Conjugated linoleic acid reduces parathyroid hormone in health and in polycystic kidney disease in rats

Hope Weiler, Susan Austin, Shirley Fitzpatrick-Wong, Evan Nitschmann, Neda Bankovic-Calic, Rebecca Mollard, Harold Aukema, and Malcolm Ogborn

ABSTRACT

Background: Feeding conjugated linoleic acid (CLA) is reported to reduce prostaglandin E$_2$ synthesis, which is required for parathyroid hormone (PTH) release.

Objective: This study was undertaken to determine whether CLA would suppress hyperparathyroidism and the resulting high-turnover bone disease in a rat model of polycystic kidney disease (PKD).

Design: Outcome measurements were conducted after 8 wk of feeding diets supplemented with and without CLA (1% of dietary fat) to Han:SPRD-cy male rats ($n = 52$). PTH, bone formation, and resorption were assessed in addition to femur bone mass with use of dual-energy X-ray absorptiometry.

Results: CLA feeding resulted in attenuation of PTH concentrations in both PKD-affected and nonaffected rats (by 60%) but did not significantly alter bone formation and resorption.

Conclusion: Reduction in PTH may open possibilities for CLA as an adjunctive therapy in secondary hyperparathyroidism. Am J Clin Nutr 2004;79(suppl):1186S–9S.

KEY WORDS Polycystic kidney disease, bone metabolism, parathyroid hormone, conjugated linoleic acid, Han:SPRD-cy rats

INTRODUCTION

Recently, dietary conjugated linoleic acid (CLA) was suggested as a potential therapy to reduce inflammation by way of modulation of eicosanoid formation in a variety of chronic diseases (1). Dietary supplementation with CLA is reflected in tissue fatty acid composition (2, 3) and displaces linoleic acid and arachidonic acid, resulting in less synthesis of prostaglandin E$_2$ (PGE$_2$) (3). These actions could be beneficial to inflammatory conditions such as polycystic kidney disease (PKD) in which elevated tissue arachidonic acid has been reported in addition to elevated parathyroid hormone (PTH) and reduced bone mass (4, 5). Because CLA is capable of reducing PGE$_2$ (3), it could, thus, have potential as an adjuvant to PKD-induced metabolic bone disease through reductions in PTH synthesis and suppression of bone turnover. This theory is based on the fact that PGE$_2$ is involved in PTH release (6) and also bone metabolism (3). In our previous study, the Han:SPRD-cy rat, a genetic model of PKD, readily responded to a fatty acid–based intervention as noted by slower disease progression (4). Therefore, the present study was conducted, in parallel with one to examine if feeding CLA would also attenuate progression of PKD (7), to explore if such feeding would also suppress hyperparathyroidism and high-turnover bone disease.

MATERIALS AND METHODS

Animals and diet

Details of the study design and animal model have been reported in Ogborn et al (7). Briefly, 52 male Han:SPRD-cy weanling rats (source was the colony maintained by M Ogborn) were randomly selected within litters to receive identical diets but made with either corn oil (7% by weight) as the control diet or the experimental diet with corn oil + CLA (1% of diet by weight; Bioriginal Food and Science Corp, Saskatoon, SK, Canada). The CLA was 59.5% pure; thus, 1.68 g was added to each 100 g of diet to achieve the 1% of diet by weight with the remainder of the 7% of fat provided by corn oil. The CLA isomers in the product were 18:2 trans-9,trans-12; C18:2 cis-9,trans-11; and C18:2 trans-10,cis-12. Diets were fed for 8 wk beginning at weaning age (3 wk). The day before the end of study, 6-h urine collections were conducted with use of metabolic cages. Animals were anesthetized after 8 wk of study with use of sodium pentobarbital (65 mg/kg intraperitoneal), followed by measurement of whole body weight and then cardiac puncture to obtain blood and, thus, exsanguinate the rat before excision of tissues for analysis. Confirmation of PKD is reported separately (7).

Diaphyses of left femurs were excised, and marrows were removed with use of saline before bone organ culture as described by Dekel et al (8). Briefly, diaphyses were incubated in Hanks balanced salt solution (Sigma, St Louis) for 2 h at 37 °C in a shaking water bath, followed by removal of bone and rapid freezing of solution. Kidney organ culture was also conducted to...
represent soft tissue in PKD and healthy states with use of the same method with exception of incubation over 1 h. Samples were stored at −80°C until duplicate analysis of PGE₂ by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). To minimize interference of the Hanks balanced salt solution with the alkaline phosphatase enzyme (according to manufacturer’s specifications), standards were reconstituted with use of this solution as opposed to the buffer provided with the kit. In addition, this assay cross-reacts with PGE₁ (70%) and PGE₃ (16.3%). For PGE₂ the coefficient of variation (CV) was <15%.

Right femurs were excised and cleaned of soft tissue for measurement of weight and length. Femurs were then measured for bone mineral content, bone area, and bone mineral density in situ with use of dual-energy X-ray absorptiometry (4500A; Hologic, Bedford, MA; small animal software option for excised femurs).

Plasma PTH (1–84) was measured with use of an ELISA (Joldon, Burlington, Canada). The average CV was <10%. Plasma calcitriol was measured with use of a radioimmunoassay (DiaSorin, Stillwater, MN). Plasma osteocalcin was measured with use of a radioimmunoassay (DiaSorin). Triplicate analysis of samples yielded an average CV of 1.7% (9). Plasma ratlaps was measured in duplicate with use of an ELISA (Osteometer BioTech, Herlev Hovedgade, Denmark). This fragment of type I collagen is released during bone resorption, and the assay is designed specifically for use in rats. Urine creatinine was measured colorimetrically with use of a kit (555-A; Sigma). Triplicate analysis of all samples yielded a CV <10%.

Statistical analyses

Differences and interaction effects between and among groups were detected by two-factor analysis of variance with use of dietary treatments (corn and corn + CLA) and PKD status (unaffected and affected) with use of SigmaStat statistical software (Jandel Scientific, San Rafael, CA). $P < 0.05$ was accepted as significant. Post hoc analysis was conducted when appropriate (ie, interaction effects) with use of the Bonferroni all pairwise comparison test. Data are presented as $\bar{x} \pm$ SEM unless otherwise stated.

RESULTS

After 8 wk of feeding, of the 52 rats studied, 35 were classified as having PKD. No difference was observed in weight of rats because of diet, but rats with PKD weighed −5% less ($P = 0.006$; Table 1). Main effects of PKD were observed to elevate plasma PTH ($P < 0.001$; Figure 1), but the CLA diet caused a 60% reduction as a main effect ($P = 0.001$). Plasma osteocalcin ($P < 0.001$; Figure 2) and ratlaps were higher with PKD ($P < 0.001$; Figure 2), and no effect of diet or PKD compared with diet interactions were observed. Plasma calcitriol was not affected by PKD or diet (data not shown). Ex vivo release of PGE₂ from bone was not affected by diet or PKD (Table 1) in contrast to our previous study (9). Release of PGE₂ from kidney expressed as microgram per gram was reduced as a main effect of CLA (CLA $0.30 \pm 0.03$ compared with control $0.39 \pm 0.03$, $P = 0.03$). When expressed as microgram per total kidney weight, an interaction effect between diet and CLA ($P = 0.009$) was observed such that PGE₂ was only higher in the PKD-affected rats fed the control diet (Figure 3).

The effect of PKD and diet on bone mass was assessed by measuring size of the femur bone as well as mineral mass as previously conducted (9). In this group of rats, femur length was not different among groups, but a main effect of PKD was observed to reduce femur weight ($P = 0.048$), bone area ($P = 0.001$), and bone mineral content ($P = 0.004$) (Table 1). Main effects of PKD on femur bone mineral density did not reach

### Table 1: Body weight and femur characteristics of Han:SPRD-cy rats with and without polycystic kidney disease (PKD) after 8 wk of feeding control diets or the same diet supplemented with 1% by weight of conjugated linoleic acid (CLA)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Affected</th>
<th>Unaffected</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>351 ± 6</td>
<td>350 ± 6</td>
<td>Diet PKD 0.914</td>
</tr>
<tr>
<td></td>
<td>(n = 18)</td>
<td>(n = 17)</td>
<td>Diet × PKD 0.771</td>
</tr>
<tr>
<td>Femur weight (g)</td>
<td>0.783 ± 0.013</td>
<td>0.768 ± 0.013</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Femur length (cm)</td>
<td>3.57 ± 0.02</td>
<td>3.53 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Femur PGE₂ (ng/g)</td>
<td>16.7 ± 1.7</td>
<td>13.3 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Femur area (cm²)</td>
<td>1.77 ± 0.02</td>
<td>1.73 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Femur BMC (g)</td>
<td>0.361 ± 0.006</td>
<td>0.352 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>Femur BMD (g/cm²)</td>
<td>0.204 ± 0.001</td>
<td>0.203 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

$\dagger$ All values are $\bar{x} \pm$ SEM. PGE₂, prostaglandin E₂; BMC, bone mineral content; BMD, bone mineral density.
statistical significance ($P = 0.052$; Table 1). No effects of CLA on bone were observed.

**DISCUSSION**

Addition of CLA to the diet (at only 1% by weight) of rats with and without PKD reduced PTH to 60% of the value observed in the respective dietary control group. These results suggest that dietary interventions with CLA could be most useful to children (10, 11) and adults (12–14) with hyperparathyroidism secondary to chronic renal failure and also to patients who continue to experience hyperparathyroidism after transplantation (15). Thus, CLA could prove to be a useful adjuvant to current therapies in chronic renal disease. Whether reduction in PTH in otherwise healthy states confers benefit or harm to bone metabolism requires further investigation before CLA can be safely used as a nutraceutical for other purposes.

Dietary CLA was suggested as a potential nutraceutical capable of moderating cyclooxygenase-2 activity or expression and reduced eicosanoid synthesis (16). Thus, it is logical to anticipate benefits in PKD status of the Han:SPRD-cy rat in which inflammation is common (17). We observed lower release of PGE$_2$ from kidney of rats with PKD and fed CLA. CLA was also reported to reduce release of PGE$_2$ from liver tissue (18) and cancer cells (19). If CLA limits PGE$_2$ release from all soft tissues, this action would help explain the lower PTH that results from CLA feeding in both healthy and PKD rats. Part of the mechanism involved in release of PTH from the parathyroid gland depends on PGE$_2$ (6). If CLA reduces PGE$_2$ biosynthesis in parathyroid tissue as well as kidney, then release of PTH would be limited. This hypothesis requires clarification through in vivo and in vitro studies.

The reductions in body weight and bone mass previously observed with PKD (9) were replicated in this study. However, body weight of the PKD rats was 95% the weight of unaffected rats in this study in contrast to 86% in our previous study (9). Bone mass expressed as bone mineral content was reduced by PKD to 94% of unaffected rats. Again, this value is not as severely reduced as previously reported at 85% for bone mineral content (9). Thus, the PKD in this study appears to be milder than expected with respect to growth and bone. However, it appears that bone mass is in proportion to body size. In humans with mild-to-moderate chronic renal insufficiency, bone mass is not reduced after correction for body size, age, and sex (20). Smaller reduction in bone mass, however, does not imply less risk of fracture, because measurements of bone mass with use of dual-energy X-ray absorptiometry are not predictive of fracture risk in patients with renal failure (21).

Interestingly, release of PGE$_2$ from femur in our study did not show the expected reductions as a result of CLA supplementation. In a report by Li and Watkins (3), reduced release of PGE$_2$ from bone as a result of CLA supplementation was only significant for tibia and not femur. Therefore, lack of effect in the femur is not entirely surprising. In addition, we did not observe lower release of PGE$_2$ from femur in rats with PKD or with feeding CLA (22). This finding was unexpected, because our previous study reported lower values for PGE$_2$ release from femurs of rats with PKD (9). On the basis of weight and biochemical assessment of PKD, the rats in this study were less severely affected at the time of sample collection, and, perhaps, reductions in bone PGE$_2$ had not yet evolved.
Osteoblast activity was elevated in PKD to values 120% of those from unaffected rats, consistent with our previous report in which osteocalcin in PKD states was 129% of control (9). In this study we chose to use a newer marker of osteoclast activity called ratlaps that is specific for a C-terminal telopeptide of rat type I collagen. In rats with PKD, ratlaps was elevated, suggesting elevated bone resorption as previously noted (9). Dietary CLA, however, did not attenuate the high-turnover bone disease. Likewise, the reduction in PTH in the unaffected rats did not result in reductions in bone turnover. In addition, plasma calcitriol was not different among groups with and without PKD despite the reductions in PTH in the CLA groups.

In summary, dietary CLA supplemented at 1% of the diet by weight offers potential to reduce hyperparathyroidism secondary to renal disease. Because CLA is also known to reduce atherosclerosis and inflammation in experimental animal models (1), it could prove beneficial in the overall management of human renal disease (23). The reduction in PTH in healthy rats implies that diets containing CLA could affect bone health in both health and disease states.

The authors had no conflicts of interest.

REFERENCES