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Research Article

Differential Effects of Sub-Chronic Administration of Fish Oil and Chia Seed Oil on Adipokine Gene Expression in Healthy Mice

Fikri Ahmad Fauzi[1,2], Albert Khemabodhi[2,3], Salza Nurbaiti Salamah[2,3], Vanessa Ayu Sumirat[1], Tanendri Arrizqiyani[2,4,5], Siti Nur Fatimah[6], Mas Rizky A. A. Syamsunarno[2,7]*

[1]Master Program of Biotechnology, Graduate School, Universitas Padjadjaran, Bandung, Indonesia; [2]Metabolic Medicine Research Group, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia; [3]Undergraduate Program of Medical Science, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia; [4]Doctoral Program of Biotechnology, Graduate School, Universitas Padjadjaran, Bandung, Indonesia; [5]Medical Laboratory of Technology, Universitas Bakti Husada, Tasikmalaya, Indonesia; [6]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia; [7]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia.

Abstract | Background: White adipose tissue, particularly the epididymal depot, regulates energy homeostasis in mice by synthesizing and secretion of adipokines, which impact insulin sensitivity, lipid metabolism, and inflammatory processes, such as lipolysis, which can lead to metabolic disease. Omega-3 fatty acids (DHA, EPA, ALA) have been shown to exert beneficial effects on metabolic health. However, while numerous studies have demonstrated the beneficial effects of omega-3 fatty acids on adipokine modulation and metabolic outcomes, the majority of this research has been conducted in models of obesity or metabolic pathology. Consequently, the precise mechanisms underlying their influence on adipokine gene expression in healthy physiological conditions remain incompletely understood. Aim: This study investigates the differential effects of fish oil (FO) and chia seed oil (CSO) on adipokine gene expression related to metabolic regulation (*Fabp4, Lep, Adipoq)* and epididymal white adipose tissue (eWAT) characteristics of healthy mice. Method: Healthy mice (n=6/group) were randomly assigned to three groups: control, FO, and CSO. Each oil treatment was delivered orally 5 µl/gBW for 4 weeks (28 days). Body weight was monitored during last week. At the end of the intervention period, mice were euthanized, and eWAT was collected for weight measurement, histological analysis, and gene expression analysis using RT-qPCR. Results and Discussion: The results indicated that neither oil significantly affected body weight ($p = 0.545 > 0.05$), eWAT weight ($p = 0.5129 > 0.05$), or adipocyte number ($p = 0.6575 > 0.05$). FO resulted in an observed increase in *Fabp4* gene expression (1.32-fold), while CSO showed *a* tendency for decrease (0.24-fold) compared to the control group ($p = 0.9306 > 0.05$); Both FO and CSO supplementation exhibited a trend of reduced expression of *Adipoq* (0.01-fold in FO; 0.03-fold in CSO) and *Lep* (0.21-fold in FO; 0.26-fold in CSO) though changes were not statistically significant for *Lep* ($p = 0.0836 > 0.05$) and *Adipoq* ($p = 0.1006 > 0.05$). Conclusion: Short-term supplementation with whole fish oil or chia-seed oil modulates expression pathways in healthy mice, influencing early transcriptional changes linked to metaflammation and metabolic homeostasis without promoting adipose tissue expansion. These results support the safety and potential contribution of whole-oil dietary strategies for maintaining adipose tissue health and understanding early molecular events leading to metabolic disease.

Keywords | Adipokines, Adiponectin, Chia-seed oil, FABP4, Fish oil, Leptin

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***Correspondence** | Mas Rizky A. A. Syamsunarno, Metabolic Medicine Research Group, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia; **Email:** rizky@unpad.ac.id
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INTRODUCTION

White adipose tissue (WAT), particularly the epididymal depot (eWAT) in mice, is a central regulator of systemic energy balance through the secretion of adipokines, signaling molecules like hormones secreted by adipose tissue, that affect metabolism adipokines, such as fatty acid binding protein 4 (FABP4), leptin, and adiponectin (Hafiane, 2024; Kamareddine *et al*., 2021). These adipokines orchestrate metabolic processes including insulin sensitivity, lipid metabolism, and their dysregulation are implicated in the pathogenesis of metabolic disorders (Hanifah *et al*., 2024; Recinella *et al*., 2020). Among them, FABP4, which is highly expressed in eWAT, has emerged as a critical intracellular lipid chaperone and its unique role in fatty acid transport and metabolism within adipocytes makes it a key target for understanding dietary lipid effects on adipose tissue function (Furuhashi, 2019). FABP4 is a novel predictive biomarker for metabolic syndrome (Mets), a cluster of cardiovascular risk factors including central obesity, insulin resistance, dyslipidemia, and hypertension. Beyond that, FABP4 contributes to the pathogenesis of numerous other conditions, such as atherosclerotic diseases, heart failure, non-alcoholic steatohepatitis, ischemic stroke, liver fibrosis, lupus, thalassemia, and various cancers (Fianza *et al*., 2021; Hamijoyo *et al*., 2021; Li *et al*., 2021). While preclinical studies show promising results for targeting FABP4 therapeutically, its clinical implications remain unclear, with some paradoxical findings suggesting a need for further research into FABP4 expression and inhibition in both animal models and humans to confirm its reliability as a biomarker and therapeutic target (Li *et al*., 2021).

In metabolic disorders, elevated leptin from increased fat mass leads to leptin resistance and promotes *metaflammation*-a chronic, low-grade inflammatory state in metabolic tissues that underlie Mets, insulin resistance, and other metabolic diseases (Xiong *et al*., 2023), by stimulating pro-inflammatory cytokines, contributing to insulin resistance and cardiovascular disease. Conversely, adiponectin, an anti-inflammatory and insulin-sensitizing adipokine, is reduced in these conditions and offers protection against metabolic dysfunction. The crucial balance between these two, reflected by an increased leptin/adiponectin ratio, signifies dysfunctional adipose tissue, heightened inflammation, and greater metabolic risk, correlating more strongly with insulin resistance and metabolic syndrome severity than individual adipokine levels (Kamareddine *et al*., 2021; Kobayashi *et al*., 2020; Raman and Khanal, 2021).

Dietary oils from animal and plant sources are important modulators of adipokine gene expression. Fish oil, rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and chia-seed oil, high in alpha-linolenic acid (ALA), differ in fatty acid composition, metabolic conversion, and biological effects (Rodway *et al*., 2023; Wang *et al*., 2024). Although both EPA/DHA and ALA are omega-3 fatty acids, their conversion efficiency, membrane incorporation, and downstream signaling diverge, resulting in distinct metabolic responses (Hanifah *et al*., 2024). While numerous studies have demonstrated the beneficial effects of omega-3 fatty acids on adipokine regulation and metabolic outcomes, the majority of this research has been conducted in models of obesity, metabolic syndrome, or other pathological states (Dao *et al*., 2024; Hanifah *et al*., 2024; L. Hill *et al*., 2020; Pauls, 2020). As a result, there is a significant gap in our understanding of how animal- and plant-derived omega-3 oils influence adipokine gene expression and adipose tissue function under healthy physiological conditions.

Syamsunarno *et al*. (2019) highlighted that adipose tissue gene regulation and metabolic flexibility are influenced by health status and dietary context, suggesting that identical nutrients may yield different outcomes in healthy versus unhealthy individuals. However, the mechanisms by which EPA/DHA and ALA modulate adipokine gene expression, especially in healthy models, remains unclear, with inconsistent findings reported between plant- and animal-derived omega-3s (Khodadustan *et al*., 2020; Napier *et al*., 2019; Pauls *et al*., 2020).

These inconsistencies may stem from differences in experimental models, health status, and the complex composition of dietary oils beyond their omega-3 content (Michaeloudes *et al*., 2023). For example, plant-based oils such as chia-seed oil and virgin coconut oil contain a variety of bioactive components that can influence lipid metabolism and inflammation independently of omega-3s (Hanifah *et al*., 2024; Hiroyuki *et al*., 2022). This underscores the importance of studying whole oils rather than isolated fatty acids.

Another layer of complexity is introduced by *metaflammation*. Even in healthy states, changes in adipokine gene expression may signal early shifts in the inflammatory and metabolic environment of adipose tissue. Previous work by Syamsunarno *et al*. (2019 and 2021) and others have underscored the importance of metabolic context and intervention timing in shaping these responses (Hanifah *et al*., 2024; Hiroyuki *et al*., 2022; Syamsunarno *et al*., 2021, 2019), but direct comparative studies in healthy models are rare.

Chia-seed oil, notable for its high ALA content among plant-based oils, and fish oil, as a source of EPA/DHA, provide an opportunity to examine these differences (Ofori-Mensah *et al*., 2020). While ALA requires enzymatic conversion to EPA/DHA (Wang *et al*., 2024; Wu *et al*., 2019), the efficiency of this process is limited, and the physiological effects of each whole oil in general remain

incompletely understood, particularly regarding their influence on *Fabp4* gene expression and the metabolic response in healthy states.

To address these knowledge gaps, this study aims to compare the effects of sub-chronic (4-week) administration of salmon fish oil (FO, rich in DHA/EPA) and chia seed oil (CSO, rich in ALA) on *Fabp4*, *Lep*, and *Adipoq* mRNA expression in eWAT of healthy mice. By elucidating the differential gene regulatory effects of these oils, this research seeks to advance our understanding of how animal- and plant-based dietary fats modulate metabolic and inflammatory pathways in healthy adipose tissue, with implications for primary prevention of metabolic disease. We hypothesized that both FO and CSO would influence adipokine gene expression.

MATERIALS AND METHODS

Materials

Animal-based and plant-based oils: Animal-based oil used in this treatment is salmon fish oil (FO) from Wellness manufacture, Indonesia. For Plant-based oil used chia-seed oil (CSO) from Tamba Sanji Wani manufacture, Indonesia. Each oil treatment was delivered orally 5 µl/gBW for each mouse based on optimization for optimum dose which can give the impact to metabolic changes from the previous studies (Syamsunarno *et al*., 2019).

Experimental design: A laboratory experiment with a complete-randomized design (CRD) to study the effect of fish oil and chia-seed oil given to healthy mice was conducted at the Animal Laboratory of the Faculty of Medicine, Universitas Padjadjaran. This experiment was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran with Protocol No. 1379/UN6.KEP/EC/2023.

Three oil-treatment group will be assigned to the mice: group FO (fish oil 5 µl/gBW/day + standard diet), group CSO (chia seed oil, 5 µl/gBW/day + standard diet), and group Control (distilled water, 5 µl/gBW/day + standard diet). The rationale for the dosage was to prevent the stomach from being full, which would lower mouse hunger and increase the resultant bias (Syamsunarno *et al*., 2019). After a 6-hour fast, distilled water and oil were administered via oral gavage (at 1 p.m.). The treatment was administered daily for 4 weeks (28 days). Body weight was recorded on the last week. On the 28th day, animals were anesthetized with ketamin then euthanized by cervical-dislocation, and the epididymal white adipose tissue (eWAT) was carefully dissected, weighed, and immediately stored for subsequent gene expression and histological analyses. The tissue samples were then processed according to standard protocols to perform RT-qPCR and histological analysis. All procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Animals: A total of 30 swiss-webster mice aged 4-6 weeks were obtained from PT. Biofarma Bandung, Indonesia. Swiss Webster mice are widely used in metabolic disease research due to their susceptibility to diet-induced obesity and metabolic syndrome (Logan *et al*., 2020). Male Swiss Webster mice are more prone to diabetes, while females show protection against hyperglycemia despite developing hepatic steatosis and adipose fibrosis (Chan *et al*., 2021). Animals sent to the Faculty of Medicine, Universitas Padjadjaran, where they will be acclimatized in cages at the controlled-temperature room, 12/12 light/dark cycle, for 7-14 days and fed and watered *ad libitum* until they reach an inclusion criteria which healthy and weighing 20-25 grams. Mice that meet the inclusion criteria (18 mouse, n=6/group) will then be randomized and a cage map will be made based on treatment group.

RNA isolation and gene expression: Tissues were isolated and stored at -80°C. eWAT were minced and isolated using Zymo Research Quick RNA Miniprep plus (No. catalog R1057T) kit. mRNA concentration was measured using a Nanodrop 2000 Thermo Scientific LGM-EIII-06. mRNA was synthesized into cDNA following to Meridian Bioscience (SensiFast cDNA Synthesis) kit procedure. Two-step RT-qPCR was performed for gene expression according to Meridian Bioscience (SensiFast Sybr No-Rox) kit procedure and using mice-specific primers against the genes (*Fabp4*) FABP4 (NM_024406); (*Lep*) leptin (NM_008493); (*Adipoq*) adiponectin (NM_009605); and (*Gapdh*) GAPDH (NM_008084) as housekeeping gene control. RT-qPCR analysis was conducted using triple replicates (triplo) for each sample (n=6). Furthermore, the expression levels of *Fabp4*, *Lep*, and *Adipoq* genes were normalized to the transcription level of the *Gapdh* gene using the Livaks formula. All primer used are listed in the Table 1.

Table 1: Primer used for gene expression.

Gene	PRIMER	REF.
Fabp4	F: tttggtcaccatccggtcag R: cccgccatctagggttatga	(Choi and Lee 2021)
Adipoq	F: agggagagaaaggagatgcag R: cagacttgggctcccacctc	(Schmid *et al*. 2021)
Lep	F: aggatctgaggggtgatgtg R: aggtgaccaaggtggcatag	(Schmid *et al*. 2021)
Gapdh (as reference gene)	F: tgtccgtcgtggatctgac R: agggagatgctcagtgttgg	(Schmid *et al*. 2021)

Histological analysis of eWAT: The epididymal white adipose tissue fixed in 10% neutral-buffered formalin

solution. After fixation, the tissue is dehydrated through a graded ethanol series, cleared with xylene, and infiltrated with molten paraffin at 60°C in multiple changes. The tissue is then embedded in paraffin molds and allowed to solidify. Thin sections (~5 µm) are cut using a microtome, floated on a warm water bath, and mounted onto adhesive-coated slides. Slides are dried overnight before deparaffinization with xylene and rehydration through descending ethanol concentrations. The staining technique using Haematoxylin-eosin (HE) dye. Histological sections were examined under a light microscope (40x) using OPTIKA microscope and six images per field of view were captured by OPTIKA Pro View software. Subsequently, image processing was conducted using ImageJ software. Adipocytes were manually selected from these images. Adipocytes counted automatically using ADIPOQ-Analyzer plugin in ImageJ according to the software's protocol (Sieckmann *et al.*, 2022). The adipocyte count was then normalized by dividing it by the cross-sectional area of the observed region to obtain cells per area^2.

Statistical Analysis

All data were tabulated and statistically analyzed using One-Way ANOVA test and Tukey test. If the parametric test requirements (normality and/or homogeneity) are not met, then the data is analyzed with the Kruskal-Wallis non-parametric test, then can be continued with Dunn's test by between groups. The value of meaningfulness or test significance results is expressed with $p<0.05$ (Kang, 2021; Syamsunarno *et al.*, 2019). GraphPad Prism v9.5.1 was used as the software for analysis. The experimental design is shown in Figure 1.

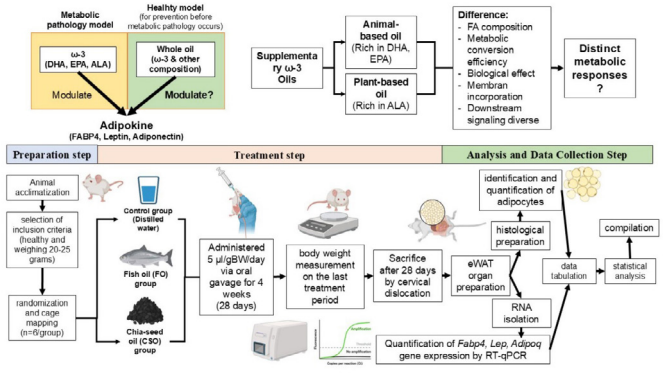

Figure 1: Experimental design.

RESULT AND DISCUSSION

Research on the comparison of salmon fish oil (FO) and chia seed oil (CSO) has been conducted. Body weight was measured last week to see changes in body weight due to treatment.

Figure 2 summarizes the effect of fish oil and chia-seed oil supplementation on body weight over a four-week period. Figure 2 did not reveal any significant differences in body weight (p = 0.545 > 0.05) between the control group (34.83 ± 1.2 g) and either the FO (33 ± 1.6 g) or CSO groups (33.33 ± 0.6 g) suggested that neither FO nor CSO administration significantly impacted body weight in the mice under these experimental conditions. This finding suggests that both FO and CSO can be safely consumed routinely without significant weight gain concerns. This indicates that both fish oil and chia-seed oil do not increase body weight.

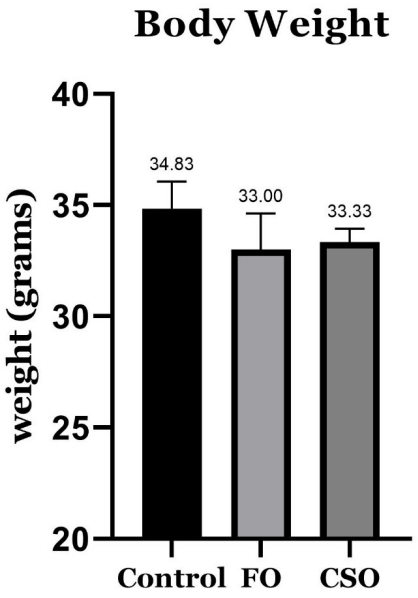

Figure 2: Effect of fish oil and chia-seed oil on body weight at last intervention period week-4). **Note:** Mice were fed by standard (Control), fish oil (FO), or chia-seed oil (CSO) and sacrificed at week 4. All data are means ± SEM; n = 6. No significanly different for one-way ANOVA from any treatment.

Figure 3 demonstrates the impact of fish oil (FO) and chia-seed oil (CSO) supplementation on epididymal white adipose tissue (eWAT) in mice. Quantification of adipocyte cell density (2A) showed no significant differences between the Control (8,091±1,667 cells/area^2), FO (7,650±0,669 cells/area^2), and CSO (8,337±1,352 cells/area^2) groups (p=0.6575 > 0.05). This lack of significant change was further supported by panel 2B, which demonstrated that epididymal white adipose tissue (eWAT) weight did not substantially differ across the treatment groups (p=0.5129 > 0.05). Visual examination of hematoxylin-eosin stained eWAT sections (2C) revealed a slight qualitative difference in adipocyte size distribution between the Control and CSO groups. However, this visual trend did not result in statistically significant quantitative changes in adipocyte cell density, as indicated in Panel A. Overall, these results suggest that neither fish oil (FO) nor chia seed oil (CSO) supplementation, at the tested dosage and duration in healthy mice, significantly impacted eWAT adipocyte cell

density or organ weight over the four-week experimental period.

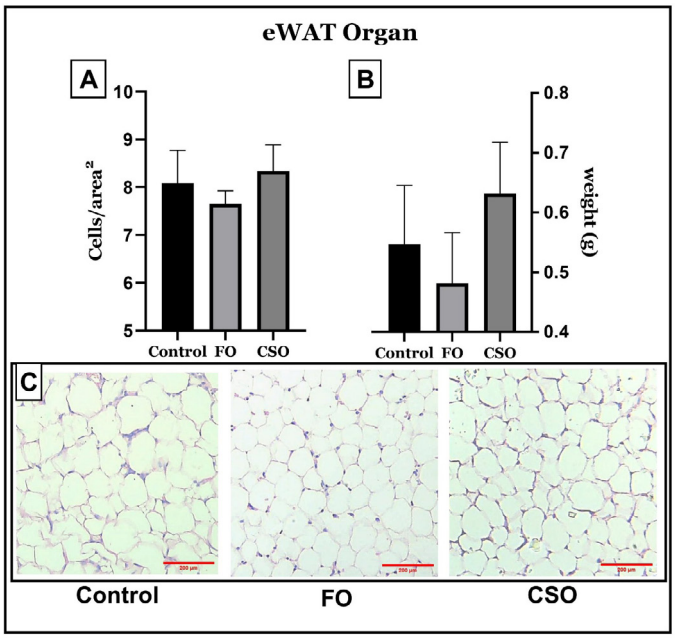

Figure 3: Effect of fish oil and chia-seed oil on epididymal white adipose tissue (eWAT) weight and number of adipocyte. **Note:** Mice were fed by standard (Control), fish oil (FO), or chia-seed oil (CSO) and sacrificed at week 4. Adipocyte number were calculated by ADIPOQ-Analyzer plugin (A). eWAT organs evaluated after sacrificed (B). All data are means ± SEM; n = 6. Hematoxylin-eosin staining for morphometry of adipocytes (C) as evaluated in (A). Bar represents 200 µm and observed at 40x magnification (C). No significanly different for one-way ANOVA from any treatment.

Based on Figure 4, FO supplementation increased *Fabp4* gene expression 1,32-fold, while CSO supplementation decreased its expression 0,24-fold compared to the control group. Despite observed alterations in gene expression, statistical analysis revealed these changes were not significant (p=0.9306 > 0.05). Both FO and CSO supplementation reduced the expression of *Adipoq* (0,01-fold in FO; 0,03-fold in CSO) and *Lep* (0,21-fold in FO; 0,26-fold in CSO). Statistical analysis showed that although changes in gene expression were seen, they were not significant for *Lep* (p=0.0836 > 0.05) and *Adipoq* (p=0.1006 > 0.05). These results suggest that dietary supplementation with fish oil and chia-seed oil may have distinct regulatory effects on genes associated with adipocyte function and metabolism. Although these observed changes did not reach statistical significance within the scope of this study. The inverse relationship observed in the FO group, where *Fabp4* expression showed an increase while *Adipoq* expression showed a decrease, hints at a potential regulatory connection between these two genes. Conversely, in the CSO group, the expression of *Fabp4*, *Lep*, and *Adipoq* all showed a tendency for decrease. Moreover, although both FO and CSO exhibited a trend of reduced *Lep* expression, the magnitude of this reduction did not directly correspond to the observed changes in *Fabp4* expression, which was observed to be upregulated by FO but remained observed to be unchanged by CSO.

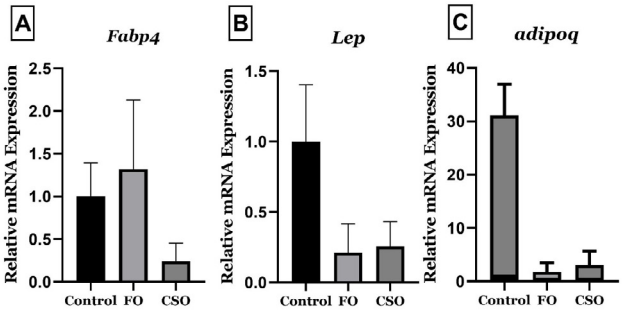

Figure 4: Gene expression of Fabp4, Adipoq, and Lep in epididymial white adipose tissue (eWAT) of mice fed with oil treatment. **Note:** Data were normalize to the reference gene, Gapdh in (A,B,C), and expressed relative to (Control) group. Fish oil treatment group (FO). Chia-seed oil treatment group (CSO). Data are means ± SEM; n = 4-5 (A,B,C). Data are box plot median (C). No significant different for one-way ANOVA from any treatment.

This study provides new evidence that sub-chronic supplementation with fish oil (FO, rich in EPA/DHA) and chia-seed oil (CSO, rich in ALA) differentially modulate the gene expression of *Fabp4*, *Lep*, and *Adipoq* in epididymal white adipose tissue (eWAT) of healthy mice, without significantly affecting body weight, eWAT mass, or adipocyte number.

Effects on Body Weight, eWAT Weight and Adipocyte Number

Neither FO nor CSO supplementation resulted in significant changes in body weight, eWAT weight, or adipocyte number over the four-week intervention. This aligns with previous studies indicating that omega-3 supplementation, particularly in healthy or non-obese models, does not necessarily induce weight gain or loss but may exert subtler effects on metabolic health (Rodway *et al.*, 2023; Syamsunarno *et al.*, 2019). The absence of significant changes in adipocyte cell density or eWAT weight suggests that both oils, at preventive doses, do not promote adipose tissue hyperplasia or hypertrophy in healthy animals. This is consistent with reports that short-term omega-3 supplementation does not promote hyperplastic expansion under physiological conditions (Hiroyuki *et al.*, 2022; Syamsunarno *et al.*, 2019). Hyperplasia, defined as an increase in adipocyte number is a physiological adaptation that can help accommodate excess energy storage and is generally considered metabolically favorable compared to hypertrophy (Xiong *et al.*, 2023). Our findings indicate that FO and CSO does

not drive excessive adipose tissue remodeling or expansion under non-pathological conditions, supporting their safety for routine preventive use.

The four-week intervention period in this study is relevant to the early phases of *metaflammation*, while the present study did not directly measure inflammatory mediators, the observed changes in adipokine gene expression may reflect subtle shifts in the adipose tissue microenvironment that precede overt inflammatory remodeling. Recent research suggests that even in the absence of overt inflammation, dietary omega-3 fatty acids can influence the expression of genes involved in immune signaling and metabolic regulation, contributing to the maintenance of metabolic homeostasis (Kalupahana *et al*., 2020).

Differential Modulation of Adipokine Gene Expression by Dietary Oils and Interplay of Pathways

The gene expression changes observed in this study reflect the complex interplay between omega-3 fatty acids, nuclear receptor activation, and metabolic signaling pathways. The adipokines *Fabp4*, *Lep*, and *Adipoq* are central to lipid metabolism, adipogenesis, and inflammatory processes in adipose tissue, and their expression is broadly regulated by the peroxisome proliferator-activated receptor (PPAR) signaling pathway and key transcription factors such as PPARγ, CCAAT/enhancer-binding protein (C/EBP), Activating Protein 2 alpha (AP2α) (Lin Y *et al*., 2023; Liu *et al*., 2020; Zargar *et al*., 2023). While our study did not include direct measurements of protein levels or signaling pathway activation, the observed patterns provide insights into the potential differential effects of fish oil (FO) and chia seed oil (CSO) at the transcriptional level.

Fish oil, rich in preformed eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), directly activates nuclear receptors like PPARγ, which can influence lipid metabolism and inflammation (Calder, 2020; Fisk *et al*., 2022). This direct action may explain the observed tendencies in gene expression. The observed increase in *Fabp4* expression with FO could be linked to an enhanced fatty acid oxidation or mobilization within adipocytes, as *Fabp4* acts as a lipid chaperone facilitating fatty acid transport and is secreted from adipocytes via a non-classical pathway, participating in *metaflammation* (Furuhashi, 2019). Concurrently, the observed decrease in *Adipoq* expression by FO is particularly intriguing given that although PPARγ activation is generally associated with increased *Adipoq* expression, high doses or specific types of fatty acids can have complex, sometimes paradoxical effects on PPARγ activity, potentially leading to suppressing *Adipoq* (Martins *et al*., 2020). This inverse relationship suggests that FO's effects may involve complex regulatory feedback or distinct signaling cascades. For instance, while *Fabp4* upregulation might indicate increased lipid flux or a subtle pro-inflammatory signal, the concomitant *Adipoq* reduction could reflect early responses to transient metabolic or inflammatory stress, as *Adipoq* is known for its anti-inflammatory properties and its role in regulating glucose and fatty acid breakdown, typically downregulated by inflammatory signaling pathways (DasNandy *et al*., 2022). This highlights that different fatty acids or their metabolites may selectively impact distinct signaling cascades within adipocytes.

In contrast, chia-seed oil, primarily a source of alpha-linolenic acid (ALA), requires enzymatic conversion to EPA and DHA, a process with limited efficiency in mice (Takic *et al*., 2022). Therefore, CSO's effects are likely driven more by ALA itself or its specific metabolic products and other bioactive components present in the whole oil (e.g., polyphenols, phytosterols), which can influence lipid metabolism and inflammation independently of omega-3s (Hiroyuki *et al*., 2022). The observed tendency for decrease in *Fabp4*, *Lep*, and *Adipoq* expression with CSO suggests a different regulatory mechanism compared to FO. *Lep* is crucial for regulating energy balance by inhibiting hunger and is involved in inflammatory responses and adipocyte differentiation (Lin Y *et al*., 2023; Reis *et al*., 2024; Sugiyama *et al*., 2022). The synchronized tendency for downregulation of all three adipokines with CSO might point to a broader, more suppressive effect on general adipokine synthesis or secretion, possibly through a different modulation of PPAR/sterol regulatory element-binding protein (SREBP) pathways or other yet-to-be-identified mechanisms by ALA or its co-components. This contrasts with FO's seemingly opposing effects on *Fabp4* and *Adipoq*. This supports the notion that evaluating the physiological effects of whole oils, rather than isolated fatty acids, more accurately reflects real-world dietary exposures and outcomes, as also suggested by Hiroyuki *et al*. (2022).

Overall, the observed differential patterns in *Fabp4*, *Lep*, and *Adipoq* expression suggest that fish oil and chia-seed oil, due to their distinct fatty acid profiles and accessory bioactive compounds, interact uniquely with adipocyte metabolic and inflammatory pathways. This phenomenon may reflect complex regulatory feedback, compensatory mechanisms, or alterations in lipid turnover involving adipokine genes and other metabolic mediators (Lin X *et al*., 2023). These findings underscore that while both oils may contribute to metabolic health, their specific mechanisms at the gene expression level appear to diverge, influencing the intricate cross-talk among these critical adipokines.

Study Implications and Limitations

Overall, these results suggest that both FO and CSO can modulate adipokine gene expression in healthy adipose tissue without inducing adverse morphological changes. This is consistent with the concept of metabolic flexibil-

ity, where nutrient signaling and gene regulation are influenced by both health status and dietary composition (Syamsunarno *et al*., 2019). While our findings contribute to understanding early transcriptional responses, further evidence linking the observed changes to functional metabolic improvements is needed before making direct claims about preventive strategies for metabolic disease, especially considering that some gene expression alterations did not reach statistical significance. Future studies should include longer intervention periods, female and metabolically challenged models, and assessment of circulating adipokine and metabolic outcomes such as protein level, insulin sensitivity and lipid profiles. Further investigation into molecular mechanisms, including post-transcriptional regulation and signaling pathways, is warranted to deepen understanding. Additionally, assessing inflammatory cytokines (e.g., TNF-α, IL-6, MCP-1) and adipose tissue immune cell markers could clarify the mechanistic links between omega-3 supplementation, adipokine gene regulation, and adipose remodeling, particularly under metabolic stress conditions.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study demonstrates that 4-week supplementation with fish oil or chia-seed oil (5 µl/gBW) elicits differential modulation of *Fabp4*, *Lep*, and *Adipoq* gene expression in healthy mice, mediated through nuclear receptor pathways and influenced by both PUFA profiles and other bioactive components. These findings underscore that even short-term dietary interventions can initiate early transcriptional changes linked to *metaflammation* and metabolic homeostasis, highlighting their potential to influence regulate adipokine signaling pathways essential for metabolic health. By clarifying how whole oils-beyond isolated fatty acids-affect key adipogenic and inflammatory genes, this work advances the understanding of the potential for dietary oils in early metabolic disease prevention strategies, particularly through targeting adipokine-related mechanisms. Importantly, neither oil induced excessive adipose tissue remodeling or expansion, confirming their safety for preventive use in healthy conditions.

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NOVELTY STATEMENT

This study directly addresses a critical gap by comparing how whole dietary oils—fish oil (animal-derived) and chia-seed oil (plant-derived)—modulate key adipokine gene expression within healthy adipose tissue, a context notably absent in most existing research focused on disease models. Our findings uniquely reveal how these complex oil matrices influence early gene regulatory events in white adipose tissue linked to *metaflammation*, an initial phase in metabolic disease development. Specifically, this research highlights that CSO demonstrates better *Fabp4* gene modulation, indicating its promising potential as a preventive strategy for diseases targeting FABP4. This offers crucial insight into the preventive potential of whole-oil dietary strategies for maintaining metabolic health and mitigating early metabolic syndrome triggers.

AUTHOR'S CONTRIBUTIONS

Fikri Ahmad led the study, managing data, analysis, visualization, and writing. Albert Kemabodhi and Salza Nurbaiti helped with data, writing, and review. Vanessa Ayu contributed to data, methods, and validation. Tanendri Arrizqiyani contributed to writing, analysis, methods, validation, and review. Siti Nur and Mas Rizky oversaw the study's design, methods, supervision, and validation, with Mas Rizky also editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

REFERENCES

Calder PC (2020). n-3 PUFA and inflammation: from membrane to nucleus and from bench to bedside. Proc. Nutr. Soc., 79(4): 1–13. https://doi.org/10.1017/S0029665120007077

Chan AML, Ng AMH, Mohd Yunus MH, Idrus RBH, Law JX, Yazid MD, Chin KY, Shamsuddin SA and Lokanathan Y (2021). Recent developments in rodent models of high-fructose diet-induced metabolic syndrome: A systematic review. Nutrients, 13(8): 2497. https://doi.org/10.3390/nu13082497

Choi RY, Lee MK (2021). *Polygonum multiflorum* Thunb. Hot Water Extract Reverses High-Fat Diet-Induced Lipid Metabolism of White and Brown Adipose Tissues in Obese Mice. Plants (Basel, Switzerland): 10(8). https://doi.org/10.3390/plants10081509

Schmid A, Roderfeld M, Gehl J, Roeb E, Nist A, Chung HR, Stiewe T, Karrasch T, Schäffler A (2021). C1q/TNF-Related Protein 3 (CTRP-3) Deficiency of Adipocytes Affects White Adipose Tissue Mass but Not Systemic CTRP-3 Concentrations. Int. J. Mol. Sci., 22(4). https://doi.org/10.3390/ijms22041670

Dao T, Thomas J, Chen YC, Nicholls S, Bubb K (2024). High-dose specific omega-3 polyunsaturated fatty acid increases plaque stability in tandem-stenosis ApoE-deficient mice fed high fat-diet. Atherosclerosis, 395(117742): 117742. https://doi.org/10.1016/j.atherosclerosis.2024.117742

DasNandy A, Patil VS, Hegde HV, Harish DR, Roy S (2022).

Elucidating type 2 diabetes mellitus risk factor by promoting lipid metabolism with gymnemagenin: An in vitro and in silico approach. Front. Pharmacol., 13: 1074342. https://doi.org/10.3389/fphar.2022.1074342

Fianza PI, Rahmawati A, Afifah S, Praptama S, Ghozali M, Sihite TA, Setiabudi D, Syamsunarno MRAA, Fucharoen S, Panigoro R (2021). Correlation between Serum Fatty Acid Binding Protein 4 (FABP4) Levels and Cardiac Function in Patients with Thalassemia Major. Dis. Markers, 2021: 5130628. https://doi.org/10.1155/2021/5130628

Fisk HL, Childs CE, Miles EA, Ayres R, Noakes PS, Paras-Chavez C, Kuda O, Kopecký J, Antoun E, Lillycrop Kam, Calder PC (2022). Modification of subcutaneous white adipose tissue inflammation by omega-3 fatty acids is limited in human obesity-a double blind, randomised clinical trial. EBioMedicine, 77(103909): 103909. https://doi.org/10.1016/j.ebiom.2022.103909

Furuhashi M (2019). Fatty acid-binding protein 4 in cardiovascular and metabolic diseases. J. Atheroscler. Thromb., 26(3): 216–232. https://doi.org/10.5551/jat.48710

Hafiane A (2024). Adiponectin-mediated regulation of the adiponectin cascade in cardiovascular disease: Updates. Biochem. Biophys. Res. Commun., 694: 149406. https://doi.org/10.1016/j.bbrc.2023.149406

Hamijoyo L, Putri M, Alita VR, Atik N, Syamsunarno MRAA (2021). Fatty acid binding protein 4 (FABP4) and metabolic-related parameters in systemic lupus erythematosus active and non-active episode. Lupus, 30(7): 1133–1139. https://doi.org/10.1177/09612033211006908

Hanifah CP, Sulistiyorini I, Sumirat VA, Anggraeni N, Putri M, Ghozali M, Syamsunarno MRAA (2024). FABP4 and metabolite profile in lipopolysaccharide-induced mice model treated with *Moringa oleifera* ethanol leaf extract. Int. J. Clin. Pharmacol. Re., 8(3): 196. https://doi.org/10.15416/pcpr.v8i3.50860

Hiroyuki A, Sastya Qomariyuti R, Nur Islami N, Sumirat VA, Anggraeni N, Syamsunarno MRAA (2022). The comparison of *Moringa oleifera* seeds oil and fish oil in organ weight and blood haematological profile in subchronic male mice. Adv. Anim. Vet. Sci., 10(10): 2265-2274. https://doi.org/10.17582/journal.aavs/2022/10.10.2265.2274

Kalupahana NS, Goonapienuwala BL, Moustaid-Moussa N (2020). Omega-3 fatty acids and adipose tissue: Inflammation and browning. Annu. Rev. Nutr., 40(1): 25–49. https://doi.org/10.1146/annurev-nutr-122319-034142

Kamareddine L, Ghantous CM, Allouch S, Al-Ashmar SA, Anlar G, Kannan S, Djouhri L, Korashy HM, Agouni A, Zeidan A (2021). Between inflammation and autophagy: The role of Leptin-adiponectin axis in cardiac remodeling. J. Inflamm. Res., 14: 5349–5365. https://doi.org/10.2147/JIR.S322231

Kang HJ (2021). The effect of dietary interventions on metabolic parameters in mice: A randomized controlled trial. Sci. Rep., 11: 12345. https://doi.org/10.1038/s41598-021-91729-2

Khodadustan E, Jafari T, Mahmoodnia L, Karami M, Momeni A, Dehkordi AH, Memarzadeh MR (2020). Effect of animal versus plant omega-3 polyunsaturated fatty acids on serum level of fetuin-A in patients with chronic renal failure; a randomized double-blinded parallel clinical trial. J. Ren. Inj. Prev., 10(2): e08. https://doi.org/10.34172/jrip.2022.22752

Kobayashi M, Uta S, Otsubo M, Deguchi Y, Tagawa R, Mizunoe Y, Nakagawa Y, Shimano H, Higami Y (2020). Srebp-1c/Fgf21/pgc-1α axis regulated by Leptin signaling in adipocytes-possible mechanism of caloric restriction-associated metabolic remodeling of white adipose tissue. Nutrients, 12(7): 2054. https://doi.org/10.3390/nu12072054

L. Hill J, M. Wyman J, M. Godwin S, A. Beech L, K. Buddington R, R.Sutter T, Ringwald-Smith K, van der Merwe M (2020). Dietary omega-3 fatty acids reduce adiposity and alter glucocorticoid-associated transcripts in epididymal white adipose tissue of C57BL/6 male mice raised on a high fat diet. J. Obes. Chronic Dis., 4(1): 13–22. https://doi.org/10.17756/jocd.2020-033

Li HL, Wu X, Xu A, Hoo RLC (2021). A-FABP in Metabolic Diseases and the Therapeutic Implications: An Update. Int. J. Mol. Sci., 22(17). https://doi.org/10.3390/ijms22179386

Lin X, Qu J, Yin L, Wang R, Wang X (2023). Aerobic exercise-induced decrease of chemerin improved glucose and lipid metabolism and fatty liver of diabetes mice through key metabolism enzymes and proteins. Biochimica et Biophysica Acta. Mol. Cell Biol. Lipids, 1868(12): 159409. https://doi.org/10.1016/j.bbalip.2023.159409

Lin Y, Qu L, Wu J, Pu M, Huang Y, Cao Y (2023). Identification of adipogenesis subgroups and immune infiltration characteristics in diabetic peripheral neuropathy. J. Immunol. Res., 2023: 3673094. https://doi.org/10.1155/2023/3673094

Liu R, Liu X, Bai X, Xiao C, Dong Y (2020). Different expression of lipid metabolism-related genes in Shandong black cattle and Luxi cattle based on transcriptome analysis. Sci. Rep., 10(1): 21915. https://doi.org/10.1038/s41598-020-79086-4

Logan IE, Bobe G, Miranda CL, Vasquez-Perez S, Choi J, Lowry MB, Sharpton TJ, Morgun A, Maier CS, Stevens JF, Shulzhenko N, Gombart AF (2020). Germ-free Swiss Webster mice on a high-fat diet develop obesity, hyperglycemia, and dyslipidemia. Microorganisms, 8(4): 520. https://doi.org/10.3390/microorganisms8040520

Martins FF, Aguila MB, Mandarim-de-Lacerda CA (2020). Eicosapentaenoic and docosapentaenoic acids lessen the expression of PPARγ/Cidec affecting adipogenesis in cultured 3T3-L1 adipocytes. Acta Histochem., 122(2): 151504. https://doi.org/10.1016/j.acthis.2020.151504

Michaeloudes C, Christodoulides S, Christodoulou P, Kyriakou TC, Patrikios I, Stephanou A (2023). Variability in the clinical effects of the omega-3 polyunsaturated fatty acids DHA and EPA in cardiovascular disease-possible causes and future considerations. Nutrients, 15(22): 4830. https://doi.org/10.3390/nu15224830

Napier JA, Olsen RE, Tocher DR (2019). Update on GM canola crops as novel sources of omega-3 fish oils. Plant Biotechnol. J., 17(4): 703–705. https://doi.org/10.1111/pbi.13045

Ofori-Mensah S, Yıldız M, Arslan M, Eldem V, Gelibolu S (2020). Substitution of fish oil with camelina or chia oils in gilthead sea bream (*Sparus aurata*, L.) diets: Effect on growth performance, fatty acid composition, haematology and gene expression. Aquacult. Nutr., 26(6): 1943–1957. https://doi.org/10.1111/anu.13136

Pauls SD (2020). Omega-3 fatty acids and adipose tissue remodeling: evidence from animal models. J. Lipid Res., 8: 1135–1147.

Pauls S, Pascoe C, Rodway L, Taylor C, Aukema H, Zahradka P (2020). Distinct transcriptional signatures of monocytes treated with α-linolenic acid and docosahexaenoic acid. Curr. Dev. Nutr., 4(nzaa058_028): nzaa058_028. https://doi.org/10.1093/cdn/nzaa058_028

Raman P, Khanal S (2021). Leptin in Atherosclerosis: Focus on Macrophages, Endothelial and Smooth Muscle Cells. Int. J. Mol. Sci, 22(11): 578966.

Recinella L, Orlando G, Ferrante C, Chiavaroli A, Brunetti L, Leone S (2020). Adipokines: New potential therapeutic target for obesity and metabolic, rheumatic, and cardiovascular diseases. Front. Physiol., 11: 578966. https://doi.org/10.3389/fphys.2020.578966

Reis IA, Baldassini WA, Ramírez-Zamudio GD, de Farias IMSC, Chiaratti MR, Pereira Junior S, Nociti RP, Carvalho PHV, Curi RA, Pereira GL, Chardulo LAL, Neto ORM (2024). Muscle tissue transcriptome of F1 Angus-Nellore bulls and steers feedlot finished: impacts on intramuscular fat deposition. BMC Genomics, 25(1): 1178. https://doi.org/10.1186/s12864-024-11066-8

Rodway LA, Pauls SD, Pascoe CD, Aukema HM, Taylor CG, Zahradka P (2023). Distinct effects of α-linolenic acid and docosahexaenoic acid on the expression of genes related to cholesterol metabolism and the response to infection in THP-1 monocytes and immune cells of obese humans. Biomedecine and Pharmacotherapie. Biomed. Pharmacother., 159(114167): 114167. https://doi.org/10.1016/j.biopha.2022.114167

Sieckmann K, Winnerling N, Huebecker M, Leyendecker P, Juliana Silva Ribeiro D, Gnad T, Pfeifer A, Wachten D, Hansen JN (2022). AdipoQ-a simple, open-source software to quantify adipocyte morphology and function in tissues and in vitro. Mol. Biol. Cell, 33(12): br22. https://doi.org/10.1091/mbc.E21-11-0592

Sugiyama Y, Shimokawa F, Sugiyama K, Kobayashi T, Yamashita Y, Kazama K, Onda K, Funaba M, Murakami M (2022). Relationships between the expression of adipose genes and profiles of hospitalized dogs. Vet. Res. Commun., 46(4): 1239–1244. https://doi.org/10.1007/s11259-022-09989-2

Syamsunarno MRAA, Alia F, Anggraeni N, Sumirat VA, Praptama S, Atik N (2021). Ethanol extract from *Moringa oleifera* leaves modulates brown adipose tissue and bone morphogenetic protein 7 in high-fat diet mice. Vet. World, 14(5): 1234–1240. https://doi.org/10.14202/vetworld.2021.1234-1240

Syamsunarno MRAA, Amalia F, Ariyanto EF, Shahib MN (2019). Short-term effects of virgin coconut oil and cod liver oil treatment in blood metabolite profile of mice. Res. J. Chem. Environ., 23(12): 124–128.

Takic M, Pokimica B, Petrovic-Oggiano G, Popovic T (2022). Effects of dietary α-linolenic acid treatment and the efficiency of its conversion to eicosapentaenoic and docosahexaenoic acids in obesity and related diseases. Molecules (Basel, Switzerland): 27(14): 4471. https://doi.org/10.3390/molecules27144471

Wang L, Cheng C, Yu X, Guo L, Wan X, Xu J, Xiang X, Yang J, Kang J, Deng Q (2024). Conversion of α-linolenic acid into n-3 long-chain polyunsaturated fatty acids: bioavailability and dietary regulation. Crit. Rev. Food Sci. Nutr., 1–33. https://doi.org/10.1080/10408398.2024.2442064

Wu Y, Liao W, Dawuda MM, Hu L, Yu J (2019). 5-Aminolevulinic acid (ALA) biosynthetic and metabolic pathways and its role in higher plants: a review. Plant Growth Regul., 87(2): 357–374. https://doi.org/10.1007/s10725-018-0463-8

Xiong P, Zhang F, Liu F, Zhao J, Huang X, Luo D, Guo J (2023). Metaflammation in glucolipid metabolic disorders: Pathogenesis and treatment. Biomedecine and Pharmacotherapie, Biomed. Pharmacother., 161(114545): 114545. https://doi.org/10.1016/j.biopha.2023.114545

Zargar S, Wani TA, Rizwan Ahamad S (2023). An insight into wheat germ oil nutrition, identification of its bioactive constituents and computer-aided multidimensional data analysis of its potential anti-inflammatory effect via molecular connections. Life (Basel, Switzerland): 13(2). https://doi.org/10.3390/life13020526