

## Omega-3 effectiveness against viability extended random skin flap in rats



Rifqi Kurniawan<sup>1\*</sup>, Sitti Rizalijana<sup>2</sup>, Beta Subakti Nata'atmadja<sup>2</sup>

### ABSTRACT

**Background:** Grafts and flaps are commonly used for wound closure which can be caused by trauma, chronic disease, post excision tumors, burns, and infections. Reconstructive can fail because of the poor wound bed, radiation, vascular insufficiency and reperfusion injury (IR). EPA and DHA (omega-3) are new compounds that have a strong effect on controlling inflammation in the resolution phase. Some studies say that EPA and DHA (omega-3) are described not only to lower plasma triglyceride levels but also have anti-inflammatory effects and improve endothelial function, all of which mediate anti-atherogenic effects. This study aimed to prove whether preoperative omega-3 administration could increase the viability of extended random skin flaps.

**Method:** This is an experimental post-test-only control group design. 24 rats were divided into 4 groups. The control group (I and II) only had an extended random skin flap, and the sample was taken on the 3rd day (Group I) and 7th day (Group II) and the treatment group (III and IV). Omega was administered according to the animal dose for 21 days. The extended random skin flap was performed, and the sample was taken 3rd day (Group III) and 7th day (Group IV) and the viability of the extended random skin flap in the study was assessed from the expression of VEGF, the number of capillaries and clinical examination assessed from survival area.

**Results:** Independent T test for each pair in groups I and III showed a significant difference between the mean number of blood vessels in group I and the average number of blood vessels in group III,  $P=0.000$ ,  $p<0.005$ , a significant difference between the mean number of blood vessels in group II and the mean number of blood vessels in group II. group IV blood vessels obtained  $P = 0.000$ ,  $p < 0.005$ . Mann-Whitney test found a significant difference between the intensity of VEGF in groups I and III with a value of  $p=0.031$  ( $p<0.05$ ) and a significant difference in VEGF expression in groups II and IV with  $p=0.038$  ( $p<0.05$ ). The results of the statistical test of survival area independent T-test for pairs in groups I and III obtained  $p: 0.353$  ( $p>0.05$ ), and the Mann-Whitney test for groups II and IV obtained  $p: 0.749$  ( $p>0.05$ ).

**Conclusion:** Consumption of omega 3 oral before surgery increased the viability of the extended random skin flap by histopathologically increasing VEGF expression and capillary count, and omega 3 oral administration before surgery did not increase the viability of the extended random skin flap assessed from the survival area on the first week.

**Keywords:** Extended random skin flap, Omega 3, the viability of flap.

**Cite This Article:** Kurniawan, R., Rizalijana, S., Nata'atmadja, B.S. 2022. Omega-3 effectiveness against viability extended random skin flap in rats. *Bali Medical Journal* 11(2): 813-817. DOI: 10.15562/bmj.v11i2.3471

<sup>1</sup>Resident of Plastic Reconstructive and Aesthetic Surgery Department, Faculty of Medicine, Universitas Airlangga, East Java, Surabaya, Indonesia;

<sup>2</sup>Plastic Reconstructive and Aesthetic Surgery Department, Faculty of Medicine, Universitas Airlangga, East Java, Surabaya, Indonesia;

\*Corresponding author:

Rifqi Kurniawan;

Resident of Plastic Reconstructive and Aesthetic Surgery Department, Faculty of Medicine, Universitas Airlangga, East Java, Surabaya, Indonesia;

[rifqibeeboy@gmail.com](mailto:rifqibeeboy@gmail.com)

Received: 2022-06-25

Accepted: 2022-08-01

Published: 2022-08-14

### INTRODUCTION

Grafts and flaps are commonly used for wound closure which can be caused by trauma, chronic disease, post excision tumors, burns, and infections. Despite careful planning and execution, Reconstructive can fail because of the poor wound bed, radiation, vascular insufficiency and reperfusion injury (IR). These recurring complications can make in tissue loss, costly surgery, additional costs that must be paid due to the operation and length of treatment carried out, and negative psychosocial effects on the patient.<sup>1</sup> Therefore, prophylactic

measures to prevent flap failure will be urgently needed in reconstructive surgery.

Flaps have their own blood supply. Flaps are usually required to cover poorly vascularized recipient areas, cover vital structures, and reconstruct eyelids, lips, and ears. Nose and cheeks, and provide padding for prominent body parts. The flap was chosen because of its better aesthetic results, for example, a defect in the nose can be closed with a skin graft, but it will leave a surgical scar that looks like a patch. Random skin flaps may require incisions around adjacent nasal tissues but are aesthetically preferable in the long run. There is no better tissue to replace nasal

tissue than the nose tissue itself.

Random skin flaps consist of skin and subcutaneous tissue transferred from one part of the body to another by vascular pedicles attached to the body that serve as a food supply. Proper flap planning is critical to the success of the operation. All possible donors for the flap should be considered as the most suitable option that can be selected. Reverse flap planning is an important principle. The pattern of the defect is transferred to a piece of cloth or paper then the steps in the surgical procedure are performed in reverse order. This pattern is used until the donor site reaches the defect. The flap is designed to

be slightly longer than required, as some of the lengths will be lost in the rotation process, and slight bending and tension can avoid kinking the flap's blood supply. The process is repeated, ensuring each time the pedicle is held in a fixed position and not allowed to shift with the flap. The principle is to measure twice and cut once because it's easier to cut a slightly long flap than it is to add a small one.<sup>2</sup>

Polyunsaturated fatty acids (PUFA) are long chains of carbon with two or more double bonds. The two main omega-3 long-chain PUFAs are docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). Supplementation with omega-3 PUFAs has been reported to have several beneficial effects on cardiovascular diseases, such as reducing cardiovascular mortality, improving lipid profile, anti-inflammatory, antiarrhythmic, decreasing thromboxane levels, and vasodilating mechanisms. Omega-3 polyunsaturated fatty acids (PUFA) must be obtained from the diet, such as from fish oil and such as flaxseed or walnuts. The American Heart Association and the National Heart Foundation of Australia recommend a dose of DHA and EPA of 1,000 mg/day.<sup>3</sup>

EPA and DHA (omega-3) are new compounds that have a strong effect on controlling inflammation in the resolution phase. At the cellular level, it can reduce neutrophil infiltration and regulate the regulation of cytokines and ROS (reactive oxygen species), as well as decrease the magnitude of the inflammatory response.<sup>4</sup>

Omega-3 polyunsaturated fatty acids come from certain fish species. These fish accumulate omega-3 fatty acids by consuming microalgae, plankton, or other small fish. Cold-water fish, such as salmon, herring, mackerel, anchovies, and sardines, are examples of fish with the highest levels of omega-3 fatty acids per gram. Significantly increased levels of omega-3 fatty acids in blood serum have been shown in humans, especially after eating salmon.<sup>5</sup>

Polyunsaturated fatty acids (PUFA) can also reduce the activation of the NF- $\kappa$ B pathway, reducing the production of proinflammatory mediators (cytokines) in contrast to the omega-6 fatty acid, arachidonic acid (AA), which is known as

a stimulator of NF- $\kappa$ B activity. They also prevent the degradation and subsequent translocation of the NF- $\kappa$ B complex to the nucleus, where it induces transcription of proinflammatory cytokines.<sup>6</sup>

The above data is also strengthened by the researchers who concluded that EPA and DHA reduce oxidative stress-induced DNA damage in vascular endothelial cells through NRF2-mediated regulation of antioxidant responses. This confirms the statement that omega-3 can be said to be an antioxidant.<sup>7</sup>

Fish oil has been used to treat a number of conditions, including asthma, diabetes mellitus, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, hyperlipidemia and cardiovascular disease. With regard to cardiovascular disease, fish oil is most often used to treat hypertriglyceridemia. When doctors refer to the use of fish oil, they are generally referring to omega-3 fatty acids, also known as polyunsaturated fatty acids (PUFAs). These specific omega-3 fatty acids include docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 22:5n-3). For the most part, neither DHA nor EPA cause clinically relevant side effects but do affect platelet function, so some clinicians feel omega 3 may pose a greater risk of bleeding, especially during surgical procedures or when administering other drugs known to affect platelet coagulation and aggregation.<sup>8</sup>

Some studies say that EPA and DHA (omega-3) are described not only to lower plasma triglyceride levels but also have anti-inflammatory effects and improve endothelial function, all of which mediate anti-atherogenic effects,<sup>7</sup> although omega-3 fatty acids can reduce platelet aggregation, omega-3 fatty acids can reduce platelet aggregation. Omega-3 does not contribute directly to increased bleeding, and then the recommendation states that it is not necessary to discontinue diet omega-3 before surgery; it is hoped that omega-3 can increase the viability of the extended random skin flap.<sup>9</sup>

The aim of this study was to prove whether preoperative omega-3 administration could increase the viability of extended random skin flaps.

## METHOD

This is an experimental posttest-only control group design. Omega-3 orally is an independent variable, and the dependent variable is the viability of extended random skin flap as seen from VEGF expression, number of capillaries expressed on immunohistochemical examination and percentage of survival area.

24 rats were divided into 4 groups, experimental animals in this study were white male rats (*Rattus norvegicus*) Wistar strain, healthy, 2-3 months old and weighing 250-350 grams. The control group (I and II) only had an extended random skin flap, and the sample was taken on the 3rd day (Group I), the other sample was taken on the 7th day (Group II) and the treatment group (III and IV). Omega-3 was administered according to the animal dose for 21 days and performed extended random skin flap, and the sample was taken 3rd day (Group III) and 7th day (Group IV).

The basic design for an extended random skin flap is a rectangular flap, the elevation of the skin and subcutaneous tissue on the rat's back with a length: width ratio of 6:2 with a pedicle on the cranial side of the rat.

Omega-3 used in this study is Omega-3 capsules with the brand Omeheart produced by Robinson Pharma Inc. Omega-3 was given for 21 days before the extended random skin flap was performed with doses of 3.2g EPA and 2.2g DHA, adjusted for animal doses. After three weeks of supplementation with 3.2g EPA and 2.2g DHA, an increase in the EPA content of neutrophils and monocytes was reported. The anti-inflammatory effect of fish oil is partially mediated by inhibiting the 5-lipoxygenase pathway in neutrophils and monocytes and inhibiting leukotriene B4 (LTB4)-a mediated function of leukotriene B5 (LTB5). In addition, omega-3 lowering interleukin IL-1 and IL-6 inhibits inflammation.<sup>10</sup>

The specimen is taken with a size of 1 x 1 cm at the distal part of the flap. Then the rats were sacrificed by means of decapitation, and then specimens were then given Hematoxylin-eosin (HE) staining to assess the number of capillaries

and immunohistochemical staining using VEGF antibodies under a light microscope to assess the amount of growth factor in the specimen.

The survival area is a viable flap area. Viable areas were observed visually. The compromised area is seen from the brownish or grayish color of the flap, and the necrotic area is seen from the black and hard flap. The survival area was calculated using the application on a cm<sup>2</sup> scale, and the percentage of survival area to the flap area was calculated using the *imitowound-digital woundcare* version 2.0.6 application.

## RESULTS

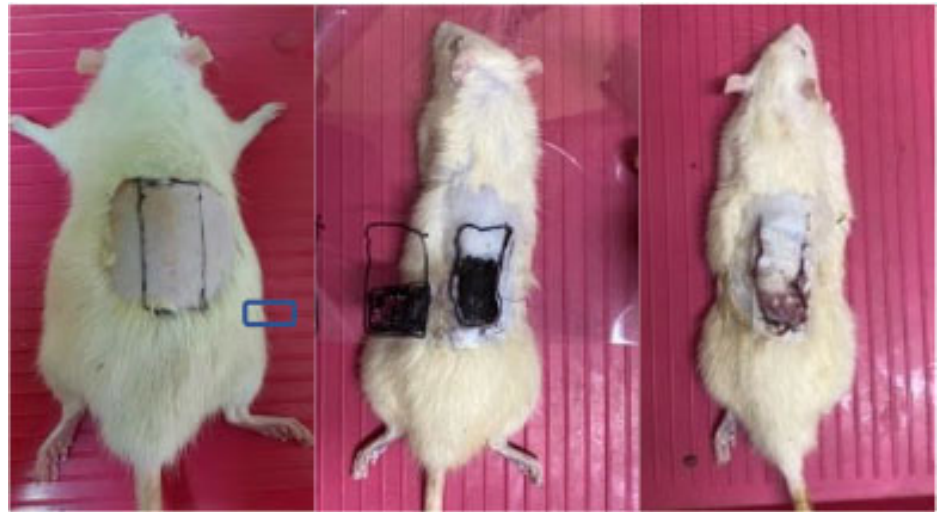
Experimental animals in this study weighed at least 250 grams, and no experimental animals died during the research process (Figure 1). The research is located in the laboratory of the Faculty of Veterinary Medicine and the Laboratory of Anatomical Pathology, Universitas Airlangga, Surabaya.

### Number of capillaries

The number of capillaries was examined by hematoxylin-eosin staining and assessed by independent reviewers under 400x magnification on each preparation. Histopathological pictures of capillaries in the control group and the treatment group on day 3 and day 7 are shown in the image below (Figure 2).

The homogeneity test was carried out on test groups I and III,  $p = 0.205$  ( $p > 0.05$ ), in test groups II and IV,  $p = 0.951$  ( $p > 0.05$ ) and in test groups III and IV,  $p: 0.951$  ( $p > 0.05$ ) so that it can be concluded that all data are homogeneous and the independent T test can be performed parametrically (Table 1).

The results of the independent T test for each pair in groups I and III showed a significant difference between the mean number of blood vessels in group I and the average number of blood vessels in group III,  $P=0.000$ ,  $p<0.005$ , a significant difference between the mean number of blood vessels in group II and the mean number of blood vessels in Group IV blood vessels obtained  $P=0.000$ ,  $p<0.005$  and the significant difference between the mean number of blood vessels in group III and the mean number of blood vessels in



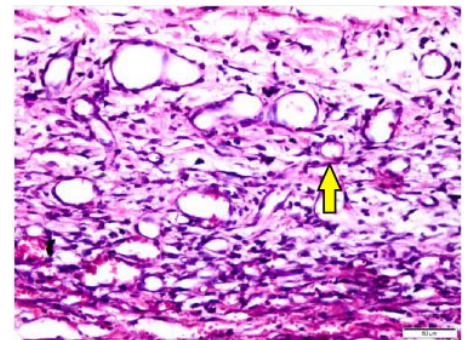
**Figure 1.** Flap elevation, survival area, marked with transparent paper and sample on the distal flap.

group IV were  $P=0.000$ ,  $p<0.005$ . Thus the mean number of blood vessels in group III was significantly more than in group I, the average number of blood vessels in group II was significantly more than in group IV, and the average number of blood vessels in group IV was significantly more than in group III.

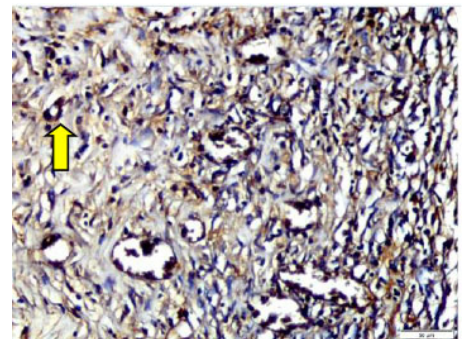
### VEGF Expression

Vascular endothelial growth factor (VEGF) is the most potent angiogenic agent. Its receptors are found only on endothelial cells and are expressed under hypoxic conditions following endothelial damage. Vascular endothelial growth factor (VEGF) has been shown to increase blood flow and skin flap survival in mice.<sup>11</sup>

Immunohistochemical staining for vascular endothelial growth factor antibodies in this study used an antibody reagent for mice VEGF (C-1):sc-7269 produced by Santacruz biotechnology. Inc. The staining process is in accordance with the protocol at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga. Readings by reviewers using a microscope and calculating the average percentage of VEGF expression from 5 fields of view, then calculating the multiplication between the percentage score of cells/immunoreactive areas and the color intensity score. Assessment of the graded intensity score are 0 (negative, there is no brown color), 1 (weak, light brown), 2 (moderate, brown), and 3 (strong, dark



**Figure 2.** The vascular endothelium (arrows), 400x magnification, HE staining in the treatment group.



**Figure 3.** Microscopic image of IHC with VEGF antibody, strong intensity in the cytoplasm of vascular endothelial cells in this study with 400x magnification

brown) (Figure 3).

VEGF overexpression was assessed from the results of brown staining on

**Table 1. The average number of capillaries.**

	Control	Treatment	p
Day 3	13,17 ± 1,04	34,23 ± 1,84	0,00
Day 7	27,83 ± 3,94	52,17 ± 4,24	0,00

**Table 2. VEGF Expression.**

	Control	Treatment	p
Day 3	4,42	8,58	0,031
Day 7	4,50	8,50	0,038

**Table 3. Survival area.**

	Control	treatment	p
Day 3	9,37 ± 3,54	7,60 ± 2,69	0,095
Day 7	7,48 ± 2,28	8,24 ± 3,53	0,467

the cytoplasm. Assessment of VEGF immunoreactivity is assessed by adding up the results of the area score with the intensity score so that the VEGF immunoreactivity score is obtained.<sup>12</sup>

Non-parametric statistical tests were conducted to determine whether there were significant differences between each control and treatment (groups I and III, II and IV, III and IV).

Mann-Whitney test found a significant difference between the intensity of VEGF in groups I and III with a value of  $p=0.031$  ( $p<0.05$ ) and a significant difference in the intensity of VEGF in groups II and IV with  $p=0.038$  ( $p<0.05$ ), but there was no difference in the intensity of VEGF in the treatment groups III and IV  $p=0.338$  ( $p>0.05$ ) (Table 2). Thus, the intensity was stronger in subjects receiving omega 3 (Groups III and IV) compared to the control group (Groups I and II).

### Survival Area

The Kolmogorov-Smirnov normality test was found in group I and III tests  $p = 0.095$  ( $p> 0.05$ ), normal data distribution with homogeneous data  $p: 0.467$  ( $p> 0.05$ ) so that the independent T test could be performed parametrically. The results of the independent T-test for pairs in groups I and III obtained  $p: 0.353$  ( $p>0.05$ ), so there was no significant difference between the mean survival area of group I (control) and group III (treatment).

The Kolmogorov-Smirnov normality test was obtained in the group II and IV tests, and the p-value was obtained:  $0.005$  ( $p<0.05$ ) so that the data was not normally distributed, so the non-parametric Mann-

Whitney test was carried out, and the results were  $p: 0.749$  ( $p>0.05$ ), so it can be concluded that there is no significant difference in the area of survival in group II (control) and group IV (treatment) (Table 3).

## DISCUSSION

The data and analysis in this study showed a significant difference in the number of capillaries per field of view and the intensity of VEGF between the control group (I and II) and the treatment group (II and IV). Both treatment groups were jointly made a random flap with the same method and technique, but groups III and IV received the same dose of oral omega-3, while groups I and II did not. VEGF is directly or indirectly implicated as a significant factor in wound healing as early as possible after trauma. Induced by inflammatory cells and local conditions in the wound, VEGF has the potential to alleviate tissue hypoxia and metabolic deprivation by promoting early angiogenesis and endothelial cell function. Maximum activity occurs in the windows period of about 3 to 7 days after injury. When the wound occurs, granulation angiogenesis stops and the blood vessels as endothelial cells undergo apoptosis.<sup>13</sup>

Damage to the vessel wall causes platelets to adhere to the exposed subendothelium, become activated, and release platelet-activating mediators such as thromboxane A2 (TxA2) and adenosine diphosphate (ADP) that amplify the platelet response induced by agonists such as collagen.<sup>14</sup>

When DHA and EPA production is increased, it causes the production of prostaglandin I2 (PGI2 or prostacyclin), which has vasodilating and local antiplatelet effects also increases and TXA2 production decreases where TXA2 functions to activate platelets. It is important to note that this process also occurs in the vascular endothelial layer leading to an imbalance in the formation of antiplatelet eicosanoids (PGI2) with TXA2.<sup>15</sup>

The action of omega-3 fatty acids is also able to suppress platelet-activating factor (PAF), which is a strong platelet activator so that it reduces platelet aggregation and causes an increase in bleeding time, a reduction in ADP (adenosine diphosphate), collagen and epinephrine.<sup>16</sup> So histopathologically, it showed a significant difference in the number of capillaries per field of view and the intensity of VEGF between the control group (I and II) and the treatment group (II and IV).

Omega 3 was found to increase the viability of the extended random skin flap from a histopathological perspective, but there was no significant difference in the mean survival area of control (groups I and II) and treatment (groups III and IV) on the first week, this could be due to the calculation of survival area involving compromised to necrotic areas were defined as bluish, gray to blackish areas on the distal side of the flap, where there may be capillaries and VEGF expression formed where receptors are found only on endothelial cells and are expressed in hypoxic conditions following endothelial damage. And VEGF expresses developing blood vessels, and their receptors are found exclusively on endothelial cells. When tissues are subjected to hypoxia or endothelial damage, VEGF protein expression is regulated to endothelial cells.<sup>11</sup> Maximum activity occurs in the windows period of about 3 to 7 days after injury.<sup>13</sup>

However, the first to the third day of the flap is characterized by an increase in the number and caliber of blood vessels. Furthermore, until the first week, there is an increase in the size and reorientation of blood vessels. In the second to the third week, there was no significant increase

in blood vessels. At the beginning of the second week, there is a progressive regression, and by the fourth week, all blood vessel diameters begin to shrink.<sup>17</sup> So that the demarcation of the necrotic area is more clearly visible if the measurement is carried out in the second week (H-14) when there is no longer an increase in the number of blood vessels and progressive regression of blood vessels occurs.

The limitation of this research is the use of tools to calculate survival area, which is used as an application, not a medical device that has been verified.

## CONCLUSION

Omega 3 oral before surgery increased the viability of the extended random skin flap histopathologically by increasing VEGF expression and capillary count, and administration of oral omega 3 before surgery has not increased the viability of the extended random skin flap assessed from the survival area in the first week.

Suggestions for further research are conducting a similar study with sampling to calculate the mean survival area carried out in the second week, where the second to the third week, there was no longer an increase in the number of blood vessels, and there was a progressive regression of blood vessels.

## RESEARCH ETHICS

This research has been approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga with ethic number 2.KE.128.11.2021 and has carefully studied the proposed animal use protocol.

## CONFLICT OF INTEREST

There is no conflict of interest in writing this research report.

## FUNDING

This research was conducted without grants, sponsors, or other sources of funding.

## AUTHOR CONTRIBUTION

All authors have the same contribution in writing the report on the results of this study, from the stage of proposal preparation, data search, and data analysis, to the interpretation of research data and presentation of the final report.

## REFERENCES

- Francis, A., & Baynosa, R.C. Hyperbaric oxygen therapy for the compromised graft or flap. *Advance in Wound Care*. 2017;6(1). pp. 23–32. <https://doi.org/10.1089/wound.2016.0707>.
- Thorne, C.H. Techniques and principles of plastic surgery. In: C.H. Thorne, K.C. Chung, A.K. Gosain, G.C. Gurtner, B.J. Mehrara, J.P. Rubin, & S.L. Spear, eds. *Grabb & Smith's Plastic Surgery*, 7th edn. Philadelphia: Lippincott Williams & Wilkins Publisher. 2004. pp. 1–12.
- McEwen, B.J., Morel-Koop, M.C., Chen, W., Tofler, G.H., Ward, C.M et al. Effects of omega-3 polyunsaturated fatty acids on platelet function in healthy subjects and subjects with cardiovascular disease. *Seminars in Thrombosis & Hemostasis*. 2013;39(1). pp. 25–32. PMID: 23329646. <https://doi.org/10.1055/s-0032-1333309>.
- Serhan, C.N., Hong, S., Gronert, K., Colgan, S.P., Devchand, P.R., Mirick, G., Moussignac, R.L. et al. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *Journal of Experimental Medicine*. 2002;196(8). pp. 1025–1037. <https://doi.org/10.1084/jem.20020760>.
- Gajos, G., Rostoff, P., Undas, A., & Piwowarska, W. Effects of polyunsaturated omega-3 fatty acids on responsiveness to dual antiplatelet therapy in patients undergoing percutaneous coronary intervention: the OMEGA-PCI (OMEGA-3 fatty acids after PCI to modify responsiveness to dual antiplatelet therapy) study. *Journal of the American College of Cardiology*. 2010;55(16). pp. 1671–1678. <https://doi.org/10.1016/j.jacc.2009.11.080>.
- Jeromson, S., Gallagher, I.J., Galloway, S.D., & Hamilton, D.L. Omega-3 fatty acids and skeletal muscle health. *Marine Drugs*. 2015;13(11). pp. 6977–7004. PMID: 26610527. <https://doi.org/10.3390/md3116977>.
- Sakai, C., Ishida, M., Ohba, H., Yamashita, H., Uchida, H., Yoshizumi, M., Ishida, T. et al. Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells. *PLoS One*. 2017;12(11). p. e0187934. PMID: 29121093. <https://doi.org/10.1371/journal.pone.0187934>.
- Bays, H.E. Safety considerations with omega-3 fatty acid therapy. *American Journal of*

- Cardiology*. 2007;99(6A). pp. 35C–43C. <https://doi.org/10.1016/j.amjcard.2006.11.020>.
- Wachira, J.K., Larson, M.K., & Harris, W.S. n-3 fatty acids affect haemostasis but do not increase the risk of bleeding: clinical observations and mechanistic insights. *British Journal of Nutrition*. 2014;111(9). pp. 1652–1662. <https://doi.org/10.1017/S000711451300425X>.
- Simopoulos, A.P. Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. *World Review of Nutrition and Dietetics*, 2003; 92: pp. 1 – 22. PMID: 14579680. <https://doi.org/10.1159/000073788>.
- Kryger, Z., Zhang, F., Dogan, T., Cheng, C., Lineaweaver, W.C., Buncke, H.J. et al. The effects of VEGF on survival of a random flap in the rat: examination of various routes of administration. *British Journal of Plastic Surgery*. 2000;53(3). pp. 234 – 239. PMID: 10738331. <https://doi.org/10.1054/bpjs.1999.3315>.
- Kato, Y., Nishihara, H., Mohri, H., Kanno, H., Kobayashi, H., Kimura, T., Tanino, M., Terasaka, S. Tanaka, S. et al. Clinicopathological evaluation of cyclooxygenase-2 expression in meningioma: immunohistochemical analysis of 76 cases of low and high grade meningioma. *Brain Tumor Pathol Springer*. 2014;(31). pp. 23–30. doi:10.1007/s10014-012-0127-8.
- Bao, P., Kodra, A., Tomic-Canic, M., Golinko, M.S., Ehrlich, H.P., Brem, H. et al. The role of vascular endothelial growth factor in wound healing. *Journal of Surgical Research*. 2009;153(2). pp. 347–358. <https://doi.org/10.1016/j.jss.2008.04.023>.
- Hechler, B., Cattaneo, M., & Gachet, C. The P2 receptors in platelet function. *Seminars in Thrombosis & Hemostasis*. 2005;31(2). pp. 150–161. <https://doi.org/10.1055/s-2005-869520>.
- DeCaterina, R., Giannessi, D., Mazzone, A., Bermi, W., Lazzarini, G., Maffei, S., Cerri, M., Salvatore, L., Weksler, B. et al. Vascular prostacyclin is increased in patients ingesting omega-3 polyunsaturated fatty acids before coronary artery bypass graft surgery. *Circulation*. 1990;82(2). pp. 428–438. <https://doi.org/10.1161/01.cir.82.2.428>.
- Goodnight Jr., S.H., Harris, W.S., & Connor, W.E. The effects of dietary omega 3 fatty acids on platelet composition and function in man: a prospective, controlled study. *Blood*. 1981;58(5). pp. 880–885.
- Hoopes, J.E. Pedicle flaps – an overview. In: T.J. Krizek, & J.E. Hoopes, eds. *Symposium on Basic Science in Plastic Surgery*, Vol. 15, St Louis: Mosby. 1976. pp. 241 – 259.



This work is licensed under a Creative Commons Attribution