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Dietary fish oil inhibits the early stage of recovery of atrophied soleus muscle in rats via Akt–p70s6k signaling and PGF2$_\alpha$

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Abstract

Skeletal muscle recovery from disuse atrophy requires the recruitment of insulin signaling for muscle growth, which is driven by protein synthesis. Dietary fish oil, which is rich in long-chain $n-3$ polyunsaturated fatty acids, is known to enhance insulin signaling and protein metabolism. Therefore, this study was performed to evaluate whether dietary fish oil facilitates muscle recovery during remobilization after disuse atrophy. Ten days of immobilization, followed by 3 or 13 days of remobilization, were applied to the hindlimbs of rats fed corn oil [corn oil diet group as control (CO)] or fish oil [fish oil diet group (FO)] as source of dietary fat. The immobilization-induced reductions in soleus muscle weight and myosin heavy-chain content were significantly restored by 3 days of remobilization in CO. However, in FO, these muscle recovery measurements did not significantly change until 13 days of remobilization. At 3 days of remobilization, both groups had significant elevations in p70 ribosomal S6 kinase (p70s6k) activation and at a greater extent in CO than in FO. The activation of Akt was also increased on Day 3, but it was not significant in FO. Throughout the remobilization period, levels of prostaglandin F2$_\alpha$ (PGF2$_\alpha$) and cyclooxygenase-2 mRNA were significantly augmented. However, FO had a lesser increase in PGF2$_\alpha$ than CO until Day 13. These findings indicate that dietary fish oil inhibits the early stage of soleus muscle recovery after disuse atrophy by suppressing the activation of Akt–p70s6k signaling and PGF2$_\alpha$ synthesis.

Keywords: Dietary fish oil; Muscle recovery; Akt; p70s6 kinase; Prostaglandin F2$_\alpha$; Cyclooxygenase-2

1. Introduction

Skeletal muscle is a highly plastic tissue that has the ability to adapt to various mechanical stimuli. For example, disused muscle, either by immobilization or by unloading, rapidly atrophies; however, it begins to recover its original state when normal muscle mobility is resumed. Muscle recovery includes a normal hypertrophic process epitomized by increased protein synthesis and muscle regeneration. This increase in protein synthesis facilitates reaccumulation of myofibrillar protein such as the myosin heavy chain (MHC), which is the primary victim of muscle atrophy [1], followed by functional restoration of the atrophied muscle. However, such muscle recovery may occur slowly without voluntary exercise [2,3] or supplementary agents [4].

An emerging body of evidence demonstrates that insulin signaling plays a key role in triggering muscle hypertrophy in response to increased muscle activity involving remobilization [5,6]. Among constituents in insulin signaling, p70 ribosomal S6 kinase (p70s6k), a downstream target of Akt via the mammalian target of rapamycin (mTOR), critically governs muscle protein synthesis for muscle growth at the mRNA translation step [6,7]. Many studies (most of which were devoted to improvements in glucose metabolism) have confirmed that dietary fish oil rich in long-chain $n-3$ polyunsaturated fatty acid (LC$n-3$PUFA), namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), enhances insulin signaling and insulin sensitivity through various mechanisms, including modification of membrane fatty acid composition in skeletal muscle [8,9]. Recently, dietary fish oil was shown to be effective at improving insulin-mediated protein anabolism through Akt–mTOR–p70s6k signaling, with marked incorporation of LC$n-3$PUFA into muscle membrane phospholipids [10]. A similar anabolic effect of dietary fish oil on protein metabolism was further revealed by a later study in which the LC$n-3$PUFA content of muscle membranes was significantly correlated with its effects [11]. This increase in LC$n-3$PUFA content in muscle membrane phospholipids enhances membrane fluidity and affects the activation of insulin signaling intermediates by altering the function of the membrane and the membrane-associated enzymes and transmembrane proteins [9,12]. To date, however, no study has examined the effects of regular dietary fish oil consumption on insulin signaling and muscle growth during increased muscle activity such as remobilization.

The aim of this study was to determine whether dietary fish oil accelerates muscle recovery during remobilization via insulin signaling in atrophied soleus muscle. According to the results,

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dietary fish oil supplementation inhibited soleus muscle recovery by suppressing the activation of Akt–p70s6k signaling during 3 days of remobilization. To find the cause of this unexpected effect, we performed additional analyses concentrating on the anti-inflammatory functions of fish oil. Ultimately, it was shown that dietary fish oil supplementation also inhibited prostaglandin F2$_\alpha$ (PGF2$_\alpha$) synthesis, which increases during muscle recovery, at 3 days of remobilization, thus accounting for the delay in the early stage of soleus muscle recovery.

2. Materials and methods

2.1. Animals and experimental protocol

Nine-week-old male Sprague–Dawley rats (315–335 g), purchased from Orient Bio Ltd. (Seoul, Republic of Korea), were housed in individual mesh cages in a room maintained at 23±1°C with a 12 h:12 h light–dark cycle. After 3 days of acclimation, the rats (n=32) were randomly assigned to two diet groups [corn oil diet group as control (CO) or fish oil diet group (FO)] and received experimental diets with water *ad libitum* throughout the experiment. Following 14 days of feeding, one hindlimb of each rat was immobilized for 10 days and thereafter remobilized for 3 or 13 days. On the last day of immobilization (n=6 in each diet group) and remobilization (n=5 in each diet group), the animals were sacrificed by exsanguination under anesthesia after intramuscular injection of zoletil (10 mg/kg) and acepromazine (0.5 mg/kg). The soleus muscles were carefully excised, quickly weighed and then rapidly frozen in liquid nitrogen. The muscles were stored at −80°C for future analysis. The weight of each animal was recorded twice per week. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Laboratory Animal Resources at Seoul National University (SNU-080201-3).

2.2. Hindlimb immobilization and diet

Hindlimb immobilization of the rats was performed in accordance with a modified version of a previously described procedure in which the soleus muscle was most greatly atrophied [13]. The rats were anesthetized as described in Animals and Experimental Protocol and dressed in premade immobilization devices. The calf muscles of the hindlimb were maintained in a shortened position by complete plantar flexion of the ankle joint. The immobilization devices were checked daily for damage and immediately repaired as necessary. Following immobilization, the animals subjected to remobilization were allowed to return to normal ambulation by complete removal of the immobilization device. The immobilization device caused no side effect to the animals, nor was it removed from atrophy-only animals before complete death to ensure that no weight bearing occurred.

The experimental diets (Table 1) were made using a slightly modified AIN-93M diet and included 7% corn oil (wt/wt diet, corn oil diet) or 2% corn oil with 5% cod liver oil (wt/wt diet, fish oil diet). The fish oil diet was prepared everyday by adding cod liver oil to a basal mixture containing every other nutrient. The isoenergetic diets were provided to the animals everyday, and food intake was recorded. The corn oil diet and the basal mixture were kept at −20°C, and cod liver oil was preserved by encapsulation throughout the experimental period.

2.3. Western blot analysis

Half of the frozen soleus muscle was minced by surgical scissors for 1 min in ice-cold lysis buffer [50 mmol HEPES–NaOH (pH 7.5), 150 mmol NaCl, 2 mM EDTA (pH 8.0), 10 mmol sodium pyrophosphate, 10 mmol sodium fluoride, 2 mmol sodium vanadate, 1% Nonidet P-40, 10% glycerol, 2 mmol phenylmethylsulfonyl fluoride and protease inhibitor cocktail] and thereafter homogenized using a mini cordless grinder on ice for 30 s. After incubation on ice for 30 min, the homogenate was centrifuged at 12,000 rpm for 30 min to remove the insoluble pellet. The protein concentration of the supernatant was determined by the Bradford method. Samples were stored in aliquots at −80°C for future analysis.

Equal amounts of the protein samples, diluted in sample buffer, were boiled for 5 min. The samples were then resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes (Millipore, Charlottesville, VA). In all cases, the membranes and gels were stained with Ponseau S and Coomassie brilliant blue, respectively, to confirm equal loading and protein transfer. Following incubation of the membranes in blocking buffer containing either nonfat milk or bovine serum albumin, the membranes were serially washed with Tris-buffered saline Tween 20 (TBS-T) and probed with specific primary antibodies. After another serial wash with TBS-T, the membranes were incubated with horseradish peroxidase (HRP)-linked secondary antibodies (Amersham, Piscataway, NJ) and serially washed again with TBS-T. HRP activity was visualized with exposure to Kodak X-ray film using an enhanced chemiluminescence reagent (Pierce, Rockford, IL). Bands of interest were quantified with Quantity one 4.3.1 densitometric software (Bio-Rad, Hercules, CA). The primary antibodies used were purchased as follows: polyclonal phospho-Akt (Ser473), Akt, phospho-p70s6k (Thr389), p70s6k, phospho-SAPK/c-jun N-termimal kinase (JNK) (Thr183/Thr185) and SAPK/JNK from Cell Signaling Technology (Danvers, MA); polyclonal phospho-insulin receptor substrate 1 (IRS1) (Ser307), IRS1, Phosphoinositide-3-kinase (PI3K) p85, insulin receptor β subunit (IRβ) and monoclonal phosphotyrosine (PY20) from Millipore; and monoclonal MHC (fast-type) from Santa Cruz Biotechnology (Santa Cruz, CA).

2.4. Immunoprecipitation

Equal amounts of the protein samples diluted in phosphate-buffered saline (PBS) were incubated with specific primary antibodies (MHC, PY20 and PI3-kinase p85) overnight at 4°C. Immune complexes were immunoprecipitated with 20 μl of protein A/G agarose (Santa Cruz Biotechnology) at 4°C, and immunoprecipitates were washed three times with PBS at 4°C. After the final wash, the pellet samples were resuspended in 40 μl of sample buffer, boiled for 5 min and centrifuged. Equal amounts of the supernatant samples were subjected to SDS-PAGE, transfer and immunoblotting with corresponding specific primary antibodies, as described in Western Blot Analysis.

2.5. Semiquantitative RT-PCR

Total RNA was isolated from the left half of the soleus muscle using Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The concentration and purity of the total RNA were calculated by using absorbances at 260 and 280 nm, and its integrity was verified by gel electrophoresis. One microgram of template RNA was incubated with 20 pmol of reverse primer at 70°C for 5 min. After chilling, the mixture was transferred to an AccuPower RT-PCR PreMix tube (Bioneer, Deajeon, Republic of Korea) with 20 pmol of forward primer, and the tube was filled with DEPC-treated distilled water up to 20 μl. RT-PCR was then performed continuously in the tube to minimize any error by pipetting. The RT reaction for cDNA synthesis consisted of 60 min at 42°C, followed by 5 min at 94°C. PCR amplification was performed at 94°C for denaturing, at 52°C [*cyclooxygenase-2* (*COX-2*)] or 60°C (*β-actin*) for annealing, and at 72°C for extension, for 1 min, respectively. PCR was completed by 10 min at 72°C. The primer sequences were 5′-ACA CTC TAT CAC TGG CAT CC-3′ (forward) and 5′-GAA GGG ACA CCC TTT CAC AT-3′ (reverse) for *COX-2*, and 5′-CAT GTT TGA GAC CTT CAA CAC-3′ (forward) and 5′-GCC ATC TCC TGC TCG AAG TCT-3′ (reverse) for *β-actin*. PCR products were separated on 1.5% agarose gels prestained with SYBR safe DNA gel stain (Invitrogen). The bands of interest were visualized and photographed using the Molecular Imager Gel Doc XR system (Bio-Rad).

2.6. Enzyme-linked immunosorbent assay

Soleus muscle homogenates, containing 300 μg of protein per 100 μl, were used for the measurement of PGF2$_\alpha$. Indomethacin, as a prostaglandin synthase inhibitor, was added to the soleus muscle homogenates at a concentration of up to 5 μg/ml to prevent additional production of PGF2$_\alpha$. The concentration of PGF2$_\alpha$ was determined by a high-sensitivity PGF2$_\alpha$ enzyme immunoassay kit (Assay Design, Inc., Ann Arbor, MI) at a wavelength of 405 nm.

2.7. Statistical analysis

Statistical analysis was performed using SPSS 12.0 software. All values are expressed as mean±S.E. Paired t test was used to analyze differences between immobilized or remobilized soleus muscles and their respective contralateral controls in each group. Comparisons among all other groups and contralateral controls of the groups were performed using two-way ANOVA for the main effect (Diet or Recovery Day) or interactions (Diet×Recovery Day). When a significant main effect existed, individual differences between the groups and the contralateral controls of the groups were assessed using one-way ANOVA, followed by Duncan's post hoc test or unpaired t test. P<.05 was considered statistically significant.

Table 1
Composition of the experimental diets

Ingredient	Corn oil diet (g/kg diet weight)	Fish oil diet (g/kg diet weight)
Cornstarch	590.692	590.692
Casein	140	140
Sucrose	100	100
Cellulose	50	50
Corn oil	70	20
Cod liver oil	–	50
Mineral mixture	35	35
Vitamin mixture	10	10
DL-Methionine	1.8	1.8
Choline bitartrate	2.5	2.5
tert-Butylhydroquinone	0.008	0.008

Cod liver oil was obtained from General Nutrition Centers, Inc. (Pittsburgh, PA, USA). Mineral mixture, AIN-93M; vitamin mixture, AIN-93VX.

3. Results

Throughout the experimental period, the fish oil diet had no effect on body weight or food intake (data not shown). The soleus muscle recoveries in weight and MHC after immobilization are shown in Fig. 1A and B, respectively. Hindlimb immobilization induced significant decreases in soleus muscle weight normalized to body weight and MHC content in both CO and FO. However, in CO, soleus muscle weight loss was reduced sequentially at 3 and 13 days of remobilization. In addition, MHC loss was not evident from 3 days of remobilization in CO. On the other hand, in FO, these muscle recovery processes did not occur at 3 days of remobilization, as the recovery rates for soleus muscle weight and MHC content from 0 to 3 days were 5% and −17%, respectively. However, at 13 days of remobilization, normal muscle recovery occurred in FO in the same manner as in CO. No differences in contralateral soleus muscle were observed between the diet groups at the corresponding time points.

Tyrosine-phosphorylated IRβ did not change during remobilization regardless of the significant decrease in total IRβ during this period (Fig. 2A and B). Ser307-phosphorylated IRS1 protein was significantly increased at 3 days of remobilization with an increased tendency (CO, $P=.055$; FO, $P=.079$) at 13 days (Fig. 2C). These alterations in phosphorylated IRS1 (Ser307) almost coincided with alterations in phosphorylated JNK protein level during remobilization (Fig. 2D). Despite the possibility that increases in the serine phosphorylation of IRS1 following elevated JNK activity may have diminished the association of IRS1 with the p85 subunit of PI3K, as well as total IRS1 protein by degradation, the protein levels of IRS1 and IRS1 associated with p85 were sustained during remobilization (Fig. 2E and F). None of the assessed proteins in proximal insulin signaling was affected by dietary fish oil supplementation.

The levels of phosphorylation of Akt and p70s6k are illustrated in Fig. 3A and B, respectively. Significant increases in Akt and p70s6k protein activation were induced at 3 days of remobilization in CO. However, such recruitment was blunted in FO in that the phosphorylation of Akt did not increase and the degree of increase in p70s6k phosphorylation was significantly lower than that in CO. At 13 days of remobilization, the increased phosphorylations of Akt and p70s6k were normalized without reference to the diets, indicating that the enhanced activation of these molecules was an early event in the muscle recovery process during remobilization.

Analyses of *COX-2* mRNA and its enzymatic metabolite $PGF2_\alpha$ were performed to investigate the mechanism of inhibition of muscle recovery by dietary fish oil. The results are presented in Fig. 4A and B, respectively. The *COX-2* mRNA level and $PGF2_\alpha$ concentration were significantly increased throughout the remobilization process in both diet groups. However, in FO, the extent of the increase in $PGF2_\alpha$ was significantly less than that in CO at 3 days of remobilization, although *COX-2* mRNA was similarly augmented in the two diet groups at this time. At 13 days of remobilization, the increases in $PGF2_\alpha$ were not different between the diet groups.

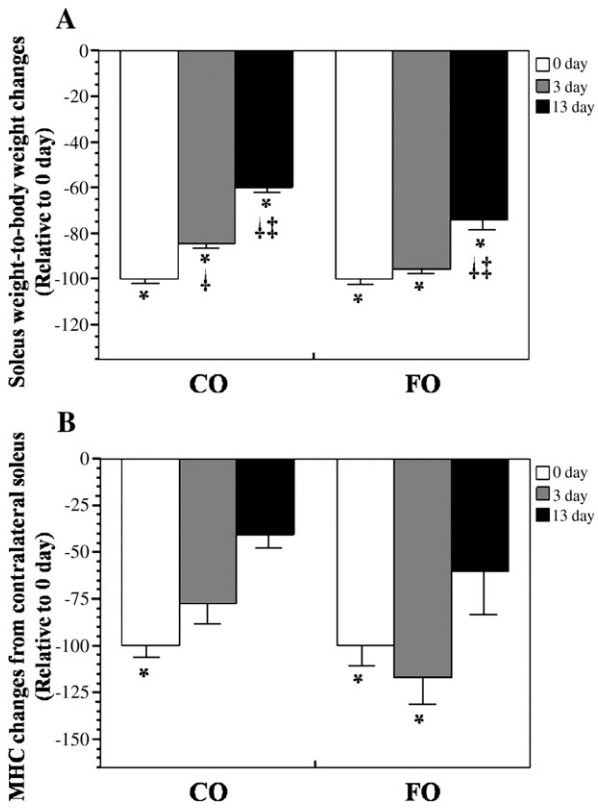

Fig. 1. Effects of remobilization and dietary fish oil on soleus muscle recovery. Dietary fish oil inhibited soleus muscle recovery at 3 days of remobilization. Soleus muscle weight-to-body weight ratio (A) and MHC content (B) were assessed immediately after 10 days of immobilization (0 day) and at 3 and 13 days of remobilization in corn-oil-fed or fish-oil-fed rats. Changes from contralateral soleus muscle were expressed as relative percentage to 0 day in each diet group. Values are expressed as mean±S.E. *Significantly different from its contralateral soleus muscle on the same day. †Significantly different from Day 0 within the same diet group. ‡Significantly different from Day 3 within the same diet group.

4. Discussion

To our knowledge, this is the first study to have sought the role of dietary fish oil in muscle recovery during remobilization after disuse. We specifically focused on the effects of dietary fish oil consumption on alterations in insulin signaling and $PGF2_\alpha$ synthesis induced by remobilization. Our data proved that dietary fish oil interfered with the early phase of soleus muscle recovery from disuse atrophy. In addition, this effect of dietary fish oil was associated with inhibition of Akt and p70s6k activation and with synthesis of $PGF2_\alpha$.

Our data on the components of proximal insulin signaling indicate that soleus muscle remobilization did not affect IRβ–IRS1–PI3K signaling in terms of protein levels. In fact, conflicting results have been produced from several studies that focused on changes in IRβ–IRS1–PI3K signaling after acute exercise [14], short-term exercise [15] and chronic exercise [16,17], as well as muscle contraction via nerve stimulation [18]. In addition, because data from muscle reuse models such as remobilization or reloading have been poorly examined, further investigation is required to elucidate the exact effect of muscle remobilization on this early insulin signaling. JNK tends to be activated with intense physical activity [19,20] in a tension-dependent manner [21], but transiently [22] in skeletal muscle. Thus, in our study, the elevation in phosphorylated JNK at 3 days of remobilization reveals that remobilization is enough to stimulate JNK for a relatively long time in soleus muscle. It is well known that the elevation of JNK activity by several pathological factors induces insulin signaling resistance in various tissues [23]. However, our results showed that the protein level of IRS1 associated with p85 was maintained at 3 days of remobilization; in addition, Akt protein, which is a downstream target of PI3K, was even activated in CO at this time. Therefore, it can be suggested that the elevation of phosphorylated JNK by remobilization-induced acute mechanical stress plays a cellular and physiological role different from that of disease states for muscle adaptation such as hypertrophy.

Despite the absence of changes in IRS1 associated with p85, Akt–p70s6k signaling was recruited during the early phase of muscle recovery from disuse atrophy, as demonstrated previously [6,24]. This result may support the notion that the Akt downstream pathway is more sensitive to mechanical stimuli in insulin signaling than its upstream pathway, as suggested previously [7]. It is noteworthy that

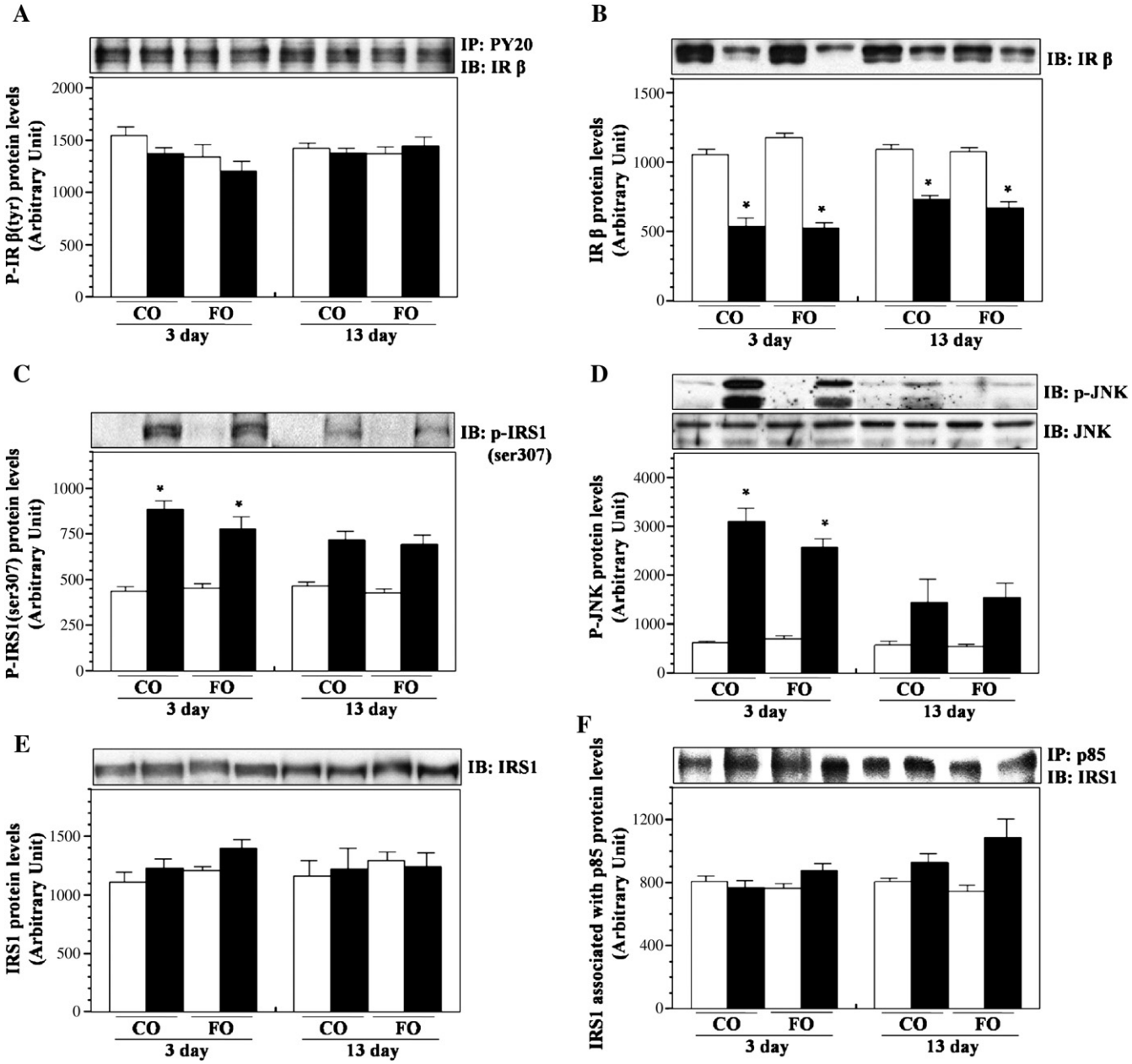

Fig. 2. Effects of remobilization and dietary fish oil on proximal insulin signaling proteins. Dietary fish oil had no effect on protein levels in proximal insulin signaling during remobilization. Protein levels of tyrosine-phosphorylated IRβ (A), total IRβ (B), Ser307-phosphorylated IRS1 (C), phosphorylated JNK (D), total IRS1 (E) and IRS1 associated with p85 (F) were assessed at 3 and 13 days of remobilization in the soleus muscles of corn-oil-fed or fish-oil-fed rats. The data for contralateral and remobilized soleus muscles are represented by open bars and black bars, respectively. Separate immunoblot bands of 3 and 13 days were derived from the same capture. The representative band is displayed on the upper panel. Values are expressed as mean±S.E. *Significantly different from its contralateral soleus muscle.

dietary fish oil consumption retarded this activation of the Akt–p70s6k pathway and subsequent muscle recovery. The early recovery process from disuse-induced muscle devastation normally includes proteolysis and increased protein synthesis for muscle remodeling [25,26]. This fact leads to the possibility that dietary fish oil inhibits muscle recovery through insufficient protein synthesis via p70s6k relative to elevated proteolysis during 3 days of remobilization. This supposition may also account for the decrease in average MHC content in FO during the early recovery period. Despite the remaining controversies on the precise role of calcineurin (CaN), some studies have suggested that CaN, which is considered an important muscular hypertrophic signaling molecule that acts via the nuclear factor of activated T cells (NFAT) for muscle cell differentiation [27] and fusion [28], is required for soleus muscle remodeling and for regrowth from atrophy at the later phase of muscle recovery [24,29]. Therefore, the significant muscle recovery that occurred in both diet groups at 13 days of remobilization without the activation of the Akt–p70s6k pathway might be somewhat attributable to the activation of the CaN pathway or others.

Our remobilization treatment promoted the synthesis of $PGF2_{\alpha}$ and *COX-2* mRNA, as formerly described in other studies employing resistance exercise [30–32], overloading [33] and myoblast stretch [34]. A growing body of evidence has documented that the *COX-2* pathway with prostaglandins plays a pivotal role in muscle growth and regeneration induced by injury [35,36], mechanical stretch [34,37] and increased loading [33,38], especially at early time points.

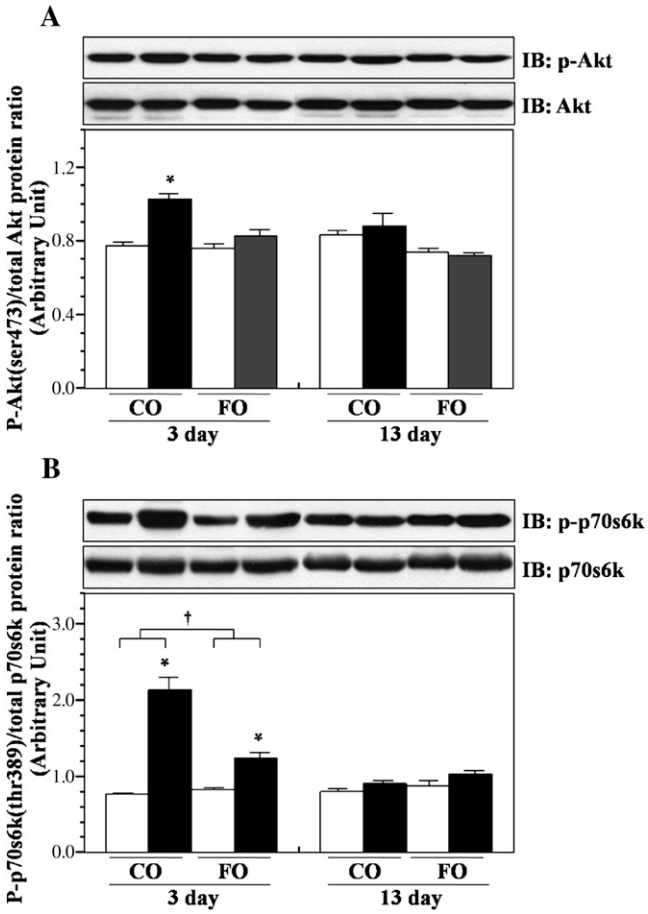

Fig. 3. Effects of remobilization and dietary fish oil on the activation of Akt and p70s6k proteins. Dietary fish oil inhibited Akt and p70s6k activation at 3 days of remobilization. Phosphorylation levels of Akt (A) and p70s6k (B) were assessed at 3 and 13 days of remobilization in the soleus muscles of corn-oil-fed or fish-oil-fed rats. Separate immunoblot bands of 3 and 13 days were derived from the same capture. Data for the contralateral and remobilized soleus muscles are represented by open bars and black bars, respectively. The representative band is displayed on the upper panel. Values are expressed as mean±S.E. *Significantly different from its contralateral soleus muscle.

Moreover, it was revealed that $PGF2_\alpha$ is more critical in muscle growth, including protein synthesis [31,39] and myotube maturation [40], than prostaglandin E_2. These results suggest that the lack of increase in $PGF2_\alpha$ synthesis at 3 days of remobilization in FO might have notably contributed to the inhibition of muscle recovery at this time. Another intriguing fact in this regard is that the impeded muscle recovery in FO was restored to the extent of that in CO at 13 days of remobilization and with increased $PGF2_\alpha$ synthesis similar to that in CO. Therefore, we speculate that soleus muscle recovery during remobilization after disuse atrophy might be regulated by the modulation of $PGF2_\alpha$ synthesis.

Although not specifically demonstrated in skeletal muscle, some studies have shown that $PGF2_\alpha$ can increase either or both phosphorylated Akt and p70s6k in smooth muscle cells [41,42] and cardiomyocytes [43]. These results imply that the $PGF2_\alpha$ synthesis suppressed by the dietary fish oil at 3 days of remobilization might inhibit muscle recovery at this time via suppression of Akt and p70s6k activation. $PGF2_\alpha$ is also involved in skeletal muscle cell growth via NFAT [44], which additionally suggests that elevation of $PGF2_\alpha$ might have facilitated the CaN–NFAT pathway mainly for late muscle recovery in CO and FO. However, for explicit delineation of the mechanisms responsible for $PGF2_\alpha$-associated muscle recovery during remobilization, additional research must be performed.

$PGF2_\alpha$, derived by *COX-2*, cannot be formed from DHA or EPA, but is formed from arachidonic acid (AA; a predominant $n-6$ fatty acid), whose release amount from the cellular membrane is determined by its content in membrane phospholipids along with the activity of phospholipase A_2 (PLA_2) [45]. This fact suggests that AA level in the muscle membrane lowered by chronic dietary fish oil intake can reduce the production of $PGF2_\alpha$ in skeletal muscle even under normal conditions, as revealed in FO on Day 13 in our results and in a previous study [46]. Furthermore, considering the fact that PLA_2 activity increases with prolonged physical exercise [47], dietary fish oil consumption, followed by a reduced level of AA, could have blunted the effect of PLA_2, which is activated in remobilization, on the dissociation of AA from the muscle membrane, consequently leading to the attenuated promotion of $PGF2_\alpha$ synthesis on Day 3. However, we are unable to suitably explain the reason why such suppression of remobilization-prompted $PGF2_\alpha$ synthesis by dietary fish oil supplementation occurring on Day 3 was not continued on Day 13.

The current study offers evidence that dietary fish oil, well known for its sensitizing effect on insulin signaling, inversely inhibits the activation of the Akt–p70s6k signaling and subsequent muscle recovery during the early period of remobilization after disuse. It was also found that this effect of dietary fish oil was associated with the suppression of remobilization-induced $PGF2_\alpha$ production. These results suggest that the anti-inflammatory function of dietary fish oil may detrimentally influence early muscle hypertrophic response, at least partly via Akt and p70s6k, to unaccustomed mechanical stimuli

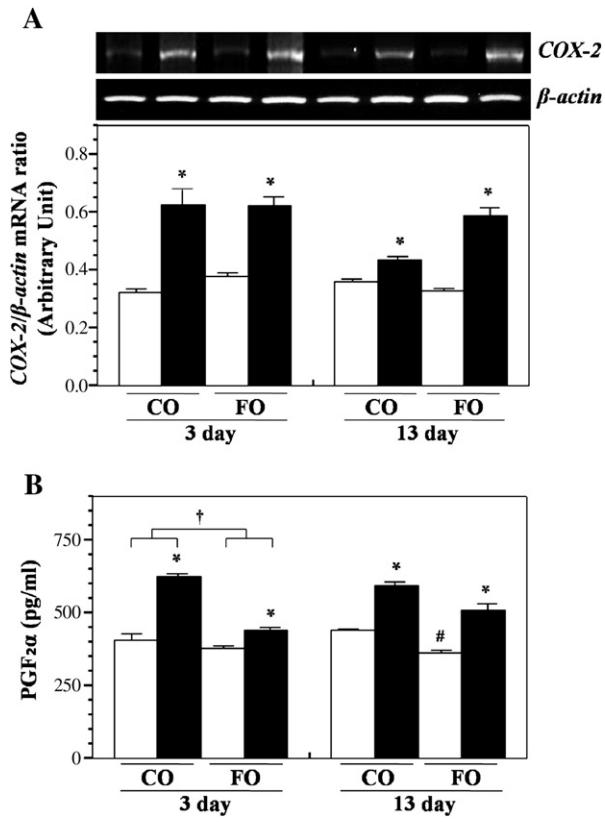

Fig. 4. Effects of remobilization and dietary fish oil on *COX-2* mRNA and $PGF2_\alpha$. Synthesis of $PGF2_\alpha$ through *COX-2* gene transcription was suppressed by dietary fish oil at 3 days of remobilization. *COX-2* mRNA levels normalized to *β-actin* (A) and $PGF2_\alpha$ levels (B) were assessed at 3 and 13 days of remobilization in the soleus muscles of corn-oil-fed or fish-oil-fed rats. Data for the contralateral and remobilized soleus muscles are represented by open bars and black bars, respectively. The representative band of *COX-2* is displayed on the upper panel. Values are expressed as mean±S.E. *Significantly different from its contralateral soleus muscle. #Significantly different from the contralateral soleus muscle in CO on Day 13.

such as remobilization, as already elucidated with respect to nonsteroidal anti-inflammatory drug use in other studies [33,36,39]. Accordingly, it is probable that patients who are in the early stage of muscle rehabilitation should be cautious of excessive consumption of fish oil. However, aside from our current report, inadequate evidence on this issue warrants additional study for more definitive conclusions.

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