Life Sciences, Medicine and Biomedicine LSMB

Research Article

Effects of EPA+DHA from yellow-stripe scad and salmon on platelet and endothelial cell-related cytokines of healthy overweight Malaysians

Yakubu Abdulrahman[1,3], Azrina Azlan[2], Loh Su Peng[2], Irmi Zarina Ismail[4], Sabariah Md Noor[1*]

[1] *Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 Serdang, Selangor.*
[2] *Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 Serdang, Selangor.*
[3] *Department of Haematology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo, University Sokoto, P.M.P 2346, Sokoto, Northern western Nigeria.*
[4] *Department of Family Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.*

https://doi.org/10.28916/lsmb.3.8.2019.28
Received 6 December 2018, Revisions received 5 July 2019, Accepted 12 August 2019, Available online 15 October 2019

Abstract

Introduction: Overweight currently has become a major global burden. Salmon is one of the major sources for fish oil to treat inflammatory related cardiovascular diseases. Yellow-stripe scad (YSS) on the other hand, is a local Malaysian fish which can be a good substitute for salmon; however, the therapeutic effects of YSS is still unclear. **Objective:** Therefore, this study compared the nutritional values EPA+DHA of YSS and salmon on body mass index (BMI), leptin and activation markers for both platelet and endothelial cell. **Methods**: Healthy overweight Malaysian adults (n=45), aged 21-55 years old, were recruited for 6-months cross-over trial study. They were randomised equally to receive eight weeks of either steamed whole YSS fish or salmon fillet, for three days per week, obtaining approximately 7000 mg EPA+DHA weekly. The diets were switched after an eight-week washout period. Baseline dietary fish intakes were similar in the two groups. Both YSS and Salmon elevated EPA+DHA level of leptin, platelet and endothelial cell phospholipid membrane (data not shown). Body mass index (BMI), serum leptin and biomarkers of platelet and endothelial cell activation (sCD40L, P-selectin, IL-1β, vWF and VCAM-1) were evaluated. **Results:** The comparison of data indicated no significant difference was observed after the treatment with YSS and salmon on pre and post BMI (p>0.05). However, there were significant differences observed in serum leptin for YSS-baseline group I and salmon-baseline group II (p<0.05). Significant changes were observed in serum P-selectin, sCD40L and IL-1β in YSS-baseline group I (p<0.05) but not in VCAM-1 (p>0.05). Significant decreased were also observed in serum vWF and VCAM-I in salmon/baseline group II (p<0.05), but not in P-selectin, sCD40L and IL-1β (p>0.05). However, there was no significant differences between YSS and salmon (P>0.05) on time and treatment in all variable after 16 week, but there was a significant effect of treatment on sCD40L from YSS and vWF from salmon (p<0.05) **Conclusion**: Both YSS and salmon could harmonized EPA+DHA into leptin, platelet and endothelial phospholipid membrane to decreased platelet and endothelial cell activation markers which may contribute to the cardioprotective effect of EPA +DHA. Thus, the health benefits of YSS fish *"ikan selar"* and salmon on leptin and prothrombotic parameter on healthy overweight adults may be similar.

Keywords: EPA/DHA; leptin; platelet and endothelial cells markers; YSS; salmon

1.0 Introduction

Overweight is a condition characterised by low-grade inflammation that promotes an elevated incidence of atherosclerosis and complex complication of the cardiovascular disease, which increases the risk of morbidity and mortality (Abdelaal, Roux, & Docherty, 2017). Currently, the prevalence of obesity in developing countries is still high. The National Health and Morbidity (NHM) of Malaysia in 2006, 2011 and 2015 reported that the prevalence of overweight and obesity in adults aged more than 18 years was 29.1% and 14.5% respectively in 2006 (Khor GL, Noor Safiza MN, Rahmah R, Jamaluddin AR, Kee CC, Geeta A, Jamaiyah H, Suzana S, Wong NF, Ahmad Ali Z, 2008), 29.4% and 15.1% respectively in 2011(Nazaimoon et al., 2011) , and finally 30.0% and 17.7% respectively in 2015 (Division, 2015). According to World Health Organization (WHO, 2016), Malaysia has lower prevalence (11.4% in males; 16.7% in females) when compared with the United States (31.7% in males; 33.9% in females) and Australia (27.5% in males; 29.8% in females) which are three-fold or four-fold higher than Asian countries, such as China (3.8% in males; 5.0% in females), India (3.7% in males; 4.2% in females), Japan (4.5% in males; 3.3% in females), and Taiwan (4.3% in males; 6.4% in females). (Division, 2015)

Evidence has demonstrated that dyslipidemia observed in overweight and obese individuals is a major risk factor of cardiovascular problems (Klop, Elte, & Cabezas, 2013). However, the mechanisms and independent risk factors involved are multifactorial. Metabolic disorder is often partnered with excessive body fat such as hypertriglyceridemia, dyslipidemia, decreased high-density lipoprotein (HDL) and impaired fasting glucose.

***** *Correspondence: Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.*
e-Mail: md_sabariah@upm.edu.my

Insulin resistance, hypertension, increased serum leptin and leptin resistance are the cluster incidence of this phenomenon in overweight adults that promotes atherosclerosis(Elbatarny & Maurice, 2005), (Corsonello, Perticone, & Malara, 2003)(Dasheng, Yanqiu, Chao, Feng, & Minxin, 2007). Inflammation activates platelets and endothelial cell, and this activation has been linked to either leptin or oxidized low-density lipoprotein (ox-LDL) apart from the natural platelet agonists such as thromboxane, collagen, ADP and thrombin. Ox-LDL and leptin whose plasma levels are elevated in overweight individual has it receptor on platelet and endothelial cell (Chatterjee et al., 2017) and (Corsonello et al., 2003) respectively. Although the major role of leptin seems to be in the regulation of body weight and energy metabolism, several pieces of evidence suggested that this hormone could be involved in other pathophysiological disorders(Jr, Leibel, Seeley, & Schwartz, 2011). That role involves the elevation of platelet and endothelial cell inflammatory cytokines such as P-selectin, sCD40 , IL-1 β, vWF and VCAM-1; which has been shown to increase in obese and overweight individuals suggesting its underlying prothrombotic event (Anwaruddin, Askari, & Topol, 2007).

Current research focused on the possible role of dietary supplementation and modification cellular phospholipid membrane from fish fillet as a replacement therapy to the orthodox drug in the overweight healthy subjects. A large body of literature has demonstrated the timely beneficial effects of fish such as mackerel, salmon, herring and cod as rich sources of EPA+DHA. These fatty fish species can supply approximately 800-1200 mg/100 g of EPA+DHA per day (Salem & Eggersdorfer, 2015). In addition, commercial fish oil containing EPA+DHA today is mainly supplied from this available source. The superiority of salmon as the first class source of EPA+DHA oil lies on the availability of the fat content in its fillet, which can supply 1200 mg of EPA+DHA per day(Kitson, Patterson, Izadi, & Stark, 2009) . Currently, there are three different species of local marine fish in Malaysia that can potentially supply the daily need for essential fish oil (EPA+DHA) (Yunus & Effendi, 2013) (Abd Aziz, Azlan, Ismail, Mohd Alinafiah, & Razman, 2013).These fishes are not only cheap but also very popular and readily available throughout the year in Malaysia. Recently data have shown that per 100 g of keris, tamban and yellow-stripe scad (*selar Kuning*) fillet can supply 551, 436 and 879 mg of EPA+DHA respectively. Among these three species, the yellow-stripe scad (YSS) showed the highest EPA+DHA content with the most beneficial nutritional value(Abd Aziz et al., 2013). Therefore, this study hypothesised that YSS, as seen in salmon EPA+DHA, will modulates the leptin, platelet and endothelial cell via the harmonisation of EPA+DHA on its phospholipid membrane to reverse leptin resistance and decrease the platelet and endothelial cell activation in obese and overweight individuals. Thus, in this study, we aimed to compare the timely effect of approximately 7000 mg EPA+DHA per week from YSS and salmon for 16 weeks, on platelet and endothelial activation-related biomarkers (sCD40L, P-selectin, IL-1β, vWF and VCAM-1) and leptin in overweight healthy subjects, before and after the treatment.

2.0 Materials and methods

2.1 Ethical approval

The study protocol was approved by the Ethics Committee for Research Involving Human Subjects UPM (JKEUPM, the acronym in Malay) and registered in the National Medical Research Register (NMRR-16-2693-3230).

2.2 Recruitment and study population

We recruited 50 healthy overweight Malaysian adults aged 21-55 years old with mean BMI of 25.3 kg/m^2, by which after advertisement and screening for suitability, all were identified as students and staff of Universiti Putra Malaysia (UPM). All participants were overweight (BMI 23-27.4 kg/m^2) individuals between 21 and 55 years of age. Exclusion criteria of this study were as follows: regular consumption of fatty fish, history of CVD, haemostasis disorders, inflammatory disease, diabetes or other significant medical history, hypertension (>140/90 mmHg), using medication to lower serum lipids, blood pressure, inflammation, menopause, pregnancy and lactation. Additionally, participants who used n-3 LCPUFA supplements regularly were required to stop using supplements for two months before inclusion. Five subjects terminated their participation from the research at the beginning of first intervention as they failed to adhere to the strict protocol of the study. Signed informed consent was obtained from subjects prior to the randomisation of their assigned numbers generated by computer software. The baseline data of the participants such as their BMI, blood pressure and fasting blood sugar were recorded to confirm they were healthy overweight individuals before commencing the dietary intervention of YSS and salmon. The participants were requested to refrain from taking omega-3 supplements and aspirin-type products 8 weeks prior to and during the study.

2.3 Experimental design

The study was a randomised, double-blind two-period crossover trial from November 2016 to May 2017. It consisted of two 8-weeks interventions which were sufficient for complete platelet turnover with 8-weeks washout period in between (Figure 1). Subjects were randomly assigned to two equal-sized YSS and salmon groups. One group was allocated to consume whole YSS while the other consumed salmon fillet. The food was given in a lunch box three times on three separate days per week for a period of 8-weeks in both interventions. To confirm if the fish were consumed by the subjects, they were requested to send image caption of fish leftover after a meal. The deviation from the diet was noted and compliance index was assumed based on the formula (Wandless *et al.*, 1979) below:

$$Compliance\ index, \% = \frac{Total\ number\ of\ lunchbox\ consumed}{Total\ number\ of\ lunchbox\ theoretically\ required} \times 100$$

Achievement of 90% compliance index with little or minor deviation was observed. Subject anthropometric measurements such as weight and height were recorded before and after the treatment. Blood sample was collected into BD vacutainer clot activator tubes from subjects after 8 hours of fasting for baseline data, as well as the data collected after the first and second interventions. Blood for human magnetic luminex assay analysis was centrifuged at 1100 *g* for 20 min at 4°C, and the serum was removed and frozen in aliquots at −80°C prior to use. Serum level for leptin and biomarkers for platelet and endothelial cells (sCD40L, P-selectin, IL-1β, vWF and VCAM-1) were measured in duplicates with human magnetic luminex assay (R&D Systems, Abingdon, United Kingdom) according to manufacturer's instructions.

2.4 YSS and salmon diet

Both whole YSS and salmon fillet were wrapped in aluminum foil and steamed cooked for 20 minutes using a double-layered steaming pot. Steaming method was adopted based on previous research that showed the process always retained the EPA+DHA content of steamed fish(Yee, Azlan, & Eng, 2018) . The fish was administered into plastic lunch box containers with white rice and vegetable for participants in their respective groups. Whole YSS was used in the study instead of YSS fillet due to its small size, which makes it clearly impracticable to make YSS fillet. To control the weight variation between whole and fillet YSS, the following procedure was conducted. The net weight of YSS fillet was determined by weighing whole YSS before and after removing the head, bones, and tail. Based on these results (data not shown), 45.1% additional weight was added to replace head, bone, and tail in this study. Whole YSS and salmon fillet were given to participants in their respective group in lunch boxes (weighed approximately 265 g/day and 246 g/day respectively) for 3 times per week. Therefore, each group consumed approximately 2300 mg of EPA+DHA per day, which would make a total of 7000 mg of EPA+DHA per week.

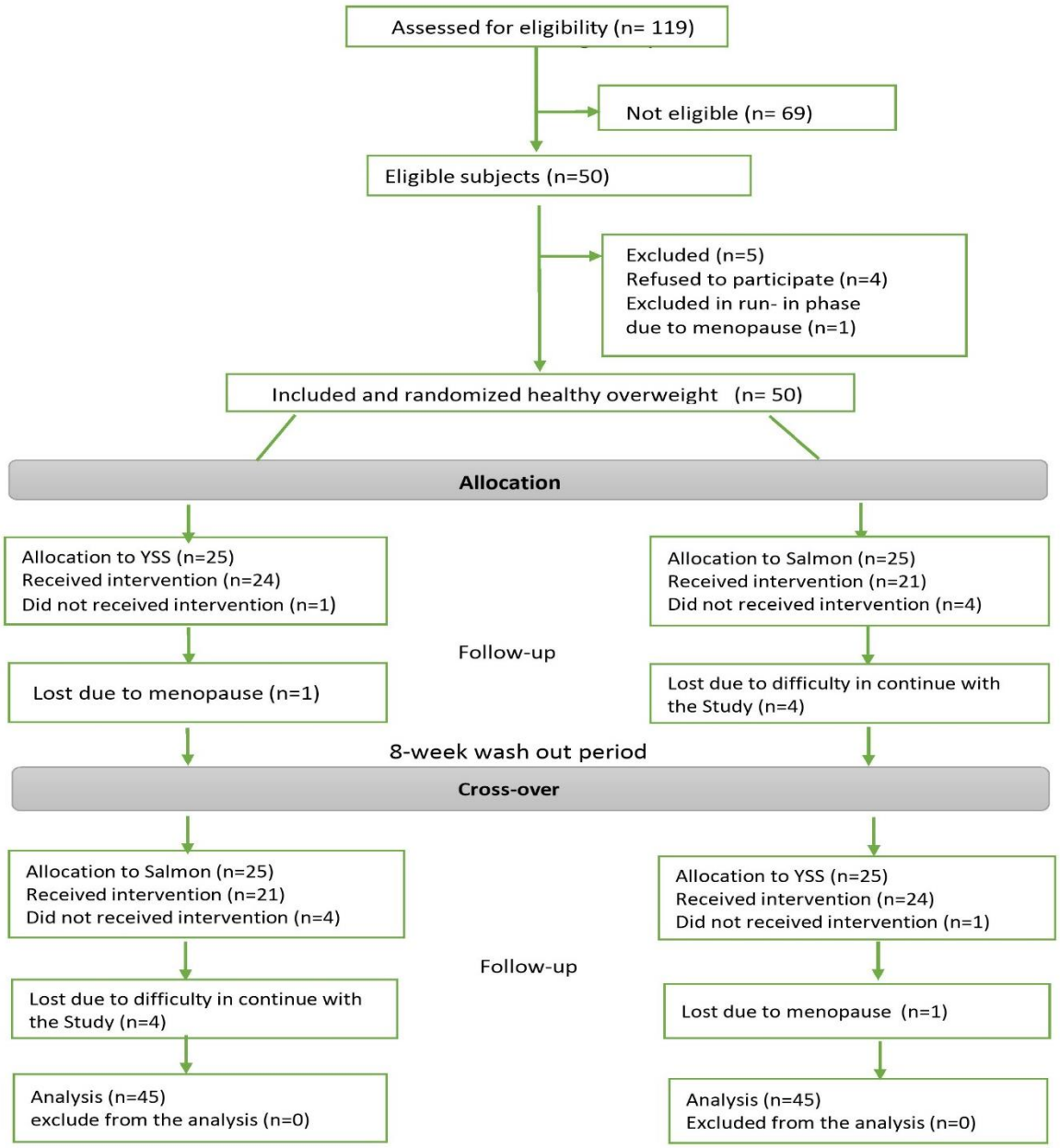

Figure 1: Phases of randomized trial

2.5 Statistical analysis

The variables were tested for normality using Shapiro-Wilk test. The analysis was done before and after treatment, and the participants were categorised into two groups according to randomised treatment assignment regardless of compliance. Data was analyzed using GraphPad Prism (version 6). Comparisons of sociodemographic characteristics were made using one-way ANOVA. Paired sample *t*-test, Wilcoxon matched-pairs signed rank test and ANCOVA or covariance of ANOVA were used to determine the changes of all variables in respective diet groups after 16 weeks. Differences in outcome between diet groups were tested using independent samples *t*-test for parametric variables and Wilcoxon rank-sum test for non-parametric variables.

3.0 Results

The demographic results of the participants (Table 1), with 5 missing values (data not shown). Out of the total number of 45 subjects, 17 were males and 33 were females, with mean age of 25.47±6.87 and 31.24±8.18 years old respectively. In regard to their ethnicity, 31 were Malays, 12 were Chinese and 2 were Indians with mean age of 31.39±7.29, 23.17±3.04, and 21.00±0.00 years old respectively. One-way ANOVA was run to find the statistical significance of mean differences in BMI of pre- and post-treatment for each diet group, and no significant differences were observed [$F(2, 109) = 0.45$, $p > 0.05$] for both YSS and salmon groups. To further evaluate the differences between YSS and salmon groups, independent samples t-test was run. No significant differences in means were observed ($t=0.497$, $r= 0.843$, $p>0.05$), even though there were clinical differences observed in YSS group.

Table 2 summarized the changes in leptin, platelet and endothelial cell activation markers after the diet treatment with YSS and salmon for the period of 16 weeks on overweight healthy Malaysian. Three groups comparison were categorised as follows: (1) YSS-baseline group I, (2) salmon-baseline group II and (3) YSS _Salmon* as group III, Time_of_trx as effect of time and treatment, and YSS _Salmon* Time_of_trx as effect of treatment only in group III; to compare each group with their respective pairs in group I and II, before and after the treatment. On the other hand, YSS _Salmon* as group III was compared as YSS and Salmon, Time_of_trx as effect of time and treatment and YSS _Salmon* Time_of_trx: as effect of treatment made by each fish on leptin was analysed as marker for overweight and obesity. The platelet and endothelial cell activation markers were analysed as marker for cardiovascular disease in overweight and obesity individuals.

Significant differences were observed in both YSS ($p<0.05$ at 0.0002) and salmon ($p<0.05$ at 0.0008) on leptin level with respective baseline in group I and II respectively. However, no significant changes were observed between YSS_Salmon* in group III ($p>0.05$ at 0.653), Time_of_trx ($p>0.05$ at 0.087) and YSS_Salmon* Time_of_trx ($p>0.05$ at 0.947).

Significant changes were observed in P-selectin ($p<0.05$ at 0.0068), sCD40L ($p<0.05$ at 0.0001) and IL-1β ($p<0.05$ at 0.0002) levels after the treatment with YSS-baseline group I for the period of 16 weeks. On the other hand, there were no significant differences observed in VCAM-1 ($p>0.05$ at 0.0568) for YSS-baseline group I for the period of 16 weeks.

In addition, no significant changes were observed in YSS_Salmon* group III ($p>0.05$ at 0.293) in sCD40L, however, significant changes in favour of YSS were observed in Time_of_trx ($p<0.05$ at 0.000) and YSS _Salmon* Time_of_trx ($p<0.05$ at 0.000)

On the other hand, significant changes were observed in leptin ($p<0.05$ at 0.0008), vWF ($p<0.05$ at 0.0001) and VCAM-1 ($p<0.05$ at 0.0006) in salmon and baseline group II for the same period of 16 weeks. However, there were no significant changes observed in P-selectin ($p>0.05$ at 0.7613), sCD40L ($p>0.05$ at 0.6905) and IL-1β ($p>0.05$ at 0.8601) in salmon and baseline group II for the period of 16 weeks. Furthermore, no significant difference was observed in YSS _Salmon* group III ($p>0.05$ at 0.117) in vWF. However, significant changes in favour salmon were observed in Time_of_trx ($p<0.05$ at 0.000) and YSS _Salmon* Time_of_trx ($p<0.05$ at 0.023) for vWF.

4.0 Discussion

The current study was a crossover, random, intervention trial that involved healthy overweight Malaysian subjects. It compared the effectiveness of treatment with YSS (local Malaysian fish) and salmon fillet (foreign imported fish) on leptin as overweight and obesity marker, platelet and endothelial activation biomarkers including P-selectin, sCD40L, IL-1β, vWF and VCAM-1 as shown in Table 2.

The consumption of YSS and salmon for 16 weeks was believed to be sufficient enough to incorporate EPA+DHA (omega-3 fatty acid) into both platelet and endothelial cell phospholipid membrane, which modified the functions of platelet and endothelial cell as well as to alter and decreased the plasma level of leptin, platelet and endothelial activation biomarkers to reduced cardiovascular event in overweight and obesity healthy adult. This is in agreement with (Baldwin, Singh, & Meyer, 2010) that shown EPA+DHA decreases platelet activation marker significantly after treatment with tuna fish oil for 14 day. Suggesting that EPA+DHA from our current study could balance omega-6-fatty acid ratio or arachidonic acid (AA) in both platelet and endothelial phospholipid membrane reduce the incident of low grade inflammation and cardiovascular related problem in overweigh and obesity if is consume regularly (figure 2)

The potential effect of both YSS-Baseline I and salmon/baseline II on BMI outcomes in gender and ethnicity showed no significant differences [$F(2, 109) = 0.45$, $p > 0.05$] after treatment in either groups I and II. Similarly, no significant differences were observed between pre and post BMI of the two groups, YSS and salmon treatment ($t=0.497$, $r= 0.843$, $p>0.05$) (Table I). These observations were consistent with previous studies that showed BMI on gender was not a potential factor to influence the effect of fish oil treatment on weight reduction (Munro & Garg, 2013)(Du, Jin, Fang, & Su, 2015). Another study by Mingay et al., 2016 showed that omega-3 fatty acid does not affect male BMI, but inversely affects female weight status after a long period of omega-3 fatty acid intervention. This suggested that treatment of overweight and obesity with omega-3 fatty acid is not necessarily effective on weight lost, and only after a longer period of intervention trial.

Serum leptin level is known to strongly correlate with the BMI in both overweight and obese individuals. Many studies suggested that the presence of leptin-resistance mechanism found in obesity explains the unguided correlation. In overweight individuals, high level of leptin is not sufficient enough to prevent physiological disturbances in energy regulation, which might indicate that overweight individuals are leptin-resistant. Thus, leptin resistance and unregulated platelet and endothelial cell activation along with low-grade inflammation in overweight and obese individuals were the major causes of tissue thrombosis and atherosclerosis (Riyahi, Tohit, Thambiah, & Ibrahim, 2018)(Davı et al., 2019), Interestingly, both YSS group I and salmon group II treatments in current study showed a significant change in plasma leptin level ($p<0.05$), and both YSS group I ($p<0.05$ at 0.0002) and salmon group II ($p<0.05$ at 0.0008) turned out to be effective on leptin with their respective baseline as shown in Table II. No significant changes were observed on leptin level in regard to YSS _Salmon* group III treatment ($p>0.05$ at 0.087), Time_of_trx ($p>0.05$ at 0.087) and YSS _Salmon* Time_of_trx ($p>0.05$ at 0.947) . Significant changes were observed between leptin in group I and II, suggesting that both fishes may have competitive EPA+DHA nutritional values. YSS and salmon diet interventions on leptin after a period of 16 weeks could attenuate leptin resistance in overweight and obesity individuals. In the central nervous system, leptin via leptin receptor (LEPRb) induces the synthesis of proopriomelanocortin (POMC), a protein inside leptin receptor that releases α-melanocyte-stimulating hormone (α-MSH) (Balthasar et al., 2004). α-MSH decreases body fat and weight by stimulating the production of activating melanocortin-3 receptor (MC3R) and melanocortin-4 receptor (MC4R)(Balthasar et al., 2004) . The MC4R-deficient mice developed obesity and leptin resistance, and MC3R knockout mice also exhibited obesity phenotypes. Therefore, genetic variation of POMC and MC4R have being identified as major contributing factor for obesity in human (Krude et al., 2014). These findings showed that the central melanocortin system is needed by leptin to promote it function as weight regulation. The current study on YSS and salmon showed that both fishes could have probably modified the POMC, MC4R and MC3R receptors to attenuate the leptin resistance and reduced the prevalence of overweight and obesity incidents.

Table 1: Subjects' sociodemographic data expressed as mean ± standard deviation

Variables	**N**	**Age**	YSS Pre-treatment (BMI-1)	YSS post-treatment (BMI-2)	Salmon Pre-treatment (BMI-3)	Salmon post-treatment (BMI-4)	*P*
	50	29.28±8.17					>0.05
Gender							
Male	17	25.47±6.87	25.14±1.49	25.14±1.49	24.98±1.67	25.09±1.49	
Female	33	31.24±8.18	25.33±1.54	25.33±.54	25.29±1.83	25.22±1.71	
Ethnicity							
Malay	31	31.39±7.29	25.36±1.66	25.36±1.66	25.32±1.91	25.31±1.70	
Chinese	12	23.17±3.04	24.93±1.21	24.93±1.21	24.88±1.47	24.88±1.56	
Indian	2	21.00±0.00	25.65±0.21	25.65±0.21	24.90±0.71	24.80±0.14	

No. of the participants (n=50), YSS=yellow-stripe scad, BMI=Body Mass Index, Mean age of 17 males and 33 females, 25.47± 6.87 and 31.24±8.18 years old respectively. No significant differences were observed between pre- and post-treatment BMI by one-way ANOVA (p >0.05) and independent-samples t-test (p >0.05), between the two diet group (YSS and Salmon).

Table 2: Changes in platelet and endothelial activation biomarkers after intervention period (16 weeks) within and between YSS and Salmon diet groups

Variable	YSS (n=42)			Salmon (n=42)			Effect of time between YSS and Salmon [c]P-value		
Platelet and endothelial activation marker	Pre-treatment	16 weeks	P-value	Pre-treatment	16 weeks	P-value	YSS Salmon*	Time of trx	YSS Salmon* Time of trx
[b]Leptin (ng/ml)	13.24 ± 6.81	11.11 ± 7.12	<0.05	12.63 ± 8.73	10.65±8.30	<0.05	ns	ns	ns
[a]P-selectin (ng/ml)	20.79 ±9.07	24.10 ± 9.28	<0.05	20.31 ± 7.46	20.79 ± 7.07	ns	ns	ns	ns
[a]sCD40L(ng/ml)	4.450 ± 2.34	6.982± 1.91	<0.05	5.285 ± 1.93	5.456 ± 2.25	ns	ns	<0.05	<0.05
[b]IL-1β (pg/ml)	13.02± 4.38	15.16 ± 3.14	<0.05	13.85 ± 4.66	13.59 ± 3.72	ns	ns	ns	ns
[b]vWF (pg/ml)	253.2 ± 60.3	228.5 ± 60.29	<0.05	315.4 ± 159.1	216.8 ± 102.6	<0.05	ns	<0.05	<0.05
[b]VCAM (ng/ml)	825.66 ± 307.4	881.69 ± 283.0	ns	756.99 ± 259.5	830.97 ± 287.4	<0.05	ns	ns	ns

All values are expressed as mean ± standard deviation. a Determined by paired-samples t-test b Determined by Wilcoxon matched-pairs signed rank test c Determined by ANCOVA. ns: not significant, trx: treatment, VCAM-1: vascular cell adhesion molecule 1, sCD40L: soluble CD40 Ligands, IL-1β: interleukin 1, beta, VWF: von Willebrand Factor, YSS Salmon*: Group, Time of trx: Effect of time and treatment, YSS _Salmon* Time of trx: Effect of treatment only.

Results of current study were in agreement with study by Pinel et al., 2016 which suggested that supplementation of EPA+ DHA at concentration of 1% w/w of EPA, for 16 weeks could preserve glucose homeostasis in obese animal and limit the body fat mass accumulation at the early stage of weight gain. Another study by (Winnicki et al., 2002) showed that the consumption of fish fillet was associated with decreased level of plasma leptin that is independent of body fat.

A dietary model of insulin resistance induced by long-term sucrose-rich diet (SRD) in rats by (Rossi et al., 2019) showed that dietary fish oil restored the plasma leptin and adiponectin levels without affecting the gene expression. Thus, this study further reemphasised that fatty fish fillet such as salmon contains omega-3 fatty acid that have an effect on the plasma leptin. This suggested that both YSS and salmon could restore the normal physiology functions of leptin to reduce appetite, regulate energy storage via exogenous modification of blood brain barrier (BBB) and phospholipid membranes with the EPA+ DHA (omega-3 fatty acids), as well as to reduce both serum leptin and platelet procoagulant activity respectively, which in turn decreased thrombus formation (Banks, Farrell, William, & Impaired, 2019) (Oh-I et al., 2005) and CVD.

Indeed, platelet and endothelial cells possessed leptin receptor that could be activated under stress. Leptin resistance in overweight and obese individuals could activate both platelet and endothelial cells to release both adhesion and signaling molecules such as P-selectin, sCD40L, IL-1β, vWF and VCAM-1, by which could further alter the coagulation haemostasis and thrombosis formation (Blann, Nadar, & Lip, 2004) . Elevated level of these proteins has been observed in obese and overweight individuals and it is believed to be one of the major risk factors of overweight and obese atherosclerosis (Ridker, Buring, & Rifai, 2001).

In the current study, significant difference was observed in P-selectin ($p<0.05$ at 0.0068) sCD40L ($p<0.05$ at 0.0001), IL-1β ($p<0.05$ at 0.0002) and vWF ($p<0.05$ at 0.0004) in YSS-baseline group I. This suggested that YSS has higher EPA/DHA as supported by Abd Aziz et al., 2013 that reported YSS contains 782.1 mg/100 g DHA in wet sample. The observed significant difference in P-selectin, sCD40L, IL-1β, and vWF after treatment with YSS-baseline group I was surprising and will decrease the higher level of P-selectin, sCD40L, IL-1β, and vWF in overweight individuals (Risk, 2009)(Beaulieu et al., 2014) . Nevertheless, it was indisputable that the improvement of plasma P-selectin, sCD40L, IL-1β and vWF following a diet with dietary fish containing omega-3 fatty acids does play a role in protecting against diseases, particularly CVD (Phang, Lincz, & Garg, 2013) (Adili et al., 2017)(Chen et al., 2000). These dietary fishes have beneficial effects on both platelet and endothelial cells, by which they could enhance the deactivation of platelet and endothelial cell activation biomarkers (adhesion molecules like P-selectin, vWF; and signaling molecules like sCD40L and IL-1β). Thus, the observed significant difference in favour of YSS-baseline treatment group I may be due to the presence of good quality of EPA+DHA in YSS fish (natural marine source) as demonstrated by (Mohanty et al., 2016),(Yunus & Effendi, 2013). This observation was in agreement with the study by Phang, Lincz and Garg, 2013, that reported higher level of EPA+DHA decreased the platelet activation markers in both men and women. EPA+DHA obtained from YSS fish consumption could modify phospholipid membranes of platelet and endothelial cells, by which will regulate the inflammatory cytokines after the activation. This is further supported by (Baldwin et al., 2010) who shown that EPA+DHA decreases platelet activation marker significantly after treatment with tuna fish oil for 14 day, suggesting that YSS could act to modified cellular phospholipid membrane to modulate platelet and endothelial signaling receptor, and to decrease low grade inflammation which is prerequisites to cardiovascular disease in overweight healthy individual. Other studies (Mozaffarian & Wu, 2011), showed that consumption of fatty fish with good quality EPA+DHA content lowers resting heart rate blood pressure and , plasma triglycerides, that might also improve cardiovascular problem via , lower inflammation, and improve vascular function, suggesting that EPA+DHA from YSS in our current study could further affect a myriad of molecular pathways, including alteration of chemical and physical properties of cellular membranes, direct interaction with and modulation of membrane channels and proteins, and further regulation of gene expression. also in-line with our study is a meta-analysis fish oil intakes showed that EPA+DHA reduced platelet activation and aggregation in twenty-four trials of CVD treatment in type-2 diabetes (1533 subjects) via platelet agonist (collagen) by 22%. (S. Cottin, Sanders, & Hall, 2011) ("Potential impact of omega-3 treatment on cardiovascular disease in type 2 diabetes," 2019). Further more in line with our current study, ("Dietary intervention with oil rich fish reduces platelet-monocyte aggregation in man," 2019). measuring platelet monocyte aggregates by flow cytometer; low doses of fish oils (1 g/d; 4 weeks) reduced platelet monocyte aggregate, however no significant changes observed in markers of platelet activation (soluble P-selectin, soluble CD40L) , suggesting that effect of EPA+DHA is a dosage depend on platelet and endothelial cell function. . Soluble CD40 ligand and IL-1β are known as the signaling molecules, by which their receptors are present on the endothelial cells. The interaction of sCD40L and IL-1β from activated platelet with its endothelial cell receptors, will activate NF-KB, which is a master regulator of inflammatory cytokines (Lindemann et al., 2001). Activated NF-KB produces an inflammatory cytokine such as MCP-1, ICAM and VCAM, which are adhesion molecules that enhance and promote the formation of atherosclerosis and cardiovascular disease (Collins & Cybulsky, 2001). Fish oil and fish diet have been known to treat inflammatory diseases due to the fact that it could reduce the level of NF-KB expression that will subsequently reduce the level of sCD40L and IL-1β (Wang & Huang, 2015). It is therefore interesting to note that high intake of YSS fish could ameliorate and protect against cardiovascular problems via modification of phospholipid membranes, whereby the receptors are present on both platelet and endothelial cells.

However, no significant changes were observed on the level P-selectin, ($p>0.05$ at 0.7613), sCD40l ($p>0.05$ at 0.6905) and IL-1β ($p>0.05$ at 0.8601) in salmon-baseline group II. YSS was shown to has high EPA+DHA content (782.1 mg/100 g wet) compared to EPA/DHA (Abd Aziz et al., 2013) content in wild salmon (629 mg/100 g)(Hamilton, Hites, & Schwager, 2005) . In addition, data obtained from United States Department of Agriculture (USDA) showed that wild salmon, farmed salmon, wild catfish, farmed catfish and Greenland halibut contain EPA+DHA at 1840, 2147.1, 236.5, 177.6, and 1177.6 mg/100 g of muscles respectively(Back, 2006) . These data showed that salmon was not the only fish with highest EPA-DHA content. In addition, longtail shad (2210 mg/100 g wet sample) was found to contain EPA+DHA at comparable amount with wild and farmed salmon (Back, 2006), which could be due to the high fat content of the fish (23.2% fat) (Seyedi, S.M., Sharifpour, I., Ramin, 2011). The non-significant results on plasma P-selectin, sCD40l and IL-1β after salmon-baseline group II treatment was in-line with study by(Colussi et al., 2014) , by which they concluded that the outcomes of fish treatment intervention are dependent on the baseline characteristic data. The beneficial changes induced by the fish may appear larger if the subject has low level of EPA/DHA. Therefore, the lower level of EPA/DHA in baseline data may be responsible for the non-significant observations in salmon fish fillet as compared to their respective base line. Furthermore, other previous studies showed that omega-3 fatty acid with less than 2 g of EPA+DHA/day failed to show its effect on inflammatory cytokine regulation (S. C. Cottin, Alsaleh, Sanders, & Hall, 2016),(Mackay et al., 2012), which may be due to insufficient dose (Bagge, Schött, & Kander, 2018)(Innes & Calder, 2018) (Asztalos et al., 2016). Thus, it is still not clear on the reason for these discrepancies, however, many studies have shown that the effects of omega-3 fatty acids are time- and dose-dependent(Venkata Krishnan, Anuradha, Bhattacharjee, & Gaiha, 2007) (Hosogoe, Ishikawa, Yokoyama, Kozuma, & Isshiki, 2017).

A good significant level of serum leptin, vWF and VCAM-1 were detected in salmon-baseline group II. However, YSS-Baseline I group showed non-significant observation in VCAM-1. Both vWF and VCAM1 are strong adhesion molecules that promote plaques development and foam cell formation. Salmon fish fillet has been known to reduce plaques, ICAM-1, VCAM-1 and MCP-1 (Parolini et al., 2014). Therefore, significant level of salmon-baseline II treatment on vWF ($p<0.05$ at 0.0001) and VCAM-1 ($p<0.05$ at 0.0006) observed in current study suggested that consumption of salmon fish fillet can provide anti-atherosclerosis property. Inflammatory cytokines such as

VCAM-1 and ICAM-1 play a crucial role in enhancing the promotion of CVD. Omega-3 fatty acid (EPA/DHA) is a ligand for peroxisome proliferator-activated receptors (PPAR), which is a nuclear transcription factor that depends on EPA+DHA and metabolism of amino acid, glucose and fat. It is known to exert anti-inflammatory potential by inhibiting the expression of proinflammatory cytokines via reducing NF-kb (Cancino et al., 2011). Although the mechanism on PPAR by fish fillet is still unknown, however, Zhu and colleague showed that marine protein may acts as ligand in PPAR to exert an anti-inflammatory effect (Zhu et al., 2010). All these results postulated that the consumption of salmon fish fillet in current study might prevent the development of CVD in overweight and obese individuals as suggested by previous studies (Seierstad et al., 2005) (Parolini et al., 2014).

In this study, the timing effect of treatment for both YSS and salmon treatments was analysed, by which YSS and salmon group III did not show any significant difference, suggesting both fishes can compete favorably in terms of nutritional value, except for sCD40L in YSS treatment and vWF in salmon treatment. Salmon fish fillet and oil are known for its cardioprotective effect in overweight and obese individuals (Parolini et al., 2014) (Seierstad et al., 2005). Current study demonstrated a significant difference on serum leptin, P-selectin, IL-1β, sCD40 and vWF levels in YSS-Baseline group I after 16 weeks intake of whole fish, suggesting that YSS can be used as treatment for cardioprotective potential as observed in salmon from previous study.

4.1 Proposed graphical abstract on the mechanism of YSS

Several reports have demonstrated that saturated fat evokes systemic inflammation and most importantly cardiovascular disorder (Acids & Risk, 2017). Prospective studies have clearly proved that overweight and obesity is a risk factor of CVD and is directly proportional to amount arachidonic acids (AA), precursor of thromboxane in platelet and endothelial phospholipid membrane, as omega-6 fatty acid or AA can be found in overweight and obese adults(Sonnweber, Pizzini, Nairz, Weiss, & Tancevski, 2018). The dietary fish fillet contains docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from different types of fish and have been shown to modify phospholipid membrane including the platelet, especially the fish fillet from salmon(Tsoupras, Lordan, Shiels, & Saha, 2019). Experimental evidence showed that omega-3 fatty acid from fish fillet ameliorated inflammation, decreased proinflammatory cytokine, inhibited signaling pathway and regulated the physical composition of inflammatory leukocytes and their recruitment into the endothelial matrix, including the free radical such as reactive oxygen species (ROS) (Calder, 2017) (Calder, 2015). Recent studies in humans showed that in addition to absolute amount of omega-6 and omega-3 fatty acid intake, higher level of omega-6 fatty acid plays an important role in overweight and obesity development of via AA eicosanoid metabolites, that predisposed healthy overweight adult to platelets and endothelial cell hyperreaction.(Simopoulos, 2016). The hyperreaction can be reversed with increased intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) via modification of both leptin resistance and leptin receptor in platelet to decrease platelet activation true reduction of arachidonic acid or omega-6 fatty acid from platelet and endothelial phospholipid membrane. Peroxisome proliferator activated receptors (PPARs) are transcription factor receptor found in nucleus, which regulate the gene expression of various molecules responsible for the metabolism of protein, carbohydrate and lipid. EPA+DHA is one of the ligands that activates PPARs.(Access, 2008) Thus, the activation of PPARs by EPA+DHA from YSS may inhibit NF-kB, as nuclear transcription factor for different cytokines and chemokines such as MCP-1, VCAM and ICAM, which are sole agent of pro-atherosclerosis as shown in Figure 2.

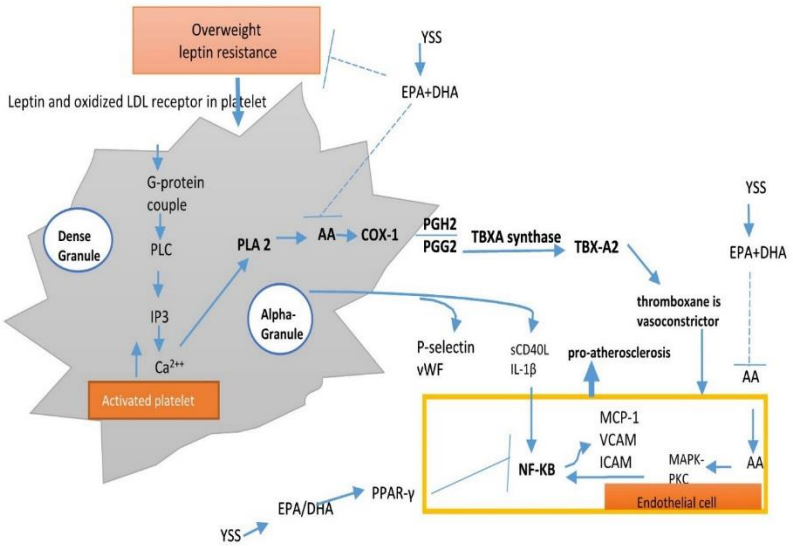

Figure2: shown that people who consumed Western diets, increase the intake of omega-6 fatty acid and decreased the omega-3 fatty acid, resulting in a large increase in the omega-6 and omega-3 ratio from 1:1 to 20:1 today or even higher. This changes in omega-6 and omega-3 ratio increase the prevalence of overweight and obesity. Experimental studies have suggested that omega-6 and omega-3 fatty acids elicit divergent effects on body fat gain through mechanisms of adipogenesis. The presence of oxidized LDL and leptin receptor in platelet phospholipid membrane could initiate cell signaling that activate platelet and endothelial to release both inflammation (sCD40L,IL-1β) and activation markers (vWF, P-Selectin) via, their phospholipids membrane that stimulates G-couple protein which in turns activate phospholipase c (PLC) and inositol 1,4,5 triphosphate (IP3), that increase the intra cellular calcium level, which further catalyzed phospholipase A2 enzymes to release Arachidonic Acids (AA). AA synthesized the release of thromboxane-A2 (TBX-A2) as vasoconstrictor in endothelial cell. EPA+DHA from YSS may inhibit leptin resistance, AA, and NF-KB via activation of ppar which is the master regulator of inflammation and EPA/DHA from YSS may also balance omega-6 and omega-3 ratio in overweight and obesity to prevention and management cardiovascular disease.

4.2 Limitation

The portion of fish given to subjects does not reflects the normal fish intake. This was done to elucidate the actual health benefit of yellow stripe scad (YSS) and salmon for 16 weeks by meeting up EPA/DHA intake recommendation (approximately 7000 mg/week) (Ministry of Health Malaysia, 2014). Indeed the amount of fish given was nearly double the recommended serving size (150g) according to (Heart

Foundation, 2008). Although the results demonstrated that short time consumption of the fish is feasible, but a longer time consumption is recommended for more sustainable effects.

5.0 Conclusion

Consumption of YSS and salmon by healthy overweight participants showed that both fishes may contribute to healthy benefit of omega-3 fatty acid (EPA/DHA). Alteration of serum leptin and activation of platelet and endothelial cells biomarkers suggested the health benefits of YSS fish (ikan selar) and salmon among healthy overweight adults may be comparable. These findings could add values to YSS, a commonly found fish in Malaysia. Further analysis on gene expression and EPA/DHA phospholipid content can be conducted to establish the health benefits of YSS.

6.0 Acknowledgements

We thank the Malaysian Ministry of Science, Technology and Innovation (Science Fund MOSTI 06- 01-04-SF2310) for their financial support and my appreciation goes to all co-researchers their contribution, support and assistance, including subjects of this study

7.0 Disclosures

The authors declare no conflicts of interest in this work

8.0 References

Abd Aziz, N., Azlan, A., Ismail, A., Mohd Alinafiah, S., & Razman, M. R. (2013). Quantitative determination of fatty acids in marine fish and shellfish from warm water of straits of malacca for nutraceutical purposes. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/284329

Abdelaal, M., Roux, C. W., & Docherty, N. G. (2017). Morbidity and mortality associated with obesity, *5*(4), 1–12. https://doi.org/10.21037/atm.2017.03.107

Access, O. (2008). peroxisome proliferator-activated receptor agonists ?, *6*, 1–6. https://doi.org/10.1186/1475-2840-7-6

Acids, S. F., & Risk, C. (2017). Saturated Fatty Acids and Cardiovascular Disease : Replacements for Saturated Fat to Reduce Cardiovascular Risk. https://doi.org/10.3390/healthcare5020029

Adili, R., Voigt, E. M., Bormann, J. L., Foss, K. N., Hurley, L. J., Meyer, E. S., … Larson, M. K. (2017). In vivo modeling of docosahexaenoic acid and eicosapentaenoic acid-mediated inhibition of both platelet function and accumulation in arterial thrombi. *Platelets*, 1–9. https://doi.org/10.1080/09537104.2017.1420154

Anwaruddin, S., Askari, A. T., & Topol, E. J. (2007). Redefining Risk in Acute Coronary Syndromes Using Molecular Medicine, *49*(3). https://doi.org/10.1016/j.jacc.2006.08.051

Asztalos, I. B., Gleason, J. A., Sever, S., Gedik, R., Asztalos, B. F., Horvath, K. V., … Schaefer, E. J. (2016). Effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular disease risk factors: a randomized clinical trial. *Metabolism: Clinical and Experimental*, *65*(11), 1636–1645. https://doi.org/10.1016/j.metabol.2016.07.010

Back, G. (2006). n Ɣ 3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits 1 – 3, *83*(May 2005), 1526–1535.

Bagge, A., Schött, U., & Kander, T. (2018). High-dose omega-3 fatty acids have no effect on platelet aggregation or coagulation measured with static and flow-based aggregation instruments and Sonoclot; an observational study in healthy volunteers. *Scandinavian Journal of Clinical and Laboratory Investigation*, *0*(0), 1–7. https://doi.org/10.1080/00365513.2018.1516477

Baldwin, K. M., Singh, I., & Meyer, B. J. (2010). Effects of Seal Oil and Tuna-Fish Oil on Platelet Parameters and Plasma Lipid Levels in Healthy Subjects, *M*, 669–681. https://doi.org/10.1007/s11745-010-3450-z

Balthasar, N., Coppari, R., Mcminn, J., Liu, S. M., Lee, C. E., Tang, V., … Lowell, B. B. (2004). Leptin Receptor Signaling in POMC Neurons Is Required for Normal Body Weight Homeostasis, *42*, 983–991.

Banks, W. A., Farrell, C. L., William, A., & Impaired, C. L. F. (2019). Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible, *63106*, 10–15.

Beaulieu, L. M., Lin, E., Mick, E., Koupenova, M., Weinberg, E. O., Carolyn, D., … Freedman, J. E. (2014). Profile During Inflammation in Mice and Humans, *34*(3), 552–564. https://doi.org/10.1161/ATVBAHA.113.302700.Interleukin

Blann, A. D., Nadar, S. K., & Lip, G. Y. H. (2004). The adhesion molecule P-selectin and cardiovascular disease, (January). https://doi.org/10.1016/j.ehj.2003.08.021

Calder, P. C. (2015). Omega-3 Fatty Acids and Inflammatory Processes, (October). https://doi.org/10.3390/nu2030355

Cancino, M., Medina, F., Varela, P., Vargas, R., Tapia, G., Luis, A., & Ferna, V. (2011). N-3 PUFA Supplementation Triggers PPAR- a Activation and PPAR- a / NF- k B Interaction : Anti-Inflammatory Implications in Liver Ischemia-Reperfusion Injury, *6*(12). https://doi.org/10.1371/journal.pone.0028502

Chatterjee, M., Rath, D., Rheinlaender, J., Walker-allgaier, B., Alnaggar, N., Zdanyte, M., … Sch, T. E. (2017). Regulation of oxidized platelet lipidome : implications for coronary artery disease, 1993–2005. https://doi.org/10.1093/eurheartj/ehx146

Chen, L. Y., Jokela, R., Li, D. Y., Bowry, A., Sandler, H., Sjöquist, M., … Mehta, J. L. (2000). Effect of stable fish oil on arterial thrombogenesis, platelet aggregation, and superoxide dismutase activity. *Journal of Cardiovascular Pharmacology*, *35*(3), 502–505. https://doi.org/10.1097/00005344-200003000-00022

Collins, T., & Cybulsky, M. I. (2001). NF- κ B in defense and disease NF- κ B : pivotal mediator or innocent bystander in atherogenesis ?, *107*(3), 255–264.

Colussi, G., Catena, C., Dialti, V., Pezzutto, F., Mos, L., & Sechi, L. A. (2014). Fish meal supplementation and ambulatory blood pressure in patients with hypertension: Relevance of baseline membrane fatty acid composition. *American Journal of Hypertension*, *27*(3), 471–481. https://doi.org/10.1093/ajh/hpt231

Corsonello, A., Perticone, F., & Malara, A. (2003). Leptin-dependent platelet aggregation in healthy , overweight and obese subjects, (March 2014). https://doi.org/10.1038/sj.ijo.0802273

Cottin, S. C., Alsaleh, A., Sanders, T. A. B., & Hall, W. L. (2016). Lack of effect of supplementation with EPA or DHA on platelet-monocyte aggregates and vascular function in healthy men. *Nutrition, Metabolism and Cardiovascular Diseases*, *26*(8), 743–751. https://doi.org/10.1016/j.numecd.2016.03.004

Cottin, S., Sanders, T. A. B., & Hall, W. L. (2011). The differential effects of EPA and DHA on cardiovascular risk factors Conference on " Nutrition and health : cell to community " Postgraduate Symposium The differential effects of EPA and DHA on cardiovascular risk factors Proceedings of the Nutrition Society, (May 2014). https://doi.org/10.1017/S0029665111000061

Dasheng, X. I. A., Yanqiu, S., Chao, L. I., Feng, Z., & Minxin, W. E. I. (2007). The change of serum leptin and its relationship with platelet membrane glycoprotein Ib in patients with coronary heart disease, *1*(4), 352–355. https://doi.org/10.1007/s11684-007-0067-6

Davı, G., Guagnano, M. T., Ciabattoni, G., Basili, S., Falco, A., Marinopiccoli, M., … Sensi, S. (2019). Role of Inflammation and Oxidant Stress, *288*(16).

Dietary intervention with oil rich fish reduces platelet-monocyte aggregation in man. (2019), (May), 2019. https://doi.org/10.1016/j.atherosclerosis.2007.04.047

Division, N. (2015). National Health & Morbidity Survey 2015; NCD Risk Factors.

Du, S., Jin, J., Fang, W., & Su, Q. (2015). Does Fish Oil Have an Anti-Obesity Effect in Overweight / Obese Adults ? A Meta-Analysis of Randomized Controlled Trials, 1–20. https://doi.org/10.1371/journal.pone.0142652

Elbatarny, H. S., & Maurice, D. H. (2005). Leptin-mediated activation of human platelets: involvement of a leptin receptor and phosphodiesterase 3A-containing cellular signaling complex. *American Journal of Physiology-Endocrinology and Metabolism*, *289*(4), E695–E702. https://doi.org/10.1152/ajpendo.00125.2005

Hamilton, M. C., Hites, R. A., & Schwager, S. J. (2005). Lipid Composition and Contaminants in Farmed and Wild Salmon, *39*(22), 8622–8629.

Heart Foundation. (2008). Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular health. *Position Statement*, (November), 1–8. https://doi.org/10.1111/j.1365-2699.2012.02686.x

Hosogoe, N., Ishikawa, S., Yokoyama, N., Kozuma, K., & Isshiki, T. (2017). Add-on Antiplatelet Effects of Eicosapentaenoic Acid With Tailored Dose Setting in Patients on Dual Antiplatelet Therapy. *International Heart Journal*, *58*(4), 481–485. https://doi.org/10.1536/ihj.16-430

Innes, J. K., & Calder, P. C. (2018). The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: A systematic review. *International Journal of Molecular Sciences*, *19*(2). https://doi.org/10.3390/ijms19020532

Jr, M. G. M., Leibel, R. L., Seeley, R. J., & Schwartz, M. W. (2011). NIH Public Access, *21*(11), 643–651. https://doi.org/10.1016/j.tem.2010.08.002.Obesity

Khor GL, Noor Safiza MN, Rahmah R, Jamaluddin AR, Kee CC, Geeta A, Jamaiyah H, Suzana S, Wong NF, Ahmad Ali Z, R. A. & A. F. Y. (2008). The Third National Health and Morbidity Survey (NHMS III) 2006: Nutritional Status of Children Aged 0 to Below 18 Years and Adults Aged 18 Years and Above. *23 Rd Scientific Conference of the Nutrition Society of Malaysia*, *14*(March), 11.

Kitson, A. P., Patterson, A. C., Izadi, H., & Stark, K. D. (2009). Pan-frying salmon in an eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) enriched margarine prevents EPA and DHA loss. *Food Chemistry*, *114*(3), 927–932. https://doi.org/10.1016/j.foodchem.2008.10.039

Klop, B., Elte, J. W. F., & Cabezas, M. C. (2013). Dyslipidemia in Obesity: Mechanisms and Potential Targets, (April). https://doi.org/10.3390/nu5041218

Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G., & Grüters, A. (2014). Severe early-onset obesity , adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans, (February). https://doi.org/10.1038/509

Lindemann, S., Tolley, N. D., Dixon, D. A., Mcintyre, T. M., Prescott, S. M., Zimmerman, G. A., & Weyrich, A. S. (2001). by regulated interleukin 1 ␣ synthesis, (September). https://doi.org/10.1083/jcb.200105058

Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes : A meta-analysis of randomized , double blind , placebo controlled trials. (2019), (May), 2019. https://doi.org/10.1016/S1567-5688(13)70005-9

Mackay, I., Ford, I., Thies, F., Fielding, S., Bachoo, P., & Brittenden, J. (2012). Effect of Omega-3 fatty acid supplementation on markers of platelet and endothelial function in patients with peripheral arterial disease. *Atherosclerosis*, *221*(2), 514–520. https://doi.org/10.1016/j.atherosclerosis.2011.12.041

Mingay, E., Veysey, M., Lucock, M., Niblett, S., King, K., Patterson, A., & Garg, M. (2016). Journal of Nutrition & Intermediary Metabolism Sex-dependent association between omega-3 index and body weight status in older Australians. *Journal of Nutrition & Intermediary Metabolism*, *5*, 70–77. https://doi.org/10.1016/j.jnim.2016.04.001

Ministry of Health Malaysia. (2014). CPG Management of acute STEMI 3rd edition, *14*, 1–106.

Mohanty, B. P., Ganguly, S., Mahanty, A., Sankar, T. V, Anandan, R., Chakraborty, K., … Sridhar, N. (2016). DHA and EPA Content and Fatty Acid Profile of 39 Food Fishes from India, *2016*. https://doi.org/10.1155/2016/4027437

Mozaffarian, D., & Wu, J. H. Y. (2011). Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, *58*(20), 2047–2067. https://doi.org/10.1016/j.jacc.2011.06.063

Munro, I. A., & Garg, M. L. (2013). Dietary supplementation with long chain omega- 3 polyunsaturated fatty acids and weight loss in obese adults. *Obesity Research & Clinical Practice*, *7*(3), e173–e181. https://doi.org/10.1016/j.orcp.2011.11.001

Nazaimoon, W., Mohamud, W., Musa, K. I., Sharifuddin, A., Frcp, K., Ismail, A., … Mrcp, I. (2011). Short Communication Prevalence of overweight and obesity among adult Malaysians : an update, *20*(May 2010), 35–41.

Oh-I, S., Shimizu, H., Sato, T., Uehara, Y., Okada, S., & Mori, M. (2005). Molecular mechanisms associated with leptin resistance: n-3 polyunsaturated fatty acids induce alterations in the tight junction of the brain. *Cell Metabolism*, *1*(5), 331–341. https://doi.org/10.1016/j.cmet.2005.04.004

Omega-3 fatty acids and inflammatory processes : From molecules to man. (2019), (May), 2019. https://doi.org/10.1042/BST20160474

Parolini, C., Vik, R., Busnelli, M., Bjørndal, B., Holm, S., Brattelid, T., … Chiesa, G. (2014). A salmon protein hydrolysate exerts lipid-independent anti-atherosclerotic activity in apoE-deficient mice. *PLoS ONE*, *9*(5), 1–9. https://doi.org/10.1371/journal.pone.0097598

Phang, M., Lincz, L. F., & Garg, M. L. (2013). Eicosapentaenoic and Docosahexaenoic Acid Supplementations Reduce Platelet Aggregation and Hemostatic Markers Differentially in Men. https://doi.org/10.3945/jn.112.171249.addition

Pinel, A., Pitois, E., Rigaudiere, J., Jouve, C., Saint-vincent, S. De, Laillet, B., … Lyon-sud, M. (2016). EPA prevents fat mass expansion and metabolic disturbances in mice fed with a Western diet, *57*. https://doi.org/10.1194/jlr.M065458

Potential impact of omega-3 treatment on cardiovascular disease in type 2 diabetes. (2019), (May), 2019. https://doi.org/10.1097/MOL.0b013e328321b3be

Ridker, P. M., Buring, J. E., & Rifai, N. (2001). Cardiovascular Events, 491–495.

Risk, M. (2009). Association Bet ween Increased Platelet P-Selectin Expression and Obesity in Patients With Type 2 Diabetes, (February). https://doi.org/10.2337/dc08-1308.

Riyahi, N., Tohit, E. R. M., Thambiah, S. C., & Ibrahim, Z. (2018). Platelet-related cytokines among normal body mass index, overweight, and obese Malaysians. *Asia Pacific Journal of Clinical Nutrition*, *27*(1), 182–188. https://doi.org/10.6133/apjcn.032017.01

Rossi, A. S., Lombardo, Y. B., Lacorte, J., Chicco, A. G., Rouault, C., Rizkalla, S. W., … Rouault, C. (2019). Dietary fish oil positively regulates plasma leptin and adiponectin levels in sucrose-fed , insulin-resistant rats, *242*(3000), 486–494. https://doi.org/10.1152/ajpregu.00846.2004.

Salem, N., & Eggersdorfer, M. (2015). Is the world supply of omega-3 fatty acids adequate for optimal human nutrition ?, 147–154. https://doi.org/10.1097/MCO.0000000000000145

Seierstad, S. L., Seljeflot, I., Johansen, O., Hansen, R., Haugen, M., Rosenlund, G., … Arnesen, H. (2005). Dietary intake of differently fed salmon; the influence on markers of human atherosclerosis. *European Journal of Clinical Investigation*, *35*(1), 52–59. https://doi.org/10.1111/j.1365-2362.2005.01443.x

Seyedi, S.M., Sharifpour, I., Ramin, M. and J. S. (2011). Proximate composition and energetic value of selected marine fish and shellfish from the West coast of Peninsular Malaysia. *International Food Research Journal*, *18*(January 2017), 137–148.

Simopoulos, A. P. (2016). An increase in the Omega-6/Omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, *8*(3), 1–17. https://doi.org/10.3390/nu8030128

Sonnweber, T., Pizzini, A., Nairz, M., Weiss, G., & Tancevski, I. (2018). Arachidonic Acid Metabolites in Cardiovascular and Metabolic Diseases. https://doi.org/10.3390/ijms19113285

Tsoupras, A., Lordan, R., Shiels, K., & Saha, S. K. (2019). In Vitro Antithrombotic Properties of Salmon (Salmo salar) Phospholipids in a Novel Food-Grade Extract, (January). https://doi.org/10.3390/md17010062

Venkata Krishnan, P., Anuradha, S., Bhattacharjee, J., & Gaiha, M. (2007). Effects of low dose omega-3 fatty acids on platelet functions and coagulation profile in Indian patients with type 2 diabetes mellitus with vascular complications: A prospective, preliminary study. *Journal, Indian Academy of Clinical Medicine*, *8*(1), 45–52.

Wang, Y., & Huang, F. (2015). N-3 polyunsaturated fatty acids and inflammation in obesity: Local effect and systemic benefit. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/581469

Winnicki, M., Somers, V. K., Accurso, V., Phillips, B. G., Puato, M., Palatini, P., & Pauletto, P. (2002). Brief Rapid Communications, 289–291. https://doi.org/10.1161/01.CIR.0000025241.01418.4D

Yee, P., Azlan, A., & Eng, H. (2018). Cooking methods affect total fatty acid composition and retention of DHA and EPA in selected fish fillets, *44*, 92–101.

Yunus, M., & Effendi, M. I. (2013). Mineral contents of selected marine fish and shellfish from the west coast of Peninsular Malaysia, *20*(1), 431–437.

Zhu, C. F., Li, G. Z., Peng, H. Bin, Zhang, F., Chen, Y., & Li, Y. (2010). Therapeutic effects of marine collagen peptides on Chinese patients with type 2 diabetes mellitus and primary hypertension. *American Journal of the Medical Sciences*, *340*(5), 360–366. https://doi.org/10.1097/MAJ.0b013e3181edfcf2