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ORIGINAL CONTRIBUTION

Short-term dietary conjugated linoleic acid supplementation does not enhance the recovery of immunodepleted dexamethasone-treated rats

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Summary *Background* Conjugated linoleic acid (CLA) has been reported to decrease fat deposition, and increase lean body mass. This has been broadly inferred to mean that CLA alters protein turnover. However, data to test the effects of CLA on protein turnover are lacking. An enhancement in immune responses by CLA has also been demonstrated. *Aim of the study* The objective of this study was to determine the potential for dietary CLA and protein intervention to improve nutritional and functional recovery in an animal model of catabolic stress and immunodepletion. *Methods* Diets varying in their protein levels in the presence or absence of CLA were tested for their effects on the recovery of glucocorticoid (intraperitoneal injection of dexamethasone, 120 mg/kg) treated rats. Following steroid injection, rats were fed 4 dietary treatments for 4 d. The diets contained 10 or 20 g/100 g protein with or without 0.5 g/100 g CLA. *Results* Dexamethasone treatment resulted in a decreased food intake and loss of weight, independent of dietary treatment. A higher number of blood monocytes occurred in rats fed the high CLA diets. The protein fractional synthesis rate in spleens of rats fed the diets containing either high proteins or CLA were higher compared to those fed diets with low protein content or without CLA, respectively. CLA, consumed post-dexamethasone treatment, did not improve protein turnover in the other tissues studied, including gut mucosa, liver, muscle and thymus. *Conclusions* The present study was performed to determine the effect of CLA in acute conditions, as opposed to a preventive approach, on the recovery from a catabolic stress with immunodepletion. Overall, no effect of short-term feeding CLA on the recovery from dexamethasone-mediated immunodepletion was observed.

Key words conjugated linoleic acid – dexamethasone – protein synthesis – white blood cells – rats

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Introduction

Conjugated linoleic acid (CLA), a class of positional and geometric conjugated dienoic isomers of octadecadienoic acid, has generated interest due to its demonstrated anticarcinogenic properties in rodent models [1, 2]. CLA has been reported to decrease fat deposition, increase lean body mass and improve feed efficiency [3]. In addition, an anabolic effect of CLA has been suggested [4]. Data on the increase in lean body mass are derived from analysis of body composition. It is inferred that the increase in lean body mass is due to CLA-mediated modulation of protein turnover. Furthermore, in severe protein catabolism, the feeding of a high protein diet is highly recommended. No data on the effects of CLA on protein turnover, nor on the role of dietary protein levels on CLA anabolic effect, are presently available.

Only a few studies investigated the effect of CLA on

immune functions. In animal experiments, improved phagocytosis [5, 6], increased mitogen-induced blastogenesis [4, 5], enhanced cytotoxic activity and macrophage killing ability [5] have been reported following CLA intake. Chew et al. [7] reported the *in vitro* effect of CLA on porcine blood lymphocytes and murine peritoneal macrophages. CLA stimulated the mitogen-induced lymphocyte proliferation, lymphocyte cytotoxic activity, and the macrophage bactericidal activity. In contrast, CLA inhibited interleukin-2 production by lymphocytes and suppressed the phagocytic activity of macrophages. In mice fed CLA, the lymphocyte proliferation response varied as a function of the stimuli [7]. Lymphocyte proliferation was enhanced in phytohemagglutinin-induced but not in concanavalin A- or lipopolysaccharide-stimulated cultures. Interleukin-2 production was also increased, whereas lymphocyte cytotoxicity was not affected by CLA treatment. In healthy women, CLA supplementation did not provide for any added benefit to their immune status [8].

Dexamethasone, a synthetic glucocorticoid with a much greater potency than endogenous glucocorticoids, mediates a catabolic response. It induces an augmentation of protein catabolism via both increased degradation and decreased synthesis of protein in several tissues [9–11]. The dexamethasone-mediated catabolic response releases muscle amino acids that serve as substrates for gluconeogenesis and for the synthesis of acute phase proteins by the liver [12]. Dexamethasone is also a potent immunosuppressive agent capable of directly affecting the function and numbers of circulating lymphocytes, macrophages and other related immune cells [13, 14]. Moreover, dexamethasone administration has resulted in significant suppression of lymphokine production and cell proliferation and it can be used as a reversible model of immunodepletion (significant diminution of function and number of immune cells) [15].

The main objective of the present work was to determine the effects of dietary intervention following a catabolic stress to improve nutritional and functional recovery in an animal model of catabolic stress and immunodepletion. More specifically, to investigate 1) the role of CLA on recovery and 2) the interaction between CLA and protein levels in rats that had been treated with dexamethasone. Four diets differing in their protein contents (10 vs. 20 g/100 g) in the presence or absence of CLA (0.5 g/100 g) were compared for their efficacy in modulating nutritional and functional recovery.

Materials and methods

Experimental design

Fifty-four Sprague Dawley rats weighing 200 g were received from Iffa-Credo (L'Arbresle, France). Rats, allocated in individual cages, had free access to rat chow diet Nafag 857 (Eberle Nafag AG, Gossau, Switzerland) and water for 3 d (adaptation period). All experiments were approved by the Ethical Committee of the Nestlé Research Center and by the Service Vétérinaire Cantonal, Lausanne, Switzerland.

The treatments consisted of four isocaloric diets containing either 10 g/100 g or 20 g/100 g protein in the absence or presence of 0.5 g/100 g CLA, which represents an important supply of this fatty acid for rats. Linoleic acid (0.5 g/100 g) was added to diets without CLA. The chemical composition of the experimental diets is presented in Table 1.

Table 1 Chemical composition of diets

Components	10% protein 0% CLA	10% protein 0.5% CLA	20% protein 0% CLA	20% protein 0.5% CLA
	(g/kg)			
Casein (87.5% protein)	114.2	114.2	228.4	228.4
Linoleic acid (18:2n-6)	5.0	–	5.0	–
CLA[1]	–	5.0	–	5.0
Soy oil	50.0	50.0	50.0	50.0
Corn starch	633.5	633.5	519.3	519.3
Sucrose	100.0	100.0	100.0	100.0
Cellulose	50.0	50.0	50.0	50.0
Myoinositol	0.3	0.3	0.3	0.3
Choline bitartrate	2.0	2.0	2.0	2.0
AIN-93M Mineral mix[2]	35.0	35.0	35.0	35.0
AIN-93-VX Vitamin mix[2]	10.0	10.0	10.0	10.0

[1] *CLA* conjugated linoleic acid represents a mixture of isomers from linoleic acid. The main isomers were 9c,11t- and 10t,12c-octadecanoic acid, representing more than 95 % of the total CLA mix.
[2] Supplied by Socochim SA, Lausanne, Switzerland [37]

After the adaptation period, rats were force-randomized by weight into 6 groups (9 rats each). Forty-five rats received an intraperitoneal (ip) injection of dexamethasone (120 mg/kg body weight in 0.5 mL). The dexamethasone dose was chosen to induce immune depletion and to produce a reversible but important protein catabolic state. Nine rats received an ip injection of saline (control rats). These rats (control group) and nine dexamethasone-treated rats (Dexa group) were killed 24 h after the ip injection. These two groups were used to confirm that dexamethasone induced significant immune depletion. All the other rats had free access to the 4 experimental diets for 4 d. Dietary treatment was initiated at the time of dexamethasone injection. Animal weights and feed intakes were measured daily.

To measure the protein fractional synthesis rate (FSR) in specific tissues at day 4 following dietary treatment, each animal received a bolus injection of 150 µmol 1-^{13}C-L-phenylalanine (99 % ^{13}C)/100 g body weight within 1 min into the lateral tail vein. The volume injected was 2 mL. Rats were sacrificed after anesthesia (isoflurane) by exsanguination in the abdominal aorta at different times after injection of the large dose of 1-^{13}C-L-phenylalanine (10, 13, 16, 19, 22, 25, 28, 31 and 34 min). Basal enrichment was obtained from untreated rats. One blood sample was also obtained in EDTA tube for cell counting and lymphocyte subset analysis.

The spleen was quickly excised in aseptic conditions and divided into two portions. The first portion was introduced in an antibiotic solution and was used for spleen cell proliferation assays; the other portion was immediately frozen and stored at –80 °C for the measurement of the spleen protein synthesis rate. Liver, thymus and quadriceps muscles were quickly excised, weighed, frozen in liquid nitrogen and stored at –80 °C until analyzed. Mucosa samples from jejunum were obtained by scraping with a glass slide, frozen in liquid nitrogen and stored at –80 °C until analyzed.

Glutathione determination in liver and muscle

Reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations were determined by HPLC (Waters, Milford, MA), using fluorometric detection, according to the method of Martin and White [16]. GSH and GSSG concentrations were calculated from individual peak area, external standard and internal standard area, and expressed in µmol/g tissue [16].

Measurement of protein fractional synthesis rate

Tissue samples from gut mucosa, thymus, spleen, liver or quadriceps muscle sample were homogenized (16000 × rpm, 30 s using a Polytron) in ten volumes of 5 g/L perchloric acid and centrifuged at 13000 × g during 25 min at 4 °C. The supernatant was neutralized by 10 mol/L KOH, centrifuged again at 13000 × g during 3 min at 4 °C, and desalted by cation exchange chromatography using AG50W-X8 resin 200–400 mesh, sodium form (Bio-Rad, Richmond, VA, USA). Amino acids were eluted with 4 mol/L NH_4OH. ^{13}C-phenylalanine enrichments were measured as the tertiary butyldimethysilyl derivative under electron-impact ionization with selective-ion monitoring on a Hewlett Packard gas chromatograph/mass spectrometer GC5890-MS 5972 MSD system (Palo Alto, CA, USA) [17].

The protein precipitate was washed twice with water and an aliquot was hydrolyzed in 6 mol/L HCl for 24 h at 110 °C. The HCl was removed by evaporation under vacuum and samples were dissolved in 0.6 mol/L HCl and purified by cation exchange chromatography as mentioned above. ^{13}C-phenylalanine enrichments was measured as the N-acetyl-n-propyl ester derivative on a Finningan "MAT 252" isotope ratio mass spectrometer (Bremen, Germany) connected to an HP5890 series II gas chromatograph (Hewlett Packard, Palo Alto, CA) via a GC combustion interface. The interface consisted of a NiO/CuO/Pt combustion furnace reactor (940 °C) and a copper reduction furnace (600 °C). Protein FSR, (%/d) was calculated as follows:

$$\text{FSR (\%/d)} = [\text{Sb}(t) - \text{Sb}[0]] \times 100/(\text{Sa } t)$$

where Sb[0] and Sb(t) are basal phenylalanine enrichment (t = 0) and phenylalanine enrichment (at time t) in tissue protein, respectively. t (days) is the time elapsed between the bolus injection and killing of the animal. Sa is the mean enrichment of tissue free phenylalanine between times 0 and t. For a given group of rats, killing the animals at different times after the tracer injection allowed us to draw a linear regression of Sa against time. Then the estimated individual values of Sa were calculated from the linear regression of the precursor enrichment against time for the whole group [18].

Hematology and lymphocyte phenotyping

Blood from the abdominal aorta was collected into K_2EDTA-coated tubes. The hematological parameters determined included white blood cell count and differential leukocyte count. These hematological parameters were analyzed according to standard procedures.

The following lymphocyte subsets were analyzed by flow cytometry in the peripheral blood as: B cells (Ki-B1R+/CD5–), T cells (Ki-B1R-/CD5+), helper or T_H cells (CD4+/CD5+) and suppressor/cytotoxic or $T_{S/C}$ cells (CD5+/CD8+). The suppliers for the various antibodies were as follows: Ki-B1R-FITC (BMA Biomedicals AG, Augst, Switzerland), CD5-PE (Serotec, Inotech AG, Dottikon, Switzerland), CD5-FITC, CD4-FITC, and CD8-PE

(Pharmingen, Becton Dickinson AG, Basel, Switzerland). Briefly, whole blood was labeled with fluorochrome-conjugated (FITC or PE) monoclonal antibodies to various lymphocyte markers. Subsequently, the red blood cells were lysed with a hypotonic buffer and the samples were immediately analyzed using a FACScan flow cytometer (Beckton Dickinson). The acquired data were then evaluated using the CellQuest® software (Beckton Dickinson).

Splenocyte proliferation

To determine the effect of dexamethasone on spleen cell proliferation, splenocytes from rats injected with dexamethasone or saline solution and killed 24 h later were prepared. The splenocytes were cultured *in vitro* for 3 d in a defined medium in the presence or absence of phytohaemaglutinin. Cells were then incubated with ^{3}H-thymidine for 6 h prior to being harvested and radioactivity counted. Results were expressed as the ratio of radioactivity measured between stimulated and non-stimulated cells.

Statistical analysis

To assess whether the protein level and the presence of CLA have an effect on weight changes and food intake, a *Linear Mixed Effect* model was used [19]. This model takes into account the repeated measurements over time for each rat. We model the protein level and the fatty acid type as fixed effects and the rats as random effect.

For the other parameters, data are expressed as mean ± SEM. Control and Dexa groups were compared using a Student's t-test, whereas for the dietary treated groups, main effects were determined by two-way ANOVA using Systat® version 7.0.1 for Windows. A mean difference was considered significant at $p < 0.05$.

Results

Food intake and body weight change

Food intake was decreased on d 2 following ip injection of dexamethasone, whereas the rats recovered their normal intake by the end of the experimental period (Fig. 1). No difference in food intake was observed either for a given day or between treatments.

All rats lost weight for 2 d following injection with dexamethasone (Fig. 2). Dexamethasone induced an average weight loss of 16 % of initial weight (35.5 g) 48 h post-injection. After d 2, weight gain resumed independently of dietary treatment. However, at the end of the experimental period, the rats were still in a negative balance of body weight gain compared to initiation of dietary treatment.

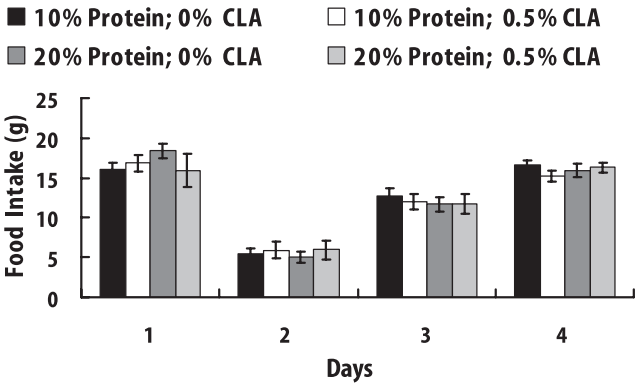

Fig. 1 Food intake of rats fed 4 d either a low (10 g/100 g) or high (20 g/100 g) protein diet with (0.5 g/100 g) or without CLA following ip injection of dexamethasone (120 mg/kg body weight). Values are means ± SEM, n = 9. No effect of dietary protein levels or presence of CLA by Linear Mixed Effect model analyses ($p > 0.05$) was observed

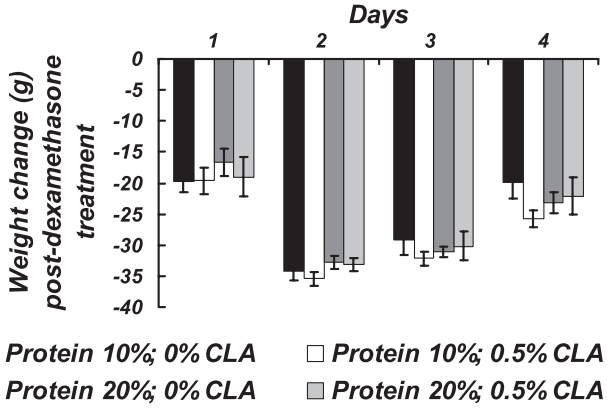

Fig. 2 Body weight change of rats fed 4 d either a low (10 g/100 g) or high (20 g/100 g) protein diet with (0.5 g/100 g) or without CLA following ip injection of dexamethasone (120 mg/kg body weight). Values are means ± SEM, n = 9. No effect of dietary protein levels or presence of CLA by Linear Mixed Effect model analyses ($p > 0.05$) was observed

Liver and thymus weights

Dexamethasone administration (120 mg/kg body weight) produced an average 17.7 % increase in liver weight by 24 h post-injection, whereas liver weight normalized during the refeeding period (Table 2). No effect of either protein level, or CLA level on liver weight was observed. Twenty-four hours post-injection, the average thymus weight was lower (42 %) in rats treated with dexamethasone compared to those receiving the saline solution. The average thymus weight of the saline-injected rats was 540 ± 34 mg (mean ± SE, n = 9). At the end of 4 d of feeding, the average thymus weights were 20–23 %

Table 2 Liver and thymus weights of rats killed 24 h following ip injection of saline solution (Control) or dexamethasone (120 mg/kg body weight; Dexa), or fed 4 d either a low (10 g/100 g) or high (20 g/100 g) protein diet with (0.5 g/100 g) or without CLA following ip injection of dexamethasone[1]

Treatment	Liver (g)	Thymus (mg)
Control	11.3±0.4	540.2±33.9
Dexa	13.3±1.8*	313.4±26.9*
10% protein; 0% CLA[2]	9.4±0.3	107.7±11.1
10% protein; 0.5% CLA	10.1±0.4	114.4±7.8
20% protein; 0% CLA	10.5±0.6	110.4±9.4
20% protein; 0.5% CLA	10.5±0.6	122.4±60.5
2-way ANOVA	p	p
Protein level	0.083	0.535
Fatty acid level	0.435	0.283
Protein x fatty acid	0.326	0.763

[1] Values are means ± SEM, n = 9.
[2] *CLA* conjugated linoleic acid.
* Significantly different from control group by Student's t-test ($p < 0.05$)

that observed in saline injected rats, irrespective of dietary treatment. Dietary treatment had no effect on thymus weight.

Reduced and oxidized liver and muscle glutathione levels

In liver, GSH and GSSG levels were lower in rats injected with dexamethasone compared to those receiving the saline solution (Table 3). Rats fed diets with the high protein level exhibited higher GSH and GSSG levels compared to rats fed diets with the low protein level. The level of dietary CLA had no effect on these concentrations.

In skeletal muscle, dexamethasone had no effect on GSH and GSSG levels 24 h post-injection (Table 3). However, this tissue exhibited lower levels of the two forms of glutathione at the end of the experimental period compared to initial values. Dietary treatment had no effect on the levels of either forms of glutathione.

Protein fractional synthesis rate

Total gut mucosa, muscle and thymus protein FSR (%/d) were significantly lower ($p < 0.05$) by 19%, 15% and 70%, respectively, in dexamethasone-treated rats, whereas the average total liver FSR was 61% higher in dexamethasone-treated rats compared to controls at 24 h post-injection (Table 4). On the other hand, total spleen FSR was unaffected by the glucocorticoid.

Dietary treatment had no effect on FSR of either gut mucosa, liver, muscle or thymus (Table 4). In these tissues, FSR values were lower at the end of dietary treatment compared to those from saline-injected rats. On the other hand, rats fed the high protein diets (20 g/100 g protein) exhibited a 22% higher spleen FSR compared to rats fed diets containing the low protein level (10 g/100 g). Similarly, rats fed the CLA diets (0.5 g/100 g) exhibited a 33% higher spleen FSR compared to rats fed diets not supplemented with CLA. Only rats fed the high protein, high CLA diet exhibited an FSR similar to values from saline-injected rats.

Blood cell counts

Dexamethasone administration produced a drastic decrease in the number of all circulating white blood cell populations and subsets as early as 24 h after injection (Table 5). Feeding the experimental diets led to a general

Table 3 Liver and muscle glutathione concentrations of rats killed 24 h following ip injection of saline solution (Control) or dexamethasone (120 mg/kg body weight; Dexa), or fed 4 d either a low (10 g/100 g) or high (20 g/100 g) protein diet with (0.5 g/100 g) or without CLA following ip injection of dexamethasone[1]

Treatment	Liver		Muscle	
	GSH (μmol/g)	GSSG (μmol/g)	GSH (μmol/g)	GSSG (μmol/g)
Control	7.38±0.30	0.096±0.008	0.84±0.05	0.020±0.004
Dexa	6.05±0.35*	0.071±0.003*	0.84±0.05	0.014±0.002
10% protein; 0% CLA[2]	4.43±0.27	0.073±0.013	0.52±0.04	0.009±0.001
10% protein; 0.5% CLA	4.42±0.23	0.053±0.005	0.52±0.02	0.008±0.000
20% protein; 0% CLA	5.35±0.18	0.072±0.005	0.54±0.03	0.009±0.001
20% protein; 0.5% CLA	5.96±0.18	0.081±0.005	0.59±0.02	0.009±0.001
2-way ANOVA	p	p	p	p
Protein level	< 0.001	< 0.001	0.13	0.58
Fatty acid level	0.18	0.83	0.40	0.70
Protein x fatty acid	0.17	0.09	0.33	0.84

[1] Values are means ± SEM, n = 9.
[2] *CLA* conjugated linoleic acid.
* Significantly different from control group by Student's t-test ($p < 0.05$)

Table 4 Fractional synthesis rate of total protein in gut mucosa, liver, muscle, spleen and thymus of rats killed 24 h following ip injection of dexamethasone (120 mg/kg body weight; Dexa), or fed 4 d either a low (10 g/100 g) or high (20 g/100 g) protein diet with (0.5 g/100 g) or without CLA following ip injection of dexamethasone[1]

Treatment	Gut mucosa	Liver	Muscle	Spleen	Thymus
Control	119.6±7.7	84.7±5.1	17.6±1.3	59.4±5.4	76.1±3.2
Dexa	96.6±6.0*	136.5±5.1*	15.0±1.4*	60.6±8.7	22.4±0.9*
10% protein; 0% CLA[2]	101.3±5.9	52.5±4.1	13.7±1.3	39.6±2.0	40.3±2.6
10% protein; 0.5% CLA	101.5±4.5	56.3±2.6	13.4±1.3	48.3±1.7	37.2±2.1
20% protein; 0% CLA	106.4±6.9	58.7±3.3	11.2±1.1	44.4±1.3	43.3±1.5
20% protein; 0.5% CLA	113.5±4.4	54.0±2.7	13.7±1.7	63.0±3.4	39.9±1.9
2-way ANOVA	p	p	p	p	p
Protein level	0.129	0.551	0.438	0.001	0.185
Fatty acid level	0.511	0.890	0.414	< 0.001	0.141
Protein x fatty acid	0.534	0.200	0.318	0.061	0.940

[1] Values are in %/d, means ± SEM, n = 9.
[2] *CLA* conjugated linoleic acid.
* Significantly different from control group by Student's t-test ($p < 0.05$)

Table 5 White bood cell populations and subsets of rats killed 24 h following ip injection of saline solution (Control) or dexamethasone (120 mg/kg body weight; Dexa)[1]

Cell type	Control	Dexa
Total white blood cells	5778±559	589±90**
Lymphocytes	4350±360	148±14**
T cells	2398±163	37±6**
T_H cells	1692±135	33±5**
$T_{S/C}$ cells	754±50	7±1**
B cells	1344±155	9±2**
Polymorphonuclear granulocytes	1008±203	402±73*
Monocytes	362±32	27±5**
Eosinophilic granulocytes	45±13	2±1*

[1] Values are number of cells/µL blood, means ± SEM, n = 9.
Significantly different from control group by Student t-test (* $p < 0.05$; ** $p < 0.001$)

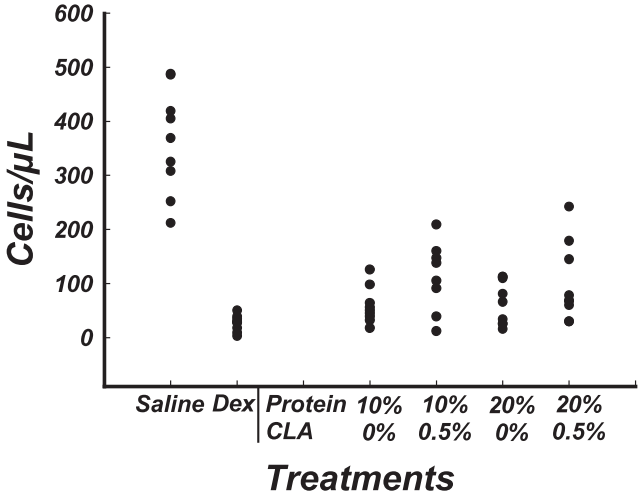

Fig. 3 Monocyte counts from rats killed 24 h following ip injection of saline solution (Control) or dexamethasone (120 mg/kg body weight; Dexa), or fed 4 d either a low (10 g/100 g) or high (20 g/100 g) protein diet with (0.5 g/100 g) or without CLA following ip injection of dexamethasone (120 mg/kg body weight). Values are means ± SEM, n = 9 except for 10 % protein, 0.5 % CLA group, n = 8. The effect of CLA was significant by 2-way ANOVA ($p < 0.05$)

recovery of all affected cell populations, although cell counts were still very far from those observed in control rats (data not shown). Except for the monocytes, no difference among the experimental groups was observed, suggesting a time related spontaneous recovery. Monocyte count was higher in the CLA fed groups compared to those fed diet without CLA (Fig. 3). All dietary treated groups considered, total white blood cell counts were 1484 ± 108 cells/µL of blood (mean ± SEM) after the 4 d feeding representing 252% of the dexamethasone-treated control (589 ± 90 cells/µL) and 26% of non-treated animals (5778 ± 559 cells/µL).

Splenocyte proliferation

The proliferation of spleen cells, cultured in the presence or absence of phytohaemaglutinin, from rats receiving dexamethasone and killed 24 h post-injection did not differ from those receiving a saline solution (data not shown). Similarly, no effect of dietary treatment on spleen cell proliferation was observed.

Discussion

The present study was performed to resolve whether short-term CLA ingestion following a stress altered protein turnover and immunologic functions in a rat model of catabolism and immunodepletion. The end goal was to assess the potential of CLA for inclusion in clinical products aimed at patients with a depleted immune system.

The administration of high doses of dexamethasone, such as in this study, has dramatic immunodepleting

and catabolic effects, resulting in a marked decrease in food intake and body weight as previously described [20–22]. The average daily food intake of rats fed the experimental diets during the following 4 d after dexamethasone administration ranged from 10.2 to 12.4 g, while food intake of control rats (not treated with dexamethasone) was 16.3 g.

Data related to the effects of CLA on food intake and growth in unstressed rats are controversial. Rats showed enhanced weight gain and improved feed efficiency (gram of body weight gain/gram of food intake) when fed a 0.5 g/100 g CLA-enriched diet [23]. On the other hand, mice fed diet supplemented with CLA at levels of 0.5 g/100 g [24] or 0.75 to 1.0 g/100 g [6, 25] exhibited reduced food intake and weight gain. Finally, mice fed CLA (0.5 to 1.5 g/100 g) exhibited lower weight gain than those fed no CLA four months following tumor promotion by phorbol ester [26]. On the other hand, a prevention by CLA feeding of growth depression in response to immune stimulation was observed for chicks, mice and rats [4, 6]. More recently, CLA limited the body weight loss associated with the autoimmune symptoms of NZB/W F1 mice in the presence of proteinuria [27]. On the other hand, body weight was lower in the CLA supplemented group compared to control group at the time proteinuria first developed. The mechanisms involved in mediating changes in body weight remain to be demonstrated. In the present study, feeding CLA acutely at the time of dexamethasone injection did not prevent the growth depression mediated by dexamethasone, and did not prevent the drastic decreased in food intake that occurred 2 d post-injection.

Dexamethasone administration produced a dramatic increase in liver weight as soon as 24 h post-injection. The liver weight was normalized 4 d after injection in rats fed the experimental diets, irrespective of the diet used. The increase in liver weight (18 %) is in a good agreement with the liver protein FSR data. Twenty-four hours after injection, liver protein FSR (%/d) was 61 % higher than the value observed in control rats. The present measurements of protein synthesis represented total liver proteins, including both fixed liver proteins and those exported, such as the acute phase proteins. Glucocorticoid treatment increases the synthesis of these inflammatory proteins [20] which would be responsible for the overall liver protein FSR increase, rather than increasing the synthesis of liver constitutive proteins. Liver weights and liver protein FSR values were either normalized or lower 4 d after dexamethasone injection, independent of dietary treatment.

Glucocorticoid administration caused a decrease in jejunum mucosa protein FSR (–19 %). This was already observed by others in pigs and rats [28, 29]; these authors reported that the protein metabolism of the small intestine is highly sensitive to dexamethasone administration producing a net protein catabolism resulting from a decreased protein synthesis and an increased protein breakdown.

Thymus weight was decreased after dexamethasone treatment and further decreased by 4 d after the dexamethasone injection (–70 %). Thymus protein FSR decreased very rapidly 24 h after glucocorticoid administration, whereas values started to increase by 4 d of dietary treatment. However, this was an effect of time on recovery as no effect of either of the dietary treatments was observed. The present results are in agreement with reported data that indicate that glucocorticoid treatment induces murine thymus apoptosis, reduces thymus weight in chicken and decreases rat thymus rRNA synthesis rate [30–32].

Spleen protein FSR was not affected by dexamethasone treatment (120 mg/kg body weight) 24 h post-injection compared to saline-injected rats. Diets containing either high levels of proteins or CLA affected spleen protein FSR relative to diets containing low levels of proteins or CLA, respectively. Only the spleen from rats fed the diet containing 20 g/100 g protein and 0.5 g/100 g CLA exhibited an FSR similar to control rats. However, it is not possible to differentiate whether this diet maintained the protein FSR following dexamethasone treatment, or if the other diets had an adverse effect during the 4 d feeding.

The levels of both GSH and GSSG in liver were decreased after dexamethasone treatment, as a result of the oxidative stress produced by the administration of high doses of glucorticoids and, probably, the low availability of amino acid precursors to support reduced GSH synthesis (especially cysteine and glutamine). In contrast, muscle GSH and GSSG were not significantly altered by dexamethasone. As expected, there was a positive effect of feeding the high protein diets (20 g/100 g protein), compared to the low protein diets (10 g/100 g protein), on the replenishment of both GSH and GSSG levels. However, the feeding period after glucocorticoid treatment was not sufficient to return to control basal values, independent of the diet used.

CLA has been reported to stimulate the immune system and to have anti-catabolic properties, however available data are limited. Miller et al. [4] fed mice semi-purified diets with and without CLA (0.5 g/100 g), or the same semi-purified diet supplemented with 0.5 % Menhaden fish oil, for a 15 day period prior to injection with LPS. Of the immune responses measured, none seemed adversely affected by CLA. In other animal experiments, improved phagocytosis [5, 6], increased mitogen-induced blastogenesis [4, 5], enhanced cytotoxic activity and macrophage killing ability [5] have been reported following CLA intake. Chew et al. [7] reported the *in vitro* effect of CLA on porcine blood lymphocytes and murine peritoneal macrophages. CLA stimulated the mitogen-induced lymphocyte proliferation, lymphocyte cytotoxic activity, and the macrophage bactericidal

activity. In contrast, CLA inhibited interleukin-2 production by lymphocytes and suppressed the phagocytic activity of macrophages [7]. In mice fed CLA, the lymphocyte proliferation response varied as a function of the stimuli [33]. Lymphocyte proliferation was enhanced in phytohemagglutinin-induced but not in concanavalin A- or lipopolysaccharide-stimulated cultures. Interleukin-2 production was also increased, whereas lymphocyte cytotoxicity was not affected by CLA treatment. In our study, the drastic decrease in all white blood cell subsets at 24 h post-dexamethasone injection confirmed the immunodepleting effect of dexamethasone [15]. Short-term feeding of CLA following dexamethasone injection resulted in a higher number of total white blood cells, polymorphonuclear cells and monocytes. However, the cell counts remained markedly depressed as compared to the untreated saline-injected control, and the CLA-mediated small effect on the monocyte count is unlikely to be clinically significant. On the other hand, in immunodepleted patients, small improvement may have health benefits. Bassaganya et al. [34] suggested that a 42 d dietary CLA supplementation preceding a disease challenge could have prevented disease-associated growth suppression in pigs based on the linear increase in percentages of CD8 + lymphocytes. Whether or not a preventive approach to CLA feeding would be efficacious remains to be determined.

Spleen cell proliferation in response to phytohemaglutinin was not influenced by dietary treatment in contrast to the study by Kunicka et al. [15]. In the latter study, dexamethasone doses of 30 mg/kg body weight were enough to produce an inhibition of spleen cell proliferation in the presence of suboptimal amounts of mitogen. A possible explanation for not observing an effect of dietary treatment on splenocyte proliferation is that cells have been cultured for 3 d in a defined medium without CLA prior to stimulation. This 3 d period may be sufficient to "wash-out" the potential effect of CLA. In this regard, it has previously been demonstrated that the culture conditions can influence the changes in cells brought about by dietary lipid manipulation, and may therefore influence the outcome of functional tests [35, 36].

In conclusion, the present study was performed to determine the effect of CLA in acute conditions, as opposed to a preventive approach, on the recovery from a catabolic stress with immunodepletion. The dexamethasone-injected rat has been used as a model. Overall, no effect of feeding CLA acutely on the recovery from dexamethasone-mediated immunodepletion was observed. The spleen protein FSR of rats fed the high protein plus CLA diet was maintained or returned to basal levels following dietary intervention. However, it is not possible to eliminate the possibility that the diets containing linoleic acid had an adverse effect resulting in a decreased protein FSR. Indeed, it must be acknowledged that the intake of linoleic acid, precursor of potent bioactive molecules, is higher in the non-CLA supplemented groups due to replacement. The small magnitude of higher numbers of monocytes that occurred in rats fed the CLA enriched diets is unlikely to have any clinical effects. Finally, the effects of feeding CLA prior to the initiation of a stress (preventive approach) remain to be determined.

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