Journal of Functional Foods 46 (2018) 250–255

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Short communications

Acute effect of fish protein hydrolysate supplementation on vascular function in healthy individuals

Thiago Silveira Alvares[a,b,c,*], Carlos Adam Conte-Junior[d], Anna Paola Pierucci[b], Gustavo Vieira de Oliveira[a,c], Elisaldo Mendes Cordeiro[a,c]

[a] Nutrition and Exercise Metabolism Research Group, Nutrition Institute, Federal University of Rio de Janeiro, Macaé, RJ, Brazil
[b] Postgraduate Program in Nutrition, Nutrition Institute, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[c] Postgraduate Program in Bioactive Products and Biosciences, Federal University of Rio de Janeiro, Macaé, RJ, Brazil
[d] Department of Food Technology, Federal Fluminense University, Niterói, RJ, Brazil

ARTICLE INFO

Keywords:
Fish protein
Functional food
Flow-mediated dilatation
Near-infrared spectroscopy
Vascular healthy

ABSTRACT

Fish protein hydrolysates (FPH) have demonstrated *in vitro* anti-hypertensive and antioxidant properties. Since hypertension and oxidative stress are key factors for endothelial dysfunction, the main purpose of the present study was to investigate the effect of FPH on vascular function. Participants consumed 5 g of FPH or a placebo (PLA) supplement. Flow-mediated dilation (FMD) and tissue O_2 saturation (StO_2) were taken at different time points over 130 min after nutritional intervention. The FPH had 80.6 ± 1.0% of protein and the major amino acid present in the FPH was glutamic acid, followed by aspartic acid and histidine. There was no significant change in FMD as well as no significant change in the StO_2 parameters between FPH and PLA supplementation. Although FPH has been shown to be a valuable source of protein, essential amino acids and antioxidants, a single dose did not promote significant changes on vascular function in healthy individuals.

1. Introduction

The development of fish protein hydrolysate (FPH) as a functional food is a relatively recent technology gaining in popularity due to the array of potential bioactive properties associated with it, including antioxidant and antihypertensive properties (Halim, Yusof & Sarbon, 2016). The beneficial role of fish consumption as a means of lowering cardiovascular diseases has been studied extensively, and observational studies have shown that fish intake is linked to reduced coronary heart disease (Goede, Geleijnse, Boer, Kromhout, & Verschuren, 2010; He et al., 2004). The key underlying pathology in cardiovascular disorders is atherosclerosis, which originates from impaired functioning of the endothelium (Gimbrone & García-Cardeña, 2016). Thus, the endothelium is a key factor for maintaining vascular health and preventing cardiovascular diseases.

Parolini et al. (2014) demonstrated that the salmon protein hydrolysate reduced atherosclerosis in apo E (-/-) mice and attenuated risk factors related to atherosclerotic disorders by acting both at vascular and systemic levels. Furthermore, studies on fish-derived peptides have shown them to be antihypertensive (Kim, Ngo, & Vo, 2012), antioxidant (García-Moreno et al., 2013), and effective in reducing plasma cholesterol and triacylglycerol levels (Nesse et al., 2014), all of which positively affect the vascular endothelium. These *in vitro* and animal studies clearly suggest the potential use of FPH to improve human vascular health.

There has been evidence demonstrating the viability of developing a food product from fish waste with potential benefit for human health (Kim et al., 2012). One study demonstrated that FPH is a suitable source of proteins for malnourished children because of the presence of di/tri peptides and balanced essential and non-essential amino acid composition (Nesse et al., 2014). Vikøren, Nygård, Lied, Rostrup, and Gudbrandsen (2013), observed beneficial effects on blood levels of glucose and LDL-cholesterol as well as glucose tolerance and body composition after FPH supplementation in overweight adults. However, none of these studies evaluated the effect of FPH on vascular function.

During the process of filleting fish, waste that is potentially nutritive is produced, creating a source of water pollution that causes serious environmental problems. In the present study, a FPH supplement was

Abbreviations: BBD, baseline brachial artery diameter; EAA, essential amino acids; FMD, , flow-mediated dilatation; FPH, fish protein hydrolysate; NIRS, near-infrared spectroscopy; PBD, peak brachial artery diameter; PLA, placebo; StO_2, tissue O_2 saturation; $StO_{2\ base}$, baseline tissue O_2 saturation; $StO_{2\ slope\_1}$, tissue O_2 desaturation rate; $StO_{2\ min}$, minimum tissue O_2 saturation; $StO_{2\ slope\_2}$, tissue O_2 resaturation rate; $StO_{2\ max}$, maximal tissue O_2 saturation; $StO_{2\ tmax}$, time to maximal tissue O_2 saturation; TAC, total antioxidant capacity; TEAC, trolox equivalent antioxidant capacity; VOT, vascular occlusion test

* Corresponding author at: Nutrition Institute, Federal University of Rio de Janeiro, Estrada do Imburo s/n, 27979-000 Macaé, Rio de Janeiro, Brazil.
E-mail address: alvares@macae.ufrj.br (T.S. Alvares).

https://doi.org/10.1016/j.jff.2018.04.066
Received 6 December 2017; Received in revised form 9 April 2018; Accepted 30 April 2018
1756-4646/

developed by using fish waste. The proximate composition, total antioxidant capacity, amino acids and biogenic amines composition of the FPH were determined, and the effect of a single oral dose of the FPH on vascular function (macro and microvascular function) in humans was investigated. Since many *in vitro* studies have demonstrated antihypertensive (Kim et al., 2012) and antioxidant properties (García-Moreno et al., 2013) of FPH our hypothesis was that FPH supplementation could positively affect the vascular function in humans.

2. Material and methods

2.1. Participants

Nine healthy adults (6 males) were recruited to participate in this study. An *a priori* power analysis for an F test (repeated measures, within-between interaction for four time points) by using G*Power software (version 3.1.9.2). On the basis of a statistical power (1 – β) of 0.80, a moderately large effect size (0.5), and an overall level of significance of 0.05, at least 8 participants were needed to detect a statistical difference. Exclusion criteria for participants included the presence of chronic diseases as determined by medical history questionnaire, hypertension, smoking, and use of vasoactive, antioxidant and/or caffeine supplements. All participants were fully informed of the nature and purpose of the investigation and provided written consent to participate. All experimental procedures were performed in accordance with the ethical standards of the Declaration of Helsinki and approved by the institutional ethics committee.

2.2. Experimental design

The study was conducted in a randomized, double-blind, crossover and placebo-controlled way. All subjects reported to the laboratory on three occasions, with at least 1-week interval between visits. The first visit was used to explain the experimental procedures and collect baseline data (Pre) of macrovascular and microvascular function parameters. In the second and third visits, the participants were randomly divided into either a fish protein hydrolysate (FPH) or placebo (PLA) supplementation and macrovascular and microvascular function were taken at different time points over 130 min after nutritional intervention (Fig. 1). The three visits were held between 07:00 and 11:00 a.m. The participants were instructed to fast for at least 8 h and restrict physical exercise and caffeine consumption before each visit. Women were advised not to report to the laboratory during the menstrual period in order to eliminate potential variations in endothelial function related to the menstrual cycle.

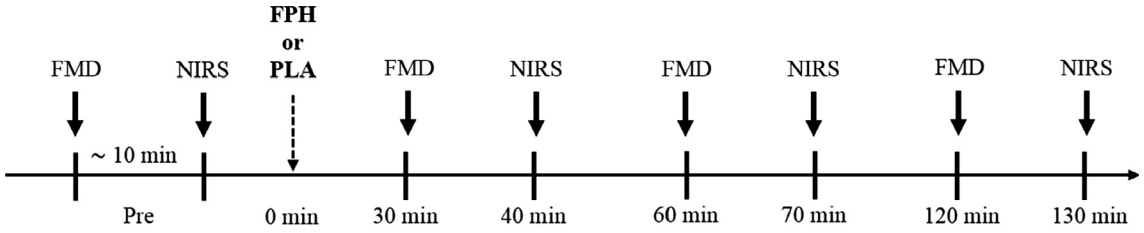
2.3. Nutritional intervention

In a double-blind and randomized manner, all participants received oral doses of either 5 g of the FPH or sucralose (as placebo - PLA). FPH was dissolved in 100 mL of water and immediately administered. PLA was offered in 6 white plastic capsules, which were administered with 100 mL of water. In order to avoid that the subjects easily detect FPH condition, all subjects were informed that in the PLA capsules also contained FPH and that the purpose of the study was to compare the effects of two different administration forms of FPH on vascular function. The FPH was prepared in our laboratory in the following manner: Fresh fish (Nile tilapia - *Oreochromis niloticus*) was filleted and the leftover processing by-products, including the frame, dark muscle, skin, small bones and fins, were used for protein hydrolysis. The fish waste was stored in a polyethylene bag at −20 °C until used for FPH production. The samples were partly thawed overnight at room temperature and blended for 2–3 min. The fish mince was dried in stove at 180 °C for one hour. The fish flour was mixed with distilled water (1:2) and adjusted to pH 7.5 with 1 M potassium hydroxide. The hydrolysis process was done in a water bath (Dubnoff orbital – NT 230, Novatecnica, Brazil) set up at 55 °C. The enzymatic hydrolysis was started by adding 2% of 2.4 L Alcalase enzyme (Novozymes, Novo Alle, DK-2880 Bagsvaerd, Denmark). After 4 h of hydrolysis, the enzyme was inactivated by heating at 90 °C for 15 min in a water bath. The mixture was then centrifuged at 8000*g* for 10 min and the supernatant was collected and filtered in 0.45 μm cellulose filter. The FPH was passed into a spray-drier (mini spray dryer B-290 advanced, Büchi Labortechnik AG, Switzerland) and the resulting powder was used for supplementation. Before giving FPH and PLA to participants, FPH was analyzed for proximate composition according to AOAC method (AOAC, 2012); total antioxidant capacity (TAC) by using the Trolox equivalent antioxidant capacity (TEAC) assay, as described by Arts, Haenen, Voss, and Bast (2004) and Deng et al. (2013); and total amino acids and biogenic amines content, respectively, according to Gatti, Gioia, Leoni, and Andreani (2010) and Bottino, Rodrigues, Ribeiro, Lazaro, and Conte-Junior (2017), by using a high-performance liquid chromatography system. Biogenic amines were evaluated in order to avoid scombrotoxin fish poisoning (Rodrigues et al., 2016).

2.4. Vascular function measurements

2.4.1. Macrovascular function

Arterial vascular responsiveness was determined as the vascular response to reactive hyperemia by measuring the flow-mediated dilation (FMD) in the brachial artery (Oliveira, Morgado, Pierucci, & Alvares, 2016; Thijssen et al., 2011). The measurement was performed by using an ultrasound (Prosound α6; Aloka Co., Tokyo, Japan) with a high-resolution linear array transducer (13 MHz) coupled with computer-assisted analysis software (e-TRACKING system, Aloka Co., Tokyo, Japan) that used an automated edge detection system for measurement of artery diameter and synchronized by 3-lead electrocardiography to the R-wave of the QRS complex. The measurement was



FPH – Fish protein hydrolysate supplementation

PLA – Placebo (sucralose) supplementation

FMD – Macrovascular function measurement

NIRS – Microvascular function measurement

Fig. 1. Experimental design of the study.