



Perch essence prevents cell death to improve skeletal muscle mass and strength: Evidence from *in vitro* and human model

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ABSTRACT

Background: Fish protein supplementation may maintain muscle strength and prevent sarcopenia as it contains a complex array of macro- and micronutrients essential for building the skeletal muscle.

Objective: The aim of this study was to evaluate the influence of perch essence (PE) supplementation on muscle mass and muscle function of through human and cell model.

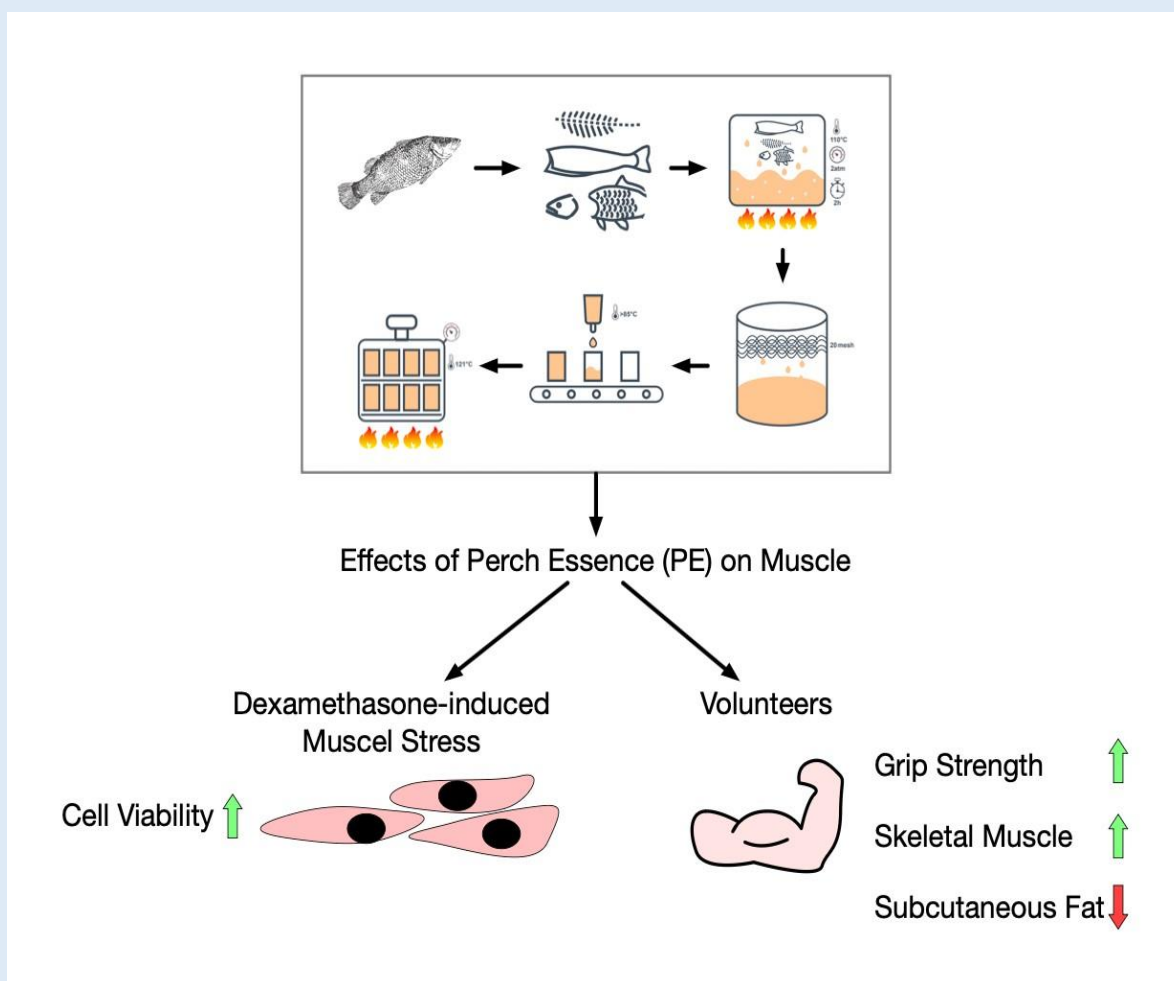
Methods: The open label clinical trial was conducted to assess the therapeutic effect of PE on muscle mass improvement. The mouse skeletal muscle cell (C2C12) model of muscle atrophy was analyzed for cell viability.

Results: Our results showed PE contained abundant branched chain amino acid, taurine, hydroxyproline and collagen.

After one month of supplementation with PE in our human model, there was a significant increase in muscle mass in the whole body and all parts of the body, with an increase of 1.55 % in the whole body, 1.79% in the trunk, 1.64% in the arms and 1.38% in the legs. The percentage of subcutaneous fat in the trunk, arms and legs also decreased significantly by 2.49%, 3.21% and 3.40% respectively. PE supplementation also improves muscle grip strength, especially with the dominant hand. The cell model results demonstrated that PE could effectively prevent skeletal muscle cell from death induced by dexamethasone.

Conclusion: This study suggests that the branched chain amino acids, taurine, hydroxyproline and collagen in PE have the potential to serve as a good source of dietary supplements for the improvement of skeletal muscle mass and strength through cell protection.

Keywords: branched chain amino acid, collagen, perch, skeletal muscle, sarcopenia



Graphical Abstract:

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INTRODUCTION

The decline of skeletal muscle mass is generally thought to be due to an imbalance between protein degradation and protein synthesis [1]. Sarcopenia is a disease characterized by progressive loss of muscle mass, strength, quality, and function that ultimately leads to functional decline and disability [2]. Dietary protein supplementation is considered an important strategy for the management and prevention of muscle atrophy or sarcopenia, especially when combined with resistance exercise [3, 4]. However, more than 30% of older adults do not meet the current RDA for daily protein intake [5]. Proteins along with amino acids are pivotal biomolecules that regulate many important metabolic pathways in the body and serve as precursors for the synthesis of essential substances [6, 7]. Therefore, proteins or amino acids from foods act as key players in preventing protein-calorie malnutrition.

Compared with plant proteins, animal proteins are easier to digest and as such provide more bioavailable amino acids to promote the synthesis of protein in the muscles [8]. Among high quality proteins, fish protein is extremely digestible and balances many regulatory factors, as well as being a plentiful source of animal protein and essential amino acids [9,10, 11]. Fish protein has been reported to promote skeletal muscle hypertrophy and protein synthesis by activating the Akt/mTOR signaling pathways [12]. In Taiwan, fish soup is a traditional dietary remedy for pregnant women or frail patients in need of fatigue relief and quick recovery [13]. Perch meat is an appealing source of physiological activities as it is rich in amino acids, peptides, and protein content, and contains 15-25% of total fish protein [14]. A previous report found that PE comprises of 32.4% peptides with a molecular weight of under 2.3 kDa, suggesting that naturally occurring bioactive peptides and proteins may treat metabolic syndrome [15]. Furthermore, PE contains a variety of high-quality

proteins and BCAA that can effectively delay swim fatigue by conserving liver glycogen and attenuating plasma TBARS, myoglobin induction by exhaustive exercise [15]. Animal studies also have shown that continuous PE supplementation for four weeks can significantly enhance muscle strength and reduce post-exercise fatigue [16]. However, the effects of perch protein on skeletal muscle have not been fully investigated in clinical trials. The aim of this study was to evaluate the effects of PE supplementation on the body composition and muscle strength of human subjects and the protection of skeletal muscle in a cellular model.

MATERIALS AND METHODS

Sample preparation: The perch (*Lates calcarifer*) used in this study was supplied by Yilan Anyong Lohas Co., Ltd. (Yilan, Taiwan). The internal organs of the perches were removed and discarded. The flesh, bone and scales of the perch were washed, and then extracted using RO water at a ratio of 1:1. The extraction was conducted at high pressure for 2-3 hours at a temperature of 110-130 °C and 1.5-3 atmospheric pressure. The resulting mixture was then centrifuged and filtered to obtain the perch extracts, which were subsequently sterilized at 121°C for 4 minutes to yield the perch essences (PE).

Analysis of protein profile and amino acids:

Hydrolyzed amino acid composition was analyzed in accordance with ISO 13903:2005 using an amino acid analyzer (Biochrom Ltd., Cambridge, UK) [14]. Extraction of free taurine with metaphosphoric acid and protein precipitation with centrifugation. Taurine content was analyzed by sodium cation exchange column, post column derivatization with o-phthalic aldehyde and detection by fluorescence at 338/425 nm [17, 18]. Hydroxyproline content was

quantified by HPLC according to the method of AOAC 982.30 with modification. The hydroxyproline content was multiplied by eight to give an approximate estimation of collagen [19].

Human Study: Recruitment of participants was through advertisements. Inclusion criteria included being aged 20 or older, not smoking, having no history of cardiovascular disease or diabetes, not taking any medication, and not pregnant. Participants were instructed to abstain from alcohol and heavy exercise 2 hours prior to the study. This study was conducted according to the recommendations of the Human Research Ethics Committee of Yuanpei University of Medical Technology, and all participants provided written informed consent. The protocol was approved by the IRB (YPU-IRB-1120223). 11 participants received 60 mL PE treatments per day for one month. Anthropometric and physiological measurements were taken on all participants during the baseline assessment. These data were obtained by trained staff following a standardized protocol. Weight and bioimpedance were measured using the Omron HBF-375 body composition monitor (OMRON Healthcare, Taiwan). BMI, body fat and lean mass percentages were estimated from these measurements. Grip strength of each hand was measured using a Camry model EH101 electronic hand dynamometer (Zhongshan Camry Electronic Co, Ltd., Guangdong, China). After resting for 10 minutes, participants measured SBP, DBP and pulse using an Omron JPN600 automated blood pressure monitor (Omron, Kyoto, Japan), and the mean of the two measurements was taken for analyses.

Cell study: Mouse C2C12 myoblasts were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and maintained at 37 °C in a humidified incubator containing 5% CO₂ [19]. The myoblast cells were

differentiated into mature muscle cells with 2% horse serum over the course of 6 days. Following this, the cells were divided into control, injury, pre-treatment (preventive), and post-treatment (therapeutic) groups. The control group only had its culture medium replaced. The injury group was incubated with 10µM DEX for 24 hours to induce injury. For the preventive group, cells were incubated with PE for an hour prior to a 24-hour incubation with 10µM DEX. The therapeutic group was first incubated with 10µM DEX for 24 hours to induce injury and then incubated with PE for an hour. Cell viability was measured according to the instructions provided in the Alamar blue product manual (Bio-RAD antibodies).

Statistical analysis of data: Statistics were analyzed using GraphPad Prism 9.5.1 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS version 26.0 (IBM, Armonk, NY, USA). The results of the experiments were expressed as the mean ± standard deviation (SD). Our analysis used the multiple paired t-test for comparison of pre- and post-treatment data within individual patients. This allowed us to assess changes in individual scores after nutraceutical treatment implementation. We used one-way ANOVA supplemented by Dunnett's multiple comparison tests to analyze the cell model data. In addition, a two-way ANOVA was used to further corroborate these findings and calculate the differences between the groups, accompanied by Šidák's multiple comparisons test. Our conclusion regarding the presence of significant differences in certain variables is strengthened by the consistency of the results from these different methods of analysis.

RESULTS

BCAA, taurine and collagen in PE: PE contains 84 mg/mL of protein and 32.4% of the PE contains peptides with a molecular weight of below 2.3 kDa [14]. The content of BCAA, taurine and collagen in PE is

shown in Table 1. The BCAA content of 663 mg/100 ml is much higher than that of mackerel and milkfish [14]. Although perch is a white meat fish, it is still rich in taurine with 69 mg/100ml. Of note is the high collagen content of PE (extrapolated from the hydroxyproline content), up to 4,792 mg/100g, as shown in Table 1. This is probably attributed to the inclusion of fish bone

and scales in our extraction technique. The same technique was used to extract milkfish and mackerel, where the hydroxyproline content of the milk extract was 297 mg/100mL, which was converted to a collagen content of 2,376 mg/100mL, whereas the mackerel extract was below the detection limit and was not detected.

Table 1. Branched amino acid and taurine contents of perch essence.

Amino acid	PE
	Concentration (mg/100g)
BCAA (Valine + Leucine + Isoleucine)	663 ± 30
Taurine	69 ± 4
Hydroxyproline	599 ± 35
Collagen*	4792 ± 284

*Collagen content can be estimated by multiplying the total hydroxyproline content in each sample by a factor of 8.

human study: The participants in this study had no previously diagnosed medical illness, except for one subject with mild hypertension. Their blood pressure, body composition and muscle grip strength were analyzed before and after a one-month course of nutraceutical therapy designed for this study. This study includes a case series of eleven participants (mean age 41.4 ± 3.3 years; male: female = 3:8) who received one oral PE treatment per day. The questionnaire revealed that 64% of the subjects had an exercise habit.

Table 2 shows the changes in various clinical data before and after one month of continuous daily supplement of PE. Our results showed there was a significant increase in muscle mass in the whole body and all parts of the body, with an increase of 1.55 % in the whole body, 1.79% in the trunk, 1.64% in the arms and 1.38% in the legs. There was also a significant decrease in the percentage of subcutaneous fat in the trunk, arms and legs by 2.49%, 3.21% and 3.40% respectively. Figure 1 shows the results in heart rate, systolic and diastolic blood pressure before and after PE consumption. Although PE contains abundant active peptides, it has been found to have an antihypertensive

effect in our previous *in vitro* studies [14], PE treatment is not expected to have a hypotensive effect in human clinical trials. However, it is also noteworthy that in one subject with moderate hypertension, one month of PE treatment reduced systolic blood pressure by 18 mmHg and diastolic blood pressure by 11 mmHg.

Although there was no statistically significant reduction in subcutaneous fat in the whole body, Figure 2 shows that there was a statistically significant reduction in subcutaneous fat of subjects with an exercise habit after one month of PE intake, especially in the legs and arms. Figure 3 shows the changes in skeletal muscle percentage in different parts of the body (whole body, trunk, arms and legs) before and after PE treatment. The results in Figure 3 show that PE is effective in increasing skeletal muscle mass in all parts of the body. The grip strength of both the right and left hand improved significantly after one month of taking PE, as shown in Figure 4. The results showed that the grip strength of the right hand was significantly higher, as the preferred hand of these subjects was the right hand.

Table 2. Clinical data of subjects before and after PE supplementation.

Variables	Pre-treatment (n=11)	Post-treatment (n=11)	p-Value
Body weight (kg)	67.36 ± 16.42	67.33 ± 17.16	>.999
Body fat percentage (%)	32.12 ± 5.63	31.16 ± 5.71	>.999
Visceral fat index	8.00 ± 5.51	7.86 ± 5.70	>.999
Basal metabolism (kcal)	1432.64 ± 301.74	1418.18 ± 300.42	.973
BMI (kg/m ²)	25.29 ± 4.96	25.25 ± 5.19	>.999
Body age (Years)	48.64 ± 13.57	48.27 ± 14.05	>.999
Heart rate (bpm)	79.82 ± 10.57	77.45 ± 9.15	>.999
Blood oxygen level (%)	96.09 ± 1.38	96.45 ± 1.63	>.999
Systolic blood pressure (mmHg)	124.45 ± 23.12	125.45 ± 17.04	.997
Diastolic blood pressure (mmHg)	82.36 ± 16.92	83.36 ± 15.71	.898
Stress level	41.18 ± 11.89	38.55 ± 15.01	.996
Subcutaneous fat (%)			
Whole body	26.41 ± 6.43	25.86 ± 6.47	.130
Trunk	23.27 ± 5.65	22.69 ± 5.68	.039
Arms	40.77 ± 11.01	39.46 ± 10.52	<.001
Legs	36.75 ± 9.29	35.50 ± 8.90	<.001
Skeletal muscle (%)			
Whole body	25.89 ± 3.89	26.29 ± 3.85	.009
Trunk	19.60 ± 3.49	19.95 ± 3.67	.047
Arms	28.08 ± 6.12	28.54 ± 5.90	.007
Legs	39.87 ± 5.92	40.42 ± 5.86	<.001
Left grip strength (kg)	27.59 ± 9.42	27.74 ± 10.13	.364
Right grip strength (kg)	29.02 ± 10.97	29.92 ± 11.12	.048

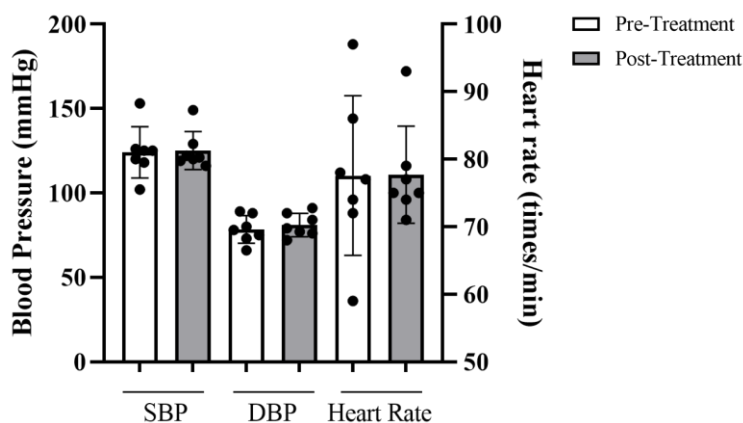


Figure 1. Change in blood pressure and heart rate after PE supplementation. SBP, Systolic blood pressure (mmHg); DBP, Diastolic blood pressure (mmHg).

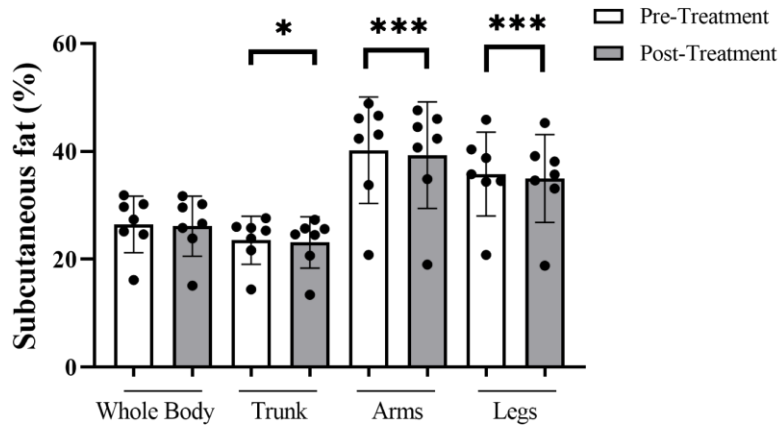


Figure 2. Change in subcutaneous fat percentage of subjects with exercise habit after PE supplementation.
 * Indicates a significant difference at the level of $p < 0.05$, *** indicates a significant difference at the level of $p < 0.001$.

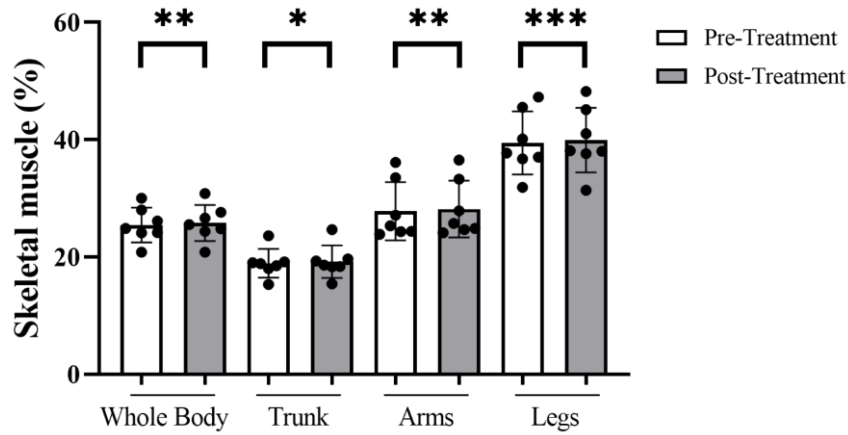


Figure 3. Comparison of skeletal muscle percentage of subjects with exercise habit after PE supplementation.
 * Indicates a significant difference at the level of $p < 0.05$, ** indicates a significant difference at the level of $p < 0.01$,
 *** indicates a significant difference at the level of $p < 0.001$.

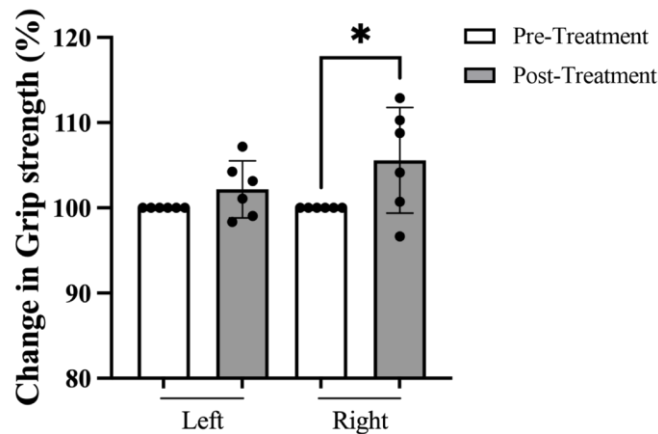


Figure 4. Changes in Grip Strength of subjects with exercise after PE supplementation.
 *Indicates a significant difference at the level of $p < 0.05$

Skeletal muscle cell (C2C12) model of muscle atrophy:

To further understand the role of PE in improving skeletal muscle mass, we developed a model of skeletal muscle cell damage by DEX. Figure 5 results demonstrates the impact of different treatment approaches on the DEX-induced muscle cytotoxicity. The results indicate that the sole administration of PE did not exhibit significant cytotoxicity (Figure 5A).

However, pre-treatment or post-treatment with PE effectively reduced muscle cell apoptosis induced by DEX (Figure 5B&C). Overall, these cell experiments suggest that PE, regardless of whether administered before or after DEX exposure, can effectively ameliorate DEX-induced muscle atrophy. This finding is significant for further research on improving muscle dystrophy symptoms and prognosis in COVID-19 patients.

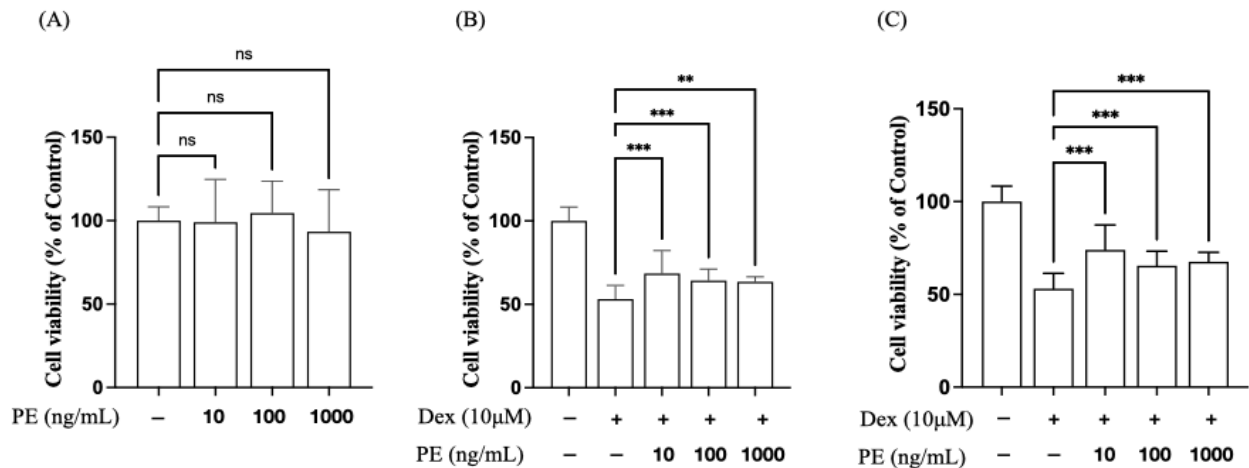


Figure 5. Effect of PE pre-treatment and post-treatment on DEX-Induced Muscle Cell Death.

The experiment involved three groups: A) PE alone, B) PE pre-treatment (preventive group), and C) PE post-treatment (therapeutic group). * indicates a significant difference at the level of $p < 0.05$. ** indicates a significant difference at the level of $p < 0.01$. *** indicates a significant difference at the level of $p < 0.001$. NS indicates not significant ($p > 0.05$).

DISCUSSION

As far as we know from fish protein studies, this is the first clinical intervention trial of concentrated perch extract, not meal, showing that fish proteins improve human skeletal muscle mass and grip strength in the absence of resistance exercise. In addition, it has been shown in cellular experiments that treatment with PE is effective in preventing the death of skeletal muscle cells induced by DEX. Daily protein meals (5 g protein/serving) containing Alaska pollack protein for 24 weeks increased skeletal muscle mass and lower-extremity muscle strength compared with whey protein consumption in healthy older women [21]. One month of PE intake in this clinical trial did not result in a significant reduction in blood pressure in accordance

with our prior study [14]. However, the results examined by a multiple paired t-test showed that PE supplementation improved subcutaneous fat, skeletal muscle mass and grip strength, particularly in subjects with exercise habits.

Compared with traditional meat products (beef, poultry, pork), fish protein contains higher levels of essential amino acids, including BCAA [11,22, 23]. Although it has been suggested that the BCAA content of perch flesh is not high [24], in our study we combined flesh, bones and scales to produce a concentrated perch extract with a high BCAA content using a high pressure, high temperature, short time extraction technique. Since BCAA are used as substrates for protein synthesis, they can stimulate the synthesis

of protein in skeletal muscle and suppress proteolysis [25-27]. Taurine, classified as conditionally essential amino acids, reverses muscle function under conditions of overuse in a number of ways, including the control of mitochondrial ROS production, the regulation of membrane potential and the induction of muscle regulatory proteins [28]. It is thought that taurine supplementation may help to reduce inflammation and improve muscle strength [29]. According to our previous studies, the amino acid composition of PE is mainly glycine, glutamic acid, alanine, and proline [14]. As key components of collagen, glycine, proline and hydroxyproline provide the sharp twist that makes the collagen helix possible. Studies indicate that endogenous synthesis of glycine, proline, and hydroxyproline is inadequate for protein synthesis, collagen production [30]. Dietary supplementation with proline and hydroxyproline enhances growth performance, collagen synthesis and muscle quality [31,32]. Moreover, collagen hydrolysates containing hydroxyproline have been shown to reduce inflammation and to promote the synthesis of collagen [33]. This study found that PE contains up to 599 mg/100g of hydroxyproline. Notably, its conversion to collagen is much higher than that of the various fish extracts we have previously studied. Through the contribution of BCAA, taurine, hydroxyproline and collagen, PE exerts effective in reducing subcutaneous fat, improving skeletal muscle mass and muscle grip strength in our human model, and preventing and treating DEX-induced cell death in C2C12 model.

Of great importance, older adults with a risk of sarcopenia have an 'anabolic resistance' that can be overcome by high quality protein intake [34-36]. In addition, dietary protein has a satiating effect [37,38], which affects older adults with low or disturbed appetite. Therefore, effective protein interventions are required to address nutritional deficiencies [39], and

fish proteins may be one reliable strategy to meet this urgent need. There have been reports that fish protein may have a greater satiating effect than other terrestrial meat protein sources. In light of this, PE via our new extraction method is prone to be more easily absorbed by frail humans with low appetite. Our study indicates that BCAA, taurine, hydroxyproline and collagen in PE have the potential to serve as a good source of dietary supplements to improve skeletal muscle mass and strength. Evidence from our *in vitro* and human model demonstrates that the effect of PE as a novel nutraceutical approach to prevent sarcopenia in the elderly could be further investigated in the large-scale clinical placebo-controlled trials.

Abbreviations: PE- perch essence; RDA-recommended daily allowance; Akt/mTOR- protein kinase B/mammalian target of rapamycin; BCAA- branched chain amino acids; RO- reverse osmosis; TBARS- 2-thiobarbituric acid reacting substances; HPLC- High Performance Liquid Chromatography; IRB - Institutional Review Board; BMI- body mass index; SBP- systolic blood pressure; DBP-diastolic blood pressure; DMEM- Dulbecco's Modified Eagle Medium; DEX-dexamethasone; ROS- reactive oxygen species.

Authors Contribution: All authors contributed to the study's conception and design. CY Hsieh, LN Chen, MH Lee and YH Ting, carried out the investigations and analyzed the outcomes. CC Lin wrote the manuscript. CC Lin, TM Wang and JF Chang directed the experiments, corrected, and edited the manuscript. All authors revised and accepted the final version of the manuscript.

Competing Interests: The authors declare no conflict of interest.

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