ORYZA OIL & FAT CHEMICAL CO. ,LTD.

ISADA Oil

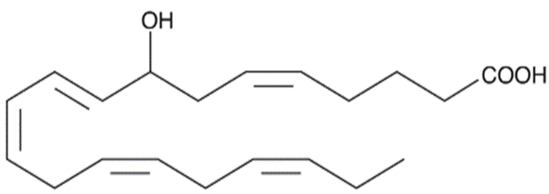
(Japanese Krill Oil)

Dietary ingredient for anti-obesity and prevention of Metabolic syndrome

■ **ISADA OIL**

（**Oil, for foods**）

Pacific krill
（Isada）



8-Hydroxy eicosapentaenoic acid
(8-HEPE)

ORYZA OIL & FAT CHEMICAL CO. ,LTD.

Ver.2.0 TK

Dietary ingredient for anti-obesity and prevention of Metabolic syndrome

ISADA Oil

(Japanese Krill Oil)

1.Introduction

Isada is a type of krill caught in the Sanriku region of Japan and its official name is pacific krill (scientific name: *Euphausia Pacifica*). Matured Isada is 1 to 2 cm in length. Isada takes one year to mature and has a lifespan of about two years. It has been reported that Isada's spawning season is from spring to summer[1)].

Even though there are more than 80 species of krill, commercial fishing is performed only for several types including Antarctic krill and Isada. Isada is well-known as a feed for cultured fish and fishing, but it has long been a popular food in Sanriku. Isada is used as a kimchi ingredient and a seasoning extract, and it is eaten by many people. In recent years, dry Isada has been produced, increasing the ways where it is used as a food.

Dr. Hidetoshi Yamada (hereinafter, Dr. Yamada) of Iwate Biology Research Center discovered 8-HEPE (8-hydroxyeicosapentaenoic acid) from Isada and clarified the anti-obesity effect and mechanism of 8-HEPE.

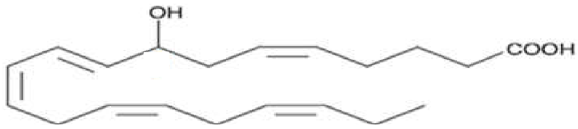
Fig. 1 Landing of Isada

Fig. 2 Isada

Oryza Oil & Fat Chemical developed ISADA Oil with an anti-obesity effect using Isada from the Sanriku shoreline of Japan.We strongly recommend "ISADA Oil" which is Krill Oil made from Japanese ingredient "Isada" containing the new component "8-HEPE".

2. New ingredient 8-HEPE

Eicosapentaenoic acid (EPA)



8-Hydroxy eicosapentaenoic acid (8-HEPE)

Fig. 3 Structural formula of EPA and 8-HEPE

8-HEPE is a compound in which the hydroxyl group is added to the eighth carbon of eicosapentaenoic acid (EPA). HEPE is generated during auto-oxidation of EPA. 8-HEPE is reported that it is also one of the compounds generated in non-specific oxidation reactions.

Formerly, there was no report on living organisms that specifically accumulate 8-HEPE. Dr. Yamada's research report indicates that Isada contains large amounts of 8-HEPE.[1)]

Table 1 8-HEPE content of seafood

Content in 100g (mg)

	ISADA (Euphausia pacifica)	Euphausia superba	Sardine	Saury	Mackerel
8-HEPE	21.8±8.8	11.4±3.4	n.d	n.d	n.d
EPA (free)	157.9±36.6	2.5±2.0	2.5±2.0	6.8±2.0	2.9±0.1

Yamada et al. Journal of Lipid Research 2014

Table 1 shows the results of measuring the 8-HEPE content of seafood. 8-HEPE was not detected in the fish. It has been reported that 8-HEPE was detected only in some crustaceans such as Isada and Antarctic krill and that Isada has the highest content.

3. Functionality of 8-HEPE

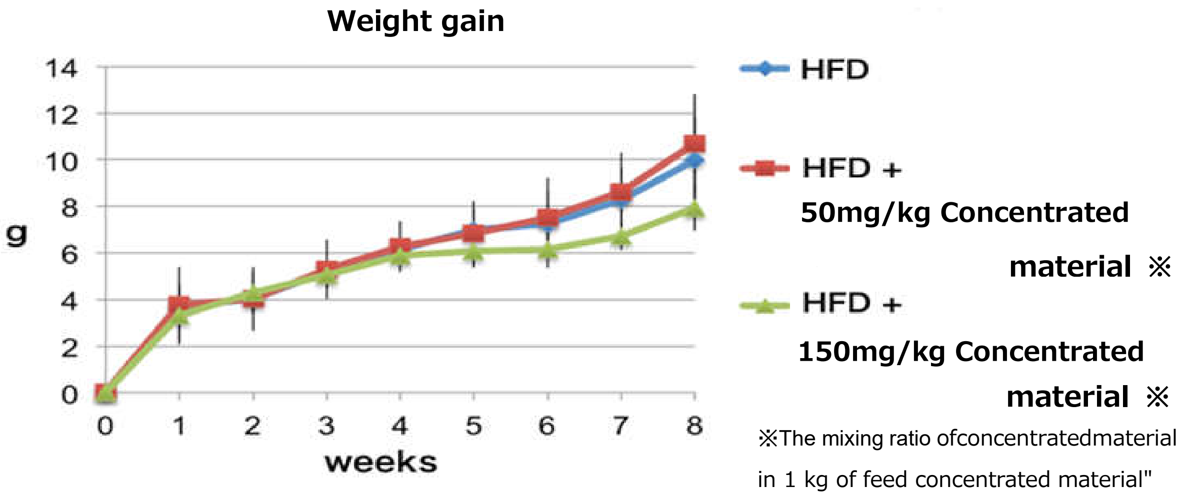


Fig. 4 Feeding experiment using concentrated 8-HEPE materials [4)]

Figure 4 shows the results of a feeding experiment using concentrated 8-HEPE materials extracted from Isada. The figure shows the changes in the weight of mice that consumed HFD (high-fat diet) including 8-HEPE feed and mice that only consumed HFD.
As a result, an effect to prevent weight increase was observed in mice that consumed feed with the concentrated material of 150 mg/kg.

The 2018 *Japan Society for Bioscience, Biotechnology, and Agrochemistry*
Quoted from "Development of new functional ingredients derived from Isada into 8-HEPE concentrated material"

Fig. 5 Abdominal CT images of mouse after the concentrated 8-HEPE material feeding test

Figure 5 shows abdominal CT images of mouse used in the test described above. The left-side image is from a mouse that only consumed HFD and the right-side image is from a mouse that consumed HFD containing 8-HEPE. Consumption of concentrated 8-HEPE material was shown to have an effect of preventing the increase of visceral fat.

4. Anti-obesity mechanism of 8-HEPE

Dr. Yamada reported that 8-HEPE activates Peroxisome Proliferator-Activated Receptor (PPAR)[3].PPAR is a type of nuclear receptor and there are three types: PPARα, γ, as well as δ. PPARα expresses in the liver or heart and it controls fat decomposition as well as the production of energy. PPARγ expresses in fat tissue and it controls the formation of fat cells and lipid metabolism. PPARδ expresses in the entire body and works to break down fat and produce energy in each tissue.

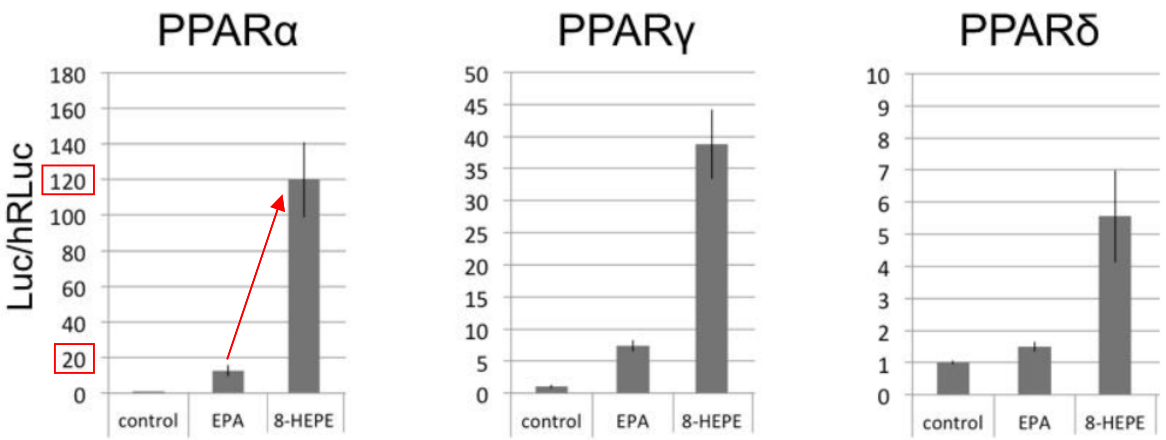


Fig. 6 Measurement of luciferase activity of EPA and 8-HEPE

Figure 6 shows the results of luciferase assay using EPA and 8-HEPE and reserach on their influence to the activity of PPARα, γ, and δ. Luciferase assay is a type of reporter assays and the PPAR activity can be measured via a method for measuring the activity of the receptor. 8-HEPE showed extremely high activity as compared to EPA at each PPAR value. Especially, a high activity was observed in PPARα and PPARγ. The activity in PPARα was 10 times higher than that of EPA.
The results above suggest that Isada's anti-obesity effect is a result of fat breakdown enhancement in the liver.

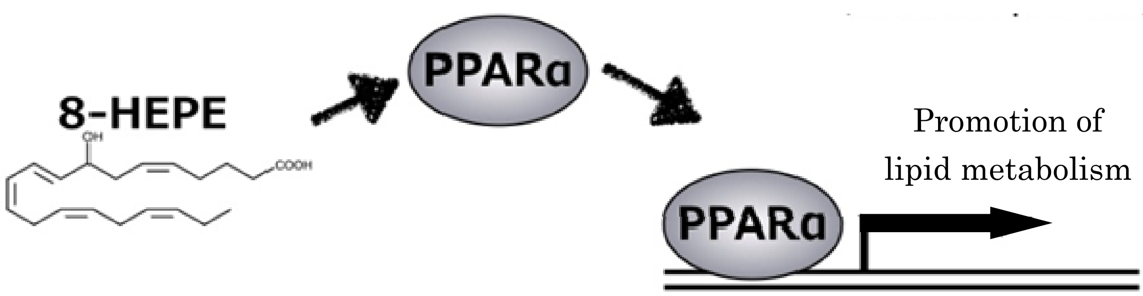


Fig. 7 Anti-obesity action of 8-HEPE

5. Anti-obesity Effect of ISADA Oil

The anti-obesity effect of 8-HEPE has been confirmed in a report by Dr. Yamada . However, there are not many reports on the anti-obesity effect of oil containing 8-HEPE produced using Isada as a raw ingredient. Oryza Oil & Fat Chemical has discovered that ISADA Oil has an anti-obesity effect.

（1） Measurement of PPAR activity using luciferase assay

The PPAR activity was measured using luciferase assay on ISADA Oil and commercially-available Krill Oil made of Antarctic krill. As a result, activation of PPARα was observed especially in ISADA Oil accompanied with concentration dependence. A tendency of activation was also observed in PPARγ as well as PPARσ (Figures 8 to 10). On the other hand, no activation was observed in PPARα, γ, or δ in Krill Oil.

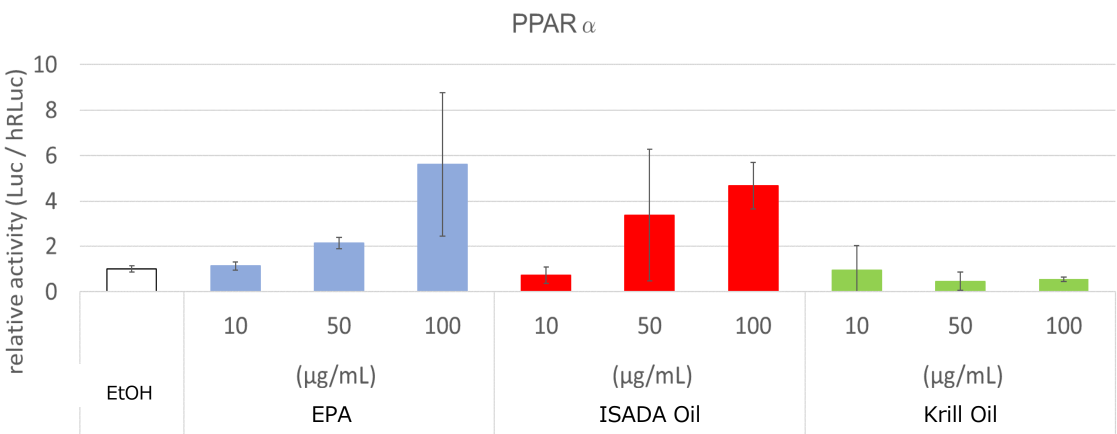


Fig. 8　Influence of ISADA Oil and Krill Oil to PPARα activity
Each value is indicated with the average value and standard error (n=3).

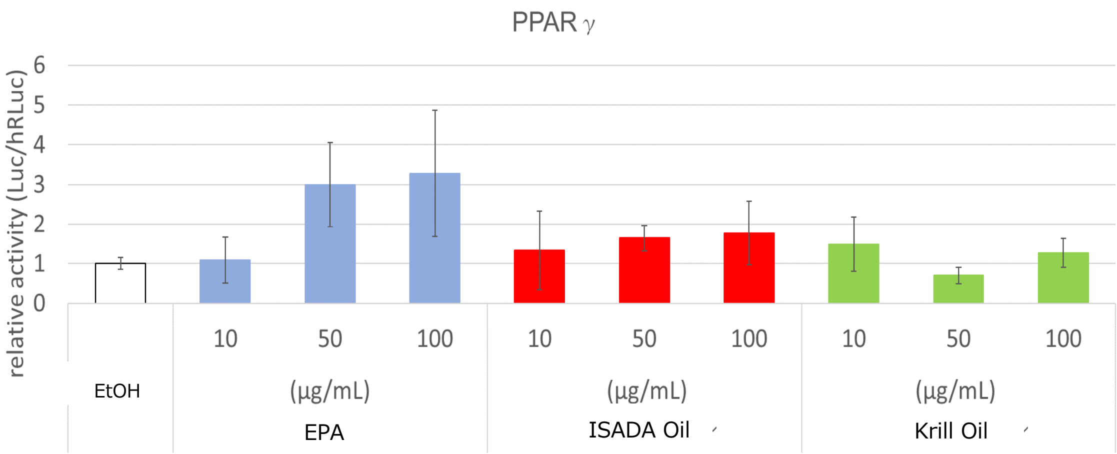


Fig. 9 Influence of ISADA Oil and Krill Oil to PPAR γ activity
Each value is indicated with the average value and standard error (n=3).

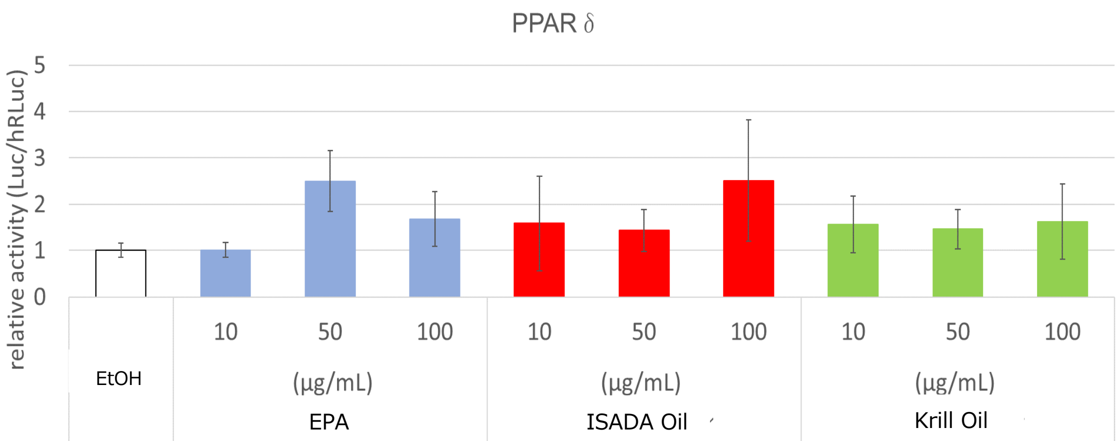


Fig. 10 Influence of ISADA Oil and Krill Oil to PPAR δ activity
Each value is indicated with the average value and standard error (n=3).

(2) Measurement of anti-obesity gene expression by RT-PCR

Mechanism of fatty acid metabolism in the body

Some of the fatty acids broken down and released into adipose tissue are (1) transported to the liver, (2) taken into mitochondria in liver cells, and then (3) oxidized in β-oxidation.

1. Pathways for fatty acids to the liver

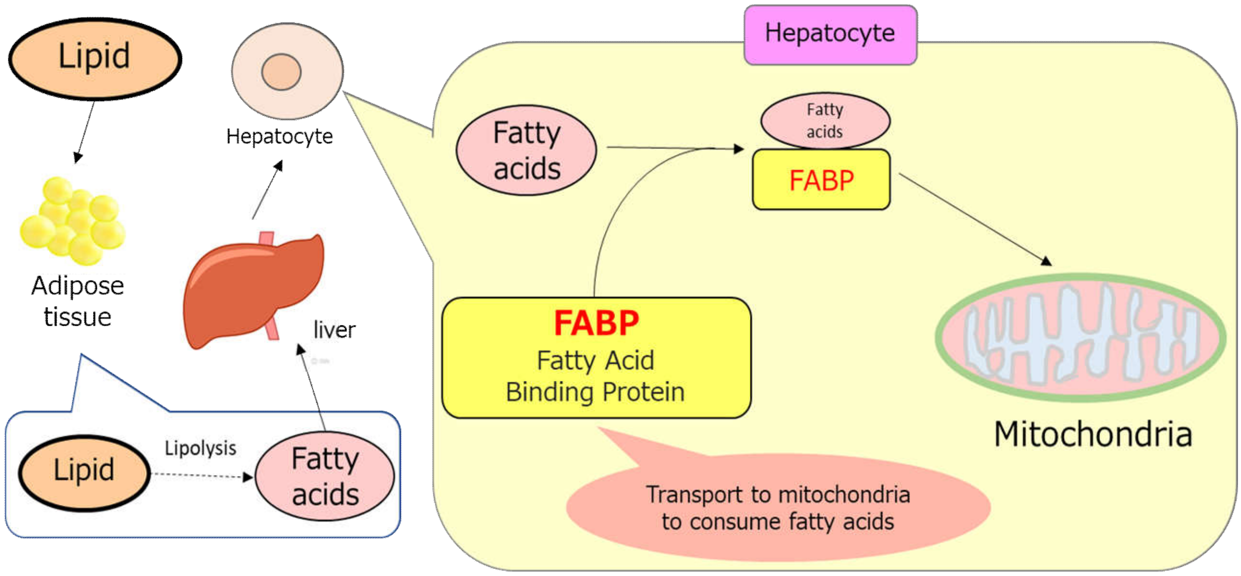


Fig. 11 Pathway from fatty acids to mitochondria in hepatocytes

Lipids in the body are broken down into fatty acids and some are transported to the liver. Fatty acids transported to the liver combine with FABP and are transported to the mitochondria in hepatocytes.
FABP is thought to protect fatty acids from metal ions and to transport them to intracellular organelles that require fatty acids.

2. Pathways in which fatty acids are taken up into the mitochondria of hepatocytes

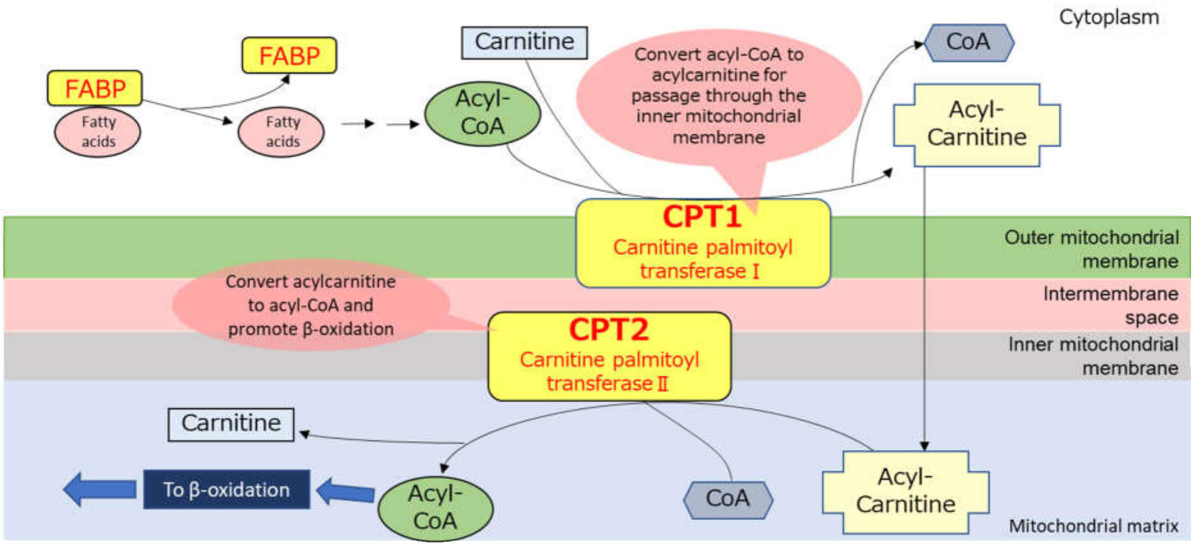


Fig. 12　Pathway in which fatty acids are incorporated into mitochondria of hepatocytes

Fatty acids transported to the mitochondria of hepatocytes are used for β-oxidation within the mitochondrial matrix, but undergo several enzymatic reactions to cross the mitochondrial inner and outer membranes.

First, they are activated by the enzyme transported to mitochondria (acyl-CoA synthetase) to be converted into acyl-CoA and then pass through the mitochondrial outer membrane to enter the inter-membrane space. As acyl-CoA cannot pass through the mitochondrial inner membrane, it is converted into acylcarnitine by carnitine palmitoyltransferase I (CPT1) in order to pass through the mitochondrial inner membrane and enter the matrix. After acylcarnitine reaches the matrix, it is converted into acyl-CoA by carnitine palmitoyltransferase II (CPT2) in order to advance to β-oxidation.

3. β - oxidation pathway diagram

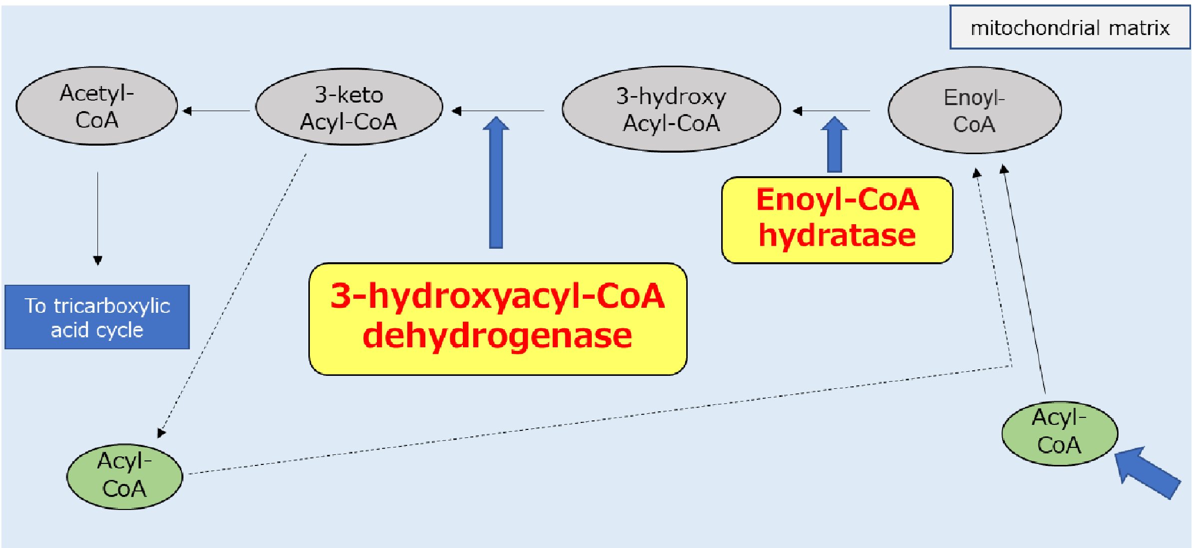


Fig. 13 β -oxidation pathway in matrix

Acyl-CoA generated by CPT2 becomes acetyl-CoA via β -oxidation and is used in the citric acid cycle. Some enzymes exist in the β -oxidation pathway as well. Enoyl-CoA hydratase adds H2O to enoyl-CoA to accelerate β -oxidation. Similarly, 3-hydroxy acyl dehydrogenase accelerates β -oxidation by oxidizing 3-hydroxyacyl-CoA.

FABP, CPT1, CPT2, enoyl-CoA hydratase, and 3-hydroxy acyl dehydrogenase are all proteins involved in fatty acid metabolism.

Genes are required for protein expression. Gene expression can be checked using RT-PCR.

Measurement of anti-obesity gene expression by RT-PCR

Oryza Oil & Fat Chemical evaluated gene expression by performing RT-PCR using five types of proteins (FABP, CPT1, CPT2, enoyl-CoA hydratase, 3-hydroxy acyl dehydrogenase) as well as CYP4A1 which is a protein that activates PPARα.（ Table 2,Fig. 14 ～ Fig. 18）

As a result, an extremely strong effect to promote the expression of three types of genes: Cyp4a1, Fabp1, and Cpt1a was observed in ISADA Oil and 8-HEPE. On the other hand, EPA and Krill Oil did not show a strong effect to promote the expression of the same genes. The effect of ISADA Oil that promotes the expression of Cyp4a1, Fabp1, and Cpt1a was presumed to be due to 8-HEPE.
In particular, regarding *Cyp4a1* and Fabp1, ISADA Oil is expected to have a higher effect to promote the expression compared to Krill Oil.

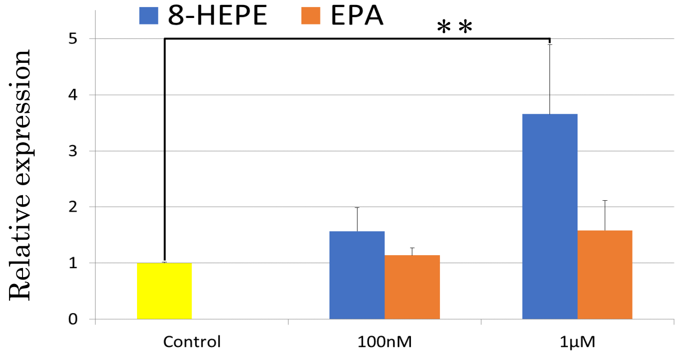
Table 2 Genes used in RT-PCR and proteins expressed

Gene	Expressed protein
Cyp4a1	CYP4A1
Fabp1	FABP1
Cpt1a	CPT1
Cpt2	CPT2
Ehhadh	Enoyl-CoA hydratase 3-hydroxyacyl-CoA dehydrogenase

[Experimental method]
FaO cells (1x105 cells/well) were cultured in Ham's F12 medium containing 10% cattle fetus for 24 hours, samples (ISADA Oil and Krill Oil) were added to the medium, and the medium was cultured for an additional 6 hours. (The control added the same amount of medium instead of the sample.) After culturing, RNAs were extracted from cells, cDNAs were generated, and actions demonstrated on the expression of each gene were evaluated by RT-PCR.

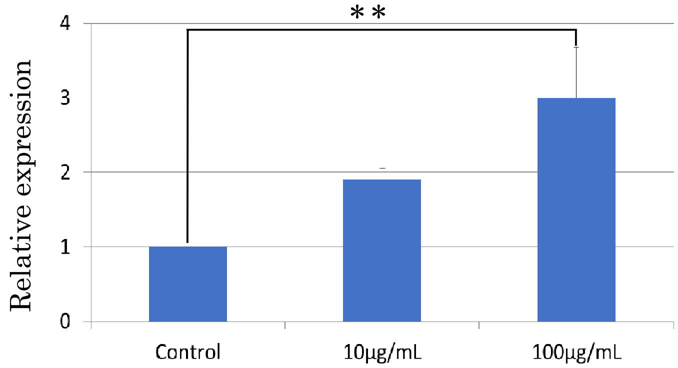
Cyp4a1 gene expression (RT-PCR)

Comparison of 8-HEPE and EPA



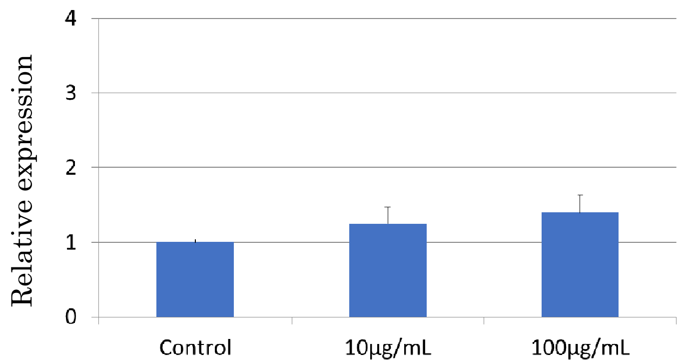
Adding 8-HEPE promoted the expression of *Cyp4a1* in a concentration-dependent manner and a significant difference was confirmed between the control and 1 μM.

ISADA Oil



Adding ISADA Oil promoted the expression of *Cyp4a1* in a concentration-dependent manner and a significant difference was confirmed between the control and 100 μg/mL.

Krill Oil



Although adding Krill Oil showed a tendency to promote the expression of *Cyp4a1*, the effect was not significant.

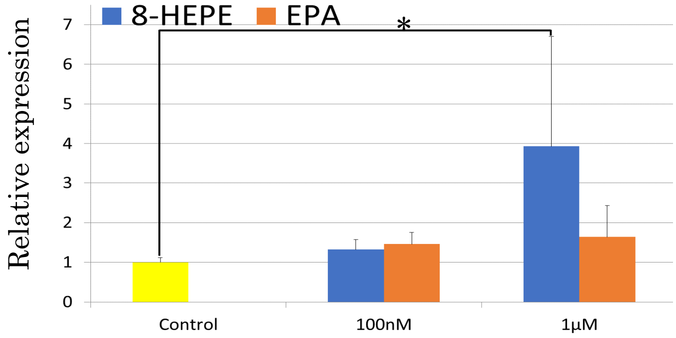
Fig. 14 Influence of ISADA Oil and Krill Oil on *Cyp4a1* gene expression

[N=3, average values ± standard error, significant difference **: $p<0.05$, *: $p<0.01$ (Dunnett's multiple comparison test)]

The expression was shown by the ratio assuming the control is 1 after beta-actin correction.

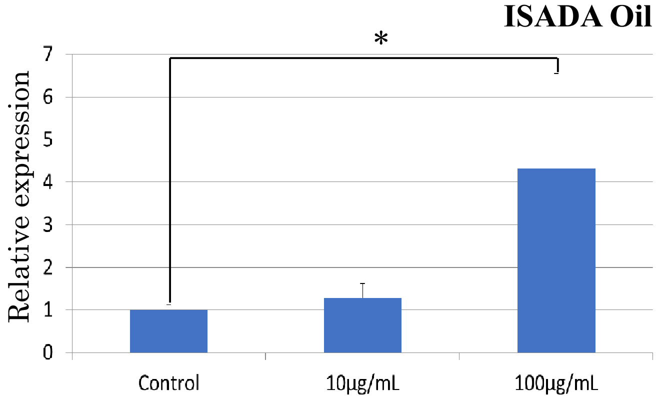
Fabp1 gene expression (RT-PCR)

Comparison of 8-HEPE and EPA



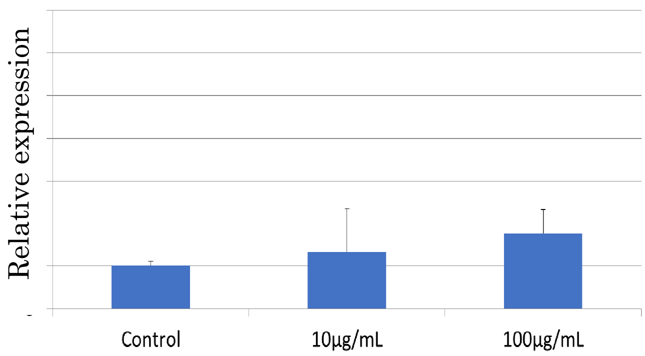
Adding 8-HEPE promoted the expression of Fabp1 in a concentration-dependent manner and a significant difference was confirmed between the control and 1 µM.

ISADA Oil



Adding 100 µg/mL of ISADA Oil promoted the expression of Fabp1 and a significant difference from the control was confirmed.

Krill Oil



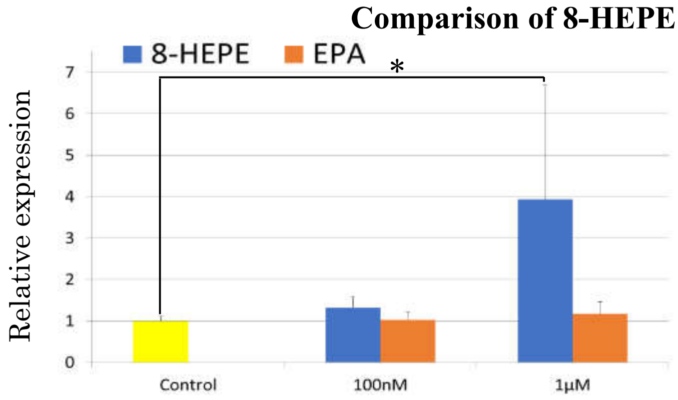
Although adding Krill Oil showed a tendency to promote the expression of *Fabp1*, the effect was not significant.

Fig. 15 Influence of ISADA Oil and Krill Oil on *Fabp1* gene expression

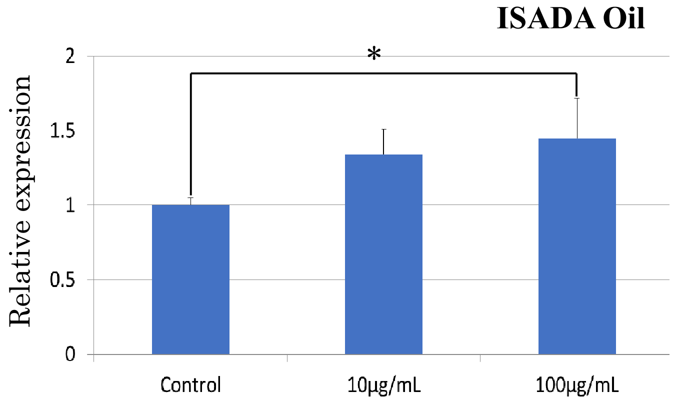
[N=3, average values ± standard error, significant difference **: $p<0.05$, *: $p<0.01$ (Dunnett's multiple comparison test)]

The expression was shown by the ratio assuming the control is 1 after beta-actin correction.

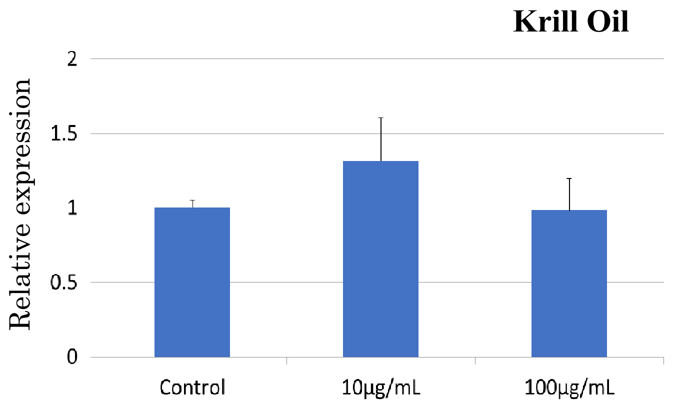
Cpt1a gene expression (RT-PCR)



Adding 8-HEPE promoted the expression of *Cpt1a* in a concentration-dependent manner and a significant difference was confirmed between the control and 1 μM.



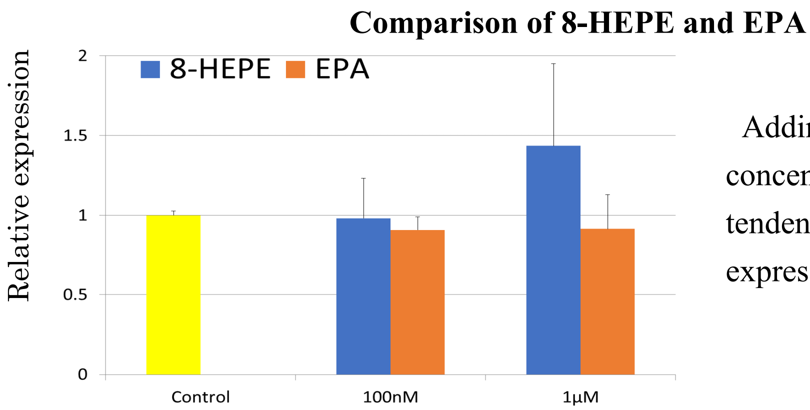
Adding ISADA Oil promoted the expression of *Cpt1a* in a concentration-dependent manner and a significant difference was confirmed between the control and 1 μM.



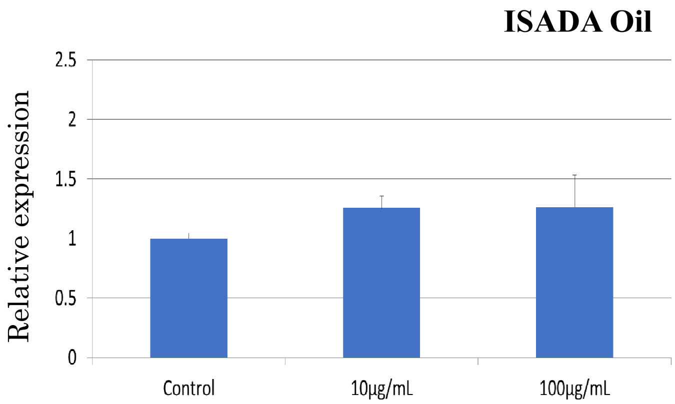
Adding Krill Oil did not show any promotion of the expression of *Cpt1a*.

Fig. 16 Influence of ISADA Oil and Krill Oil on *Cpt1a* gene expression
[N=3, average values ± standard error, significant difference **: $p<0.05$, *: $p<0.01$ (Dunnett's multiple comparison test)]
The expression was shown by the ratio assuming the control is 1 after beta-actin correction.

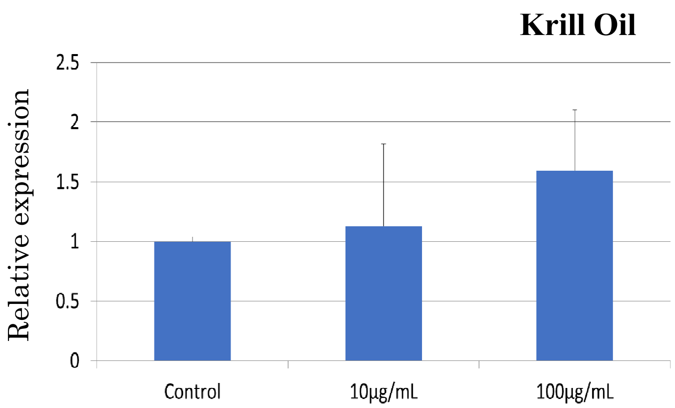
Cpt2 gene expression (RT-PCR)



Adding 8-HEPE showed a concentration-dependent tendency to promote the expression of *Cpt2*.



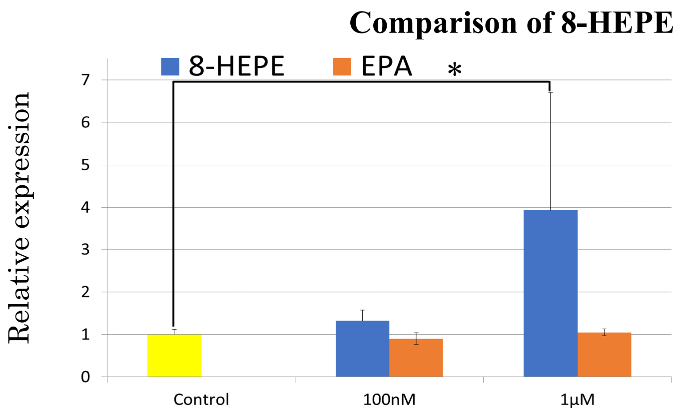
Adding ISADA Oil showed a tendency to promote the expression of *Cpt2*.



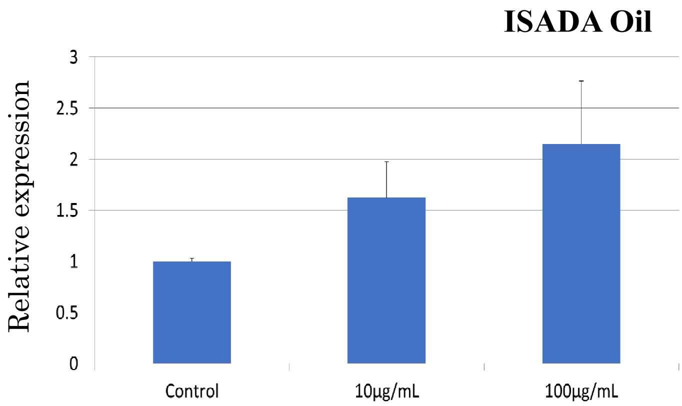
Adding Krill Oil showed a concentration-dependent tendency to promote the expression of *Cpt2*.

Fig. 17 Influence of ISADA Oil and Krill Oil on *Cpt2* gene expression
[N=3, average values ± standard error, significant difference **: $p<0.05$, *: $p<0.01$ (Dunnett's multiple comparison test)]
The expression was shown by the ratio assuming the control is 1 after beta-actin correction.

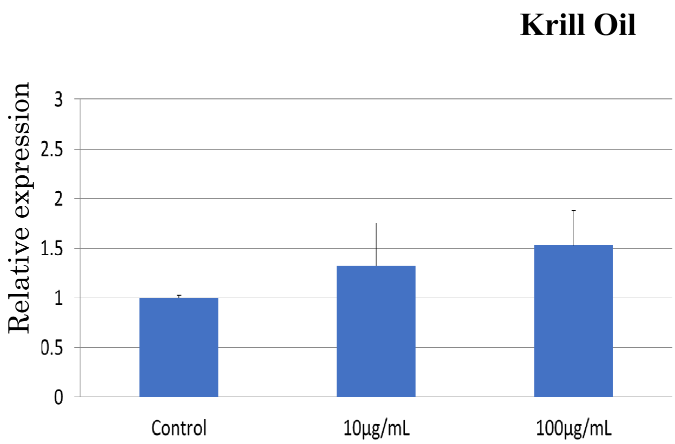
Ehhadh gene expression (RT-PCR)



Adding 8-HEPE showed a concentration-dependent tendency to promote the expression of *Ehhadh*.



Adding ISADA Oil showed a concentration-dependent tendency to promote the expression of *Ehhadh*.



Adding Krill Oil showed a concentration-dependent tendency to promote the expression of *Ehhadh*.

Fig. 18 Influence of ISADA Oil and Krill Oil on *Ehhadh* gene expression

[N=3, average values ± standard error, significant difference **: $p<0.05$, *: $p<0.01$ (Dunnett's multiple comparison test)]

The expression was shown by the ratio assuming the control is 1 after beta-actin correction.

(3) Weight increase suppression test in mouse

Since we confirmed that ISADA Oil increases anti-obesity gene expression in cell tests, we investigated the effect of ISADA Oil on the body weight of mouse.

As a result, a significant suppression of weight gain was observed in the group with a high-fat diet as well as oral administration of a large-dose of ISADA Oil as compared to the control group. The effect to suppress weight gain was not observed in the group with a high-fat diet as well as oral administration of a small-dose of ISADA Oil as compared to the control group.

This confirmed that consumption of the recommended amount of ISADA Oil has an effect to suppress weight gain.

[Experimental method]

Mice (6 weeks of age, male) were preliminary raised with normal feed for one week. Then, they were separated into four groups as shown in Table 3 in order to carry out an experiment. The mice had a free access to feed during the experimental period.

Mice were weighed daily and each concentration of sample was administered at 10 mL / kg (Table 3).Figure 19 shows the change in body weight of each group of mice.

Table 3 Conditions of the test on mouse

Group	Feed	Content of administration
Nomal	Regular Diet	Water
Control	High-fat Diet	Water
High dose	High-fat Diet	ISADA Oil (8-HEPE : 0.21mg/kg/day)
Low dose	High-fat Diet	ISADA Oil (8-HEPE : 0.07mg/kg/day)

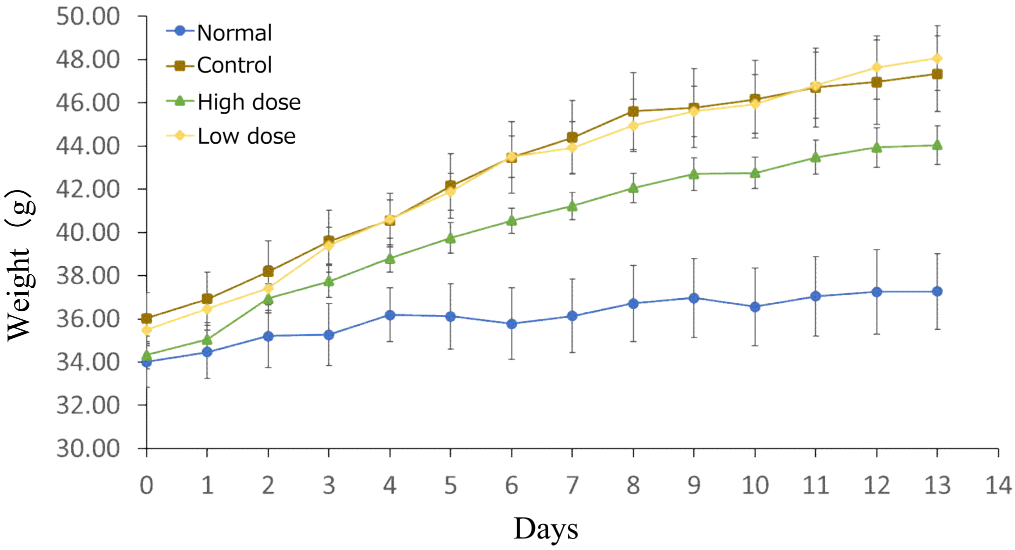


Fig. 19 Changes in mice's weight caused by the administration of ISADA Oil

（4）Clinical trial, Effect of ISADA Oil on Anti-obesity

We evaluated the anti-obesity effect of ISADA Oil in a single-blind study. All subjects ingested ISADA Oil (210mg / day) for 4 weeks. Obesity parameters and blood components were measured to evaluate anti-obesity effect. All subjects were divided into ISADA Oil 210 mg / day intake group (6 people) and placebo intake (8 people) group to evaluate obesity parameters and blood components after 4 weeks consecutive intakes.（Table 4,Fig. 20,Fig. 21）

【Test method】
1. Subjects : 14 healthy Japanese men (24-59 years old)
2. Intervention : 3 capsules containing 70 mg of ISADA Oil per day
3. Test period : 4 weeks
4. Out come : Measurement of obesity parameters
(Weight, body fat percentage, waist, hip, visceral fat)
Measurement of blood components
(Total cholesterol, LDL cholesterol,HDL cholesterol,
neutral fat, free fatty acid)

As a result of the trial, the body weight, body fat percentage, waist and hip relative values decreased in the ISADA Oil group compared to the placebo group. Regarding visceral fat, a tendency to suppress increase of visceral fat was observed in the ISADA Oil intake group versus the placebo group.

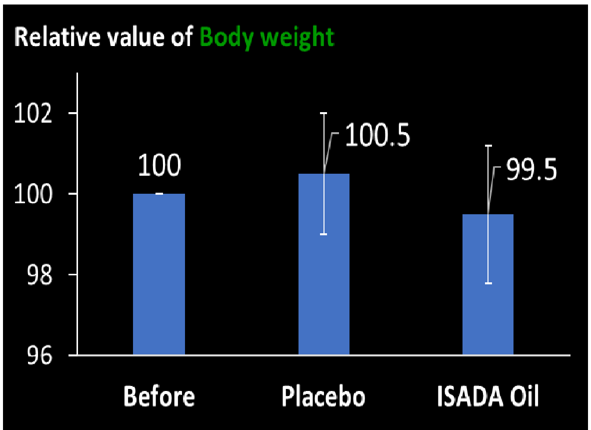
Similarly, as a result of measuring blood components, the relative values of total cholesterol and LDL cholesterol were significantly decreased in the ISADA Oil intake group compared to the placebo group, and a decrease in neutral fat and free fatty acids was observed.

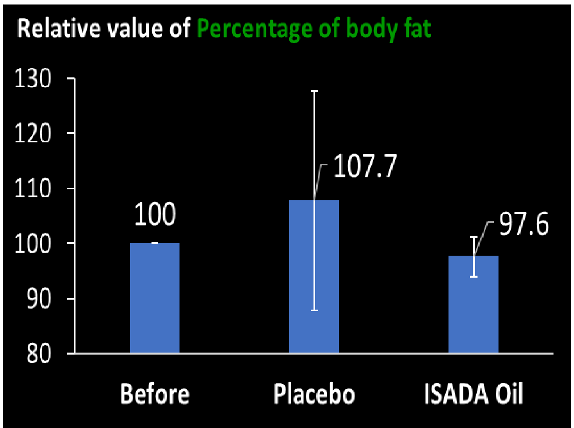
Based on the above results, it was observed that intake of ISADA Oil (210mg / day) for 4 weeks improved human obesity parameters and blood components, especially cholesterol.

Table 4 Results before and after intake of ISADA Oil (210mg / day) for 4 weeks

Obesity parameters

	Before	After (Relative value)	Change (Relative value change)
Body weight (kg)			
Placebo	61.94±9.8	62.13±10.2 (100.5±1.5)	0.3±0.9 (0.5)
ISADA Oil	**70.8±12.9**	**70.4±12.4 (99.5±1.7)**	**-0.4±1.2 (-0.5)**
Percentage of body fat (%)			
Placebo	21.51±6.5	22.41±6.6 (107.7±20.0)	1.4±3.7 (7.7)
ISADA Oil	**23.1±6.0**	**22.7±6.7 (97.6±3.7)**	**-0.4±0.9 (-2.4)**
Waist circumference (cm)			
Placebo	80.88±10.4	83.77±9.9 (103.5±3.9)	2.83±3.0 (3.5)
ISADA Oil	**89.45±8.0**	**89.72±6.7 (100.4±3.1)**	**-0.93±2.8 (0.4)**
Hip circumference (cm)			
Placebo	94.15±5.0	92.95±5.8 (99.1±3.4)	-0.88±3.3 (-0.9)
ISADA Oil	**98.83±7.7**	**97.00±6.8 (98.2±3.4)**	**-1.83±3.42 (-1.8)**
Visceral fat (cm^2)			
Placebo	66.7±46.2	84.7±46.5 (145.4±61.8)	19.6±11.4 (45.4)
ISADA Oil	100.5±52.6	108.50±34.0 (116.1±22.9)	8.00±20.5 (16.1)





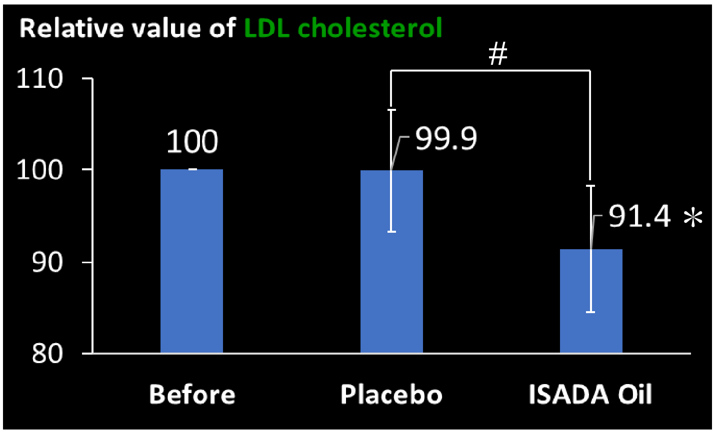
Relative values were expressed as a ratio of values before and after intake.

Fig. 20 Results before and after intake of ISADA Oil for 4 weeks (comparison of relative values)

Table 5 Results before and after intake of ISADA Oil (210mg / day) for 4 weeks

Blood components

	Before	After (Relative value)	Change (Relative value change)
Total cholesterol(mg/dL)			
Placebo	209.5±32.6	208.0±25.5 (99.9±6.6)	-1.5±14.5 (-0.1)
ISADA Oil	**217.3±39.6**	**198.0±34.6 (91.4±6.86*#)**	**-19.3±15.9 (-8.6)**
LDL cholesterol(mg/dL)			
Placebo	123.1±31.7	124.1±23.7 (102.4±9.3)	1.0±11.76 (2.4)
ISADA Oil	**132.7±33.1**	**117.2±31.4 (88.6±10.9*#)**	**-15.5±15.2 (-11.4)**
HDL cholesterol(mg/dL)			
Placebo	63.0±15.1	63.3±14.7 (100.7±3.4)	0.25±1.8 (0.7)
ISADA Oil	58.5±14.3	57.5±10.7 (99.8±9.2)	-1.00±5.3 (0.2)
Triglyceride(mg/dL)			
Placebo	80.9±46.0	83.4±54.1 (106.0±46.1)	2.5±30.9 (6.0)
ISADA Oil	**109.7±51.3**	**101.2±47.9 (94.4±14.5)**	**-8.5±19.6 (-5.6)**
Free fatty acid (mEq/L)			
Placebo	0.66±0.24	0.58±0.27 (93.8±35.5)	-0.08±0.25 (-6.2)
ISADA Oil	**0.79±0.38**	**0.63±0.27 (91.9±65.4)**	**-0.16±0.38 (-8.1)**



Comparison before and after intake (relative value)
＊: $p < 0.05$

Comparison with placebo group (relative value)
#: $p < 0.05$

Fig. 21 Results before and after intake of ISADA Oil for 4 weeks (comparison of relative values)

6. Functional components contained in ISADA Oil and other physiological activities

In addition to 8-HEPE, ISADA Oil contains omega 3 polyunsaturated fatty acids represented by EPA and DHA. It is known that large amounts of EPA and DHA are contained in Fish Oil as well. Whereas most of the EPA and DHA in Fish Oil is triacylglycerols type (TG type), Krill Oils including ISADA Oil contain a large amount of phospholipid type (PL type) EPA and DHA. PL type of EPA and DHA can increase the EPA and DHA content in blood with a small intake amount as they are efficiently absorbed in the body compared to the TG type.[1)]

Table 6 Comparison of ingredients

	EPA	DHA	8-HEPE	astaxanthin
ISADA Oil	19.10%	12.40%	0.17%	1340ppm*2
Krill Oil *1	13.12%	6.54%	0.002%	265ppm

＊1：Measured value of Antarctic krill oil

＊2：Measure free astaxanthin

Table 6 shows the results of comparing the components of ISADA Oil and commercially-available Krill Oil made with Antarctic krill under the same conditions. The results indicate that ISADA Oil contains larger amounts of 8-HEPE and docosahexaenoic acid (DHA) than Krill Oil. In addition to 8-HEPE, EPA, and DHA, ISADA Oil contains astaxanthin which is known to have antioxidant properties as well as phosphatidyl choline that is expected to improve brain functions.

Krill Oil extracted from Antarctic krill has been used in human clinical studies to improve or alleviate premenstrual syndrome and dysmenorrhea, reduce blood cholesterol and triglyceride levels in hyperlipidemic subjects. In arthritic subjects, it has been reported to reduce inflammation, including joint inflammation. In addition, it has been reported that it is expected to improve alcoholic fatty liver in animal studies, spermatogenic ability of living body, ability to maintain sperm, and improvement of brain function[5)]. Because these effects involve omega-3 polyunsaturated fatty acids, the same effects as Krill Oil can be expected from ISADA Oil.

(1) Effect to improve conditions of hyperlipidemia

Krill Oil is known to have an effect to improve conditions of hyperlipidemia and this effect is mainly due to EPA. EPA is well-known to be contained in Fish Oil. It has been medically confirmed to act on cholesterol in the body, to having an anti-thrombotic effect, lower the blood lipid levels, as well as blood pressure levels.
Krill Oil contains phospholipid-type EPA (see the previous page), enabling EPA to be absorbed efficiently in the body when compared to Fish Oil.

Table 7 Changes in blood composition caused by Krill Oil[6)]

3g Krill Oil	Time(day)/mg/dL		% Change
	0	90	
Total Cholesterol	250.52	205.67	-17.9
LDL	172.81	105.16	-39.15
HDL	64.18	102.45	59.64
Triglycerides	152.77	112.27	-26.51

Table 8 Changes in blood composition caused by Fish Oil consumption [6)]

3g Fish Oil	Time(day)/mg/dL		% Change
	0	90	
Total Cholesterol	231.15	217.55	-5.88
LDL	121.67	117.83	-4.56
HDL	56.64	59.03	4.22
Triglycerides	140.87	136.44	-3.15

Table 7 and 8 show the results of a clinical trial carried out to study the changes in blood composition caused by the consumption of Krill Oil and Fish Oil[6)]. Test subjects consumed Krill Oil or Fish Oil for 90 days and their blood composition was studied before and after the test. Test subjects were separated into the Krill Oil consumption group and the Fish Oil consumption group. The test was carried out on 60 people, and 30 people, in each respective group.

As a result, a notable decrease in the total cholesterol, LDL cholesterol, and triglycerides levels was observed in the Krill Oil group when compared to the Fish Oil group and a more notable increase in the HDL cholesterol level was observed in the Krill Oil group.

Where an increase in LDL cholesterol and triglycerides leads to hyperlipidemia, an increase in HDL cholesterol leads to a suppression of hyperlipidemia.

The results above suggest that Krill Oil has a higher effect on suppressing hyperlipidemia than Fish Oil. Like Krill Oil, ISADA Oil contains phospholipid type EPA, and is considered to have a hyperlipidemic effect.

(2) Effect to improve brain functions

Krill Oil also contains DHA which has been reported to have an effect to improve brain functions[7)].

Just like EPA, DHA exists in Krill Oil as phospholipid-type DHA so it can be absorbed more efficiently in the body than Fish Oil.

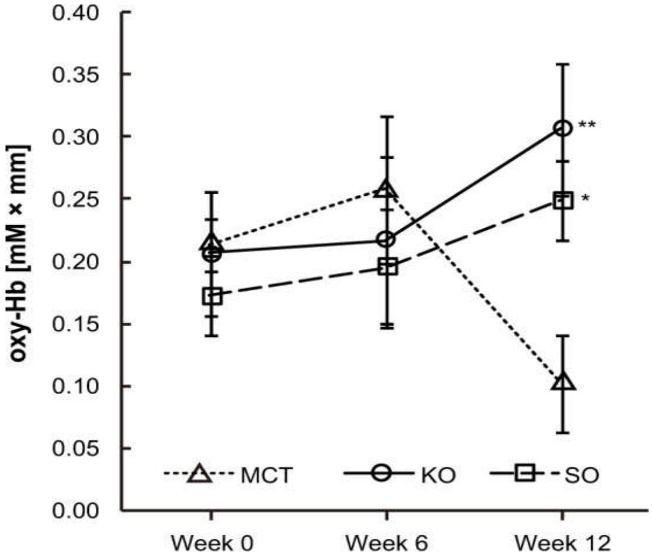
Figures 22 and 23 show the results of a clinical trial carried out to study the changes in the concentration of oxygenated hemoglobin in the brain caused by the consumption of Krill Oil[8)] . KO indicates Krill Oil, SO indicates Sardine Oil, and MCT indicates a placebo. The oxygenated hemoglobin concentration in the brain is a value that suggests activation of the brain during work. The higher the concentration of oxygenated hemoglobin in the brain, the more active the brain. This test measures the oxygenated hemoglobin concentration in the brain as the subject works.

Figure 22 shows the results of measuring the concentration of oxygenated hemoglobin in the brain while test subjects worked on a 2-back test. The 2-back task is a method used to measure the subjects' working memory (capacity to temporarily store information and process it). Working memory is involved in the memories in our daily life and it is believed that a declined working memory leads to an overall decline in memory.

Figure 23 shows the results of measuring the concentration of oxygenated hemoglobin in the brain while test subjects were taking a test using Uchida-kraepelin test paper. The Uchida-Kraepelin test is a simple calculation that is conducted to analyze the personality and behavior of the performer in addition to the calculation ability of the implementer. In this test, the Uchida-kraepelin test was used to measure the subjects' calculation ability.

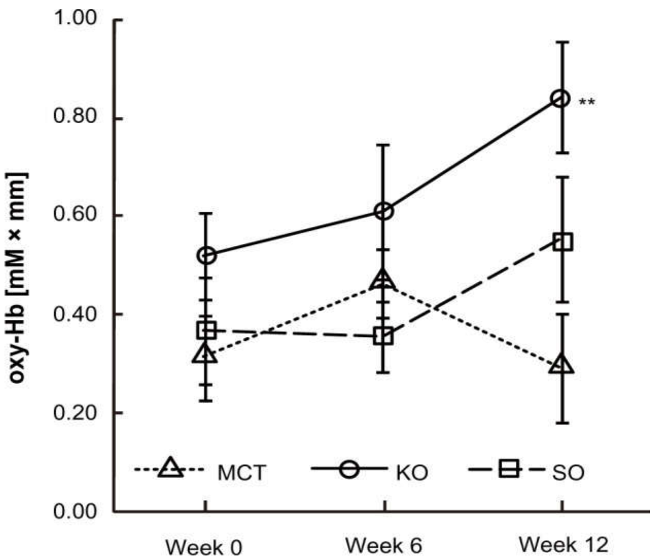
The results shown in Figures 22 and 23 show that Krill Oil has a greater effect to improve both working memory and calculation ability than Sardine Oil.

Like Krill Oil, ISADA Oil contains phospholipid-type DHA, and is considered to have potential to activate brain functions effect.



MCT：Placebo　KO：Krill Oil　SO：Sardine Oil

Fig. 22 Changes in oxyhemoglobin concentration in the brain with Krill Oil intake [5]
（Measured during 2-back task）



MCT：Placebo　KO：Krill Oil　SO：Sardine Oil

Fig. 23 Changes in oxyhemoglobin concentration in the brain with Krill Oil intake [5]
（Measured when testing with Uchida-kraepelin test）

7. References

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8. Stability of ISADA Oil

(1) ISADA Oil's stability in long-term storage

The long-term storage stability of ISADA Oil was examined. As a result, the 8-HEPE content did not decrease even after 24 months under refrigeration (10 ° C).

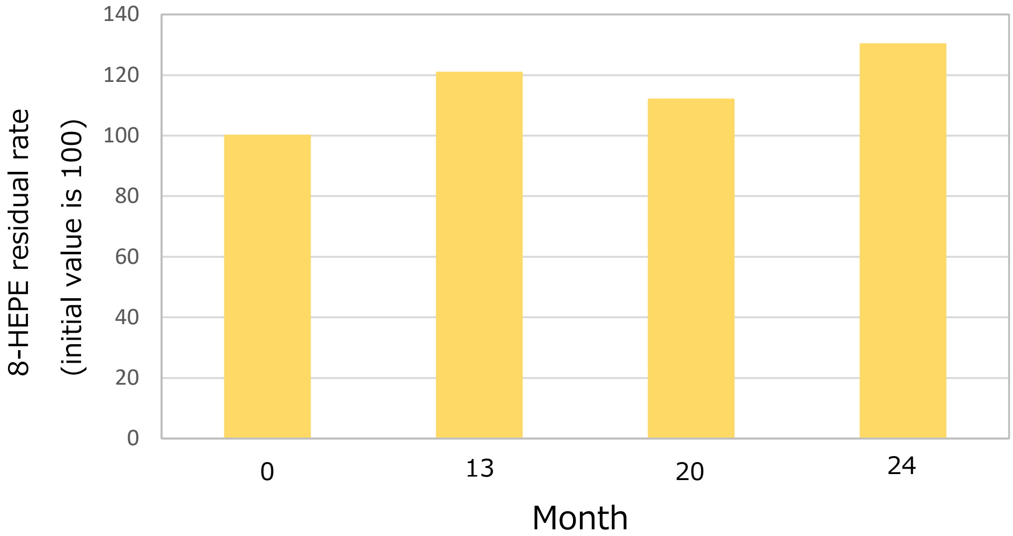

Fig. 24 ISADA Oil's stability in long-term storage

9. Nutrition profiles

Analyzed item (/100g)	ISADA Oil	Method
Water(g)	1.1g	Heating drying method under normal pressure
Protein(g)	4.6g	Kjeldahl method,nitrogen protein conversion factor：6.25
Fat(g)	98.0g	Acid decomposition method
Ash(g)	3.5g	Direct incineration method
Carbohydrate(g)	<0.1	Refer to note 1
Energy(kcal)	900kcal	Refer to note 2
Sodium(mg)	518mg	Atomic absorption spectrophotometory
Sodium chloride equivalent (g)	1.31g	Refer to note 3

The nutritional information of ISADA Oil was analyzed according to the standard in nutrition labeling (March 30, 1995 ; No. 139 Eishin) .

Note 1 Calculation : 100 - (water + protein + fat + ash)
Note 2 Energy conversion factor : Protein 4; fat 9; sugar 4; dietary fiber 2
Note 3 In terms of sodium
Test trustee: SUNATECH
Test report was issued on: April 8, 2019
Report No.: 190402096-001-01

10. Safety of ISADA Oil with No Rice Oil Added

(2) Acute toxicity（LD_{50}）

Acute Toxicity of ISADA Oil was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where ISADA Oil 2,000 mg/kg was orally given to mice (ICR, 6-week old). And the mice were observed for 14 days. No abnormalities and fatal event observed at 2,000 mg/kg. No abnormalities of organs observed under macroscopic examination upon autopsy. Thus, LD_{50} of ISADA Oil is deduced to be >2,000 mg/kg.
Tested at: BioSafety Research Center Inc.
(Formerly Food and Agricultural and Pharmaceutical Safety Evaluation Center)
Test report was issued on: April 1, 2019
Report No.: H318 (584-014)

(2) Mutagenicity (Ames test)
An Ames test was carried out on ISADA Oil with and without the presence of S9mix using Salmonella Typhimurium TA98 and TA100. At concentrations of 4.9 to 5000 µg/plate, increases in the number of mutant colonies were not observed. This result indicates that ISADA Oil does not have any mutagenicity.

11. Recommended dosage

We recommend 210 mg/day of ISADA Oil based on the result of our clinical trial.

12. Packaging

ISADA Oil

1kg、5kg	Interior packaging	: Coated can
	Exterior packaging	: Cardboard

13. Storage

Store the material in a cool, dark place avoiding high temperatures and direct sunlight.
Please use as soon as possible once opened.

14. Labeling Examples

<Food>

ISADA Oil

Labeling examples: Krill extract, Isada extract, ISADA Oil/Edible Rice Oil

PRODUCT STANDARD

PRODUCT NAME : **Krill Oil（ISADA Oil）** (FOOD)

This oil is extracted from Japanese *Euphausia pacifica* Hansen（*Euphausiidae*）.It contains not less than 0.10% of 8-HEPE (8-hydroxy eicosapentaenoic acid).

Appearance	Deep red oil with characteristic odor.	
8-HEPE	Min. 0.10 %	(HPLC-CAD)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)
(2) Inorganic arsenic	Max. 0.5 ppm	(HPLC-ICP mass spectrometry)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Krill Extract	80%
Rice Bran Oil	20%
Total	100%

Expiry date 2 years from the date of manufacturing.

Storage Store in refrigerator at 4℃, dry and dark place in an original container avoiding high temperature and sun light.

Established Date	June 20, 2019
Revised Date	—
Specification No.	G-920TK

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

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Established Date : May 16, 2019
Revised Date : October 15, 2019

ORYZA OIL & FAT CHEMICAL CO., LTD.