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Orchidectomy-induced alterations in volumetric bone density, cortical porosity and strength of femur are attenuated by dietary conjugated linoleic acid in aged guinea pigs

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ABSTRACT

Age-related osteoporosis and sarcopenia are ascribed in part to reductions in anabolic hormones. Dietary conjugated linoleic acid (CLA) improves lean and bone mass, but its impact during androgen deficiency is not known. This study tested if CLA would attenuate the effects of orchidectomy (ORX)-induced losses of bone and lean tissue. Male guinea pigs (n = 40; 70–72 weeks), were randomized into four groups: 1) SHAM + Control diet, 2) SHAM + CLA diet, 3) ORX + Control diet, 4) ORX + CLA diet. Baseline blood sampling and dual-energy X-ray absorptiometry (DXA) scans were conducted, followed by surgery 4 days later with the test diets started 7 days after baseline sampling. Serial blood sampling and DXA scans were repeated 2, 4, 8 and 16 weeks on the test diets. Body composition and areal BMD (aBMD) of whole body, lumbar spine, femur and tibia were measured using DXA. At week 16, muscle protein fractional synthesis rate (FSR), volumetric BMD (vBMD), microarchitecture and bone strength were assessed. Body weight declined after SHAM and ORX surgery, with slower recovery in the ORX group. Dietary CLA did not affect weight or lean mass, but attenuated gains in fat mass. Lean mass was stable in SHAM and reduced in ORX by 2 weeks with whole body and femur bone mineral content (BMC) reduced by 4 weeks; CLA did not alter BMC. By week 16 ORX groups had lower free testosterone and myofibrillar FSR, yet higher cortisol, osteocalcin and ionized calcium with no alterations due to CLA. ORX + Control had higher prostaglandin E_2 (PGE_2) and total alkaline phosphatase compared to SHAM + Control whereas ORX + CLA were not different from SHAM groups. Femur metaphyseal vBMD was reduced in ORX + CTRL with the reduction attenuated by CLA. Femur cortical thickness (Ct.Th.) and biomechanical strength were reduced and cortical porosity (Ct.Po.) elevated by ORX and attenuated by CLA. This androgen deficient model with a sarcopenic-osteoporotic phenotype similar to aging men responded to dietary CLA with significant benefits to femur density and strength.

Introduction

Osteoporosis and sarcopenia are age-related musculoskeletal diseases that impair physical function, increase fracture risk and decrease quality of life [1]. In epidemiological studies the age-related increase in fractures seen in women is also prevalent in men, with 13–20% of men over age 50 years at risk of osteoporotic fracture [2]. Approximately one third of all hip fractures occur in men [3] accompanied by up to 10% higher rates of one-year mortality compared to women [4,5]. Similarly, at 65–70 years the prevalence of sarcopenia ranges from 13% to 24% and exceeds 50% for those older than 80 years [6]. It is estimated that in men over the age of 50 years, whole body muscle mass and BMC are lost at a rate of 1–2% per year [7,8]. Sarcopenia is associated with lower BMD [9] and an elevated risk of all types of fractures including hip fractures in men more so than women [10,11]. Studies with more than two serial measures of both lean and bone mass are lacking to establish if reductions in lean mass precede loss of BMD in men or simply coexist as manifestations of reduced testosterone with age. However, in ORX rats,

Abbreviations: aBMD, Areal bone mineral density; BMC, Bone mineral content; BV/TV, Bone volume/total volume; CLA, Conjugated linoleic acid; Ct.Ar, Cortical area; Ct.Ar./Tt.Ar, Cortical area/total area; Ct.Po, Cortical porosity; Ct.Th, Cortical thickness; L, Distance between fulcrum; c, Distance from neutral axis to outer perimeter; DPD, Deoxypyridinoline; DXA, Dual-energy X-ray absorptiometry; W_{break}, Energy at break; FSR, Fractional synthesis rate; IL-6, Interleukin-6; iCa, Ionized calcium; LS, Lumbar spine; d_{max}, Maximum extension at maximum flexure load; F_{max}, Maximum flexure load; μCT, Micro-computed tomography; ORX, Orchidectomy; PUFA, Polyunsaturated fatty acid; PGE_2, Prostaglandin E_2; RBC, Red blood cell; ε_{max}, Strain; σ_{max}, Stress; Tb.N, Trabecular number; Tb.Sp, Trabecular separation; Tb.Th, Trabecular thickness; Tt.Ar, Trabecular area; TRACP, Tartrate-resistant acid phosphatase; 25OHD, 25-Hydroxyvitamin D; vBMD, Volumetric bone mineral density; WB, Whole body; E, Young's modulus.

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reduced whole body lean mass is evident by 2 weeks whereas loss of BMC only becomes evident after 8 weeks [12]. The age-related declines in muscle and bone are ascribed to complex physiological interactions including reductions in testosterone, often a catabolic inflammatory state [7,13] and elevated fat mass in aging men [14].

Androgens are recognized in the maintenance of muscle as well as trabecular and cortical bone mass in men [15,16]. Aging associates with declines of up to 50% of free testosterone [17]. Moreover, testosterone inhibits the production of inflammatory cytokines such as IL-6 in bone marrow-derived stromal cells whereas elevated IL-6 as a result of ORX in mice results in osteoclastogenesis and loss of bone mass [18]. Hence, with age, a decrease in testosterone potentially has direct and indirect catabolic effects on the musculoskeletal system. To counter these consequences of aging, pharmaceuticals predominate, however, benefits from nutrients such as conjugated linoleic acid (CLA) are emerging. CLA elevates lean mass [19,20], BMD [21] and bone microarchitecture in young male [22] and female mice [21] and decreases IL-6 [21]. The possible mechanisms behind improved lean mass include elevated protein fractional synthesis rate (FSR) in gastrocnemius and soleus muscle of rats [23] and in humans (18–45 years), CLA reduces the catabolic effects of resistance training on muscle [24]. In bone, CLA suppresses osteoclastogenesis and enhances bone formation in estrogen deficient mice [25]. Whether CLA could blunt muscle and bone loss in aging males has not been addressed. Dietary CLA is comprised of the natural cis-9, trans-11 CLA isomer whereas supplemental CLA also contains the trans-10, cis-12 CLA isomer which has been associated with transient reductions in BMD [26]. Thus the objective of this study was to test the hypothesis that dietary cis-9, trans-11 CLA following orchidectomy (ORX)-induced androgen deficiency prevents reductions in muscle and bone. Aged guinea pigs were selected as an otherwise healthy model for aging bone [27] with natural age related declines in lean mass [28] and suitable for dietary lipid research [29].

Methods and materials

Study protocol

Male, retired breeder guinea pigs (n = 40; 70–72 weeks of age; pigmented strain, Elm Hill Laboratories, Boston, USA) were housed singly with room temperature of 21–22 °C, relative humidity of 50% and a lighting cycle from 0600 to 1800 h. After 1 week adaptation and 7 days (−1 week) prior to dietary intervention, guinea pigs were block randomized by weight into four groups: 1) SHAM + Control diet, 2) SHAM + CLA diet, 3) ORX + Control diet, 4) ORX + CLA diet and underwent baseline DXA measurements and blood sampling (Supplemental Fig. 1). Surgical interventions were then completed (−4 days) followed by 3 days recovery prior to the start of the diet intervention (0 week). Serial DXA measurements and blood sampling were performed at weeks 2, 4, 8 and 16 on the diet. At week 16, muscle protein FSR was estimated using a flooding dose of L-[ring-$^2H_5$]phenylalanine followed by anaesthesia (AErrane® isoflurane gas, Baxter Inc., Mississauga, ON, Canada) 50 min later for blood sampling and excision of left quadriceps that were snap frozen in liquid nitrogen, animals were then exsanguinated and organ weights were obtained. Ethical approval was obtained from the McGill University Animal Care Committee and all procedures were in accordance with the Canadian Council on Animal Care [30].

Experimental diets

Prior to randomization (−1 week), the guinea pigs were fed *ad libitum* a commercial high fiber diet (product 2041, Teklad Diets, Harlan, Madison, WI, USA) for 1 week. After recovery from surgery (0 week), the control diet (product 5SUS, TestDiet, Richmond, IN, USA) or the control diet with 1% as CLA by weight (product 5ALC, TestDiet, Richmond, IN, USA) were provided *ad libitum*. The isomeric composition of the CLA oil (Lipid Nutrition, Loders Croklaan, Channahon, IL, USA) was 4:1 cis-9, trans-11:trans-10, cis-12 with negligible quantities of other fatty acids. Both diets were in pellet form, isoenergetic (Supplemental Table 1), stored at 4 °C and met the National Research Council recommendations [31]. Diet and deionized water were renewed daily and consumption monitored daily for 14 days post surgery then every 3 days.

Surgical intervention

ORX was performed as previously described [32]. Briefly, guinea pigs were anesthetized and positioned in dorsal recumbency, shaved and an incision was made to expose the linea alba. Those in the sham groups had the incision closed with tissue adhesive and recovered whereas the ORX groups had their testes, epididymis and gubernaculum excised. All guinea pigs were monitored over 14 consecutive days for weight gain; none were excluded from the study.

Blood sampling and DXA measurements

Blood from the saphenous vein was drawn (0730 to 0930 h) into lithium heparin at −1, 2, 4, 8 and 16 weeks, prior to anesthesia [33]. After centrifugation (2200*g*, 15 min), plasma was removed and stored at −80 °C. Packed RBC were twice washed with saline then stored in an equal weight of water/methanol/butylated hydroxytoluene solution to prevent polyunsaturated fatty acid (PUFA) oxidation [34]. Bone and body composition including whole body lean mass, fat mass, BMC and aBMD were measured in whole body (WB), left (L) femur and tibia as well as in lumbar spine vertebrae 1 to 4 (LS 1–4) under isoflurane anesthesia using DXA (QDR v12.5, Discovery Series 4500A, Hologic Inc., Bedford, MA, USA).

Blood and tissue fatty acid profiling

CLA (including cis-9, trans-11 and trans-10, cis-12 CLA isomers) was measured in RBC samples using the Bondia-Pons method [35] modified by use of 200 μl of RBC solution combined with 10 μl of heptadecanoic acid (C17:0, internal standard) and the addition of 600 μl of hexane after methylation. CLA in quadriceps muscle and liver was assessed using the O'Fallon method [36]. Fatty acid methyl esters were separated using a 100 m CP-Sil-88 capillary column (Varian-Chrompack, CP7489) in a Varian CP-3800 Gas Chromatograph (Varian, Inc., Walnut Creek, CA, USA) with a flame-ionization detector. Fatty acid methyl ester peaks were identified using authentic standard 461 (cat# GLC-461, Nu-Chek Prep, Inc., Elysian, MN) and cis-9, trans-11 and trans-10, cis-12 CLA (cat# UC60M and UC61M, Nu-Chek Prep, Inc., Elysian, MN) used to calculate recovery (>90%). The fatty acids were expressed as a percentage of the total fatty acids identified from dodecanoic acid (C12:0) to docosahexaenoic acid (C22:6). Based on the weight of the liver and total fatty acid content, liver % fat by weight was calculated.

Blood biochemistry

Plasma testosterone (Shanghai BlueGene Biotech Co. Ltd., Shanghai, China) and serum IL-6 (Cusabio Biotech Co. Ltd., Wuhan, China) were determined using ELISA kits. Plasma 25OHD was assessed using chemiluminescence (LIAISON® 25 OH Vitamin D Total, Liaison, DiaSorin, Stillwater, MN). Ionized calcium (iCa) was measured in heparinized capillary samples (ABL800 FLEX analyzer, Radiometer, Copenhagen, Denmark). Serum total cortisol (ADI-900-001, Enzo Life Sciences, Farmingdale, NY), PGE_2 (ADI-900-0071, Enzo Life Sciences, Farmingdale, NY), plasma N-terminal propeptide of type 1 collagen (P1NP; AC-33f1 Immunodiagnostic Systems Inc., Fountain Hills, AZ), osteocalcin (EIA 8002, Quidel Corp, San Diego, CA) and deoxypyridinoline (DPD: EIA 8032, Quidel Corp, San Diego, CA) were measured using enzyme immunoassays at week 16 and total serum alkaline phosphatase and tartrate-