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Effect of dietary fish oil on mouse testosterone level and the distribution of eicosapentaenoic acid-containing phosphatidylcholine in testicular interstitium

Nobuhiro Zaima [a,*], Saori Kinoshita [a], Nao Hieda [a], Hirona Kugo [a], Kaori Narisawa [a], Ayami Yamamoto [a], Kenichi Yanagimoto [b], Tatsuya Moriyama [a]

[a] Department of Applied Biological Chemistry, Graduate School of Agricultural Science, Kindai University, 204-3327 Nakamachi, Nara City, Nara 631-8505, Japan

[b] Human Life Science R&D Center, Nippon Suisan Kaisha, Ltd., Tokyo, Japan

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ABSTRACT

Low levels of serum testosterone are characteristically associated with diabetes, coronary atherosclerosis, obstructive sleep apnea, rheumatoid arthritis, and chronic obstructive pulmonary disease. Testosterone replacement therapy is effective against many of these disorders, indicating the importance of maintaining a healthy testosterone level. In this study, we investigated the effects of fish oil on murine testosterone metabolism and analyzed the dynamics of relevant lipids in testes by matrix-assisted laser desorption ionization mass spectrometry imaging. Testosterone was upregulated in mice that received fish oil. In the testicular interstitium, eicosapentaenoic acid-containing phosphatidylcholine was distributed characteristically. These data suggest that eicosapentaenoic acid is involved in testosterone metabolism.

1. Introduction

Testosterone is a steroid hormone secreted mainly by Leydig cells in the testicular interstitium of males. It plays an important role in the maintenance of male reproductive tissues (such as testes and prostate), spermatogenesis, and in the promotion of secondary sexual characteristics such as increase in muscle mass [1]. Although testosterone functions in biological regulation, it is also associated with several disorders. The onset and development of several diseases is prevalent in male populations with low testosterone levels. Men with low levels of serum testosterone are at twice the risk of developing diabetes compared to men with normal testosterone level [2]. Phillips et al. reported that low serum testosterone can be a risk factor in developing coronary atherosclerosis [3]. Low serum testosterone levels can also be seen in patients with obstructive sleep apnea, rheumatoid arthritis, and chronic obstructive pulmonary disease [4]. Testosterone replacement therapy is an acknowledged treatment approach for many of these disorders; thus, maintaining healthy testosterone levels can prevent disease onset or disease progression, in some instances [4].

Dietary habits can modulate testosterone metabolism. In humans, daily urinary excretion of testosterone is 13% higher with a high-fat, low-fiber diet than that with a low-fat, high-fiber diet [5]. An earlier study showed that dietary fish oil influences testosterone synthesis and alters fatty acid composition in rat testicular plasma membranes [6]. These reports collectively suggest that fatty acid composition of food affects testosterone metabolism. Nonetheless, it is unclear whether specific fatty acids get incorporated into the testicular interstitium or the fatty acids in the diet directly influence the fatty acid composition of testicular interstitium plasma membranes. The challenge in understanding these aspects is partly attributed to the difficulties in analyzing spatial distribution of specific lipids in tissues. Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) is a suitable method to analyze the characteristic lipid distribution in different tissues including the testes [7–12]. In this study, we investigated the effect of fish oil on murine testosterone metabolism and subsequently analyzed the dynamics of relevant lipids in mouse testes by MALDI-MSI. The results of this study provide some insights into the relationship between testosterone metabolism and lipid nutrition.

* Corresponding author.
E-mail address: zaima@nara.kindai.ac.jp (N. Zaima).

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2. Materials and methods

2.1. Materials

The MALDI matrix 2,5-dihydroxybenzoic acid was procured from Bruker Daltonics (Bremen, Germany). Glass slides (Fisherbrand Superfrost Plus) were purchased from Thermo Fisher Scientific (MA, USA) for an LTQ-XL linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Indium-tin oxide-coated glass slides for a time-of-flight (TOF)/TOF-type instrument (Autoflex) were procured from Bruker Daltonics. Paraformaldehyde was purchased from Nacalai Tesque (Kyoto, Japan). Carboxymethylcellulose sodium salt (low viscosity) was purchased from Sigma-Aldrich Co. (MO, USA). All other reagents used in this study were of the ultrapure grade available.

2.2. Animal experiments

All animal experiments were approved by the Institutional Animal Care and Use Committee and were conducted according to the Kindai University Animal Experimentation Regulations (approval number KAAG-25-002). Mice of the ddY strain were provided with food and water *ad libitum* in a humidity-controlled room, in a 12-hour light/12-hour dark cycle. The room temperature was maintained at 25 °C ± 1 °C. Nutritional supplements were administered via two routes: dietary and gavage. In the dietary administration groups, mice (male, 8 weeks old; Japan SLC, Inc., Hamamatsu, Japan) were randomly subdivided into two groups—control group and dietary fish oil group—and provided with food for 10 weeks (n=5). The mice in the dietary fish oil group were provided food supplemented with fish oil consisting of purified triglycerides extracted from sardines (please mention the concentration; Nippon Suisan Kaisha, Ltd., Tokyo, Japan) during this period. Dietary components and fatty-acid composition are shown in Tables 1 and 2. In gavage groups, mice (male, 15 weeks old; Japan SLC, Inc.) were provided with food (same as the control group in dietary group) for 4 weeks, and subsequently subdivided into two groups—control group and fish oil gavage administration group (n=5). Carboxymethylcellulose was orally administered to the control group for 7 d. Fish oil (Nippon Suisan Kaisha) was orally administered once a day (2285 mg/[kg d]) to the gavage group for 7 d. Body weight and food intake were measured every 3 d.

Table 1
Dietary components.

	Control group (g/100 g)	Fish oil group (g/100 g)
Choline chloride	0.2	0.2
Cystine	0.3	0.3
AIN-93 vitamin mix	1	1
AIN-93G mineral mix	3.5	3.5
Cellulose	5	5
Sucrose	10	10
Casein	22.2	22.2
Cornstarch	27.8	27.8
Lard	25	25
Olive oil	5	0
Fish oil	0	5

Table 2
Fatty acid composition.

Fatty acid	Olive oil (%)	Fish oil (%)
8:0	–	–
10:0	–	–
12:0	–	–
14:0	–	5.2
16:0	10.8	6.6
16:1	1.0	9.3
16:2	–	1.7
16:3	–	2.8
16:4	–	4.8
18:0	2.7	0.5
18:1	77.1	9.6
18:2 n-6	7.8	1.4
18:3 n-3	0.6	0.9
18:4 n-3	–	5.1
20:4 n-6	–	1.3
20:4 n-3	–	1.1
20:5 n-3	–	30.8
22:5 n-6	–	0.3
22:5 n-3	–	2.9
22:6 n-3	–	15.7

2.3. Tissue collection and preparation of tissue section

Blood samples were obtained from the inferior vena cava of mice, under anesthesia. The mice were then perfused through the left cardiac ventricle with an isotonic sodium chloride solution. The collected testes were frozen on a plate cooled with liquid nitrogen without any chemical fixation. Consecutive 10-μm sections were prepared using a cryostat (CRYOCUT CM 1850; Leica Microsystems, Wetzlar, Germany). Successive slices were mounted onto glass slides, and were used for immunohistochemical staining and analyses by means of an LTQ-XL linear ion trap mass spectrometer. Indium-tin oxide-coated glass slides were used for TOF analyses.

2.4. Immunohistochemical staining

The slices were fixed with 4% paraformaldehyde for 10 min. After rinsing in phosphate-buffered saline (PBS), endogenous horseradish peroxidase in the tissue slices was blocked using aqueous hydrogen peroxide (a 3% solution in methanol) for 8 min. After rinsing in PBS, the tissue slices were blocked with Blocking One Histo (Nacalai Tesque). The slices were incubated overnight with an antibody against testosterone (1:50 dilution; Cloud-Clone Corp., TX, USA), at 4 °C. On the following day, the slices were rinsed in PBS and incubated with a secondary antibody conjugated with horseradish peroxidase. The slides were developed with diaminobenzidine (Vector Laboratories, Burlingame, CA, USA). Quantitative analysis of the histological staining was performed using the ImageJ software (National Institutes of Health, MD, USA).

2.5. MSI

A 50 mg/mL solution of 2,5-dihydroxybenzoic acid in a methanol: water mixture (7:3, v/v) served as a matrix. The matrix solution was sprayed uniformly over the slices by means of an airbrush with a 0.2 mm caliber nozzle (Procon Boy FWA Platinum; Mr. Hobby, Tokyo, Japan). MSI was performed using a MALDI TOF/TOF-type instrument (Autoflex) and the LTQ-XL linear ion trap mass spectrometer. Autoflex was used for imaging based on the MS data, and LTQ-XL was mainly used for imaging based on tandem mass spectrometry (MS/MS) data. This was done because Autoflex is suitable for simultaneous analysis of multiple samples, whereas LTQ-XL can perform simultaneous imaging on the basis of MS and MS/MS data. The data were acquired with a step size of 50 μm in both analyses. The FlexImaging software 4.0 (Bruker Daltonics) and ImageQuest software (Thermo Fisher Scientific) were used to create two-dimensional ion density maps. Normalization of the spectra to the total ion current was also performed in the imaging software. The distribution of the MS/MS product ions was visualized without normalization.