RESEARCH ARTICLE

c9t11-Conjugated linoleic acid-rich oil fails to attenuate wasting in colon-26 tumor-induced late-stage cancer cachexia in male CD2F1 mice

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Scope: Cancer cachexia is characterized by muscle and adipose tissue wasting caused partly by chronic, systemic inflammation. Conjugated linoleic acids (CLAs) are a group of fatty acids with various properties including anti-inflammatory *cis*9, *trans*11 (c9t11)-CLA and lipid-mobilizing *trans*10, *cis*12 (t10c12)-CLA. The purpose of this study was to test whether dietary supplementation of a c9t11-CLA-rich oil (6:1 c9t11:t10c12) could attenuate wasting of muscle and adipose tissue in colon-26 adenocarcinoma-induced cachexia in mice.

Methods and results: Loss of body weight, muscle and adipose tissue mass caused by tumors were not rescued by supplementation with the c9t11-CLA-rich oil. In quadriceps muscle, c9t11-CLA-rich oil exacerbated tumor-induced gene expression of inflammatory markers tumor necrosis factor-α, IL-6 receptor and the E3 ligase MuRF-1 involved in muscle proteolysis. In epididymal adipose tissue, tumor-driven delipidation and atrophy was aggravated by the c9,t11-CLA-rich oil, demonstrated by further reduced adipocyte size and lower adiponectin expression. However, expression of inflammatory cytokines and macrophage markers were not altered by tumors, or CLA supplementation.

Conclusion: These data suggest that addition of c9t11-CLA-rich oil (0.6% c9t11, 0.1% t10c12) in diet did not ameliorate wasting in mice with cancer cachexia. Instead, it increased expression of inflammatory markers in the muscle and increased adipose delipidation.

Keywords:
Adipose atrophy / Cancer cachexia / Colon-26 adenocarcinoma / Conjugated linoleic acid / Muscle

1 Introduction

Cancer cachexia is a syndrome of involuntary and progressive weight loss in advanced cancer patients. It occurs in 30–90% of cancer patients, and negatively affects the quality of life, response to anticancer therapy and survival [1]. Cancer cachexia is characterized by systemic inflammation and believed to be mediated by pro-inflammatory cytokines produced by the tumor and the host. These elevated cytokines – tumor necrosis factor-α (TNF-α), IL-1, IL-6 and IFN-γ alter the metabolism of the host and contribute to the wasting [2]. Adipose tissue is depleted primarily through lipolysis [3]. Skeletal muscle is prominently lost mainly through ubiquitin-proteasome-mediated proteolysis [4]. Both TNF-α and IL-6 have been shown to contribute to fat loss [5–10] and muscle protein degradation *in vivo* and *in vitro* [11–13]. Therefore, pharmaceutical or nutraceutical agents with anti-inflammatory properties have been tested as candidates for cancer cachexia treatment, with promising results [14, 15].

Conjugated linoleic acid (CLA) is a group of positional and stereo-isomers of linoleic acid (18:2n-6) found in red meat and dairy products [16]. Among the 28 different

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Abbreviations: CLA, conjugated linoleic acid; **CSA,** cross-sectional area; **H&E,** hematoxylin and eosin; **MCP-1,** monocyte chemo-attractant protein-1; **TNF-α,** tumour necrosis factor-α

isomers, c9t11-CLA accounts for the majority of CLA in the diet [17]. A mixture of c9t11-CLA and t10c12-CLA (1:1 ratio) is widely used in research and is marketed for humans as a weight-loss supplement (*e.g.* Tonalin, Clarinol). Previous studies have shown that CLA has anti-carcinogenic effects in chemical models of carcinogenesis [18–20], while no such effects were found in genetic carcinogenic models [21]. Numerous studies have also demonstrated the anti-inflammatory properties of CLA [22–25]. The notion that CLA has potential benefits in cancer cachexia was supported with evidence that addition of 0.5% CLA to the diet preserved muscle mass in cachectic mice bearing Lewis Lung carcinoma [26] as well as mice with colon-26 adenocarcinoma [27]. In other studies, 0.5% CLA was protective against TNF-α-induced [22] as well as LPS-induced [23] wasting and the latter was associated with decreased plasma TNF-α levels. However, recent evidence suggests that c9t11-CLA and t10c12-CLA exhibit different biological effects. T10c12-CLA plays a role in antiadipogenesis, and might induce inflammation in white adipose tissue and lead to insulin resistance [28–34]. In contrast, c9t11-CLA inhibits inflammation and insulin resistance both *in vitro* and *in vivo* [23, 35, 36]. Whether dietary addition of an oil rich in c9t11-CLA is beneficial in cancer cachexia is still unclear. Colon-26 adenocarcinoma-induced cancer cachexia in mice is a well-studied model of cancer cachexia, where IL-6 plays a dominant role in cachexia development [37, 38]. Using this model in the present study, we hypothesized that an oil rich in c9t11-CLA would exert anti-inflammatory effects in both skeletal muscles and adipose tissue, and ameliorate wasting in mice with colon-26 adenocarcinoma induced- cachexia.

2 Materials and methods

2.1 Experimental animals and design

Five-wk-old, male CD2F1 mice (BALB/c x DBA/2; Charles River Laboratories, Wilmington, MA, USA) were housed five *per* cage at $22 \pm 0.5^{\circ}C$ on a 12 h light/dark cycle. When mice weighed approximately 20 g, they were randomized by weight to one of four groups: control diet-treated mice without tumors (PBS, $n = 10$), control diet-treated mice with tumors (Tumor, $n = 20$), CLA diet-treated mice with tumors (Tumor CLA, $n = 20$) and control diet-treated mice without tumors (Pair-fed, $n = 10$), pair-fed to match the food intake of tumor CLA mice. Considering both tumor burden and CLA may reduce food intake, the purpose of including the Pair-fed group was to exclude the confounding effect of reduced food intake on changes found in Tumor CLA and Tumor mice compared with PBS mice. On study day 0, the mice were inoculated subcutaneously in the right flank with either 1×10^6 colon-26 adenocarcinoma cells suspended in 100 μL PBS (Tumor and Tumor CLA groups) or vehicle (PBS and Pair-fed groups). Mice were maintained on isocaloric, modified AIN-93G pelleted diets (Research Diets, Brunswick, NJ, USA) containing 7% fat by weight. Diets contained either 7% soybean oil (PBS, Tumor and Pair-fed groups) or 6% soybean oil and 1% c9t11-CLA free fatty acids (C9t11-CLA FFA) (Tumor CLA group). C9t11-CLA FFA (DP999, Lipid Nutrition, Wormerveer, The Netherlands) were 73.8% total CLA isomers composed of 60.9% c9t11- and 12.8% t10c12-CLA isomers. Therefore, the levels of c9t11- and t10c12-CLA equated to 0.6 and 0.1% of diet in weight, respectively. Body weights and food intake were measured daily. When there was a 20% difference in body weight between PBS and Tumor groups (day 17 post-inoculation), mice were sacrificed by cervical dislocation. Adipose tissue, skeletal muscles, organs and tumors were then harvested, weighed, snap-frozen in liquid nitrogen and stored at $-80^{\circ}C$ until analysis. The study was conducted according to the institutional guidelines and all procedures were approved by the Institutional Animal Care and Use Committee of the Ohio State University.

2.2 Real-time RT-PCR

RNA was isolated from epididymal adipose tissue using the RNeasy® Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) and from muscle using TriZol reagent (Invitrogen, Carlsbad, CA). RNA was reverse transcribed with the High Capacity cDNA Archive Kit (ABI, Foster City, CA, USA) according to the manufacturer's protocol. RT-PCR analysis was performed with pre-designed primers from Applied Biosystems (TaqMan Gene Expression Assays). Target gene expression was normalized to the endogenous control (18S rRNA in epididymal adipose and GAPDH in skeletal muscle) and expressed as $2^{-\Delta\Delta CT}$ relative to the PBS group [39].

2.3 Histology

At necropsy, small sections of epididymal adipose tissue were fixed in 4% paraformaldehyde. Histology sections were prepared (OSU Pathology Core Facility) with a thickness of 5 μm and stained with hematoxylin and eosin (H&E) for analysis by light microscopy. For quantification of adipocyte size, three random images from each sample were captured by a blinded evaluator at a magnification of 200× using an Olympus 1 × 50 microscope (Olympus, Center Valley, PA, USA) equipped with a Pixera Pro 150ES digital camera (Pixera, Los Gatos, CA, USA). Using ImageJ software version 1.42q (NIH, Bethesda, MD), the images were converted to binary and 150–160 adipocytes from the three images *per* sample were measured for cross-sectional area (CSA) and perimeter using the "analyze particle" function.

2.4 Statistical analysis

Data are presented as mean ± SEM. Differences among PBS, Tumor and Tumor CLA groups were tested by one-way

ANOVA followed by *post hoc* Tukey's test using MINITAB15 (State College, PA, USA). Daily body weights values were measured with one-way ANOVA using repeated analysis with SYSTAT 12 (Chicago, IL, USA). Comparison of Tumor or Tumor CLA group with Pair-fed group was performed by unpaired Student's *t*-tests. Differences of $p<0.05$ were considered significant.

3 Results

3.1 Effect of CLA on body weight and tissue weight in mice with cancer cachexia

There was no significant difference among groups in initial body weight, and PBS group had significantly greater final body weight compared with the rest of the groups (Table 1). Mice in the PBS group exhibited continual growth over the course of the study (Fig. 1A). The body weight gain in both Tumor and Tumor CLA groups was positive for the first 13 days then negative throughout the rest of the study, indicating an earlier growth followed by body weight loss. As early as day 2, there was a significantly lower body weight gain in the Tumor CLA group compared with the PBS group. Starting at day 9, there was a significantly lower weight gain in the Tumor group compared with the PBS group (Fig. 1A). Because food intake is reduced in this model of cancer cachexia [37, 40], and CLA is also reported to reduce food intake in rodents [41], a group of mice on the control diet was pair-fed to the Tumor CLA group. Body weight gain in the Pair-fed group was negative throughout the study, suggesting that food restriction prevented growth in these mice. However, the body weight at the end of the study was not different between Pair-fed and the two tumor-bearing groups (Fig. 1B). Food intake was similar in the Tumor and Tumor CLA groups, and it was about 70% of that consumed by mice in PBS group (data not shown).

Both the Tumor and Tumor CLA groups had significantly lower skeletal muscle and epididymal adipose tissue masses compared with the PBS group (Table 1). Although pair-fed mice lost similar amount of body weight, their muscle and adipose tissue weights were markedly higher compared with mice in the Tumor and Tumor CLA groups, suggesting that food restriction alone was not sufficient to induce wasting. Absolute weight of heart and liver were lower in the Tumor and Tumor CLA mice compared with PBS mice. Compared with pair-fed mice, the liver weight was significantly higher in mice of Tumor and Tumor CLA groups in terms of both absolute weight and normalization to body weight. Spleen weight was also significantly higher in Tumor and Tumor CLA mice compared with PBS or Pair-fed mice, which is consistent with the increased inflammation status in tumor-bearing mice. There was no difference in tumor weight with or without the addition of CLA in the diet (Table 1).

Table 1. Body, tissue and organ weights[a)]

	Absolute weight				Percent body weight			
	PBS ($n=10$)	Tumor ($n=18$)	Tumor CLA ($n=20$)	Pair-fed ($n=10$)	PBS ($n=10$)	Tumor ($n=18$)	Tumor CLA ($n=20$)	Pair-fed ($n=10$)
Initial body weight (g)	20.8±0.5	19.8±0.4	20.8±0.2	20.8±0.4				
Final body weight (g)	23.4±0.6^{a}	17.6±0.3^{b}	17.7±0.3^{b}	17.9±0.5				
Quadriceps muscle (mg)	310.9±8.6^{a}	214.7±8.4^{b*}	225.2±7.0^{b*}	295.7±11.5	1.34±0.04	1.22±0.04*	1.27±0.03*	1.65±0.05
Gastrocnemius muscle (mg)	236.1±7.6^{a}	173.0±6.9^{b*}	177.5±3.9^{b*}	220.6±6.0	1.02±0.04	0.98±0.03*	1.00±0.02*	1.24±0.02
Tibialis anterior muscle (mg)	92.0±3.7^{a}	72.8±2.8^{b*}	77.7±3.1^{b*}	91.1±5.2	0.40±0.02	0.42±0.01*	0.44±0.02*	0.51±0.02
Epididymal adipose (mg)	734.2±52.6^{a}	101.3±28.1^{b*}	36.1±6.2^{b*}	351.5±38.3	3.12±0.17^{a}	0.55±0.15^{b*}	0.20±0.03^{b*}	1.93±0.17
Heart (mg)	117.4±4.9^{a}	91.7±2.8^{b}	98.8±3.0^{b}	99.4±2.8	0.50±0.02	0.52±0.01*	0.56±0.01	0.56±0.01
Liver (mg)	964.5±36.3^{a}	781.8±26.1^{b*}	797.2±25.8^{b*}	588.8±24.0	4.14±0.14	4.45±0.11*	4.48±0.11*	3.29±0.05
Spleen (mg)	66.8±2.6^{a}	151.4±12.7^{b*}	147.2±8.6^{b*}	46.7±3.3	0.29±0.01^{a}	0.86±0.07^{b*}	0.82±0.04^{b*}	0.26±0.02
Tumor (g)	–	1.73±0.11	1.99±0.12	–	–	9.97±0.68	11.27±0.71	--

Initial and final body weights, weights of muscles, adipose and tumor are expressed as absolute weight and as percentage of body weight (including tumor weight for tumor groups). Muscle weights are the sum of both hind limbs. Difference among PBS, tumor and tumor CLA groups were tested by one-way ANOVA and different letters indicate significant difference ($p<0.05$). Comparisons of tumor *versus* pair-fed as well as tumor CLA *versus* pair-fed were performed by unpaired *t*-tests and significant differences are denoted by*.

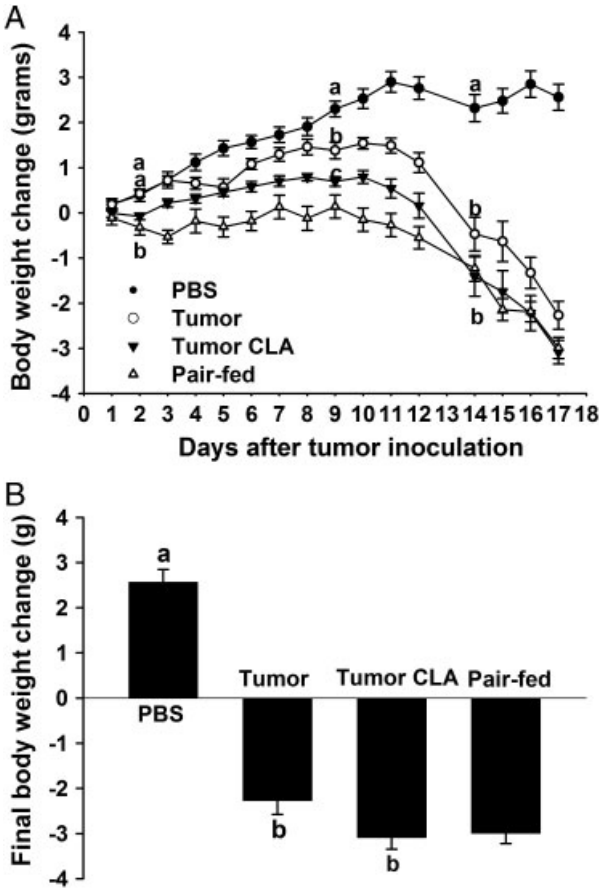

Figure 1. Body weight gain. Mice were inoculated with PBS (PBS, $n = 10$; Pair-fed, $n = 10$) or colon-26 adenocarcinoma (Tumor, $n = 20$; Tumor CLA, $n = 20$) and fed with either control diet (PBS, Pair-fed, Tumor) or CLA supplemented diet (Tumor CLA). (A) Body weight gain over time. Values represent means ± SEM. Difference among PBS, Tumor and Tumor CLA groups were tested by one-way ANOVA and different letters indicate the first day that a significant difference ($p < 0.05$) was observed. Such differences were maintained in the following days unless otherwise denoted. (B) Final body weight gain at the day of necropsy (day 17 after tumor/PBS inoculation). Significant differences among PBS, Tumor and Tumor CLA groups are denoted by different letters. Unpaired *t*-tests were performed to compare the Tumor and Tumor CLA with Pair-fed group, and no significant differences were found.

3.2 Effect of CLA on skeletal muscles in mice with cancer cachexia

CLA did not inhibit the loss of skeletal muscle mass in mice with cancer cachexia (Table 1). To determine whether CLA affected the proteolysis at the molecular level, gene expression of proteolytic markers were measured. Atrogin-1 and MuRF-1 are E3 ligases involved in proteasomal proteolysis while Bnip3 is critical in lysosomal proteolysis. These genes are induced in cancer cachexia and regulated by pro-inflammatory cytokines [40, 42]. In the quadriceps muscle, gene expression of Atrogin-1, MuRF-1 and Bnip3 were increased 290-, 29- and 95-fold in Tumor mice compared with the PBS group, respectively (Fig. 2). The addition of CLA in the diet of Tumor mice did not inhibit the induction of these proteolytic markers. Instead, expression of MuRF-1

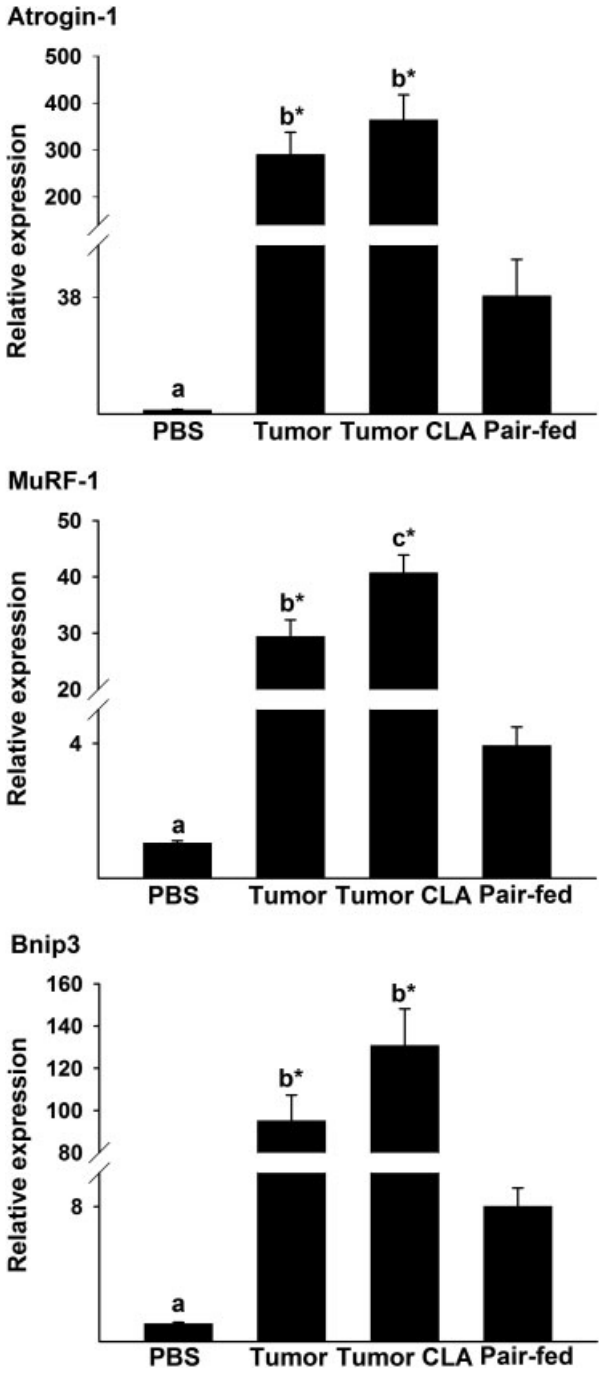

Figure 2. Expression of genes involved in proteolysis in quadriceps muscle by real-time RT-PCR. Different letters indicate significant differences ($p < 0.05$) among PBS ($n = 10$), Tumor ($n = 18$) and Tumor CLA ($n = 20$) groups tested by one-way ANOVA. * indicates a significant difference ($p < 0.05$) compared with the Pair-fed group ($n = 10$) tested by unpaired *t*-tests.

was significantly higher in the Tumor CLA group compared with the Tumor group. There was a slightly increased expression of Atrogin-1, MuRF-1 and Bnip3 in the Pair-fed group, but to a markedly smaller extent compared with the two groups of tumor-bearing mice (Fig. 2). Similar patterns were observed in transcript levels of inflammatory markers in quadriceps muscle (Fig. 3). IL-6 and IL-6 receptor mRNA expression were significantly increased by 2.5- and 26-fold, respectively, in Tumor mice compared with PBS group. Addition of CLA in tumor-bearing mice did not inhibit the induction of inflammation. On the contrary, Tumor CLA mice exhibited significantly higher induction of TNF-α, IL-6 receptor and the macrophage marker, F4/80 compared with mice in the Tumor group (Fig. 3). These data suggest that CLA supplementation may potentially increase inflammation rather than inhibit inflammation in muscles as hypothesized.

3.3 Effect of CLA on white adipose tissue in mice with cancer cachexia

Light microscopy examination of H&E stained epididymal adipose tissue revealed profound morphological alterations, including smaller adipocytes in the Tumor group compared with the PBS group, leaving enlarged interstitial space among adipocytes. Supplementation with CLA in tumor-bearing mice resulted in a larger extent of adipocyte shrinkage and delipidation (Fig. 4A). Morphometric analysis confirmed that both the CSA and perimeter of adipocytes were significantly smaller in the Tumor CLA group compared with Tumor group, and both Tumor groups had substantially reduced adipocyte size compared with the Pair-fed group (Figs. 4B and C).

In support of the morphological analysis in white adipose tissue, we found a dramatic reduction in gene expression of adipose tissue-derived adipokines in Tumor and Tumor CLA mice (Fig. 5A). Adiponectin and leptin are both markers of mature adipocytes. Adiponectin transcript levels in the Tumor and Tumor CLA groups were reduced by 66 and 78% compared with the PBS group, and both were significantly reduced compared with the Pair-fed group. Leptin transcripts in the Tumor and Tumor CLA groups were almost 100% depleted compared with the PBS mice. Expression of adiponectin and leptin mRNA in the Pair-fed group was lower compared with PBS mice, but substantially higher than the two tumor-bearing groups (Fig. 5A).

In order to explore whether adipose tissue-derived inflammatory cytokines are involved in the adipose atrophy, we detected gene expression of IL-6, TNF-α and monocyte chemo-attractant protein-1 (MCP-1). There was no significant difference in IL-6 transcript levels among the PBS, Tumor and Tumor CLA groups. Unexpectedly, gene expression of TNF-α and MCP-1 were significantly reduced in tumor-bearing mice (Tumor and Tumor CLA groups) compared with PBS mice (Fig. 5B). MCP-1 has been demonstrated to recruit macrophages to adipose tissue during inflammation [43]. Consistently, the gene expression of macrophage markers, F4/80 and CD68, were not found to be elevated in tumor-bearing mice (Fig. 5C).

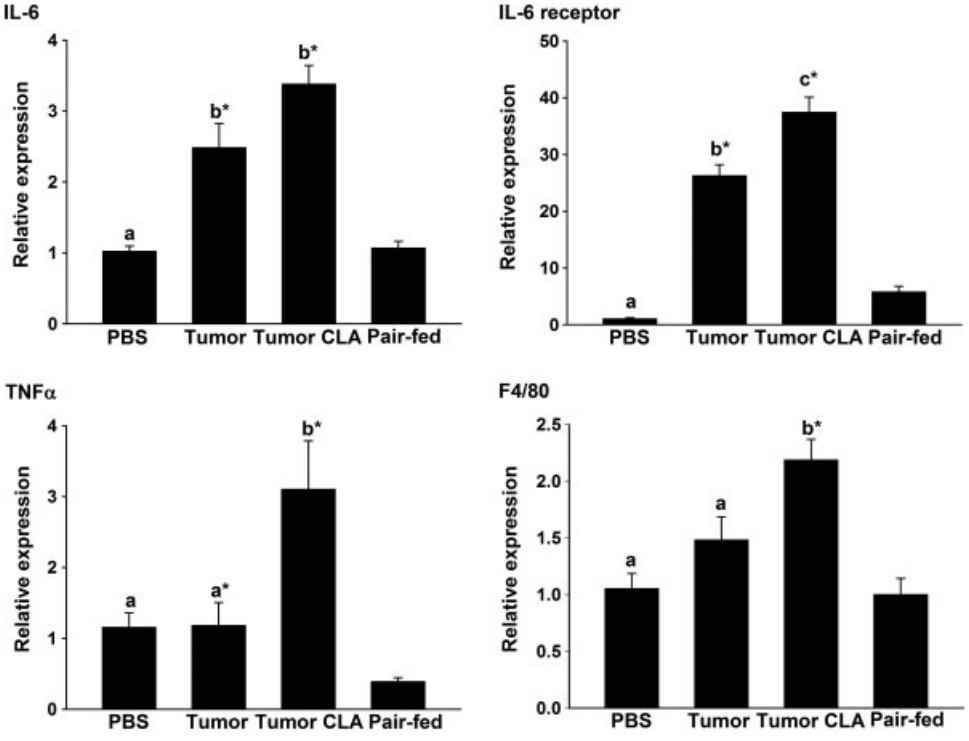

Figure 3. Expression of genes involved in inflammation in quadriceps muscle by real-time RT-PCR. Different letters indicate significant differences ($p<0.05$) among PBS ($n=10$), Tumor ($n=18$) and Tumor CLA ($n=20$) groups tested by one-way ANOVA. * indicates a significant difference ($p<0.05$) compared with the Pair-fed group ($n=10$) tested by unpaired *t*-tests.

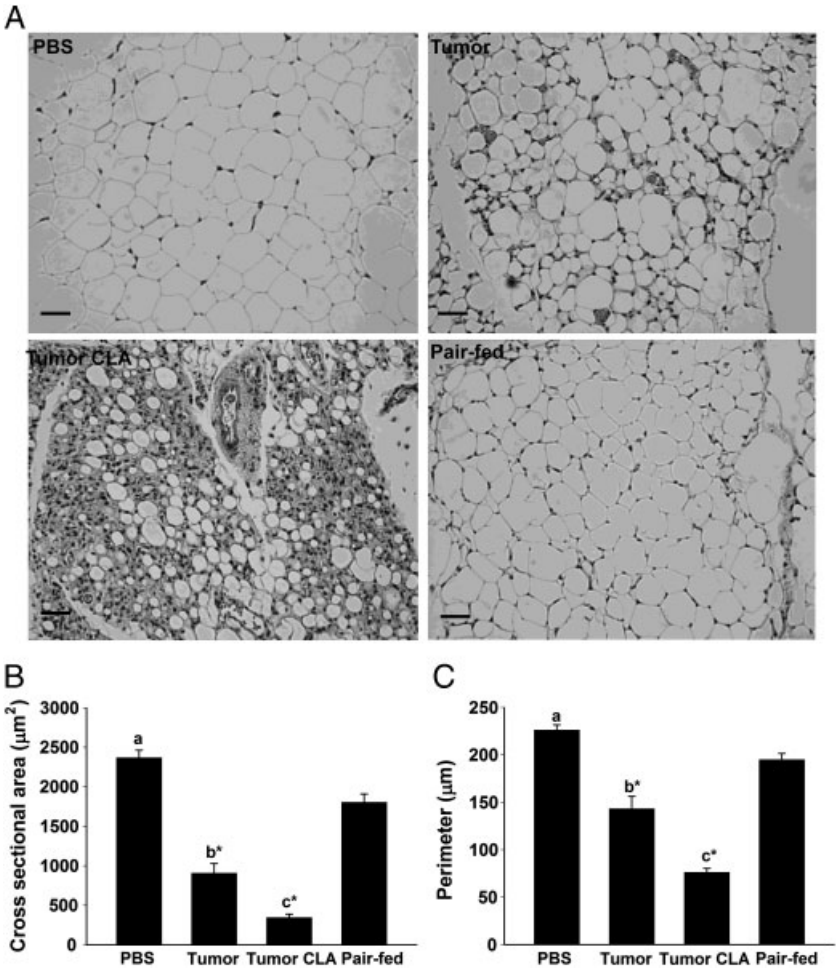

Figure 4. Morphological evaluation of epididymal adipose tissue. (A) H&E stained adipose tissue observation by light microscopy. Images are 200 × magnification. Scale bar = 50 μm (B) CSA measurement of adipocytes of epididymal adipose tissue. (C) Perimeter measurement of adipocytes of epididymal adipose tissue. Different letters indicate significant differences ($p<0.05$) among PBS ($n=10$), Tumor ($n=11$) and Tumor CLA ($n=9$) groups tested by one-way ANOVA. * indicates a significant difference ($p<0.05$) compared with the Pair-fed group ($n=10$) tested by unpaired *t*-tests.

4 Discussion

This study evaluated the effects of an oil rich in c9t11-CLA on skeletal muscle and white adipose tissue wasting in mice with cancer cachexia. We utilized male CD2F1 mice inoculated with colon-26 adenocarcinoma, a well-established model of cachexia. Cancer cachexia develops quickly in this model, characterized by ~20% body weight loss in 17 days as well as severe adipose tissue atrophy and muscle loss. Higher relative liver and spleen weight are in accordance with the increased synthesis of acute phase proteins and high inflammation status reported in cancer cachexia [44].

Total food intake over the course of the study was reduced by 30% in both groups of tumor-bearing mice compared with mice inoculated with vehicle (PBS). The pair-fed mice had a similar body weight loss as the tumor-bearing mice. However, their muscle and adipose tissue mass were substantially higher, suggesting that tumor burden induced a much greater extent of muscle and adipose tissue loss than food restriction. Additionally, the liver and spleen in pair-fed mice were smaller, and expression of inflammatory cytokines in muscle and adipose tissue was lower compared with tumor-bearing mice. Unlike the delipidated adipocytes in tumor-bearing mice, adipocytes in pair-fed mice were spherical and without interstitial space. Together, these data provide evidence that reduced food intake alone is not sufficient to induce a cachectic state.

Addition of the c9t11-CLA-rich oil at 1% of the diet (equivalent to 0.6% of c9t11-CLA and 0.1% t10c12-CLA isomers in the diet) of tumor-bearing mice did not inhibit tumor growth or improve body weight, skeletal muscle or adipose tissue weight. On the contrary, the c9t11-CLA-rich oil exacerbated tumor-induced gene expression of inflammatory markers IL-6 receptor, TNF-α and F4/80 as well as the E3 ligase MuRF-1 involved in proteolysis in quadriceps muscles. While the adipose tissue weight was nonsignificantly decreased, adipocyte size was more severely diminished in the Tumor CLA group compared with the Tumor group. In a previous study using the same animal model, dietary addition of 0.5% CLA preserved skeletal muscle mass in mice [27]. Possible reasons for the discrepancies between our results and the former study include differences in: (i) gender of the animal models; (ii) tumor growth; (iii) duration and dose of CLA supplement and/or

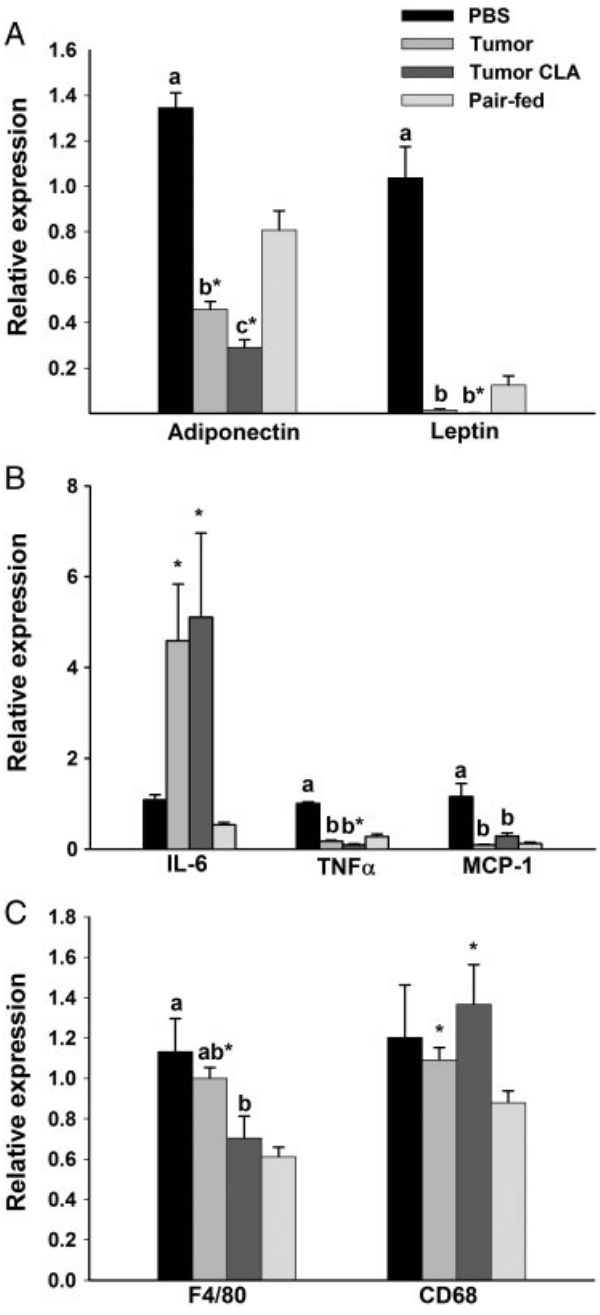

Figure 5. Real-time RT-PCR analysis of adipose tissue-derived (A) adipokines, (B) proinflammatory cytokines and (C) markers of macrophage infiltration in epididymal adipose tissue. Different letters indicate significant differences ($p<0.05$) among PBS ($n=10$), Tumor ($n=18$) and Tumor CLA ($n=20$) groups tested by one-way ANOVA. * indicates a significant difference ($p<0.05$) compared with the Pair-fed group ($n=10$) tested by unpaired *t*-tests.

(iv) composition of diet including ratio of CLA isomers and added tocopherols. The former study utilized female CD2F1 mice that appeared to be more resistant to wasting than the male mice used in our study (McCarthy D., unpublished observation). The female mice were sacrificed with tumor weight less than 5% of body weight on day 21 while in our study the tumors weighed ~10% of body weight on day 17 at necropsy. Finally, the former study initiated the 0.5% CLA (equivalent to 0.2% c9t11- and 0.2% t10c12-CLA by diet weight, personal communication with McCarthy D) diet containing 250 ppm tocopherols 2 wk before tumor inoculation while our study started the 1% CLA (equivalent to 0.6% c9t11- and 0.1% t10c12-CLA by diet weight) diet on the same day as tumor inoculation. In the literature there are reports of CLA with both positive and negative effects in animals with cancer cachexia. McCarthy and Graves and McCarthy-Beckett *et al.* reported that 0.5% of CLA in the diet preserved muscle mass in mice with Lewis Lung carcinoma, but had no effect in mice with B16 melanoma or in rats with Morris 7777 hepatoma [26, 45]. Wong *et al.* demonstrated that supplementation with 0.3% or 0.9% CLA did not affect mammary tumor growth or body weight in mice with mammary tumors. They pointed out that dietary CLA may modulate the immune defense to prevent tumorigenesis but had no obvious effect on the growth of established, aggressive tumors [46].

In our study, adipose tissue atrophy in tumor-bearing mice is associated with decreased expression levels of leptin and adiponectin, which is consistent with previous reports [40, 47–50]. Since adipose tissue also produces inflammatory cytokines including TNF-α and IL-6, they may play a role in adipose tissue loss in an autocrine and/or paracrine manner. However, it has been shown that TNF-α and IL-6 mRNA levels in white fat are unaffected in MAC16-induced cachectic mice as well as cancer cachexia patients [3, 47, 51]. Our results also showed no significant change in IL-6 mRNA level, but decreased TNF-α level. This may be explained by the late stage of cachexia when tumors accounted for about 10% of the body weight. It is possible that the adipose tissue atrophy was so severe at this stage that it could not maintain normal capacity of expressing inflammatory cytokines. Additionally, the markers of macrophages were not elevated. Our group has found evidence of increased plasma IL-6 levels in tumor-bearing mice compared with mice inoculated with PBS (unpublished data). Therefore, the delipidation of adipose tissue in tumor-bearing mice may be primarily mediated by circulating inflammatory cytokines rather than adipose-derived cytokines. Notably, adding 1% of c9t11-CLA-rich oil tended to worsen the adipose delipidation more profoundly in tumor-bearing mice, demonstrated by the further decreased adipocyte size and adiponectin expression. This could be attributed to the small amount (0.1% in diet weight) of t10c12-CLA in the diet, because t10c12-CLA is believed to decrease lipogenesis and adipogenesis in both cultured adipocytes and adipose tissue of mice [29]. We have observed that 0.1% t10c12-CLA reduced adiposity in growing healthy CD2F1 male mice (unpublished data). Others have also reported fat loss in healthy mice fed with diet containing ≤0.1% t10c12-CLA isomer [52, 53].

Generally, loss of adipose mass occurs earlier and more rapidly than muscle loss in both cancer cachexia patients and mice with colon-26 tumors [54, 55]. Greater loss of adipose compared with muscle was also seen in the tumor-bearing mice in our study. Although the loss of skeletal muscle mass in tumor-bearing mice was not altered by addition of CLA, the expression of MuRF-1 was significantly higher in the Tumor CLA group compared with the Tumor group. At the same time, markers of inflammation and macrophages were induced by CLA in muscles of tumor-bearing mice. There is evidence that macrophage infiltration into muscle could be stimulated by inflammatory cytokines [56] and contributes to muscle atrophy [57]. Therefore, it is possible as cachexia progresses further, the CLA treated tumor-bearing mice may have more severe loss of muscle mass compared with mice in the Tumor group.

In summary, supplementation with the c9t11-CLA-rich oil (0.6% c9t11 and 0.1% t10c12 CLA isomers in diet) did not ameliorate the wasting in late-stage cancer cachexia. Instead, the addition of c9t11-CLA-rich oil caused more severe adipose atrophy and further increased expression of inflammatory markers in the muscle. It is possible the lack of protective effects of CLA on cachexia in this study is attributed to the small amount of t10c12-CLA isomer in the CLA mixture, as t10c12-CLA has been reported to have antiadipogenic and proinflammatory effects in mouse models for obesity [28, 36]. In order to clarify whether t10c12-CLA is responsible for these debilitating effects, it is necessary to test the effects of a series of CLA oils with ascending concentration of t10c12-CLA isomers on cancer cachexia. The present data suggest more research is required before CLA supplementation is recommended for treatment of late stage cancer cachexia.

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The authors have declared no conflict of interest.

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