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Original Article

Effects of dietary n-3/n-6 fatty acid content on post-operative adhesions in myocardial infarction mice

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SUMMARY

Background and aim: Adhesions after thoracic surgery led to more difficult sternal re-entry and surgical dissection, blunted visibility of mediastinal tissues, potential injury to cardiovascular structures, an increased risk of surgical bleeding, and more time-consuming procedures.
Recent molecular and cell biology studies have shown that n-3 unsaturated fatty acids exert pleiotropic effects on vascular endothelial cells, inflammatory cells, and platelets, suppress lesion formation and plaque instability, prevent recurrence of vascular events and cardiac death in patients with ischemic heart disease. Since around 2010′, attention has been focused on the n-3 fatty acids and DHA of edible “seaweed oil” extracted from algae. However, no previous studies have reported how dietary containing marine algae oil and nutritional oral taking habits affect post-operative adhesions.
The purpose of this study was to investigate the effects of diets with different n-3/n-6 fatty acid content on adhesions in thoracic cavity.
Methods: Four-week-old mice were randomly divided into three groups and housed in separate cages. The groups of animals were continuously provided with three different diets for 1 month (the Control group (n=4), DHA-containing “marine algae oil” diet; the DHA group (n=4), the HF group (n=5)). MI model mice were induced by a trained animal surgical expert. After operation,

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evaluated items were 1) body weight, 2) echocardiography for cardiac function, and 3) adhesions. Evaluations of 1) and 2) were performed before MI preparation, 1 day, and 7 days after MI, and assessment of 3) was evaluated the adhesion at 7 days after MI.
Results: The results were 1) there were no significant differences in body weight among the three groups, 2) cardiac function in the HF group tended to deteriorate, and on post-operative day 7, the DHA group tended to deteriorate less than the Control group and 3) adhesions tended to be worse in the HF group and mild in the DHA group.
Conclusion: Adhesions tended to be the worst in the HF group and mild in the DHA group. The results in this study indicated that the type of diet affects adhesion levels following MI.

Introduction

Adhesions after thoracic surgery led to more difficult sternal re-entry and surgical dissection, blunted visibility of mediastinal tissues, potential injury to cardiovascular structures, an increased risk of surgical bleeding, and more time-consuming procedures [1]. For patients undergoing surgery, physical distress caused by symptoms or secondary complications can be a factor that negatively affects the patient's psychiatric condition and quality of life. The hypothesis generation study showed preliminary evidence that increased coronary artery bypass grafting (CABG) wasting may be associated with pathogen burden, inflammation, and endothelial activation [2]. Adhesion prevention in MI patients is important to avoid adverse events [3].

Adhesion formation is a process in which organs and tissues that are distant from each other adhere due to an inflammatory reaction caused by surgery [3]. The pathogenesis of postsurgical adhesions is a multistep process, and the key factors are loss of mesothelial cells, accumulation of fibrin in areas devoid of mesothelial cells, loss of normal pericardial fibrinolysis, and local inflammation [4]. And there is a growing body of evidence suggesting that alterations in macrophage function are involved in the pathophysiology of adhesion formation. It is known that the development of adhesions may be the result of an inflammatory process [5]. To avoid adverse events, it is important to conduct surgical procedures carefully [3,6–13], and uppression of cardiac fibroblast activity can be achieved by administering substances [3,7,8].

In the other hands, the risk of myocardial infarction increases in the presence of coronary risk factors such as obesity, glucose or metabolic disorders, and dyslipidemia attributable to Western diet and aging [14–16]. In recent years, polyunsaturated fatty acids have attracted attention from a dietary [17] and the n-3 series eicosapentaenoic acid (EPA) in fish is thought to suppress inflammation and arteriosclerosis, and the n-6 series arachidonic acid (AA) produces an inflammatory effect [18]. Also, long-term administration of EPA has been shown to improve the prognosis of cardiac remodeling after chronic MI by regulating the activity of pro-inflammatory M1 macrophages [19–23].

Recent molecular and cell biology studies have shown that n-3 unsaturated fatty acids exert pleiotropic effects on vascular endothelial cells, inflammatory cells, and platelets, suppress lesion formation and plaque instability, prevent recurrence of vascular events and cardiac death in patients with ischemic heart disease [24–32]. Since around 2010′, attention has been focused on the n-3 fatty acids and DHA of edible “seaweed oil” extracted from algae, because fish oil is a limited resource and fish cannot produce large amounts of long-chain omega-3 fatty acids [33–35]. However, no previous studies have reported how dietary containing marine algae oil and nutritional oral taking habits affect post-operative adhesions.

The purpose of this study was to investigate the effects of diets with different n-3/n-6 fatty acid content using algae oil on adhesions developed at the MI site in mice.

Materials and methods

Ethical issues

Animal welfare and animal protection were considered in the experimental design and the minimal number of animals was used to meet the purpose of the study. The experimental protocol incorporated ethical considerations and pain was minimized by the use of anesthesia. In addition, breeding management was performed in an appropriate environment (Yokohama City University Animal Experiment Committee : Approval number F-A-15-04, redacted).

Animals and diet characteristics

Eight-week-old male mice C57BL/6 (Japan SLC inc.) were used in this study. Four-week-old animals were purchased for pre-administration of three kinds of diet with different fatty acid content, which were fed to each group until the age of eight weeks. Thereafter, the mice (the Control group, the DHA-containing diet (DHA) group, the high-fat diet (HF) group; n = 4, 4, 5, respectively) were subjected to MI. The MI model was adopted because it results in the artificial induction of adhesions/wounds, which enables the study of adhesion formation and resolution.

Test diets

The dietary composition was based on AIN-93G. The approximate compositions of the test diets are shown in Table 1. An oil extracted from the cultured microalgae *Aurantiochytrium limacinum* (NIES-3737 strain) was used as the DHA source. The algae, a generous gift from Euglena Co. Ltd, was lyophilized and the lipid was extracted using the Bligh and Dyer method [36]. The extracted lipid contained about 30% (w/w) DHA. The compositions of the major fatty acids in the test diet are shown in Table 1.

Procedures and data collection

Feeding method

Four-week-old mice were randomly divided into three groups and housed in separate cages. The groups of animals were continuously provided with three different diets (the Control group, the DHA group, and the HF group) for 1 month. For each cage, 100 g of diet was prepared and changed twice a week. Filtered tap water in a water bottle and each kind of diet were freely available. Table 1 shows the characteristics of the feeding.

Table 1
Fatty acid composition and characteristics of each diet （g/kg）

	Control *[1]	DHA *[2]	HF *[3]
b-Cornstarch*	397	397	210
Casein*	200	200	200
a-Cornstarch*	132	132	60
Sucrose**	100	100	100
Soybean Oil***	70	26.4	70
Algal oil****	0	43.6	0
Beef tallow***	0	0	259
Cellulose Powder*	50	50	50
AIN-93G Mineral Mix.*	35	35	35
AIN-93G Vitamin Mix.*	10	10	10
L-Cystine***	3	3	3
Choline Bitartrate***	2.5	2.5	2.5
t-BHQ***	0.014	0.014	0.014

*[1]Standard diet for mice with corn starch.
*[2] NIES-3737 Oil-rich DHA-rich diet.
*[3] High-fat diet containing beef tallow.

Method for producing MI model mouse

MI model mice were induced by a trained animal surgical expert referring to a previously published method [37].

1) Put the mouse in a small container and weigh it, and then, start anesthesia with isoflurane at a concentration of 4% (200 ml/min) and reduce the dose to 3.5%, 3.0%, 2.5%, 2.0% every 30 seconds.
2) Fix the mouse on the treatment table and intubate the outer cylinder of the indwelling needle (20G) except awakening (about 15–30 seconds).
3) Confirm the synchronization of respiration and the disappearance of pain reflex with an artificial respirator (SN-480-7 manufactured by Shinano Co., Ltd.) connected to a vaporizer with 2.0% (-1.5%) anesthesia.
4) When opening the chest, use a thoracotomy device to open the 4th intercostal space, and then ligate left anterior descending coronary artery (LAD) using 7–0 nylon ligature at the point about 1–1.5 mm from the starting root of LAD without injuring LAD.
5) When closing the chest, ligate the intercostal space with 1 needle with 5–0 nylon thread, and then ligate the skin with 3 needles with 5–0 nylon thread. Turn off anesthesia, check spontaneous breathing, remove the ventilator, and extubate.
6) Wait for recovery on a hot sheet, and put it in a newly prepared cage after awakening.

Parameters evaluated

The animals were evaluated for 1) body weight, 2) echocardiography for cardiac function, and 3) adhesions. Evaluations of (1) and (2) were performed before MI preparation, 1 day after MI treatment, and 7 days after MI treatment, and assessment of (3) was evaluated the adhesion at 7 days post-MI by evaluation index of Izumi *et al.* [38].

Echocardiographic assessment [38,39]

Echocardiographic assessment was conducted to determine infarct size. The specific echocardiography method is as follows. Each mouse is weighed and placed in an anesthesia chamber where they were anesthetized with isoflurane of 4.0% (200ml/minute) at the starting point and decreased each 0.5% per 30 seconds till 2.0%. Following confirmation of anesthesia, the mouse is secured to the surgical table with tape and continuously provided with 1.0–1.5% isoflurane via a nasal mask. The animals were placed on the table so that the torso is on the midline, with the limbs spread apart. One joint forefinger less smell hair removal cream was spread and keep a few minutes on the chest, and the hair is removed from the chest. Echocardiography was then performed on the unconscious animal. Long-axis cross-sectional images of the left ventricle were obtained and measured values were obtained from the M-mode screen. A heart rate of 450–550 beats/min was maintained by adjusting the anesthetic concentration accordingly. After completion of echocardiography, the mice were allowed to regain consciousness on a heating plate prior to being returned to their home cage.

Evaluation by masson trichrome staining

Seven days after the MI procedure, the heart was dissected, fixed with formalin and alcohol, paraffin embedded, and tissue slices were prepared. Fibrosis at the MI site was visually confirmed and evaluated by Masson trichrome staining, thereby confirming the infarction.

Evaluation of adhesions (adhesion scale, izumi et al.*, 2012) [38]*

Adhesions were evaluated according to the evaluation index (adhesion severity: a modified version of the classification) of Izumi *et al.* [38]. In this evaluation, the degree of adhesion is judged based on whether blunt or sharp peeling is possible and the ratio relative to the length of thoracotomy.

Data analysis

After the collected echo data were entered into database sheet, a two-group of all combinations of EF in the Control, the DHA and the HF groups using the Mann-Whitney U test and three-groups comparison using the Kruskal-Wallis test were formed respectively. As there were no significant differences, average reference values from the prior literature [40] and all experimental animal data acquired were calculated. Pre-operative measurements of eight parameters (IVSTd, IVSTs, LVIDd, LVIDs, LVPWTd, LVPWTs (mm), EF, FS (%)) were performed on each mouse in triplicate by experienced technicians to calculate an average value (Table 2). The Wilcoxon's signed rank test was used to compare pre- and post-MI values as well as between days 1 and 7 post-MI. Between group comparisons of adhesion severity values were performed using the Kruskal-Wallis test. The statistical software SPSS 27.0 J was used for the statistical analyses. The period of this study was from August 22, 2018 to the end of November 2018.

Table 2
List of abbreviations

IVSTd	intraventricular septal thickness in diastole
IVSTs	intraventricular septal thickness in systole
LVIDd	left ventricle internal diameter in diastole
LVIDs	left ventricle internal diameter in systole
LVPWTd	left ventricular posterior wall thickness in diastole
LVPWTs	left ventricular posterior wall thickness in systole
EF	ejection fraction
FS	fractional shortening

Echocardiographic evaluation measured six parameters: IVSTd, IVSTs, LVIDd, LVIDs, LVPWTd, and LVPWTs. EF and FS were obtained using the Cubed method. Values of EDV, ESV, EF, and FS were calculated according to the following formulae, EDV (Left ventricular end-diastolic volume: Cubed method) =LVDdv3, ESV (Left ventricular end systolic volume: Cubed method) = LVDsv3, EF=(EDV-ESV)/EDV and FS=(LVDdv-LVDsv)/LVDdv×100.

Results

Effect of diet on weight

Changes in body weight of the animals in the Control, the DHA, and the HF groups are as shown in Figure 1. The average body weight at the beginning of the diet administration period (4 weeks old), before surgery (8 weeks old), 1 and 7 days after surgery were 12.59 g, 24.17 g, 22.30 g, and 24.20 g in the Control group, respectively. The average body weight in the DHA group was 12.93 g, 25.73 g, 24.31 g, and 25.65 g, and that in the HF group was 12.41 g, 25.16 g, 23.79 g, and 24.66 g. Additionally, no statistical differences were observed between the three groups at the start of diet administration through to the day of MI treatment. Furthermore, on the 1st post-operative day, the Control, the DHA and the HF groups lost 1.88 g, 1.42 g, and 1.38 g, respectively, with no significant differences in body weight observed one week later.

Effect of diet on echocardiographic parameters

Changes in the echocardiography parameters for the Control, the DHA, and the HF groups are shown in Figures 2–4.

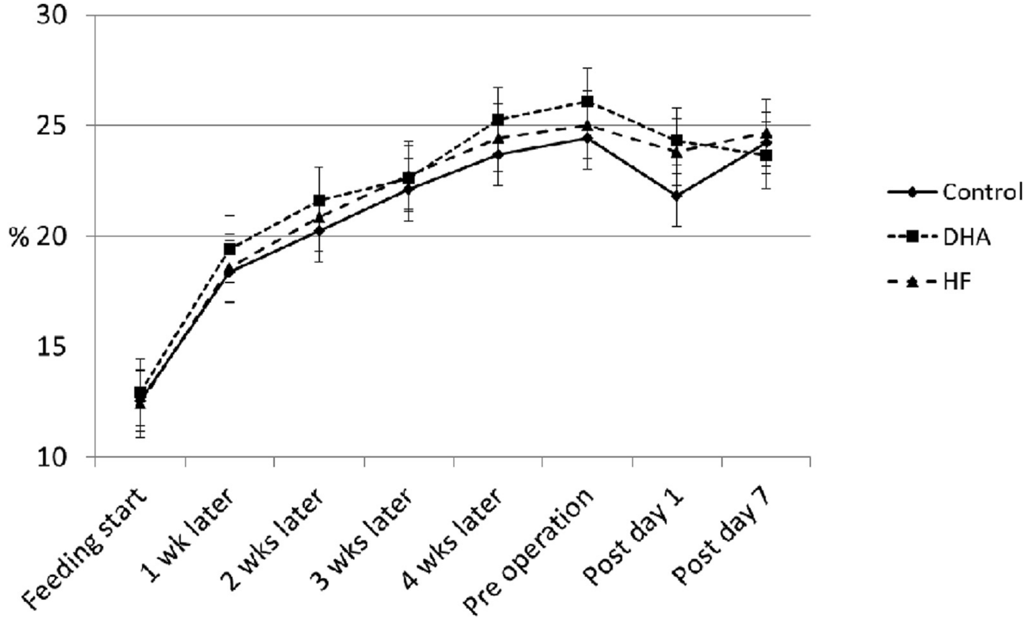

Figure 1. Changes in body weight of the animals in the Control, the DHA, and the HF groups are as shown. Data were expressed as mean±SD. No statistical differences were observed between the three groups at the start of diet administration through to the day of MI treatment, on the 1st post-operative day, in body weight observed one week later.

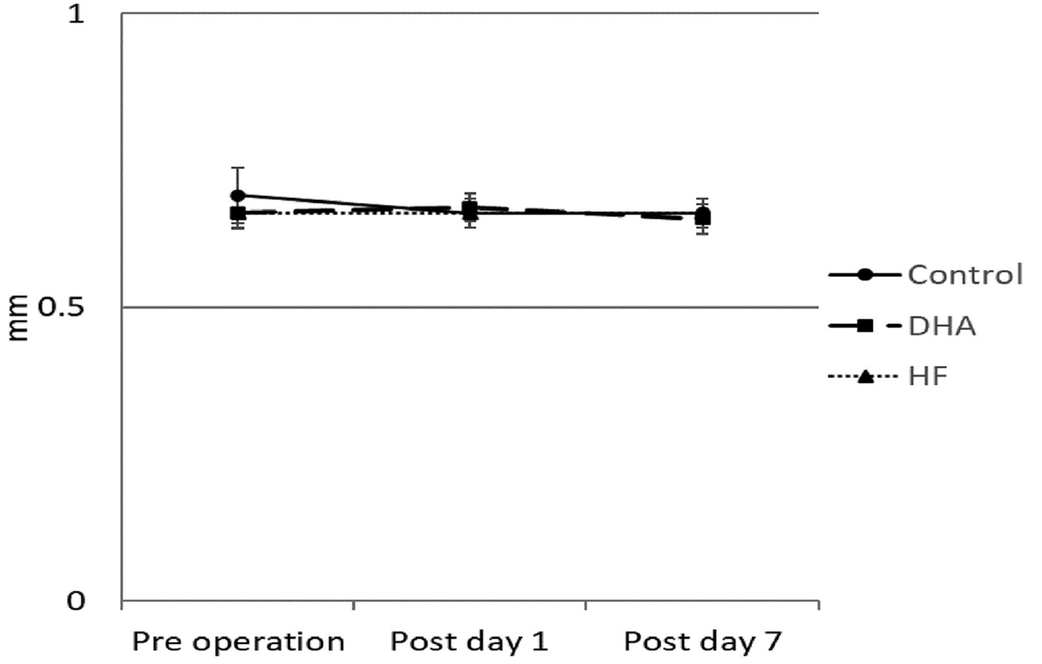

Figure 2. IVSTd is a measure of the average wall thickness, and values obtained before treatment, and on post-operative days 1 and 7. Data were expressed as mean±SD. No significant differences were observed between the groups at the pre- and post-operative times.

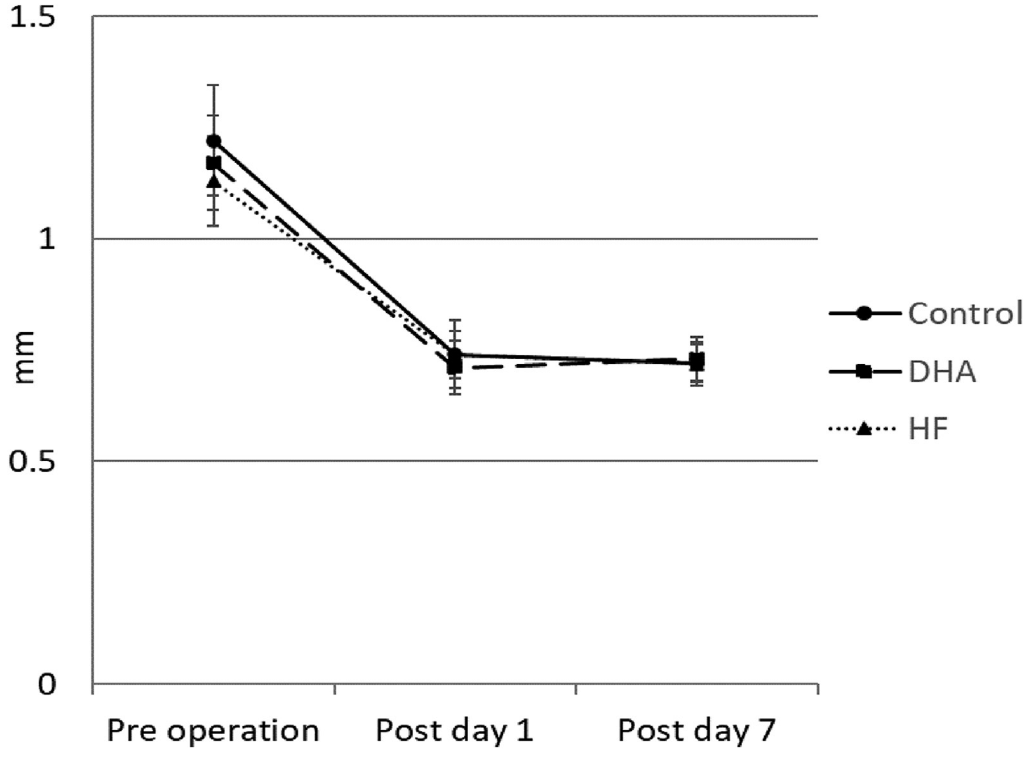

Figure 3. IVSTs values obtained before treatment, and on post-operative days 1 and 7. Data were expressed as mean±SD. No significant differences were observed between the groups at the pre- and post-operative times.

Effect of diet on IVSTd

IVSTd is a measure of the average wall thickness, and values obtained before treatment, and on post-operative days 1 and 7 were 0.69 mm, 0.66 mm, and 0.66 mm in the Control group, 0.66 mm, 0.66 mm, and 0.65 mm in the DHA group, and 0.67 mm, 0.66 mm, and 0.66 mm in the HF group. No significant differences were observed between the groups at the pre- and post-operative times.

Effect of diet on IVSTs

IVSTs values obtained before treatment, and on post-operative days 1 and 7 were 1.22 mm, 0.75 mm, and 0.72 mm in the Control group, 1.16 mm, 0.70 mm, and 0.74 mm in the DHA group, and 1.15 mm, 0.73 mm, and 0.70 mm in the HF group. No significant differences were observed between the groups at the pre- and post-operative times.

Effect of diet on EF

EF values obtained before treatment, and on post-operative days 1 and 7 were 77.28%, 46.05%, and 42.87% in the Control group, 78.08%, 49.6%, and 49.08% in the DHA group, and 77.07%, 48.37%, and 46.17% in the HF group. A significant decrease in EF, relative to the pre-treatment value, was observed in the HF group on post-operative days 1 and 7. Furthermore, when comparing the three groups, the mean EF value was significantly lower in the Control group than in the DHA group on post-operative day 7 (p <0.05).

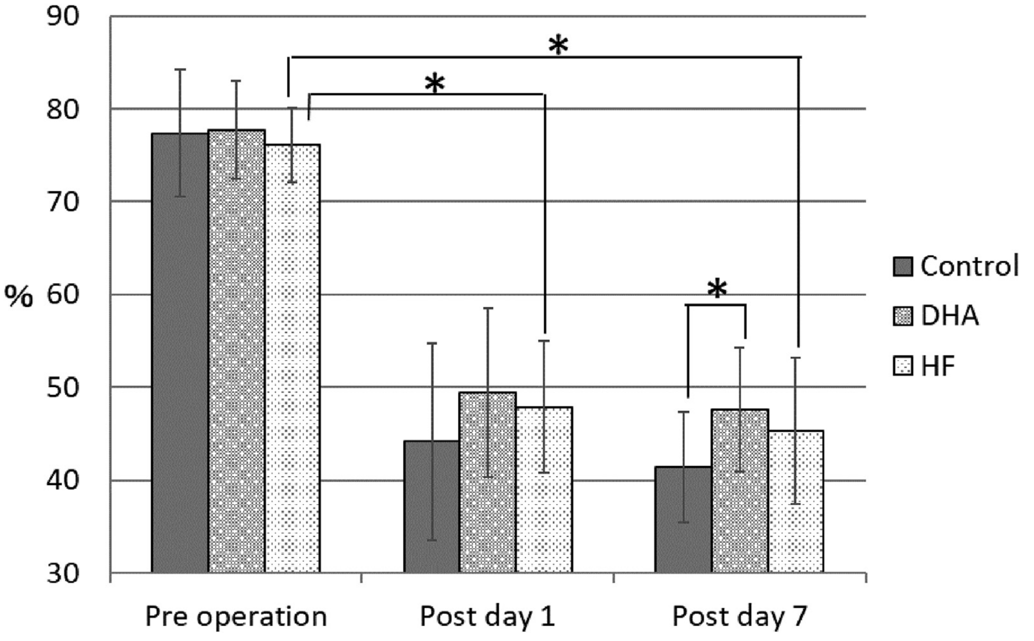

Figure 4. EF values obtained before treatment, and on post-operative days 1 and 7. Data were expressed as mean±SD. A significant decrease in EF, relative to the pre-treatment value, was observed in the HF group on post-operative days 1 and 7 (p <0.05). Comparing the three groups, the mean EF value was significantly lower in the Control group than in the DHA group on post-operative day 7 (p <0.05).
* Wilcoxon signed rank test and Mann-Whitney U test.

Evaluation by masson trichrome staining and adhesion severity

On post-operative day 7, the fibrosis at the MI site was evaluated by Masson trichrome staining, and infarction was confirmed. Adhesion severity in the Control group was Grade 1 in 1 (25%) and Grade 3 in 3 (75%) cases, while that in the DHA group was Grade 2 in 3 (75%) and Grade 3 in 1 (25%), and that in the HF group was Grade 3 in all cases (Table 3). Comparing the treatment groups indicated that the grade of adhesions tended to be worse in the HF group, but no significant difference was observed between any combinations of two groups. Regarding observational evaluation, the adhesion and pericardial fat in the DHA group were visually assessed as being smoother than in the HF group (Figure 5).

Discussion

These results of the present study demonstrated the effects of diets with different n-3/n-6 fatty acid content on adhesions at the site of myocardial infarction (MI) in mice. Since around 2010′, attention has been focused on the n-3 fatty acids and DHA of edible “seaweed oil” extracted from algae, because fish

Table 3
Adhesion severity of each group

	Control (n=4)	DHA (n=4)	HF (n=5)
Grade1	1 (25%)	0	0
Grade2	0	3 (75%)	0
Grade3	3 (75%)	1 (25%)	5 (100%)

*There is no Grade 0 and Grade 4.

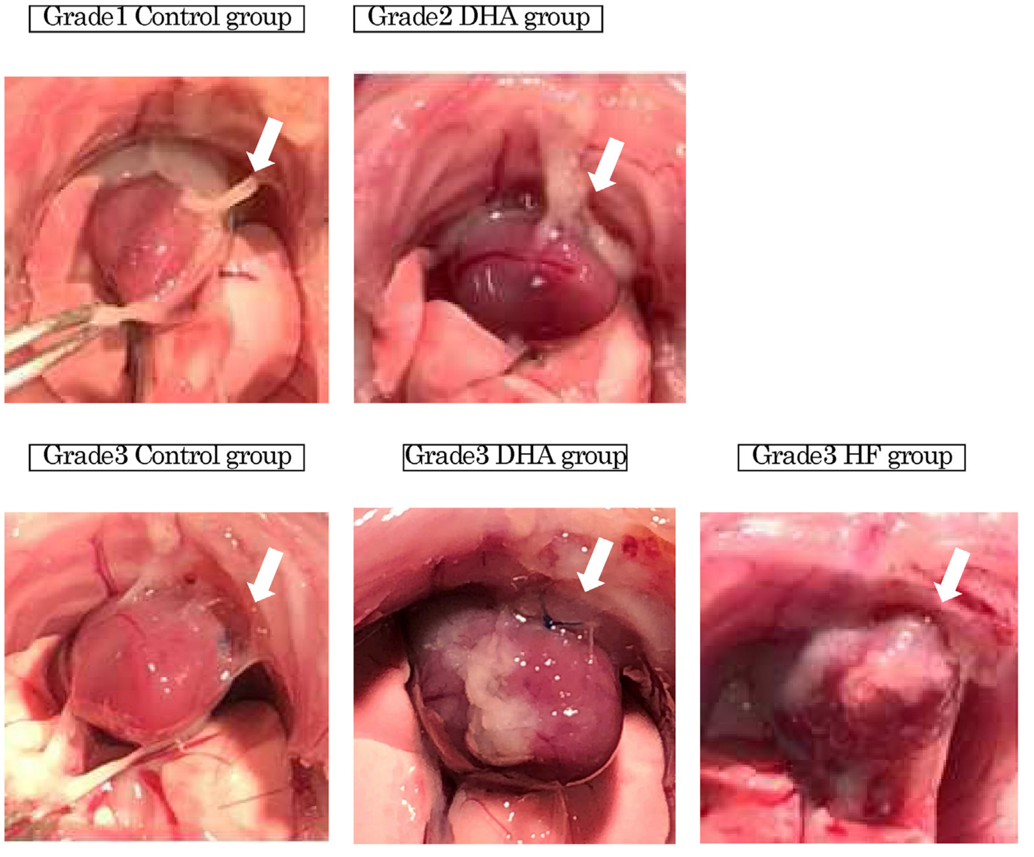

Figure 5. On post-operative day 7, comparing the treatment groups among the Control group, the DHA group, and the HF group, indicated that the grade of Adhesion severity [38] tended to be worse in the HF group, but no significant difference was observed between any combinations of two groups. Regarding observational evaluation, the adhesion and pericardial fat in the DHA group were visually assessed as being smoother than in the HF group.

oil is a limited resource and fish cannot produce large amounts of long-chain omega-3 fatty acids [33–35]. Basically, almost oral taking algae is not digested. From this reason, in this study the diet containing “marine algae oil” was selected for the DHA group.

Weight evaluation

Average body weight was observed to gradually increase in all experimental groups. There were no differences in weight change among three groups prior to MI treatment. In all groups, average weight decreased by about 5–11% on the 1st day after treatment and returned to a weight similar to the pre-MI weight by the 7th day in the Control and the HF groups. In contrast, the weight of the DHA group did not recover as quickly. However, there were no significant differences in weight change among three groups on the 7th day after MI.

Changes in cardiac function in measured echocardiographic values

Tanaka *et al.* reported an index value for IVSTd in allogeneic mice of 0.7 ± 0.1 mm [40]. In this experiment, the mean values of IVSTd among the groups ranged from 0.663 to 0.685 mm, and the average values after one week were 0.651–0.663, which was within the normal variation of the index value. Similarly, the index value for IVSTs reported by Minegishi *et al.* was 1.21 ± 0.03 mm [41], and the average IVSTs before surgery ranged from 1.148 to 1.224 mm in all groups, and 0.701–0.737 mm after one week. As described above, the average values measured before MI were similar in all three groups, the values on days 1 and 7 post-MI were different, with no subsequent changes observed between days 1 and 7. Thus, the data indicate that there was no movement in the front wall and no change in the thickness after MI attributable to the dietary differences.

Additionally, significant decreases in EF, which is an indicator of cardiac function, were observed in the HF group before the treatment, as well as on days 1 and 7 post-MI. On the 7th day post-MI, the mean EF value was significantly lower in the Control group compared to the DHA group ($p < 0.05$).

As described above, wall thickness did not differ among the three groups; however, differences in cardiac function were observed among the groups at each assessment point. The HF group was fed a diet rich in beef tallow. A significant increase in the total amount of ceramide (intercellular lipids) produced in the heart results in differences in signals for cell death and autophagy, which are believed to affect myocardial remodeling [42,43]. It is thought that the absence of an observed effect on wall thickness was due to the final evaluation being conducted at 7 days post-MI, which may be an insufficient duration for myocardial remodeling to occur. Moreover, excess accumulation of ceramide is believed to cause blood circulation disorders [44], intracellular abnormalities, or reactions that may affect cardiac outcomes. In future studies, it will be important to conduct studies that evaluate minute changes in heart cells.

The state of adhesion

Evaluation of adhesion tissue formation between the heart and the chest wall was performed on the 7th day after MI using the adhesion severity scale of Izumi *et al.* [38]. In the Control group, Grade 3 (75%) adhesions were the most prevalent. In comparison, Grade 2 (75%) adhesions were the most prevalent in the DHA group, whereas all HF animals were categorized as Grade 3. Statistical comparison of the treatment groups indicated that no significant differences were found between any two groups. However, the level of adhesions was high in the HF group, while the adhesion tended to be less prominent in the DHA group. The adhesions and pericardial fat in the DHA group were visually assessed as being smoother than in the HF group. Moreover, it is possible that the components constituting adipose tissue and adhesions are different. Serhan *et al.* reported that metabolites derived from n-3 fatty acids in human report are present in the exudate during late stage inflammation, and that these metabolites produce an anti-inflammatory effect [45]. These findings indicate that it is possible that the state of the surrounding adipose tissue and the constituents of the adhesion may affect the degree of adhesion formation. The results of this research showed that taking the fatty diet not only HF but also DHA accumulate the fat in thoracic cavity comparing normal diet. Even taking DHA, unlimited fat intake can be possible to be a risk factor of potential adhesions. In this study, the DHA diet of containing "marine algae oil" was used after changed almost 60% of soybean oil to "marine algae oil". It will be important in future studies to further examine the pericardial fat component in each group and find the detailed mechanism to prevent to occur postoperative complication related to adhesion. And it is needed that an appropriate fatty-acid containing of the DHA diet will be fixed.

As shown above, there were no differences in body weight, IVSTd, and IVSTs among the three groups, whereas EF was diminished in the HF group, and EF in the DHA group was significantly higher than the Control group on day 7 post-MI. This suggests that the consumption of a DHA-containing diet may improve the situation related to EF. In addition, the degree of adhesions in the DHA group tended to improve slightly, suggesting that the difference in fatty acid content may affect adhesions. Since the 1980s, it has been shown that adhesions have various causes. Examination of how factors related to patients' lifestyle choices, such as diet and nutrition, affects a beneficial inflammatory balance on prevention of cardiovascular disease [45–47] will lead to practical suggestions for nursing concreate support in the future. It is necessary to continue experimental studies using MI model mice to evaluate various factors affecting adhesion formation.

Limitations and strengths of the study

This experimental research was a novel mouse study in the field of nursing that used daily diet containing DHA to address clinically relevant questions. The surgical techniques used in this research were highly technical and required a high level of surgical training. We are exploring how to further develop this project. In future research, it is necessary to evaluate the effect of diet preparation, extend the study to include molecular biology, and to utilize a more appropriate research design that focuses on evidence-based preventive nursing intervention.

Conclusion

The purpose of this study was to investigate the effect of diets with different n-3/n-6 fatty acid content on adhesions at the site of MI in an experimental mouse MI model.

1) Body weight change among the three groups parallelly decreased on the 1st day post-MI.
2) Cardiac function in the HF group tended to deteriorate, and on the 7th day post-MI, the DHA group tended to deteriorate less than the Control group.
3) Adhesions tended to be the worst in the HF group and mild in the DHA group.

Author's contributions

Y.C. was responsible for the whole study conception and design with a role of an experimental procedures, a performer of making MI and echography, and a writer of this manuscript in English. I.N. assisted to collect the data and wrote draft text in Japanese. K.I. made each type of diet for this mice experiment and supply for this research. T.S. also supported for K.I. joined in this research as experiment technicians. T.I. made critical opinions to the manuscript. Y.C. also obtained a national funding as a priority investigator.

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Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

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References

[1] Kaneko Y, Hirata Y, Achiwa I, Morishita H, Soto H, Kobayahsi J. Adhesion barrier reduces postoperative adhesions after cardiac surgery. Asian Cardiovascular and Thoracic Annals 2012;20:257–62.
[2] Pamela SM, Lorraine SE, Joyce NG, Martinez-Maza O, Corvera-Tindel T, Magpantay L, et al. Exhaustion, immune-inflammation, and pathogen burden after cardiac surgery: An exploratory study. Eur Cardiovasc Nurs 2014;13(3):211–20.
[3] Nkere UU. Postoperative adhesion formation and the use of adhesion preventing techniques in cardiac and general surgery. ASAIO Journal 2000;46:654–6.
[4] Cannata A, Petrella D, Russo FC, Bruschi G, Fratto P, Gambacorta M, et al. Postsurgical intrapericardial adhesion: Mechanisms of formation and prevention. The Annals of Thoracic Surgery 2013;95:1818–26.
[5] Ar'Rajab A, Dawidson I, Sentementes J, Sikes P, Harris R, Mileski W. Enhancement of peritoneal macrophages reduces postoperativce peritoneal adhesion formation. Journal of Surgical Research 1995;58:307–12.
[6] Milgalter E, Uretzky G, Siberman S, Appelbaum Y, Shimon DV, Kopolovic J, et al. Pericardial meshing: An effective method for prevention of pericardial adhesions and epicardial reaction after cardiac operations. The Journal of Thoracic and Cardiovascular Surgery 1985;90:281–6.

[7] Reikerås O, Nordstrand K, Sørlie D. Use of Dextran to prevent pericardial adhesions caused by maize starch powder. European Surgical Research 1987;19:62–4.
[8] Gabbay S, Guindy AM, Andrews JF, Amato JJ, Seaver P, Khan MY. New outlook on pericardial substitution after open heart operations. The Annals of Thoracic Surgery 1989;48:803–12.
[9] Zapolanski A, Fishman NH, Bronstein MN, Ellertson DG, O'Connell TJ, Siegel S. Modified pericardial closure to protect cardiovascular structures during sternal reentry. The Annals of Thoracic Surgery 1990;50:665–6.
[10] Gabbay S. The need for intensive study of pericardial substitution after open heart surgery (Editorial). American Society for Artificial Internal Organs Transactions 1990;36:789–91.
[11] Thompson JN, Whawell SA. The pathogenesis and prevention of adhesion formation. British Journal of Surgery 1995;82:3–5.
[12] Holmdahl L, Risberg B, Beck DE, Burns JW, Chegini N, diZerega GS, et al. Adhesions: pathogenesis and prevention-panel discussion and summary. Eur J Surg Suppl 1997;(577):56–62.
[13] Nkere UU, Whawell SA, Sarraf CE, Schofield JB, O'Keefe PA. Pericardial substitution after cardiopulmonary bypass surgery: A trial of an absorbable patch. The Thoracic and Cardiovascular Surgeon 1998;46:77–83.
[14] Lorgeril MD, Salen P, Martin J, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon. Diet Heart Study. Circulation 1999;99(6): 779–85.
[15] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937–52.
[16] Anand SS, Islam S, Rosengren A, Rosengren A, Franzosi GM, Steyn K, et al. Risk factors for myocardial infarction in women and men: insights from the INTERHEART study. European Heart Journal 2008;29:932–40.
[17] Innes JK, Calder PC. Marine Omega-3(N-3) Fatty Acids for Cardiovascular Health: An Update for 2020. International Journal of Molecular Sciences 2020;21:1362.
[18] Ito R, Satoh-Asahara N, Yamakage H, Sasaki Y, Odori S, Kono S, et al. An increase in the EPA/AA ratio is associated with improved arterial stiffness in obese patients with dyslipidemia. Journal of Atherosclerosis and Thrombosis 2014;21: 248–60. 20.
[19] Williams HJ, Fisher EA, Greaves DR. Macrophage differentiation and function in atherosclerosis: opportunities for therapeutic intervention? Journal of Innate Immunity 2012;4:498–508.
[20] Takamura M, Kurokawa K, Ootsuji H, Inoue O, Okada H, Nomura A, et al. Long-term administration of eicosapentaenoic acid improves post-myocardial infarction cardiac remodeling in mice by regulating macrophage polarization. Journal of the American Heart Association 2017;6:e004560.
[21] Jain AP, Aggarwal KK, Zhang PY. Omega-3 fatty acids and cardiovascular disease. European Review for Medical and Pharmacological Sciences 2015;19:441–5.
[22] Li D, Wahlqvist LM, Sinclair JA. Advances in n-3 polyunsaturated fatty acid nutrition. Asia Pac J Clin Nutr 2019;28(1):1–5.
[23] Magnus JC, Lee HP, Bonaventura J, Zemla R, Gomez LJ, Ramirez HM, et al. Ultrapotent chemogenetics for research and potential clinical applications. Science 2019;12(3436):364.
[24] Singh RB, Mengi SA, Xu YJ, Arneja SA, Dhalla SN. Pathogenesis of atherosclerosis: A multifactorial process. Experimental and Clinical Cardiology 2002;7:40–53.
[25] Parker MC, Wilson MS, Menzies D, Sunderland G, Thompson JN, Clark DN, et al. Colorectal surgery: the risk and burden of adhesion-related complications. Colorectal Disease 2004;vol. 6:506–11.
[26] Attard JP, MacLean AR. Adhesive small bowel obstruction: epidemiology, biology and prevention. Canadian Journal of Surgery 2007;50:291–300.
[27] Chang LT, Yuen CM, Sun CK, Wu CJ, Sheu JJ, Chua S, et al. Role of stromal cell-derived factor -1 alpha, level and value of circulating interleukin -10 and endothelial progenitor cells in patients with acuter myocardial infarction undergoing primary coronary angioplasty. Circ J 2009;73(6):1097–104.
[28] Catena F, Saverio SD, Kelly MD, Biffl LW, Ansaloni L, Mandalà V, et al. Bologna guidelines for diagnosis and management of adhesive small bowel obstruction (ASBO): 2010 evidence-based guidelines of the world society of emergency surgery. World Journal of Emergency Surgery 2011;6:5.
[29] Calder PC. The role of marine omega-3 (n-3) fatty acids in inflammatory processes., atherosclerosis and plaque stability. Molecular Nutrition & Food Research 2012;6:1073–80.
[30] Riccioni G, Sblendorio V. Atherosclerosis: from biology to pharmacological treatment. Journal of Geriatric Cardiology 2012; 9:305–17.
[31] Chang CL, Deckelbaum RJ. Omega-3 fatty acids: mechanisms underlying “protective effects” in atherosclerosis. Current Opinion in Lipidology 2013;24:345–50.
[32] de Castro Leão M, Raffin Pohlmann A, de Cristo Soares Alves A, Helena Poliselli Farsky S, Klimuk Uchiyama M, Araki K, et al. Docosahexaenoic acid nanoencapsulated with anti-PECAM-1 as co-therapy for atherosclerosis regression. Eur J Pharm Biopharm 2021;159:99–107. 35.
[33] Doughman SD, Krupanidhi S, Sanjeevi CB. Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. Curr Diabetes Rev 2007;3(3):198–203. 32.
[34] Arterburn LM, Oken HA, Bailey Hall E, Hamersley J, Kuratko CN, Hoffman JP. Algal-oil capsules and cooked salmon: nutritionally equivalent sources of docosahexaenoic acid. J Am Diet Assoc 2008;108(7):1204–9. 33.
[35] Lenihan-Geels G, Bishop KS, Ferguson LR. Alternative sources of omega-3 fats: can we find a sustainable substitute for fish? Nutrients 2013;5(4):1301–15. 34.
[36] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 1959;37:911–7.
[37] Tarnavski O, McMullen JR, Schinke M, Nie Q, Kong S, Izumo S. Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. Physiological Genomics 2004; 16:349–60.
[38] Izumi Y, Takahashi Y, Kohno M, Nomori H. Cross-linked poly (Gamma-Glutamic Acid) attenuates pleural and chest wall adhesions in a mouse thoracotomy model. European Surgical Research 2012;48:93–8.

[39] Zhang Y, Takagawa J, Sievers RE, Khan FM, Viswanathan NM, Springer LM, et al. Validation of the wall motions score and myocardial performance indexes as novel techniques to assess cardiac function in mice after myocardial infarction. American Journal of Physiology Heart and Circulatory Physiology 2007;292:H1187–92.
[40] Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, et al. Transthoracic echocardiography in models of cardiac disease in the mouse. Circulation 1996;94:1109–17.
[41] Minegishi S, Ishigami T, Kawamura H, Kino T, Chen L, Nakashima-Sasaki R, et al. An isoform of Nedd4-2 plays a pivotal role in electrophysiological cardiac abnormalities. Int Mol Sci 2017;18(6):1268.
[42] Russo SB, Tidhar R, Futerman AH, Cowart AL. Myristate-derived d16 : 0sphingolipids constitute a cardiac sphingolipid pool with distinct synthetic routes and functional properties. Journal of Biological Chemistry 2013;288:13397–409.
[43] Russo SB, Baicu CF, Laer VA, Geng T, Kasiganesan H, Zile RM, et al. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. The Journal of Clinical Investigation 2012;122:3919–30.
[44] Obata Y, Kita S, Koyama Y, Fukuda S, Takeda H, Takahashi M, et al. Adipo-Nectin/T-cadherin system enhances exosome biogenesis and decreases cellular ceramides by exosomal release. JCI Insight 2018;3:e99680.
[45] Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. The Journal of Experimental Medicine 2000;192:1197–204.
[46] Bäck M, Hansson GK. Omega-3 fatty acids, cardiovascular risk, and the resolution of inflammation. FASEB Journal 2019; 2033:1536–9.
[47] Miller PS, Evangelista LS, Giger JN, Martinez-Maza O, Corvera-Tindel T, Magpantay L, et al. Exhaustion, immuno-inflammation, and pathogen burden after cardiac surgery: An exploratory study. European Journal of Cardiovascular Nursing 2015;13:211–20.