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Dietary conjugated linoleic acid in the *cis*-9, *trans*-11 isoform reduces parathyroid hormone in male, but not female, rats

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Abstract

Previously, a mixture of conjugated linoleic acid (CLA) isoforms reduced parathyroid hormone (PTH) in male rats over 8 weeks. The objective herein was to determine which isoform caused the reduction in PTH; whether the effect was sex specific; and whether CLA-induced reductions in PTH were sustained. Male and female weanling rats (n=48) were randomized to a control diet or one made with 0.5% of the diet as *cis*-9, *trans*-11 (c9,t11) CLA, 0.5% of the diet as *trans*-10, *cis*-12 (t10,c12) CLA or these CLA in a mixture. Measurements made after 4, 8 and 16 weeks were body weight, bioactive PTH, ionized Ca, whole-body and regional bone mineral density (BMD) using dual-energy X-ray absorptiometry. With the use of a factorial design, a sex×c9,t11 CLA interaction was observed that reduced PTH (139.5±63.9 vs. 95.8±42.4 pg/ml, P=.02) in male rats only. No other effects of c9,t11 CLA were observed. Regarding t10,c12 CLA, no interaction effects were observed, but a main effect was observed to reduce lumbar spine BMD (0.265±0.044 vs. 0.255±0.044 g/cm^2, P<.01) along with reduced retention of Ca and P at Week 4. No other dietary effects were observed. In summary, the c9,t11 CLA isoform is responsible for reduced PTH and this effect is sex specific; this was true whether fed as a pure isomer or mixed with an equal amount of t10, c12 CLA. Whether such reductions in PTH might be observed in females lacking sex hormones such as ovariectomized rats and also in humans is required to expand health implications of dietary CLA.

Keywords: Conjugated linoleic acid; Parathyroid hormone; Rat; Bone mineral density

1. Introduction

To date a main focus of research related to dietary conjugated linoleic acid (CLA) has been on body composition, particularly lean and fat mass [1], and less so regarding bone mass and metabolism as the primary outcomes. There are a number of mechanisms by which CLA might affect bone. Dietary supplementation with CLA is reflected in tissue fatty acid composition and displaces linoleic acid and arachidonic acid [2]. One result is reduced synthesis of prostaglandin E_2 (PGE_2) that depends on release of arachidonic acid from the plasma membrane phospholipids [2]. Prostaglandins play major roles in cell signaling in organs such as bone [2] and the parathyroid gland [3]. The parathyroid gland has a major impact on the endocrine control of bone metabolism and mineralization. Recently, we conducted a study whereby feeding a CLA mixture (1% of the diet by weight) to male rats for 8 weeks did not alter bone formation and resorption, femur bone mineral density (BMD) or release of PGE_2 from femur, but suppressed parathyroid hormone (PTH) to 60% of control values in both normal and hyperparathyroid states [4]. The mixture of CLA used in the study was 19% *cis*-9, *trans*-11 (c9,t11) and 28% *trans*-10, *cis*-12 (t10,c12) CLA. Thus the results may have been due to combined effects of the two CLA isomers or specific to only one isomer. Additionally, the reduced PTH may or may not have been sustained and to date whether females experience a similar reduction in PTH following exposure to CLA has not been tested.

Other studies of dietary CLA have not yet included measurement of PTH and thus whether PTH is reduced in association with bone or other health outcomes is not clear.

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Nonetheless, it has long been accepted that continuously elevated PTH leads to bone loss whereas intermittent elevations lead to increases in bone mass [5]. PTH follows a circadian rhythm reaching the lowest nadir between 0930 and 1100 hours followed by a small peak in the afternoon, another smaller nadir at 2100 and the primary peak at 0314 hours [6,7]. These diurnal patterns do not seem to change with age in premenopausal women and men [8]. Reductions in PTH in the order of 30% to 40% in the rodent CLA study were measured at the morning nadir [4]. The reduced PTH might be related to the effects of CLA on calcium absorption since another study in rats reported higher net and fractional calcium absorption with CLA supplementation [9]. In women fed a high calcium diet (2414 vs. 815 mg/day) PTH was 40% lower [10]. In another study, 150 mg of calcium supplementation with each meal plus 450 mg prior to bed in healthy men also results in reduced PTH in the morning compared to placebo [11]. Enhanced calcium absorption plus lower PTH would support higher bone mass.

Higher dietary CLA is associated with higher BMD of the forearm in postmenopausal women [4]. In mice, feeding mixed CLA increases bone mass in young males [12] and reduces age-associated bone loss in females [13]. In osteoblast-like cells, the c9,t11 CLA isomer increases the number and size of mineralized bone nodules while the t10, c12 CLA isomer did not [14]. Based on these studies it appears that the dietary form of CLA, c9,t11, might be responsible for reduced PTH and enhanced bone mass.

The objectives of the proposed research in male and female rats fed CLA (c9,t11 and/or t10,c12 CLA) from 4 to 20 weeks of life were to (1) determine which isoform caused the reduction in PTH; (2) whether the effect was sex specific; and (3) whether CLA-induced reductions in PTH were sustained over longer periods of time. A secondary objective was to determine the physiological response to CLA by measuring other variables including bone mass, PGE_2, ionized serum Ca and Ca/P retention to help explain any reductions in PTH or alterations in bone.

2. Materials and methods

2.1. Study protocol and diets

This study was approved by the University of Manitoba Committee on Animal Care and conformed to Canadian Council on Animal Care guidelines [15]. Sprague-Dawley rats (24 male and 24 female) were randomized at 3 weeks of age to receive one of four diets between 4 and 20 weeks of age. Between Weeks 3 and 4 of life, animals were acclimatized to the housing conditions and fed the control diet. Animals were housed in same-sex pairs and food disappearance monitored three times weekly over the 16-week period. The diets all contained 84 g total fat/kg diet to ensure that essential fatty acids were not compromised at the expense of adding CLA. The diets were as follows: (1) control AIN-93 diets [16] made with soybean oil (n-6/n-3 ratio ×7:1); (2) control diet combined with 0.5% c9,t11 CLA; (3) control diet combined with 0.5% t10,c12 CLA and (4) control diet combined with 0.5% c9,t11 CLA+0.5% t10,c12 CLA in fatty acid form. These CLA mixtures were adjusted so that a single isomer represented 0.5% of the diet by weight. The CLA isomers were all in free fatty acid form and provided in kind from Lipid Nutrition, a division of Loders Croklaan (Channahon, IL, USA). The t10,c12 CLA product was 69.8% t10,c12 with total CLA at 83.1%; the c9,t11 product was 61.7% c9,t11 and total CLA at 73.2%; and the pre-mixed CLA was 74.5% c9,t11 and t10,c12 CLA with total CLA at 80.6% (Loders Croklaan Inc., certificate of analyses). Total Ca/kg diet was 5.1 g/kg and total P/kg diet was 46.08 g/kg based on mineral content of casein and mineral mix (Harland Teklad, certificate of analyses).

2.2. Study measurements: growth and bone

Weight was measured weekly for assessment of growth. At 4, 8 and 16 weeks after consuming the diets, rats were then anaesthetized using isofluorane gas for measurement of bone mass including whole body, lumbar spine, femur and tibia using a small animal program and dual-energy X-ray absorptiometry (DXA; 4500A Elite Series, Hologic, Bedford, MA, USA). Whole-body length from tip of nose to base of tail was also measured in the anesthetized state. The DXA measurements have been validated using identical hardware and software for rats 130 g in weight and higher for whole-body assessment [17] and in rodents as small as mice for high-resolution regional scans [18]. After each blood sampling and DXA, the rats continued on the feeding trial until 20 weeks of age.

2.3. Biomarkers of bone metabolism

At each of the three time points, a blood sample of no more than 10% blood volume was taken from the saphenous vein, between 0800 and 1000 hours to control for diurnal variations, and separated to obtain serum for determination of serum PTH, osteocalcin and C-telopeptide of Type 1 collagen (CTx). Both bioactive and intact PTH were measured using an ELISA (Alpco Diagnostics, Windham, NH, USA), osteocalcin using an ELISA (Osteometer, Nordic Bioscience, Herlev, Denmark), in addition to urinary CTx using an ELISA (Ratlaps, Osteometer, Nordic Bioscience). All of these protein assays are specific to rodents. Regarding serum ionized Ca, samples were measured within 4 h of collection using a Nova analyzer (Model 11, Nova Biomedical) and a CV <1.6 over the study period. In the last 5 days of each study phase, rats were housed in metabolic cages and mass balance studies conducted by measuring disappearance of food and excretion of nutrients over the last 3 days; the first 2 days are adaptation to the new housing. Minerals (Ca, P) were measured in the 72-h pooled samples of urine and feces following digestion in nitric acid and using inductively coupled plasma optical emission spectroscopy (Varian Liberty 200, Varian Canada).