PLOS ONE

RESEARCH ARTICLE

Increased intestinal-fatty acid binding protein in obesity-associated type 2 diabetes mellitus

Dicky L. Tahapary[1,2☯]*, Atikah I. Fatya[3,4☯], Farid Kurniawan[1,2], Cicilia Marcella[2], Ikhwan Rinaldi[3,4,5], Tri J. E. Tarigan[1,2], Dante S. Harbuwono[1,2], Em Yunir[1,2], Pradana Soewondo[1,2‡]*, Dyah Purnamasari[1,2‡]

1 Division of Endocrinology, Metabolism, and Diabetes, Dep artment of Internal Medicine, Faculty of Medicine Universitas Indonesia, Depok City, Indonesia, **2** Metabolic, Cardiovascular, and Aging Research Cluster, The Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, **3** Department of Internal Medicine, Fa culty of Medicine Universitas Indonesia, Jakarta, Indonesia, **4** Division of Hematology and Medical Oncology, Depa rtment of Internal Medicine, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, **5** Clinical Epidemiology and Evidence-based Medicine Unit, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

☯ These authors contributed equally to this work.
‡ PS and DP also contributed equally to this work.
* dicky.tahapary@ui.ac.id (DLT); pradana.soewondo@ui.ac.id (PS)

Citation: Tahapary DL, Fatya AI, Kurniawan F, Marcella C, Rinaldi I, Tarigan TJE, et al. (2023) Increased intestinal-fatty acid binding protein in obesity-associated type 2 diabetes mellitus. PLoS ONE 18(1): e0279915. https://doi.org/10.1371/journal.pone.0279915

Editor: Yasin Hasan Balcioglu, Istanbul Bakirkoy Prof Dr Mazhar Osman Ruh Sagligi ve Sinir Hastaliklari Egitim ve Arastirma Hastanesi, TURKEY

Received: July 17, 2022

Accepted: December 18, 2022

Published: January 26, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: DLT recieved grant from the Ministry of Research and Technology Republic of Indonesia [PUPTN Dikti NKB-2766/UN2.RST/HKP.05.00.2020]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

Obesity is a traditional risk factor for type 2 diabetes mellitus (T2DM). However, recent studies reported that metabolically unhealthy obesity (MUO) exerts a higher risk of developing T2DM than metabolically healthy obesity (MHO) because of its higher state of insulin resistance. This may happen due to metabolic endotoxemia through gut dysbiosis and increased intestinal permeability. Our study aimed to know the association of intestinal permeability using intestinal fatty acid-binding protein (I-FABP) with obesity-related T2DM patients in Indonesia.

Methods

This was a cross-sectional study that recruited 63 participants with obesity defined using body mass index (BMI) classification for the Asia-Pacific population (BMI $\geq$25 kg/m2). All participants were then grouped into T2DM and non-T2DM based on American Diabetes Association (ADA) diagnostic criteria. The I-FABP levels were measured using the enzyme-linked immunosorbent assay method.

Results

The I-FABP level of T2DM group was higher compared to non-T2DM group, namely 2.82 (1.23) ng/mL vs. 1.78 (0.81) ng/mL ($p<0.001$; mean difference 1.033 with 95% CI 0.51–1.55). This difference was not attenuated even after adjustment for age. The fitted regression model using linear regression was: i-FABP = 1.787+1.034*(DM) (R^2 = 18.20%, standardized ß = 0.442, $p<0.001$).

Competing interests: The authors have declared that no competing interests exist.

Conclusions

This study underscores the association of intestinal permeability with T2DM in people with obesity and supports the evidence of the potential role of intestinal permeability in the pathogenesis of obesity-related T2DM.

Introduction

The increasing prevalence of obesity is in line with the rise in obesity-related cardiometabolic complications, including type 2 diabetes mellitus (T2DM) [1]. However, not all people with obesity have a similar risk for T2DM. Around 10–20% of people belong to metabolically healthy obesity (MHO) with a lower relative risk of T2DM [2, 3]. Metabolically healthy obesity and metabolically unhealthy obesity (MUO) differ in fat distribution, ectopic fat deposition, inflammatory markers, adipocyte dysfunction and insulin resistance [2]. The discrepancy in insulin resistance between these two indicates other factors that influence the mechanism of insulin resistance in obesity. One of the potential factors influencing this mechanism is intestinal dysbiosis through increased intestinal permeability [4].

Increased intestinal permeability, which is directly correlated with intestinal dysbiosis, may trigger chronic inflammation and insulin resistance through metabolic endotoxemia [4, 5]. Animal studies have shown an association between intestinal permeability and insulin resistance in obesity; however, human studies have been inconsistent [6–8]. Increased intestinal fatty acid-binding protein (I-FABP) in plasma reflects enterocyte loss and is inversely proportional to the degree of intestinal villi atrophy [9, 10]. This marker is suitable for obesity, which has rapid enterocyte turnover and shortened intestinal villi [6]. It has also been reported as an intestinal permeability marker in inflammatory and metabolic diseases, including T2DM [11–15]. Nonetheless, the published paper regarding its use in obesity-related T2DM is still rare and limited to Caucasians only [8]. Increased I-FABP level in people with obesity who exerted chronic hyperglycemia has been reported. However, their study only included severely obese patients who were candidates for bariatric surgery (body mass index [BMI] >40 kg/m^2). They also used hemoglobin A1c [HbA1c] $>6\%$ as a cut-off for chronic hyperglycemia, which was in the range for prediabetes, despite some of the patients having long-standing diabetes and using medication [8].

To our knowledge, the available data were limited to Caucasians. Thus, this is the first study that investigated intestinal permeability in people with a lower degree of obesity in relation to their diabetes status in Asian population. Asians and Caucasians differ in terms of defining obesity according to the BMI cut-off since Asians have a higher body fat percentage and more visceral fat despite having relatively similar BMI [16, 17]. This study aims to investigate the association of I-FABP level, as an intestinal permeability marker, with obesity-related T2DM in Indonesia.

Materials and methods

Ethics statement

This research has been approved by the Health Research Ethics Committee, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo Hospital (FMUI-RSCM) no. KET-480/UN2.F1/ETIK/PPM.00.02/2021, which was granted on 19th May 2021. All patients provided their written informed consent.

Participants

This was a cross-sectional study using secondary data from our larger study on gut microbiota profile in various spectrum of dysglycemia patients that has not been published yet. The study recruited participants with obesity based on BMI for Asia-Pacific population (BMI ≥25 kg/m2) aged 18 to 60 years old in FMUI-RSCM from July 2018 to August 2019. The medical history taken from interview and questionnaires as well as measurements from initial visit were used as baseline data. Participants with chronic gastrointestinal disorders, severe kidney and liver disorders, autoimmune diseases, history of taking steroids, non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics in the past month, pregnant or breastfeeding, were excluded. Participants included in this study underwent laboratory tests which were further grouped into T2DM and non-T2DM. Patients' comorbidities such as hypertension and dyslipidemia were also evaluated.

Anthropometric and biochemical measurements

Data used in the present study were extracted from our larger study. As in the larger study, all participants enrolled were measured for anthropometric parameters including weight (kg), height (cm), waist circumference (cm), hip circumference (cm) and waist-hip ratio (WHR) by standardized methods. Systolic and diastolic blood pressure was measured twice using a validated device and the measurements' average was calculated. Blood samples were drawn after an overnight fasting and kept in the freezer with a temperature of -80˚C. Biochemical profiles being measured were fasting plasma glucose (FPG), HbA1c, fasting insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG).

The serum level of I-FABP was measured using the enzyme-linked immunosorbent assay (ELISA) method (R&D systems-DY3078 DuoSet ELISA for FABP2) at the Metabolic, Cardiovascular and Aging Cluster, The Indonesian Medical Education and Research Institute, Faculty of Medicine Universitas Indonesia (IMERI-FMUI). The assay ranged from 31.2 to 2,000 pg/mL.

BMI classification

Body mass index is calculated as weight (kilograms) divided by the square of the height (meters^2) [18]. According to Asia-Pacific cut-off points, [16] as also recommended by national clinical guideline on type 2 diabetes mellitus management in Indonesian adults by The Indonesian Society of Endocrinology (PERKENI), [19] the BMI classification is as follow: underweight (<18.5 kg/m^2), normal weight (18.5–22.9 kg/m^2), overweight (23–24.9 kg/m^2), obese (≥25 kg/m^2). Obese groups are further categorized into obese I (25–29.9 kg/m^2), obese II (30–34.9 kg/m^2) and obese III/morbid obese ((≥35 kg/m^2).

Type 2 diabetes mellitus grouping

Participants were grouped according to American Diabetes Association (ADA) criteria for diabetes: T2DM (FPG ≥126 mg/dL and/or HbA1c ≥6.5% and/or history of DM/diabetes treatment) or non-T2DM (FPG <126 mg/dL and HbA1c <6.5% and no history of DM/diabetes treatment) [19, 20].

Hypertension and dyslipidemia definition

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or on antihypertensive medication. Whereas, dyslipidemia was defined as

abnormal lipid metabolism indicated by the levels of TC ≥200 mg/dL, LDL-C ≥100 mg/dL, HDL-C <40 mg/dL and/or TG ≥150 mg/dL or on lipid-lowering treatment.

Statistical analysis

The data were analyzed from May to June 2021 and the statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) IBM version 28.0. Numerical data were presented as mean ± standard deviation (SD) if normally distributed or as median (minimum-maximum) if not normally distributed. The mean difference of I-FABP level between two groups was analyzed using linear regression, which was further adjusted for confounding. The correlation between I-FABP and HbA1c levels was analyzed using Spearman correlation. A comparison of I-FABP level between subjects who consumed and did not consume lipid-lowering therapy was analyzed using independent T-test. A p-value of <0.05 was considered statistically significant.

Results

We recruited 63 subjects with obesity, of which 34 subjects had T2DM. The majority of subjects were women (82.53%), aged >45 years (63.50%), obesity grade I (54.00%) and central obesity (93.70%). The T2DM group were older (p<0.001), had more hypertension (p = 0.049), lower LDL cholesterol (p = 0.013), and as predicted, higher FPG (p<0.001) and HbA1c (p<0.001) levels. (**Table 1**) In this group, 2 subjects were newly diagnosed with T2DM and had no history of antidiabetic therapy, while 70.58% (24/34) of subjects had been diagnosed with T2DM in less than 5 years and 17.65% (6/34) had T2DM for more than 10 years. Most subjects did not have any history of diabetic complications. Among them, 14 patients consumed only 1 oral anti-diabetic drugs (OAD), 12 subjects on dual OADs therapy, 2 subjects had combination of OADs and insulin therapy and 2 others did not take anti-diabetic medication.

The mean I-FABP level in the group with T2DM was higher, namely 2.82 (1.23) ng/mL vs. 1.78 (0.81) ng/mL (p<0.001; mean difference 1.033 with 95% CI 0.51–1.55). (**Fig 1**) We analyzed the mean difference between two groups using linear regression and the fitted regression model was: i-FABP = 1.787+1.034*(DM) (R^2 = 18.20%, standardized ß = 0.442, p<0.001). Other confounding factors that might interfere the result such as age and sex were analyzed. This difference was still significant even after adjustment for age difference. The fitted regression model after adjustment for age was: i-FABP = 0.470+0.757*(DM)+0.031*(age). The overall regression was statistically significant (R^2 = 22.6%, standardized ß = 0.324, p = 0.012).

We also analyzed the correlation between I-FABP and HbA1c levels using Spearman correlation to evaluate the relationship of gut permeability with hyperglycemia. The I-FABP level was positively correlated with the HbA1c level, however the association was weak (r = 0.251, p = 0.047). To see if there was an effect of lipid-lowering therapy on I-FABP level, we compared the I-FABP levels between subjects who consumed lipid-lowering therapy and those who did not. There was no significant difference found between those groups (p = 0.608).

Discussion

This study observed a higher I-FABP level among participants with obesity and T2DM in comparison to those without T2DM. This result supports the evidence on the potential role of intestinal permeability in the pathogenesis of T2DM in participants with obesity [8].

Our study supports previous studies in other countries that reported increased I-FABP, as gut integrity marker, in T2DM [12–15]. These findings suggest that increased intestinal permeability due to intestinal dysbiosis is associated with systemic inflammation and insulin

Table 1. Subject characteristics.

Variable	T2DM (n = 34)	Non-T2DM (n = 29)	P value
Age (year)	50.65 ± 6.32	41.86 ± 11.16	<**0.001**[a]
• >45 years old (%)	76.50	48.30	**0.021**[b]
Female (%)	85.30	79.30	
BMI (kg/m^2)	29.80 (25.11–42.50)	29.84 (25.73–39.10)	0.478[c]
Obesity			0.228[b]
• Grade I (%)	55.90	51.70	
• Grade II (%)	26.50	41.40	
• Grade III (%)	7.60	6.90	
Waist (cm)	94.53 ± 10.68	93.16 ± 10.43	0.612[a]
WHR	0.88 (0.80–1.03)	0.88 (0.81–1.01)	0.815[c]
Central obesity (%)	97.10	89.70	0.250[b]
Dyslipidemia (%)	52.90[#]	31.00[$]	0.08[b]
Hypertension (%)	44.10[*]	20.70[&]	**0.049**[b]
FPG (mg/dL)	130.00 (83.00–311.00)	89.00 (75.00–105.00)	<**0.001**[c]
HbA1c (%)	7.50 (4.70–13.20)	5.70 (4.70–6.40)	<**0.001**[c]
Fasting insulin (mIU/L)	11.06 ± 5.88	9.58 ± 4.11	0.262[a]
I-FABP (ng/mL)	2.82 ± 1.23	1.78 ± 0.81	<**0.001**[a]

Data presented as mean ± SD, median (min-max), or proportion (%).

T2DM: type 2 diabetes mellitus, BMI: body mass index, WHR: waist-to-hip ration, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c.

[a]: linear regression

[b]: Chi-square test

[c]: Mann-whitney test.

[#]: 29.4% subjects were on lipid-lowering therapy in T2DM group

[$]:13.8% subjects were on lipid-lowering therapy in Non-T2DM group

[*]: 26.5% subjects were on antihypertensive therapy in T2DM group

[&]: 17.2% subjects were on antihypertensive therapy in Non-T2DM group

https://doi.org/10.1371/journal.pone.0279915.t001

resistance, [6, 12, 21] potentially leading to T2DM. It has been reported that increased lipopolysaccharide (LPS) level in T2DM patients was associated with pro-inflammatory cytokines [22]. There was an increase in C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and a predominant T helper 1 (Th 1) pathway [13, 21, 22]. It has also been reported that the association of I-FABP levels with CRP, blood glucose and TG levels were still significant even after adjustment for age and BMI [15]. The impairment of intestinal integrity might provide the influx of LPS to the systemic circulation and induce metabolic endotoxemia, resulting in low-grade systemic inflammation and consequently, insulin resistance.

Interestingly, this study mean I-FABP serum level was higher than in previous studies. This might be due to the fact that all subjects in this study had obesity, and most of them also had abdominal obesity. Furthermore, this might also be influenced by the differences in genetics, race and geographical location, which contributed to different enterotypes, and types of physical activity and diet, thus resulting in the differences in intestinal permeability. Intestinal permeability is closely associated with intestinal microbiota composition [23].

Geographical location determines the dominant enterotype, where healthy Indonesians belong to enterotype II, which is dominated by the genus Prevotella. This finding is different from the dominant enterotype in other Asian countries such as Japan, China, and Taiwan, which belongs to enterotype I, and western countries which are more abundant in Bacteroides

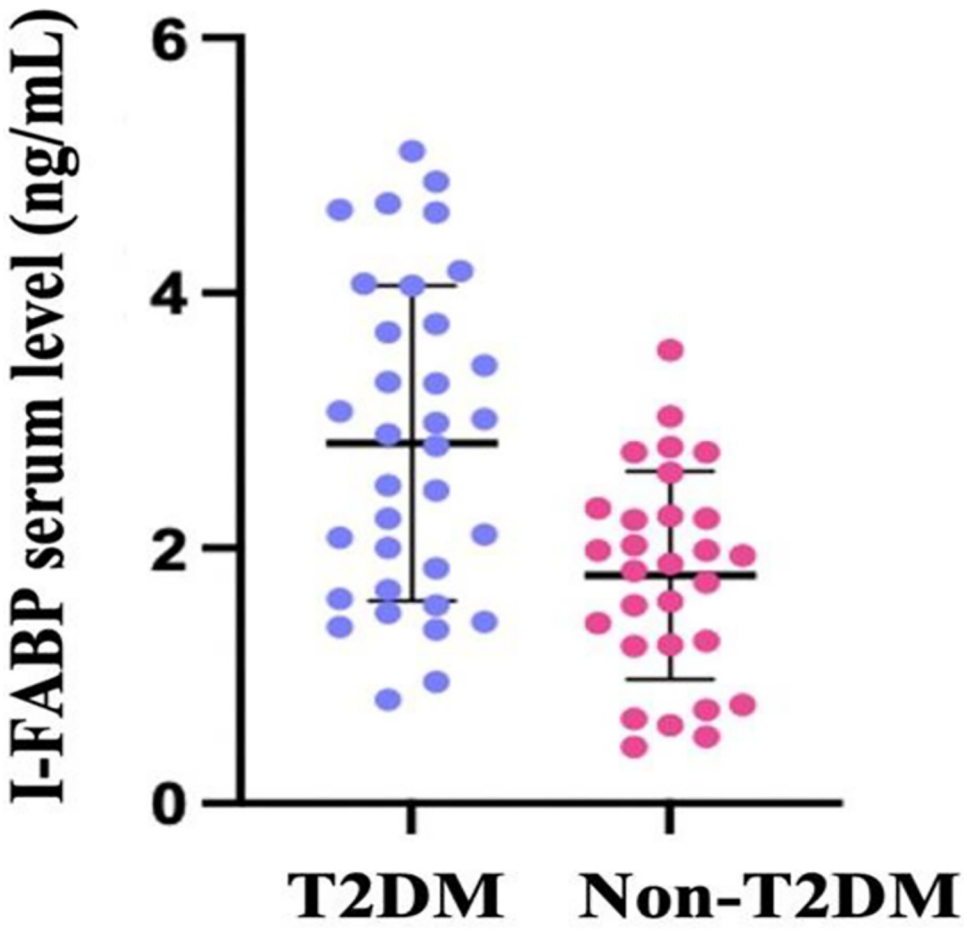

Fig 1. Serum level of I-FABP in T2DM and non-T2DM groups, were presented in dot-plot form with mean (SD).

https://doi.org/10.1371/journal.pone.0279915.g001

(enterotype III) [24]. Westernized diet, which commonly comprises high-fat, high-carbohydrate, and low-fiber, is correlated with increases in Firmicutes and Proteobacteria. In contrast, an increase in the Firmicutes/Bacteroidetes ratio is associated with higher BMI, intestinal permeability, and insulin resistance [6, 25].

The frequency of physical activity also affects microbiota composition. Several studies reported that daily physical activity increased the diversity of Firmicutes bacteria, which then improves intestinal permeability by producing tight-junction protein [23]. In this study and previous studies, there was no analysis of the diet and activity patterns of the subjects. However, the results of this study were in line with changes in dietary patterns and lifestyles in Indonesia, especially in urban areas, which adopt a Westernized diet and a sedentary lifestyle, thereby potentially changing the composition of the gut microbiota and increasing intestinal permeability in people with obesity.

Other factors that contribute to variations in intestinal permeability are age and sex [23]. Intestinal dysbiosis is higher in older age, which is in line with the results of research by Rahayu et al who reported a reduced gut microbiota diversity and an increase in the proportion of Enterobacteriaceae, Coliform and Escherichia coli in the elderly population in Indonesia [26]. From multivariate analysis, the I-FABP levels were independently associated with either T2DM or age. This is consistent with the previous study in which I-FABP levels were associated with T2DM, even after controlling the confounding variables like age and BMI [12].

Intestinal-FABP is specifically synthesized in enterocytes and released to systemic circulation whenever enterocyte apoptosis/necrosis occurs [10]. Increased I-FABP serum levels indicate greater gut integrity loss in T2DM patients. This is in line with some intestinal mucosal changes in obesity and T2DM, including increased small intestinal enterocyte mass as well as the length of crypt and villi due to faster enterocyte turnover [6, 15]. However, I-FABP serum levels were correlated with duodenal *FABP2* gene expression thus, there was a possibility that high I-FABP serum levels were due to its increased pool production rather than increased enterocyte loss [15]. It has been previously postulated that the I-FABP serum levels increase in people with morbid obesity and chronic hyperglycemia representing higher enterocyte loss through the role of *FABP2* gene expression [8]. Meanwhile, others have reported that the production of I-FABP was determined partly by duodenal *FABP2* gene expression, but its serum concentration was affected by BMI and insulin resistance. It is suggested that there was an increase in enterocyte loss in the presence of insulin resistance, particularly in uncontrolled diabetes [15]. In contrast to the previous study, [27] our study observed a weak positive correlation between I-FABP and HbA1c levels, which supports the potential effect of uncontrolled diabetes on enterocyte loss. In the opposite direction, our finding also aligns with the hypothesis that metabolic endotoxemia triggers insulin resistance and hyperglycemia through the disrupted intestinal barrier [4]. Another interesting view is that long-standing hyperglycemia leads to disrupted gut epithelial integrity [28]. Therefore, further evaluation should be addressed to evaluate the causal relationship between those two.

Despite being the first study to assess the association of I-FABP level, as an intestinal permeability marker, with obesity-related T2DM in Indonesia, there were some limitations in the study. Firstly, this is a cross-sectional study, thus no causal inference can be generated. Secondly, it did not analyze the effect of diet, physical activity and antidiabetic drugs. We also did not assess the inflammation markers, as previous studies have showed increased I-FABP level was related to systemic inflammation. Furthermore, as most participants were women, whether this association found in men needs further study.

In conclusion, the findings of this study support an association between intestinal permeability and obesity-related T2DM, which highlights the involvement of the intestinal barrier in the pathophysiology of T2DM in obesity. This indicates the potential role of using one of the intestinal permeability markers to predict the risk of developing T2DM in obesity in the future and is likely to help classifying them as MHO and MUO. Furthermore, improving the intestinal barrier is promising to be one of the treatment strategies to prevent obesity-related complications.

Supporting information

S1 Data.
(XLSX)

Author Contributions

Conceptualization: Dicky L. Tahapary, Atikah I. Fatya, Cicilia Marcella, Pradana Soewondo, Dyah Purnamasari.

Data curation: Dicky L. Tahapary, Atikah I. Fatya, Farid Kurniawan, Cicilia Marcella.

Formal analysis: Atikah I. Fatya, Farid Kurniawan, Ikhwan Rinaldi, Dyah Purnamasari.

Funding acquisition: Dicky L. Tahapary, Em Yunir, Pradana Soewondo, Dyah Purnamasari.

Methodology: Dicky L. Tahapary, Atikah I. Fatya, Farid Kurniawan, Cicilia Marcella, Pradana Soewondo, Dyah Purnamasari.

Supervision: Dicky L. Tahapary.

Writing – original draft: Dicky L. Tahapary, Atikah I. Fatya, Cicilia Marcella.

Writing – review & editing: Dicky L. Tahapary, Atikah I. Fatya, Farid Kurniawan, Cicilia Marcella, Tri J. E. Tarigan, Dante S. Harbuwono, Em Yunir, Pradana Soewondo, Dyah Purnamasari.

References

1. Bluher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol. 2019; 15(5):288–98. https://doi.org/10.1038/s41574-019-0176-8 PMID: 30814686
2. Bluher M. Metabolically Healthy Obesity. Endocr Rev. 2020; 41(3):405–20.
3. Fingeret M, Marques-Vidal P, Vollenweider P. Incidence of type 2 diabetes, hypertension, and dyslipidemia in metabolically healthy obese and non-obese. Nutr Metab Cardiovasc Dis. 2018; 28(10):1036–44. https://doi.org/10.1016/j.numecd.2018.06.011 PMID: 30139688
4. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007; 56(7):1761–72. https://doi.org/10.2337/db06-1491 PMID: 17456850
5. Allam-Ndoul B, Castonguay-Paradis S, Veilleux A. Gut Microbiota and Intestinal Trans-Epithelial Permeability. Int J Mol Sci. 2020; 21(17):1–14. https://doi.org/10.3390/ijms21176402 PMID: 32899147
6. Nagpal R, Newman TM, Wang S, Jain S, Lovato JF, Yadav H. Obesity-Linked Gut Microbiome Dysbiosis Associated with Derangements in Gut Permeability and Intestinal Cellular Homeostasis Independent of Diet. J Diabetes Res. 2018; 2018:1–9. https://doi.org/10.1155/2018/3462092 PMID: 30250849
7. Genser L, Aguanno D, Soula HA, Dong L, Trystram L, Assmann K, et al. Increased jejunal permeability in human obesity is revealed by a lipid challenge and is linked to inflammation and type 2 diabetes. J Pathol. 2018; 246(2):217–30. https://doi.org/10.1002/path.5134 PMID: 29984492
8. Verdam FJ, Greve JW, Roosta S, van Eijk H, Bouvy N, Buurman WA, et al. Small intestinal alterations in severely obese hyperglycemic subjects. J Clin Endocrinol Metab. 2011; 96(2):E379–83. https://doi.org/10.1210/jc.2010-1333 PMID: 21084402
9. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009; 9 (11):799–809. https://doi.org/10.1038/nri2653 PMID: 19855405
10. Vreugdenhil AC, Wolters VM, Adriaanse MP, Neucker AMVD, Bijnen AAV, Houwen R, et al. Additional value of serum I-FABP levels for evaluating celiac disease activity in children. Scandinavian Journal of Gastroenterology. 2011; 46:1435–41. https://doi.org/10.3109/00365521.2011.627447 PMID: 22029621
11. Sarikaya M, Ergul B, Dogan Z, Filik L, Can M, Arslan L. Intestinal fatty acid binding protein (I-FABP) as a promising test for Crohn's disease: a preliminary study. Clin Lab. 2015; 61(1–2):87–91. https://doi.org/10.7754/clin.lab.2014.140518 PMID: 25807642
12. Cox AJ, Zhang P, Bowden DW, Devereaux B, Davoren PM, Cripps AW, et al. Increased intestinal permeability as a risk factor for type 2 diabetes. Diabetes Metab. 2017; 43(2):163–6. https://doi.org/10.1016/j.diabet.2016.09.004 PMID: 27745826
13. Hoffmanova I, Sanchez D, Habova V, Andel M, Tuckova L, Tlaskalova-Hogenova H. Serological markers of enterocyte damage and apoptosis in patients with celiac disease, autoimmune diabetes mellitus and diabetes mellitus type 2. Physiol Res. 2015; 64(4):537–46. https://doi.org/10.33549/physiolres.932916 PMID: 25470519
14. Yuan JH, Xie QS, Chen GC, Huang CL, Yu T, Chen QK, et al. Impaired intestinal barrier function in type 2 diabetic patients measured by serum LPS, Zonulin, and IFABP. J Diabetes Complications. 2021; 35 (2):1–10. https://doi.org/10.1016/j.jdiacomp.2020.107766 PMID: 33168395
15. Lalande C, Drouin-Chartier JP, Tremblay AJ, Couture P, Veilleux A. Plasma biomarkers of small intestine adaptations in obesity-related metabolic alterations. Diabetol Metab Syndr. 2020; 12:1–11.
16. WHO. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363:157–63. https://doi.org/10.1016/S0140-6736(03)15268-3 PMID: 14726171

17. Guricci S, Hartriyanti Y, Hautvast J, Deurenberg P. Relationship between body fat and body mass index: differences between Indonesians and Dutch Caucasians. Eur J Clin Nutr. 1998; 52:779–83. https://doi.org/10.1038/sj.ejcn.1600637 PMID: 9846588

18. NHLBI. The practical guide identification, evaluation, and treatment of overweight and obesity in adults. National Heart, Lung, and Blood Institute; 2000.

19. PERKENI. Pedoman pengelolaan dan pencegahan diabetes mellitus tipe 2 dewasa di Indonesia 2019. Soelistijo SA, Lindarto D, Decroli E, Permana H, Sucipto KW, Kusnadi Y, et al., editors: PB Perkeni; 2019.

20. American Diabetes A. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021; 44(Suppl 1):S15–S33. https://doi.org/10.2337/dc21-S002 PMID: 33298413

21. Horton F, Wright J, Smith L, Hinton P, Robertson M. Increased intestinal permeability to oral chromium (51Cr)-EDTA in human Type 2 diabetes. Diabetic Medicine. 2014; 31:559–63. https://doi.org/10.1111/dme.12360 PMID: 24236770

22. Jayashree B, Bibin YS, Prabhu D, Shanthirani CS, Gokulakrishnan K, Lakshmi BS, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. Mol Cell Biochem. 2014; 388(1–2):203–10. https://doi.org/10.1007/s11010-013-1911-4 PMID: 24347174

23. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms. 2019; 7(1):1–22. https://doi.org/10.3390/microorganisms7010014 PMID: 30634578

24. Mobeen F, Sharma V, Tulika P. Enterotype Variations of the Healthy Human Gut Microbiome in Different Geographical Regions. Bioinformation. 2018; 14(9):560–73. https://doi.org/10.6026/97320630014560 PMID: 31223215

25. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA. 2010; 107(33):14691–6. https://doi.org/10.1073/pnas.1005963107 PMID: 20679230

26. Rahayu ES, Utami T, Mariyatun M, Hasan PN, Kamil RZ, Setyawan RH, et al. Gut microbiota profile in healthy Indonesians. World J Gastroenterol. 2019; 25(12):1478–91. https://doi.org/10.3748/wjg.v25.i12.1478 PMID: 30948911

27. Tsai IT, Wu CC, Hung WC, Lee TL, Hsuan CF, Wei CT, et al. FABP1 and FABP2 as markers of diabetic nephropathy. Int J Med Sci. 2020; 17(15):2338–45. https://doi.org/10.7150/ijms.49078 PMID: 32922199

28. Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, Blacher E, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science. 2018; 359(6382):1376–83. https://doi.org/10.1126/science.aar3318 PMID: 29519916