moreover, *mTORC1* stimulation may lead to hyperinsulinemia by impeding autophagy in pancreatic β -cells (37). Therefore, *mTORC1* overactivation may contribute to metabolic disorders. Another gene identified was colony stimulating factor 3 (*CSF3*), which appears to play a role in obesity and insulin resistance. Patients with morbid obesity had a greater abundance of *CSF3* in their adipose tissues (38), and exposure to higher levels of *CSF3* induced marked insulin desensitization in human adipocytes and myotubes (39). *Pleckstrin* (*PLEK*) appeared in both eQTM look-ups and is involved in platelet activation. Although the knowledge about *PLEK* in the context of cardiovascular disease is limited, a potential role has been suggested in atherosclerosis (40). *FRAT1*, *CSF3*, *PLEK*, and other eQTMs overlapped with CpGs identified in the overweight/obese models,

which may indicate that these effects are present and/or more pronounced in people with higher BMI (9).

Although the role of GI and GL on the pathways discussed above requires further investigation, the results of the MR analysis suggest that in children and adolescents with overweight/obesity, GL affects CpG cg24892433 methylation, which, in turn, is causally related to T2D risk.

We can only speculate about the nature of the observation that BMI stratification revealed a higher number of associations compared with the whole-sample analysis. A potential explanation is that certain selection factors (e.g., genetic and/or environmental factors) are more enriched in the stratified groups, leading to (stronger) associations between dietary GI or GL and DNAm. Indeed, the vast majority of identified CpGs had the same direction of effects in the stratified and the whole-sample analysis, whereas effect sizes in the latter were attenuated.

The major limitations of this study include its modest to low sample size, especially in the BMI-stratified analysis. We followed a consortium-wide effort to maximize the sample sizes, but the studies were partly limited by the availability of data on consumed foods, which are required to fully calculate the dietary indices. Heterogeneity arising from the various dietary resources across the cohorts (e.g., FFQ vs. dietary records, type of FFQ [non- or semi-quantitative], and/or the number of collected food items with carbohydrate content) may have influenced the GI and GL scores; we attempted to minimize this by using a harmonized calculation approach. Misreporting of food consumption is a known concern and may have affected the associations. A larger fraction of our findings showed considerable heterogeneity, which can be expected given the inherent heterogeneity of methods of the dietary assessments, the availability of certain probes across cohorts, the modest to low sample sizes per cohort, and the age range from 4.5 to 17 years, because age can influence DNAm. Last, the possibility remains that other factors not accounted for affected the associations.

The major strengths of the study include the harmonized approach to derive the GI and GL values across cohorts and the use of eQTM data from similar age groups to evaluate the functional properties of DNAm sites in blood and, especially, adipose tissue. Unfortunately, eQTM data for

childhood and adolescence are rare and omit key metabolic tissues (e.g., the liver). Because key findings of our analysis involved genes with major roles in the liver, a look-up in hepatic eQTM data would have given more insights into tissue-specific CpG–transcript relations.

In conclusion, this meta-analysis revealed 537 blood DNAm sites associated with dietary GI and GL in young participants—the vast majority after BMI stratification. These may be considered potential markers of dietary response. Although the functional importance of the identified CpGs needs to be further defined, multiple CpGs appear to play regulatory roles in the expression of genes involved in the impairment of metabolism and obesity development (e.g., *FRAT1*, *CSF3*, *WDR27*). More analyses with larger sample sizes and complementary designs are required to support our observations and to explore (reverse) causality of these findings and (tissue-specific) functionality of identified CpG–gene relations.

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