Indonesian Journal of Clinical Pharmacy, June 2020
Vol. 9 Iss. 2, pg 137–146
ISSN: 2252–6218, e-ISSN: 2337-5701
Available online at:
http://ijcp.or.id
DOI: 10.15416/ijcp.2020.9.2.137

Research Article

Effect of Fish Oil, Virgin Coconut Oil, and Used-Cooking Oil Consumption on Mice Hematological Profile

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Abstract
Humans require macronutrients and micronutrients to fulfill daily energy requirements, and triglyceride is a notable example, belonging to the fat family. This is particularly consumed frequently, and is composed of glycerol, and the fatty acid, specifically differentiated into unsaturated, saturated, trans, and cis forms. Furthermore, these constituents are known to play many roles in the body, including in the hematopoietic process. This involves oxidation and consequently stem cell differentiation into many blood cells in long-term, although the effect short-term is currently unknown. The study aimed, therefore, to investigate the effect of short-term intake of different fatty acid types on hematological profile in an animal model, conducted at the Animal Laboratory, Universitas Padjadjaran in October 2018. In addition, each group comprised 6 mice, orally administered distilled water as control (Group A), fish oil (Group B), virgin coconut oil (Group C), and used-cooking oil (Group D), at a dose of 5 µl/g body weight/day for 2 weeks. Subsequently, analysis was performed using blood measurement with hematology analyzer. The results showed lower white blood cell (WBC) count in Group B compared to D ($p<0.05$), alongside lymphocyte count ($p<0.01$). Moreover, the WBC in Group C was lower than D ($p<0.01$), also observed in lymphocyte count ($p<0.001$), % lymphocyte ($p<0.01$), while the % granulocyte count was higher than group D ($p<0.01$). Therefore, the highest total leukocyte and lymphocyte number among the other groups, as well as higher percentage of differential lymphocyte count was observed with mice provided with used-cooking oil compared to coconut oil, alongside a lower percentage of differential granulocyte count ($p<0.05$). However, fatty acid intake in group A, B, C, and D had no significant impact on RBC and platelet parameters. In conclusion, used-cooking oil induces a change in hematological profiles compared to fish oil and virgin coconut oil, featuring the increased total white blood cells and lymphocyte, as well as reduced % granulocyte.

Keywords: Fatty acid, hematological profile, leucocyte

Efek Pemberian Minyak Ikan, Minyak Kelapa Murni, dan Minyak Jelantah Terhadap Profil Hematologi Mencit

Abstrak
Manusia membutuhkan makronutrien dan mikronutrien untuk memenuhi kebutuhan energi harian. Salah satu sumber makronutrien adalah trigliserida yang merupakan salah satu jenis lemak yang paling sering dikonsumsi. Senyawa ini tersusun atas asam lemak dan gliserol. Terdapat banyak jenis asam lemak seperti asam lemak jenuh, asam lemak tidak jenuh, asam lemak trans, dan asam lemak cis. Rantai asam lemak memiliki banyak peran dalam tubuh, salah satunya adalah hematopoiesis sel stem. Pada konsumsi lemak jangka panjang, hematopoiesis ini terjadi melalui oksidasi asam lemak yang selanjutnya akan menstimulasi diferensiasi sel stem menjadi sel-sel darah di perifer, tetapi efeknya dalam jangka pendek belum diketahui. Oleh karena itu, penelitian ini bertujuan untuk menginvestigasi efek jangka pendek dari konsumsi berbagai jenis asam lemak terhadap profil hematologi mencit yang dilakukan di Laboratorium Hewan, Universitas Padjadjaran pada Oktober 2018. Mencit diberikan air suling sebagai kontrol (Grup A), minyak ikan (Grup B), minyak kelapa murni (Grup C), dan minyak jelantah (Grup D) dengan dosis 5µl/g berat badan/hari secara oral selama dua minggu. Profil hematologi diukur menggunakan *hematology analyzer*. Hasilnya, grup B memiliki jumlah leukosit lebih rendah dibandingkan grup D ($p<0,05$) dan limfosit yang lebih rendah dibandingkan grup D ($p<0,01$). Grup C memiliki jumlah leukosit lebih rendah dibandingkan grup D ($p<0,01$), jumlah limfosit yang lebih rendah dibandingkan grup D ($p<0,001$), % limfosit lebih rendah dibanding grup D ($p<0,01$), dan % granulosit lebih tinggi dibanding grup D ($p<0,01$). Selain itu, konsumsi asam lemak pada grup A, B, C, dan D tidak memengaruhi indeks RBC dan platelet secara signifikan. Sebagai simpulan, minyak jelantah memberikan efek terhadap perubahan profil hematologi mencit dibandingkan minyak ikan dan minyak kelapa murni, yaitu meningkatkan leukosit dan limfosit dan menurunkan % granulosit.

Kata kunci: Asam lemak, leukosit, profil hematologi

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Submitted: 11th September 2019, Accepted: 6th May 2020, Published: 29st June 2020

Introduction

Humans need macronutrients and micronutrients in order to fulfill their energy requirements. The macronutrient consisted of carbohydrates, protein, and fat. Fat is divided based on different chemical structures into triglyceride, phospholipid, and sterol. Triglyceride is fat that consumed daily by a human. Fat is arranged of three fatty acid chains bind with a glycerol chain. Based on the number of double bond, fatty acids are divided into unsaturated fatty acids and saturated fatty acids (SFAs). Unsaturated fatty acids are classified into monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Fatty acids are also distinguished by hydrogen configuration in the double bond as cis- and trans- configuration. Fatty acids have many roles such as health improvement, involved in the pathophysiology of several chronic illnesses, and hematopoietic stem cells (HSCs) maintenance.[1–3]

Fatty acids involvement in diseases can be identified in several rich-fat diet studies. Obesity is the most well-known impact of long-term consumption of the western diet which high in fat foods.[4,5] Moreover, the development of long-term metabolic disorder such as type 2 diabetes is linked with the high-fat diet.[6] Furthermore, the type of diet can induce gut microbial imbalance which leads to several diseases such as ulcerative colitis and atherosclerosis.[7,8] It is believed that the imbalance of gut microbiome takes place in the development of the diseases.[7,8] However, the risk of ulcerative colitis is reduced in long-term high intake of n-3 PUFAs.[7] Another recent interesting study is about the risk of cardiovascular disease which is more influenced by the type of fat rather than the amount of fat.[9] The risk of cardiovascular disease (CVD) is significantly reduced if SFA is replaced by MUFA and/or PUFA rather than carbohydrate.[9] Therefore, many recent studies establish that type of fatty acid affects health by contributing to the development of several chronic inflammatory diseases. However, the acute effect of fat diet is rarely discussed and needs further study.

Fatty acids have a function to maintain HSC by supplying substrate in Fatty Acid Oxidation (FAO). The oxidation contributes to HSC maintenance through the PML-PPARδ-FAO pathway by increasing stem cell asymmetric division. The asymmetric division results in one copy of the stem cell and one differentiated cell. The differentiated cell will transform into all mature blood lineage. This stem cell division is influenced by many factors, including exogenous nutrient availability.[10] Therefore, high consumption of fat can increase fatty acid availability in cells, and then influence stem cell differentiation.

Peroxisome Proliferator-Activated Receptor-δ (PPAR-δ), encoded by the PPAR-D gene, is the main PPAR expressed on HSC. As the nuclear receptor important in fatty acid sensing and metabolic pathway regulation, PPAR-δ has a profound role in the activation and regulation of fatty acid oxidation, however, the mechanism is still not well understood.[10] Besides its role in HSC maintenance, PPARs affect the anti-inflammatory and pro-inflammatory process.[2,11] For example, many studies on n-3 PUFAs such as EPA, DHA, and DPA in fish oil resulted in preventive and curative effect of PUFAs on CHD, diabetes, hypertension, hyperlipidemia, arthritis, asthma, and COPD.[12–18] It is believed that EPA and DHA n-3 PUFAs have an anti-inflammatory effect through the production of protectin, resolvin, activation of PPAR-α, and inhibition of arachidonic acid metabolism.[12,19]

Another study of marine n-3 PUFA supplementation in humans showed a negative effect on platelet activation. It inhibits platelet aggregation in females, whereas only EPA consumption can reduce platelet activation in males.[20,21] This phenomenon explained by

the reduced concentration of phospholipid arachidonic acid (omega-6 fatty acid) and physicochemical changes of the platelet membrane.[20] This structure changes can influence mean platelet volume (MPV) that acts as an indicator of coronary artery disease (CAD), since larger MPV associated with risk of CAD.[22] Therefore, n-3 PUFA consumption can reduce the risk of CAD. Although the study of this fatty acid on platelet already exist, assessment of n-3 PUFA's impact on other hematological profile is still limited so further examination is needed.

The pro-inflammatory effect of PPARs is demonstrated in the SFA study,[11] which shows that SFA is pro-inflammatory and amplify the monocyte or macrophage that can contribute to the pathophysiology of several inflammatory diseases like atherosclerosis and diabetes.[11,23,24] SFAs, including virgin coconut oil, are usually consumed by Indonesian as cooking oil. It is usually used repeatedly in the deep frying method. This used-cooking oil is high in trans fatty acids and predisposes to thermal oxidation.[25,26] It produces reactive free radicals, e.g. ROS, that cause oxidative stress and tissue injury which stimulate cytokine pro-inflammatory release.[25,27] This pro-inflammatory effect highly probable mediated by PPARs.[11] Therefore, the consumption of virgin coconut oil rich in SFA and used-cooking-oil can affect the hematological profile, especially inflammatory cells.

Based on the background, we hypothesized that the administration of virgin coconut oil rich in SFAs, fish-oil rich in n-3 PUFAs, and used-cooking oil rich in trans fatty acid can activate HSC maintenance and affect the hematological profile in animal model, especially inflammatory cells. Thus, the purpose of this study was to investigate the acute effect of virgin coconut oil rich in SFAs, fish-oil rich in n-3 PUFAs, and used-cooking oil rich in trans fatty acid by comparing each hematological profile in mice.

Methods

This study was permitted by the Health Research Ethics Committee of Universitas Padjadjaran, Bandung, Indonesia no. 840/UN6.KEP/EC/2019. All experiments were conducted based on Reduce, Refine, Replace (3R) and 5 Freedom (5F) principles.

Animals

Twenty four male white mice (*Mus musculus*) with bodyweight ranged between 30 g to 44 g (bodyweight average 38,8±2,71 g) and aged 8 to 10 weeks old were obtained from Bio Farma Company, Bandung, Indonesia. The number of samples was determined by Mead's Equation method. Mice were maintained in cages at the controlled-temperature room, 12/12 light/dark cycle, and *ad-libitum* accessed for water and food before and during treatment. Mice were adapted for one week before the experiment. Mice were divided into four groups; group A as control (distilled water 5 µL/gBW/day), group B (fish oil 5 µL/gBW/day), group C (virgin coconut oil 5 µL/gBW/day), and group D (used-cooking oil 5 µL/gBW/day). Fish oil was cod liver oil. Used cooking oil was virgin coconut oil that has cooked 6 times repeatedly. The rationality of dose was to prevent the stomach to become full that might reduce mouse appetite and further made the resulting bias. The distilled water and oil were given by intragastric tube after 6 hours fast (at 03.00 pm). The treatment was conducted for 14 days.

Hematology assessment

After 14 days of treatment, mice were euthanized by intraperitoneal injection of 200 µl ketamine:xylazine:saline (1:1:8). Mice were fasted overnight prior euthanized. Blood was drawn from the heart approximately 100 µl into a vacutainer tube containing potassium EDTA. Blood was further analyzed with an automatic hematology analyzer to measure 18

whole blood parameters at the Biochemistry Laboratory Faculty of Pharmacy Universitas Padjadjaran.

Statistical analysis

Multiple group comparisons performed with the GraphPad Prism program. Non-normally distributed data was examined by Kruskal Wallis and followed with Dunn's multiple comparison test. Meanwhile, normally distributed data were analyzed by one-way ANOVA and followed with Tukey's multiple comparison test. A p-value of <0.05 was considered as statistically significant.

Results

To determine whether oils affect the hematology profile, the 18 parameters value resulted from the hematology analyzer was evaluated. Numerous results of leucocyte absolute cell number and differential cell count of each group of oil shown in Figure 1. Using ANOVA, a significant effect of oil consumption shown in the total WBC number. Post hoc comparisons using Tukey's test pointed out that the total WBC number of used-cooking oil consumption was significantly higher than the control (p=0.0058), fish oil (p=0.0147),

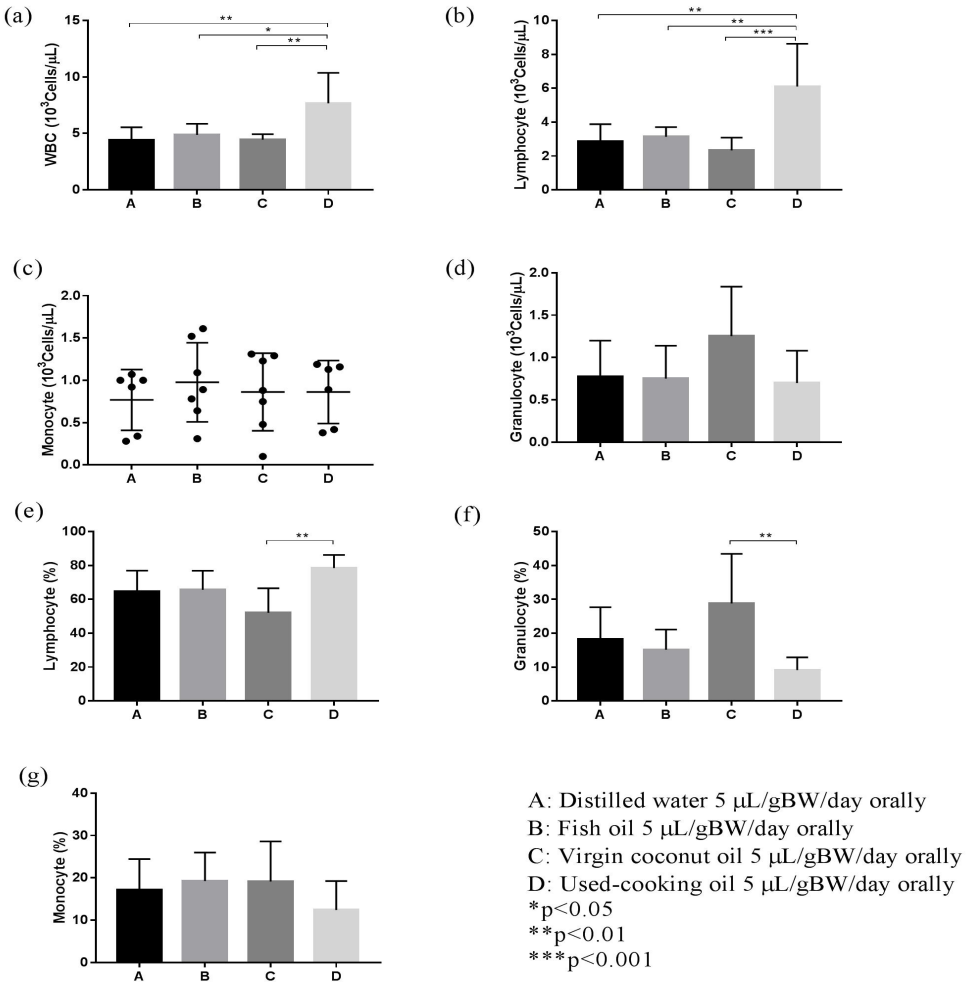


Figure 1 The Effect of Fish Oil, Coconut Oil, and Used-Cooking Oil after 14 Days of Treatment on White Blood Cell Parameters

WBC=White Blood Cell

and coconut oil (p=0.0045) groups (7.66±2.69 vs 4.40±1.13 vs 4.86±0.99 vs 4.43±0.49 10^3 cells/µl respectively). By contrast, the total WBC number of the control group did not significantly differ from the fish oil and virgin coconut oil groups.

The corresponding result can be found on a total lymphocyte number (Figure 1d). The mean value of WBC count of each group was as follows: group A=4.40±1.13 10^3 cells/µl, group B=4.86±0.99 10^3 cells/µl, group C=4.43±0.498 10^3 cells/µl, and group D=7.66±2.69 10^3 cells/µl. Using ANOVA, a significant effect of oil consumption shown in the total lymphocyte numbers (p=0.0004). Post hoc comparisons using Tukey's test pointed out that the total lymphocyte number of used-cooking oil consumption was significantly higher than the control (p=0.0028), fish oil (p=0.0045), and coconut oil (p=0.0004) groups. However, the total lymphocyte number of the control group did not significantly differ from the fish oil and virgin coconut oil groups.

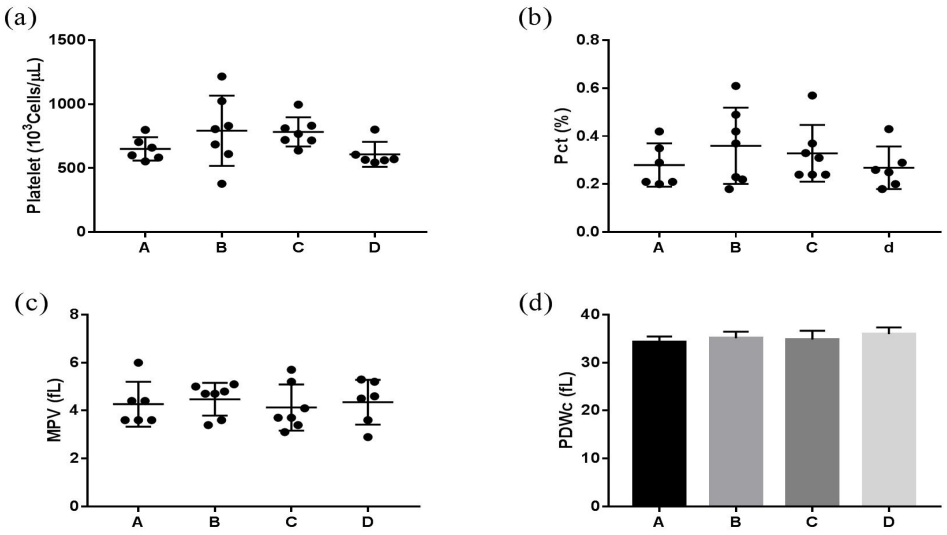
Another significant effects are found in some differential cell counts. Using ANOVA, a significant effect of oil consumption showed in differential granulocyte count (p=0.0093) and differential lymphocyte count (p=0.0062) (Figure 1e and f). Post hoc comparisons using Tukey's test pointed out that differential granulocyte count of the used-cooking oil group was significantly lower than the coconut oil group (9.13±3.74 vs 28.83±14.62 % respectively, p=0.0065). Meanwhile, the differential lymphocyte count of the used-cooking oil group was significantly higher than the coconut oil group (p=0.0031). By contrast, the differential granulocyte count and differential lymphocyte count of other comparisons did not significantly differ from each other.

There was no statistically significant effect discovered on granulocyte, monocyte, and differential monocyte count. However, some differences found in the groups. Figure 1b shows that coconut oil has the highest mean number of granulocyte among the other groups (1.25 ±0.58 10^3 cells/µl), whilst the other has pretty much the same amount of it. Figure 1c points out similar amount of monocyte in the groups, even though the fish oil group has the highest number (0.97±0.46 10^3 cells/µl) and control group has the lowest number (0.76±0.35 10^3 cells/µl). Mice that were given coconut oil has the highest granulocyte percentage (28.83±14.62 %) and used-cooking oil has the lowest granulocyte percentage (9.13±3.74 %) (Figure 1f).

Figure 2 shows the effect of oils on platelet parameters (platelet count, mean platelet volume, platelet crit, and platelet distribution width). It showed the nearly same amount of platelet parameter values resulted from control, fish oil, coconut oil, and used-cooking oil group, even though no significant effect. A similar result can be found in Figure 3 which shows the effect of oils on red blood cell parameters (total red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration). Our study showed that there was no difference in all red blood cells parameters values among the group.

Discussion

NF-kB is a primary regulator of acute and chronic inflammation through increased expression of IL-1, IL-6, and TNF-α in mesenchymal stem cell (MSC). The regulator then inhibits the expression of PPAR-γ associated with MSC adipogenesis reduction. The increase of inflammatory process and low adipogenesis in MSC induce the development of HSC in the bone marrow which can affect peripheral blood parameters. This hematopoiesis takes place particularly in chronic consumption of high fatty acid diet.[26] Short-term consumption of



A: Distilled water 5 µL/gBW/day orally
B: Fish oil 5 µL/gBW/day orally
C: Virgin coconut oil 5 µL/gBW/day orally
D: Used-cooking oil 5 µL/gBW/day orally
*p<0.05
**p<0.01
***p<0.001

Figure 2 The Effect of Fish Oil, Coconut Oil, and Used-Cooking Oil after 14 Days of Treatment on Platelet Parameters

Pct=Plateletcrit; MPV=Mean Platelet Volume; PDWc=Platelet Distribution Width

fatty acid has a different result. In a study conducted by Keisuke Ito, et al. (2012), mice were administered GW (PPAR-δ activator) daily for 28 days. There was no alteration in the proliferation and differentiation of HSC in bone marrow.[2] As in our study, it might be suggested that short term of fatty acid consumption did not activate HSC proliferation and differentiation, resulted in no difference in red blood cell and platelet parameters. Therefore, no significant alteration and effect observed on peripheral blood parameters, except for some leucocyte parameters.

Significant results in some leucocyte parameters in our study are probably influenced by the regulatory mechanism of lipid in the inflammatory reaction. Some lipids such as SFA and different classes of PUFA can induce inflammation that is predominantly signaling by PPARs. It is stimulated by eicosanoid, the product of essential fatty acid metabolism, that has pro-inflammatory effect.[11]

The highest total number of WBC and lymphocyte on the used-cooking oil group among the other groups indicates that the immune system is more active in used-cooking oil consumption. This result corresponds with the previous study, the intake of virgin coconut oil heated 10 times significantly increased the inflammatory biomarkers.[28] The inflammation probably due to the biomarkers and cell & tissue injury caused by reactive oxygen species (ROS) production and antioxidant enzymes deletion in the intake of used-cooking oil contained trans fatty acids (TFA).[28,29]

In this experiment, used-cooking oil was defined as repeatedly heated oil by more than 6 times. The oil underwent a high-temperature deep-frying process. This process makes the

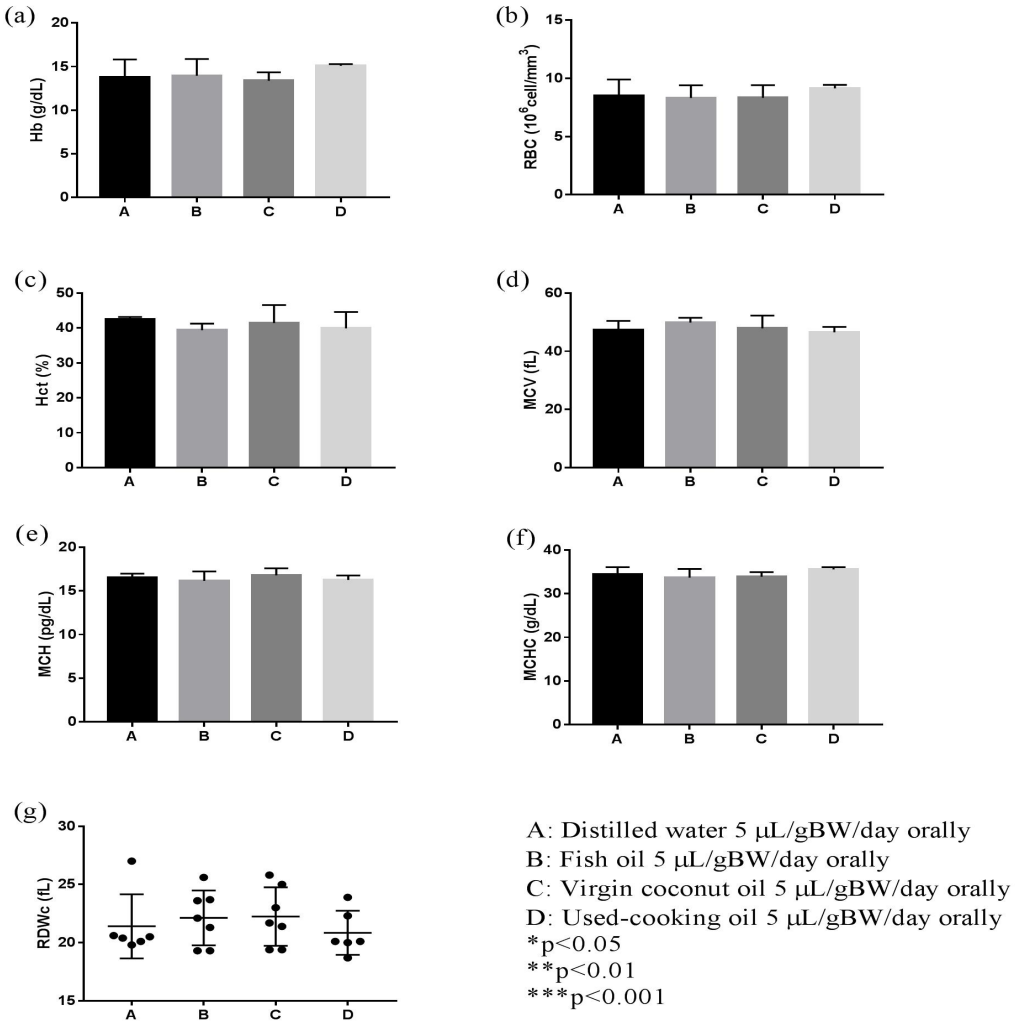


Figure 3 The Effect of Fish Oil, Coconut Oil, and Used-Cooking Oil after 14 Days of Treatment on Red Blood Cell Parameters

Hb=Hemoglobin; RBC=Red Blood Cell; Hct=Hematocrit; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; RDWc=Red Cell Distribution Width

fatty acid susceptible to various chemical reactions such as isomerization and thermal oxidation which produce reactive free radicals.[20,28] Unsaturated fatty acid and cis fatty acid are unstable, thus isomerization can occur easier on them resulting in SFA and TFA, respectively.[3,20] This is corroborated by the enhancement of TFA and SFA on subsequent heating or frying oil.[29] On the other study, the establishment of TFA starts at the second use of cooking oil and it enhances concomitantly with the continous use of oil.[20] In our study, peripheral WBC and lymphocyte number in used-cooking oil consumption had the highest concentration among the other groups.[28] Our results emphasized that direct consumption of used-cooking oil has a maleficent effect on health.

The previous study showed that trans fatty acid play role in cardiovascular and metabolic disease.[14,30] One of the reasons is the high reactivity of the fatty acid that further triggered inflammation process that can contribute to diseases event such as atherosclerosis.[31]

However, previous studies were mostly conducted in the community or clinical setting.[14,32] The activation of the inflammation process, showed by high white blood cells, is already triggered in short-term consumption. It is suggested that the process of metabolic and cardiovascular disease already started from the beginning of the high consumption of trans fatty acids. However, this study did not check the direct impact of used-cooking oil in metabolic or cardiovascular diseases. Further investigation using the animal model in metabolic diseases such as LDLR−/− mice (low-density lipoprotein, LDL, receptor) is suggested to find the correlation between used-cooking oil and metabolic and cardiovascular disease event.

Conclusions

In summary, this study verified that short-term oil consumption affects peripheral leucocyte parameters. Used-cooking oil induces change in hematological profiles compared to fish oil and virgin coconut oil, i.e., increases total white blood cells and lymphocyte and reduces % granulocyte.

Funding

This research was supported by Research Grant from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (No. 3854/UN6.C/LT/2019).

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Rodwell VW, Bender D, Botham KM, Kennelly PJ, Weil PA. Harpers illustrated biochemistry 30th Edition. US: McGraw-Hill Education; 2015.
2. Ito K, Carracedo A, Weiss D, Arai F, Ala U, Avigan DE, et al. A PML–PPAR-δ pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat Med. 2012;18(9):1350–8. doi: 10.1038/nm.2882
3. Whitney EN, Rolfes SR. Understanding Nutrition. 14th Edition. USA: Cengage Learning; 2015.
4. Freeman LR, Haley-Zitlin V, Rosenberger DS, Granholm AC. Damaging effects of a high-fat diet to the brain and cognition: A review of proposed mechanisms. Nutr Neurosci. 2014;17(6):241–51. doi: 10.1179/1476830513Y.000000009
5. Murphy EA, Velazquez KT, Herbert KM. Influence of high-fat-diet on gut microbiota: A driving force for chronic disease risk. Curr Opin Clin Nutr Metab Care. 2015;18(5):515–20. doi: 10.1097/MCO.0000000000000209
6. Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, et al. High-fructose and high-fat diet-induced disorders in rats: Impact on diabetes risk, hepatic and vascular complications. Nutr Metab. 2016;13(1):15. doi: 10.1186/s12986-016-0074-1
7. Ananthakrishnan AN, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Fuchs CS, et al. Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. Gut. 2014;63(5):776–84. doi: 10.1136/gutjnl-2013-305304
8. Li D, Zhang L, Dong F, Liu Y, Li N, Li H, et al. Metabonomic changes associated with atherosclerosis progression for LDLR–/– mice. J Proteome Res. 2015;14(5):2237-54. doi: 10.1021/acs.jproteome.5b00032
9. Wang DD, Hu FB. Dietary fat and risk of cardiovascular disease: Recent controversies and advances. Annu Rev

Nutr. 2017;37:423-46. doi: 10.1146/annurev-nutr-071816-064614
10. Pouzolles M, Ob–roglu L, Taylor N, Zimmermann VS. Hematopoietic stem cell lineage specification. Curr Opin Hematol. 2016;23(4):311–7. doi: 10.1097/MOH.0000000000000260
11. Le Menn G, Neels JG. Regulation of immune cell function by PPARs and the connection with metabolic and neurodegenerative diseases. Int J Mol Sci. 2018;19(6):1575. doi: 10.3390/ijms19061575
12. Innes JK, Calder PC. Omega-6 fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids. 2018;132:41–8. doi: 10.1016/j.plefa.2018.03.004
13. Bird JK, Calder PC, Eggersdorfer M. The role of n-3 long-chain polyunsaturated fatty acids in cardiovascular disease prevention, and interactions with statins. Nutrients. 2018;10(6):775. doi: 10.3390/nu10060775
14. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, et al. Intake of saturated and trans unsaturated fatty acids and risk of all-cause mortality, cardiovascular disease, and type 2 diabetes: Systematic review and meta-analysis of observational studies. BMJ. 2015;351:h3978. doi: 10.1136/bmj.h3978
15. Miyata J, Arita M. Role of omega-3 fatty acids and their metabolites in asthma and allergic diseases. Allergol Int. 2015; 64(1):27–34. doi: 10.1016/j.alit.2014.08.003
16. Sacks FM, Lichtenstein AH, Wu JH, Appel LJ, Creager MA, Kris-Etherton, PM, et al. Dietary fats and cardiovascular disease: A presidential advisory from the American Heart Association. Circulation. 2017;136(3):e1–23. doi: 10.1161/CIR.0000000000000510
17. The ORIGIN Trial Investigators. n–3 Fatty acids and cardiovascular outcomes in patients with dysglycemia. N Engl J Med. 2012;367(4):309–18. doi: 10.1056/NEJMoa1203859
18. Turchini GM, Ng WK, Tocher DR. Fish oil replacement and alternative lipid sources in aquaculture feeds. 1st Ed. Boca Raton: CRC Press; 2010.
19. Zúñiga J, Cancino M, Medina F, Varela P, Vargas R, Tapia G, et al. N-3 PUFA supplementation triggers PPAR-α activation and PPAR-α/NF-κB interaction: anti-inflammatory implications in liver ischemia-reperfusion injury. PloS One. 2011;6(12):e28502. doi: 10.1371/journal.pone.0028502
20. Phang M, Garg ML, Sinclair AJ. Inhibition of platelet aggregation by omega-3 polyunsaturated fatty acids is gender-specific—Redefining platelet response to fish oils. Prostaglandins Leukot Essent Fatty Acidss. 2009;81(1):35–40. doi: 101016/j.plefa.2009.05.001
21. Phang M, Sinclair A, Lincz L, Garg M. Gender-specific inhibition of platelet aggregation following omega-3 fatty acid supplementation. Nutr Metab Cardiovasc Diss. 2012;22(2):109–14. doi: 10.1016/j.numecd.2010.04.012
22. Sansanayudh N, Anothaisintawee T, Muntham D, McEvoy M, Attia J, Thakkinstian A. Mean platelet volume and coronary artery disease: A systematic review and meta-analysis. Int H Cardiol. 2014;175(3):433–40. doi: 10.1016/j.ijcard.2014.06.028
23. Fife B. Health properties of coconut oil. Agro Food Ind Hi Tec. 2013;24(3):7–10.
24. Hunter JE, Zhang J, Kris-Etherton PM. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: A systematic review. Am J Clin Nutr. 2010;91(1):46–63. doi: 10.3945/ajcn.2009.27661
25. Nurul-Iman BS, Kamisah Y, Jaarin K, Qodriyah HMS. Virgin coconut oil

prevents blood pressure elevation and improves endothelial functions in rats fed with repeatedly heated palm oil. Evid Based Complementary Altern Med. 2013;2013:629329. doi: 10.1155/2013/629329

26. Bhardwaj S, Passi SJ, Misra A, Pant KK, Anwar K, Pandey RM, et al. Effect of heating/reheating of fats/oils, as used by Asian Indians, on trans fatty acid formation. Food Chem. 2016;212:663–70. doi: 10.1016/j.foodchem.2016.06.021
27. Cortez M, Carmo LS, Rogero MM, Borelli P, Fock RA. A high-fat diet increases IL-1, IL-6, and TNF-α production by increasing NF-κB and attenuating PPAR-γ expression in bone marrow mesenchymal stem cells. Inflammation. 2013;36(2):379–86. doi: 10.1007/s10753-012-9557-z
28. Hamsi MA, Othman F, Das S, Kamisah Y, Thent ZC, Qodriyah HMS, et al. Effect of consumption of fresh and heated virgin coconut oil on the blood pressure and inflammatory biomarkers: an experimental study in Sprague Dawley rats. Alexandria J Med 2015;51(1):53–63. doi: 10.1016/j.ajme.2014.02.002
29. Srivastava S, Singh M, George J, Bhui K, Saxena AM, Shukla Y. Genotoxic and carcinogenic risks associated with the dietary consumption of repeatedly heated coconut oil. Br J Nutr. 2010;104(9):1343–52. doi: 10.1017/S0007114510002229
30. Lichtenstein AH. Dietary trans fatty acids and cardiovascular disease risk: Past and present. Curr Atheroscler Rep. 2014;16(8):433. doi: 10.1007/s11883-014-0433-1
31. Monguchi T, Hara T, Hasokawa M, Nakajima H, Mori K, Toh R, et al. Excessive intake of trans fatty acid accelerates atherosclerosis through promoting inflammation and oxidative stress in a mouse model of hyperlipidemia. J Cardiol. 2017;70(2):121–7. doi: 10.1016/j.jjcc.2016.12.012
32. Ahmed SH, Kharroubi W, Kaoubaa N, Zarrouk A, Batbout F, Gamra H, et al. Correlation of trans fatty acids with the severity of coronary artery disease lesions. Lipids Health Dis. 2018;17(1):52. doi: 10.1186/s12944-018-0699-3