Effect of yellow stripe scad (YSS) fish consumption on platelet microparticles markers: Can YYS fish be like salmon in overweight healthy individual?

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ABSTRACT
Overweight and obesity is currently a growing burden, according several research data from National Health and Morbidity Survey (NHMS) in Malaysia, that in 1999 overweight were at 16.6%, obesity 4.5%; in 2006, overweight were at 29.1%, obesity 14%, in 2011 overweight were, 29.4%, obesity 15.1% and in 2015 overweight were 30%, obesity 17.7%. Suggesting that there is parallel prevalence increase, in this sedentary lifestyle in 2015 compared to 1999. Overweight and obesity affects multiple factor such as shifting lifestyles, urbanization, rising income and influence the genetic make-up of life. Currently, it is a great concern in Malaysia, because it is a risk factor for most inflammatory disease such as cardiovascular disease, cancer and type 2 diabetes, stroke and chronic inflammatory disease. Recent information by Malaysian health minister, won that the country is facing overweight and obesity pandemic problem showing that half of Malaysian population were either overweight or obese. In addition, the latest estimates from the World Health Organization (WHO), show that almost 14% of the country’s citizens fall under the “obese” category in Malaysia. A further 40% are overweight. Evidence has shown that PMPs interact with sub-endothelial matrix to mobilise monocytes and neutrophils that favour foam cells formation. PMPs play an important role in the transport and delivery of bioactive molecules that can signal inflammation and promote aberrant angiogenesis in diseases such as atherosclerosis, diabetic retinopathy and cancers in overweight. PMPs may affect target cells either by stimulating them via surface-expressed ligands or by transferring surface receptors from one cell to the others. The modulation effect of omega-3 fatty acid (EPA/DHA) from salmon on platelets and endothelial cell markers has been established. American Heart Foundation in collaboration with Committee on Evaluation, Prevention and Detection recommends 250 to 300 mg of cooked salmon per day for the treatment of high blood pressure and cardiovascular diseases. Yellow stripe scad (YSS) is a local Malaysian fish, recently identified to increase HDL-C in overweight subjects. However, the atheroprotective effect of omega-3 fatty acid from YSS on PMPs markers in overweight and obese healthy individuals is still unclear. Thus, the aim of this article is to explore the nutritional value (EPA/DHA) of YSS Fish fillet on platelet microparticles markers that predetermine overweight and obesity risk factors of atherosclerosis.

Key words: overweight, leptin, platelet activation, platelet microparticles markers, YSS

INTRODUCTION
Overweight and obesity in Malaysia
Overweight/obesity is a growing concern in Malaysia as diseases such as type 2 diabetes, cancer, cardiovascular disease, stroke and chronic diseases are reaching worrying levels, however, a well-balanced diet has vital roles in maintaining normal weight, promoting an overall healthy life and preventing chronic inflammatory disorders such as atherosclerosis, heart diseases, cancers and post-operative morbidity¹. World Health Organization (WHO) in 2016 has shown that over 1.9 billion adults aged ≥ 18 years were overweight, whereas more than 650 million aged ≥ 18 years were obese. Recently, Malaysia was ranked as the country with second highest overweight population in South East Asia²,³. Current data from National Health and Morbidity Survey have shown that the prevalence rate of overweight male and female in Malaysia between 2011 and 2015 were at 29.4% and 30.0%, respectively, and the obesity prevalence rate were at 15.1% and 17.7%, respectively⁴-⁷. The presence of activated platelets in overweight subjects generates complex reactions that put healthy subjects at risk of pro-thrombotic state⁸. Atherosclerosis is an inflammatory disease characterized by abnormal lipid metabolism and storage disorder, that cause the infiltration of neutrophil, monocytes and macrophages into the endothelial matrix, via platelet...
activation or PMPs markers on the vascular arterial wall, for foam cell formation. Foam cells are lipid rich core of fat laden, which are formed as a result of monocytes recruitment into the endothelial matrix in the earlier stage of atherosclerotic formation. Several studies have shown that omega-3 fatty acid (EPA+DHA) inhibits expression of platelet activation, PMPs and their related makers, leucocyte recruitment and foam cell formation. Salmon fish fillet is well-known for its cardio-protective properties. This review article highlights the complex interactions between overweight with platelets activation, PMPs and its related makers on endothelial cells and vascular smooth muscle cells (VSMC) for the pathogenesis of atherosclerosis. The dietary values of local Malaysian fish, the salmon (EPA/DHA) in comparison to foreign fish, the salmon (EPA/DHA) on PMPs markers to prevent atherosclerosis will also be discussed.

**Association between Overweight, Leptin and Platelet Activation**

Overweight is a low grade chronic inflammatory disorder that has been linked to chronic degenerative disease. Several literatures associated this metabolic disorder to excessive adipose tissue that is commonly accompanied by hypertriglyceridemia, hyperinsulinemia, insulin-resistance, decreased HDL cholesterol and hypertension. The occurrence of these multiple factors triggers the atherosclerotic lesions in the endothelium. However, another possible mechanism is involved, i.e. haemostatic imbalance, particularly platelet hyper-reactivity, which aggravates the pathogenesis of atherosclerosis. The latter hypothesis elicits the search for specific circulating factors that may be responsible for the enhanced amplification of acute thrombotic reciprocation to vascular endothelial bed injury in overweight and obese individuals. Overweight and obesity can be characterised as leptin resistance. Leptin is an adiponectin hormone produced by OB gene and consists of 167-amino acids, functioning as fat and energy storage regulator in mammals. Leptin acts directly in hypothalamic receptors to reduce food appetite and to enhance the energy expenditure level by regulating both fatty acid and glucose metabolism in normal subjects. In fact, studies have shown that high concentration of leptin demonstrates signs of leptin resistance, and the ob/ob mouse that lacks functional leptin, due to nonsense mutation in codon 105 ob — gene, develops severe hyperplasia and excessive obesity. Thus, leptin plays a significant role in this diet-related disorder. Previous literatures also reported that leptin has complex biological roles more/less than the body weight modulation; i.e. leptin was reported to regulate angiogenesis, immune function, fertility, and bone formation, and the presence of leptin receptors can be found in various types of tissues. Human platelets were shown to possess the long form of leptin receptors and high levels of leptin were shown to act collegially along with ADP to foster platelet hyper-reaction in-vitro. These observations have shown the possibility that leptin resistance may contribute to atherosclerotic lesion that alters the haemostatic balance in overweight subjects (Figure 1).

**Activated platelets release inflammation markers and platelet microparticles (PMPs)**

Activated platelets release multiple platelet activation markers, which are associated with vascular inflammation and thrombosis that include soluble CD40 ligand (sCD40L), P-selectin, Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), thromboxane-A2 (TXA2) and platelet factor 4 (PF-4). RANTES mobilises T-cells and monocytes to the injured endothelial cells/VSMC via platelet P-selectin to stimulate monocyte migration and arrest into the vascular endothelium. PDGF is a platelet growth factor that plays significant roles in proliferation, migration and stimulation of VSMC in the presence of serotonin and TGF-β. PDGF also acts as monocyte and neutrophil chemotactic factor and signalling via the platelet-derived growth factor receptor (PDGFR) pathway. This pathway plays a significant role in VSMC migration and proliferation, which is a primary characteristic of atherosclerosis. PF-4 is a subfamily of CXC chemokine that promotes the differentiation of monocytes into foam cell formation through the interaction of oxidized low-density lipoproteins (LDL) and injured endothelial cells. It also protects the oxidized LDL receptors from degradation. sCD40L is a soluble protein released from alpha granules of activated platelets with the structural resemblance to TNF-α superfamily, which is found on the exposed surface of activated platelets. sCD40L increases the stability of new platelet aggregation and facilitates the inflammatory mechanism that involves vascular endothelial cells and reactive oxygen species (ROS). This leads to the expression of adhesion molecules such as Intercellular Adhesion Molecule-1 (ICAM-1), E-selectin and Vascular Cell Adhesion Molecule-1 (VCAM-1) in both VSMC and endothelial cells, including the secretion of matrix metalloproteinase (MMPs), procoagulant factors,
Figure 1: Proposed leptin-dependent pathway of platelet activation in healthy overweight that releases platelet microparticles and markers; P-selectin (CD62P), CD42a(Gplb) and GPIb-V-IX, CD63, platelet endothelium adhesion molecule-1(PECAM-1(CD31), integrin glycoprotein (GP) IIb/IIIa, (CD41 and CD61).
cytokines and chemokines. Activated platelets also release PMPs for their normal physiological function. The small size and structure of PMPs make them an important cargo in platelet cell communication and a delivery agent of platelet — borne bio-active molecules such as platelet activation markers, platelet inflammation cytokines, signalling molecules, growth factors, and microRNAs (miRNA). An increased level of plasma PMPs was shown to occur in subjects with few pathological conditions such as overweight with type 2 diabetes or non-overweight with type 2 diabetes. In contrast, overweight subjects in the absence of diabetic condition shows no effect of PMPs markers, but an increased level of PMPs without markers was observed. Increased levels of specific positive PMPs markers in subjects with a diabetic condition may provide a potential pathway by which PMPs contribute to the pathogenesis of atherosclerosis and cardiovascular events.

Janowska-Wieczorek and colleagues have shown that haematopoietic stem-progenitor cells expressed PMPs membrane receptors such as CD62, CD41, PAR-1 and CXCR4 that encourage the engraftment of progenitor cells to the endothelium. This observation suggests that PMPs from activated platelets play a significant role in stem cell transplantation and recipient cells, which affects the cell function of the recipients. PMPs engulf cytoplasmic membrane during their formation to acquire protein and RNA from the cytosol of the parent cells. Multiple evidence have suggested that following the attachment or fusion of PMPs to the target cells, PMPs will transfer the RNA and protein content to the recipient cells. This process facilitates the recipient cell to internalise PMPs via endocytosis or through receptor-ligand interactions, which suggests that PMPs could reprogram a target cells. Ratajczak et al., shown that microparticles derived from embryonic stem cells reprogrammed the haematopoietic stem cell progenitors via the horizontal transfer of protein and mRNA delivery. Other studies have shown that microparticles from endothelial cell induced angiogenesis in both in-vivo and in-vitro via horizontal transfer of mRNA to human macrovascular and microvascular endothelium. The CD41+ microparticles are known to have coagulant properties, however, the anticoagulant properties are still unknown.

Pathophysiology of PMPs and its related markers in atherosclerosis

PMPs are known to play an important haemostatic role, by which the inability of platelets to generate PMPs was linked to bleeding episode (Castaman’s defect). Secondly, platelets in patient with Scott’s syndrome shows impaired ability to generate PMPs and displays a bleeding diathesis. PMPs were demonstrated to play significant roles in the activation of vascular endothelial cells, exposure of PMPs to endothelial phospholipase A2, and stimulation of arachidonic acid release from both platelets and endothelial cells, which are subsequently metabolised to produce thromboxane A2. The trans-activation of both platelets and endothelial cells further promotes the interactions of monocyte endothelial cells. Berckmans and colleagues reported that the presence of PMPs in healthy individuals, particularly the CD41+ microparticles, are known to generate thrombin. CD41+ microparticles are highly procoagulant in nature. The presence of CD41+ receptors on PMPs may contribute to the pathogenesis of arterial thrombosis disease. Several literatures have demonstrated that circulating PMPs may provide a potential diagnostic and prognostic makers of atherosclerotic vascular disease. CD63-exposing and P-selectin from PMPs reflect the platelet activation markers in myocardial infarction and peripheral arterial disease. Michelsen et al., have shown that increased levels of PMPs was observed in myocardial infarction survivors. PMPs have been shown to be independent of large PMPs, soluble CD40 ligand (sCD40L) and plasma thrombin-antithrombin complexes in patients with myocardial infarction, but not in healthy controls. Another study by Chironi et al. demonstrated that the diameter of both internal and external carotid artery were negatively correlated with PMPs derived from platelets, endothelial cells and leucocytes. It is, however, still unclear about the effect of omega-3 fatty acid (EPA/DHA) from YSS fish fillet on PMPs (CD41) and its related markers such as P-selectin (CD62P), CD42a (GpIb), GpIb-V-IX, CD63, platelet endothelium adhesion molecule-1 (PECAM-1(CD31)), integrin glycoprotein (GP) IIb/IIIa.

Platelet microparticles (PMPs) in endothelial cells and atherosclerosis

Endothelial cells (ECs) are the barrier that maintains the balance between the circulatory molecules and the cells. It is the key modulator of vascular homeostasis, which plays an essential role in signal transduction that affects the reorganization of vascular wall physical composition. ECs release certain vascular - balanced chemical molecules such as nitric oxide (NO), endothelia, prostaglandin I2 and endothelium-derived relaxing factor that maintains the integrity of
Atherosclerosis is an inflammatory disease linked to platelet microparticles (PMPs) that is characterised with inflammation, adhesion, and lipid deposition and procoagulation. Endothelial damage influences the adhesion of PMPs to vascular endothelial cell and induces the proliferation, apoptosis and transmigration endothelial cell. The endothelial cell mobilizes proteins such as ICAM-1, GPIIb/GPIIIa, and RANTES. The presence of PMPs increases the adhesion of molecules to mobilize monocytes and leucocytes into subendothelial layer to enhance vascular inflammation in the endothelial lesion. For example, iCAM-1, MPO, IL-8 and IL-10 are known to be involved in this process. Besides that, ox-LDL can easily activates platelet through CD36, called scavenger receptor, to release PMPs and dysfunctional HDL to form the bases of atherosclerosis that can be inhibited by DHA and EPA via PMPs markers.
weight subjects can be obtained using flow cytometry and the therapeutic effect of omega-3 fatty acid (EPA/DHA) from YSS fish fillet on these markers will be monumental to treat these diet-related disorders (Figure 3).

**Selected platelet microparticles (PMPs) marker in atherosclerosis**

P-selectin (CD62P): is an adhesion molecule that links PMPs to endothelial cells (ECs). Increased levels of PMPs and P-selectin in blood circulation is a sign of diabetes, hyperlipidemia, hypertension and overweight. P-selectin in diabetes patients are known to be associated with thrombotic events, such as retinopathy, coronary heart disease and atherosclerosis. The PMPs level is associated with platelet activation by expression of platelet P-selectin\(^\text{67}\). It is indeed, to note that, PMPs target in cardiovascular treatment are still limited\(^\text{68}\). PMPs with omega-3 fatty from YSS may modulate P-selectin levels as compared to those from salmon in both platelet and ECs.

CD42a (GpIb) and GPIb-V-IX, or GPIb\(_{\alpha}\)/Ib\(_{\beta}\)/V/IX : are also known as CD42a/CD42b/CD42d/CD42a respectively, are four of the glycoproteins belong to leucine-rich family. It facilitates platelets adhesion to vWF during vascular endothelial damage\(^\text{69}\). Deficiency of one of these four proteins can lead to Bernard-Soulier syndrome, which is a severe blood bleeding disorder. The interaction between vWF and GPIb-V-IX\(^\text{70}\) signals the transduction to activate platelet integrin (GPIIb/IIIa). This mechanism was observed in Apoe\(^{-/-}\) mice injected with GPIb\(_{\alpha}\), by which a drastic reduction in atherosclerosis lesion size was observed\(^\text{71}\). The observation indicates that GPIb\(_{\alpha}\) binding to vWF on endothelial cell is highly pro-inflammatory.

**PMPs and platelet integrin aIIb \(3\) (GpIIb-IIIa):** Platelet integrin belongs to the fibrinogen receptor family that is crucial for platelet activation, which can be found on the platelet membrane and the CD41/CD61 complex. Platelet integrin is also known as glycoprotein (GP) Ila/IIIa. The combination of CD41 and CD61 is associated with non-covalent bonding with fibrinogen, which is the basis of platelet aggregation\(^\text{72}\). Thus, the presence of this receptor (Ila/IIIa) in PMPs during acute coronary disorder allows the binding of PMPs to vascular wall, which will initiate a signal transduction pathway such as extracellular signal-regulated kinase, phosphoinositide 3-kinase (PI3-kinase) pathways, and activation of pertussis-toxin-sensitive G protein\(^\text{73}\). Studies have shown the association between high levels of PMPs with various diseases such as sickle cell disease, malignant tumour formation, thrombocytopenic purpura, cardiovascular diseases and haemolytic disorders\(^\text{65,74}\).

**Modulation effects of fish fillet (EPA and DHA) on platelet microparticles (PMPs) markers**

A meta-analysis of randomised clinical intervention trials have shown that omega-3 fatty acid (EPA/DHA) reduced the procoagulant function of platelets and cardiovascular disease (CVD)\(^\text{75}\). Other studies have shown a significant beneficial effects of EPA/DHA on platelet functions in type 2 DM and coagulation profile in patients with vascular complications, with marginal favourable effects on the glycaemic status and lipid profiles\(^\text{76}\). A pilot study of omega-3 fatty acid supplementation have shown the occurrence of attenuated platelet activation even among patients that took aspirin or aspirin plus clopidogrel\(^\text{77}\). Omega-3 fatty acid (EPA/DHA) exerts a gender-based dependent effects on platelet microparticles and platelet activation but not on MP levels. With regards to thrombotic disease risk, males may benefit more from EPA supplementation\(^\text{78}\). Other effects of EPA/DHA include protection against CVD by reducing serum triacylglycerides (TAG)\(^\text{79}\), heart rate\(^\text{80}\), blood pressure (BP)\(^\text{81}\), and serum homocysteine levels\(^\text{82}\), as well as regulating and resolving inflammatory process\(^\text{83}\) as shown in randomized trials with fish oil supplementation. However, other reports have shown that omega-3 have no effect on platelet aggregation\(^\text{84}\). Despite so, there is a paucity of available data on yellow stripe scad (YSS) as a source of omega-3 fatty acid in overweight healthy subjects.

**Current available data**

In human, immune cells such as monocytes, lymphocytes, neutrophils and platelets are regarded as inflammatory cells that protect the body against foreign invaders. High levels of omega-3 fatty acid (EPA/DHA) in the phospholipid membrane allows the cells to function effectively. However, in overweight individuals, the level of EPA/DHA is compromised with the higher level of omega-6 fatty acid (Arachonic acid) at 10-20% that decreases docosa-hexaenoic acid (DHA) to 2–4% and eicosapentaenoic acid (EPA) to 0.5–1%\(^\text{85,86}\). Even so, the level of omega-3 fatty acid in phospholipid membrane can be altered as omega-3 fatty acid can be obtained from fish and plant sources, despite the fact that the
Figure 3: PMPs are microvesicles with approximately 0.1-1 μm in size and they are released from activated platelets with the aid of physiological agonists such as collagen or thrombin. PMPs express some function markers such as P-selectin (62P), adhesion receptor, GP IIb/IIIa (CD41), PAR-1, GPIb, and CXCR4. PMPs also contain different coagulation proteins and have a role in the haemostatic response to inflammation. PMPs activate the endothelial and stimulate cytokine and adhesion molecules to release their content, promoting the contraction of VSMC. PMPs were found in high concentration in patients with acute vascular syndromes, obesity, diabetes mellitus and atherosclerosis.

...body does not produce omega-3 fatty acid. Currently, three types of fish samples were identified to contain a desirable amount of omega-3 fatty acids (EPA/DHA). Yellow stripe scad (YSS) showed the highest DHA with a Polyunsaturated/Saturated (P/S) = 1.7, moonfish showed the highest ALA with a P/S = 1.0, and longtail shad showed the highest EPA with P/S = 0.4. The intake of omega-3 fatty acid from fish fillets, especially from salmon, was shown to change the ratio of omega-6 to omega-3 fatty acid in favour of omega-3 phospholipid membrane that modulates the cellular inflammation via blood cells in overweight and obese subjects. Previous study by Abdulazeez et al., 2013 has shown that omega-3 fatty acid content in most Malaysian fishes contain lower EPA that falls between 2.7-343.0 mg/100 g wet sample compared to wild salmon (629 mg/100 g). A study by Chang WL et al., 2017 showed that YSS could increase HDL-C levels in overweight subjects further suggesting that YSS fish fillets are comparable to wild salmon, and the omega-3 fatty acid in salmon fish fillets was shown to be cardioprotective. American Heart Foundation recommends the intake of omega-3 fatty acid at 250–300 mg/day for heart disease patients and it is indeed, to note that majority of fish oil capsule production contains salmon. Despite so, the effect omega-3 fatty acid (EPA/DHA) from YSS on PMPs and thrombosis is still unclear. Therefore, it is promising that the consumption of YSS at 250–300 mg/day with reference to recommendation by American Heart Foundation may ameliorates overweight/obesity-related risk factors such as platelet activation, PMPs and cardiovascular diseases.

CONCLUSION

This review article emphasised the role of EPA/DHA in overweight and obesity subjects on PMPs-related biomarkers. The EPA/DHA content in fishes differs from one fish to another in terms of quantity and quality. Despite so, several studies have shown the comparisons between EPA/DHA content of salmon and other fatty fishes. DHA is quantitatively the most important omega-3 PUFA in salmon and has consistently been shown to have unique and indispensable...
roles in platelet phospholipid membrane and cardiovascular diseases. Overweight and obesity subjects were shown to have deficits in EPA/DHA levels due to the higher levels of omega-6 fatty acid (arachidonic acid) on the platelet cellular membrane. Therefore, it is therapeutically promising. Intervention studies in 2017 have shown that EPA and DHA from YSS in-crease d the levels of HDL-C in overweight subjects, suggesting that the consumption of YSS at 250-300 mg/day could reduce the levels of platelet activation, PMPs marker, atherosclerosis and other related risk factors in overweight healthy people.

**ABBREVIATIONS**

ADP: Adenosine diphosphate  
BP: Blood Pressure  
CD41/CD61: integrin glycol protein (GP) IIb/IIIa  
CD41*: Platelet microparticle marker  
CD42a: GpIb, GPIb-V-IX,  
CD62: P-selectin  
CD62E: E-selectin  
CVD: Cardiovascular Disease  
CXCR4: CD184 chemokine  
CXC: chemokine  
HDL-C: High-Density Lipoprotein Cholesterol  
ICAM-1: Intercellular Adhesion Molecule-1  
IL-1β: Interleukin-1 B  
LDL-C: Low-density lipoprotein (LDL) cholesterol  
MCP-1: Monocyte Chemoattractant Protein-1  
mirRNA: microRNAs  
MMPs: Matrix metalloproteinases  
NO: Nitric Oxide  
OB-gene: Obesity gene  
PAR1: Protease-Activated Receptors-1  
PDGF: Platelet-Derived Growth Factor  
PDGFR: Platelet-Derived Growth Factor Receptor  
PECAM: Platelet Endothelium Adhesion Molecule-1 (CD31),  
PF4: Platelet factor 4  
P13: Phosphatidylinositol3-kinase  
PMPs: Platelet Micro-Particles  
RANTES: Regulated on Activation Normal T-cell Expressed and Secreted  
ROS: Reactive Oxygen Species  
sCD40L: Soluble CD40 ligand  
TAG: Triacylglycerides  
TGF-β: Transforming growth factor-β  
TNF-α: Tumor Necrosis Factor alpha  
TXA2: Thromboxane-A2  
VCAM-1: Vascular Cell Adhesion Molecule-1  
VSMC: Vascular Smooth Muscle Cells  
VWF: Von Willebrand Factor  
WHO: World Health Organization  
YSS: Yellow Stripe Scad

**COMPETING INTERESTS**

The authors declare no conflicts of interest regarding the publication of this paper.

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