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Lipidomic Profiling Disturbance as Good Follow up Index in Obese Diabetic Patients

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Metabolite profiling, or metabolomics, has become a powerful approach that has been widely adopted for clinical diagnostics. This study aimed to evaluate the metabolomics profile in obese and obese diabetic patients as the early predictor of diabetes with obese patients.
Subjects and Methods: This study was conducted on fifty-four from one hundred and sixty unrelated individuals. Participants were mainly 35–70 years of age were classified to four groups are normal, obese and obese diabetic, obese.
Results: It was found a significant increase in mean values of LDL concentration in obese as compared to healthy group. It also found that, in obese subjects with the metabolic syndrome and T2D, oxidative stress is increased and the redox state is a potentially useful therapy. Some lipid metabolism-related metabolites, including saturated fatty acides, palmitate and stearate and

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unsaturated fatty acid archidonic acid, have been identified in diabetic, which indicate a dysregulation of lipid metabolites in diabetic subjects.
Conclusion: In conclusion, metabolomics of lipid intermediate was considered as good index of metabolic syndrome and diabetes and can be taken in consideration for follow up and treatment.

Keywords: Obesity; diabetic; metabolomics.

1. INTRODUCTION

The omic sciences of systems biology, including genomics, transcriptomics, proteomics, and metabolomics, have been in existence for decades, whereas much attention has been focused on their development and application in the last several years [1]. Metabolomics can be defined as the "comprehensive characterization of the small molecule metabolites in a biological system". In turn, such small metabolites represent the outcome of gene expression and define the biochemical phenotype of a cell, tissue, organ and organism [2].

The small metabolites include the intermediates and end products of metabolism, and they compass both primary metabolites (e.g. sugars, amino acids, fatty acids and organic acids) and secondary metabolites (e.g. phenylpropanoids and alkaloids). Metabolomics can also characterize the dynamic metabolome, reflecting changes in the abundance of small molecules during development and in response to external stresses. Metabolomics can provide an overview of the metabolic status and global biochemical events associated with a cellular or biological system [3].

Lipidomics is a specialized subset of metabolomics that evaluates lipid profiles [4]. Lipids play many important roles in cancer processes including invasion, migration, and proliferation [5]. Another subset of the metabolomics field focuses on using labeled substrates (e.g., ^{13}C labeled glucose) to define metabolic fluxes or biomarkers in disease states. This approach enables us to further our understanding of the metabolism in disease or drug responses by following the metabolism of labeled substrates into their pathway products within specific times. For example, glucose can undergo glycolysis to lactate or be shunted through the pentose phosphate pathway to form ribose, and the ^{13}C labeled carbons in glucose can reveal how much goes into each pathway. This information provides a better understanding of the pathways that are upregulated or downregulated and can define metabolic phenotypes in disease states [6].

Although various methodologies have been employed, two technologies have prevailed as the core methodologies of metabolite profiling: nuclear magnetic resonance NMR [7]and mass spectrometry MS, with the latter coupled to an array of separation techniques including GC and LC [8]. NMR allows the rapid, high-throughput and automated analysis of crude extracts, and the quantitative detection of many different groups of metabolites [9]. Providing also structural information including stereochemical details [10] However, NMR is less sensitive than MS-based approaches and NMR data have been metaphorically compared to 'the tip of the iceberg', with LC-MS providing details of the much larger, submerged portion [11]. Recently, attention has concentrated on the identification of biomarkers thanks to metabolomic analyses by means of which it is possible to better define the mechanisms at the base of obesity and prevent its onset [12].

Obesity is characterized by abnormal or excessive fat accumulation that is the result of a chronic imbalance between energy intake and energy expenditure. It poses a substantial health risk, as obesity is linked to several common diseases, such as type 2 diabetes, cardiovascular disease, stroke, arthritis, and several types of cancer. The prevalence of obesity and its associated co morbidities has steadily increased over the last 40-50 years, highlighting the pathogenic relevance of an obesogenic environment. Because obesity, insulin resistance, diabetes, dyslipidaemia and fatty liver tend to co-occur in the same individual, it has been useful to refer to this cluster of manifestations as 'metabolic syndrome' [13].

Obesity is a disorder of the whole body and obviously involves metabolic changes, but the actual alterations in metabolism during obesity and any dysfunction associated with obesity at the level of individual organs or cellular organelles are not yet clearly understood [14]. As metabolomics can readily detect subtle changes in the metabolic network, it is uniquely poised to increase our understanding of obesity and obesity-related diseases [15].

This study aimed to evaluate the metabolomics profile in obese and obese diabetic patients as the early predictor of diabetes with obese patients.

2. SUBJECTS AND METHODS

This study was approved by the Bioethical and Research Committee- Faculty of Medicine- King Abdulaziz University(KAU) and an oral voluntary consent from all the participated subjects was obtained. All subjects were collected from King Abdulaziz University Hospital of Saudi Arabia, Jeddah. Four groups were recruited in this study type 2 diabetes, obese, obese-diabetes and control.

Inclusion Criteria:

- Saudis men and women
- Body mass index (BMI) ≤18.5-24.9 kg/m^2 for control and diabetic and BMI ≥30 kg/m^2 for obese and obese/diabetic.

Exclusion criteria:

- Pregnancy and currently breastfeeding
- Patients had BMI =25.0-29.9,
- Chronic diseases (cancer, heart disease, hepatitis, renal inadequacy, respiratory failure, or other diseases that will affect the clinical observations and biological indicators).

2.1 Study Design

This study was conducted on fifty-four from one hundred and sixty unrelated individuals. Participants were mainly 35–70 years of age at the time of recruitment between 2015 and 2016 at King Abdulaziz University Hospital (KAAUH). The subjects were classified to six groups are normal, obese and obese diabetic, obese hypertension, normal diabetic and normal hypertension based on determination of their Body Mass Index (BMI). Blood samples were collected from the individuals on EDTA tubes, and serum samples was separated and subjected to following analysis. Thyroid profile, Diabetic markersandMetabolomics (lipolysis and lipogenesis) by GCMS.

2.2 Anthropometric Assessment

Height and weight measurements were obtained from each participant. BMI was calculated to classify as per the groups.

2.3 Body Mass Index (BMI)

Body Mass Index (BMI) was calculated by the equation:

BMI = Weight in kg/height in m^2

Using BMI, the subjects were graded for obesity using a Garrow and Webster (1985), and nomogram of WHO (1995).

Table 1. BMI classification

Subjects	BMI
Normal	18.5-24.9
Obesity Grade I	30.0-34.9
Obesity Grade II	35.0-39.9
Obesity Grade III	≥ 40

2.4 Sample Collection

After 10-12 hours overnight fasting, about five milliliters of blood drawn from each participant using standard venipuncture technique in plain

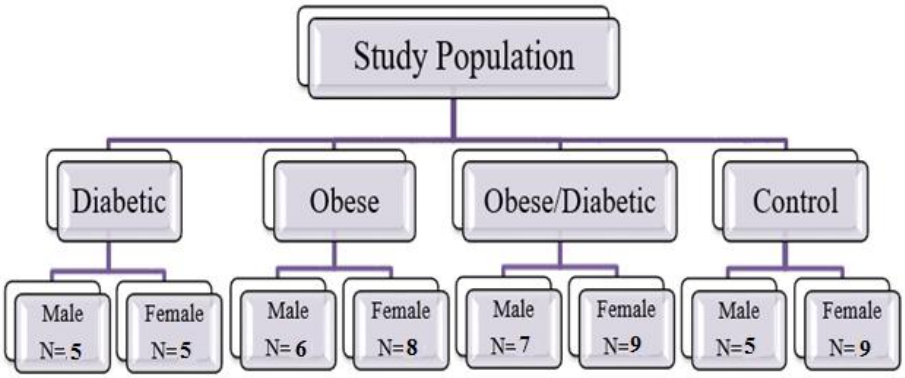

Fig. 1. Study population chart

vacutainer tubes for serum separation. Plain vacutainer tubes left at room temperature for about 30 minutes for blood clotting. The serum was separated by centrifugation at 3500 rpm for 10 minutes in a Sigma centrifuge, UK. The serum was separated into Eppendorf tubes and stored at -80°C for further analysis. Serum samples were lyophilized to remove water and to obtain a powder for easily shipping to the University of California, Davis (UC-Davis) for GCTOF-MS and LCQTOF-MS analysis.

- Data is log 2 normalized and auto scaled.
- No outlier detected according to 95% confidence region.
- No clear distinction between four treatment groups (control, diabetic, obese, obese/diabetic)
- Three clusters (highlighted with numbers) are detected. It is recommended to examine the meta data to explore the reason of the separation.

2.5 Univariate Analysis

- Data is log 2 normalized.
- Both parametric *(para)* and non-parametric *(non_para)* test are applied to the Treatment
- Post hoc analysis are used to perform pairwise comparisons between any of the two groups
- Fold change is given between any of the two groups.
- Boxplots are provide in the boxplots.tar.gz file (using raw data).

3. RESULTS AND DISCUSSION

Metabolomics is the latest of the "omics" technologies that employs state of the art analytical instrumentation in conjunction with pattern recognition techniques to monitor and discover metabolic changes in subjects related to disease status or in response to a medical or external intervention. Global metabolomics alterations reflect changes due to environmental factors, genetic variation and regulation, changes in gut microflora, and altered kinetic activity or levels of enzymes. Therefore, metabolomics alterations represent changes in the phenotype and molecular physiology [16].

This study investigated the relationship between LDL, a marker of systemic oxidative stress and obesity (Table 2). We found a significant increase in mean values of LDL concentration in obese as compared to healthy group. Our results are in agreement with those reported by Velagapudi et al. [22] who found that LDL level were significantly higher in overweight/obese vs. normal weight children also oxo-LDL is significantly associated with adiposity and with insulin resistance, independent of body fatness. It was suggested that systemic oxidative stress may be a novel risk factor for T2D and obesity. Increased lipid peroxidation has been reported in obese people. It has also been reported that, in obese subjects with the metabolic syndrome and T2D, oxidative stress is increased and the redox state is a potentially useful therapy. The possible explanation for the positive relationship between obesity and LDL is the impaired antioxidant defense of HDL in abdominally obese subjects as the antioxidant action of HDL components prevents LDL oxidation and renders LDL resistant to oxidation [17].

Our results are in agreement with those reported by who found the release of free radicals is enhanced from conditions such as hyperglycaemia, ischaemia, infection and would be available to oxidize the particle. It was found that, the amount of oxidized phospholipid on LDL apo B100 appears to be a good marker of atherosclerotic progression. Increased oxidative stress, reflected by elevated levels of oxidized LDL, may precede the development of insulin resistance [28] and therefore be important in the early pathophysiology of type 2 diabetes mellitus [18].

In this study (Fig. 2) and (Tables 3, 4), some lipid metabolism-related metabolites, including saturated fatty acides, palmitate and stearate and unsaturated fatty acid archidonic acid, have been identified in diabetic, which indicate a dysregulation of lipid metabolites in diabetic subjects. Metabolites of the phospholipids and fatty acid pathway are associated with diabetes. Phosphatidylcholines (PCs) products or metabolites are the important components of lipid bilayer of cells, as well as being involved in metabolism and signaling (Fig. 3). Similar to previous studies, this study also shows that the obese subjects contribute to abnormal lipid metabolism in diabetic organism [19-23]. An earlier study suggested that the peroxisome-proliferator activated receptors (PPAR), involved in lipid homeostasis and metabolism, were activated by statin treatment [24]. Thus, we can conclude that the improvement of lipid profile and metabolism may be due to these active ingredients. In conclusion, metabolomics of lipid

intermediate was considered as good index of metabolic syndrome and diabetes and can be taken in consideration for follow up and treatment.

Table 2. Lipid profile in all studied groups (Mean ± SD)

Definition	Control	Obese	DM	Obese and diabetic	*p-value*
T-Chol (mg/dl)	175.4 ± 24.9	189.1 ± 39.2	190.9 ± 39.5	164.5 ± 64.6	NS
TG(mg/dl)	60.1 ± 21.2	107.6 ± 52.4	174.9 ± 104.2	116 ± 51.4	<0.001(a,b,c,d) NS (b,d)
HDL-C (mg/dl)	55.8 ± 10.3	45.9 ± 10.7	50.1 ± 18.1	33.4 ± 12.5	<0.001(a,b)(a,d)(b,d)(c,d)
LDL-C (mg/dl)	102.5 ± 22.2	118.6 ± 25.2	103.7 ± 30.8	108.5 ± 56.6	<0.001(a,b)

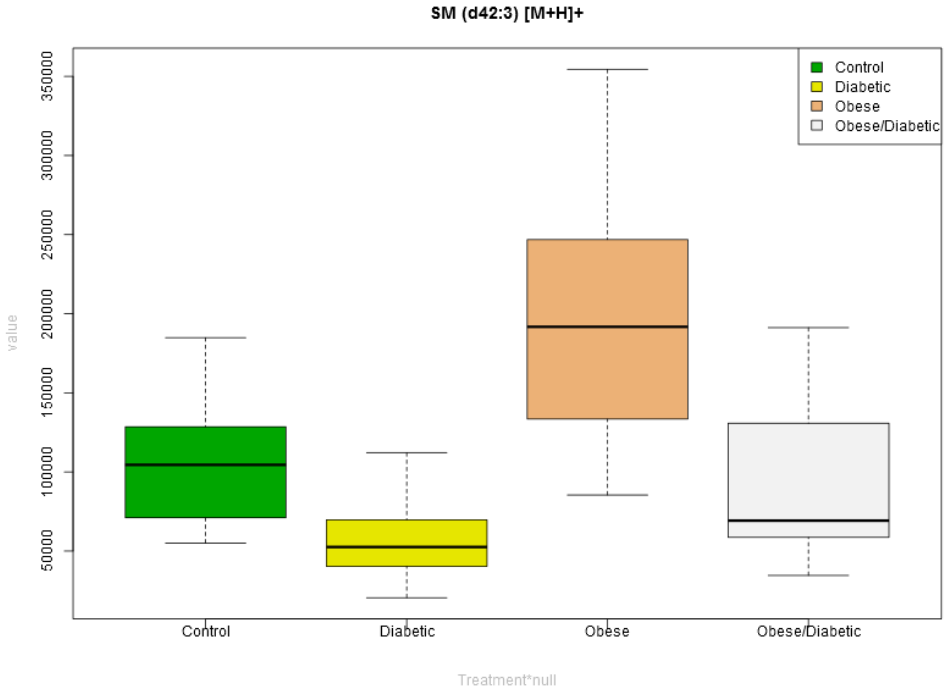

Fig. 2. A total of 174 (184*) compounds were found to be different on a p-value cutoff of 0.05 using Games-Howell post hoc analysis (Dunn's procedure*). The 10 known compounds with smallest p value are given

Table 3. The games-Howell post hoc test all possible combinations of group differences when the homogeneity of variances is not assumed

Annotation	nonpara_p_posthoc__Control_Diabetic	fold change (Control_Diabetic)
SM (d42:3) [M+H]+	0.00	1.79
SM (d39:1) [M+H]+	0.01	1.57
TG(50:3) [M+NH4]+	0.02	0.43
SM (d42:2) [M+H]+	0.03	2.08
SM (d38:1) [M+H]+	0.08	1.52
SM (d38:2) [M+H]+	0.10	1.72
4-acetamidobutyric acid	0.12	2.91
SM (d40:2) [M+H]+	0.13	1.69
1-methyladenosineAglL	0.15	0.98
Urea [M+H]+	0.15	1.23

Table 4. Pair wise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented

Annotation	nonpara_p_posthoc__Control_Diabetic	fold change (Control_Diabetic)
SM (d42:3) [M+H]+	0.00	1.79
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TG(50:3) [M+NH4]+	0.01	0.43
SM (d38:2) [M+H]+	0.02	1.72
SM (d38:1) [M+H]+	0.03	1.52
SM (d40:2) [M+H]+	0.03	1.69
SM (d36:2) [M+H]+	0.10	1.51
4-acetamidobutyric acid	0.12	2.91
l-glutamineAglL	0.17	1.09

fold change < 1 ⇔ control < diabetic (increase)
fold change > 1 ⇔ control > diabetic (decrease)

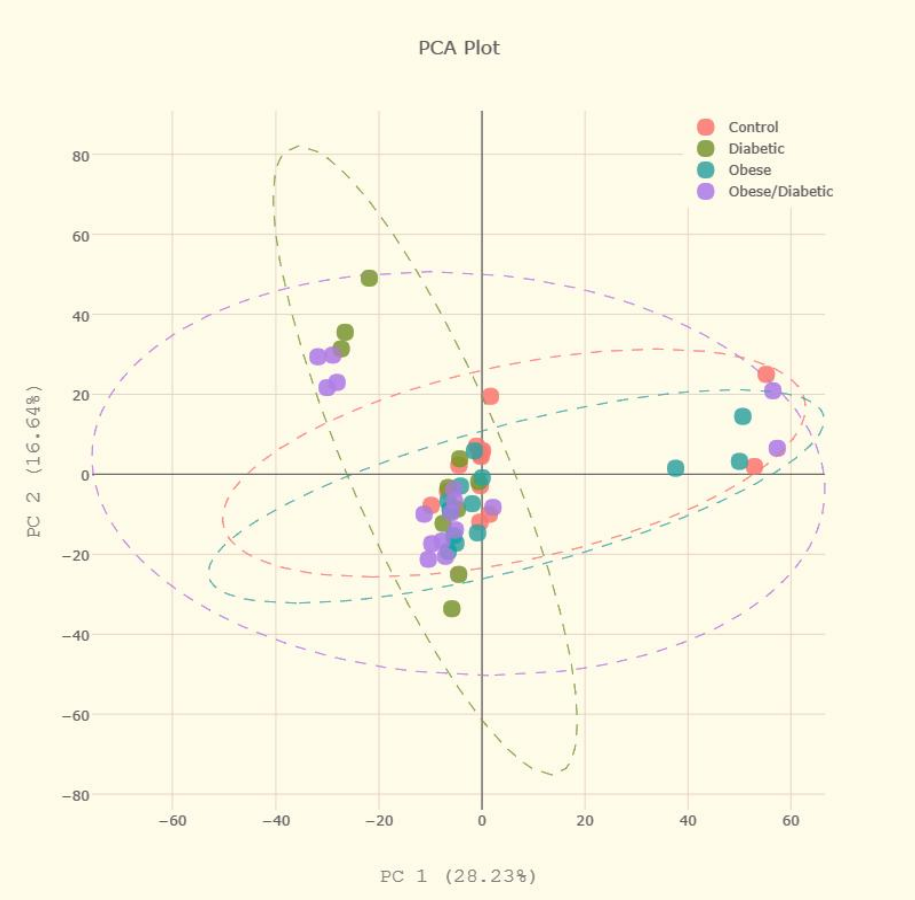

Fig. 3. Principal component analysis (PCA) showing association of different parameters

4. CONCLUSION

Metabolomics of lipid intermediate was considered as good index of metabolic syndrome and diabetes and can be taken in consideration for follow up and treatment.

CONSENT AND ETHICAL APPROVAL

This study was approved by the Bioethical and Research Committee- Faculty of Medicine- King Abdulaziz University (KAU) and a written voluntary consent from all the participated

subjects was obtained. All subjects were collected from King Abdulaziz University Hospital of Saudi Arabia, Jeddah.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Holmes E, Wilson ID, Nicholson JK. Metabolic phenotyping in health and disease. Cell. 2008;134:714–717.
2. Mauro Commisso a, Pamela Strazzer a, Ketti Toffali, Matteo Stocchero, Flavia Guzzo. 2013;4(5):e201301007.
3. Montoya G, Strauss V, Fabian E, Kamp H, Mellert W, Walk T, Looser R, Herold M, Krennrich G, Peter E, van Ravenzwaay B. Abstracts / Toxicology Letters. 221S. 2013; S59–S256.
4. Denkert C, Budczies J, Kind T, Weichert W, Tablack P, Sehouli J, Niesporek S, Konsgen D, Dietel M, Fiehn O. Mass spectrometry- based metabolic profiling reveals different metabolite patterns in invasive ovarian carcinomas and ovarian borderline tumors. Cancer Res. 2006;66: 10795–10804.
5. Lindon JC, Holmes E, Nicholson JK. Metabonomics techniques and applications to pharmaceutical research & development. Pharmacol Res. 2006;23:1075–1088.
6. Schauer N, Fernie AR. Plant metabolomics: Towards biological function and mechanism. Trends Plant Sci. 2006; 11:508–516.
7. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal. Chem. 2006;78: 779–787.
8. Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. Nat. Rev. Micro. 2005;3:431–438.
9. Wenk MR. Lipidomics: New tools and applications. Cell. 2010;143:888–895.
10. Fernandis AZ, Wenk MR. Lipid-based biomarkers of cancer. J. Chrom. B. 2009;877:2830–2835.
11. Zhang GF, Sadhukhan S, Tochtrop GP, Brunengraber H. Metabolomics, pathway regulation, and pathway discovery. J. Biol. Chem. 2011;286:23631–23635.
12. Jennifer L. Spratlin, Natalie J. Serkova, S. Gail Eckhardt. Clin Cancer Res. 2009;15: 431-440.
13. Catenacci VA, Hill JO, Wyatt HR. The obesity epidemic. Clin. Chest Med. 2009; 30:415-444.
14. Saarni SE, Saarni SI, Fogelholm M, Heliovaara M, Perala J, Suvisaari J, Lonnqvist J. Body composition in psychotic disorders: A general population survey. Psychol. Med. 2009;39:801-810.
15. Suvisaari JM, Saarni SI, Perala J, Suvisaari JV, Harkanen T, Lonnqvist J, Reunanen A. Metabolic syndrome among persons with schizophrenia and other psychotic disorders in a general population survey. J. Clin. Psychiatry. 2007;68:1045-1055.
16. Illig T, Gieger C, Zhai G, Misch-Margl R, Wang-Sattler W, Prehn R, Altmaier C, Kastenmüller E, Kato G, Mewes BS, et al. A genomewide perspective of genetic variation in human metabolism. Nat. Genet. 2010;42:137-141.
17. Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, Ebbels T, De Iorio M, Brown IJ, Veselkov KA, et al. Human metabolic phenotype diversity and its association with diet and blood pressure. Nature. 2008;453:396-400.
18. Nikkila J, Sysi-Aho M, Ermolov A, Seppanen-Laakso T, Simell O, Kaski S, Oresic M. Gender-dependent progression of systemic metabolic states in early childhood. Mol. Syst. Biol. 2008;4:197.
19. Maeba, R., Maeda, T., Kinoshita, M., Takao, K., Takenaka, H., Kusano, J., Yoshimura, N., Takeoka, Y., Yasuda, D., Okazaki, T. et al. (2007). Plasmalogens in human serum positively correlate with high-density lipoprotein and decrease with aging. J. Atheroscler. Thromb. 14, 12-18.
20. Oresic M, Simell S, Sysi-Aho M, N¿nt¿-Salonen K, Sepp¿nen-Laakso T, Parikka V, Katajamaa M, Hekkala A, Mattila I, Keskinen P, et al. Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. J. Exp. Med. 2008;205:2975-2984.
21. Martin FPJ, Dumas ME, Wang Y, Legido-Quigley C, Yap IKS, Tang H, Zirah S, Murphy GM, Cloarec O, Lindon JC, et al. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol. Syst. Biol. 2007;3:112.

22. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu PV, Yetukuri L, Islam S, Felin J, Perkins R, Borén J, Oresic M, et al. The gut microbiota modulates host energy and lipid metabolism in mice. J. Lipid Res. 2010;51:1101-1112.
23. Martin FPJ, Dumas ME, Wang Y, Legido-Quigley C, Yap IKS, Tang H, Zirah S, Murphy GM, Cloarec O, Lindon JC, et al. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol. Syst. Biol. 2007;3:112.
24. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu PV, Yetukuri L, Islam S, Felin J, Perkins R, Borén J, Oresic M, et al. The gut micro biota modulates host energy and lipid metabolism in mice. J. Lipid Res. 2010; 51:1101-1112.

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