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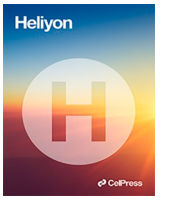

Effects of dietary adjustment of *n*-3:*n*-6 fatty-acid ratio to 1:2 on anti-inflammatory and insulin-signaling pathways in ovariectomized mice with high fat diet-induced obesity

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A R T I C L E I N F O

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A B S T R A C T

Estrogen deficiency increases the secretion of inflammatory mediators and can lead to obesity. Consequently, estrogen deficiency can cause metabolic syndrome, particularly insulin resistance during menopause. Both fish oil and perilla oil contain *n*-3 fatty acids, which may regulate several inflammatory cytokines. Additionally, adjusting the dietary *n*-3:*n*-6 fatty-acid ratio to 1:2 may help treat or prevent chronic diseases. Therefore, we investigated the effect of anti-inflammatory and insulin-signaling pathways, not solely in relation to the (*n*-3:*n*-6 fatty-acid ratio at 1:2), but also considering the origin of *n*-3 fatty acids found in fish oil and perilla oil, in a mouse model of estrogen deficiency induced by ovariectomy and obesity induced by a high-fat diet (HFD). Female C57BL/6J mice were divided into five groups: sham mice on a normal diet; ovariectomized (OVX) mice on a normal diet (OC); OVX mice on a HFD plus lard oil (OL), fish oil (OF), or perilla oil (OP). The dietary *n*-3:*n*-6 ratio in the OF and OP groups was adjusted to 1:2. The results showed OF group exhibited significantly lower abdominal adipose tissue weight, fewer liver lipid droplets, and smaller uterine adipocytes, compared with the OL group. Compared with the OL group, the OF and OP groups exhibited higher oral glucose tolerance and lower serum alanine aminotransferase activity, triacylglycerol levels, and total cholesterol levels. Hepatic *JAK2*, *STAT3*, and *SOCS3* mRNA expression and p–NF–κB p65 and IL-6 levels were significantly lower in the OF and OP groups than in the OL group. Only the OF group exhibited an increase in *PI3K* and *Akt* mRNA expression, decrease in *GLUT2* mRNA expression, and considerable elevation of *p*-Akt. Both fish and perilla oil reduced inflammatory signaling markers. However, only fish oil improved insulin signaling (*PI3K*, *Akt*, and *GLUT2*). Our data suggest that fish oil can alleviate insulin signaling through activating the PI3K-Akt-GLUT2 cascade signaling pathway.

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1. Introduction

Postmenopause is associated with lower lipase activity in the abdomen and buttocks; the resultant accumulation of lipids can often lead to obesity [1]. Humans and animals with obesity exhibit higher levels of serum tumor necrosis factor-α (TNF-α), interleukin-1β (IL)-1β, and IL-6 produced by adipose tissue–derived macrophages, which can lead to chronic inflammation [2]. IL-6 is involved in the inflammatory and insulin-signaling pathways. Numerous inflammatory factors play a pivotal role in initiating inflammation, impacting lipid metabolism and insulin receptor signaling [3]. These factors contribute to reduced insulin sensitivity and the onset of insulin resistance (IR). Once insulin engages with the insulin receptor, it activates insulin receptor substrate-1 (IRS-1), which in turn triggers downstream signal transduction pathways [4]. The primary routes for insulin signal transduction are the phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) pathways, collectively known as the PI3K/AKT signaling pathways. Consequently, glucose transporters (GLUTs) cannot migrate to the cell membrane to uptake and utilize glucose [5,6].

Polyunsaturated fatty acids (PUFAs) are divided *n*-3 and *n*-6 fatty acids. *n*-3 and *n*-6 fatty acids are precursors to signaling molecules, but they have opposite effects. The major *n*-6 fatty acid is arachidonic acid, and the metabolic products of arachidonic acid (2-series prostaglandins and 4-series leukotrienes) are inflammatory, atherogenic, and prothrombotic effects [7,8]. The major of *n*-3 fatty acids from animal source are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and these fatty acids-derived metabolites alleviate the proinflammatory effects of *n*-6 fatty acids [8]. The 2-series prostaglandins (PGs) are converted from arachidonic acid (*n*-6 fatty acid) which catalyzed by cyclooxygenases (COXs). Among these prostaglandins, PGE2 can cause insulin resistance in hepatocytes by a mechanism distinct from but synergistically with IL-6 [7]. *n*-3 and *n*-6 fatty acids are ligands and modulators for the nuclear receptors NF-κB, PPAR and SREBP-1c. An animal study showed that a high ratio of dietary *n*-3/*n*-6 PUFAs improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 pathway [9]. Therefore, lower *n*-6/*n*-3 PUFA could down-regulate inflammatory genes and lipid synthesis. *n*-3 fatty acid could down-regulate inflammatory genes and lipid synthesis, and stimulate fatty acid degradation [8]. Studies have shown that *n*-3 fatty acids can relieve metabolic syndrome by improving inflammatory response [10–12]. *n*-3 fatty acids reduce inflammation and improve saturated fatty-induced insulin resistance, while *n*-6 PUFA promotes insulin resistance [13]. DHA is considered more beneficial for obesity than EPA [14,15]. Higher lipid levels of DHA but not EPA or alpha-linolenic acid (ALA) are associated with slower progression of coronary artery disease in postmenopausal women [16]. In addition, premenopausal women have higher DHA levels than postmenopausal women [17] and it has been shown to be more antithrombotic than EPA [18]. DHA supplementation has been shown to reduce triglycerides (TG) in a dose-dependent manner in healthy postmenopausal women [19]. Postmenopausal patients with type 2 diabetes also have lower DHA levels than their healthy controls [17]. Siriwardhana et al. [13] reported that mice fed the EPA-diet could prevent and improve obesity and insulin resistant induced by a high saturated fatty acids diet.

In Westernized diets, the main dietary PUFAs are *n*-6 fatty acids. Fish oil is an animal source of *n*-3 fatty acids, mainly are EPA and DHA. A clinical trial demonstrated the benefits of dietary supplementation with fish oil in several cases of inflammatory and cardiovascular disease in humans [20] Perilla oil is rich in *n*-3 fatty acids, specifically ALA. Lee et al. demonstrated that perilla oil could protect kidney LLC-PK sub 1 cells by alleviating oxidative stress [21]. Park et al. noted inhibition of the secretion of IL-1β and IL-6 in ALA-treated pancreatic acinar cells exposed to hydrogen peroxide (H_2O_2) [22]. Some studies have focused on the effects of fish and perilla oil under normal physical conditions; however, the effects of fish oil and perilla oil, the source of *n*-3 fatty acids from animal and plant, on the anti-inflammatory and insulin-signaling pathways during menopause remain unclear. Liu et al. [9] compared the effect of a high *n*-3/*n*-6 fatty acid ratio (1:1) or a low *n*-3/*n*-6 fatty acid ratio (1:4), and they found that increasing *n*-3/*n*-6 fatty acid ratio can prevent obesity and insulin resistance. Moreover, adjusting the dietary *n*-3:*n*-6 fatty-acid ratio to 1:2 may be effective against chronic diseases [23]. Our previous study showed adjusting the fish oil-containing dietary *n*-3:*n*-6 fatty-acid ratio to 1:2 attenuated leukocyte infiltration into tissues in diabetic mice with sepsis [24]. Therefore, we examined the effects of anti-inflammatory and insulin-signaling pathways not on the ratio (*n*-3:*n*-6 fatty-acid ratio to 1:2) but on the source of *n*-3 fatty acids in fish oil and perilla oil in estrogen-deficient ovariectomized (OVX) mice with high-fat diet (HFD)-induced obesity.

2. Materials and methods

2.1. Animals and study design

Female C57BL/6J mice ($n = 40$; 6 weeks old) were purchased from the Taiwan National Laboratory Animal Center and housed at room temperature (23 °C ± 2 °C) and 50% ± 10% humidity with a 12-h light–dark cycle. The mice were fed a modified AIN-93 diet containing 620 g/kg of cornstarch, 100 g/kg of sucrose, 140 g/kg of casein, 2 g/kg of L-cysteine, 40 g/kg of corn oil, 50 g/kg of cellulose, 35 g/kg of mineral mixture, 10 g/kg of vitamin mixture, and 3 g/kg of choline bitartrate. After 1 week of acclimation, the 7-week-mice underwent either bilateral laparotomy (control group [C], $n = 8$) or bilateral oophorectomy (OVX groups, $n = 32$). The OVX mice were further randomly divided into four groups ($n = 8$ each) to receive (1) a normal diet (OC), (2) an HFD plus lard oil (OL), (3) an HFD plus fish oil (OF), or (4) an HFD plus perilla oil (OP). Two weeks after recovery from surgery, the mice were administered the designated diet intervention for 8 weeks. The mice in the OL, OF, and OP groups were fed a 60% kcal HFD (dietary composition is presented in Supplementary Table 1). The dietary *n*-3:*n*-6 ratio in the OF and OP groups was 1:2. After 8 weeks of intervention, the mice were killed, and their tissue was stored at −80 °C for further analysis. Liver and abdominal and uterine adipose tissue was collected, weighed, and pathologically examined. The total RNA and protein were extracted from the liver tissue and analyzed. All animal experiments adhered to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Taipei Medical

University. The IACUC Ethics Committee approved this study under protocol LAC-2015-0407.

2.2. Gas chromatography-mass spectrometry

The fatty-acid profiles of the dietary oils were obtained through gas chromatography (GC). Lard oil, fish oil, or perilla oil (200 μL) was added to 3 mL of 0.05% butylated hydroxytoluene in methanol and *n*-hexane (v/v = 4:1). After thorough mixing, the solution was placed on ice, and 0.3 mL of acetyl chloride was slowly added. The aliquot was incubated at 95 °C for 60 min and then centrifuged at 2000×*g* for 15 min. Thereafter, 0.2 mL of chloroform was added to the supernatant, and the mixture was vortexed and centrifuged at 2500×*g* for 15 min. The supernatant was collected and dried, and 100 μL of *n*-hexane was added. Lard, fish, and perilla oil were analyzed with a Thermo Scientific TRACE GC Ultra equipped with column (30 m × 0.32 mm × 0.2 μm) of 90% biscyanopropyl and 10% phenylcyanopropyl polysiloxane. The GC oven temperature was programmed to increase from 160 °C to 240 °C at 1.5 °C/min and was maintained at 240 °C for 10 min. The injector temperature was 260 °C, the flow rate of the carrier gas (i.e., helium) was 30 mL/min, and the split ratio was 1:10; 1-μL samples were injected into a GC mass spectrometer. The fatty-acid compositions were calculated using the Chrom-Card data system (Supplementary Table 2).

2.3. Oral gavage glucose tolerance test

After 7 weeks of diet intervention, the mice fasted for 14–16 h, and fasting blood glucose and insulin were analyzed. Subsequently, a glucose solution (2 g/kg of body weight) was administered orally, and blood was collected 30, 60, 90, and 120 min afterward. Glucose and insulin levels were determined using an autoanalyzer (Hitachi 7060, Hitachi, Japan). The glucose area under the curve (AUC) was calculated using the following formula: 0.25 × fasting value + 0.5 × 30-min value + 0.75 × 60-min value + 0.5 × 120-h value. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: serum insulin (mmol/L) × blood glucose (mmol/L)/22.5.

2.4. Detection of serum biomarkers

The serum was collected and prepared through centrifugation at 1500×*g* at 4 °C for 15 min. After 8 weeks of diet intervention, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), triacylglycerol (TG), and total cholesterol (TC) were analyzed using an autoanalyzer. Serum estradiol was analyzed using a radioimmunoassay kit (Diagnostic Products, Los Angeles, CA, USA).

2.5. Hematoxylin and eosin staining

Liver tissue and uterine adipose tissue were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm-thick slices, as described in our previous study [25]. The tissue sections were stained with hematoxylin and eosin and examined under a light microscope equipped with a charge-coupled device camera (Olympus BX51, Olympus, Tokyo, Japan).

2.6. Real-time reverse transcription polymerase chain reaction analysis of mRNA expression

We performed total RNA extraction and real-time reverse transcription polymerase chain reaction (RT-PCR) analysis as described in our previous study (Hou et al., 2012). Total RNA was extracted from the liver tissue with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and converted into cDNA with a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit. This cDNA was used for RT-PCR on an ABI 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA) to evaluate the expression of seven genes (*JAK2*, *STAT3*, *SOCS3*, *IRS-1*, *PI3K*, *Akt*, and *GLUT2*), and β-actin cDNA was used as an internal control for quantification. The primers are presented in Supplementary Table 3.

2.7. Western blot analysis of protein expression

Protein levels in liver tissue was measured through Western blotting, in a similar manner to that used in our previous study [26]. Protein was extracted from the liver tissues with radioimmunoprecipitation assay lysis buffer, and p–NF–κB (1:1000, ab28856, Abcam, Cambridge, UK), Akt (1:1000, #9272, Cell Signaling Technology, Danvers, MA, USA), *p*-Akt(1:1000, #9271 Cell Signaling Technology) andβ-actin(1:1000, ab8227, Abcam) levels were determined through Western blot analysis.

2.8. Enzyme-linked immunosorbent assay

IL-6 levels in liver tissue were measured using an e-Bioscience Mouse IL-6 Ready-SET-Go! enzyme-linked immunosorbent assay kit according to the manufacturer's instructions.

2.9. Statistical analysis

Experimental values are expressed as the mean ± standard error of the mean ($n = 8$). The data are analyzed with a parametric *t*-test

based on their normality of distribution by Shapiro-Wilk test or non-parametric Mann-Whitney test for comparing C and OC groups. In addition, the parametric one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test or non-parametric Kruskall-Wallis test followed by Dunn's multiple comparison tests is used among OL, OF, and OP groups. A p-value of <0.05 is considered statistically significant.

3. Results

3.1. Effects of lard, fish, and perilla oil on body weight and liver, abdominal adipose tissue, and uterine adipose tissue weight in OVX mice with HFD-induced obesity

There was no significant difference in energy intake among the four groups of OVX mice. Body weight and liver, abdominal adipose tissue, and uterine adipose tissue weight were measured at the end of the study (Table 1). The OVX mice exhibited significantly increased body weight and liver, abdominal adipose tissue, and uterine adipose tissue weight compared with the C mice ($p < 0.05$). Additionally, the OL, OF, and OP groups exhibited significantly greater body weight, abdominal adipose tissue weight, and uterine adipose tissue weight than did the OC group. The body weights of the OL, OF, and OP groups were 34.20 ± 1.19, 30.20 ± 0.61, and 32.71 ± 0.34 g, respectively. The abdominal adipose tissue weights of the OL, OF, and OP groups were 1.27 ± 0.20, 0.76 ± 0.05, and 1.23 ± 0.09 g, respectively. Among the HFD groups, the OF group exhibited the lowest body weight and abdominal adipose tissue weight. The body weights of the OF group was 11.7% ($p < 0.05$) and 7.7% ($p < 0.05$) lower than those of the OL and OP groups, respectively, and the abdominal adipose tissue weight of the OF group was 40.2% ($p < 0.05$) and 38.2% ($p < 0.05$) lower than those of the OL and OP groups, respectively.

3.2. Effects of lard, fish, and perilla oil on serum biomarkers in OVX mice with HFD-induced obesity

The effects of the various oils on the oral glucose tolerance and of the OVX mice and the AUC are shown in Fig. 1A and B, respectively. Oral glucose tolerance decreased significantly in the HFD-treated OVX mice, compared with the normal diet–treated OVX mice. Among the HFD-treated OVX groups, the OF group exhibited the highest oral glucose tolerance, followed by the OP and OL groups. No significant difference was observed in the glucose AUC between the C and OC groups. Additionally, the AUCs for the OL, OF, and OP groups were significantly larger than that of the OC group. Among the OL, OF, and OP groups, the OF group had the smallest AUC. The effects of various sources of n-3 fatty acids on the fasting serum insulin, glucose, and HOMA-IR of the OVX mice are shown in Fig. 1C, D, and 1E, respectively. No significant differences were observed in serum fasting insulin or glucose among the OL, OF, and OP groups (Fig. 1C and D). The HOMA-IR of the OF group was slightly lower than that of the OL group; however, no significant difference was observed among the OL, OF, and OP groups (Fig. 1E). Fig. 2 shows the serum ALT, AST, TG, and TC levels of each group. No significant difference was observed between the C and OC groups. However, compared with lard oil, fish oil significantly decreased the ALT activity and the levels of TG and TC in the OVX mice with HFD-induced obesity. The results for perilla oil were similar to those for fish oil.

3.3. Effects of lard, fish, and perilla oil on liver appearance and histochemical staining of liver and uterine adipose tissue in OVX mice with HFD-induced obesity

As presented in Fig. 3A and B, the livers of the OC and OL groups were grossly enlarged and pale. The OL group demonstrated considerable hepatomegaly and fatty liver formation, compared with the OC group; however, the OF and OP groups exhibited significantly less hepatomegaly and fatty liver formation, compared with the OL and OC groups (Fig. 3B). As shown in Fig. 3C, the number of large uterine adipocytes in the OC group was considerably greater than that in the C group. However, the OL, OF, and OP groups exhibited significantly larger adipocytes than the OC group. Additionally, histological analyses revealed that the OL group had larger adipocytes than the OF and OP groups. These results indicated that treating OVX mice with fish oil and perilla oil for 8 weeks can slow the accumulation of macrovesicular fat in the liver and inhibit the formation of uterine adipose tissue.

Table 1
Effects of lard, fish, and perilla oil on body, liver, and abdominal and uterine adipose tissue weight in OVX mice with HFD-induced obesity.

	C	OC	OL	OF	OP
Body weight	22.38 ± 0.26*	27.14 ± 0.83	34.20 ± 1.19[a]	30.20 ± 0.61[b]	32.71 ± 0.34[a]
Liver weight	0.90 ± 0.02*	1.09 ± 0.04	0.98 ± 0.02[a]	0.95 ± 0.02[a]	0.94 ± 0.02[a]
Abdominal adipose weight	n/a*	0.49 ± 0.04	1.27 ± 0.20[a]	0.76 ± 0.05[b]	1.23 ± 0.09[a]
Uterine adipose tissue weight	0.21 ± 0.03*	0.84 ± 0.10	1.81 ± 0.13[a]	1.53 ± 0.11[a]	1.68 ± 0.22[a]

C: Sham mice on a normal diet; OC: OVX mice on a normal diet; OL: OVX mice on an HFD plus lard oil; OF: OVX mice on an HFD plus fish oil; OP: OVX mice on an HFD plus perilla oil. Data are expressed as mean ± SE ($n = 8$). * significant difference between C and OC groups at $p < 0.05$ using Student's t-test. Letters (a,b) indicate significant difference at $p < 0.05$ using one-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons among OL, OF and OP groups.

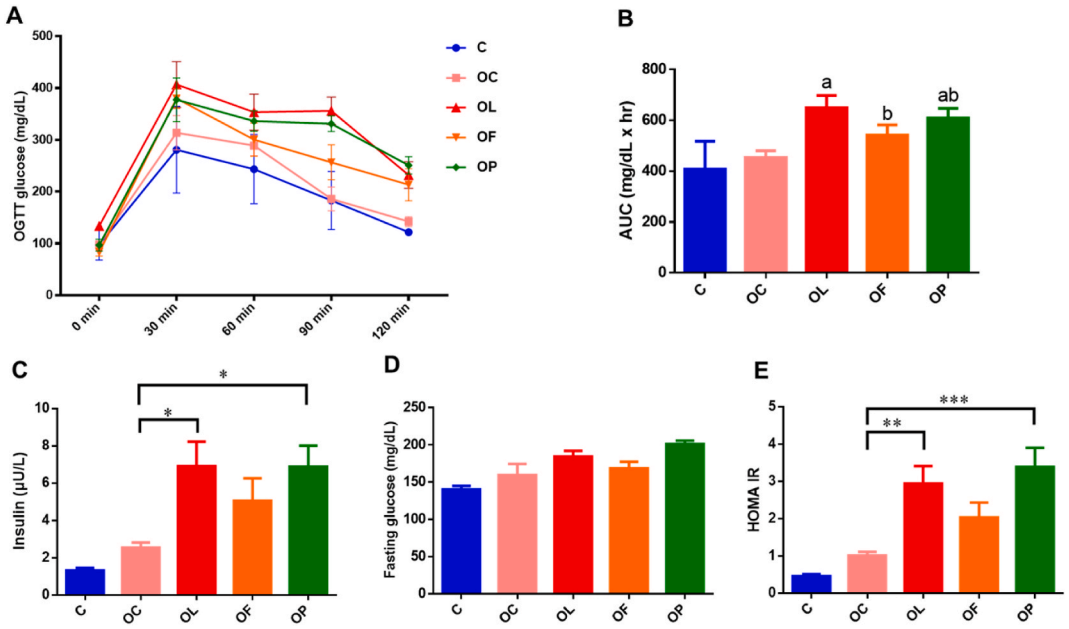


Fig. 1. Effects of lard, fish, and perilla oil on (A) oral glucose tolerance, (B) AUC, (C) insulin, (D) fasting glucose, and (E) HOMA-IR in OVX mice with HFD-induced obesity. C57BL/6J mice were divided into five groups ($n = 8$): (1) C, (2) OC, (3) OL, (4) OF, and (5) OP mice. HOMA-IR was calculated using the following formula: serum insulin (mmol/L) × blood glucose (mmol/L)/22.5. Data are expressed as mean ± SE ($n = 8$). The asterisk (*) shows a significant difference between the two groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Bars with different letters indicated significant difference at $p < 0.05$ in one-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons among OL, OF and OP groups.

3.4. *Effects of lard, fish, and perilla oil on the anti-inflammatory pathway in OVX mice with HFD-induced obesity*

The expression of mRNA of genes involved in the anti-inflammatory pathway in the liver (*JAK2*, *STAT3*, and *SOCS3*) was assessed using real-time RT-PCR (Fig. 4A). *JAK2* and *STAT3* mRNA expression was considerably lower in the OF and OP groups than in the OL group ($p < 0.05$); however, *SOCS3* mRNA expression was only significantly lower in the OF group ($p < 0.05$). The effects of various oils on liver p–NF–κB p65 (Ser 536) and IL-6 expression in the OVX mice are shown in Fig. 4B and C. Both the OF and OP groups exhibited lower *JAK2*, *STAT3*, and *SOCS3* mRNA expression and p–NF–κB and IL-6 levels.

3.5. *Effects of lard, fish, and perilla oil on the insulin-transduction pathway in OVX mice with HFD-induced obesity*

Fig. 5A presents the effects of fish and perilla oil on the insulin-transduction pathway in the liver. *IRS-1*, *PI3K*, *Akt*, and *GLUT2* mRNA expression was analyzed. No significant differences were observed in *IRS*-1 mRNA expression among the groups. *PI3K* mRNA expression was considerably higher in both the OF and OP groups than it was in the OL group ($p < 0.05$). Compared with the OL group, the OF group demonstrated significantly higher *Akt* mRNA expression and lower *GLUT2* mRNA expression ($p < 0.05$). Additionally, the OF group had significantly increased *p*-Akt (Fig. 5B). However, no significant differences were observed between the OL and OP groups. These results indicate that fish oil can regulate the insulin-signaling pathway by increasing *PI3K* and *Akt* mRNA expression and reducing *GLUT2* mRNA expression (see Fig. 6).

4. Discussion

Postmenopause is associated with a high obesity rate. In the present study, the effects of various sources of *n*-3 fatty acids in fish oil and perilla oil (by adjusting the same *n*-3:*n*-6 fatty-acid ratio to 1:2) on the anti-inflammatory and insulin-signaling pathways were examined in OVX mice with HFD-induced obesity. To mimic the postmenopausal state in rodents, the ovaries are usually removed (ovariectomy; OVX). OVX in rodents leads to increased total body weight as in postmenopausal women [27–30], total body fat gain [28], and impaired insulin sensitivity and glucose regulation [31]. Combining HFD with OVX further increases body weight gain in female rodents compared to OVX alone [27–29], and the loss of estrogen may increase the risk of T2D in females, especially when combined with HFD [32]. Bilateral oophorectomy resulted in a decrease in serum estradiol (Supplementary Fig. 1). Thus, this surgery appropriately mimics estradiol deficiencies among postmenopausal women. As shown in Table 1, the body weight, liver weight, and abdominal and uterine adipose tissue weight of the OC group were greater than those of the C group. Lipase activity decreases in women during menopause [1]. Because of this decrease in lipase activity, fat tends to acuminate in the abdominal region and buttocks and near the gonadal glands [1]. In this study, an HFD of lard oil, fish oil, or perilla oil significantly increased the body weight and

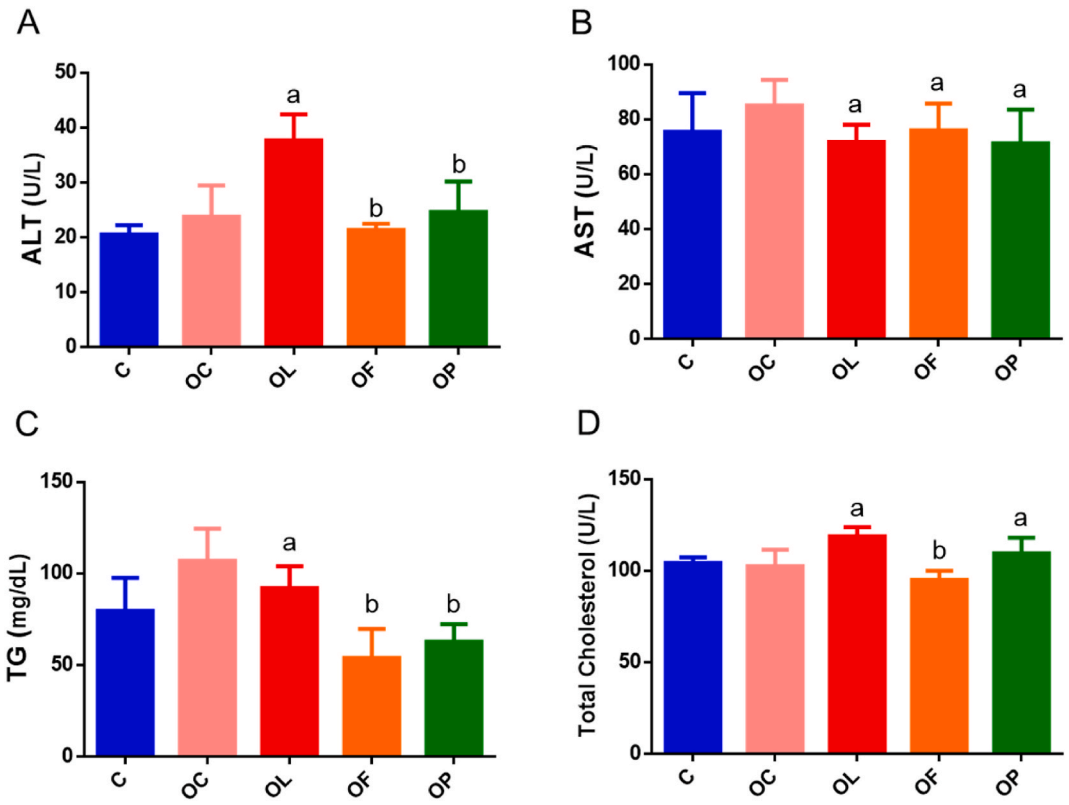
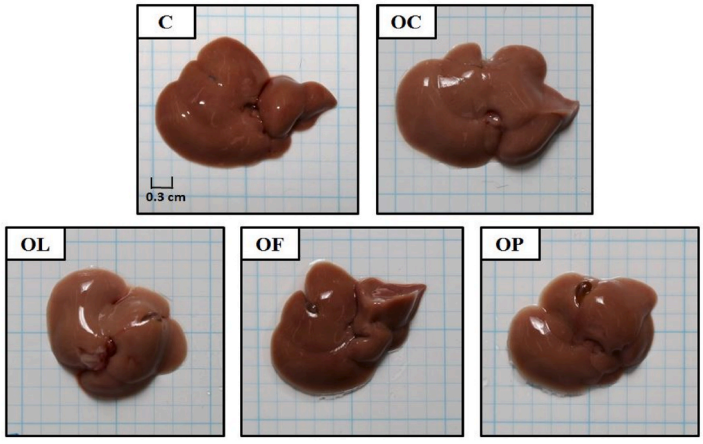


Fig. 2. Effects of lard, fish, and perilla oil on (A) ALT, (B) AST, (C) TG, and (D) TC in OVX mice with HFD-induced obesity. C57BL/6J mice were divided into five groups ($n = 8$): (1) C, (2) OC, (3) OL, (4) OF, and (5) OP mice. Data are expressed as mean ± standard error ($n = 8$). Bars with different letters indicated significant difference at $p < 0.05$ in one-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons among OL, OF and OP groups.

abdominal and uterine adipose tissue weight of OVX mice (Table 1). Among the three groups of OVX mice with HFD-induced obesity, body and abdominal adipose tissue weight were the lowest in the OF group (Table 1). Furthermore, compared with the C group, the OVX mice exhibited greater accumulation of liver lipid droplets and larger uterine adipocytes. Compared with the OC mice, the OL mice had greater liver lipid droplet accumulation and larger uterine adipocytes. However, the OF and OP groups exhibited significantly fewer liver lipid droplets and had smaller uterine adipocytes, compared with the OL mice (Fig. 3). Studies have shown that fish oil and perilla oil can prevent excessive growth of white adipose tissue in rats with HFD-induced obesity [33]. Ruzickova et al. found that EPA and DHA increase the peroxisomal β-oxidation of fatty acids in muscles and the liver [34]. Therefore, we hypothesized that fish oil, which is rich in EPA and DHA, could reduce abdominal adipose tissue weight, uterine adipocyte size, and liver fat deposition by elevating the peroxisomal β-oxidation of fatty acids. Additionally, fish oil reduced the fat content of the liver to alleviate liver damage in OVX mice; thus, AST levels were lower in the OF group than in the OL group (Fig. 2A). Both fish oil and perilla oil reduced serum TG and TC concentrations (Fig. 2C and D), which is consistent with the results of other studies [35,36]. Studies have shown [35,36] that both fish oil and perilla oil can reduce serum TG concentrations; however, another study concluded that only fish oil affects serum TG concentrations. Nakatani et al. observed that fish oil reduced the liver sterol regulatory element–binding proteins (SREBPs) in mice with HFD-induced obesity [37]. SREBPs are upstream-signaling proteins that regulate cholesterol synthesis; therefore, fish oil presumably reduces serum cholesterol by decreasing SREBPs. As shown in Fig. 1, the AUC of the OF group were lower than those of the OL group, and HOMA-IR of the OF group were slightly lower than those of the OL group. One study indicated that 3- or 6- week intervention with fish oil or ALA could alleviate insulin resistance [38]. The animal employed in other studies had normal physiological conditions; however, the mice in the present study had estradiol deficiency.

Treatment with fish oil and perilla oil considerably reduced p–NF–κB and IL-6 levels; *JAK2*, *STAT3*, and *SOCS3* mRNA expression was also significantly lower after fish or perilla oil treatment than it was after lard oil treatment (Fig. 4). Osborn and Olefsky demonstrated that EPA and DHA activate G protein–coupled receptor 120 on the cell membrane inhibits the phosphorylation of NF-κB, thereby reducing IL-6 levels [39]. Wang et al. reported that EPA and DHA significantly suppressed the production of TNF-α, IL-6, and MCP-1 more than saturated fatty acids did [40]. Park et al. determined that ALA reduced the production of IL-1β and IL-6 in H_2O_2-treated pancreatic acinar cells [22]. In a study of rats fed partially hydrogenated vegetable fat, Rao and Lokesh demonstrated that the downregulation of NF-κB by *n*-3-rich linseed oil was modulated by PPARγ activation, eicosanoid cascade, and the secretion of cytokines by macrophages [41].

A



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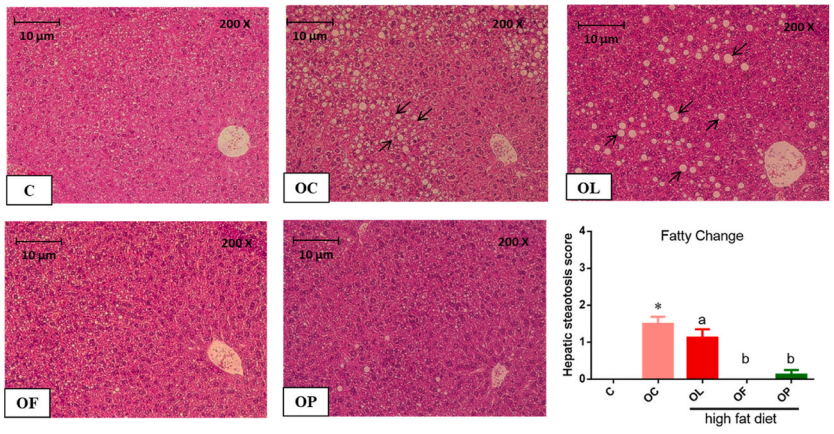

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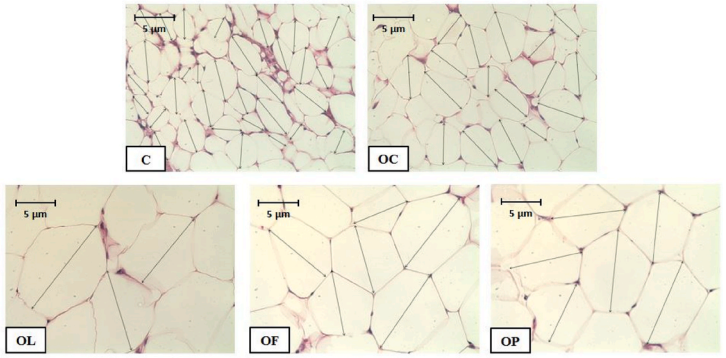

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Fig. 3. Effects of lard, fish, and perilla oil on (A) liver appearance and the hematoxylin and eosin staining results of histologically sectioned (B) liver and (C) uterine adipose tissue OVX mice with HFD-induced obesity. C57BL/6J mice were divided into five groups ($n = 8$): (1) C, (2) OC, (3) OL, (4) OF, and (5) OP groups. Scale bar for liver tissue = 10 μm, and scale bar for uterine adipose tissue = 5 μm. Data were expressed as mean ± standard error ($n = 5$). *, differ significantly at $p < 0.05$ between C group vs OC group by a non-parametric Mann-Whitney test. Bars with different letters indicated significant difference at $p < 0.05$ in non-parametric Kruskall-Wallis test followed by Dunn's multiple among OL, OF and OP groups.

IL-6 activates downstream JAK; subsequently, STAT is transferred to the nucleus to promote the transcription of SOCS proteins. SOCS proteins suppress IRS and inhibit the activation of PI3K and Akt. Consequently, GLUT cannot migrate to the cell membrane to uptake glucose [5,6]. Ai et al. [42] administered an HFD (20% lard oil) to Wistar rats for 10 days and found significantly increased *GLUT2* mRNA expression in the small intestine. Arnés et al. found that the *GLUT2* mRNA expression of Wistar rats with type 2 diabetes was significantly higher than that of normal rats [43] Therefore, an HFD or insulin resistance may increase *GLUT2* mRNA expression. As shown in Figs. 5A and 1B, *GLUT2* mRNA expression and the AUC were increased in the OL group. Therefore, lard oil increases insulin resistance and decreases insulin sensitivity, which can prevent cellular glucose uptake. Thus, *GLUT2* mRNA expression increases to maintain cellular glucose uptake. However, the *GLUT2* mRNA expression of the OF group was lower than that of the OL group (Fig. 5A). Among the OVX groups, only the OF group demonstrated increased phosphorylation of Akt in the insulin-transduction pathway (Fig. 5B). Zhu et al. [44] indicated that activation of the PI3K-Akt-PPARs-GLUT2 cascade signaling pathway ameliorates metabolic disorders, thereby attenuating insulin resistance. Our data suggest that fish oil can alleviate insulin signaling through activating the PI3K-Akt-GLUT2 cascade signaling pathway. Samane et al. found that feeding rats an HFD (50% total fat) with 15% of the fat replaced by fish oil for 4 weeks significantly increased *p*-Akt in adipose tissue, the gastrocnemius, and the liver [45]. An animal study showed that although perilla oil had a lower blood lipid effect, it cannot improve insulin resistance under a high-fat diet feeding [46]. In the present study also found that the HOMA-IR of the OP group was higher than those in the OC group. Therefore, it can be seen from the insulin signal pathway that its downstream Akt was not activated. In a high-fat diet (60% kcal from fat) feeding animal study, perilla oil can reduce inflammation by activating NF-κB [47]. The results of the study also showed that perilla oil could reduce the inflammatory response but could not improve insulin resistance in menopausal animals fed with a high-fat diet. While both fish oil and perilla oil reduced inflammation in the same diet with a 1:2 ratio of *n*-3:*n*-6 fatty acids, they had different effects on modulating insulin signaling in this model of menopause. In this study, fish oil resulted in significantly higher *p*-Akt than lard or perilla oil; this result may be attributed to improved insulin signaling by fish oil. One previous study showed that perilla oil increased the hepatic *n*-3 fatty acids proportion almost exclusively as α-linolenic acid. On the other hand, fish oil increased the ratio of EPA and DHA in hepatic lipid fraction [35]. Furthermore, a previous study showed that the fatty acid composition of blood in mice changed after dietary oil interventions [48]. The experimental diet in the present study was designed with a ratio of *n*-3:*n*-6 fatty acids of 1:2. However, the sources of dietary oils were different, which made the composition of *n*-3 fatty acids different. Thus, it may also the reason for the different insulin signaling pathways by these two different dietary oils of *n*-3 fatty acid. In addition, we hypothesized that EPA and DHA might exert competitive effects on enzymes attributed to downstream metabolites in inflammation and alleviate insulin signaling.

A limitation of this study is that in order to adjust the ratio of *n*-3:*n*-6 fatty acids to 1:2, we used corn oil, lard oil, fish oil and perilla oil for adjustment. Corn oil is rich in n-6 fatty acids, its *n*-3:*n*-6 ratio is 1:82, and it also contains other fatty acids. Therefore, S:M:P (saturated fatty acids: monounsaturated fatty acids: polyunsaturated fatty acids) was different between the four groups. Dietary composition is complex, and it is impossible to only consume dietary fat from a single source. Since this study adjusted the diet with a *n*-3:*n*-6 fatty acid ratio to 1:2, it may also be the reason for the inconsistency of S:M:P. In addition, the animal models provide valuable insights, they might not perfectly replicate human physiology. In the future direction, translate findings to human studies to determine if the observed effects are consistent in human physiology and provide clinical relevance.

5. Conclusion

Adjusting the dietary *n*-3:*n*-6 fatty-acid ratio to 1:2 with fish oil or perilla oil can reduces inflammatory signaling markers; however, only fish oil can mediate the insulin-signaling pathway in OVX mice with HFD-induced obesity. Fish oil and perilla oil significantly reduced the phosphorylation of NF-κB, IL-6 concentrations, and *JAK2*, *STAT3*, and *SOCS3* mRNA expression to inhibit inflammation. Second, fish oil reduced the AUC and HOMA-IR by increasing P13K and Akt expression, decreasing GLUT2 expression, and considerably elevating *p*-Akt levels. Therefore, fish oil can alleviate insulin signaling through activating the PI3K-Akt-GLUT2 cascade signaling pathway. These results indicate that fish oil reduces symptoms of obesity, including body weight gain and abdominal and uterine adipose tissue accumulation. Moreover, fish oil improves the inflammatory response and insulin signaling, as indicated by our OVX mouse model of obesity.

Ethics statement

All animal experiments adhered to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Taipei Medical University. The IACUC Ethics Committee approved this study under protocol LAC-2015-0407.

Author contribution statement

Yu-Tang Tung: Conceived and designed the experiments; Wrote the paper.

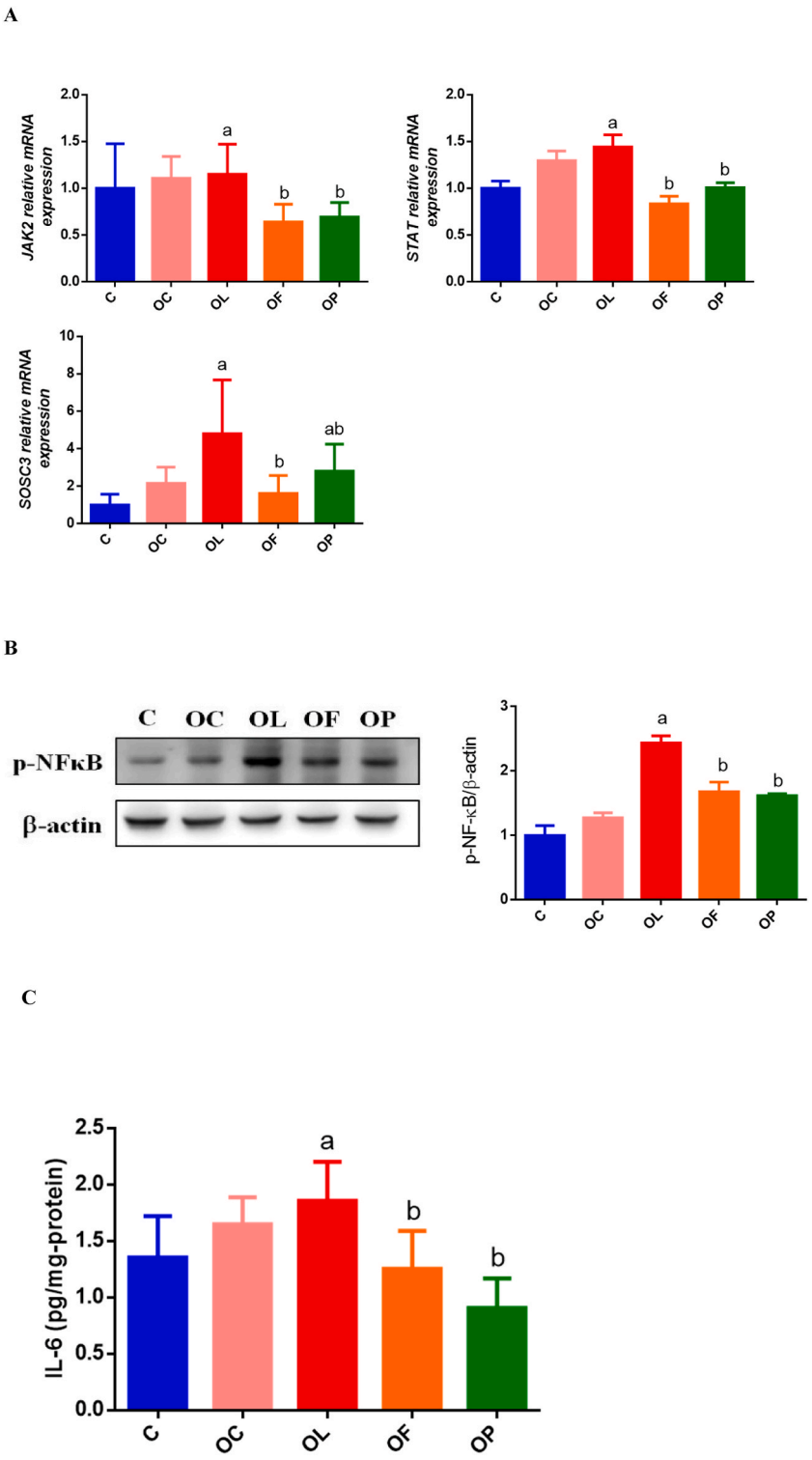

Fig. 4. Effects of lard, fish, and perilla oil on the anti-inflammatory pathway in liver of OVX mice with HFD-induced obesity: (A) expression of genes involved in inflammation (*JAK2*, *STAT3*, and *SOCS3*) and levels of (B) NF-κB and (C) IL-6. C57BL/6J mice were divided into five groups ($n = 8$): (1) C, (2) OC, (3) OL, (4) OF, and (5) OP groups. Data are expressed as mean ± standard error ($n = 8$). Bars with different letters indicated significant difference at $p < 0.05$ using one-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons among OL, OF and OP groups.

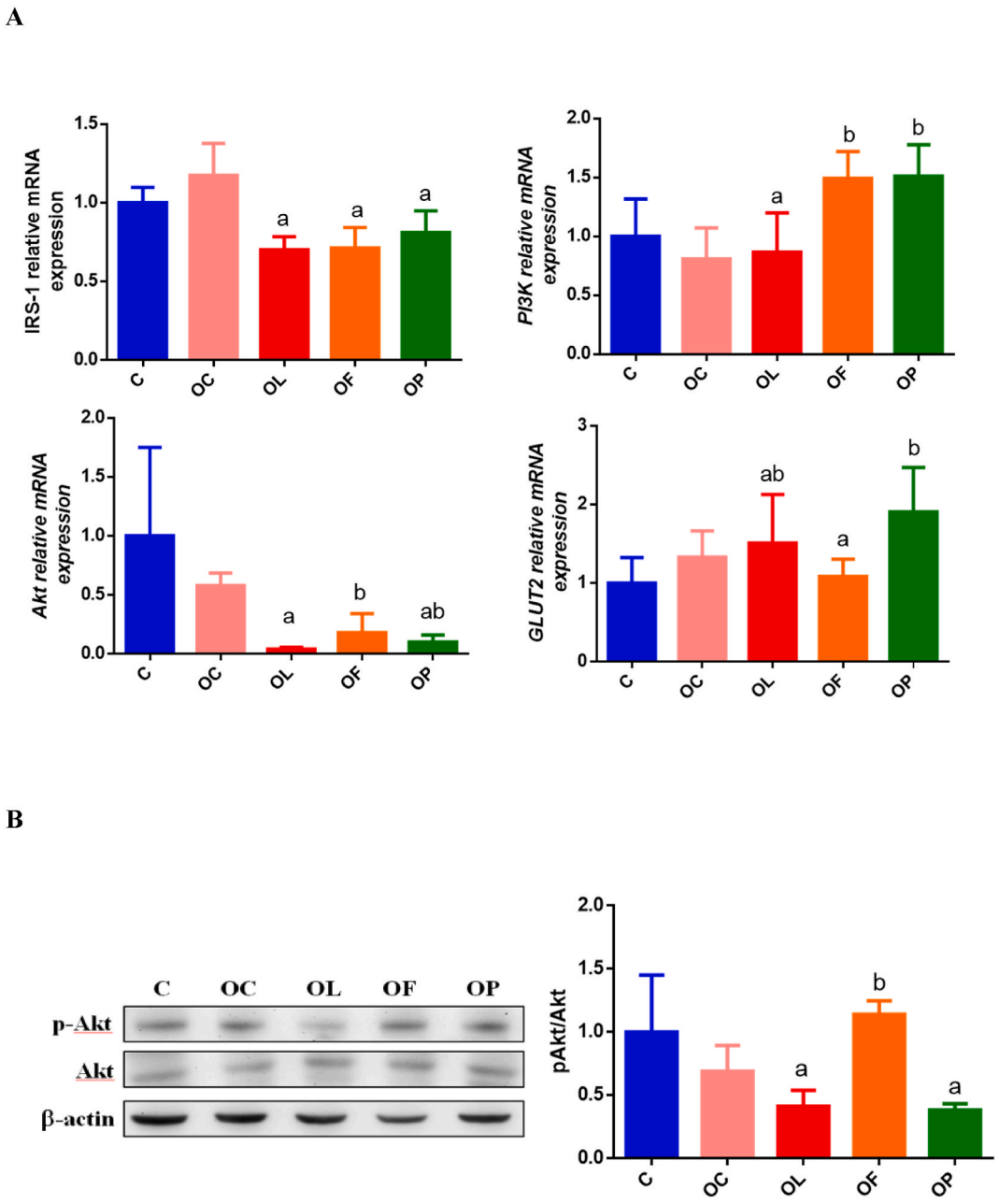

Fig. 5. Effects of lard, fish, and perilla oil on insulin transduction pathway in liver of OVX mice with HFD-induced obesity: (A) expression of genes involved in insulin transduction (*IRS-1*, *PI3K*, *Akt*, and *GLUT2*) ($n = 8$), and (B) levels of *p*-Akt. C57BL/6J mice were divided into five groups ($n = 3$): (1) C, (2) OC, (3) OL, (4) OF, and (5) OP groups. Data are expressed as mean ± standard error. Bars with different letters indicated significant difference at $p < 0.05$ using one-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons among OL, OF and OP groups.

Ya-Ling Chen, Tsorng-Harn Fong: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Tzu-Yu Fan: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Wan-Chun Chiu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

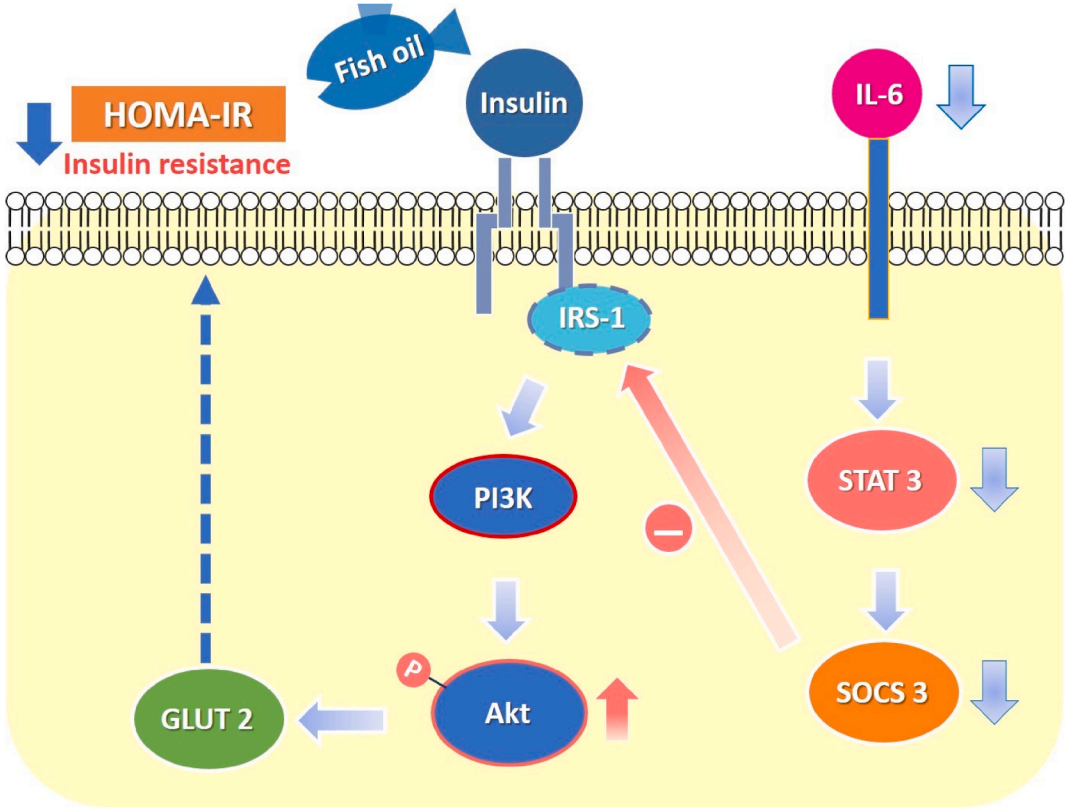

Fig. 6. Proposed mechanism of fish oil–mediated modulation of anti-inflammatory and insulin-signaling pathways in estrogen-deficient OVX mice with HFD-induced obesity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20451.

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