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Improvement of bone formation biomarkers after 1-year consumption with milk fortified with eicosapentaenoic acid, docosahexaenoic acid, oleic acid, and selected vitamins

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Abstract

The hypothesis of this study was that the replacement of regular milk with fortified milk in hyperlipidemic adults for 1 year would improve bone biomarkers. The fortified milk contained eicosapentaenoic acid and docosahexaenoic acid from fish oils, oleic acid, vitamins A, B_6, and E, as well as folic acid. We believe that the fortified milk will improve the blood fatty acid profile and vitamin status in subjects to benefit bone health biomarkers. From the 84 patients who accepted to participate, 11 of these were excluded for the presence of metabolic diseases and 1 was excluded for noncompliance with the protocol. Seventy-two hyperlipidemic patients (35-65 years) were randomly divided between 2 study groups. The supplement group (E; n = 39) consumed 0.5 L/d of fortified milk that contained fish oil, oleic acid, and vitamins. The control group (C; n = 33) consumed 0.5 L/d of semiskimmed milk containing the same amount of total fat. Blood samples were taken at T_0, T_3, T_6, and T_{12} months to determine plasma fatty acids, vitamins B_6, E, and 25-hydroxyvitamin D and serum folate, calcium, soluble osteoprotegerin (OPG), soluble receptor activator of NF-κB ligand (RANKL), osteocalcin, parathormone, type I collagen carboxy-terminal telopeptide, and malondialdehyde. After 1 year, the E group showed a significant increase in plasma eicosapentaenoic acid (42%), docosahexaenoic acid (60%), vitamin B6 (38%), OPG (18%), RANKL (7%), OPG/RANKL (10%), red blood cell folate (21%), serum folate (53%), calcium (4%), vitamin D (11%), and osteocalcin (22%). Dietary supplementation with the fortified milk drink improved nutritional status and bone formation markers in adult hyperlipidemic patients.

Keywords: Bone; Polyunsaturated fatty acids; EPA; DHA; Fortified milk; Oleic acid; Vitamins; Human

Abbreviations: C, control group; BMI, Body mass index; CTX, type I collagen carboxy-terminal telopeptide; E, fortified milk group; HPLC, high-performance liquid chromatography; iPTH, intact parathormone; n-3 PUFA, n-3 polyunsaturated fatty acids; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of NF-κB ligand; RBC, red blood cell.

1. Introduction

There is a wealth of evidence from epidemiologic and clinical studies that diet and nutrition play important roles in the prevention of chronic diseases, including cardiovascular

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disease and osteoporosis [1]. Osteoporosis, which affects many millions of people around the world, is characterized by bone fragility that can lead to bone fracture. An adequate intake of calcium and vitamin D has proven to be an important factor in the prevention of osteoporosis [2], and other dietary nutrients may also exert a positive influence [3]. The consumption of long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may influence bone growth and remodeling in humans [4]. Thus, studies have reported that DHA and EPA intake may have beneficial effects on bone, inhibiting bone resorption and promoting bone formation [4,5]. The precise mechanism for which the n-3 PUFA can have an effect on bone remodeling is unknown, but one hypothesis is that EPA and DHA may have a beneficial effect by modulating the osteoprotegerin (OPG)/receptor activator of nuclear factor κB (RANK) balance toward bone formation [6,7].

In bone remodeling, key roles are played by the activity of osteoblasts (involved in bone formation) and osteoclasts (involved in bone resorption) and by the balance between OPG and nuclear factor κB (RANK) signaling [8]. OPG, a soluble cytokine produced by osteoblasts acts by competing with RANK, a surface molecule of osteoclasts that binds to receptor activator of NF-κB ligand (RANKL), which is present on osteoblasts and involved in bone remodeling via an osteoclast activation mechanism [9]. It has been shown that inflammatory signals can modulate the OPG/RANKL system by decreasing OPG and increasing RANK levels, thereby producing bone resorption. Therefore, EPA and DHA may benefit bone due to their well-known anti-inflammatory actions.

Dairy products are an excellent source of bioavailable calcium and vitamin D. An oil blend containing EPA and DHA from fish oils, olive oil, vitamins B6 and E, and folic acid was produced and included in skimmed milk to create a milk drink with one third of the saturated fatty acids contained in semiskimmed milk but with the same amount of total fat but the presence of EPA and DHA. The hypothesis for this study is that the replacement of regular milk with fortified milk for 1 year will improve bone status in hyperlipidemic adults. The fortified milk, containing EPA and DHA from fish oils, oleic acid, vitamins A, B_6, and E, and folic acid, may improve the fatty acid profile in blood, vitamin status, and bone biomarkers.

2. Methods and materials

2.1. Experimental design

The present study was part of a larger study designed to evaluate the effects of dietary fatty acids and vitamins on cardiovascular disease risk factors, blood fatty acids, and nutritional status in hyperlipidemic adults. From that study, 84 volunteers accepted to participate in this subproject. The increased amount of blood needed for this study was the limiting factor for not all the patients of the main study could participate in this one. This longitudinal, controlled, randomized, and double-blinded study of a 12-month nutritional intervention was performed in hyperlipidemic adults referred to the Department of Endocrinology of the San Cecilio University Hospital in Granada (Spain). Inclusion criteria were age between 30 and 65 years and blood triacylglycerols more than 150 mg/dL and/or low-density lipoprotein cholesterol between 130 and 159 mg/dL. Exclusion criteria were presence of heart disease or endocrine or metabolic disorders (eg, hypothyroidism or diabetes) and receipt of medication, fish oil, or vitamin supplements, from 3 months before the study to the finish of the nutritional intervention.

From the 84 patients who accepted to participate, 11 of these were excluded for the presence of metabolic diseases and 1 was excluded for noncompliance with the protocol. The remaining 72 patients were randomly assigned to 1 of 2 intervention groups: 33 to the semiskimmed milk group (control group; C) and 39 to the fortified milk drink group (group E; Fig. 1). The fortified supplement group (E; n = 39) was administered with 500 mL/d of a milk product (Puleva Omega3; Puleva Food S.L., Granada, Spain) fortified with EPA, DHA (from fish oils), oleic acid, vitamins A, B_6, D, and E, and folic acid and having a comparable total fat comparable to that of standard semiskimmed milk (1.9 g/100 mL). The control group (C; n = 33) was supplied with 500 mL/d of regular semiskimmed milk with added vitamins A and D (Table 1).

The milk products were produced and packaged in white 500-mL carton containers by Puleva Biotech S.A. (Granada, Spain) so that patients and researchers were blinded to their content. Patients were instructed to consume the milk products in 2 × 250 mL doses at the beginning and at the end of the day and not to change their physical activities or usual diet. Compliance with the consumption protocol during the intervention period was monitored and ensured by regular telephone calls and collection of the empty containers. Dietary intake was assessed at baseline and at the end of the study by means of a self-administered 7-day food frequency questionnaire, using Nutriber Software to estimate the intake of nutrients.

2.2. Ethical statement

The study complied with the Helsinki Declaration (2004), EEC Good Clinical Practice recommendations (document 111/3976/88, July 1990), and current Spanish legislation regulating clinical research in humans (RD 561/1993 on clinical trials). The protocol was approved by the ethical committee of "San Cecilio" University Hospital (Granada, Spain), and all participants gave their written consent.

2.3. Blood sampling and clinical examination

Interviews with patients, including anamnesis and clinical examination, were carried out in the hospital at the beginning of the study (T_0) and after 3 (T_3), 6 (T_6), and 12 months (T_{12}).