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Original Research Article

The Association Between Intake of Saturated, Monounsaturated, and Polyunsaturated Fatty Acids with Bifidobacterium Abundance Among Obese Adults Without Metabolic Syndrome

Euodia Sinthika[1], Ninik Rustanti[1*], Endang Sri Lestari[2]

[1]*Department of Nutrition Sciences, Faculty of Medicine, Universitas Diponegoro, Indonesia*
[2]*Department of Clinical Microbiology, Faculty of Medicine, Universitas Diponegoro, Indonesia*

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Abstract

Background: *Bifidobacterium* is a key gut microbe that contributes to host metabolism, immunity, and intestinal integrity through SCFA production. Dietary fats are known to modulate gut microbiota, but evidence on the effects of specific fat types—SFA, MUFA, PUFA—on *Bifidobacterium* in obese adults without metabolic syndrome remains limited.

Objectives: To examine the association between intake of dietary fat types and the abundance of *Bifidobacterium* among obese adults without metabolic syndrome**.**

Methods: A cross-sectional study was conducted in Semarang, Indonesia, involving 60 obese adults (BMI ≥ 25 kg/m²) without metabolic syndrome. Dietary intake was assessed using a validated SQ-FFQ, and *Bifidobacterium* abundance was quantified using qPCR from fecal samples. Correlation and multivariate linear regression - adjusted for age, sex, and energy intake - were used to assess associations between variables.

Results: Saturated fat intake was moderately and negatively correlated with *Bifidobacterium* levels ($r = –0.464$; $p < 0.001$), while total fat intake also showed a statistically significant, but weaker, negative correlation ($r = –0.346$; $p = 0.007$). PUFA intake showed a weak but statistically significant positive correlation ($r = 0.269$; $p = 0.037$), whereas MUFA intake was not significantly associated. Multivariate analysis identified SFA as an independent negative predictor of *Bifidobacterium* abundance.

Conclusion: High intake of saturated fat is associated with decreased *Bifidobacterium* levels even in obese adults without metabolic syndrome, whereas PUFA may exert modest protective effects. These findings suggest that the type of dietary fat, rather than its quantity, plays a key role in modulating gut microbiota composition.

Keywords: *Saturated Fats; MUFA; PUFA; Bifidobacterium; Obesity*
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INTRODUCTION

The human gastrointestinal system has a complex and diverse microbial population referred to as the gut microbiota, which is crucial for sustaining host health.[1] These microbes participate in the metabolism of nutrients, regulation of the immunity, and maintenance of the intestinal barrier.[2–4] Among the beneficial bacterial genera is *Bifidobacterium* which has antimicrobial effects, short-chain fatty acids (SCFA) production, and pathogen inhibition.[5] The evidence shows that the reduction of *Bifidobacterium* abundance is linked to obesity and metabolic dysfunction.[6]

Obesity is a global health challenge linked to chronic low-grade inflammation, insulin resistance, and increased risk of non-communicable diseases.[6] However, a subset of people with obesity who are metabolically healthy—that is, they have normal blood pressure, glucose, and lipid levels—these individuals are often referred to in the literature as metabolically healthy obese, although the definition variesare.[7]

*Corresponding author:
E-mail: *ninik.rustanti@fk.undip.ac.id*
(Ninik Rustanti)

The gut microbiota composition in obese adults without metabolic syndrome may differ from that of metabolically unhealthy obese counterparts, but this remains an understudied area.[7,8] Studying obese adults without metabolic syndrome reveals patterns associated with adiposity that appear early in the progression of the condition without the accompanying metabolic complications.[7,9]

Diet is one of the most powerful modulators of the gut microbiota.[10,11] While the effects of dietary fiber and prebiotics on microbial diversity and function have been extensively studied, the role of dietary fat subtypes—saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)—is less clearly defined.[12,13] SFAs, commonly found in animal fats and processed foods, have been associated with a reduction in beneficial gut bacteria and promotion of endotoxemia in animal studies.[13,14] In contrast, unsaturated fats, including MUFAs and omega-3 PUFAs, are associated with anti-inflammatory effects and improved gut microbiota composition.[15,16]

Recent studies suggest that the quantity and type of dietary fat intake can differentially influence the composition of the gut microbiota.[17,18] For example, omega-3 PUFA supplementation has been shown to increase *Bifidobacterium* levels and ameliorate inflammation in a mouse model of metabolic syndrome.[19] Similarly, in humans a diet rich in MUFAs was associated with increased gut microbial diversity and a higher abundance of SCFA-producing bacteria.[20] Despite this growing body of evidence, studies specifically examining *Bifidobacterium* in relation to distinct dietary fat subtypes—particularly in metabolically healthy obese populations—remain scarce.[13]

Furthermore, most existing research on diet–microbiota interactions relies on 16S rRNA sequencing to infer relative abundance.[21] This technique offers valuable ecological insights but fails to provide exact measurement of particular taxa.[22] Few studies have employed quantitative PCR (qPCR) methods with standard curves to measure *Bifidobacterium* in absolute terms, despite the fact that such measures can provide more accurate microbial assessments.[23] This methodological constraint leads to discrepancies in the reported relationships between dietary fat and microbial abundance.[22]

The lack of studies investigating the link between the composition of dietary fat and the abundance of *Bifidobacterium* in metabolically healthy obese individuals is a crucial gap in the literature. Considering the importance of *Bifidobacterium* in the maintenance of gut balance and the prevention of inflammation, knowing the impact of various fat types on its abundance among metabolically healthy obese individuals may help in the formulation of strategies designed to mitigate metabolic decline with age. This is why the focus of this study is to assess how the intake of SFA, MUFA, and PUFA relates to the abundance of *Bifidobacterium* in the microbiome of obese adults who do not have metabolic syndrome.

MATERIALS AND METHODS

Study Design and Participants

A total of 60 participants (40 females and 20 males) aged 19–44 years with a body mass index (BMI) > 25 kg/m², based on WHO's Asia-Pacific BMI cutoff for Asian populations, were included in this cross-sectional study, which was conducted in Semarang city, Indonesia from October 2024 to December 2024. Participants were screened through an initial interview and health history questionnaire conducted by trained enumerators to verify inclusion and exclusion criteria. Participants were eligible if they were not pregnant or breastfeeding, had not consumed dietary supplements, prebiotics, or probiotics in the past month, had no diagnosed metabolic syndrome or chronic diseases such as coronary heart disease, and were not undergoing a weight loss diet. Participants were excluded if they experienced acute diarrhea one day before sample collection or consumed supplements, prebiotics, or probiotics one to three days prior to the study. Written informed consent was obtained from all participants.

Anthropometric and Dietary Assessment

Anthropometric measurements were conducted using a GEA Medical stadiometer and a bioelectrical impedance analyzer (BIA, TANITA DC-360, Tokyo, Japan). Body mass index (BMI) values were directly obtained from the BIA analysis. Dietary intake was assessed through face-to-face interviews using a semi-quantitative food frequency questionnaire (SQ-FFQ), which evaluated habitual intake during the past month, including sources of SFA, MUFA, and PUFA. Nutrient intake data from the SQ-FFQ were analyzed using Nutrisurvey software with the Indonesian food composition database. The SQ-FFQ used in this study was adapted from a previously validated instrument developed for use in the Indonesian adult population.[24]

Fecal Sample Collection and DNA Extraction

Participants collected fecal samples in the morning and submitted them to the laboratory within three hours. Each sample (100 mg) was mixed with 1 mL of DNA/RNA stabilization solution and stored at –20°C until processing. DNA was extracted using the Favorgen® DNA Stool Isolation Kit according to the manufacturer's protocol. DNA concentration and purity were measured using a NanoDrop spectrophotometer, and only samples with A260/A280 purity ratios between 1.8 and 2.0 were included for further analysis.

Quantification of *Bifidobacterium* by qPCR

Quantitative real-time PCR (qPCR) was conducted to determine the abundance of *Bifidobacterium* in stool samples. The amplification used SMOBIO® Fast qPCR Master Mix and genus-specific[25] forward primer 5′-GGGTGGTAATGCCGGATG-3′ and reverse primer 5′-TAAGCGATGGACTTTCACACC-3′. Each reaction was run in duplicate. A standard curve was generated using seven 10-fold serial dilutions of DNA extracted from pure *Bifidobacterium* culture. Ct values obtained from participant samples were converted to log CFU/g of feces using the linear regression equation: $y = –0.2801x + 12.332$, with a coefficient of determination $R^2 = 0.9938$.

Table 1. Participant Characteristic and *Bifidobacterium* Abundance

Characteristics	N	Mean	SD	Min	Max
Age (yr)	60	25,72	±5,89	19	45
Body Weight (kg)	60	77,6	±17,39	56	163,2
Height (cm)	60	160,75	±8,87	144,3	178
BMI (kg/m$^{2)}$	60	29,93	±5,58	25,1	60,7
Waist Circumference (cm)	60	94,1	±13.1	79	161
Bifidobacterium (log CFU/g)	60	9,08	±0.99	6,09	10,71

Table 2. Dietary Intake

Intake	Mean	SD	Min	Max
Energy (kcal)	1674,77	±366,80	969,3	2607,3
Carbohydrate (g)	168,59	±38,26	88	281,8
Protein (g)	74,55	±24,46	24,8	152,54
Total Fats (g)	73,45	±21,66	29,9	141,93
a. Saturated Fats (g)	39,50	±13,62	14	71,1
b. MUFA(g)	17,15	±7,28	4	39,55
c. PUFA(g)	17,11	±5,87	3.3	28,50
Total Fiber(g)	9,29	±3,67	3,5	19,7
a. Soluble Fiber(g)	4,42	±1,90	1,1	9,7
b. Insoluble Fiber(g)	4,8	±2,02	1,3	10,0

Statistical Analysis

All statistical analyses were performed using SPSS version 25. Descriptive statistics were used to summarize participant characteristics and dietary intake. Data distribution was tested using the Kolmogorov-Smirnov test. Pearson or Spearman correlation and linear regression analyses were used to assess the association between dietary fat intake (SFA, MUFA, PUFA) and *Bifidobacterium* abundance. A p-value less than 0.05 was considered statistically significant.

Ethical clearance

The study protocol (Ethical Clearance number 457/EC/KEPK/FK-UNDIP/VIII/2024) was approved by the Ethics Committee of the Faculty of Medicine, Universitas Diponegoro.

RESULTS

Based on the analysis of participant characteristics in Table 1, the study participants were predominantly young adults (mean age 25.72 ± 5.89 years), with all individuals classified as obese. The mean BMI of 29.93 ± 5.58 kg/m² places most subjects in the Class I obesity category. As shown in Table 1 the mean absolute abundance of *Bifidobacterium* was 9.08 ± 0.99 log CFU/g, which aligns with levels considered adequate for gut homeostasis in healthy individuals. According to previous clinical microbiota benchmarks, *Bifidobacterium* populations >8 log CFU/g are generally indicative of a eubiotic (balanced) intestinal profile.[26]

The mean daily energy intake of participants in Table 2 was 1674.77 ± 366.80 kcal, carbohydrate (mean 197.6 g/day) and protein intake (mean 55.5 g/day) which is below Indonesian Recommended Dietary Allowance (AKG 2019) according to sex and age [27]. The results showed that mean energy intake represented approximately 76% of the recommended level, while protein and carbohydrate intakes corresponded to 85% and 54%, respectively, of the AKG

Despite the low energy intake, total fat consumption averaged 73.45 ± 21.66 g/day, exceeding the AKG, and contributing 39–45% of total energy—surpassing WHO's recommended 20–35%. Saturated fat intake was particularly high, averaging 39.5 g/day, which corresponds to approximately 179% of the AKG limit for males (22.2 g/day) and 198% of the AKG limit for females (20 g/day). In contrast, MUFA (17.15 ± 7.28 g/day) and PUFA (17.11 ± 5.87 g/day) were within generally acceptable ranges. WHO recommends 15–20% of energy from MUFA and 6–11% from PUFA, which corresponds to approximately 33–44 g MUFA and 13–24 g PUFA/day on a 2000 kcal diet.[28]

Regarding fiber, the total fiber intake was only 9.29 ± 3.67 g/day, far below the AKG recommendations of 37 g/day for males and 32 g/day for females. Soluble fiber was 4.42 ± 1.90 g/day and insoluble fiber was 4.80 ± 2.00 g/day, both well below the ideal ranges of ~8–10 g/day (soluble) and 24–27 g/day (insoluble). This suggests poor fiber intake both in type and quantity, which may negatively affect gut microbiota diversity, particularly in the context of this study focusing on *Bifidobacterium.*[29] These findings indicate a pattern of overall low energy, carbohydrate, protein, and fiber intake, paired with disproportionately high total and saturated fat consumption, suggesting an imbalanced dietary pattern among participants.[17]

Table 3 demonstrates a moderate-to-strong inverse relationship between saturated fat intake and *Bifidobacterium* ($r = –0.464$, $p < 0.001$), as well as total fat intake ($r = –0.346$, $p = 0.007$). These findings are consistent with previous literature indicating that high SFA disrupts microbial equilibrium and lowers colonization of beneficial bacteria[30]. As observed in Table 3, PUFA intake showed a weak but significant positive correlation ($r = 0.269$, $p = 0.037$), aligning with omega-3/6 literature indicating anti-inflammatory and microbiota-supportive properties.[14,19] Additionally, soluble fiber intake (mean 4.42 ± 1.90 g/day) was still below the RDI, which recommends 5–10 g/day of soluble fiber. Despite the shortfall, soluble fiber still exhibited a

positive correlation with *Bifidobacterium* ($r = 0.509$, $p < 0.001$), confirming its key role as a fermentable substrate for gut bacteria.[29,31]

Multivariate linear regression in Table 4 confirmed that saturated fat intake are significant negative predictors of *Bifidobacterium* abundance. Saturated fat ($B = –0.024$, $p = 0.030$) remained a consistent dietary predictor of reduced *Bifidobacterium*, even when controlling for other macronutrients. This finding supports the hypothesis that high saturated fat intake, even in energy-deficient or metabolically healthy individuals, contributes to early gut microbial shifts, potentially preceding metabolic complications.[32,33]

DISCUSSION

This study investigated the association between dietary intake of SFA, MUFA, and PUFA with the abundance of *Bifidobacterium* in the gut microbiota of metabolically healthy obese adults. Even though the participants did not present with clinical signs of metabolic syndrome, their diet showed signs of potential microbial dysregulation concerning *Bifidobacterium* abundance. This highlights that microbiota alterations may occur prior to the clinical onset of metabolic disorders.

Saturated fat consumption was linked detrimentally and significantly with *Bifidobacterium* abundance.[18,34] These results support prior studies that propose high SFA-containing diets can promote detrimental changes in gut microbiota that decreases *Bifidobacterium* and increases inflammatory taxa such as *Bilophila wadsworthia*.[35,36] Mechanistically, saturated fat may cause these changes due to increased bile acid release, oxidative damage, and epithelial tissue injury, all of which create a habitat that is less favorable for colonization by commensal microbes.[17] Additionally, the average intake of saturated fat in our population exceeded AKG recommendations, indicating that even in individuals with preserved metabolic parameters, a high SFA intake could contribute to early dysbiosis.[28,37] Saturated fat also stood out as an independent negative predictor in the regression model, reinforcing its critical impact on gut microbial homeostasis.[18]

Additional studies provide further support for the observed association between high saturated fat intake and microbial imbalance[38–40]. In animal models, diets rich in saturated fats have been shown to reduce the abundance of *Bifidobacterium* while increasing the presence of lipopolysaccharide (LPS)-producing gram-negative bacteria. This shift leads to endotoxemia and low-grade systemic inflammation.[32,33] Inflammation, in turn, can impair the gut mucosal environment, further inhibiting colonization of commensal genera such as *Bifidobacterium*.[41] These findings suggest that dietary saturated fats may not only reduce microbial diversity but may also directly impair protective microbial populations.

The source of saturated fats may also influence microbial outcomes.[42,43] Diets dominated by animal-based saturated fats appear more detrimental to the microbiota than those with plant-based sources.[44] In the present study, no differentiation was made between fat

Table 3. Correlation Between Fat Intake and *Bifidobacterium* Abundance

Genera	Dietary Intake	*p Value*	r
Bifidobacterium	Energy	0,144	-0,191*
	Carbohydrate	0,792	-0,035*
	Protein	0,986	0,002*
	Total Fats	0,007	-0,346*
	Saturated fats	<0,001	-0,464**
	MUFA	0,085	-0,224*
	PUFA	0,037	0,269
	Total fibers	<0,001	0,470*
	Solube fiber	<0,001	0,509*
	Unsolube Fiber	0,006	0,353*

*p<0,05: Pearson Test, **p<0,05: Rank-Spearman Test

Table 4. Regression and Risk Markers

Genera	Variable	Coefisien B (unstandardized)	*p Value
Bifidobacterium	Age	-0,016	0,144
	BMI	-0,041	0,583
	Waist Circumference	-0,001	0,892
	Energy	0,001	0,079
	Carbohydrate	0,003	0,196
	Protein	0,001	0,840
	Total Fats	0,013	0,170
	Saturated fats	-0,024	0,030
	MUFA	-0,018	0,349
	PUFA	0,031	0,176
	Total fibers	0,280	0,373
	Solube fibes	-0,225	0,484
	Unsolube Fibers	-0,237	0,440

*Significant *p value*<0,05

sources. Therefore, future analyses should evaluate whether specific food categories—such as processed meats, dairy fats, or tropical oils—exert distinct effects on *Bifidobacterium*.

In comparison, PUFA consumption showed a weak but significant positive correlation with *Bifidobacterium* abundance. Even though the regression model did not classify PUFA as a significant independent predictor, its association appears to strengthen the argument that PUFAs, and especially omega-3 fatty acids, reinforce the diversity of gut microbes and the proliferations of beneficial genera[19]. Omega-3 fatty acids, a major subgroup of PUFAs, have demonstrated potential in maintaining epithelial tight junction integrity and reducing oxidative stress.[19,45] It has been demonstrated that PUFAs promote anti-inflammatory responses and enhance the activity of SCFA-producing bacteria, which may include *Bifidobacterium*[45,46]. Nonetheless, the impact of PUFAs is likely modified by other factors such as the quantity and quality of the fats—omega-3 vs omega-6—as well as interactions with other dietary components like fiber.[47] In clinical settings, omega-3 supplementation has been associated with increased fecal *Bifidobacterium* abundance and elevated short-chain fatty acid concentrations.[48] These changes are linked to improved markers of gut barrier function and systemic metabolic health. The potential mechanisms include anti-inflammatory effects and modulation of gut barrier function, which may create a more favorable environment for beneficial microbes. Although the positive relationship between PUFA and *Bifidobacterium* was modest in this study, it may be amplified under conditions of increased PUFA intake or when combined with adequate fiber. PUFAs and dietary fiber may act synergistically by supplying substrates and modulating luminal conditions that favor beneficial microbes[49,50]. This hypothesis remains to be tested in future trials.

Monounsaturated fat intake, although within the recommended range, did not show a significant correlation with *Bifidobacterium* levels. which was also observed in other studies. The findings most recent are consistent with other publications that reported either no or inconsistent effect of MUFAs on gut microbiota composition.[13] The discrepancy in the impact of MUFAs could be attributed to the fat's origin: plant-based sources, like olive oil, are rich in bioactive compounds while animal fats can also contain SFA.[44] Without specific dietary pattern data, the effects of MUFA could not be separated based on food sources, the lack of association may represent an erosion of potentially positive impacts from MUFA-rich foods**.**

The absence of a significant association between MUFA and *Bifidobacterium* may reflect the complexity of MUFA interactions with the gut ecosystem. MUFAs, particularly oleic acid, have been associated with anti-inflammatory properties and improved metabolic markers.[51] However, their effects on microbial composition are often subtle and context-dependent. In some interventions, olive oil–based MUFA diets have increased *Bifidobacterium* and *Lactobacillus* levels.[20] In other cases, MUFA effects were negligible. These discrepancies may be due to variations in phenolic compounds, fermentation status, or interactions with other macronutrients.[52]

The primary microbial outcome of this study was the absolute abundance of *Bifidobacterium*. This genus is recognized for its beneficial effects on intestinal health. *Bifidobacterium* plays a role in producing acetate, inhibiting pathogens, supporting mucosal immunity, and maintaining the integrity of the gut barrier.[5,29] High levels of *Bifidobacterium* have been associated with metabolic health, while low levels have been linked to dysbiosis. The mean *Bifidobacterium* abundance observed in this study was 9.08 ± 0.99 log CFU/g, which falls within the range considered adequate for eubiosis. However, approximately 20% of participants had levels below 8 log CFU/g. This threshold has been used in clinical studies as an indicator of early dysbiosis, particularly in populations with chronic inflammatory conditions.[26,30]

The use of quantitative PCR with a standard curve allowed for precise estimation of bacterial counts. This method provides higher accuracy than relative abundance estimates derived from 16S rRNA sequencing.[25,34] Expressing results in log CFU/g offers clinically relevant insights and facilitates comparison across studies.[23] The use of genus-specific primers targeting Bifidobacterium ensured specificity, which strengthens the validity of the findings.

Taken together, the results suggest that saturated fat intake is negatively associated with Bifidobacterium abundance, while those with greater PUFA consumption generally showed a pattern of higher *Bifidobacterium* abundance. MUFA intake does not appear to have a strong or consistent relationship with *Bifidobacterium* in this context. These observations reinforce the idea that different types of dietary fat may influence gut microbiota composition in distinct ways.[17,44] Even in individuals who are metabolically healthy, dietary fat composition can influence key microbial populations.

The findings of this study highlight an important principle in nutritional microbiology. Not all dietary fats exert equal effects on the gut microbiota[13]. The molecular structure, food matrix, and co-consumed nutrients all influence how fats interact with microbial populations. Saturated fats, especially from animal sources, tend to decrease *Bifidobacterium* levels.[17,44] PUFAs, particularly omega-3 fatty acids, may promote their growth.[19] MUFAs show inconsistent effects, possibly due to source variation and dietary context.[15]

Importantly, this study focused exclusively on individuals without metabolic syndrome. This design allows for clearer interpretation of diet–microbiota relationships without interference from hyperglycemia, insulin resistance, or dyslipidemia. The results suggest that even in a metabolically "healthy" state, the quality of dietary fat intake can influence gut microbial ecology. Early reductions in *Bifidobacterium* may precede clinical signs of metabolic dysfunction.[53] Therefore, maintaining appropriate fat balance may serve as a strategy to preserve microbiota health and delay metabolic complications.

In public health nutrition, these findings support existing recommendations to limit saturated fat intake and increase consumption of healthy unsaturated fats. However, the cross-sectional nature of this study limits the ability to establish causality in the observed

associations. Moreover, individual variability in baseline gut microbiota composition may have influenced the microbial response to dietary fat intake. Despite these limitations, interventions targeting fat types may improve microbial profiles before metabolic disease develops. Future studies should investigate whether lowering saturated fat intake can positively modulate *Bifidobacterium* levels and improve host metabolic outcomes. Such trials would clarify the therapeutic potential of dietary fat modulation in microbiota-focused prevention strategies.

CONCLUSION

A high intake of saturated fat leads to a reduction in *Bifidobacterium* abundance, even among metabolically healthy obese individuals. PUFAs appear to offer a mild protective effect, while MUFAs show little to no influence. These findings underscore the importance of dietary fat quality as a key factor shaping the gut microbiota ecosystem. Public nutrition guidelines should prioritize lowering saturated fat intake and encouraging greater consumption of PUFAs to help prevent gut dysbiosis and long-term metabolic disturbances.

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