의학박사 학위논문

고도비만 소아 및 청소년에서 체중 감량 중재 반응에 따른 대사체 및 유전체 분석

Metabolomic and Genetic Signatures for the Effects of Weight loss Interventions on Severe Obesity in Children and Adolescents

2021 년 2 월

서울대학교 대학원
임상의과학과
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ABSTRACT

Metabolomic and Genetic Signatures for the Effects of Weight Loss Interventions on Severe Obesity in Children and Adolescents

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Objective:

Childhood obesity has increased worldwide, and many clinical and public interventions have attempted to reduce morbidities. However, there are no reliable biomarkers to identify high-risk groups or good response groups to specific interventions. This study aimed to determine the metabolomic and genetic signatures associated with weight control interventions in obese children.

Methods:

Forty obese children from the "Intervention for Children and Adolescent Obesity via Activity and Nutrition (ICAAN)" cohort were selected according to intervention responses. Based on changes in BMI (body mass index) z-scores, half of the children were responders (n=20), and the others were non-responders (n=20).

Physiological data and blood samples were collected at baseline, and after 6 and 18 months of intervention. A total of 120 samples were collected, and the metabolites of serum samples were quantitatively analyzed using CE-TOFMS (capillary electrophoresis time of-flight mass spectrometry). We performed a secondary analysis using the Metaboanalyst™ program to build a relevant metabolite and molecular genetic network for significant metabolites. For genomic analysis, the genome list obtained from GWAS (genome-wide association study) data and a literature review with IPA (ingenuity pathway analysis) were compared in 123 subjects in the intervention study.

Results:

A total of 194 metabolites in 120 samples were detected based on an HMT (human metabolome technologies) standard library. Nine metabolites (1-or 3-Methylhistidine, 3-Hydroxypropionic acid, 8- or 2-Hydroxyoctanoic acid-1, alanine, aspartic acid, cystine, indole-3-acetic acid, N-acetylalanine, and tyrosine) showed significant changes at 18 months from baseline in responders and non-responders ($p<0.05$). After reanalyzing the data using the Metaboanalyst program, 13 metabolites (1-Methyl-4-imidazoleacetic acid, 10-Hydroxydecanoic acid, 3-Indoxylsulfuric acid, 2- or 8-Hydroxyoctanoic acid-2, allo-threonine, azelaic acid, disulfiram, indole-3-acetic acid, N-Acetyllysine-1 or -2, N-acetylornithine, succinic acid, and XC0065) showed significant differences between responders and non-responders at baseline ($p<0.05$), and six metabolites (2-oxooctanoic acid, glucose, isocitric acid, lauric acid, sulfotyrosine, and XC0126) showed significant differences between baseline and 6months samples in responders and non-responders ($p<0.05$). In addition, nine metabolites (1- or 3-Methylhistidine,

alanine, aspartic acid, cystine, glycocholic acid, homovanillic acid, piperidine, sulfotyrosine, and tyrosine) showed significant changes between baseline and 18 months in responders and non-responders (p<0.05). After 18 months of intervention, 63 metabolites showed significant differences regardless of response (FDR (false discovery rate) -adjusted p-value <0.05). Combining the results of the above analyses, alanine, aspartic acid, and cystine were significantly changed by the intervention after 18 months. We also selected genomic indicators by analyzing the key literature and analyzing metabolite networks. As a result, significant p-values were observed in IRS1 rs2943641 and FAIM2 rs7138803 in the GWAS of the extended 123 intervention study subjects.

Conclusions:

Our study showed significantly different metabolomic profiles for alanine, aspartic acid, and cystine in responder and non-responder groups after 18 months, according to the interventions. In addition, various analytical methods and validations were attempted to select metabolites showing differences between the two groups at each time point. We also performed an integrated analysis of metabolomic and genomic results, and as a result, we found that IRS1 rs2943641 and FAIM2 rs7138803 were associated with intervention effect in obesity. This result calls for further research on biomarkers related to specific treatments or interventions in a larger group of obese children. It can also be used as a predictor of the effects of obesity interventions.

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Keywords: biomarkers; metabolomics; genomics; obesity; pediatric

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LIST OF ABBREVIATIONS

BCAA	Branched-chain amino acids
BMI	Body mass index
CE-TOFMS	Capillary electrophoresis time of flight mass spectrometry
FAIM2	Fas apoptotic inhibitory molecule 2
FDR	False discovery rate
GWAS	Genome-wide association study
HCA	Hierarchical cluster analysis
HMT	Human Metabolome Technologies, Inc.
ICAAN	Intervention for Children and Adolescent Obesity via Activity and Nutrition
IPA	Ingenuity pathway analysis
IRS1	Insulin receptor substrate 1
kNN	k-means nearest neighbor
PCA	Principal component analysis
SNP	Single nucleotide polymorphisms

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INTRODUCTION

The prevalence of childhood obesity has increased worldwide[1]. Child and adolescent obesity rate in some countries exceeded 30%. According to statistics published by the Korean Ministry of Education, the Korean obesity rate in 2018 also reached 25%. Childhood and adolescent obesity are related to anthropometric and metabolic changes such as metabolic syndrome, including dyslipidemia, hypertension, and insulin resistance[2]. Adolescent obesity is associated with several cancers and cardiovascular disease in adulthood[3,4]. Thus, obesity is a severe health problem, and exacts social and economic costs.

Obesity is caused by various complex factors, of which genetic and epigenetic factors are the major causes. These factors affect the lipiodome, metabolome, and proteome, and various omics studies have investigated the causes of obesity[5]. Knowing the cause and risk factors of obesity is important not only for understanding pathogenesis but also for finding effective personalized treatment. Metabolomics is an advanced analysis technique and data processing tool that uses many metabolites in human tissues or biofluids. Metabolites are small molecules, substrates, intermediates, and end-products of cellular regulatory processes, which play important roles in cellular and physiological energetics, structure, and signaling[6]. The concept of the metabotype is based on cluster analysis, where individuals with similar metabolite patterns are grouped in clusters. Metabotyping is set to play a key role in the development and delivery of personalized nutrition[7]. Therefore, metabotyping of obese

patients will play an essential role in developing a new understanding and treatment of obesity.

As obesity rates increased, metabolic studies that reveal metabolomic signatures of obese patients and study metabolic changes such as inflammation or oxidative stress associated with obesity are increasing[8]. However, few studies have investigated childhood obesity, especially those involving weight loss interventions[9-13]. The prepubertal obese children study reported that urine trimethylamine N-oxide (TMAO) decreased after lifestyle intervention. Of the 32 identified metabolites, xanthosine, 3-hydroxyisovalerate, and dimethylglycine were altered following intervention[14]. In the other overweight adolescent study, after an eight-week exercise program, the urine concentrations of pantothenic acid, glyceric acid, l-ascorbic, xanthine, and adenosine were increased compared to the normal weight group[15].

Genetic factor studies in obesity have been performed in various ethnic groups, ages, and genomic variation associated with interventional effects[16-18]. The effect of obesity gene variability on weight change is only 2.4%, and only 1.6% when correcting for age, sex, and initial weight[19]. Thus, obesity is affected by multifactorial traits and -omics studies that combine genetic and metabolomic causes are critical. However, no integrated -omics study of weight loss effects in childhood obesity exists.

This study analyzed metabolites at different intervention intervals by selecting responders and non-responders and identifying significantly different metabolites. We confirmed significant genetic variation between responders and non-responders and considered the relationship of this variation to metabolites and clinical indicators.

From this study, metabolomic and genomic information could be used to identify biomarkers related to childhood obesity and develop future personalized treatments.

MATERIALS AND METHODS

Study Population

We extracted 40 obese subject (body mass index [BMI]>97th percentile for age and sex) from 242 patients in the 'Intervention for Children and Adolescent Obesity via Activity and Nutrition (ICAAN) study' cohort based on intervention responses. This study was designed as a multidisciplinary intervention test to prevent excessive weight gain and to improve several health indices in children and adolescents with obesity in Korea[20]. Participants were aged from 6 to 17 years old and more than the 95th percentile of age- and sex-specific BMI according to the 2007 Korean National Growth Charts. This study is a follow-up for up to 24 months after interventions, and they were randomly divided into 3 groups and received interventions, including the usual care, exercise, and nutrition feedback group. Each group has a similar portion (usual care group (n=84, 34.7%; exercise group (n=74, 30.6%); nutrition feedback group (n=84, 34.7%)), and all groups receive five category interventions; nutrition, physical activity, group activity, parental education, and self monitoring. The exercise group included the contents of the usual care group and added weekly exercise class and activity feedback. The nutrition feedback group received an additional individual nutrition feedback including the usual care group contents[21,22]. Patients with obesity-related hereditary diseases or other underlying disease were excluded. Of the 242 patients, 163 were followed up 6 months after weight intervention, and 111 were followed up 18 months later. A total of 131 participants were dropped out during the intervention,

mainly due to busy schedule, no response, no willingness, or busy schedule of parents. A flowchart of the study participants can be found in the Supplementary data **(Fig. 1).** We observed follow-up data at baseline, 6 months, and 18 months after the intervention to observe the intervention effects trends. When the BMI z-score was checked after 18 months, participants were selected in two groups according to the change in BMI z-scores. 20 subjects who presented significant intervention were assigned to the target group (responder, n=20). The others were non-responders (n=20) who had minimal weight loss. The changes in responder BMI z-scores were <-0.45, and >-0.1 in the non-responder group, respectively. Random sampling was not possible due to the limitation of the number of samples, so it was set in consideration of the portion and the number of samples of each intervention group. The non-responder group was selected according to the number of responders, and no patients gained weight due to the intervention, so the patients with the least weight change were selected. It was confirmed that there were no significant differences according to sex, age and type of intervention. Physiological data and blood samples were collected at baseline, and 6 and 18 months after the intervention in responder and non-responder group, for a total of 120 collected samples. Metabolic and genomic analyses of the samples were analyzed in two groups.

Sample preparation

The 120 samples were transported from SNU to HMT (Human Metabolome Technologies, Inc.) via Young-In Frontier Co., Ltd. In HMT, stored in a deep freezer below -80 ℃. Each 50 μL sample was mixed with 200 μL of methanol containing internal standards (20 μM).

Milli-Q water (150 μL) was then added and mixed thoroughly. The solution (300 μL) was filtered through a 5-kDa cut-off filter (ULTRAFREE-MC-PHCC, Human Metabolome Technologies, Yamagata, Japan) remove macromolecules. The filtrate was centrifugally concentrated and resuspended in 50 μL of ultrapure water immediately before measurement. The compounds were measured in the cation and anion modes of CE-TOFMS (capillary electrophoresis time of-flight mass spectrometry) based on metabolome analysis equipped with an Agilent CE-TOFMS system (Agilent Technologies Inc.) and a fused silica capillary i.d. 50 μM × 80 cm.

Metabolomic data processing and Statistical Analysis

Peaks detected by CE-TOFMS analysis were extracted using automatic integration software (MasterHands ver. 2.17.4.19, Keio University; Tokyo, Japan) to obtain peak information including m/z, migration time (MT), and peak area. The peak area was then converted to the relative peak area. The peak detection limit was determined based on the signal-noise ratio; S/N = 3. Putative metabolites were then assigned from HMT's standard library and known-unknown peak library based on m/z and MT. The tolerance was ±0.5 min for MT and ±10 ppm in m/z. If several peaks were assigned the same candidate, the candidate was given the branch number. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed using statistical analysis software (developed by HMT). The peak profiles with putative metabolites

were represented on metabolic pathway maps using VANTED (Visualization and Analysis of Networks containing Experimental Data) software[23]. The pathway map was prepared based on the metabolic pathways that are known to exist in human cells.

We performed a secondary analysis using the Metaboanalyst program to build a relevant metabolite and molecular genetic network for significant metabolites[24]. The comparative data analysis by HMT calculated all except 'N.D' (not detected) data and was based on welch's t-test. To include these missing values, we used the minimum value imputation method to increase the sample volume. Any missing values were assumed to be below the detection limits and could be imputed with the compound minimum data by the kNN (k means nearest neighbor) method[25]. In addition, MetaMapp 2017 (http://metamapp.fiehnlab.ucdavis.edu/) and Cytoscape version 3.7.2 (https://cytoscape.org/index.html) were used for network analysis related to metabolic changes by the weight loss intervention.

Genomic Analysis and SNPs selection

For genomic analysis, genomic DNA was extracted from the subject's peripheral blood samples and genotyping was performed using the Axiom^{TM} Precision Medicine Research Array (PMRA) chip (Thermo Fisher Scientific, Waltham, MA, USA). GWAS data of 39 subjects from 40 patients (group 2) were included from the ICAAN intervention study cohort. A total of 902,560 single nucleotide polymorphisms (SNPs) was available. The exclusion criteria for quality control for the imputed data were minor allele frequency <0.0, missing genotype rate >0.1, the missing rate per person >0.1, and Hardy-Weinberg equilibrium p-value <0.001 were excluded. In total,

293,713 SNPs remained in our analysis.

To include more samples, 154 patients with SNP information at 6 months follow-up were selected from the intervention study. Based on the changes in BMI z-scores, 41 responders and 82 non-responders were selected and GWAS was performed on a total of 123 subjects (group 1). After the quality control processing similar to group 1, 281,745 of 902,560 SNPs remained.

To increase the yield of the genomic analysis, a PubMed literature search was performed to select candidate SNPs and compare them with the genome list obtained from GWAS data in our group. Articles published between 2007 and 2019 were included. The representative search terms used were 'genetics', 'genomics', 'genome' "AND" 'obesity', 'weight loss intervention', 'obesity intervention', 'BMI change' "AND" 'child', 'children', 'adolescent'. We reviewed approximately 60 studies and selected the top 13 according to the selection criteria. The selection criteria were scored to determine studies with a total score of 5 or higher: 1) intervention study (obesity intervention -3/ obesity observation -2/ other-1), 2) race (Asia -2/other-1), 3) age (children -2/ adult -1). We excluded syndromic obesity and the monogenic causes of obesity. Seven final candidate genes and 12 SNPs were selected considering the study with the highest score, the top-ranked journal with an impact factor over 10, and the gene with more than two significant reports. A list of selected article reviews can be found in the Supplementary data **(Table S1).** To further study genomics, we selected more candidate genes and SNPs by reflecting the molecules obtained through metabolic network analysis. Finally, we selected 26 candidate SNPs and 18 genes from the reference review and compared group 1 and

group 2 subject's GWAS results.

In addition, the relationship between the significant genotype of SNPs and the change in clinical indicators of the subjects was confirmed.

Ingenuity Pathway Analysis

IPA (Ingenuity Systems Inc., Redwood City, CA) is a software platform that offers biological analysis such as an 'Upstream Regulator Analysis', 'Mechanistic Networks', 'Causal Network Analysis' and 'Downstream Effects Analysis' using a large number of biological data[26]. The IPA is the leading pathway analysis to a vast amount of biological information regularly updated for all chemicals modeled as drugs or clinical candidates. IPA can quickly visualize complex -omics data and perform insightful data analysis and identify the most significant pathways in various biological systems. It has been used to reveal the relationship between obesity and clozapine[27]. We constructed a network in IPA using metabolite data significantly analyzed by Metaboanalyst, baseline, and 6 and 18 -month post-intervention intervals in the responder and non-responder groups. In addition, a genetic literature search related to obesity was performed using major compounds in this network.

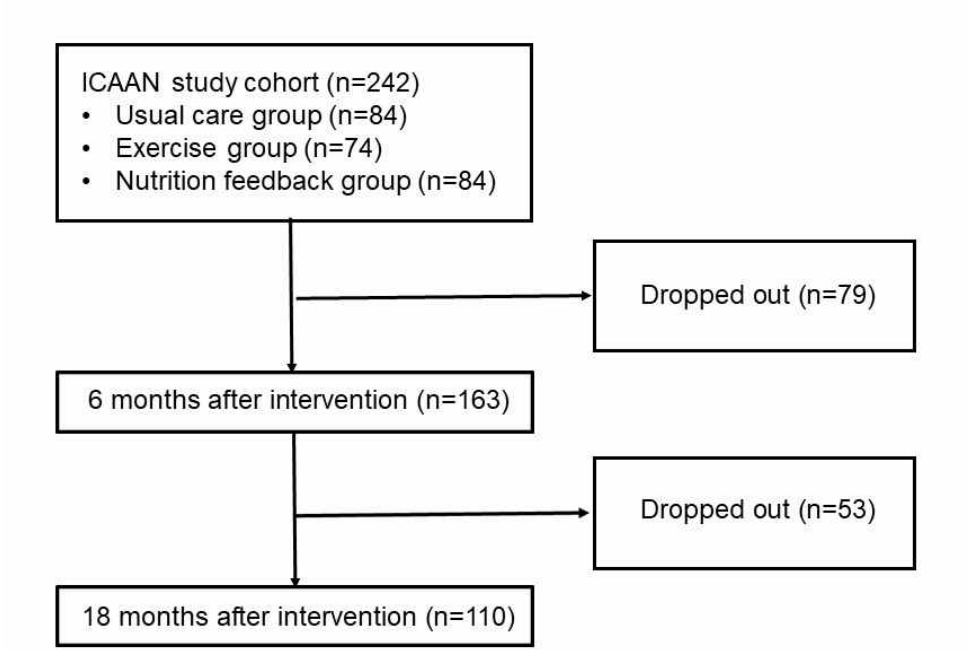


Figure 1. A flowchart of the study participants.

RESULTS

Targeted metabolomic analysis by CE-TOFMS

A total of 20 responders, and 20 non-responders were selected after 18 months of intervention based on BMI z-score changes. Samples were collected before intervention, and 6 months (six time point), and 18 months after intervention (18 time point); thus, a total 120 serum samples were analyzed. Thirty-nine of 40 patients yielded SNP information, and **Table 1** presents their BMI z-scores and characteristics. The changes in responder BMI z-scores were < -0.45, and > -0.1 in the non-responder group. A total of 194 metabolites were identified using CE-TOFMS by HMT **(Table S2)**. Of the 194 metabolites analyzed, 111 metabolites in cation mode and 83 metabolites in anion mode, and nine metabolites (1- or 3-methylhistidine, 3-hydroxypropionic acid, 8- or 2-hydroxyoctanoic acid-1, alanine, aspartic acid, cystine, indole-3-acetic acid, N-acetylalanine, and tyrosine) were significantly different between responders and non-responders before and 18 months after intervention ($p<0.05$) **(Table 2)**. PCA showed no significant metabolite differences between the two groups, but the HCA showed a different distribution in 18-month metabolites than the 0 and 6-month time points **(Fig. 2).** In the pathway map of candidate metabolites, five out of nine metabolites (alanine, aspartic acid, cystine, indole-3-acetic acid, and tyrosine) were present in the map; the detected metabolite pathway maps are shown in **Fig. 3.**

We performed additional comparative analysis and validation of HMT data using the Metaboanalyst program. After missing value

imputation using the k-NN (K-nearest neighbor) method, we selected new putative metabolites, which showed differences from previous results. Thirteen metabolites (1-methyl-4-imidazoleacetic acid, 10-hydroxydecanoic acid, 3-indoxylsulfuric acid, 2- or 8-hydroxyoctanoic acid-2, allo-threonine, azelaic acid, disulfiram, indole-3-acetic acid, N-acetyllysine-1 or -2, N-acetylornithine, succinic acid, and XC0065) showed significant differences between responders and non-responders at baseline ($p<0.05$). Six metabolites (2-oxooctanoic acid, glucose, isocitric acid, lauric acid, sulfotyrosine, and XC0126) showed significant changes between baseline and 6months post-intervention in responders and non-responders ($p<0.05$). In addition, nine metabolites (1- or 3-methylhistidine, alanine, aspartic acid, cystine, glycocholic acid, homovanillic acid, piperidine, sulfotyrosine, and tyrosine) also showed significant changes from baseline after 18months in responders and non-responders ($p<0.05$) **(Table 3).** We performed a spaghetti plot using the relative peak areas of these putative metabolites, which indicated the trends of change for each metabolite according to the intervention duration **(Fig. 4).**

HMT performed further analysis on metabolites changed by weight loss interventions regardless of response. Sixty-eight metabolites were significantly changed from baseline after 18 months, and 63 identified metabolites were selected, including 15 amino acids, 11 carboxylic acids, six fatty acids, four carbohydrates, four nucleotides and others (FDR-adjusted p <0.05) **(Table 4).** Metabolic pathway analysis was performed on these metabolites **(Table 5)**, and network and quantitative changes were observed using MetaMapp 2017 and

Cytoscape version 3.7.2 programs **(Fig. 5)**. As a result of pathway analysis, 36 metabolic pathways were identified, and the top three pathway with high impact were 1) alanine, aspartate and glutamate metabolism; 2) phenylalanine, tyrosine and tryptophan biosynthesis; and 3) D-glutamine and D-glutamate metabolism.

Of the 63 metabolites, 55 were amenable to network analysis, and four (prostaglandin F2α, L-glutamine, 3-hydroxypropionic acid, and azelaic acid) showed statistical significance for longitudinal variation, especially exercise intervention (p <0.05).

In summary, the altered metabolites in all analysis including HMT analysis, additional analysis by Metaboanalyst, and longitudinal change analysis were alanine (fold-change (FC) 0.91), aspartic acid (FC 0.47), and cystine (FC 3.49) **(Fig. 6).** Alanine tended to decrease in responders after 18 months of intervention (p-value 0.013, ratio 0.9), and aspartic acid also tended to decrease in both groups (p-value 0.028, ratio 1.2). However, cystine tended to increase after 18 months of intervention in both groups (p-value 0.021, ratio 1.4) **(Table 2, Fig. 4)**.

Table 1. Characteristics of 39 obese study patients who had SNP information.

		Responder group (n=19)		Non-responder group (n=20)	
Age (years)		11.3 ± 1.9		11.0 ± 2.4	
Sex (%)	Male	13	(68.4)	8	(40.0)
	Female	6	(31.6)	12	(60.0)
Intervention type (%)	Exercise + usual	7	(36.8)	8	(40.0)
	Nutrition + usual	9	(47.4)	6	(30.0)
	Usual group	3	(15.8)	6	(30.0)
BMI z-score	0 (baseline)	3.03± 1.12		2.96± 0.92	
	6 time point	2.8±1.32		2.93± 0.94	
	18 time point	2.08± 1.36		3.33± 0.94	
Difference of BMI z-score	0_6 time point	-0.22 ± 0.43		-0.03± 0.19	
	6_18 time point	-0.73± 0.63		0.40 ± 0.36	
	0_18 time point	-0.95± 0.44		0.38± 0.32	

Table 2. The relative intensity of putative metabolites and comparative analysis. Putative metabolites from the HMT standard library; nine metabolites showed significant changes between baseline and 18-month samples.

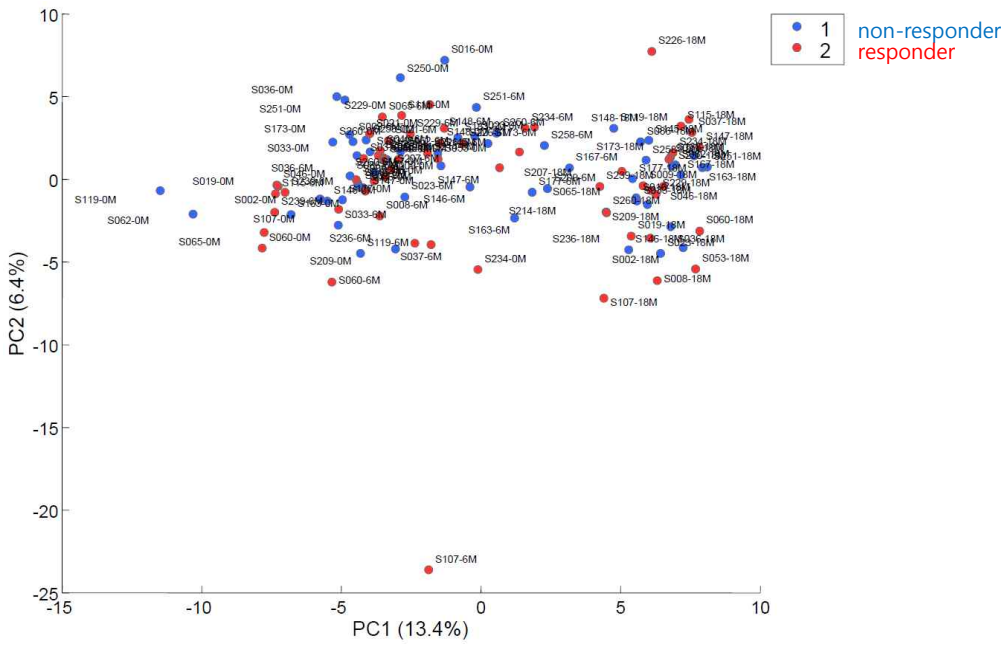
Compound name	Relative area[a]								Comparative analysis			
	Non-responder				Responder				Responder vs non-responder Baseline (0 month)		Responder vs non-responder Intervention after 18 months (18 month)	
	0 (month)		18 (month)		0 (month)		18 (month)					
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Ratio[b]	*p*-value[c]	Ratio	*p*-value
1-Methylhistidine 3-Methylhistidine	2.6E-03	2.1E-03	1.6E-03	7.4E-04	2.4E-03	2.5E-03	3.0E-03	2.9E-03	0.9	0.798	1.9	0.049
3-Hydroxypropionic acid	N.A.	N.A.	1.3E-03	2.3E-04	N.A.	N.A.	1.0E-03	6.8E-05	N.A.	N.A.	0.8	0.018
8-Hydroxyoctanoic acid 2-Hydroxyoctanoic acid	1.9E-04	6.5E-05	1.3E-04	2.0E-05	1.6E-04	5.2E-05	2.6E-04	1.8E-05	0.8	0.169	1.9	0.022
Alanine	1.1E-01	1.8E-02	1.1E-01	1.6E-02	1.1E-01	1.5E-02	9.1E-02	2.1E-02	1.0	0.865	0.9	0.013
Aspartic acid	6.4E-03	3.0E-03	2.3E-03	7.3E-04	4.6E-03	2.9E-03	2.9E-03	7.3E-04	0.7	0.069	1.2	0.028
Cystine	3.6E-04	3.0E-04	1.4E-03	8.1E-04	6.2E-04	5.1E-04	2.0E-03	8.1E-04	1.7	0.068	1.4	0.021
Indole-3-acetic acid	1.5E-04	4.5E-05	1.5E-04	3.7E-05	2.1E-04	1.1E-04	2.0E-04	8.3E-05	1.4	0.066	1.4	0.031

N-Acetylalanine	2.4E-04	6.2E-05	2.4E-04	3.0E-05	2.3E-04	6.6E-05	2.9E-04	3.9E-05	1.0	0.889	1.2	0.028
Tyrosine	2.3E-02	3.5E-03	2.4E-02	3.1E-03	2.6E-02	4.0E-03	2.2E-02	4.4E-03	1.1	0.057	0.9	0.028

[a]Relative Peak Area =Metabolite Peak Area/Internal Standard Peak Area × Sample Amount. [b]The ratio is of computed by using averaged detection values. The latter was used as denominator. [c]The p-value is computed by Welch's t-test (p <0.05).

Abbreviation: S.D., standard deviation; N.A. (Not Available), The calculation was impossible because of insufficience of the data.

(A)



(B)

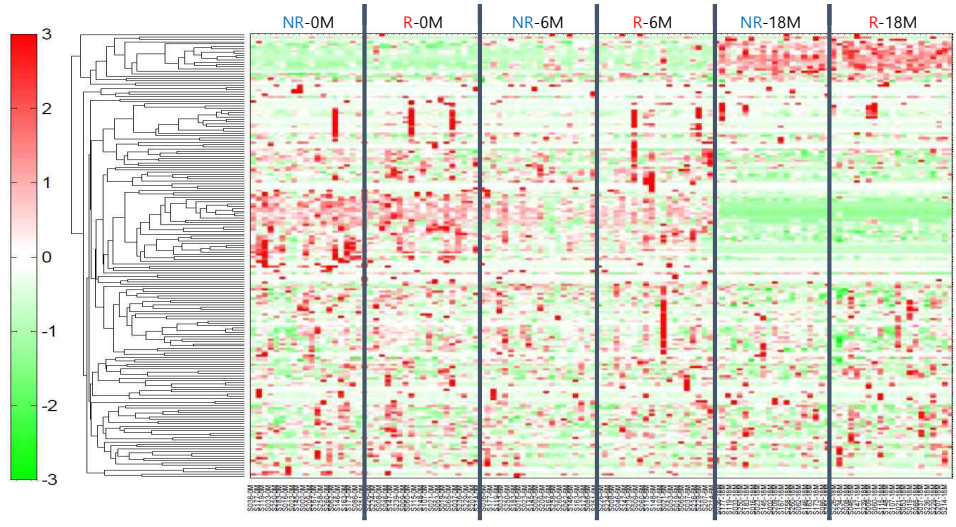


Figure 2. (A) Principal component analysis of all samples. PC1 and PC2 show first and second principal components, respectively. The number in parentheses is the contribution rate, and the plot labels are

sample names. (B) Hierarchical cluster analysis results of all samples. HCA is performed at peaks, and the distances between peaks are displayed in tree diagrams (NR: non-responder, R: responder)

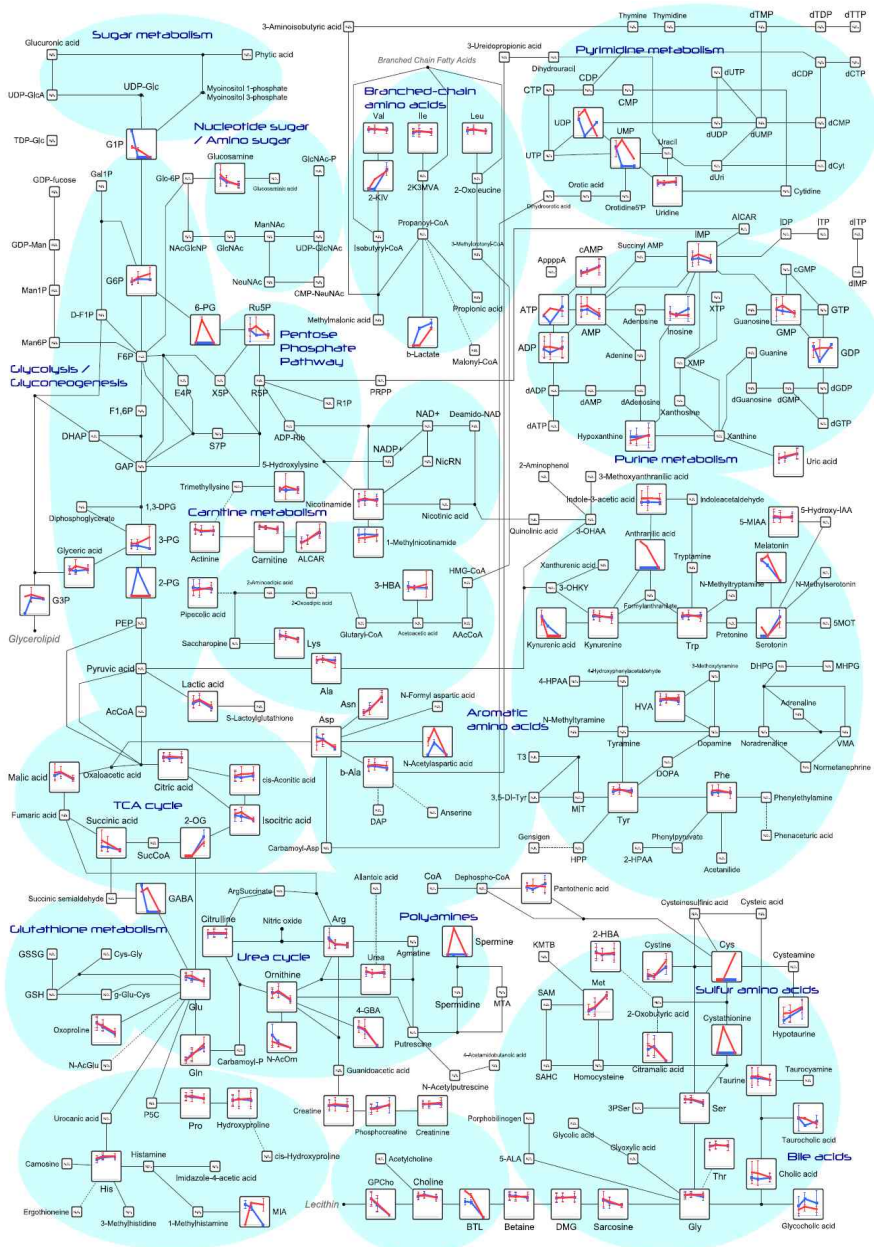
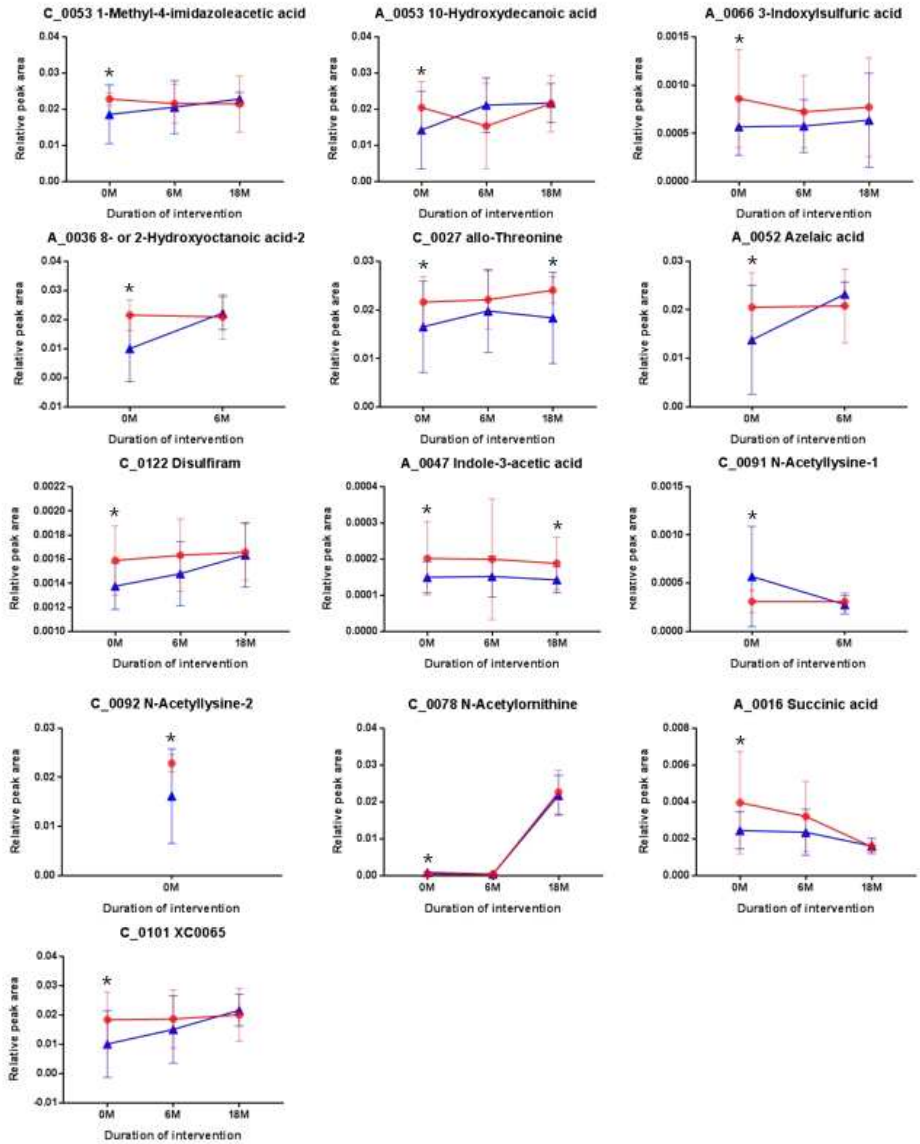
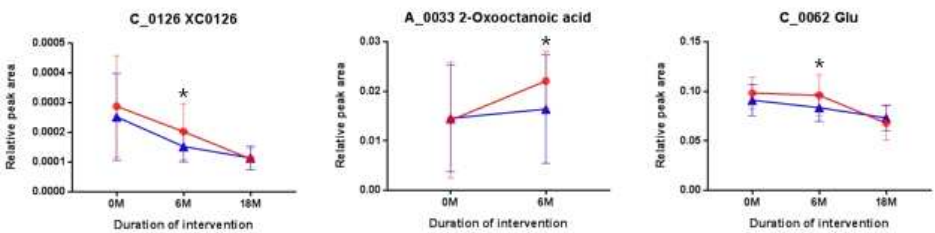


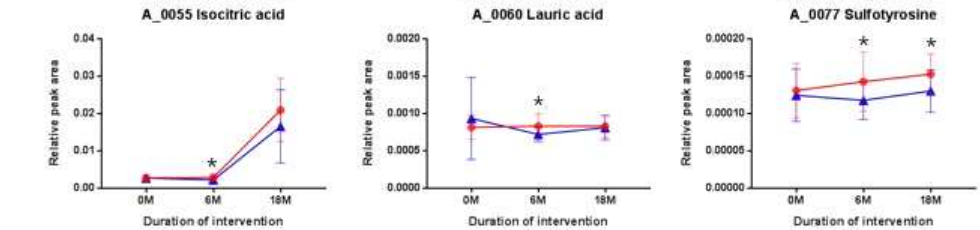
Figure 3. In HMT standard metabolites, detected metabolites in this study are plotted on the pathway map. The lines represent the relative areas of each metabolites at 0, 6, 18-month time points for non-responders (blue), and responders (red), respectively.

(A)


C_0053 1-Methyl-4-imidazoleacetic acid
A_0053 10-Hydroxydecanoic acid
A_0066 3-Indoxylsulfuric acid
A_0036 8- or 2-Hydroxyoctanoic acid-2
C_0027 allo-Threonine
A_0052 Azelaic acid
C_0122 Disulfiram
A_0047 Indole-3-acetic acid
C_0091 N-Acetyllysine-1
C_0092 N-Acetyllysine-2
C_0078 N-Acetylornithine
A_0016 Succinic acid
C_0101 XC0065
Relative peak area
Duration of intervention
0M
6M
18M

(B)


C_0126 XC0126
A_0033 2-Oxooctanoic acid
C_0062 Glu
Relative peak area
Duration of intervention
0M
6M
18M



(C)

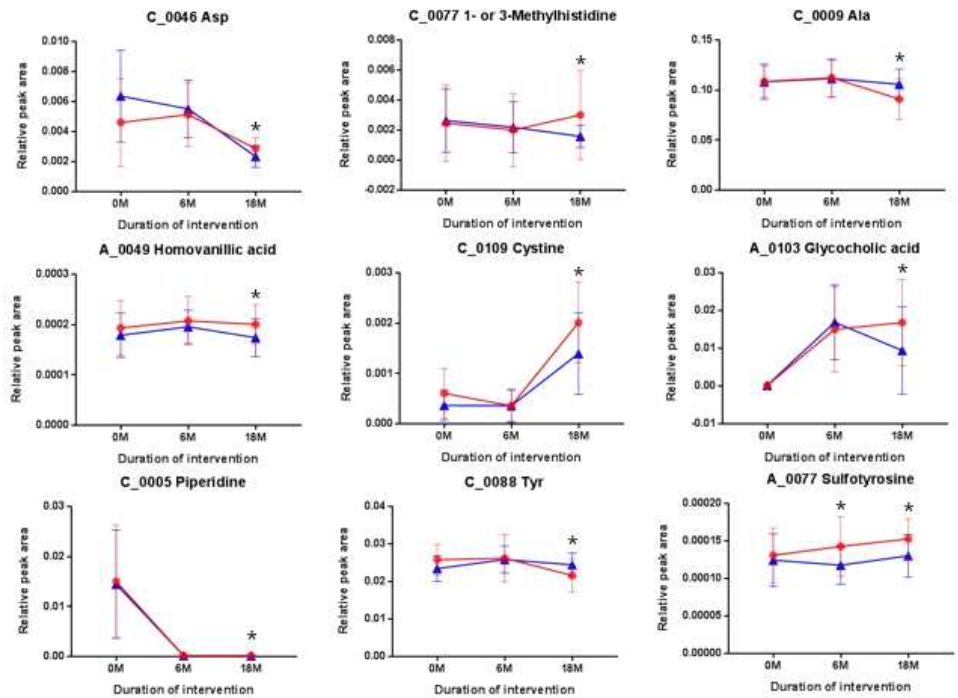


Figure 4. Spaghetti plot of newly selected significant metabolites analyzed by Metaboanalyst. (A) Significant metabolites at baseline between weight loss responders and non-responders; (B) Significant metabolites at 6 months compared with baseline between intervention groups; (C) Significant metabolites at 18 months compared with baseline between intervention groups. (blue, non-responders; red, responders; $*p < 0.05$)

Table 3. New selected metabolites after missing value imputation and comparative analysis.

Putative metabolites	Comparative analysis (p-value)[a] (non-responders vs. responders)		
Compound name[b]	Baseline	Intervention after 6 months	Intervention after 18 months
1-Methyl-4-imidazoleacetic acid	0.033334*	0.63399	0.45285
10-Hydroxydecanoic acid	0.037269*	0.073615	0.9436
3-Indoxylsulfuric acid	0.033219*	0.16402	0.39574
8-Hydroxyoctanoic acid-2 2-Hydroxyoctanoic acid-2	0.00031199*	0.54113	
allo-Threonine	0.04382*	0.3242	0.016014*
Azelaic acid	0.031105*	0.18751	
Disulfiram	0.0091874*	0.09607	0.78549
Indole-3-acetic acid	0.044537*	0.23846	0.016913*
N-Acetyllysine-1	0.040309*	0.33015	
N-Acetyllysine-2	0.0062093*		
N-Acetylornithine	0.023157*	0.60341	0.63014
Succinic acid	0.031116*	0.10099	0.70946
2-Oxooctanoic acid	0.917	0.04989*	
Glutamic acid	0.16814	0.033127*	0.29025
Isocitric acid	0.76393	0.018274*	0.13955
Lauric acid	0.35806	0.015064*	0.69241
Sulfotyrosine	0.57326	0.024036*	0.014555*
1-Methylhistidine 3-Methylhistidine	0.81297	0.78386	0.048642*
Alanine	0.96193	0.89453	0.013367*
Aspartic acid	0.070155	0.55228	0.029478*
Cystine	0.073209	0.97186	0.020345*
Glycocholic acid	0.39682	0.58489	0.048734*
Homovanillic acid	0.36147	0.36708	0.035362*
Piperidine	0.87267	0.97264	0.014093*
Tyrosine	0.055373	0.83144	0.024063*

[a]The p-value was computed by Welch's t-test (*p<0.05).[b]Unknown metabolites were excluded.

Table 4. List of metabolites that changed significantly after 18 months of intervention relative to baseline (FDR-adjusted p-value <0.05).

number	compound name	FDR-adjusted p-value	number	compound name	FDR-adjusted p-value
amino acid			*carnitines*		
1	Asparagine	9.65E-19	35	Carnitine	4.69E-07
2	Glutamine	5.55E-16	36	O-Acetylcarnitine	1.36E-11
3	S-Methylcysteine	1.62E-12	37	Octanoylcarnitine	9.96E-04
4	Cystine	1.43E-09	*fatty acids*		
5	Glutamic acid	2.45E-09	38	iso, Valeric acid	2.07E-08
6	Ornithine	3.38E-08	39	8-Hydroxyoctanoic acid-1	2.07E-08
7	Aspartic acid	4.12E-06	40	Citramalic acid	6.36E-07
8	Lysine	7.47E-06	41	Hexanoic acid	1.33E-04
9	Serine	2.80E-05	42	8-Hydroxyoctanoic acid-2	7.38E-04
10	Arginine	1.54E-03	43	Azelaic acid	7.27E-03
11	Histidine	2.80E-03	*ketoacids*		
12	Proline	2.43E-02	44	2-Oxoisovaleric acid	1.16E-23
13	Alanine	2.58E-02	45	4-Methyl-2-oxovaleric acid	1.93E-19
14	Phenylalanine	2.69E-02	46	2-Oxoglutaric acid	4.91E-04
15	Methionine	7.15E-23	*nucleotides*		
carboxylic acids			47	1-Methyladenosine	3.59E-10
16	Methionine sulfoxide	3.86E-18	48	8-Hydroxy-2′-deoxyguanosine	1.56E-03
17	N-Acetyllysine-1	6.80E-17	49	GMP	2.27E-03
18	4-Guanidinobutyric acid	6.52E-10	50	AMP	2.25E-04
19	5-Oxoproline	7.12E-10	*cholines*		

20	N-Acetyllysine-3	2.25E-04	51	Choline	4.88E-03
21	Sarcosine	3.69E-04	52	Glycerophosphocholine	5.06E-17
22	Symmetric dimethylarginine	1.22E-03	53	Phosphorylcholine	8.03E-05
23	Isobutyric acid, Butyric acid	7.38E-04	*TCA derivatives*		
24	N-Acetylornithine	9.63E-03	54	Isocitric acid	2.03E-06
25	Penicillamine	2.42E-02	55	Succinic acid	5.21E-04
26	Creatinine	3.07E-03	*organic acids*		
carbohydrates			56	Lactic acid	2.62E-04
27	Glucosamine	2.89E-06	57	3-Hydroxypropionic acid	5.18E-03
28	3-Phosphoglyceric acid	3.92E-05	58	Malic acid	6.56E-04
29	Mucic acid	5.03E-04	*sulfinic acid*		
30	Threonic acid	4.79E-03	59	Hypotaurine	1.41E-02
organic oxygen			*prostaglandin*		
31	Kynurenine	2.01E-02	60	Prostaglandin F2α	1.35E-02
organic nitrogen			*others*		
32	N-Methylputrescine	1.28E-02	61	Ser-Glu	2.29E-09
*xanthine*s			62	2-Oxooctanoic acid	2.71E-04
33	Uric acid	5.41E-06	63	Tyr-Arg_divalent	1.68E-03
34	Hypoxanthine	4.39E-02			

Table 5. Analysis of metabolic pathways associated with metabolic changes by weight loss intervention.

Metabolic pathway	Total[a]	Expected	Hits[b]	Raw p-value[c]	Holm adjusted p-value[d]	FDR adjusted p-value[e]	Impact[f]
Aminoacyl-tRNA biosynthesis	48	1.55	12	9.52E-09	7.99E-07	7.99E-07	0.17
Arginine biosynthesis	14	0.45	7	6.85E-08	5.69E-06	2.88E-06	0.25
Alanine, aspartate and glutamate metabolism	28	0.90	7	1.67E-05	1.37E-03	4.68E-04	0.58
D-Glutamine and D-glutamate metabolism	6	0.19	3	5.91E-04	4.78E-02	1.24E-02	0.50
Glyoxylate and dicarboxylate metabolism	32	1.03	5	2.97E-03	2.38E-01	4.44E-02	0.04
Citrate cycle (TCA cycle)	20	0.65	4	3.17E-03	2.51E-01	4.44E-02	0.18
Arginine and proline metabolism	38	1.23	5	6.40E-03	4.99E-01	7.68E-02	0.33
Butanoate metabolism	15	0.48	3	1.09E-02	8.42E-01	1.15E-01	0
Histidine metabolism	16	0.52	3	1.32E-02	1.00E+00	1.18E-01	0.22
Nitrogen metabolism	6	0.19	2	1.41E-02	1	1.18E-01	0
beta-Alanine metabolism	21	0.68	3	2.79E-02	1	2.13E-01	0
Purine metabolism	65	2.10	5	5.47E-02	1	3.79E-01	0.15
Glutathione metabolism	28	0.90	3	5.87E-02	1	3.79E-01	0.03
Cysteine and methionine metabolism	33	1.06	3	8.75E-02	1	4.90E-01	0.13
Glycine, serine	33	1.06	3	8.75E-02	1	4.90E-01	0.31

and threonine metabolism							
Glycerophospholipid metabolism	36	1.16	3	1.07E-01	1	5.62E-01	0.08
Phenylalanine, tyrosine and tryptophan biosynthesis	4	0.13	1	1.23E-01	1	5.75E-01	0.50
Pantothenate and CoA biosynthesis	19	0.61	2	1.23E-01	1	5.75E-01	0
Pyruvate metabolism	22	0.71	2	1.57E-01	1	6.92E-01	0.03
Propanoate metabolism	23	0.74	2	1.68E-01	1	7.06E-01	0
Valine, leucine and isoleucine biosynthesis	8	0.26	1	2.31E-01	1	8.83E-01	0
Taurine and hypotaurine metabolism	8	0.26	1	2.31E-01	1	8.83E-01	0.29
Biotin metabolism	10	0.32	1	2.80E-01	1	9.81E-01	0
Phenylalanine metabolism	10	0.32	1	2.80E-01	1	9.81E-01	0.36
Nicotinate and nicotinamide metabolism	15	0.48	1	3.90E-01	1	1	0
Selenocompound metabolism	20	0.65	1	4.83E-01	1	1	0
Ether lipid metabolism	20	0.65	1	4.83E-01	1	1	0
Sphingolipid metabolism	21	0.68	1	5.00E-01	1	1	0
Lysine degradation	25	0.81	1	5.62E-01	1	1	0
Glycolysis / Gluconeogenesis	26	0.84	1	5.77E-01	1	1	0
Porphyrin and chlorophyll metabolism	30	0.97	1	6.30E-01	1	1	0
Arachidonic acid metabolism	36	1.16	1	6.97E-01	1	1	0
Amino sugar and	37	1.19	1	7.07E-01	1	1	0

nucleotide sugar metabolism							
Pyrimidine metabolism	39	1.26	1	7.26E-01	1	1	0
Valine, leucine and isoleucine degradation	40	1.29	1	7.35E-01	1	1	0.01
Tryptophan metabolism	41	1.32	1	7.44E-01	1	1	0.09

[a]Total is the total number of compounds in the pathway; [b]the Hits is the actually matched number from the user uploaded data; [c]the Raw p is the original p value calculated from the enrichment analysis; [d]the Holm p is the p value adjusted by Holm-Bonferroni method; [e]the FDR p is the p value adjusted using False Discovery Rate; [f]the Impact is the pathway impact value calculated from pathway topology analysis.

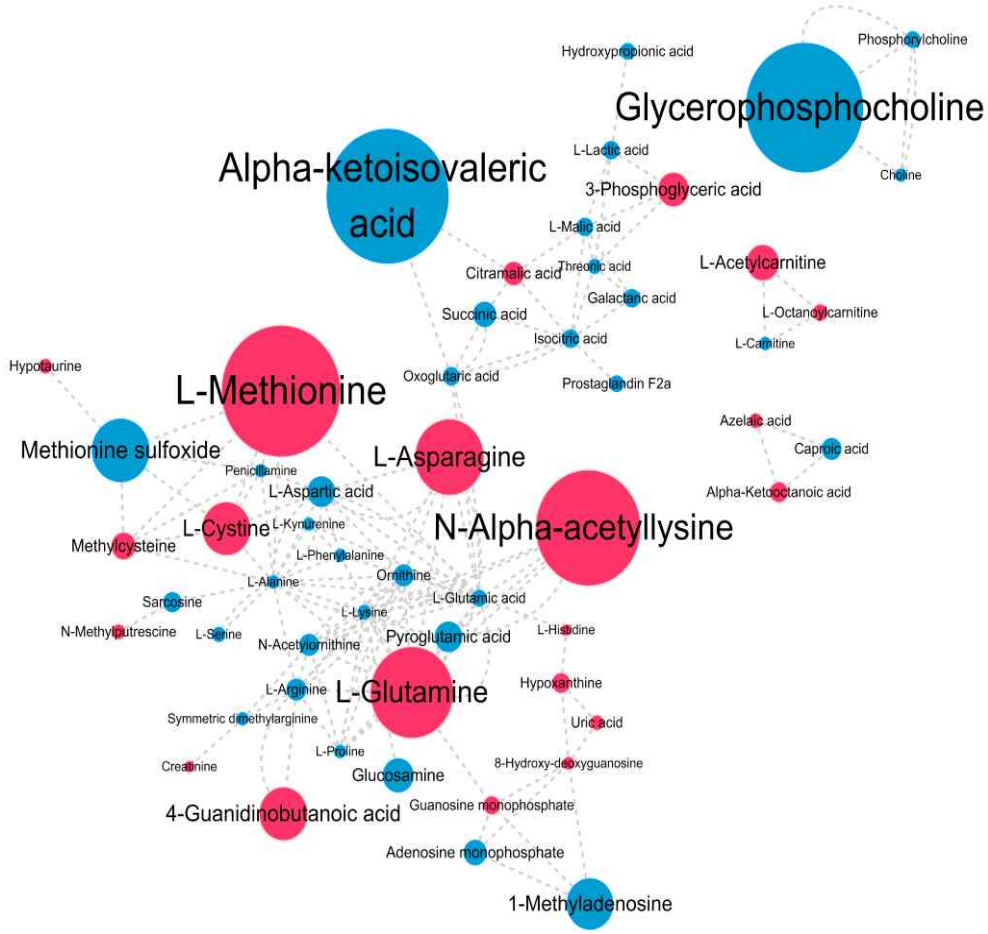


Figure 5. Network assay of metabolites changed by the weight loss intervention. All metabolites have an FDR adjusted p-value <0.05. (Node size: fold change; node color: red (fold change > 1), blue (fold change < 1))

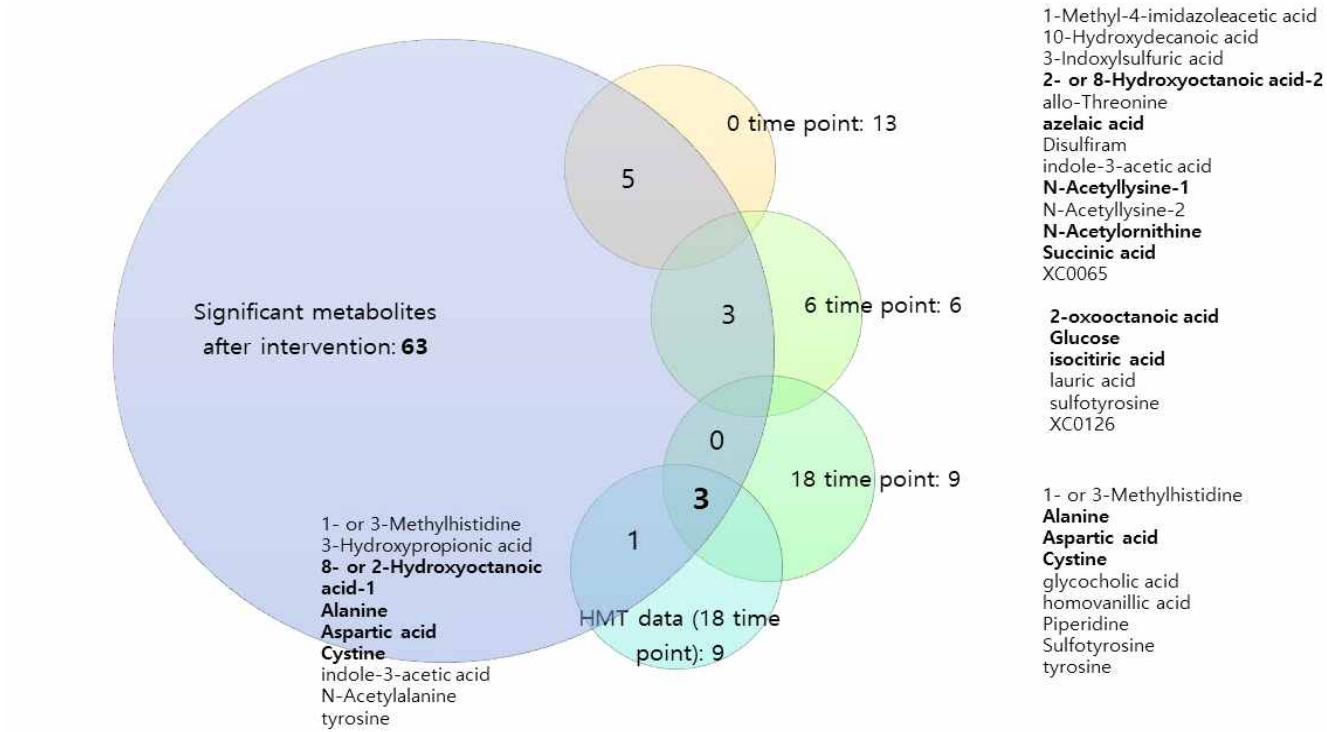


Figure 6. Venn diagram of metabolites selected by HMT analysis, additional analysis by Metaboanalyst, and longitudinal change analysis. Of 194 metabolites, 63 were significant metabolites after the intervention, and 13 represented significant changes between responders and non-responders at baseline. Six were significant at six time points, and 18 were significant at the 18-month time point. Alanine, aspartic acid, cystine were meaningful in all analyses.

Genomic analysis

To detect SNPs that were significantly associated with childhood obesity interventions, GWAS analysis was performed and the candidate genes and SNPs identified in previous studies involving obesity were replicated. The genomes of 39 patients with SNP information were analyzed in 40 study populations (19 were responders and 20 were non-responders; group 2). Their sex, intervention type, and BMI z-score were analyzed, and 902,560 SNPs were identified, but no significance was noted from the SNP list after quality control. In addition, we performed the same SNP analysis on a larger sample size, which was 123 out of 242 patients from the ICAAN intervention study (41 were responders and 82 were non-responders; group 1), and no significant results were obtained from GWAS information. They were selected based on the BMI z-score change between baseline and 6 months after the intervention, and their characteristics are shown in the **Table 6.**

For further analysis to replicate obesity intervention associated gene, candidate genes and SNPs (7 genes, 12 SNPs) were selected through a reference review considering race, age, obesity intervention, and impact factor of the journal[16-18,28-30]. As a result of the analysis in group 1 and group 2, p-values were detected in FTO, SEC16B, and TFAP2B gene, but neither the FDR-adjusted p-value nor the p-value was significant **(Table 7, Fig. 7)**.

Genomic analysis integrating metabolic pathway analysis by IPA

Using IPA, a networks can be obtained at each time point for metabolites data significantly analyzed in Metaboanalyst before and after intervention in the responders and non-responders groups. In the baseline predicted network of the biomarkers, nitric oxide, TNF, BCL2 were main molecules in the network when comparing the responders and non-responders group. Triacylglycerol, L-glutamic acid were in network of 6 months after intervention, and IL1B, CDKN1A, EGFR, hydrogen peroxide were in network of 18 months after intervention **(Fig. 8).**

Based on the key molecule information obtained from this network analysis, a new target gene and SNP list for genome analysis was established through a PubMed and OMIM literature review (https://www.ncbi.nlm.nih.gov/omim) [19,29,31-41]. A total of 17 genes and 26 SNPs were selected **(Table 8),** and 123 subjects (group 1) and 39 subjects (group 2) from the childhood obesity intervention study were identified to match these gene lists. In group 2, 14 out of 26 SNPs were confirmed in the GWAS analysis results, but no SNPs yielded significant p-value. However, in group 1, 13 SNPs out of 26 SNPs were identified, of which IRS1 (rs2943641) and FAIM2 (rs7138803) showed significance (p <0.05). The OR value of IRS1 (rs2943641) was 3.075, the OR value of FAIM2 (rs7138803) was 2.038, and the FDR adjusted p-value was not satisfied. **(Table 9).**

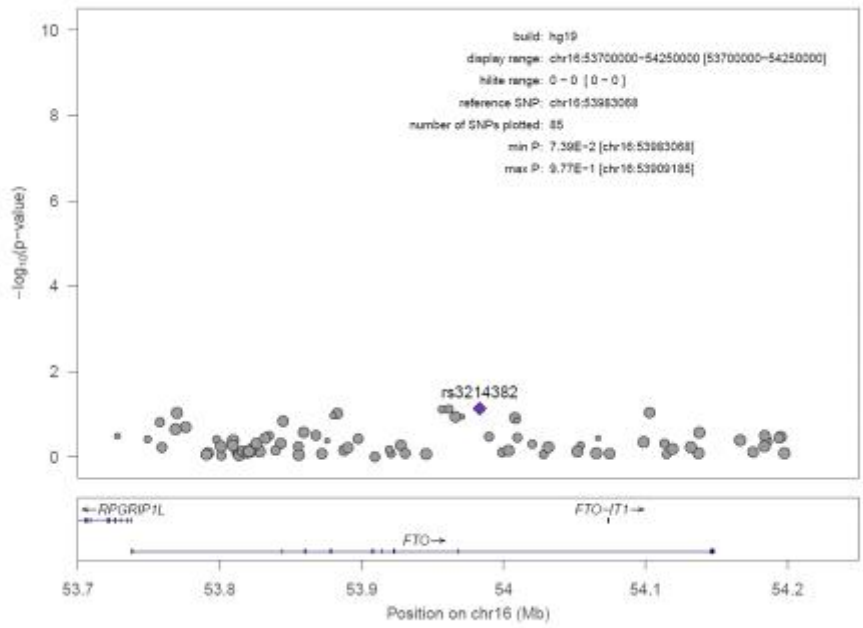
Table 6. Baseline characteristics of 123 patients and the list of SNPs identified in the group.

		Responder group (n=41)		Non-responder group (n=82)		P-value
Age (years)		11.6 ± 2.2		10.9 ± 1.7		0.05
Sex (%)	Male	29	(70.7)	49	(59.8)	0.23
	Female	12	(29.3)	33	(40.2)	
Intervention type (%)	Exercise + usual	20	(48.8)	23	(28.1)	0.05
	Nutrition + usual	13	(31.7)	30	(36.6)	
	Usual	8	(19.5)	29	(35.4)	
BMI_z-score	0 (baseline)	3.02± 0.90		2.84± 0.82		0.29
	6 time point	2.8±1.32		2.99± 0.90		<0.01*
	0-6 time point	-0.52± 0.26		0.14± 0.18		<0.01*

Table 7. Gene and SNPs list and result from 123 subjects.

Gene name	Chromosome	SNP	p-value	FDR
FTO	16	rs1421085	0.8945	0.9353
FTO	16	rs9939609	0.7538	0.9353
FTO	16	rs8050136	0.7538	0.9353
FTO	16	rs17817449	0.8865	0.9353
SEC16B	1	rs543874	0.8863	0.9353
TFAP2B	6	rs987237	0.3829	0.9353
RBSG4	1	rs1027493		
RBSG4	1	rs870879		
RBSG4	1	rs873822		
MIR486/NKX6-3	8	rs6981587		
MC4R	18	rs12970134		
MC4R	18	rs571312		

(A)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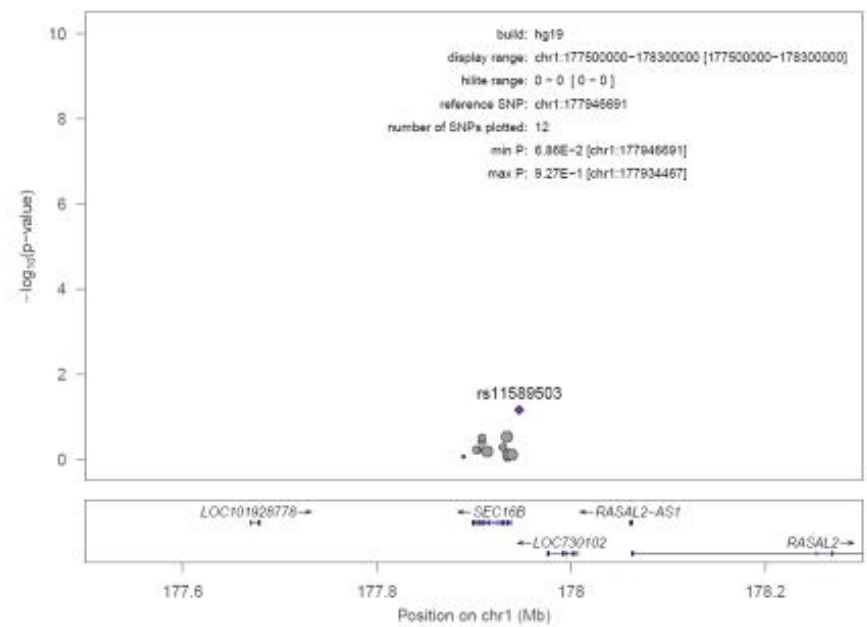
hilite range: 0 - 0 [0 - 0]
reference SNP: chr16:53983068
number of SNPs plotted: 85
min P: 7.39E-2 [chr16:53983068]
max P: 9.77E-1 [chr16:53909185]
rs3214382
-log10(p-value)
RPGRIP1L
FTO-IT1
FTO
53.7
53.8
53.9
54
54.1
54.2
Position on chr16 (Mb)

(B)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hilite range: 0 - 0 [0 - 0]
reference SNP: chr1:177946691
number of SNPs plotted: 12
min P: 6.86E-2 [chr1:177946691]
max P: 9.27E-1 [chr1:177934467]
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-log10(p-value)
LOC101928778
SEC16B
RASAL2-AS1
LOC730102
RASAL2
177.6
177.8
178
178.2
Position on chr1 (Mb)

(C)

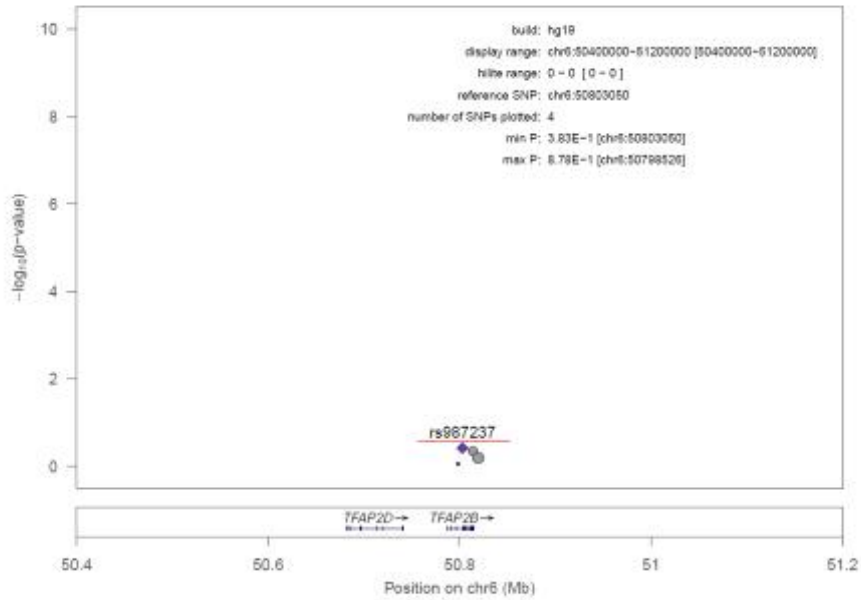

Figure 7. LocusZoom plot for the FTO, SEC16B, and TFAP2B genes in 123 subjects with a *p*-value in genetic analysis. (A) FTO (B) SEC16B (C) TFAP2B.

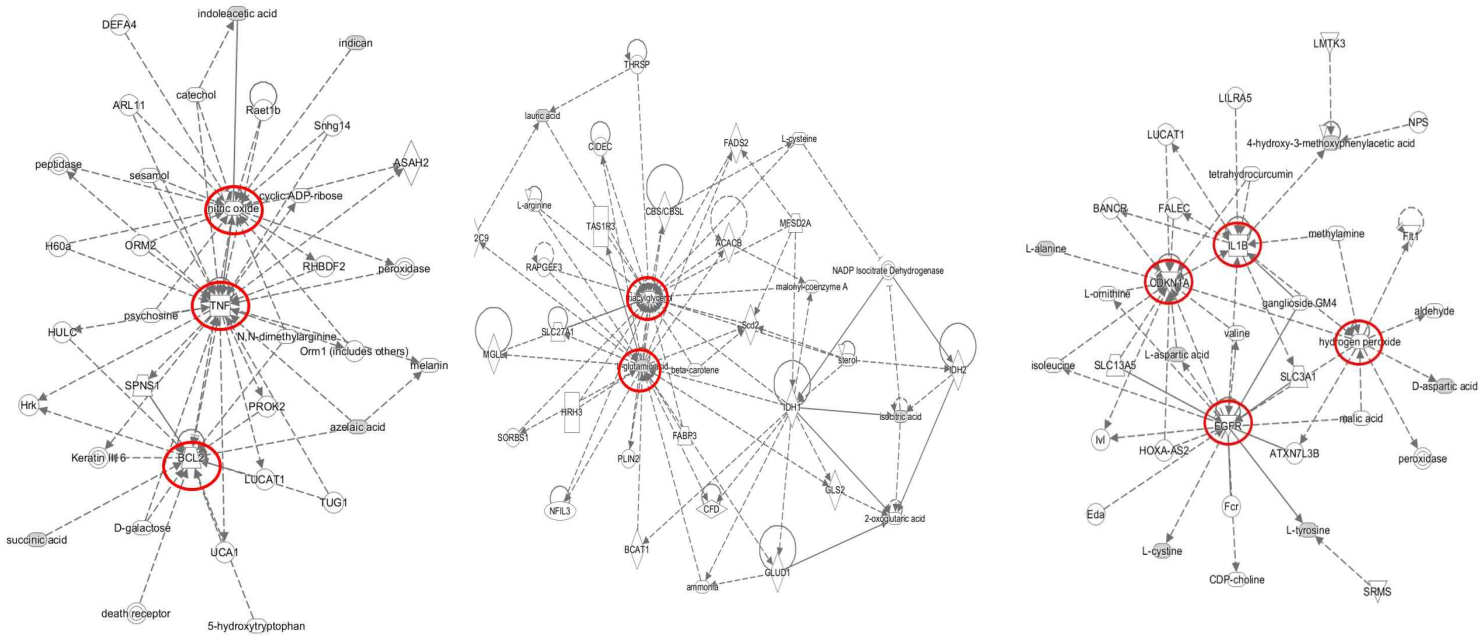

Figure 8. Significant metabolite pathways and networks analysis by IPA in pre-intervention responders and non-responders. (A) baseline (B) 6 months after intervention (C) 18 months after intervention. Molecular interactions between connected molecules represent direct (solid line) or indirect (dotted line).

Table 8. The list of 17 new target genes and 26 SNPs for genetic analysis.

No.	Candidate gene (17)	SNPs (26)
1	SH2B1	rs7359397
2	FLJ35779	rs2112347
3	KCTD15	rs29941
4	NRXN3	rs10150332
5	RPL27A	rs4929949
6	CADM2	rs13078807
7	BDNF	rs10767664
8	BDNF	rs6265
9	BDNF	rs2030323
10	IRS1	rs2943645
11	IRS1	rs2943641
12	LEP	rs7799039
13	FAIM2	rs7138803
14	MRAP2	rs587777046
15	PTEN	Heterozygous c.202T>C; p.(Tyr68His), Heterozygous c.512A>G; p.(Gln171Arg)
16	ACE	I/D
17	VEGF	rs833061
18	TNF alpha	rs1800629
19	MC4R	rs17782313
20	MC4R	rs2229616
21	MC4R	rs12970134
22	MC4R	rs571312
23	FTO	rs1421085
24	FTO	rs9939609
25	FTO	rs8050136
26	FTO	rs17817449

Table 9. Identified gene and SNP list for the replication of candidate genes in the study group. Thirteen of 26 genes and SNPs in the analysis of group 1, and 2 were significant (* $p<0.05$).

		Group 2 (subject n=39) (responder 19/ non-responder 20)			Group 1 (subject n=123) (responder 41/non-responder 82)		
Gene	SNP	OR	p-value	FDR adjust p-value	OR	p-value	FDR adjust p-value
SH2B1	rs7359397	0.73	0.713	0.992	0.61	0.313	0.978
RPL27A	rs4929949	0.88	0.802	1.000	-	-	-
BDNF	rs6265	1.09	0.866	1.000	1.59	0.116	0.977
BDNF	rs2030323	0.95	0.922	1.000	1.49	0.175	0.977
IRS1	**rs2943641**	2.82	0.388	0.975	3.08	**0.03496***	0.977
FAIM2	**rs7138803**	2.98	0.087	0.975	2.04	**0.02218***	0.977
TNF-α	rs1800629	0.72	0.685	0.990	0.73	0.538	0.984
MC4R	rs17782313	1.50	0.488	0.978	0.78	0.470	0.981
MC4R	rs12970134	2.95	0.097	0.975	1.06	0.871	0.996
MC4R	rs571312	1.25	0.724	0.994	0.65	0.230	0.977
FTO	rs1421085	0.30	0.147	0.975	0.95	0.895	0.997
FTO	rs9939609	0.48	0.374	0.975	0.88	0.754	0.992
FTO	rs8050136	0.48	0.374	0.975	0.88	0.754	0.992
FTO	rs17817449	0.57	0.503	0.979	0.94	0.887	0.997

The association between IRS1, the FAIM2 genotype, and clinical characteristics

The genotype frequency of IRS1 variant in responders is CC (80.5%), TC (17.1%), and TT (2.4%), and in non-responders, CC (91.5%) and TC (8.5%). The minor allele frequency (T-allele) was found to be 0.11 in the responders and 0.043 in the non-responders, which is higher in the responders. In FAIM2 variant, the genotype frequency is AA (9.8%), AG (43.9%), and GG (46.3%) in responders, and AA (7.3%), AG (24.4%), and GG (68.3%) in non-responders. The minor allele frequency (A-allele) is 0.317 in the responders and 0.195 in the non-responders, which is also higher in responders.

When analyzing whether there is significant clinical parameters between genotype, only weight change in responder showed significant difference between CC and TC genotypes (p=0.006), and HDL change was 0.051 of p-value at the IRS1 (rs 2943641) locus **(Table 10)**. The clinical parameters used the difference between baseline and 6 months after intervention, and included bmi z-score, weight, insulin, adiponectin, c-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting blood sugar (FBS), gamma-glutamyl transferase (GGT), tryglyceride (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL). At the FAIM2 (rs7138803) locus, only the BMI z-score change in responders was found a significant p-value between AA and GG genotypes (p=0.003), and there were no other significant phenotype associated with the genotypes in both responders and non-responders **(Table 11)**.

Table 12 shows the clinical parameters that showed significant

changes before and after 6 months of intervention within each genotype of responders and non-responders by paired-test. In responders, differences in BMI z-score, weight loss, AST, ALT and adiponectin showed significant changes after intervention in IRS1 CC type, and BMI z-score, ALT and adiponectin showed significant changes in FAIM2 AG type. In non-responders, except for BMI z-score and weight change, only adiponectin change was observed significant in IRS1 CC type.

Table 10. Comparison of various phenotypes among the different genotypes at IRS1 rs2943641 in responders and non-responders.

IRS1 rs2943641	Responder (n=41)				Non-responder (n=82)		
	CC (n=33)	TC (n=7)	TT (n=1)	p-value[a]	CC (n=75)	TC (n=7)	p-value
△BMI z-score[b]	-0.557±0.278	-0.389±0.053	-0.3437693	0.094	0.135±0.186	0.216±0.099	0.077
△weight change (kg)	-1.782±3.325	0.886±0.951	-2.8	**0.019**	4.799±2.61	4.471±1.069	0.855
△FBS (mg/dl)	-0.03±14.152	5.143±6.362	13	0.263	1.053±12.942	0.429±9.199	0.797
△insulin (μU/ml)	1.333±19.488	-0.086±14.464	0.2	0.943	1.145±18.352	4.457±15.144	0.507
△CRP (mg/dl)	-0.391±2.887	1.726±5.489	-0.33	0.983	0.129±3.096	0.16±2.155	0.875
△AST (IU/L)	-10.788±24.507	-18.714±38.517	3	0.625	-0.613±17.837	3.143±14.427	0.365
△ALT (IU/L)	-25.818±56.01	-36±77.812	1	0.873	-0.933±38.652	8±45.177	0.255
△GGT (IU/L)	-2.97±24.067	-10.143±14.668	-1	0.735	-1.04±20.607	6±15.166	0.232
△HDL (mg/dl)	1.818±16.417	-12.143±13.946	13	**0.051**	-0.8±16.673	-1.857±14.645	0.678
△LDL (mg/dl)	5.121±43.472	-16.286±25.663	-100	0.117	-5.387±31.878	-3.714±24.081	0.765
△TG (mg/dl)	14.697±76.603	17.714±46.846	-56	0.431	0.653±77.744	-12.429±57.885	0.829
△Adiponectin (μg/ml)	3.555±3.125	4.114±4.388	8	0.372	1.168±5.45	-1.5±3.379	0.129

[a]P-values analyzed using the Kruskal-Wallis test.

[b]delta (△) values are the differences between after 6 months of intervention and baseline.

Table 11. Comparison of various phenotypes among the different genotypes at FAIM2 rs7138803 in responders and non-responders.

FAIM2 rs7138803	Responder (n=41)				Non-responder (n=82)			
	GG (n=19)	AG (n=18)	AA (n=4)	p-value[a]	GG (n=56)	AG (n=20)	AA (n=6)	p-value
△BMI z-score[b]	-0.471±0.292	-0.516±0.194	-0.804±0.22	**0.005**	0.138±0.192	0.168±0.165	0.094±0.139	0.488
△weight change (kg)	-0.826±3.722	-1.411±2.621	-3.575±1.94	0.057	4.829±2.746	4.955±1.78	3.617±2.359	0.332
△FBS (mg/dl)	0.105±15.502	1.556±11.057	4.5±12.61	0.768	1.214±14.013	2.85±8.074	-7.167±8.886	0.096
△insulin (μU/ml)	-0.358±19.473	1.056±18.086	7.85±16.447	0.572	3.652±19.239	-3.175±14.108	-3.983±16.445	0.598
△CRP (mg/dl)	0.486±4.125	-0.63±2.979	0.24±1.221	0.11	0.252±2.579	-0.26±4.275	0.31±2.003	0.569
△AST (IU/L)	-5.842±18.936	-15.778±31.193	-22.25±37.933	0.739	-0.339±17.282	0.4±20.364	-2.167±10.458	0.432
△ALT (IU/L)	-16.895±51.197	-35.722±63.74	-34.75±78.134	0.779	-0.357±39.121	1.95±44.988	-5.5±9.182	0.215
△GGT (IU/L)	-2.632±26.932	-6.056±18.306	-2.75±20.516	0.891	0.304±21.062	-2.3±20.888	-1.167±8.134	0.964
△HDL (mg/dl)	-0.895±17.188	2.111±15.613	-8.25±20.597	0.746	-1.268±17.648	-2.5±13.805	8±10.77	0.272
△LDL (mg/dl)	-11.579±32.995	5.056±54.009	21±32.609	0.366	-6.482±33.864	-4.3±22.607	3.167±32.811	0.698
△TG (mg/dl)	-6.105±76.47	28.722±69.222	38±42.253	0.187	-1.036±75.904	9.65±76.843	-28.833±80.626	0.697
△Adiponectin (μg/ml)	3.405±3.497	4.056±3.457	4.1±2.618	0.818	0.854±5.523	1.565±4.778	-0.333±6.044	0.682

[a]P-values analyzed using the Kruskal-Wallis test.

[b]delta(△) values are the differences between after 6 months of intervention and baseline.

Table 12. P-value of comparison before and after 6 months of weight intervention in each genotype.

*p-value / genotype	zsbmi6 - zsbmi0	weight6 - weight0	FBS6 - FBS0	Insulin6 - Insulin0	CRP6 - CRP0	AST6 - AST0	ALT6 - ALT0	GGT6 - GGT0	HDL6 - HDL0	LDL6 - LDL0	Triglyceride6 - Triglyceride0	Adiponectin6 - Adiponectin0
responder IRS1 CC	**<0.001**	**0.007**	0.965	0.575	0.893	**0.016**	**0.014**	0.137	0.754	0.963	0.581	**<0.001**
responder IRS1 TC	**0.018**	0.063	0.075	0.612	1	0.115	0.176	0.173	0.075	0.128	0.31	0.051
non-responder IRS1 CC	**<0.001**	**<0.001**	0.1	0.886	0.276	0.8	0.942	0.87	0.884	0.159	0.983	**0.015**
non-responder IRS1 TC	**0.018**	**0.018**	0.671	0.398	0.866	0.31	0.463	0.345	0.735	1	0.933	0.176
responder FAIM2 AA	0.068	0.068	0.593	0.465	0.465	0.273	0.465	0.854	0.715	0.273	0.068	0.066
responder FAIM2 AG	**<0.001**	0.122	0.234	0.983	0.085	0.044	**0.029**	0.147	0.938	0.636	0.256	**<0.001**
responder FAIM2 GG	**<0.001**	0.777	0.913	0.085	0.344	0.09	0.083	0.091	0.809	0.165	0.546	**0.002**
non-responder FAIM2 AA	0.116	**0.028**	0.116	0.753	0.753	0.916	0.246	0.893	0.173	0.916	0.6	0.917
non-responder FAIM2 AG	**0.001**	**<0.001**	0.259	0.629	0.629	0.344	0.239	0.765	0.408	0.409	0.837	0.167
non-responder FAIM2 GG	**<0.001**	**<0.001**	0.069	0.374	0.127	0.629	0.834	0.996	0.969	0.181	0.987	0.07

* P-values analyzed using the Wilcoxon pair test.

DISCUSSION

In previous studies, metabolites or genomes associated with childhood obesity or BMI compared to controls were described[8,29,31,39]. We further analyzed metabolites showing significant differences according to the weight intervention responses in severely obese pediatric patients in various ways and significant genome result was found by comparisons with a patient's GWAS data.

The metabolomic signature associated with obesity intervention

This study determined that in children with obesity, alanine, aspartic acid, and cystine are representative metabolites with significant differences between the responder and non-responder groups 18 months after weight intervention. We also showed statistically significant baseline metabolites and metabolic pathway after 18 months of intervention in severely obese children. Alanine, aspartate and glutamate metabolism pathway showing the highest impact is related to these three metabolites. Alanine is clustered with central carbon metabolism, urea cycle related metabolism, and branched-chain amino acids (BCAA), and the aromatic amino acids pathway. Aspartic acid is also clustered with central carbon metabolism, urea cycle related metabolism, and nucleotide metabolism. According to HMT analysis, cystine is related to urea cycle-related metabolism, lipid and amino acid metabolism, and metabolism of coenzymes. BCAA is strongly associated with insulin resistance, type 2 diabetes, and metabolic diseases, and alanine is one of the byproducts of BCAA

catabolism[42]. When glucose is first produced during BCAA catabolism, it accumulates and increases pyruvate's transamination to alanine. Zheng et al. (2016) found the amino acid profile change from the POUND LOST and DIRECT trial, which confirmed metabolite changes after a diet weight loss intervention of 2 years in adult obese patients. In both trials, weight loss was directly related to concurrent reduction of BCAAs (leucine, isoleucine), aromatic AAs (tyrosine, phenylalanine), and other amino acids (alanine, sarcosine, hydroxyproline and methionine)[43]. They showed that alanine reductions were significantly related to improved insulin resistance, independent of weight loss, in both trials (both $p < 0.05$). Another diet-induced weight-loss intervention study also found that decreased BCAA levels were associated with reduced BMIs[44].

Although the mechanism of BCAA involvement in metabolic disease is unclear, it is closely related to insulin resistance and adipose tissue catabolism. A recent study showed an association between activation of BCAA ketoacid dehydrogenase (BCKD) and insulin resistance development[45]. When BCKD expression decreases in adipose tissue, circulating BCAA increases, along with the uptake of BCAAs from the muscle that insulin facilitates, increasing insulin resistance as a result[46]. Lipids and BCAA play a role in the development of obesity-related insulin resistance. The rise in circulating BCAA is driven by an obesity-related decline in their catabolism in adipose tissue[42]. Therefore, in our study, the significant change in alanine in the subjects with greater weight change for intervention may be related to insulin resistance and mechanistically associated with BCAA. Considering the BCAA trend in our study, there was no significant difference between responders and

non-responders. However, leucine decreased to a ratio of 0.9 compared with baseline and 18 months after intervention in the responder group and had a significant p-value (p=0.028).

Alanine is one of the 49 BMI-associated metabolites and is also significantly and positively associated with insulin resistance, and several amino acids have been linked to BMI[47]. Alanine and aspartic acid were also included, and each metabolite was related to BMI 5.3% and 7.0%, respectively, from 427 unrelated individuals of European ancestry participating in the Health Nucleus cohort study[47]. Alanine and aspartate levels were dramatically elevated in obese versus lean participants in another study, which showed increased catabolism of BCAA and correlated with insulin resistance in obese humans[48]. That study also found that Insulin Receptor Substrate 1 (IRS1) phosphorylation at serine 302 in skeletal muscle was increased in BCAA-fed rats compared with other groups. BCAA contributes to the development of obesity-associated insulin resistance. It is accompanied by serine and tyrosine phosphorylation of IRS1, which is related to the genome analysis results in our study.

Aspartate, like pyruvic acid, is an amino acid associated with the tricarboxylic acid cycle. Metabolic shift in pyruvic acid decrease after weight intervention in overweight pre-adolescent and obese women[15,49]. Since aspartate is generated from glycolytic intermediates in the tricarboxylic acid cycle, the low aspartate in the responder group that is effective in weight loss is considered physically active. This effect is related to decreased pyruvic acid in a previous study.

In a Japanese obese adult study, obesity was associated with higher cystine and glutamate levels but lower glycine levels[50]. In another study, BCAA and cystine levels increased with obesity or

type 2 diabetes, and alanine increased with obesity and metabolic illness. Increased oxidative stress can elevate cystine levels, and cysteine inhibits tyrosine aminotransferase activity[51]. A high-cystine diet in a mouse model increased visceral fat, inhibited metabolic rate, and decreased glucose tolerance[52]. High cystine intake promotes adiposity, suggesting that plasma cystine levels may also be associated with human obesity. However, it is difficult to explain the increase in cystine after 18 months in the response group. Another study showed that cysteine decreases slightly after weight loss[53]. Considering why cystine increases after weight intervention, total cysteine, which contains cystine, increases with age in obese children[54]. In addition, after acute exercise, cysteine increased via gutathione biosynthesis[55]. However, this change in cystine after weight loss intervention requires further validation.

When we compared the results of our studies with those of adult obesity, there are some similarities and differences, which may be due to pubertal development or differing gut microbiomes or dietary intake changes. During the pubertal stage, insulin sensitivity decreases by 50% and can be associated with increased total body lipolysis and decreased glucose oxidation[56]. These alterations may have affected our study's metabolic changes, and other studies have shown that metabolomics profiles change due to increased adiposity measures in post-pubertal male groups[57]. The microbiota contributes to the biosynthesis of amino acids, meaning that the same foods may contribute different caloric and nutrient bioavailability to different people[58]. Gut microbial profiling of individuals with insulin resistance and insulin sensitivity is associated with different host dietary intervention responses and weight changes. Hippurate, a metabolomics

marker, is associated with changes in fasting plasma glucose levels and insulin secretion, which is also closely related to the gut microbiome[59]. Dietary intake may also affect amino acid levels and obesity. Xu et al. (2018) revealed metabolite differences according to milk nutritional composition in obese subjects with metabolic syndrome and found that orotate, leucine, isoleucine and adenine are significant biomarkers[60]. In obese adults, a low glycemic diet increased serine and decreased tyrosine, leucine and valine levels compared with the high-glycemic-index diet or low fat diet[61]. According to a study on grain amino acid composition of wheat-related species showed that most amino acids for wheat-related species were lysine (2.74%), threonine (2.83%), phenylalanine (4.17%), isoleucine (3.42%), valine (3.90%), histidine (2.81%), glutamic acid (29.96%), proline (9.12%), glycine (3.59%), alanine (3.37%), and cysteine (1.57%)[62]. Wheat intake and glycemic diet are closely related to our dietary culture, so we thought that there might be differences in metabolites depending on the amount and type of diet.

Genetic analysis in the weight loss response and non-response groups

We failed to replicate the obesity-related SNPs in the first analysis of 39 patients (group 2), but in a further breakdown of 123 patients (group 1), we found 13 of the 26 SNPs related to obesity. We observed that rs2943641 in the IRS1 gene (OR 3.08) and rs7138803 in the FAIM2 gene (OR 2.04) were significantly associated with childhood obesity intervention responses in the Korean population.

Thus, considering each odds ratio, IRS1 rs2943641 or FAIM2 rs7138803 genetic variation tended to be improved by 3.08 times and 2.04 times, respectively.

Insulin receptor substrate 1 (IRS 1) is an adipose locus that increases fasting insulin, plays an important role in activating phosphatidylinositol 3-kinase (PI 3-kinase)[63]. IRS1 regulates gene expression, phosphotyrosine dephosphorylation, protein degradation, and serine phosphorylation[64]. The PI 3-kinase pathway activates long-form receptor (LRb)-associated JAK2 signaling by binding leptin to LRb and increases phosphorylation of IRS1 through this process[65]. Leptin is secreted from adipose tissues and stimulates serine or tyrosine phosphorylation of IRS1. Therefore, the bio-signal action of leptin is increased by PI 3-kinase. PI 3-kinase phosphorylates the plasma membrane phospholipids that subsequently stimulate Akt (protein kinase B) by promoting phosphorylation at tyrosine and serine, so IRS/PI 3-kinase/Akt pathway is required for insulin regulation of glucose homeostasis[66]. Furthermore, rs2943641 in IRS1 is a type 2 diabetes susceptibility locus associated with increased fasting and glucose-stimulated hyperinsulinemia and impaired insulin sensitivity[67]. IRS1 rs 2943641 is associated with insulin resistance and hyperinsulinemia in French, Danish and Finnish population-based cohorts and reducing IRS1 protein levels and PI3-kinase activity in skeletal muscle *in vivo*[68]. Serine phosphorylation sites of IRS1 are replaced by alanine, which increases tyrosine autophosphorylation and insulin signaling, and can be considered to be consistent with the metabolic analysis results in this study[69].

BCAAs induce chronic phosphorylation of mTOR, JNK, and IRS1, contributing to the development of insulin resistance-related IRS1[70].

Huang et al. (2018) suggested a mechanism for the relationship between IRS1 and insulin resistance. When IRS1 expression decreases, subcutaneous fat storage decreases, and ectopic fat deposition increases. Finally, insulin resistance, dyslipidemia increases, and adiponectin decrease[32]. As a result, the risk of T2DM and cardiovascular disease increases[71].

Unfortunately, the only clinical parameter that showed a significant difference between the genotype of the IRS1 variant was the weight change of the responder, and it is already known that certain genotypes are associated with obesity. In a population-based cohort study using European GWAS data, the C allele of rs2943641 of IRS1 increases the risk of T2DM and affects glucose-stimulated hyperinsulinemia and insulin sensitivity[68]. In another study, A allele of FAIM2 rs 7138803 was found to be associated with high obesity and T2DM risk[37,72]. Thus, the significant difference in bmi z-score change between GG and AA type of FAIM2 variant in responder means that risk allele is associated in the intervention response. However, large-scale further long-term studies are needed to analyze why responders with high minor allele frequency (MAF) have better weight change intervention outcomes.

Fas apoptotic inhibitory molecule 2 (FAIM2) is regulated by the nutritional state and the methylation levels of the FAIM2 promoter and is associated with obesity, but the mechanisms are unclear[73]. In Qi et al. (2012), the FAIM2 gene interacts with dietary carbohydrates to increase BMI[74,29]. FAIM2 is an anti-apoptotic gene that encodes Apo1, a protein that inhibits apoptotic signals at the Fas receptor, and is involved in adipocyte apoptosis and appetite control nerve development[75,76].

Neuronal FAIM2 expression is regulated by the PI3-kinase/Akt pathway, related to IRS1[77]. FAIM2 also acts as a calcium leak channel in the endoplasmic reticulum, and the amino acid essential for this activity is aspartate[78]. FAIM2 rs 7138803 was first reported to be associated with obesity in Caucasians, and a polymorphism related to obesity and type 2 diabetes in Asia, such as China and Japan[37,79,80]. Another study showed that SNPs are related to coronary artery disease and obesity, and we found this polymorphism in our patients[81]. Wu et al. (2015) revealed that the methylation level of the FAIM2 promoter is significantly associated with the degree of physical activity in obese and lean children[73]. Finally, FAIM2 rs 7138803 reduces the expression of FAIM2 and induces apoptosis of more adipocytes, thereby promoting the recruitment of macrophages in adipose tissue. Increased macrophages cause the production of cytokines such as TNF-α and IL-6, which may increase inflammation and insulin resistance, thus increasing the risk of obesity risk[78,82]. TNF-α regulates IL-6 production in adipocytes, directly affects the insulin signaling cascade, and is also an important molecule at the six month time points in this study. TNF-α and IL-6 are also associated with IRS1, diminishing tyrosine phosphorylation, and IRS1 expression in adipose tissue in lean and healthy people compared to obese patients[77]. Therefore, these genetic variations can predict obesity, but it can also be expected that obese children will have an excellent response to weight control interventions.

This study's was limited by the availability of subjects for longitudinal studies due to many dropouts from the childhood obesity intervention cohort. So, there is a limitation of the small number of samples, and there is a possibility that there is a selection bias in the

results. Lower family functioning, exercise group, lower initial attendance rate, and non-self-referral pathways were significantly associated with 6-month dropouts, and lower family functioning and lower initial attendance rates were associated with late dropouts in our cohort study[83]. Thus, it would be important to focus on these factors to reduce the dropout rate in further intervention cohort research. In addition, this study did not analyze the factors related to adolescent age while observing changes in long-term follow-up during the intervention. Metabolic changes were most pronounced after 18 months in the heatmap, but the probability that hormonal changes and other factors affected the metabolites was not considered. Therefore, additional studies are required to analyze other factors, such as social and environmental factors, in addition to metabolites and genomes. In fact, poor family function was associated with higher level of depressive symptoms in this childhood obesity cohort[84]. Further research is needed on how these factors affect intervention outcomes in children. Also, within the same intervention group, the compliance of patients and their parents may have affected the intervention effect. It is also a limitation that the patient ′s hospital visit time and participation level are not considered in the study. In addition, factors such as diet could not be limited to homogenous, which may have influenced metabolite results. Especially, metabolite is affected by sampling time or whether to eat at the time of sample, so it will be important to control these factors and follow the trend of metabolites. Finally, it is necessary to classify metabolites through metabotyping, and long-term metabolic changes for weight intervention through continuous follow-up in the obesity cohort should be examined.

However, this study may facilitate larger investigations to obtain more significant metabolites and genomes associated with childhood obesity intervention in the future. Notably, it is the first study to analyze metabolites with genomes according to effects in the obesity intervention in children.

In conclusion, it is clinically meaningful that there are various metabolic changes after obesity intervention, and it is important that indicators that vary depending on the intervention effect have been identified. Our data suggest that differences in insulin resistance and amino acid metabolism in adipose tissue led to differences in metabolites and responses to weight loss intervention in obese children. Our research may ultimately prevent and treat obesity in children and adolescents by screening genetic indicators for obesity and monitoring prevention and intervention effects using more precise indicators. We identified biomarkers related to obesity gene pathway networks, and the prevention and management of obesity based on integrated omics information that can serve as indicators for intervention evaluations. Metabolic and genetic signatures could be used as clinical tools for understanding obesity pathogenesis and predicting obesity intervention effects.

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Supplement data

Table S1. A list of top 13 article reviews selected by literature searching for candidate genes and SNPs.

No	Authors/Title	Journal	Contents	intervention/observation/other (3/2/1)	Race (Asia/other) (2/1)	Children/ Adult (2/1)	Total score
1	Valsesia A, Wang QP, Gheldof N, et al. Genome-wide gene-based analyses of **weight loss interventions** identify a potential role for NKX6.3 in metabolism.	Nat Commun 2019;10: 540.	Two loci close to NKX6.3/MIR486 and RBSG4 are identified in the Canadian discovery cohort (n = 1166) and replicated in the DiOGenes cohort (n = 789). Modulation of HGTX (NKX6.3 ortholog) levels in Drosophila melanogaster leads to significantly altered triglyceride levels.	3 (weight loss intervention)	1	1	5
2	Drabsch T, Holzapfel C. A Scientific Perspective of Personalised Gene-Based Dietary Recommendations for **Weight Management.**	Nutrients 2019;11.	* Genetics and Obesity-> FABP2, PPARG, FTO, TMEM18, MC4R * Genetics and Weight Loss-> Results of that meta-analysis showed that people carrying the FTO risk allele of SNP rs9939609 achieved a	3 (weight management)	1	1	5

			similar weight loss compared to non-risk allele carriers after dietary intervention. * Genetics and Dietary Intake-> FTO SNP rs421085, RARB SNP rs7619139 etc.				
3	Leon-Mimila P, Villamil-Ramirez H, Lopez-Contreras BE, et al. Low Salivary Amylase Gene (AMY1) Copy Number Is Associated with Obesity and Gut Prevotella Abundance in **Mexican Children** and **Adults.**	Nutrients 2018;10.	1q11 CNV(copy number variants) was significantly associated with obesity in children, but not in adults. Only AMY1 CNV was significantly associated with obesity in both age groups.	2	1	2	5
4	Yoo KH, Yim HE, Bae ES, et al. Genetic Contributions to Childhood Obesity: Association of Candidate Gene Polymorphisms and Overweight/Obesity in **Korean Preschool Children.**	J Korean Med Sci 2017;32: 1997-2004.	* A total of 96 control, 48 overweight, and 46 obese preschool children were genotyped for ACE, AT2, VEGF, TGF-β1, and TNF-α polymorphisms.	2	2 (Korea)	2	6
5	Wu YY, Lye S, Briollais	Int J	EXBF acts antagonistically to the FTO rs9939609	2	1	2	5

	L. The role of early life growth development, the FTO gene and exclusive breastfeeding on child BMI trajectories.	Epidemiol 2017;46:1512-1522.	risk allele and by the **age of 15,** the predicted reduction in BMI after 5 months of EXBF is 0.56 kg/m2 [95% confidence interval (CI) 0.11-1.01; P = 0.003] and 1.14 kg/m2 (95% CI 0.67-1.62; P < 0.0001) in boys and girls, respectively. EXBF influences early life growth development and thus plays a critical role in preventing the risks of overweight and obesity even when those are exacerbated by genetic factors.				
6	Wang S, Song J, Yang Y, et al. Rs12970134 near MC4R is associated with appetite and beverage intake in **overweight and obese children**: A family-based association study in **Chinese population.**	PLoS One 2017;12:e0177983.	rs12970134 near MC4R was associated with appetite and beverage intake, and food responsiveness could mediate the effect of rs12970134 on beverage intake in overweight and obese Chinese children population.	2	2	2	6
7	Song QY, Song JY, Wang Y, et al. Association Study of Three Gene Polymorphisms Recently	Obes Facts 2017;10:179-190	* A total of 3,922 children, including 2,191 normal-weight, 873 overweight and 858 obese children, from three independent studies were included in the study.	2	2	2	6

	Identified by a Genome-Wide Association Study with Obesity-Related Phenotypes in **Chinese Children.**	.	* The pooled odds ratios of the A-allele of rs564343 in PACS1 for obesity and severe obesity were 1.180 (p = 0.03) and 1.312 (p = 0.004), respectively. * We showed for the first time that the rs564343 in PACS1 was associated with risk of severe obesity in a non-European population.				
8	Dina C, Meyre D, Gallina S et al. Variation in FTO conbtributes to **childhood obesity** and severe adult obesity	Nat Genet. 2007 Jun;39(6):724-6.	French childhood obesity1: rs1421085 (OR = 1.28), rs17817449 (OR = 1.25) French childhood obesity 2a: rs1421085 (OR = 1.47), rs17817449 (OR = 1.52) German childhood obesity: rs1421085 (OR = 1.69), rs17817449 (OR = 1.65)	2	1	2	5
9	Garver WS, Newman SB, Gonzales-Pacheco DM, et al. The genetics of **childhood obesity** and interaction with dietary macronutrients. (Review)	Genes Nutr. 2013;8(3):271 - 287.	The objective of this article is to provide a review on the origins, mechanisms, and health consequences of obesity susceptibility genes and interaction with dietary macronutrients that predispose to childhood obesity.	2	1	2	5
10	Monnereau C, Vogelezang S, Kruithof CJ, et al. Associations of genetic risk scores based on adult	BMC Genet 2016;17:120.	A genetic risk score based on 97 SNPs related to adult BMI was associated with peak weight velocity during infancy and general and abdominal fat measurements at the age of 6 years.	2	1	2	5

	adiposity pathways with **childhood** growth and adiposity measures.		* For all but one SNPs identified for childhood BMI, information was available in our dataset. We used rs3751812 as a proxy for rs1421085 (R 2 = 0.93, D' = 0.97)				
11	Graff M, North KE, Richardson AS, et al. BMI loci and **longitudinal BMI** from adolescence to **young adulthood** in an ethnically diverse cohort.	Int J Obes (Lond) 2017;41: 759-768 .	* 5,962 European American (EA), 2,080 African American (AA), and 1,582 Hispanic American (HA) individuals from the National Longitudinal Study of Adolescent to Adult Health (Add Health) * We found SNPs in/near FTO, MC4R, MTCH2, TFAP2B, SEC16B, and TMEM18 were significantly associated ($p<0.0015 \approx 0.05/34$) with BMI change in EA and the ancestry-combined meta-analysis. * No SNPs were significant after Bonferroni correction in AA or HA, although 5 SNPs in AA and 4 SNPs in HA were nominally significant ($p<0.05$). In EA and the ancestry-combined meta-analysis, rs3817334 near MTCH2 showed larger effects in younger respondents, while rs987237 near TFAP2B, showed larger effects in older respondents across all Waves.	3 (change in BMI)	1	2	6

12	Livingstone KM, Celis-Morales C, Papandonatos GD, et al. FTO genotype and **weight loss:** systematic review and meta-analysis of 9563 individual participant data from eight randomised controlled trials.	Bmj 2016;354:i4707.	* systematic review and meta-analysis (n=9563) The minor allele for the fat mass and obesity associated gene (FTO) rs9939609 is linked to increased risk of obesity. Carriage of the FTO minor allele has no effect on the efficacy of lifestyle and drug related **weight loss interventions.**	3 (weight loss intervention)	1	1	5
13	Song JY, Song QY, Wang S, et al. Physical Activity and Sedentary Behaviors Modify the Association between Melanocortin 4 Receptor Gene Variant and Obesity in **Chinese Children and Adolescents.**	PLoS One 2017;12:e0170062.	* Two common variants (rs12970134 and rs17782313) near MC4R were genotyped in 2179 children and adolescents aged 7–18 years in Beijing of China. * we found the significant interaction of rs12970134 and physical activity/sedentary behaviors on BMI (Pinteraction = 0.043). The rs12970134 was found to be associated with BMI only in children with physical activity<1h/d and sedentary behaviors ≥2h/d (BMI: β = 1.27 kg/m2, 95%CI: 0.10–2.45, P = 0.034).	2	2	2	6

Table S2. A total of 194 candidate metabolites were comparatively analyzed by HMT using the CE-TOF MS method.

ID[a]	Compound name	Pathway label	Responder vs non-responder ratio[b] (baseline - 18 time point)	P-value[c](baseline - 18 time point)
C_0053	1-Methyl-4-imidazoleacetic acid	MIA	1<	N.A.
C_0117	1-Methyladenosine	1-Methyladenosine	0.9	0.401
C_0077	1-Methylhistidine 3-Methylhistidine	1-Methylhistidine 3-Methylhistidine	1.9	0.049
C_0050	1-Methylnicotinamide	1-Methylnicotinamide	1.0	0.738
A_0053	10-Hydroxydecanoic acid	10-Hydroxydecanoic acid	0.8	N.A.
C_0015	2-Aminoisobutyric acid 2-Aminobutyric acid	2-Aminoisobutyric acid 2-Aminobutyric acid	1.2	0.150
A_0010	2-Hydroxybutyric acid	2-HBA	1.1	0.582
A_0008	2-Hydroxyisobutyric acid	2-Hydroxyisobutyric acid	<1	N.A.
A_0018	2-Hydroxyvaleric acid	2-Hydroxyvaleric acid	0.8	0.159
A_0029	2-Oxoglutaric acid	2-OG	0.7	0.105
A_0013	2-Oxoisovaleric acid	2-KIV	0.9	0.319
A_0033	2-Oxooctanoic acid	2-Oxooctanoic acid	N.A.	N.A.
A_0050	2-Phosphoglyceric acid	2-PG	N.A.	N.A.
C_0023	3-Amino-2-piperidone	3-Amino-2-piperidone	N.A.	N.A.
C_0013	3-Aminobutyric acid	3-Aminobutyric acid	1.1	0.446
A_0009	3-Hydroxybutyric acid	3-HBA	3.4	0.224
A_0005	3-Hydroxypropionic acid	b-Lactate	0.8	0.018
A_0062	3-Indolebutyric acid	3-Indolebutyric acid	N.A.	N.A.
A_0066	3-Indoxylsulfuric acid	3-Indoxylsulfuric acid	1.2	0.389
A_0032	3-Phenylpropionic acid	3-Phenylpropionic acid	N.A.	N.A.
A_0051	3-Phosphoglyceric acid	3-PG	3.6	0.217

C_0057	4-Guanidinobutyric acid	4-GBA	N.A.	N.A.
A_0021	4-Methyl-2-oxovaleric acid 3-Methyl-2-oxovaleric acid	2-Oxoleucine 2K3MVA	1.0	0.741
C_0073	5-Hydroxylysine	5-Hydroxylysine	1.0	0.845
A_0063	5-Methoxyindoleacetic acid	5-MIAA	0.9	0.509
A_0020	5-Oxoproline	Oxoproline	1.2	0.177
A_0079	6-Phosphogluconic acid	6-PG	N.A.	N.A.
C_0118	8-Hydroxy-2′-deoxyguanosine	8-Hydroxy-2′-deoxyguanosine	1.0	0.518
A_0035	8-Hydroxyoctanoic acid-1 2-Hydroxyoctanoicacid-1	8-Hydroxyoctanoic acid 2-Hydroxyoctanoic acid	1.9	0.022
A_0036	8-Hydroxyoctanoic acid-2 2-Hydroxyoctanoicacid-2	8-Hydroxyoctanoic acid 2-Hydroxyoctanoic acid	N.A.	N.A.
A_0101	ADP	ADP	0.8	0.547
C_0009	Ala	Ala	0.9	0.013
C_0084	Alliin	Alliin	0.9	0.631
C_0027	*allo*-Threonine	allo-Threonine	<1	N.A.
C_0041	Alloisoleucine	Alloisoleucine	1.2	0.081
A_0088	AMP	AMP	1.3	0.132
C_0051	Anthranilic acid	Anthranilic acid	N.A.	N.A.
C_0080	Arg	Arg	1.0	0.938
A_0073	Ascorbate 2-sulfate	Ascorbate 2-sulfate	1.2	0.117
C_0044	Asn	Asn	1.1	0.482
C_0046	Asp	Asp	1.2	0.028
A_0109	ATP	ATP	0.7	0.536
A_0052	Azelaic acid	Azelaic acid	N.A.	N.A.
C_0025	Betaine	Betaine	1.1	0.279
C_0030	Betaine aldehyde_+H_2O	BTL	N.A.	N.A.

C_0068	Betonicine	Betonicine	1<	N.A.
C_0105	Butyrylcarnitine	Butyrylcarnitine	1.1	0.678
C_0094	Caffeine	Caffeine	<1	N.A.
A_0085	cAMP	cAMP	1.0	0.842
C_0072	Carnitine	Carnitine	1.0	0.823
A_0099	Cholic acid	Cholic acid	1.1	0.787
C_0016	Choline	Choline	1.0	0.883
A_0045	*cis*-Aconitic acid	cis-Aconitic acid	1.1	0.420
A_0030	Citramalic acid	Citramalic acid	N.A.	N.A.
A_0056	Citric acid	Citric acid	1.0	0.673
C_0082	Citrulline	Citrulline	1.0	0.637
C_0040	Creatine	Creatine	1.1	0.412
C_0022	Creatinine	Creatinine	1.1	0.343
C_0031	Cys	Cys	1<	N.A.
C_0103	Cystathionine	Cystathionine	N.A.	N.A.
C_0109	Cystine	Cystine	1.4	0.021
C_0018	Diethanolamine	Diethanolamine	0.8	0.305
C_0122	Disulfiram	Disulfiram	1.0	0.614
C_0054	Ectoine	Ectoine	N.A.	N.A.
C_0002	Ethanolamine	Ethanolamine	1.0	0.587
A_0027	Ethanolamine phosphate	EAP	1.0	0.985
C_0012	GABA	GABA	N.A.	N.A.
A_0102	GDP	GDP	0.8	N.A.
C_0060	Gln	Gln	1.2	0.083
C_0062	Glu	Glu	0.9	0.306
C_0085	Glucosamine	Glucosamine	1.0	0.779
A_0074	Glucose 1-phosphate	G1P	N.A.	N.A.
A_0075	Glucose 6-phosphate	G6P	1.4	N.A.
A_0057	Glucuronic acid Galacturonic acid	Glucuronic acid Galacturonic acid	0.8	N.A.
C_0003	Gly	Gly	1.1	0.072
A_0011	Glyceric acid	Glyceric acid	1.0	0.597
C_0010	Glycerol	Glycerol	1.0	0.969

A_0043	Glycerol 3-phosphate	G3P	1.1	N.A.
C_0114	Glycerophosphocholine	GPCho	1.1	0.326
A_0103	Glycocholic acid	Glycocholic acid	0.5	0.141
A_0092	GMP	GMP	0.9	N.A.
C_0081	Guanidinosuccinic acid	Guanidinosuccinic acid	1.1	0.784
A_0022	Heptanoic acid	Heptanoic acid	1<	N.A.
A_0014	Hexanoic acid	Hexanoic acid	1.4	N.A.
A_0048	Hippuric acid	Hippuric acid	1.3	0.375
C_0066	His	His	1.0	0.668
C_0093	Homocitrulline	Homocitrulline	0.5	N.A.
A_0049	Homovanillic acid	HVA	1.1	0.172
C_0039	Hydroxyproline	Hydroxyproline	1.1	0.452
C_0019	Hypotaurine	Hypotaurine	1.1	0.431
C_0049	Hypoxanthine	Hypoxanthine	1.0	0.915
C_0042	Ile	Ile	1.0	0.967
C_0067	Imidazolelactic acid	Imidazolelactic acid	1.1	0.356
A_0089	IMP	IMP	1.3	0.149
A_0047	Indole-3-acetic acid	Indole-3-acetic acid	1.4	0.031
C_0071	Indole-3-ethanol	Indole-3-ethanol	1.2	0.551
C_0116	Inosine	Inosine	0.3	0.399
A_0019	Isethionic acid	Isethionic acid	1.1	0.456
A_0004	Isobutyric acid Butyricacid	Isobutyric acid Butyricacid	1<	N.A.
A_0055	Isocitric acid	Isocitric acid	1.0	0.817
A_0007	Isovaleric acid Valeric acid	Isovaleric acid Valeric acid	0.9	N.A.
A_0054	Kynurenic acid	Kynurenic acid	N.A.	N.A.
C_0099	Kynurenine	Kynurenine	1.0	0.668
A_0006	Lactic acid	Lactic acid	1.2	0.122
A_0060	Lauric acid	Lauric acid	1.0	0.628
C_0043	Leu	Leu	1.0	0.735
C_0061	Lys	Lys	1.0	0.355
A_0025	Malic acid	Malic acid	1.1	0.372
C_0107	Melatonin	Melatonin	N.A.	N.A.

C_0064	Met	Met	1.0	0.743
C_0038	Metformin	Metformin	N.A.	N.A.
C_0074	Methionine sulfoxide	Methionine sulfoxide	1.0	0.858
A_0064	Mucic acid	Mucic acid	1.2	0.284
A_0068	Myristoleic acid	Myristoleic acid	1.1	0.908
C_0014	*N*,*N*-Dimethylglycine	DMG	1.0	0.681
A_0024	*N*-Acetyl-β-alanine	N-Acetyl-b-alanine	1<	N.A.
A_0023	*N*-Acetylalanine	N-Acetylalanine	1.2	0.028
A_0046	*N*-Acetylaspartic acid	N-Acetylaspartic acid	N.A.	N.A.
C_0102	*N*-Acetylgalactosamine *N*-Acetylmannosamine *N*-Acetylglucosamine	N-Acetylgalactosamin e ManNAc GlcNAc	<1	N.A.
A_0015	*N*-Acetylglycine	N-Acetylglycine	N.A.	N.A.
C_0091	N-Acetyllysine-1	N-Acetyllysine	N.A.	N.A.
C_0092	N-Acetyllysine-2	N-Acetyllysine	N.A.	N.A.
C_0090	N-Acetyllysine-3	N-Acetyllysine	N.A.	N.A.
A_0081	*N*-Acetylmuramic acid	N-Acetylmuramic acid	1.4	0.426
C_0078	*N*-Acetylornithine	N-AcOrn	1.1	N.A.
C_0037	*N*-Methylproline	N-Methylproline	1.6	0.175
C_0011	*N*-Methylputrescine	N-Methylputrescine	N.A.	N.A.
A_0078	N^2-Phenylacetylglutam ine	N2-Phenylacetylgluta mine	1.0	0.902
C_0079	N^5-Ethylglutamine	N5-Ethylglutamine	0.9	0.461
C_0069	N^6-Methyllysine	N6-Methyllysine	1.0	0.975
C_0032	Nicotinamide	Nicotinamide	0.9	0.326
C_0097	*O*-Acetylcarnitine	ALCAR	1.1	0.397
C_0070	*O*-Acetylhomoserine 2-Aminoadipicacid	O-Acetylhomoserine 2-Aminoadipicacid	1.0	0.847
C_0120	Octanoylcarnitine	Octanoylcarnitine	1.0	0.868
C_0121	Ophthalmic acid	Ophthalmic acid	<1	N.A.
C_0045	Ornithine	Ornithine	1.1	0.431
A_0067	Pantothenic acid	Pantothenic acid	0.8	0.662
C_0087	Paraxanthine	Paraxanthine	<1	N.A.

A_0034	Pelargonic acid	Pelargonic acid	1.1	0.086
C_0063	Penicillamine	Penicillamine	N.A.	N.A.
A_0038	Perillic acid	Perillic acid	1.2	N.A.
C_0075	Phe	Phe	1.0	0.871
A_0065	Phosphocreatine	Phosphocreatine	1.0	0.911
C_0089	Phosphorylcholine	Phosphorylcholine	1.2	0.242
C_0036	Pipecolic acid	Pipecolic acid	1.1	0.241
C_0005	Piperidine	Piperidine	1.3	0.095
C_0024	Pro	Pro	1.0	0.957
A_0090	Prostaglandin E_2	Prostaglandin E2	N.A.	N.A.
A_0091	Prostaglandin F_{2a}	Prostaglandin F2a	1<	N.A.
A_0070	Ribulose 5-phosphate	Ru5P	0.7	0.656
C_0047	*S*-Methylcysteine	S-Methylcysteine	1.1	0.481
C_0007	Sarcosine	Sarcosine	1.0	0.928
C_0095	SDMA	SDMA	1.0	0.685
A_0061	Sebacic acid	Sebacic acid	N.A.	N.A.
C_0017	Ser	Ser	1.1	0.152
C_0108	Ser-Glu	Ser-Glu	N.A.	N.A.
C_0083	Serotonin	Serotonin	0.7	0.447
C_0096	Spermine	Spermine	N.A.	N.A.
C_0056	Stachydrine	Stachydrine	1.1	0.823
A_0016	Succinic acid	Succinic acid	1.0	0.669
A_0077	Sulfotyrosine	Sulfotyrosine	1.1	0.077
A_0031	Tartaric acid	Tartaric acid	N.A.	N.A.
C_0033	Taurine	Taurine	1.0	0.823
A_0110	Taurocholic acid	Taurocholic acid	0.7	0.459
A_0037	Terephthalic acid	Terephthalic acid	1.1	0.085
C_0113	Tetrahydrouridine	Tetrahydrouridine	N.A.	N.A.
C_0086	Theobromine	Theobromine	0.8	N.A.
C_0028	Thr	Thr	1.0	0.905
A_0026	Threonic acid	Threonic acid	1.1	0.272
C_0004	Trimethylamine *N*-oxide	Trimethylamine N-oxide	1.0	0.986
C_0098	Trp	Trp	0.9	0.166
C_0088	Tyr	Tyr	0.9	0.028

C_0076	Tyr-Arg_divalent	Tyr-Arg_divalent	N.A.	N.A.
A_0097	UDP	UDP	<1	N.A.
A_0084	UMP	UMP	1<	N.A.
C_0001	Urea	Urea	1.1	0.385
A_0040	Uric acid	Uric acid	1.0	0.799
C_0112	Uridine	Uridine	1.0	0.326
C_0026	Val	Val	1.0	0.529
A_0028	XA0004	XA0004	1.0	0.915
A_0044	XA0013	XA0013	1.4	0.345
A_0069	XA0027	XA0027	0.8	0.406
A_0072	XA0033	XA0033	1.0	0.914
C_0035	XC0016	XC0016	1.3	0.106
C_0055	XC0029	XC0029	1.1	0.863
C_0100	XC0061	XC0061	1.0	0.902
C_0101	XC0065	XC0065	1.6	N.A.
C_0123	XC0120	XC0120	1.3	0.067
C_0126	XC0126	XC0126	1.0	0.776
C_0127	XC0132	XC0132	1.0	0.876
C_0008	β-Ala	b-Ala	1.1	0.082
A_0017	β-Hydroxyisovaleric acid	b-Hydroxyisovaleric acid	1.2	0.203
C_0058	ɣ-Butyrobetaine	Actinine	1.0	0.745
C_0106	ɣ-Glu-2-aminobutyric acid	g-Glu-2-aminobutyric acid	1.1	N.A.

[a]ID consists of the analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively. [b]The ratio is computed using averaged detection values, and the latter is used as the denominator. [c]The p-value is computed using Welch's t-test. (*<0.05, **<0.01, ***<0.001)

Abbreviation: N.A. (not available), the calculation was impossible because of insufficient data.

국문초록

연구 목적: 소아 및 청소년기 비만은 전 세계적으로 증가하는 추세이며, 이환율을 줄이기 위해 많은 임상 치료 및 중재가 이루어지고 있다. 그러나 체중중재에 대해 중재효과가 우수한 반응군 또는 비만 고위험군을 발굴하기 위한 효과적인 바이오마커는 없는 실정이다. 따라서 본 연구에서는 아동, 청소년 비만환자에서 중재 효과를 판정 및 예측하는데 필요한 대사체 및 유전체 지표의 발굴을 목표로 한다.

연구 방법: 아동, 청소년 자원을 활용한 비만 대사체 정보를 생산하기 위해 '고도비만의 소아청소년 장기추적 중재연구 (ICAAN)'에서 중재 반응에 따라 총 40명의 대상자가 선정되었다. 추적 18개월까지 BMI z-score 변화를 기준으로 체중감소가 현저한 대상자 20명 (responder)과 체중감소가 미미한 대상자 20명 (non-responder)을 선정하였으며, 중재 기간별 (중재 전, 중재 6개월 후, 중재 18개월 후) 에 해당하는 각각의 혈청시료와 생리학적 데이터를 수집하였다. 총 40명을 대상으로 120개의 샘플을 수집하고 CE-TOFMS 방법을 사용해 혈청 시료의 대사체의 정량분석을 시행하였고, Metaboanalyst 프로그램을 통해 2차 분석을 수행하여 관련 대사체 및 분자유전학적 네트워크를 구축하였다. 또한 IPA 및 문헌검색을 통해 기 확보된 유전체와 대사체 정보를 통합하여 유전체 분석을 시행하였고, 대상 확대를 위해 코호트 내 중재 6개월 후 BMI z-score를 기준으로 선별된 비만 환자 123명 (반응군 41명, 비반응군 82명)의 대상에서 GWAS 정보를 비교 분석 및 유의미한 SNP의 유전형을 구분하여 임상지표를 분석 하였다.

연구 결과: 총 120개의 혈청시료를 HMT에 의뢰한 결과 총 194개의 대사체가 발굴되었다. 9개의 대사체 (1-or 3-Methylhistidine, 3-Hydroxypropionic acid, 8-or 2-Hydroxyoctanoic acid-1, alanine, aspartic acid, cystine, indole-3-acetic acid, N-Acetylalanine, tyrosine)

는 체중중재 전과 18개월 후를 비교하였을 때 반응군과 비반응군간 유의미한 차이를 보였다 ($p<0.05$). Metaboanalyst 프로그램을 통한 재분석 후, 13개의 대사체 (1-Methyl-4-imidazoleacetic acid, 10-Hydroxydecanoic acid, 3-Indoxylsulfuric acid, 2- or 8-Hydroxyoctanoic acid-2, allo-Threonine, azelaic acid, disulfiram, indole-3-acetic acid, N-Acetyllysine-1 or -2, N-Acetylornithine, Succinic acid, XC0065) 가 체중중재 전 반응군과 비반응군간 유의미한 차이를 보였고, 6개의 대사체 (2-oxooctanoic acid, glucose, isocitiric acid, lauric acid, sulfotyrosine, XC0126) 가 체중중재 전과 6개월 후를 비교하였을 때 반응군과 비반응군간 유의미한 차이를 보였다 ($p<0.05$). 추가로, 9개의 대사체 (1- or 3-Methylhistidine, alanine, aspartic acid, cystine, glycocholic acid, homovanillic acid, piperidine, sulfotyrosine, tyrosine)가 체중중재 전과 18개월 후를 비교하였을 때 두 그룹간 유의미한 차이를 보였다 ($p<0.05$). 또한 중재 18개월 후, 총 63개의 대사체가 중재 자체에 의해 유의미한 변화를 보였다 (FDR-adjusted p-value <0.05). 상기 분석 결과를 종합하였을 때, alanine, aspartic acid, cystine 이 중재 18개월 후 반응군간 유의미한 차이를 보이는 대사체로 선정되었다. 또한 주요문헌을 검색하고 대사체 네트워크를 통합 분석하여 유전체 분석 지표를 선정하였다. 결과적으로, 코호트 내 123명의 GWAS 정보 분석에서 IRS1 rs2943641 과 FAIM2 rs7138803 이 중재 반응군에서 유의미한 차이를 보였다 ($p<0.05$).

결론: 본 연구에서는 체중 중재 18개월 후 반응군 및 비반응군 비교시 alanine, aspartic acid, cystine 이 유의미하게 차이를 보이는 대사체로 발굴되었다. 그리고 각 시점에서 두 그룹간 대사체 차이를 확인하기 위해 다양한 분석 방법 및 검증이 시도되었다. 또한 대사체 및 유전체 통합 분석을 통해 연구대상자에서 비만 중재와 관련된 IRS1 rs2943641 와 FAIM2 rs7138803를 확인하였다. 이 결과는 아동, 청소년 비만에서 특정 치료 또는 중재에 대한 평가의 모니터링으로 사용가능한 바이오 마커의

연구에 사용될 수 있을 것이다. 또한 비만 중재 효과에 대한 예측 지표 발굴에 사용될 수 있을 것으로 기대된다.

주요어 : 대사체, 유전체, 비만, 소아, 청소년, 체중 중재, 비만지표
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