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Journal of Nutritional Biochemistry 17 (2006) 735–741
Journal of Nutritional Biochemistry

t10,c12 conjugated linoleic acid induces compensatory growth after immune challenge☆

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Received 20 October 2005; received in revised form 19 December 2005; accepted 20 December 2005

Abstract

Previous work demonstrated that feeding commercial preparations of conjugated linoleic acid (CLA) [a 50:50 mixture of c9,t11 and t10,c12 CLA (cCLA)] partially overcame lipopolysaccharide (LPS)-induced growth depression. The objective of this study was to determine which CLA isomer was responsible for the reduction of LPS-induced growth depression. Dietary cCLA supplementation for 3 weeks protected mice from LPS-induced weight loss 24 h after injection compared to mice fed isocaloric and isonitrogenous control diets supplemented with either corn oil (CO) or a mixture of CO and olive oil. Dietary c9,t11 or t10,c12 CLA led to body weight loss intermediate to controls and cCLA. After LPS-induced weight loss, the t10,c12 CLA fed mice regained weight faster than the control or c9,t11 CLA fed mice. Dietary t10,c12 CLA and cCLA reduced plasma tumor necrosis factor 2 h after LPS stimulation. While neither c9,t11 nor t10,c12 CLA isomers alone protected from immune-induced weight loss, the t10,c12 CLA isomer induced compensatory gain.
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Keywords: CLA; Endotoxin; Tumor necrosis factor; TNF; Cachexia; Immune stress; NF-κB; PPARγ

1. Introduction

Cachexia is a condition characterized by the general state of body weight wasting and anorexia that is a result of immune stimulation [1]. Many chronic and/or inflammatory conditions such as infection, cancer, acquired immunodeficiency syndrome or rheumatoid arthritis result in a cachectic response [1–3]. Cachexia can be induced experimentally by injection of bacterial lipopolysaccharide (LPS) [4,5], and tumor necrosis factor (TNF) is a key proinflammatory mediator of cachexia [6]. Administration of TNF is sufficient to induce a cachetic state, while inhibition of TNF, by neutralizing antibodies or soluble TNF receptors, attenuates cachexia [6]. One pathway by which LPS induces wasting is through the binding of LPS or TNF to their respective surface receptors (TLR4 or TNFR2), which activate the proinflammatory NF-κB signaling cascade [7]. In unstimulated cells, the NF-κB element resides in the cytoplasm as a heterodimer (p50, p65 or RelA) in association with its inhibitor proteins (IκBα, IκBβ, or IκBγ) in an inactive form. TLR4 or TNFR receptor binding leads to activation of the IκB kinases, leading to the ubiqutin-proteosome-mediated degradation of IκBs, thus, exposing the nuclear translocation element of the NF-κB heterodimer. The freed NF-κB translocates to the nucleus and mediates transcription of various inflammatory mediators including IL-1, TNF and cyclooxygenase-2 (COX-2) [7].

Conjugated linoleic acids (CLAs) are a group of geometric and positional isomers of linoleic acid. Conjugated linoleic acid occurs naturally in lipids derived from ruminant sources, especially milk and cheese [8]. The two most widely studied isomers of CLA, c9,t11 and t10,c12, are found in an approximately 50:50 ratio in commercial preparations (cCLA). In 1987, a ground beef derivative, later described

☆ This work was partially supported by funding from the Cooperative State Research, Education and Extension Service (Food and Agricultural Sciences National Needs Graduate Fellowship), U.S. Department of Agriculture; the College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI; unrestricted gifts from BASF; and patent royalties from the Wisconsin Alumni Research Foundation.

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doi:10.1016/j.jnutbio.2005.12.004

Table 1
Experimental diet composition

Mouse diet					
Ingredient (g/100 g)	OO	c9,t11 CLA	t10,c12 CLA	CLA90	CO
Sucrose	47.6	47.6	47.6	47.6	47.6
Casein	21	21	21	21	21
Corn starch	15	15	15	15	15
CO	5.75	5.75	5.75	5.5	6
Cellulose	5	5	5	5	5
AIN-76 mineral mix[a]	3.5	3.5	3.5	3.5	3.5
AIN-76 vitamin mix[b]	1	1	1	1	1
Calcium carbonate	0.4	0.4	0.4	0.4	0.4
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
OO	0.25	–	–	–	–
c9,t11 CLA	–	0.25	–	–	–
t10,c12 CLA	–	–	0.25	–	–
CLA90	–	–	–	0.5	–

CLA90, 50:50 mixture of c9,t11 and t10,c12 CLA.

[a] Supplied per kilogram of premix: 500 g calcium phosphate, dibasic ($CaHPO_4$), 220 g potassium citrate, monohydrate ($K_3C_6H_5O_7 \cdot H_2O$), 74 g sodium chloride, 52 g potassium sulfate, 24 g magnesium oxide, 6 g ferric citrate (16–17% Fe), 3.5 g manganous carbonate (43–48% Mn), 1.6 g zinc carbonate (70% ZnO), 0.55 g chromium potassium sulfate [$CrK(SO_4)_2 \cdot 12H_2O$], 0.3 g cupric carbonate (53–55% Cu), 0.01 g potassium iodate (KIO_3), 0.01 g sodium selenite ($Na_2SeO_3 \cdot 5H_2O$), 118.03 g sucrose, finely powdered.

[b] Supplied per kilogram of premix: 3 g nicotinic acid or nicotinamide, 1.6 g calcium *d*-pantothenate, 0.7 g pyridoxine–HCL, 0.6 g thiamin–HCl, 0.6 g riboflavin, 0.2 g folic acid, 0.02 g *d*-biotin, 0.001 g cyanocobalamin (vitamin B_{12}), 120,000 RE retinyl palmitate or acetate (vitamin A), 5000 IU α-tocopheryl acetate (vitamin E), 0.0025 g cholecalciferol (vitamin D_3), 0.005 g menaquinone (vitamin K) finely powdered sucrose to make 1000 g. All diets contained 0.001 g per 100 g ethoxyquin as a preservative.

as CLA (predominately c9,t11 CLA), was found to have anticarcinogenic properties [9,10]. Since then, dietary cCLA has been shown to prevent body fat accumulation [11,12], have antiatherosclerogenic properties [13,14] and prevent immune-induced cachexia [15–19].

Commercially prepared CLA feeding consistently reduced immune-induced wasting in various animal models. Feeding cCLA partially prevented LPS-induced weight loss in chickens, rats and mice [15–17,20]. In a model of systemic lupus erythematosis, cCLA supplementation reduced body weight wasting that is characteristic of end stage disease [17]. In a murine model of rheumatoid arthritis, cCLA supplementation reduced body weight loss due to immunization of Type II collagen in Freund's complete adjuvant [21]. Muscle wasting due to colon-26 adenocarcinoma (cancer cachexia) was prevented by dietary cCLA [22]. Commercially prepared CLA also prevented body weight loss due to intravenous administration of TNF [23]. Mechanisms by which CLAs influence inflammation and hence immune-induced weight loss may be through its role in modulation of eicosanoid synthesis via COX inhibition, cellular regulation by ligation of peroxisome proliferator-activated receptors (PPARs) and/or modulation of the NF-κB signal transduction pathway [19,23–25].

Although it is well established that dietary cCLA (mixed isomers) reduces immune-induced weight loss, no report has appeared on the isomer-specific effect of CLA on immune-induced weight loss in vivo. It is therefore the goal of this study to determine which isomer of CLA, c9,t11 or t10,c12 CLA inhibits LPS-induced weight loss.

2. Materials and methods

2.1. Animals

Eight-week-old male BALB/c mice were obtained from Harlan (Indianapolis, IN) and maintained in accordance with protocols approved by the College of Agricultural and Life Sciences Animal Care and Use Committee, University of Wisconsin, Madison, WI. Animals were acclimated to their environment for 48 h before being randomly assigned to diet treatments ($n=6$ per group). Diet treatments consisted of a powdered 99% complete diet (TD94060; Harlan-Teklad, Madison, WI) supplemented with 1% oil (Table 1). In Experiment 1, there were four diet/injection groups: (1) 0.25% c9,t11 CLA (Natural Lipids, Hovdebygda, Norway)+LPS; (2) 0.25% t10,c12 CLA (Natural Lipids)+LPS; (3) 0.25% olive oil (OO)+LPS; (4) 0.25% OO+vehicle injection. In Experiment 2 there were five diet/injection groups: (1) 0.25% c9,t11 CLA+LPS; (2) 0.25% t10,c12 CLA+LPS, (3) a 50:50 CLA isomer mixture (cCLA, Natural Lipids)+LPS; (4) corn oil (CO)+LPS; and (5) CO+vehicle injection. Supplemental oil purity (Table 2) was assessed by gas chromatography using an HP-88 100-m fused silica capillary column (0.25 mm i.d., 0.2-μm film thickness) as previously described [26]. The total fat composition of the final diet was 6%, where 5.75% or 5.5% came from CO and 0.25% came from CLA isomers or OO, or 0.5% came from cCLA. (The rate of 0.5% cCLA was chosen to match CLA isomer content to that of diets fed

Table 2
Fatty acid composition of oils used in diet preparations

Fatty acid (%)	Test oil				
	c9,t11 CLA	t10,c12 CLA	cCLA	OO	CO
c16:0	n/d	n/d	0.6	15.3	12.8
c18:0	n/d	n/d	n/d	n/d	2.4
c18:1 c9	4.9	n/d	6.4	74.0	29.1
c18:1 c11	n/d	n/d	0.3	n/d	0.8
c18:2 c9,c12	n/d	n/d	0.3	10.6	53.2
c18:2 c9,t11	91.8	3.8	42.7	n/d	n/d
c18:2 t10,c12	2.2	94.7	43.5	n/d	n/d
c18:2 cc[a]	0.8	1.5	1.9	n/d	n/d
c18:2 tt[b]	0.3	n/d	4.1	n/d	n/d
c18:3 c9, c12, c15	n/d	n/d	n/d	n/d	1.2
c20:0	n/d	n/d	n/d	n/d	0.5

Fatty acids not listed were not detectable under the assay conditions. Columns may not add to exactly 100% due to rounding. n/d=not detectable.

[a] Conjugated cis, cis isomers.

[b] Conjugated trans, trans isomers.

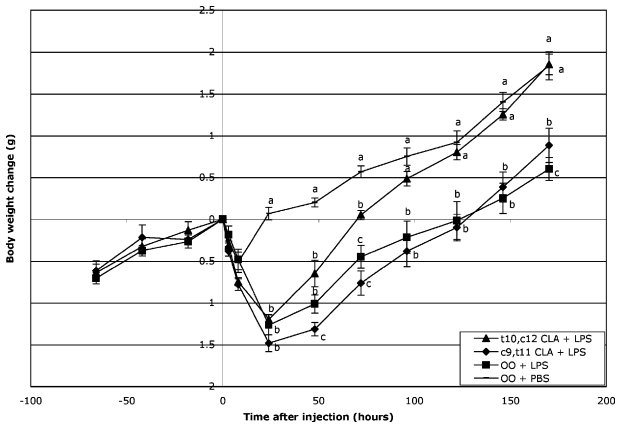

Fig. 1. Body weight change normalized to body weight at time of LPS injection as an immune challenge. Male BALB/c mice (six per group) were fed diets supplemented with 0.25% c9,t11 or t10,c12 CLA or OO for 15 days prior to intraperitoneal injection of 1 mg/kg LPS. Body weight was monitored after LPS injection. Data were analyzed with SAS proc mixed accounting for autocorrelation. Uncommon letter superscripts were significantly different within a period ($P<.05$).

purified isomers.) Animals were fed test diets for 21 days prior to stimulation. On Day 0, mice were injected intraperitoneally with 1 mg/kg bacterial LPS (*Escherichia coli* O55:B5; Sigma, St. Louis, MO) dissolved in sterile phosphate-buffered saline (PBS) (0.137 M NaCl, 0.008 M Na_2HPO_4, 0.0027 M KCl, 0.0015 M KH_2PO_4, pH 7.4) or the sterile PBS vehicle. In Experiment 2, 1 and 2 h after LPS stimulation, 100 μl of blood was drawn from the retroorbital plexus vein using heparinized capillary tubes while animals were under isoflurane anesthesia. Cumulative body weight change postinjection was measured at 3, 8, 12 and 24 h after injections and daily thereafter.

2.2. Plasma tumor necrosis factor

Blood samples collected in Experiment 2 were centrifuged at 10,000×*g* for 5 min. Plasma was collected and stored at −80°C until analyzed by ELISA. Opti-EIA murine TNF ELISA kit (BD Pharmingen, San Jose, CA) was used to measure plasma TNF concentration according the manufacture's instructions. Briefly, Nunc MaxiSorb 96-well plates (Fisher Scientific, Pittsburgh, PA) were coated with antimouse TNF capture antibody overnight and blocked with 1% bovine serum albumin in PBS. Diluted plasma samples were added to the plate and incubated at room temperature for 2 h. The plate was washed five times. Biotin-conjugated anti-TNF antibody and streptavidin-conjugated horseradish peroxidase were added to the plate and incubated at room temperature for 1 h. The plate was washed seven times. The substrate solution containing tetramethylbenzidine and hydrogen peroxide (BD Pharmingen) was incubated with the plate for exactly 15 min prior to addition of 1 M H_3PO_4 to stop the reaction. Optical density was read at 450 nm with a reference wavelength of 600 nm on an ELx808 Ultra Microplate Reader (Bio-Tek Instruments INC, Winooski, VT). The sample TNF concentration was calculated based on a standard curve generated using known concentrations of recombinant murine TNF.

2.3. Statistics

Body weight data were analyzed using the mixed procedure accounting for autocorrelation of repeated measures. ANOVA and least significant differences procedures were performed on data from plasma TNF concentration. Differences were considered significant at $P<.05$.

3. Results

In the first study, mice were fed c9,t11 CLA, t10,c12 CLA or OO for 3 weeks prior to LPS stimulation. Fig. 1 shows body weight change normalized to body weight at the time of LPS stimulation. During the first 8 h after injection with LPS or PBS, mice in all dietary and injection treatment groups lost weight. Mice injected with LPS, but not PBS, continued to lose weight for 24 h after stimulation. All LPS-injected groups lost approximately 6% of their initial body weight by 24 h after injection. In the days following maximal weight loss, the t10,c12 CLA fed mice regained weight faster than did either the c9,t11 CLA or OO fed mice, such that by 72 h post-LPS injection, differences in cumulative body weight change were significant. The cumulative body weight change in the t10,c12 CLA fed group was no longer statistically different from the OO fed PBS-injected control group 96 h after LPS stimulation. The growth rate for the 3 days prior to LPS stimulation was 0.24 g/day across all groups. Given the growth rate of 0.24 g/day, the cumulative body weight change of the mice in the c9,t11 CLA and OO fed LPS-stimulated groups lagged 3 days of growth behind the PBS-injected group for the duration of the trial (Fig. 1).

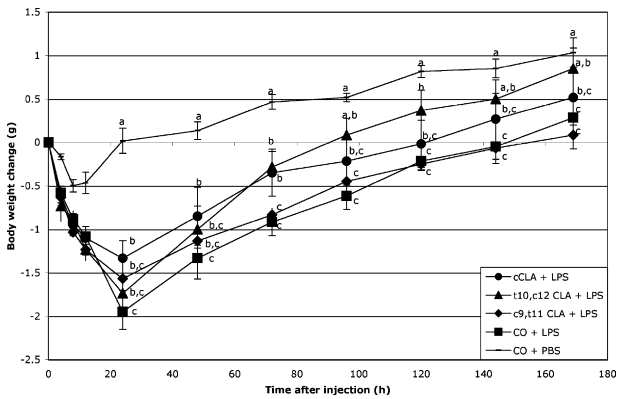

Fig. 2. Body weight change after injection of LPS as an immune challenge. Male BALB/c mice (six per group) were fed diets supplemented with 0.25% c9,t11 or t10,c12 CLA, 0.5% of a 50:50 isomer mixture (cCLA) or CO for 21 days prior to intraperitoneal injection of 1 mg/kg LPS in PBS or PBS alone. Body weight was monitored daily after LPS injection. Data were analyzed with SAS proc mixed accounting for autocorrelation. Uncommon letter superscripts were significantly different within a period ($P<.05$).

Since the results of the first trial showed that individual isomers of CLA did not protect against immune-induced weight loss, and since a 50:50 mixture of c9,t11 and t10,c12 CLA (cCLA) has been previously shown to reduce weight loss 24 h after LPS injection [16], a second trial was conducted, which included cCLA as a treatment. In the second experiment, mice in all diet and injection groups lost weight in the first 8 h after injection. Lipopolysaccharide-injected, but not PBS-injected, mice continued to lose weight for the first 24 h after injection (Fig. 2), as observed in the first experiment. Dietary cCLA partially protected mice from LPS-stimulated weight loss compared to the CO fed mice at the 24-h time point. Weight loss in the c9,t11 and t10,c12 CLA isomer fed/LPS-injected groups was intermediate to the LPS-injected cCLA and CO fed groups (Fig. 2). After the first 24 h, mice in the t10,c12 group began to regain weight such that by 72 h after LPS injection, the cumulative body weight change of the t10,c12 fed mice was significantly greater than that of the c9, t10 CLA or CO fed and LPS-injected mice. The t10,c12 CLA fed mice regained body weight such that the cumulative body weight change post-LPS injection of the t10,c12 CLA fed mice was no longer statistically different from PBS-injected animals at the 96-h time point (Fig. 2). Although the cCLA fed mice lost less weight 24 h after injection, the cumulative body weight change post-LPS injection never became statistically similar to that of the CO fed PBS-injected group. Growth rate in the cCLA fed mice following peak weight loss paralleled that of the CO and c9,t11 CLA fed mice (Fig. 2).

Lipopolysaccharide injection causes increases in plasma TNF concentration, peaking 1 to 2 h after intraperitoneal injection across all dietary treatments [17]. In blood samples collected at 1 h after LPS simulation, c9,t11 and t10,c12 CLA and CO fed mice had TNF concentrations of 6 ng/ml plasma, while the cCLA fed mice had plasma TNF concentrations of 3 ng/ml (Table 3). Two hours after LPS stimulation, plasma TNF concentration was reduced in all groups and cCLA fed mice had significantly lower TNF concentrations (1.9 ng/ml) than did the CO (4.3 ng/ml) fed LPS-injected mice. The t10,c12 CLA fed mice had an intermediate plasma TNF concentration 2 h after LPS injection (2.3 ng/ml), while the c9,t11 CLA fed mice had plasma TNF concentrations (4.0 ng/ml) similar to the LPS-injected CO fed mice (Table 3).

Table 3
Plasma TNF concentration 1 and 2 h after LPS injection

Diet/injection treatment	Plasma TNF (ng/ml)		S.E.	
	1 h	2 h	1 h	2 h
cCLA+LPS	2.93[a]	1.94[a,c]	0.74	0.57
t10,c12 CLA+LPS	5.74[b]	2.34[a,b,c]	0.90	0.29
c9,t11 CLA+LPS	5.20[b]	4.04[a,b]	0.97	0.97
CO+LPS	5.87[b]	4.37[b]	0.81	1.24
CO+PBS	0.47[c]	0.40[c]	0.19	0.16

Uncommon superscripts represent significant differences ($P<.05$).

4. Discussion

The aim of this study was to examine the isomer-specific effects of CLA on the immune-induced weight loss model of cachexia. Miller et al. [16] previously used the endotoxin/wasting model to demonstrate that CLA reduces immune-induced wasting. In the Miller study, mice were fed a 50:50 mixture of c9,t11 and t10,c12 CLA (cCLA) for 15 days prior to an immune challenge with LPS. Mice fed cCLA lost less weight 24 h after LPS injection compared to the CO or fish oil fed LPS-injected mice, and cCLA fed mice regained weight back to controls levels by 72 h [16]. Other studies support a role of CLA in protecting animals from immune-induced cachexia [15–17,19,21,27]. In the LPS-induced weight loss model of cachexia, animals' maximum weight loss occurred 24 h after the inflammatory stimulus. Weight loss in cCLA fed animals 24 h after LPS stimulation was approximately half of the weight loss seen in control fed animals [15,16]. Consistent with literature findings, this study showed that mice fed a CO diet and injected with LPS lost up to 8.6% of their initial weight, while mixed isomers of cCLA partially overcame this weight loss. (Mice fed cCLA lost 5.6% of initial body weight.) The c9,t11 and t10,c12 CLA fed groups were intermediate between CO and cCLA fed groups 24 h after LPS injection, losing 7.7% and 6.8% of their initial body weight, respectively (Fig. 2). Surprisingly, neither c9,t11 nor t10,c12 CLA when fed alone prevented weight loss at 24 h after LPS injection, while the 50:50 mixture of the isomers did prevent weight loss at 24 h. Recent evidence from an inflammatory bowel disease model has also shown antiinflammatory effects with the 50:50 mixture of CLA and no antiinflammatory effect when the mice were fed the individual isomers. At this time, no explanation can be provided, which explains why the isomers together are more effective than c9,t11 or t10,c12 CLA alone. However, the protective effect of the combined isomers on preventing LPS-induced weight loss is consistent with previous findings [16,17,22,28].

While LPS and the inflammatory cytokine TNF generate signals for inflammation via different membrane receptors (TLR4 and TNFR2, respectively), the pathways converge by activating the nuclear transcription factor NF-κB [29,30]. Passive immunization against TNF attenuates the toxic effects of LPS [31], while administration of a dose of TNF mimics the toxic effect of LPS administration [32]. While it is established that mixed isomers of CLA inhibit LPS-induced weight loss and plasma TNF concentration in vivo [16,17], it is not yet clear which isomer of CLA is responsible for these effects. c9,t11 CLA inhibited LPS-induced TNF in cultured 264.7 macrophages in vitro [17]. However, c9,t11 CLA did not inhibit LPS-induced TNF release in ex vivo cultured human PBMCs [33], in ex vivo cultured splenocytes [34] or in ex vivo cultured macrophages in response to LPS [35]. Data from the current study seem to indicate that c9,t11 CLA does not reduce serum TNF concentration either 1 or 2 h after an LPS stimulus in

vivo. In the case of ex vivo cultured macrophages, t10,c12 CLA was found to be the important isomer in the reduction of TNF production in response to LPS challenge [35]. The in vivo data from this study are consistent with the reports that mixed isomers of CLA inhibit serum TNF concentration after LPS exposure [17,23,35]; however, t10,c12 CLA only inhibited plasma TNF concentration 2 h after LPS injection. Tumor necrosis factor released in response to an inflammatory process can be the result of either preformed TNF release or TNF resulting from new synthesis via an NF-κB-mediated mechanism. The current study design does not allow us to address whether CLA's effect on TNF release is via preformed or through new synthesis mediated by the NF-κB signal transduction pathway.

Another signal transduction pathway by which TNF production is controlled is through the PPAR gamma (PPARγ). Evidence for a PPARγ-dependent mechanism for mixed isomers of CLA in a model of inflammatory bowel disease has been presented [28]. While a mixture of CLA isomers was shown to produce a pronounced effect on inflammation in that model, individual isomers had no effect. It is difficult to explain if CLA's regulation of inflammation is via a PPARγ-dependent mechanism since both c9,t11 and t10,c12 CLA activate PPARγ to a similar extent [24,36]. Recent evidence indicates the t10,c12 and not the c9,t11 CLA isomer was responsible for inhibiting protein expression in vivo, presumably through the regulation of NF-κB-mediated signal transduction [25]. Hence, it is possible that the antiinflammatory effect of CLA is independent of PPARγ effects on the NF-κB signaling pathway.

Miller et al. [16] showed that animals fed mixed isomers of CLA regained weight back to nonstimulated control levels by 72 h after LPS injection, while CO fed or fish oil fed mice did not completely recover body weight during the experiment. In the current study, mice fed t10,c12 CLA recovered LPS-induced weight loss back to the level of the nonstimulated control, while c9,t11 CLA, cCLA and CO fed mice did not (Figs. 1 and 2). Mice fed t10,c12 CLA compensated for lost weight by recovering initial body weight by 70 h after LPS injection (in Experiment 1, Fig. 1) and regaining body weight back to the same level as nonstimulated (PBS-injected) mice. Mice fed c9,t11 CLA or CO took more than twice as long to recover initial body weight and lagged the PBS-injected mice for the duration of both experiments (Figs. 1 and 2). It is unlikely that reduction in plasma TNF concentration was responsible for the observed compensatory gain seen in the t10,c12 CLA fed mice since the compensatory gain in the t10,c12 CLA fed mice persisted for the duration of the experiments, while plasma TNF concentration peaked after 1 h after LPS exposure and is not detectable in plasma after 24 h [37]. Secondly, if reductions in plasma TNF were responsible for compensatory gain, the cCLA fed mice should also have regained weight back to the nonstimulated control level. However, cCLA fed mice never regained weight back to the level of the PBS-injected control mice.

Another possible explanation for the compensatory gain is that t10,c12 CLA affects another mediator downstream from TNF in the signaling cascade. NF-κB activation is known to cause severe muscle wasting in mice [38]. Cyclooxygenase-2, under the control of the NF-κB signaling pathway, produces prostaglandin E_2 [39]. Prostaglandin E_2 is known to be catabolic to muscle tissue [40]. Furthermore the COX inhibitor indomethacin will attenuate LPS toxicity [41] and cancer cachexia-related muscle wasting [42]. Evidence suggests that the t10,c12 CLA isomer, but not c9,t11 CLA, inhibited NF-κB signal transduction and COX protein expression and enzymatic activity both in vitro and in vivo in immune-related tissues and cells [25]. The regulation of NF-κB by t10,c12 CLA in adipose tissue has now been reported to be counter to what was found in immune-related tissue. The t10,c12 CLA isomer up-regulated NF-κB signaling and related gene products (i.e., TNF) in adipose tissues [43]. The inhibitory effect of CLA on NF-κB-dependent products in immune-related tissues/cells during an inflammatory process is supported in vivo by CLA's down-regulation of plasma TNF, iNOS [17], IL-1β [44] and decreased expression of COX-2 in lung [25]. Likewise, CLA's, particularly the t10,c12 CLA isomer, inhibition of adipocyte lipoprotein lipase [45] and fat accumulation in adipose [11] would support an up regulation of NF-κB-related products as described by Chung et al. [43]. Hence, tissue-specific regulation of NF-κB by t10,c12 CLA reported by Li et al. [25] and Chung et al. [43] is consistent with the dietary effects of CLA in animal studies. Both research groups suggest that CLA's regulation of NF-κB appears to be an unidentified upstream event. It is upstream of NF-κB where the opposing regulation of NF-κB in immune vs. adipose tissue may be found.

In conclusion, individual isomers of CLA exhibit discrete effects on cumulative body weight change after immune challenge with LPS. Neither c9,t11 nor t10,c12 CLA isomer alone was able to reduce immune-induced weight loss 24 h after stimulation to the same extent as a 50:50 mixture of the two isomers. This study confirms that cCLA reduced plasma TNF after LPS administration and suggests that dietary t10,c12 CLA may also reduce LPS-induced plasma TNF in vivo. Only dietary t10,c12 CLA feeding resulted in compensatory growth after immune-induced weight loss, an effect that may be due to attenuation of inflammatory signaling via the NF-κB pathway.

Acknowledgments

The authors would like to acknowledge Alexander Butz for his tireless efforts in carrying out these experiments. This work was partially supported by funding from the Cooperative State Research, Education and Extension Service (Food and Agricultural Sciences National Needs Graduate Fellowship), U.S. Department of Agriculture; the College of Agricultural and Life Sciences, University of Wisconsin,

Madison, WI; unrestricted gifts from BASF; and patent royalties from the Wisconsin Alumni Research Foundation.

References

[1] Kotler DP. Cachexia. Ann Intern Med 2000;133:622–34.

[2] Kotler DP, Tierney AR, Wang J, Pierson Jr RN. Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. Am J Clin Nutr 1989;50:444–7.

[3] Rall LC, Roubenoff R. Rheumatoid cachexia: metabolic abnormalities, mechanisms and interventions. Rheumatology 2004;43: 1219–23.

[4] Klasing KC, Austic RE. Changes in protein synthesis due to an inflammatory challenge. Proc Soc Exp Biol Med 1984;176:285–91.

[5] Sharma RJ, Macallan DC, Sedgwick P, Remick DG, Griffin GE. Kinetics of endotoxin-induced acute-phase protein gene expression and its modulation by TNF-alpha monoclonal antibody. Am J Physiol Regul Integr Comp Physiol 1992;262:R786–93.

[6] Beutler B, Cerami A. Tumor necrosis, cachexia, shock, and inflammation — a common mediator. Annu Rev Biochem 1988;57: 505–18.

[7] Caamano J, Hunter CA. NF-{kappa}B family of transcription factors: central regulators of innate and adaptive immune functions. Clin Microbiol Rev 2002;15:414–29.

[8] Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compos Anal 1992;5:185–97.

[9] Ha Y, Grimm N, Pariza M. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 1987;8:1881–7.

[10] Ip C, Chin SF, Scimeca JA, Pariza MW. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res 1991;51: 6118–24.

[11] Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. Lipids 1997;32:853–8.

[12] Park Y, Storkson JM, Albright KJ, Liu W, Pariza M. Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 1999;34:235–41.

[13] Lee NL, Krichevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 1994;108:19–25.

[14] McLeod RS, LeBlanc AM, Langille MA, Mitchell PL, Currie DL. Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. Am J Clin Nutr 2004;79: 1169–74.

[15] Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poult Sci 1993;72:1301–5.

[16] Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem Biophys Res Commun 1994;198: 1107–12.

[17] Yang M, Cook ME. Dietary conjugated linoleic acid decreased cachexia, macrophage tumor necrosis factor-alpha production, and modifies splenocyte cytokines production. Exp Biol Med 2003; 228:51–8.

[18] Yang M, Cook ME. Dietary CLA decreased weight loss and extended survival following the onset of kidney failure in NZB/W F1 mice. Lipids 2003;38:21–4.

[19] Hontecillas R, Wannemeulher MJ, Zimmerman DR, Hutto DL, Wilson JH, Ahn DU, et al. Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid. J Nutr 2002; 132:2019–27.

[20] Yokota T, Meka CS, Kouro T, Medina KL, Igarashi H, Takahashi M, et al. Adiponectin, a fat cell product, influences the earliest lymphocyte precursors in bone marrow cultures by activation of the cyclooxygenase-prostaglandin pathway in stromal cells. J Immunol 2003;171:5091–9.

[21] Butz DE, Cook ME. CLA modulates immune-induced cachexia after immunization with arthritogenic collagen type II. FASEB J 2002; 16:A985.

[22] Graves E, Hitt A, Pariza MW, Cook ME, McCarthy DO. Conjugated linoleic acid preserves gastrocnemius muscle mass in mice bearing the colon-26 adenocarcinoma. Res Nurs Health 2005;28:48–55.

[23] Pariza MW, Park Y, Cook ME. Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc Soc Exp Biol Med 2000; 223:8–13.

[24] Yu Y, Correll PH, Vanden Heuvel JP. Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR[gamma]-dependent mechanism. Biochim Biphys Acta Mol Cell Biol Lipids 2002;1581:89.

[25] Li G, Barnes D, Butz D, Bjorling D, Cook ME. 10t, 12c-conjugated linoleic acid inhibits lipopolysaccharide-induced cyclooxygenase expression in vitro and in vivo. J Lipid Res 2005;46:2134–42.

[26] Kramer JK, Blackadar CB, Zhou J. Evaluation of two GC columns (60-m SUPELCOWAX 10 and 100-m CP Sil 88) for analysis of milk fat with emphasis on CLA, 18:1, 18:2 and 18:3 isomers, and short- and long-chain FA. Lipids 2002;37:823–35.

[27] Bassaganya-Riera J, Hontecillas-Magarzo R, Bregendahl K, Wannemuehler MJ, Zimmerman DR. Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. J Anim Sci 2001;79:714–21.

[28] Bassaganya-Riera J, Reynolds K, Martino-Catt S, Cui Y, Hennighausen L, Gonzalez F, et al. Activation of PPAR [947] and [948] by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. Gastroenterology 2004;127:777.

[29] Kawai T, Akira S. Toll-like receptor downstream signaling. Arthritis Res Ther 2005;7:12–9.

[30] Lin C-C, Hsiao L-D, Chien C-S, Lee C-W, Hsieh J-T, Yang C-M. Tumor necrosis factor-[alpha]-induced cyclooxygenase-2 expression in human tracheal smooth muscle cells: involvement of p42/p44 and p38 mitogen-activated protein kinases and nuclear factor-[kappa]B. Cell Signal 2004;16:597–607.

[31] Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 1985;229:869–71.

[32] Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, et al. Shock and tissue injury induced by recombinant human cachectin. Science 1986;234:470–4.

[33] Albers R, van der Wielen RPJ, Brink EJ, Hendriks HFJ, Dorovska-Taran VN, Mohede ICM. Effects of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. Eur J Clin Nutr 2003;57:595–603.

[34] Yamasaki M, Chujo H, Hirao A, Koyanagi N, Okamoto T, Tojo N, et al. Immunoglobulin and cytokine production from spleen lymphocytes is modulated in C57BL/6J mice by dietary *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid. J Nutr 2003;133:784–8.

[35] Changhua L, Jindong Y, Defa L, Lidan Z, Shiyan Q, Jianjun X. Conjugated linoleic acid attenuates the production and gene expression of proinflammatory cytokines in weaned pigs challenged with lipopolysaccharide. J Nutr 2005;135:239–44.

[36] Clement L, Poirier H, Niot I, Bocher V, Guerre-Millo M, Krief S, et al. Dietary *trans*-10, *cis*-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J Lipid Res 2002;43:1400–9.

[37] Yang M. Dietary conjugated linoleic acid decreases cachexia, macrophage TNF-{alpha} production and modifies splenocyte cytokines production, in Conjugated linoleic acid (CLA) prolongs the survival and reduces cachexia of the autoimmune NZB/W F1 mouse: role of cytokine regulation by CLA in body weight wasting and murine systemic lupus erythematosus. Madison (Wis): University of Wisconsin; 2002. p. 92–124.

[38] Cai D, Frantz JD, Tawa JNE, Melendez PA, Oh B-C, Lidov HGW, et al. IKK[beta]/NF-[kappa]B activation causes severe muscle wasting in mice. Cell 2004;119:285.
[39] Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LBA, et al. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063–73.
[40] Rodemann H, Goldberg A. Arachidonic acid, prostaglandin E2 and F2 alpha influence rates of protein turnover in skeletal and cardiac muscle. J Biol Chem 1982;257:1632–8.
[41] Johnson RW, von Borell E. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. J Anim Sci 1994;72:309–14.
[42] Hitt A, Graves E, McCarthy DO. Indomethacin preserves muscle mass and reduces levels of E3 ligases and TNF receptor type 1 in the gastrocnemius muscle of tumor-bearing mice. Res Nurs Health 2005; 28:56–66.
[43] Chung S, Brown JM, Provo JN, Hopkins R, McIntosh MK. Conjugated linoleic acid promotes human adipocyte insulin resistance through NF{kappa}B-dependent cytokine production. J Biol Chem 2005;280:38445–56.
[44] Turek JJ, Li Y, Schoenlein IA, Allen KGD, Watkins BA. Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n-6:n-3 fatty acid ratio. J Nutr Biochem 1998;9:258–66.
[45] Xu X, Storkson J, Kim S, Sugimoto K, Park Y, Pariza MW. Short-term intake of conjugated linoleic acid inhibits lipoprotein lipase and glucose metabolism but does not enhance lipolysis in mouse adipose tissue. J Nutr 2003;133:663–7.