

# Basic Science for Sustainable Marine Development

## PROCEEDING

INTERNATIONAL SEMINAR 2015

Ambon, 3-4 June 2015

Organized by  
Faculty of Mathematics and Natural Sciences  
Pattimura University



# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

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October 2015

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## **Welcoming Address by The Organizing Committee**

The honorable, the rector of Pattimura University

The honorable, the vice rector of academic affair, Pattimura University

The honorable, the vice rector of administration and financial affair, Pattimura University

The honorable, the vice rector of planning, cooperation and information affair, Pattimura University

The honorable, all the deans in Pattimura University

The honorable, the key note speakers and other guests.

We have to thank The Almighty God for the blessings that allow this International seminar can be held today. This is the first seminar about MIPA Science in which the Faculty of MIPA Pattimura University becomes the host. The seminar under the title Basic Science for Sustainable Marine Development will be carried out on 3 June 2015 at Rectorate Building, the second floor. There are 250 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

This International seminar is supported by the amazing people who always give financial as well as moral supports. My special thanks refer to the rector of Pattimura University, Prof. Dr. Thomas Pentury, M.Si, and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, M. Si. I also would like to express my deepest gratitude to Dr. Kotaro Ichikawa, the director of CSEAS Kyoto University, Prof. Bohari M. Yamin, University of Kebangsaan Malaysia, Prof. Dr. Budi Nurani Ruchjana (Prisident of Indonesian Mathematical Society/Indo-MS), Dr. Ir. A. Syailatua, M.Sc (Director of LIPI Ambon), and Hendry Ishak Elim, PhD as the key note speakers. We expect that this international seminar can give valuable information and contribution especially in developing basic science for sustainable marine development in the future.

Last but not least, we realize that as human we have weaknesses in holding this seminar, but personally I believe that there are pearls behind this seminar. Thank you very much.

Chairman

Dr. Netty Siahaya, M.Si.

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## **Opening Remarks By Dean of Mathematic and Natural Science Faculty**

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 17<sup>th</sup> anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title Basic Science for Sustainable Marine Development. The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Dean of Mathematic and Natural Science Faculty

Prof. Dr. Pieter Kakisina, M.Si.

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## **$\alpha$ -Glucosidase Inhibition Activity of Several Fatty Acid Compounds**

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### **ABSTRACT**

The research about inhibition activity for  $\alpha$ -glucosidase of some fatty acid compounds have been done. Saturated fatty acid such as lauric acid (12:0), palmitic acid(16:0), stearic acid (18:0), and unsaturated fatty acid like oleic acid (18:1), linoleic acid (18:2),  $\alpha$ -linoleic acid, and compounds mixture which contain fatty acid such as Virgin Coconut Oil (VCO), olive oil and shark liver oil. The method used to *in vitro* test antidiabetic activity is inhibition toward  $\alpha$ -glucosidase. Acarbose is used as positive control. Inhibition activity toward  $\alpha$ -glucosidase of fatty acid compounds: lauric acid (50.22%), palmitic acid (49.39%), stearic acid (41.30%), oleic acid (87.17%), linoleic acid (89.24%),  $\alpha$ -linoleic acid (89.55%), compounds mixture which contain fatty acid: Virgin Coconut Oil (VCO) (55.42%), olive oil (24.55%) and shark liver oil (61,60%). It can be concluded that the increase number of double bonds of fatty acid can lead the increased of antidiabetic activity and bond extension of saturated fatty acid can be able to reduce antidiabetic activity and also inhibition activity of single fatty acid compound has no effect toward mixture inhibition, but it highly depend on interaction of each components of mixture itself.

**Keywords:** *Inhibition activity,  $\alpha$ -glucosidase, saturated fatty acid, unsaturated fatty acid*

### **INTRODUCTION**

Glucosidase is a key enzyme which involved in metabolism of carbohydrates, located at the edge of cell surface of the small intestine, and formation process of glycoproteins and glycolipids. Glucosidase works by breaking down carbohydrates into glucose in human intestine. Compounds that can inhibit glucosidase activity is compound that has potential as an antidiabetic, because it is able to reduce blood sugar levels (Rout et al., 2009; Gholamhoseinian et al., 2008).

$\alpha$ -glucosidase is an enzyme that body's needed in process of carbohydrate metabolism, which located at the edge of the cell surface of the small intestine and as a key enzyme in the metabolism, also needed in the formation process of glycoproteins and glycolipids (Lee.DS, et al, 2001). This enzyme works on  $\alpha$ -1,4 glycosidic bond to break down carbohydrates into glucose in human intestine. Compounds which can inhibit the activity of these enzymes indicates that the compound has potential as antidiabetic that can reduce blood sugar levels (Yoshikawa, M., et al, 2001).



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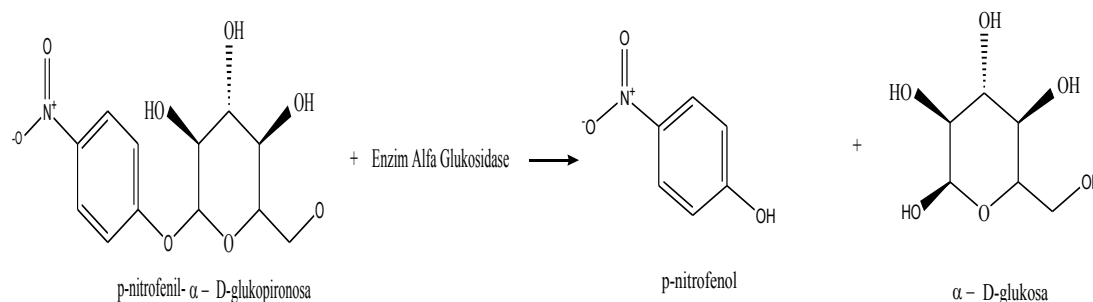


Figure 1. The reaction mechanism  $\alpha$ -glucosidase (Yoshikawa, M., et al, 2001)

$\alpha$ -glucosidase inhibitors also inhibit the  $\alpha$ -amylase enzyme in pancreas in addition to inhibit the bond between the membrane of small intestine by the  $\alpha$ -glucosidase.  $\alpha$ -amylase in pancreas has its function to hydrolyze starch into oligosakarida in lumen of small intestine. Enzyme inhibition in this system reduces the rate of digestion of carbohydrates, resulted lack of absorbed glucose because the carbohydrates are not broken down into glucose molecules (Samantha, J., et al, 2009).

The weakness of  $\alpha$ -glucosidase inhibitor commercially now are side effects like diarrhea, bloating, gas, and skin disorders (Bischoff, et al, 1985). To avoid or reduce bad impact of drugs and also to provide more selection of drugs, it still need to find new inhibitors of  $\alpha$ -glucosidase for further drugs development (Lam, et al, 2008).

Diabetes mellitus is a degenerative disease which characterized by the presence of glucose levels that exceed normal values (hyperglycemia) due to the body lacks of insulin both in absolute and relative (Bnounham et al., 2002). Diabetes mellitus is clinically divided into Insulin Dependent Diabetes Mellitus (IDDM) and Non-Insulin Dependent Diabetes Mellitus (NIDDM) (Bnounham et al., 2006).

According to data of WHO, Indonesia takes 4<sup>th</sup> rank in the number of people in the world with diabetes. Diabetes mellitus is a cause of inadequate insulin activity, due to reduced insulin secretion (IDDM) or due to insulin resistance in sensitive tissues to insulin (NIDDM) (Adnyana, 2004). According to Bhat et al. (2008), control of hyperglycemia for patients with diabetes mellitus, such as through the tractus gastro-intestinal, in peripheral, suppress hepatic glucose production, increase glucose uptake in peripheral (depends / on not depends on the existence of insulin) and also therapeutic approach in the form inhibition of hydrolysis carbohydrate Enzyme such as  $\alpha$ -amylase (Nickavar & Nasibeh, 2009) and  $\alpha$ -glucosidase to slow down the absorption of glucose and antidiabetic drugs consumption.

Fatty acids and glycerol, are the main constituent of vegetable oil or fat and as a raw material for all lipids in all living things. This acid is easy to be found in cooking oil (cooking), margarine, or animal fats and determine its nutritional value. By nature, fatty acids can be shaped freely (because fat is hydrolyzed) or bound as glycerides.

Fatty acid is a weak acid and dissociates in water partially. Generally liquid-phase or solid in room temperature (27 °C). The longer of the chain of atoms C, the fatty acid will be easier to freeze and also more soluble. Saturated fatty acids are more stable (not easily to react) than unsaturated fatty acids. Double bonds in unsaturated fatty acids easily react with oxygen (easily oxidized).

The existence of double bonds in the unsaturated fatty acids making it has two forms: *cis* and *trans*. All fatty acids of natural vegetable only have a *cis* form (denoted with a "Z"). *Trans* fatty acids (*trans* fatty acids, denoted by "E") only produced by the waste from animals or made synthetically. As the result of polarization of H atom, *cis* fatty acids having a curved

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chain. While in trans fatty acids, because of the H atoms opposite so it does not sustain a strong polarization effects and the chain relatively straight.

The purpose of this study was to determine the antidiabetic activity of several compounds of fatty acids, fatty acid mixture and the influence of the double bond and the bond extension to the antidiabetic activity.

## METHODS

### *Materials and Methods*

Materials of this research are fatty acid compounds; lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2),  $\alpha$ -linolenic acid. A mixture of compounds that contain fatty acids: Virgin coconut oil (VCO), olive oil, shark liver oil.

### *Test of Antidiabetic Activity*

*In vitro* antidiabetic activity test was carried out using  $\alpha$ -glucosidase (Saijyo et al., 2008). A total of 1 mg of  $\alpha$ -glucosidase was dissolved in 1000  $\mu$ L of phosphate buffer (pH 7). Then 12  $\mu$ L of enzyme solution was diluted in 30  $\mu$ L of phosphate buffer before being used for testing. Total of 250  $\mu$ L of 20 mM-*p*-nitrophenil- $\alpha$ -D glukopiranosida, 475  $\mu$ L of 100 mM phosphate buffer, and 25  $\mu$ L sample solution was dissolved in DMSO. After a homogeneous solution was incubated for 5 min at 37 °C, and then added 250  $\mu$ L of solution of  $\alpha$ -glucosidase enzymes, incubation continued for 25 minutes. The reaction was stopped by addition of 1 mL of 0.2 M Na<sub>2</sub>CO<sub>3</sub>. The amount of *p*-nitrophenol which released was measured at a wavelength,  $\lambda$  = 400 nm. Furthermore, the ability of inhibition calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{OD test} - \text{OD blanko}}{\text{COD test} - \text{COD blanko}} \times 100\%$$

OD test shows absorbance of the sample with the addition of enzymes, OD blank is the absorbance of the sample without addition of enzymes, COD test absorbance control with the addition of enzymes and COD blank is the absorbance control without the addition of enzymes.

## RESULTS AND DISCUSSION

Antidiabetic activity of several compounds of fatty acids can be seen by how much its inhibition to  $\alpha$ -glucosidase. Fatty acid compounds with the percentage of inhibition value above 50%, has potential as an inhibitor of  $\alpha$ -glucosidase. Inhibitory activity against  $\alpha$ -glucosidase enzyme of some compounds of fatty acids, both saturated fatty acids such as lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0) and unsaturated fatty acids such as oleic acid (18:1), linoleic acid (18:2),  $\alpha$ -linolenic acid (18:3) and acarbose as a positive control served on Tabel.1.

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Table 1. Inhibition activity of  $\alpha$ -glucosidase acarbose and some fatty acid compounds

No	Sampel	COD Test – COD Blank	OD Test-OD Blank	% Inhibition
1	Acarbose	0.1409	0.1354	96.09
2	Lauric Acid (12:0)	0.1409	0.0707	50.22
3	Palmitic Acid (16:0)	0.1409	0.0696	49.39
4	Stearic Acid (18:0)	0.1409	0.0582	41.30
5	Oleic Acid (18:1)	0.1409	0.1220	87.17
6	Linoleic Acid (18:2)	0.1409	0.1257	89.24
7	$\alpha$ -Linoleic Acid (18:3)	0.1409	0.0868	89.55

In this study, acarbose compound used as a positive control. Acarbose is the main compound of oral antidiabetic drugs of  $\alpha$ -glucosidase inhibitor group. Acarbose is pseudotetrasakarida, a natural microbial products derived from broth culture *Actinoplanes* strains SE 50. Acarbose works by competitive reversible towards  $\alpha$ -glucosidase in the brush border of mucosa of the small intestine, so that hydrolysis process of oligosaccharides into monosaccharides not occurred. This drug works to reduce the absorption of glucose in small intestine, so have effect to decrease blood glucose levels after meals.

Unsaturated fatty acids compounds such as oleic acid (18:1), linoleic acid (18:2),  $\alpha$ -linolenic acid (18:3), has inhibition value 87.17%, 89.24 %, 89.55% respectively, thus potential as agents of  $\alpha$ -glucosidase inhibitor. Increasing number of double bonds in fatty acid compounds, giving a tendency to increased inhibitory activity against  $\alpha$ -Glucosidase. The greater number of double bonds in a fatty acid compound resulted in increasingly polarized H atoms to a curved form structure that can obstruct the bond between the carbohydrates with the active site of  $\alpha$ -glucosidase. Inhibition of this bond resulted not immediate in hydrolysis reaction of carbohydrates into glucose.

These results are consistent with previous studies, which an increasing number of double bonds of fatty acids lead to an increase the potential inhibition of various enzymes such as topoisomerase (Suzuki, et al., 2000), hialuronidases (Suzuki, et al., 2002), and telomerase (Eitsuka, et al., 2005).

In the study conducted by The Han Nguyen et al., (2011) isolated two unsaturated fatty acids, namely, acid 7 (Z)-octadecanoic and acid Acid 7 (Z), 10 (Z)-octadecanoic of body wall of sea cucumbers (*Stichopus japonicus*) found that these two compounds have inhibitory to the  $\alpha$ -Glucosidase in *Saccharomyces cerevisiae* with  $IC_{50}$  with each values by 0.51  $\mu$ g/ $\mu$ L and 0.49  $\mu$ g/ $\mu$ L and the inhibition of the  $\alpha$ -Glucosidase in *Bacillus stearothermophilus* 0.51  $\mu$ g/ $\mu$ L and 0.49  $\mu$ g/ $\mu$ L. This study showed that the compound 7 (Z)-octadecanoic have inhibitory activity value smaller than compound 7 (Z), 10 (Z)-octadecanoic.

One approach of therapy to preventing diabetes mellitus is to inhibit the absorption of glucose by inhibiting  $\alpha$ -glucosidase. In this study, two fatty acids with strong inhibitory activity toward  $\alpha$ -glucosidase enzyme, acid 7 (Z)-octadecanoic and Acid 7 (Z), 10 (Z)-octadecanoic, purified and identified from sea cucumbers. Therefore, sea cucumbers fatty acids could potentially to be developed as a new natural *nutraceutical* for the management of type-2 diabetes (The Han Nguyen, et al, 2011).

Compounds of lauric acid (12:0), palmitic (16:0) and stearic acid (18:0) are saturated fatty acid compound which consists of each 16 and 18 C atoms. Inhibitory activity of compounds such as 50.22% lauric acid, palmitic acid 49.39% and 41.30% for stearic acid. The extension of the bond on saturated fatty acid turns down the activity of  $\alpha$ -Glucosidase

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inhibition. The third compounds are not active in inhibit  $\alpha$ -glucosidase so have no potential as an antidiabetic.

The study which conducted by Rachel Lennon and colleagues stated that palmitic fatty acids and saturated fatty acids can increase cellular insulin because of an increase of cellular glucose levels (Rachel Lennon, et al, 2009). *In vivo* testing conducted by Wissal el Assaad and his colleagues found an increase in palmitic and stearic fatty acids and both of them synergy in the death of pancreatic  $\beta$ -cells which furthermore turn will affect to reduction of insulin resistance (Wissal el Assaad, et al., 2003).

In this study, testing for  $\alpha$ -glucosidase inhibitory activity toward some mixture of compounds which contain lots of fatty acids, such as virgin coconut oil, olive oil and shark liver oil, presented in Table 2.

Comparison in composition of fatty acid compounds constituent of a mixture effected against  $\alpha$ -Glucosidase inhibition activity. The activity of single fatty acid compound has not affect to mixture inhibition activity but dependent on interaction of each component of mixture compounds. *Coconut Virgin Oil* (VCO) which is composed of lauric acid (50%), myristic (17%), palmitic (9%) (Sulistyowati, 2009) has 55.42% inhibition. Value inhibition activity of compounds in a mixture of VCO is higher if compared with the inhibitory activity of these compounds singly.

Table 2. Inhibition of  $\alpha$ -Glucosidase Activity Acarbose and some Compound Mixed Fatty Acids

No	Sampel	COD Test – COD Blank	OD Test-OD Blank	% Inhibition
1	Acarbose	0.1409	0.1354	96.09
2	Virgin Coconut Oil	0.1409	0.0781	55.42
3	Olive Oil	0.1409	0.0346	24.55
4	Shark Liver Oil	0.1409	0.0868	61.60

Olive oil consist of 69.7% unsaturated fatty acid compounds and dominated by fatty acids compounds with one double bond (MUFA), such as 83% oleic acid (Sulistyowati, 2009), only have 24.55% of the activity.  $\alpha$ -Glucosidase inhibition activity of oleic acid is 87.18%, but sustain decreased inhibition activity when it composed as mixture in olive oil.

Shark liver oil consists of 75% unsaturated fatty acid compound long chain (PUFA); linolenic (18:3), linoleic (18:2), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) and 25% unsaturated fatty acid compounds with one double bond (MUFA), has antidiabetic activity amounted to 61.60%.  $\alpha$ -Glucosidase inhibition activity of several compounds in a single constituent is;  $\alpha$ -linolenic acid compound (18:3) 89.55%, linoleic acid compound (18:2) 89.24% and 87.18% for oleic compound. Inhibitory activity decreased when arranged as mixture in shark liver oil.

Interaction compounds of constituent a drug can give effect to the drug activity. In pharmacodynamic interactions drug component may cause some effects like, additive interaction will cause twice effect of drug activity. Synergistic interactions or potentiation will give the effect of drug to amplify or increase the effects of other drugs. The antagonist interaction will give the deny effect one another (Gerry S, et al., 2009).

With the same comprehension can be investigated how the influence of compound interaction with other compounds in a mixture and their influence toward inhibition activity. This study conducted to see how the antidiabetic activity of a single fatty acid compound and its activity in the mixture of compounds. High and low inhibitory activity against single fatty

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acid compounds and in mixtures compounds compared with acarbose compounds, presented in Figure 1.

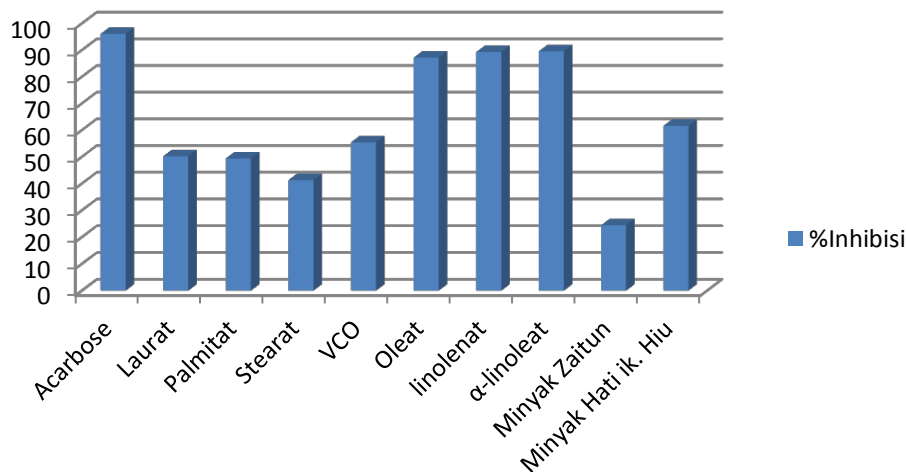


Figure 1.  $\alpha$ -Glucosidase inhibitory activity fatty acid compounds in single and in mixture.

Inhibition activity against  $\alpha$ -glucosidase VCO by 85.45% is higher if compared with activity of lauric acid, palmitic and stearic which is the main constituent of VCO. This increased activity caused of VCO consists of components of compound that interact in a synergistic.

$\alpha$ -glucosidase inhibitory activity of olive oil as 24.55%, very small if compared with the value of inhibition compounds oleic acid, linoleic and  $\alpha$ -linolenic acid. This decrease activity caused by the interaction between of components of olive oil constituent compounds which antagonistic to give deny effect one another.

Shark liver oil has inhibitory activity of 61.60%. This value is very small if compared with the value of inhibition compounds oleic acid, linoleic and  $\alpha$ -linolenic acid. This decrease activity due to the interaction between the components of the shark live oil constituent compounds is not additive and synergistic but antagonistic contrary, thereby give deny effect one another.

However, the value of inhibition activity of shark liver oil is greater when compared with a mixture of fatty acid compounds such as VCO and olive oil.

## CONCLUSIONS

Inhibition activity toward  $\alpha$ -glucosidase enzyme several single fatty acid compounds; lauric 50.22%, palmitic 49.39%, stearic 41.30%, oleic 87.17%, linoleic 88.24%,  $\alpha$ -linolenic acid 89.55%. Inhibition activity of mixture compounds which containing fatty acid: virgin coconut oil (VCO) 55.24%, olive oil 24.55%, shark liver oil 61.60%. Inhibition activity of a single fatty acid compound did not affect to the mixture inhibitory activity thereof, but it depends on each component of constituent mixture. Increasing number of double bonds of fatty acids caused increased inhibitory activity against  $\alpha$ -glucosidase. The extension of the bond on the saturated fatty acid turns down the activity of  $\alpha$ -glucosidase inhibition.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

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## SUGGESTIONS

This study should be continued to determine inhibition activity of  $\alpha$ -glucosidase of unsaturated fatty acids compounds long chain (PUFAs) other and *in vivo* advanced research.

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