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Trans-10,*cis*-12-conjugated linoleic acid worsens renal pathology and alters cyclooxygenase derived oxylipins in obesity-associated nephropathy☆

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Abstract

Dietary conjugated linoleic acid (CLA) reduces indicators of early renal disease progression and the associated elevated cyclooxygenase (COX) levels in young obese rats with obesity-associated nephropathy (OAN). Therefore, renal function and injury and COX and its metabolites were assessed in obese *fa/fa* Zucker rats with more advanced renal disease. Obese rats at 16 weeks of age were provided with either *cis*(c)9, *trans*(t)11 (*fa/fa*-9,11) or t10,c12 (*fa/fa*-10,12) CLA for 8 weeks, and compared to lean (lean-CTL) and obese (*fa/fa*-CTL) rats provided the control diet without CLA. Obese rats displayed significantly reduced renal function and increased renal injury compared to lean rats. In the obese rat groups, glomerular hypertrophy was reduced in both CLA-supplemented groups. While all other measures of renal function or injury were not different in *fa/fa*-9,11 compared to *fa/fa*-CTL rats, the *fa/fa*-10,12 rats had greater renal hypertrophy, glomerular fibrosis, fibrosis, tubular casts and macrophage infiltration compared to the *fa/fa*-CTL and *fa/fa*-9,11 groups. The *fa/fa*-10,12 group also had elevated levels of renal COX1, which was associated with increased levels of two oxylipins produced by this enzyme, 6-keto-prostaglandin $F_{1\alpha}$, and thromboxane B_2. Renal linoleic acid and its lipoxygenase products also were lower in obese compared to lean rats, but CLA supplementation had no effect on these or any other lipoxygenase oxylipins. In summary, supplementation with c9,t11 CLA did not improve more advanced OAN and t10,c12 CLA worsened the renal pathology. Altered production of select COX1 derived oxylipins was associated with the detrimental effect of the t10,c12 isomer.

Keywords: Conjugated linoleic acid isomers; Kidney pathology; Obesity; Oxylipins; COX

1. Introduction

Obesity is strongly associated with renal injury and an increased risk of progression to end-stage renal disease (ESRD) in both older and younger adults [1–5]. Since both obesity and ESRD are escalating at alarming rates [6–8], treatments that can reduce obesity and the accompanying renal disease risk are needed. One such potential treatment in obesity-associated nephropathy (OAN) is conjugated linoleic acid (CLA), which consists of a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. The two most common isomers are the *cis*(c)-9,*trans*(t)-11 form, which is found predominantly in dairy products and meat of ruminant animals, and the t10,c12 form, which is produced from linoleic acid for use in nutritional supplements. CLA supplements have been targeted for reducing weight and body fat, although more recent evidence suggests that the use of CLA for weight loss may not be as strong as initially believed [9,10]. However, CLA also has been reported to have a number of other health effects, including antitumorigenic, proapoptotic and antimetastatic effects in cancer cells, and antiatherogenic and cardioprotective effects in experimental animal models [11–16].

Abbreviations: c, *cis*; BW, body weight; CLA, conjugated linoleic acid; COX, cyclooxygenase; ESRD, end-stage renal disease; *fa/fa*-9,11, obese Zucker rats given c9,t11 CLA diet; *fa/fa*-10,12, obese Zucker rats given t10,c12 CLA diet; *fa/fa*-CTL, obese Zucker rats given control diet; H&E, hematoxylin and eosin; HODE, hydroxyoctadecadienoic acid; HETE, hydroxyeicosatetraenoic acid; IL, interleukin; lean-CTL, lean Zucker rats given control diet; LOX, lipoxygenase; MGA, mean glomerular area; MGV, mean glomerular volume; ND, not detected; OAN, obesity-associated nephropathy; PAS, periodic acid-Schiff; PG, prostaglandin; t, *trans*; TNF, tumor necrosis factor; TXB_2, thromboxane B_2.

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There also is evidence for beneficial effects of CLA in a number of models of chronic kidney disease. In autoimmune NZB/W F1 mice, CLA supplementation initiated with onset of renal failure was able to slow disease progression [17]. Dietary CLA supplementation in the Han:SPRD-*cy* model of polycystic kidney disease reduces renal inflammation, cell proliferation, fibrosis and oxidative damage [18–20]. In our previous studies in the obese *fa/fa* Zucker rat, 8-week supplementation of young rats with t10,c12 CLA alone or in combination with the c9,t11 isomer resulted in a reduction in glomerular hypertrophy [21], which is one of the earliest signs of OAN [22–25]. The JCR:LA-cp rat is another model of obesity that exhibits renal injury and provides dietary CLA to weanling rats for 8 weeks reduced urinary albumin levels and glomerulosclerosis [26]. However, whether this apparent beneficial effect in young rats would result in renal disease reduction in the long term with more advanced OAN is not known.

Obesity is a proinflammatory state [27], and CLA isomers have been reported to have specific effects on adipose tissue and pro- and anti-inflammatory molecules. We also have shown that t10,c12 CLA decreases adipocyte dysfunction by reducing cell size and increasing cell number in epididymal adipose tissue of *fa/fa* Zucker rats [16,28], and it is generally believed that t10,c12 CLA is responsible for effects on body composition [29–32]. Animal models of inflammation and obesity have shown that a mixture of CLA isomers increases anti-inflammatory cytokines and decreases proinflammatory cytokines [33–36]. On the other hand, studies in humans have documented elevated levels of C-reactive protein with t10,c12 CLA and elevated isoprostanes with supplementation with either isomer [37,38]. Others have reported that t10,c12 CLA promotes adipose tissue inflammation in mouse adipose tissue, 3T3–L1 preadipocytes and primary human adipocytes [39–42]. The effect of dietary CLA isomers on the kidney within the context of the obese state remains to be determined.

CLA may mediate some of its effects by altering oxylipin levels, as has been demonstrated in several tissues [42–47]. Oxylipins are oxygenated fatty acid metabolites that have many physiological functions in the body and in the kidney including modulation of inflammation, as well as regulation of renal hemodynamics, water and salt transport, renin secretion and hypertension [48,49]. Cyclooxygenase (COX)-derived oxylipins have been studied the most, and renal COX levels have been shown to be altered in the *fa/fa* Zucker rat [50–52] as well as other forms of renal disease [48,49]. In a previous study, we showed that dietary CLA in young *fa/fa* Zucker rats mitigated some of these COX abnormalities along with the reductions in glomerular hypertrophy [21]. CLA also had similar COX oxylipin-lowering effects along with slowing disease progression in the Han:SPRD-*cy* rat model of polycystic kidney disease [20]. In the obese JCR:LA-cp rat, however, COX oxylipins are not altered, but the levels of several lipoxygenase (LOX)-derived oxylipins are elevated in diseased kidneys [53]. The LOX oxylipins have not been examined in the *fa/fa* Zucker rat.

Therefore, to examine the effects of CLA isomers on more advanced OAN, older *fa/fa* Zucker rats were provided with c9,t11 and t10,c12 CLA isomers to determine their effects on renal pathology and to relate these effects to potential changes in COX and LOX oxylipins.

2. Methods

2.1. Animal model and diet

After 1 week of acclimatization, obese *fa/fa* Zucker rats (Harlan Laboratories, Indianapolis, IN, USA) at 16 weeks of age were placed in one of three dietary groups: obese *fa/fa* Zucker control (*fa/fa*-Ctrl), obese *fa/fa* Zucker provided c9,t11 CLA (*fa/fa*-9,11) or obese *fa/fa* Zucker provided t10,c12 CLA (*fa/fa*-10,12). Rats were provided these diets *ad libitum* for 8 weeks (n=10/group). In addition, a lean (*fa/+*) Zucker rat group (n=10) served as the lean control (lean-CTL). The *fa/fa*-CTL and lean-CTL groups were provided a control diet based on the AIN-93G formulation [54], and the CLA groups were provided the same diet containing 0.4% (wt/wt) c9,t11 or t10,c12 CLA from a synthetic source (95% purity; Natural ASA, Hovdebygda, Norway), as previously described [55]. The CLA replaced an equal amount of soybean oil so that all diets had the same oil content (8.5% wt/wt) and were isocaloric.

2.2. Sample collection

Forty-eight hours before termination, rats were placed in metabolic cages for 12 h, and urine samples were collected and stored at −80°C until analyzed. Rats were fasted for 12 h prior to termination, anesthetized with CO_2 and terminated by exsanguination via decapitation. Body weight and length were used to calculate body mass index, as described and validated for the rat [56]. Trunk blood was collected and centrifuged at 2000×*g* for 20 min to obtain serum and stored at −80°C. The right kidneys were removed, weighed, flash-frozen and stored at −80°C for fatty acid, oxylipin and immunoblotting analysis. Left kidneys were removed, weighed, and bisected in the coronal plane through the hilum of the kidney and fixed in 10% buffered neutral formalin for 48 h before embedding in paraffin wax for histological, morphometric and immunohistochemical analyses. Perirenal fat depots were also dissected out and weighed. All animal procedures and care were examined by the University of Manitoba Animal Care Committee and were within the guidelines of the Canadian Council on Animal Care. Other assessments of these animals for blood pressure and adipose function have been previously reported [16].

2.3. Biochemical analyses

Urinary protein concentrations were determined using the Bradford method for total protein determination [57]. Commercial kits were used to determine serum and urine creatinine (Sigma, Oakville, Canada), serum glycated hemoglobin (HbA1c) and glucose (Biosafe Laboratories, Chicago, IL, USA) and serum cholesterol and triglycerides (BioPacific Diagnostics, Charlottetown, Canada).

2.4. Histological analyses

Paraffin-embedded kidneys were sectioned at 3–5 μm and processed using our previously described methods for histological analyses [58,59]. Sections for mean glomerular volume (MGV) and tubular calcification were stained with hematoxylin and eosin; those for quantitative analysis of fibrosis were stained with Sirius red (an adaptation of Masson's trichrome stain). Sections for tubular atrophy, dilation and tubular cast were stained with periodic acid-Schiff (PAS). Renal macrophage inflammation was detected using a 1:50 dilution of a mouse anti-rat monocyte/macrophage monoclonal antibody (MAB1435; Chemicon International, Temecula, CA, USA).

Image analysis and morphometry were performed using our previously described methods [58,59]. Briefly, 20–50 images were captured using a Spot Junior CCD camera on an Olympus BX60 microscope by random stage movement through the sections and were subsequently analyzed using the Image-Pro Plus software version 6.0 (Media Cybernetics, Silver Spring, MD, USA). The observer was blinded to dietary treatment, although differences between sections from lean and obese rats were obvious upon microscopic examination.

To determine MGV, 150 randomly selected glomeruli were examined by light microscopy under low power (10× objective). MGV was calculated by measuring the maximum diameter of each glomerular tuft using Image-Pro Plus Software 6.0 measurement tools. The diameters (μm) were converted to MGV using the following equations: mean glomerular area (MGA)=πr^2 and MGV=1.25